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ORIGINAL ARTICLE

Mercury in Tree Swallow Food, Eggs, Bodies, and Feathers at Acadia National Park, Maine, and an EPA Superfund Site, Ayer, Massachusetts

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Abstract We monitored nest boxes during 1997–1999 at Acadia National Park, Mt. Desert Island, ME and at an old-field site in Orono, ME to determine mercury (Hg) uptake in tree swallow (*Tachycineta bicolor*) eggs, tissues, and food boluses. Also, in 1998–1999 we monitored nest boxes at Grove Pond and Plow Shop Pond at a U.S. Environmental Protection Agency Superfund site in Ayer, MA. We recorded breeding success at all locations. On average among locations, total mercury (THg) biomagnified 2 to 4-fold from food to eggs and 9 to 18-fold from food to feathers. These are minimum values because the proportion of transferable methyl mercury (MeHg) of the THg in insects varies (i.e., 35%–95% of THg) in food boluses. THg was highest in food boluses at Aunt Betty Pond at Acadia, whereas THg in eggs was highest at the Superfund site. A few eggs from nests at each of these locations exceeded the threshold (i.e., 800–1,000 ng/g, wet wt.) of embryotoxicity established for Hg. Hatching success was 88.9% to 100% among locations, but five eggs failed to hatch from 4 of the 11 clutches in which an egg exceeded this threshold. MeHg in feathers was highest in tree swallows at Aunt Betty Pond and the concentration of THg in bodies was related to the concentration in feathers. Transfer of an average of 80%–92% of the Hg in bodies to feathers may have enhanced nestling survival. Residues of Hg in tissues of tree swallows in the Northeast seem higher than those of the Midwest.

Keywords Acadia National Park · Biomagnification · Embryotoxicity · Hatching success · Fledging success · ME · Mercury · Methyl mercury · MA · Superfund site · *Tachycineta bicolor* · Tree swallow

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1 Introduction

Contamination of water resources in Acadia National Park (ANP) are attributed to non-point sources of atmospheric deposition of acids and toxic substances, including heavy metals, because lakes sampled are remote from points of mercury discharge (Akielaszek & Haines, 1981). Concentrations of mercury ranged from 530 to 2,800 ng/g in five chain pickerel (*Esox niger*) and 1,000 ng/g in one white perch (*Morone americana*) from Seal Cove Pond and mercury averaged (LS Means) 2,100 ng/g in seven smallmouth

bass (*Micropterus dolomieu*) from Hodgdon Pond with mercury levels of 3,900, 3,400, 2,800, and 2,300 ng/g in individual fish (T.A. Haines, unpublished data; see also Bank, Burgess, Evers, & Loftin, 2005). The aquatic invertebrates that fish eat are also eaten by the insectivorous tree swallow (*Tachycineta bicolor*) (Quinney & Ankney, 1985; Holroyd, 1983). Primary foods of nestling tree swallows in Ontario were mayflies (Ephemeroptera, *Hexagenia*), aquatic Diptera (especially Nematocera midges), dragonflies (Odonota), and Lepidoptera (Holroyd, 1983). At another location in Ontario, important prey species in nestling tree swallow diets were chironomid midges (35%), several mayfly genera, and homopterans, but horseflies (*Tabanus*, 48%) and burrowing mayflies (*Hexagenia*, 13.4%) were most important on a dry weight basis (Blancher & McNicol, 1991). Aquatic insects readily take up mercury (Hall, Rosenberg, & Wiens, 1998; Tremblay, Lucotte, Meili, Cloutier, & Pichet, 1996). Because tree swallows breed extensively throughout North America, accept boxes for nesting (Robertson & Rendell, 1990), and have been used as a monitor of MeHg dynamics related to experimental acidification of wetlands (St. Louis et al., 1996), the tree swallow is recognized as a new model organism for research (Jones, 2003).

We sampled tree swallows and their food during 1997–1999 in ANP watersheds, which complemented the ongoing watershed-scale research on mercury biogeochemistry and mass balance as part of EPA-PRIMENet (Kahl et al., 2005). We collected eggs, nestlings, feathers from nestlings, and food boluses brought to nestlings at boxes erected near ANP ponds and at an inland field site in Orono, ME, which served as a reference site. We included an Environmental Protection Agency (EPA) designated Superfund site (Ayer, MA) as a second reference site to determine uptake of mercury, other metals (i.e., cadmium, lead, chromium) and arsenic. Residues of these latter elements will be reported elsewhere. We related amounts of total mercury in tissues to measures of reproductive output. Specific objectives were: (1) To determine concentrations of total mercury (THg) in eggs, whole carcasses of nestlings, feathers, and food boluses brought to nestlings by adults, (2) to relate THg residues in tree swallow tissue to hatching and fledging success, and (3) to relate THg residues in tree swallow tissues to water chemistry and wetland characteristics among sites.

2 Materials and Methods

2.1 Sampling sites

We erected nest boxes at three locations (Figure 1). One site was along margins of a 1.2 ha field and an adjacent 0.4-ha residential lot on Bennoch Road, 1 mile north of Orono, ME (*Lat*. 44.8992°N–*Long*. 68.6820°W). Although an inland terrestrial site, a shallow freshwater wetland (~0.5 ha) and the Stillwater River – a branch of the Penobscot River – were within 500 m of nest boxes. The other Maine sites were on Mt. Desert Island at Acadia National Park (Hodgdon Pond, *Lat*. 44.3164°N–*Long*. 68.3979°W; Seal Cove Pond, *Lat*. 44.2955°N–*Long*. 68.4000°W; Aunt Betty Pond, *Lat*. 44.3704°N–*Long*. 68.2752°W). Characteristics of these ponds varied widely (Table I) but the ponds represented three park habitats where mercury in fish is extremely high. The other reference

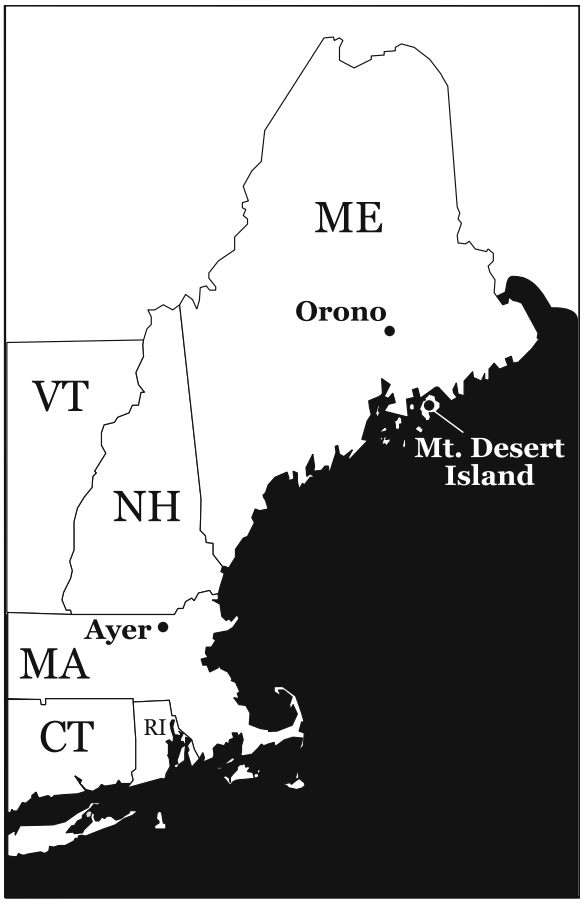

Figure 1 Locations of sampling sites (Orono and Mt. Desert, ME; Ayer, MA) to determine mercury in tree swallow food boluses and tissues, 1997–1999.

Table I Water chemistry characteristics of Aunt Betty Pond, Hodgdon Pond, and Seal Cove, Acadia National Park, Mt. Desert Island, Maine

Pond	Area (ha)	Max. depth (m)	Temp. (°C)	CLpH[a] (SU)	ANC (μeq/l)	Sp. cond. (μS/cm)	T. color Hazen (PCU)	DOC (mg/l)	Diss. O_2 (mg/l)
Hodgdon[b]	14.2	6.7	23	6.30	66.2	37	28	5.9	7
Seal Cove[b]	114.6	13.4	13	6.63	61.7	39	9	3.9	4.5
Aunt Betty[c]	13.8	2.1	17–25	5.95	132	32.5	39	9.5	2.1–7.8
Pond	Ca (mg/l)	Mg (mg/l)	K (mg/l)	Na (mg/l)	Si (mg/l)	Al (μg/l)	Cl (mg/l)	SO_4 (mg/l)	Watershed: Lake area ratio (ha)
Hodgdon	1.44	0.73	0.29	4.07	1.6	59	5.18	2.88	28:1
Seal Cove	1.66	0.71	0.29	4.32	0.7	39	5.93	3.60	8:1
Aunt Betty	2.52	0.54	0.07	3.17	0.5	45	3.87	1.10	22:1

[a] CLpH = closed cell pH, samples stored in syringe at 4 °C < 48 before analysis.

[b] Data for May or June, 1995 (Burgess, 1997).

[c] Data mostly for 24 July 1995 (Kahl, 1996); temperature and dissolved oxygen data for 17 June 1994 from files of Acadia National Park, Water Resources Database (B. Gawley, personal communication).

site was Ayer, MA (Grove Pond, *Lat*. 42.5528°N–*Long*. 71.5860°W; Plow Shop Pond, *Lat*. 42.5556°N–*Long*. 71.5930°W), which is on the former Fort Devens military base, now a U.S. EPA-designated Superfund site. The shallow (24.3 ha) Grove Pond was the site of a tannery and associated dump where arsenic, chromium, lead, and mercury were used and discharged into the pond until 1953. Four other upstream ponds drain into Grove Pond that flows into the shallow (12.2 ha) Plow Shop Pond, formerly adjacent to a railroad roundhouse and an industrial park. Plow Shop Pond also borders on and receives seepage from Shepley's Hill Landfill on Fort Devens (ABB Environmental Services, Inc., 1995). In 1998, surficial sediments in both ponds were highly contaminated with biologically active methyl mercury (MeHg) (mean ± SE, ng/g, dry wt): Grove Pond, 21.5 ± 7.1, ranging to 70.4; Plow Shop, 36.6 ± 8.8, ranging to 81.9. (T.A. Haines, unpublished data).

2.2 Collection of data

At all sites we erected Knowlton-type nest boxes (Bluebird Association of Maine, 1995). We maintained 16 boxes at Orono in 1997 and 1998; 12 boxes at Hodgdon Pond, ANP in 1997–1999, 12 boxes at Seal Cove Pond, ANP in 1997 and 1998, and 5 boxes in 1998 and 16 boxes in 1999 at Aunt Betty Pond, ANP. At Ayer, MA we maintained 12 boxes in 1998 and 16 boxes in 1999 at Grove Pond, and 13 boxes in 1998 and 9 boxes in 1999 at Plow Shop Pond. We placed boxes on iron posts equipped with a 1-m long by 10.2-cm diameter plastic drainpipe suspended freely by wire just beneath the box to deter mammalian predators. Boxes were placed during early April before birds arrived; egg laying was not expected until May (Stutchbury & Robertson, 1987).

We monitored boxes each day in spring to record initial nest building and egg-laying dates at the Maine sites, but only every 2–3 days at the Massachusetts site. We observed females to determine age (i.e., second-year [SY] *versus* after-second year [ASY]), based on brown body color of SYs or bright blue color of ASYs (Hussel, 1983); age at breeding may affect productivity (DeSteven, 1978).

At the ME sites we collected the third egg of each clutch on the day the egg was laid. At the MA study site we collected the first three eggs from each clutch after four eggs were laid for a composite sample. In 1997–1998, we collected one nestling ≥14 days old or with the wing chord ≥70 mm; in 1999 we attempted to collect nestlings closer to fledging at 16 days old or with the wing chord ≥80 mm.

At all study areas we obtained food boluses from nestlings (Quinney & Ankney, 1985) by use of ligatures (Moore, 1983), a plastic-coated wire that prevented nestlings from swallowing the food bolus. We placed ligatures on a few or all nestlings per brood for 10 to 139 min at 6–9 days after hatch (Blancher & McNicol, 1991). We kept food boluses on ice and stored them in glass vials that had been soaked overnight in 50% HNO_3, rinsed three times with deionized water, and air-dried. At the MA sites in 1998 we collected, but did not analyze food boluses, instead we attempted to

identify to order or family the array of invertebrate taxa being fed to nestlings. In 1999, we again collected food boluses from as many nestlings as logistically possible to amass the 1-g sample required for analyses. Because individual food boluses often weighed <0.1 to 0.4 g, we combined food boluses from 1 to 3 broods to obtain the required 1-g sample. When we combined food boluses from broods, the nestling collected from the brood that contributed most grams of food was used to determine degree of biomagnification of THg from food to eggs, carcasses, and feathers. We prepared tissues with dissecting instruments that we rinsed in 5% nitric acid and deionized water between each sample. *Eggs*: To remove contents, we allowed eggs to start thawing, stippled the shell with a scalpel, then separated the shell and placed the semi-solid contents in a pre-weighed, 3-dram vial. We obtained weight of egg contents by reweighing the vial. *Feathers*: We completely defeathered carcasses of nestlings by hand picking while they were partly frozen; feathers were sealed in a plastic zip-lock bag. We weighed dry feathers, rinsed them three times in deionized water, extracted excess water, and froze them. *Carcass*: We weighed carcasses in the field when collected and we reweighed them before and after defeathering them. We removed the proventriculus and ventriculus (gizzard) of each specimen and we washed out and preserved the contents. After rinsing these organs in deionized water we returned them to the carcass and froze them in plastic bags until we analyzed them.

2.3 Analyses for mercury, quality assurance, and quality control

We analyzed tissue samples for THg following standard operating procedures of the Orono Field Station, Leetown Science Center (SOP F10.9; Microwave acid digestion of biological tissue for elemental analysis-Hg, revised 6/3/96: SOP F10.11; Analysis of solutions for total mercury with the Merlin Cold Vapor Atomic Fluorescence Spectrometer). The quality assurance program for samples included analyses of reagent blanks, duplicate analyses of sample splits, matrix spikes, and certified, standard reference materials (SRMs).

We homogenized each sample individually, digested the sample by microwave, and analyzed sub-samples for percent moisture and THg. We determined moisture content by drying ~2-g sub-samples to constant weight at 105 °C. We digested by microwave radiation ~1.5 g of wet tissue in a 1:1 mixture of nitric acid and hydrogen peroxide in a Teflon pressure reactor. We then added stannous chloride to reduce mercury that we determined by Merlin Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) (Haines, Komov, & Jagoe, 1992). We determined the amount of methyl mercury (MeHg) in feather samples from three nestlings at each of four sites (Hodgdon, Aunt Betty, Grove, and Plow Shop ponds) by CVAFS following University of Maine Sawyer Environmental Research Center SOPs: Alkaline Digestion and Solvent Extraction of Methyl Mercury from Sediment and Biological Samples, Second Revision, June 30, 2003; Methyl mercury in Water by Aqueous Ethylation, Purge and Trap, Gas Chromatography and Cold Vapor Atomic Fluorescence Spectrometry (Modified Draft EPA Method 1630), Second Revision, January 1, 2003.

2.4 Data analyses

We used SAS (SAS Institute, 1990) to test for differences in THg among locations for tree swallow tissues, food boluses, and degree of biomagnification from food boluses to carcasses, and to feathers. Because few birds nested in 1997 when the boxes were first erected and because of missing data for some variables among years and individual boxes, we performed ANOVA with the model of ponds with boxes nested within ponds. We tested for the effect of ponds on $\log_e$-transformed response variables and treated years as replicate measurements of individual boxes. When $P \leq 0.05$ for a variable in the overall model, we evaluated differences among individual ponds by Least Square means and Tukey's HSD multiple comparisons test.

We used GLM, ANOVA to analyze for differences among ponds for means of Julian date of first egg laid, Julian date for young fledged, percent of eggs that hatched, and percent of young fledged. We performed a logit transformation (SAS Institute, 1990) on percent eggs hatched after assigning 1 to the 0.0% values and 99 to the 100% values. Mean logit differences among ponds were tested by ANOVA and Tukey's test. A separate analysis was performed for years 1998 and 1999, excluding 1997, which had few samples and missing data in several cells, for the response variables Julian date of first eggs laid, and for Julian date

for young fledged. We used a two-sample *t*-test to determine if either the mean amount of THg in eggs or if Julian date of first egg laid was different between clutches with <100% hatchability and those with 100% hatchability. After log transformations, we regressed concentrations of Hg in feathers on concentrations of Hg in carcasses.

We obtained total nanograms per sample by multiplying by weight (g) of the sample by the nanogram per gram of Hg per sample. We summed amounts of Hg in carcasses and feathers and calculated the percentage of total burden of Hg that was in feathers. We estimated contribution of egg Hg to total body burden of nestlings by dividing total body burden into amounts of Hg in the third egg (ME sites) or the mean of the three eggs collected from each clutch at MA sites. We used univariate *F*-tests with Least Square means and Tukey's HSD multiple comparisons to test for differences in *ng* of Hg for variables (i.e., eggs, bodies, feathers, percent in feathers, percent from egg) among ponds for years 1998 and 1999 combined.

3 Results

3.1 Mercury in tissues

Average amounts of THg were different among sites for food boluses ($F_{5,\ 34}$ = 3.02, P = 0.02), eggs ($F_{5,\ 57}$ = 8.15, P = 0.0001) (Figure 2a–b), carcasses ($F_{5,\ 57}$ = 8.77, P = 0.0001), and feathers ($F_{5,\ 56}$ = 20.01, P = 0.0001) (Figure 2c–d). Total mercury residues for eggs within the same clutch increased by 26% to 52% for three clutches, and decreased by 0.01% to 35.9% for three other clutches at ME sites. Among three clutches in the same box at Grove Pond, MA, THg increased by 156% from clutches 1 to 2, and declined 2% from clutches 2 to 3. In 1997, an unhatched egg from Seal Cove Pond contained 251 ng/g of THg and two unhatched eggs from Hodgdon Pond contained 408 and 1,023 ng/g of THg, respectively. In 1999, an infertile egg from Plow Shop Pond contained 154 ng/g of THg.

Total nanograms of Hg in birds (feathered bodies) averaged 4,471 to 13,590 among locations and years (Table II) and differences among ponds were similar to that based on nanogram per gram (see Figure 2a–b, Figure 2c–d). We detected no differences among sites and years in percentages (range of means; 78% to 92%) of total body Hg that was in the feathers, except that the percentage at Orono was lower ($P \leq 0.037$) than at other sites (Table II). The estimated average contribution of the egg to body burden was 2.4%–23.9% with lower values at Orono and Aunt Betty Pond compared with other sites ($P \leq 0.003$). For all samples combined Hg in feathers was associated with Hg in carcasses (ANOVA, $F_{1,\ 82}$ = 45.0, P = 0.0001, r^2 = 0.35; Figure 3).

3.2 Biomagnification of Hg

We detected no differences among ponds in minimum levels of magnification of Hg from food boluses to eggs ($F_{5,\ 32}$ = 1.44, P = 0.238), to carcasses ($F_{5,\ 34}$ = 1.06, P = 0.402), or to feathers ($F_{5,\ 34}$ = 0.89, P = 0.499) (Table III). Average increases of Hg from food boluses to eggs ranged from 1.9 times higher (Plow Shop Pond) to 4.2 times higher (Hodgdon Pond); in carcasses means ranged from 0.25 times lower (Seal Cove Pond) to 0.41 times lower (Hodgdon Pond); and in feathers means ranged from 9.3 times higher (Plow Shop Pond) to 18.4 times higher (Hodgdon Pond).

3.3 Reproductive performance

All but two of the nesting females were after-second-year birds. Performance of the two second-year females was similar to that of the after-second-year females and these two were retained in the data set. Females initiated laying eggs at different times among sites ($F_{5,\ 60}$ = 13.6, P = 0.001). At Ayer, MA (Grove Pond and Plow Shop) swallows began laying 4–6 days earlier (Ps $\leq$ 0.017) than at Orono, and 11–15 days earlier (Ps $\leq$ 0.016) than at Acadia National Park, ME (Aunt Betty Pond, Hodgdon Pond, Seal Cove Pond). Egg laying at Orono began an average of 4–9 days earlier (Ps $\leq$ 0.016) than at Acadia. On average, laying of the first egg also was six days earlier ($F_{1,\ 52}$ = 11.8, P = 0.001) in 1999 than in 1998. Similarly, mean fledging dates of the broods among locations were different ($F_{5,\ 55}$ = 19.7, P = 0.001). Differences in fledging dates among sites were nearly identical to those for initial egg laying; Ayer, MA was 5–8 days earlier than at Orono, and 10–15 days earlier than at Acadia; Orono was 5–8 days earlier than at Acadia. Average fledging date was 10 days earlier in 1999 than in 1998 ($F_{1,\ 50}$ = 60.8,

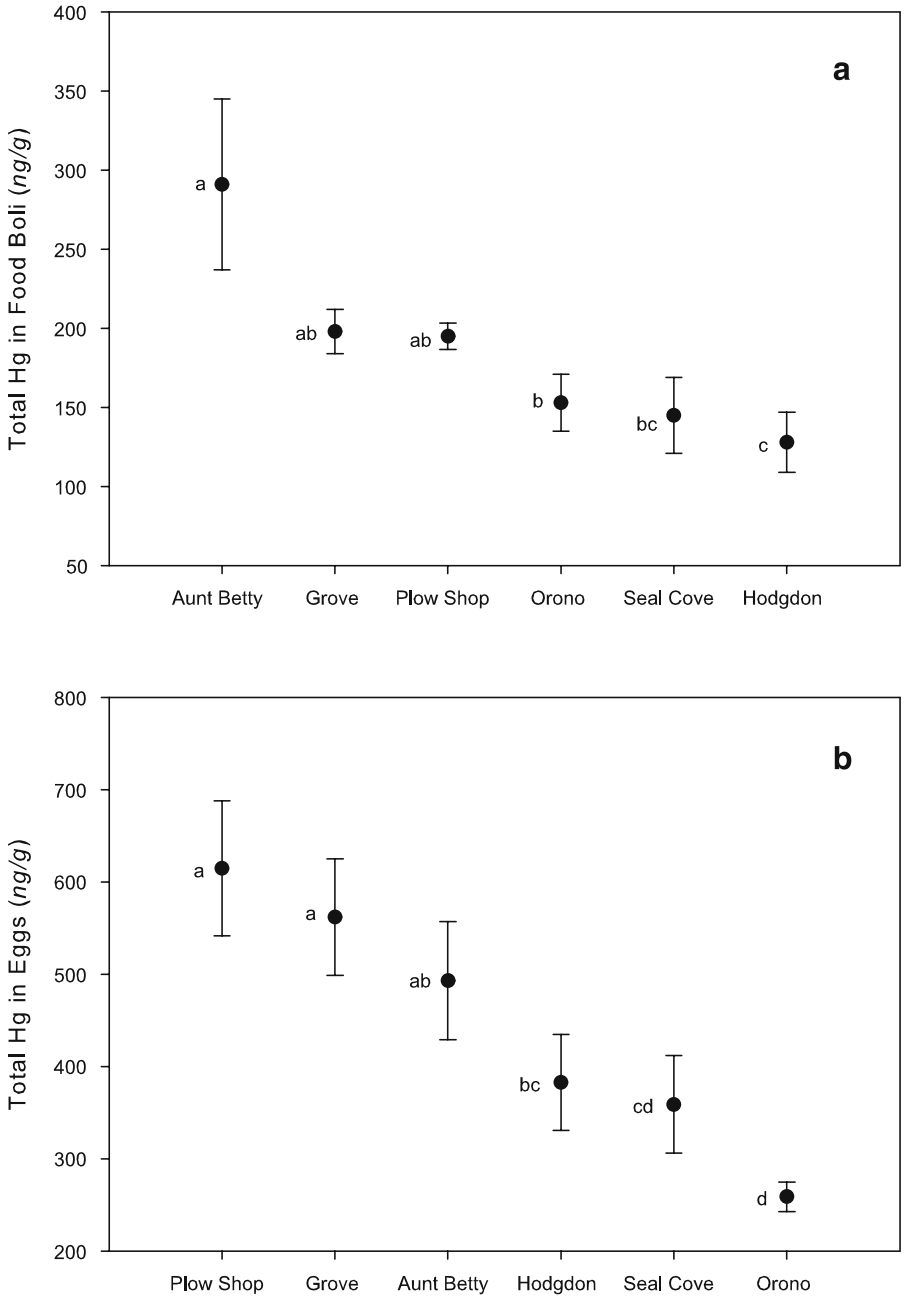

Figure 2 a–b Mean (*SE*) amounts of THg in food boluses and tree swallow eggs collected in Orono, ME (old field), Mt. Desert, ME (Aunt Betty, Hodgdon, and Seal Cove ponds) and Ayer, MA (Grove and Plow Shop ponds), 1997–1999. Different letters signify statistical differences of means among sampling sites.

P = 0.0001) and a pond*year interaction was evident ($F_{3,\ 50}$ = 3.58, P = 0.02).

We detected no differences ($F_{5,\ 56}$ = 0.67, P = 0.65) among ponds in mean percent of eggs that hatched in a clutch. Mean percentage of nestlings that fledged (85.2%), however, was lower for the Orono site than for any other pond ($F_{5,\ 54}$ = 2.42, P = 0.048) except for Plow Shop Pond (P = 0.144), which fledged 93.7% of the nestlings. No other differences in percentage of hatchlings fledged were detected among ponds.

We detected no differences between the concentration of THg in eggs of clutches in which all eggs hatched and in those clutches in which not all eggs hatched (t_{80} = 0.243, P = 0.809). Neither the mean Julian date when eggs were laid (t_{92} = 0.607, P = 0.545) nor Julian date when nestlings fledged (t_{58} = 0.666, P = 0.508) was different between clutches with 100% hatched and

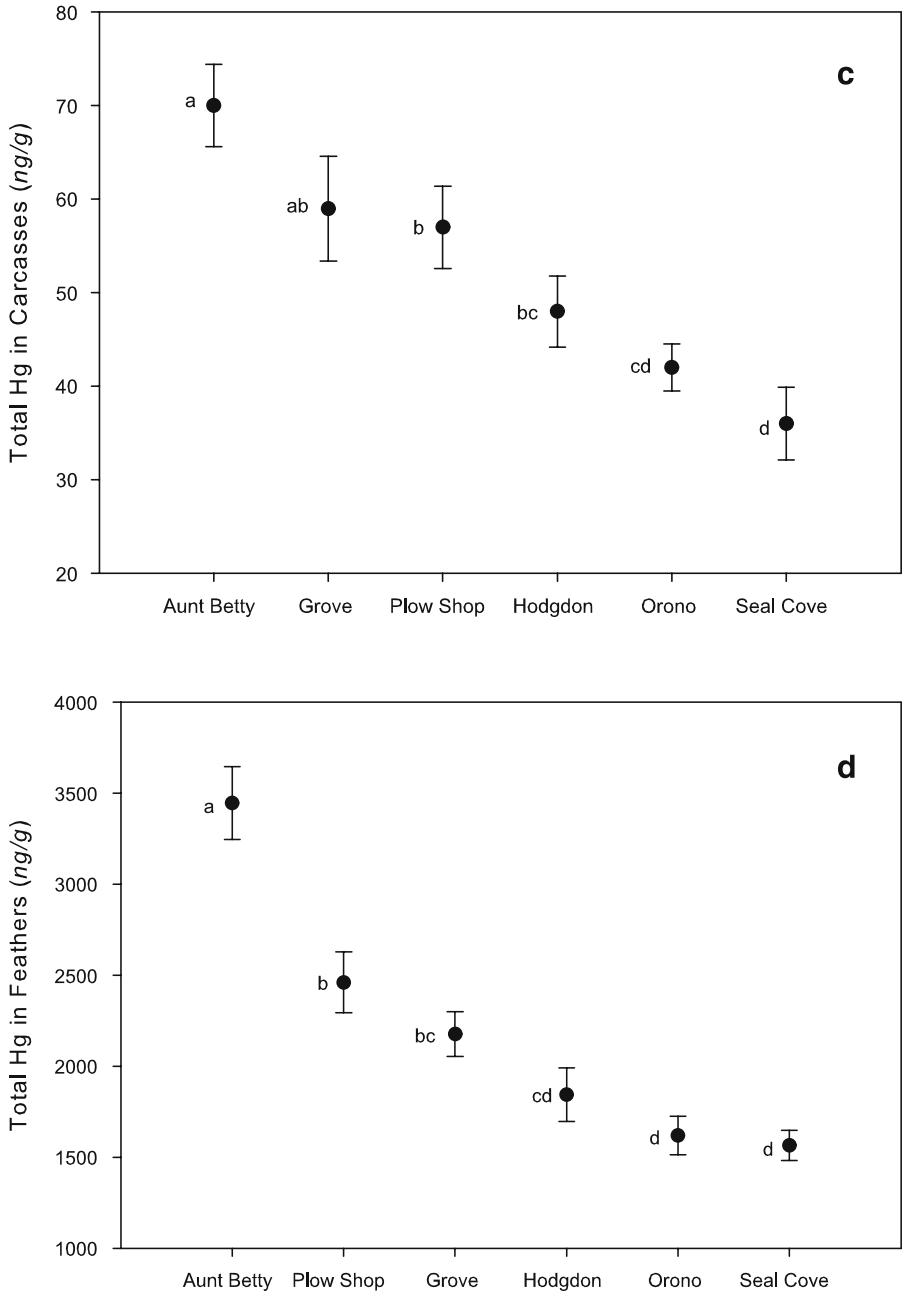

Figure 2 c-d Mean (SE) amounts of THg in tree swallow carcasses and feathers collected in Orono, ME (old field), Mt. Desert, ME (Aunt Betty, Hodgdon and Seal Cove ponds) and in Ayer, MA (Grove and Plow Shop ponds), 1997-1999. Different letters signify statistical differences of means among sampling sites.

those with <100% hatched. The percentage change in amount of THg in eggs from the same clutch, related to the order in which the eggs were laid, was inconsistent, ranging from +52% to −36%, with no difference for one set of consecutively laid eggs.

Quality assurance procedures for analyses of THg in tree swallow tissues included analyzing Standard Reference Materials (SRM): (lobster hepatopancreas [TORT-2] for food boluses, egg, and carcass tissue; dogfish muscle [DORM-2] for eggs, carcasses, and feathers; albacore muscle [TUNA] for eggs, carcasses, and feathers; and blanks, matrix spikes, and duplicates. Mean ± SD (n) for combined tissues for each SRM used were: TORT-2, 266 ± 39 (26); DORM-2, 4,516 ± 276 (16); TUNA, 810 ± 126 (5). Relative Percent Difference (RPD) between duplicates ranged from a low of 10.0 ± 10.7% for feathers to a high of 16.3 ± 12.3% for carcasses. Percent recoveries of spikes were 113 ± 12.4 (n = 5) for food boluses, 101 ± 11.4 (n = 9) for eggs, 99 ± 16.9 (n = 14) for carcasses, and 123 ±

Table II Total mercury burdens in tree swallow eggs and nestlings (sum of Hg in bodies and feathers) from sites in Maine and Massachusetts, 1997–1999

Location	Year	Mean ± SE of Hg (ng), *n*				Percent of Hg in feathers, *n*		Percent of Hg from egg, *n*	
		Egg		Carcass and feathers					
ME									
Orono	1997	437 ± 30	5	5,569 ± 531	4	84.0 ± 2.9	4	8.1 ± 1.3	4
	1998	410 ± 37	12	4,471 ± 260	12	82.2 ± 1.3	12	9.4 ± 0.9	12
Hodgdon	1997	430	1	4,296	1	77.9	1	10.0	1
	1998	707 ± 119	4	4,892 ± 953	4	85.4 ± 2.6	4	15.9 ± 3.8	4
	1999	580 ± 132	6	4,978 ± 383	7	81.0 ± 1.5	7	10.2 ± 2.7	5
Seal Cove	1997	677 ± 112	3	5,072 ± 375	4	83.5 ± 0.4	4	12.4 ± 1.6	3
	1998	495 ± 109	5	5,135 ± 724	4	89.4 ± 2.2	4	12.4 ± 4.0	4
Aunt Betty	1998	316 ± 101	2	13,590 ± 728	2	92.2 ± 1.2	2	2.4 ± 0.9	2
	1999	783 ± 94	14	8,796 ± 799	13	84.0 ± 0.8	13	8.9 ± 0.7	13
MA									
Grove	1998	1,280 ± 176	6	5,682 ± 501	8	85.4 ± 1.4	8	23.9 ± 3.8	6
	1999	675 ± 80	14	6,029 ± 448	13	80.5 ± 1.3	13	11.4 ± 1.4	12
Plow Shop	1998	1,015 ± 90	4	6,845 ± 257	4	84.8 ± 0.8	4	15.0 ± 2.0	4
	1999	788 ± 148	9	6,730 ± 662	8	82.8 ± 2.1	8	11.0 ± 2.3	8

39.8 (n = 11) for feathers. With few exceptions analytical results were within acceptable limits; two TUNA (albacore muscle) SRMs associated with two samples of feathers were 8.1% and 8.7% low, and this SRM associated with a carcass analyses was 24.2% low. Two blanks exceeded 0.1 ng/g, but other quality assurance data were acceptable. The limit of detection for THg was 0.06 ng/l and the concentrations of THg in all sample digestates far exceeded the instrument detection limit. Forty-four of 48 blanks were below the detection limit, 4 were greater (i.e., 0.1, 0.16, 0.39, and 25.2 ng/g). Standard Reference Materials for MeHg were 103.3% of IAEA-356 (marine sediment) and 115% of TORT-2. The sample for Ongoing Precision and Recovery was 90.5%, the RPD was 2.4%, and two blanks were zero; all values were within acceptable limits. The detection limit for the MeHg method was 4.0 ng/g.

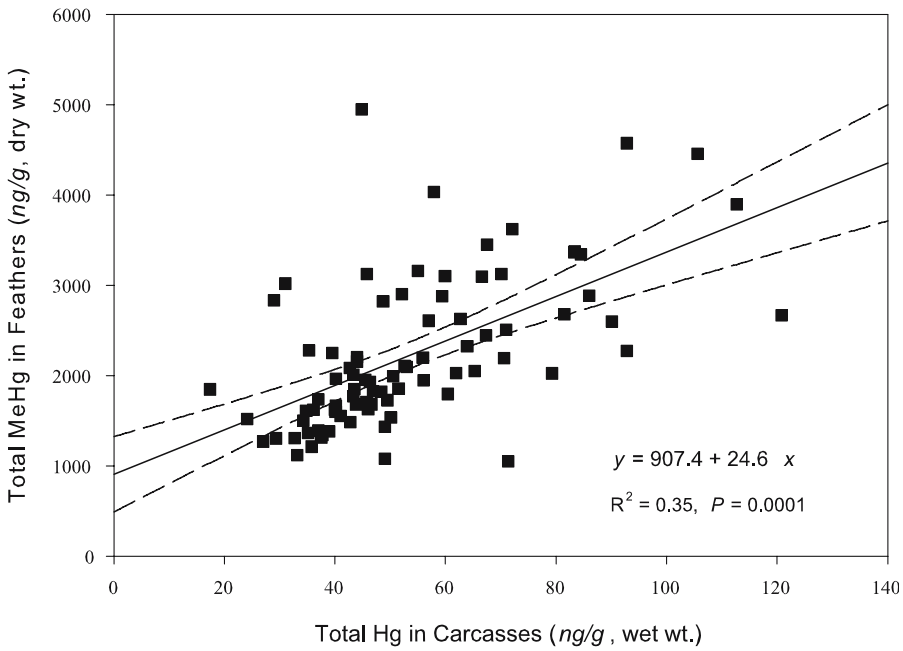

Figure 3 Regression of MeHg concentration in feathers (dry wt.) on THg concentration in carcasses (wet wt.) of 84 tree swallow nestlings collected in ME and MA, 1997–1999.

Table III Minimum biomagnification values of total mercury from food boli to eggs, carcasses, and feathers for tree swallows at Acadia National Park, Mt. Desert Island, Maine, Orono, Maine, and at an EPA Superfund site, Ayer, Massachusetts, 1997–1999

Location	Mean ± SE, *n* (range), ng/g, wet wt.							
	Food Bolus		Biomagnification[a] (Tissue THg/Food Boli THg)					
			Egg		Carcass		Feathers	
Acadia NP, ME								
Aunt Betty Pd	291 ± 54 (124–527)	8	2.2 ± 0.53 (0.65–4.9)	8	0.33 ± 0.07 (0.16–0.59)	8	14.6 ± 3.1 (5.7–29.1)	8
Hodgdon Pd	128 ± 19 (49–211)	8	4.2 ± 1.2 (1.04–10.7)	7	0.41 ± 0.07 (0.27–0.88)	8	18.4 ± 3.4 (8.5–36.0)	8
Seal Cove Pd	145 ± 24 (70–248)	6	3.03 ± 0.86 (1.19–6.8)	6	0.25 ± 0.04 (0.15–0.38)	6	12.6 ± 1.7 (6.6–18.1)	6
Orono, ME (field)	153 ± 18 (40–268)	11	2.05 ± 0.55 (0.77–7.3)	11	0.35 ± 0.09 (0.17–1.15)	11	14.0 ± 3.1 (6.3–40.9)	11
Ayer, MA								
Grove Pd	198 ± 14 (141–273)	10	2.5 ± 0.46 (1.26–4.9)	9	0.38 ± 0.05 (0.18–0.70)	10	12.2 ± 1.6 (7.6–24.4)	10
Plow Shop Pd	195 ± 8.3 (187–212)	3	1.9 ± 0.34 (1.60–2.6)	3	0.30 ± 0.07 (0.20–0.44)	3	9.3 ± 1.1 (7.0–10.7)	3

[a] Because food boluses contain mostly aquatic insects, which vary widely (35% to 95%) in the amount of THg that is MeHg, our calculations based on THg in food boluses represent minimum values of biomagnification. Values > 10 are rounded to nearest whole number.

4 Discussion

4.1 Mercury in tissues

Most mercury in the environment is inorganic mercury, but becomes biologically active after conversion to MeHg by aquatic organisms (Jensen & Jernelov, 1969). Bryan (1979) reported that mercury is the only heavy metal that biomagnifies up food chains. Although mercury compounds may be stimulatory, they are not essential for any specific function of living tissues (Bryan, 1979; Luckey & Venugopal, 1978). Most Hg in animal tissue is the biologically active form (MeHg) that is preferentially bound to protein (Thompson, 1996) and not readily eliminated as is inorganic mercury. Exposure of birds is to MeHg, which accounts for nearly 100% in tissues (Thompson & Furness, 1989); 70%–93% of the body burden of Hg is in the feathers (Burger, Rodgers, & Gochfeld 1993). Gerrard and St. Louis (2001) reported for tree swallows in Ontario a near 1:1 relationship between concentrations of THg and concentrations of MeHg in seven tissues, including eggs, carcasses, and feathers, which corroborates other studies that most THg in tissues is MeHg. Our analyses of feathers from ME and MA indicated that all THg was MeHg, thus, Hg in eggs and carcasses probably can be considered as primarily MeHg. Mercury in food boluses that contained mostly insects cannot be assumed as being all MeHg, because amounts of MeHg in invertebrates vary among insect orders (Tremblay et al., 1996) and functional feeding groups (Hall et al., 1998).

4.2 Biomagnification of Hg

Our values for biomagnification are minimal because they are based on food boluses content of THg, not MeHg, the biologically active form. Because proportions of MeHg vary by functional feeding groups of insects [i.e., 35% to 50% MeHg in detritivores-grazers (dipterans, ephemeropterans, trichopterans), but 70%–95% MeHg in predators (heteropterans, coleopterans, odonates); Tremblay et al., 1996], we can determine only minimum values. Based on THg of food boluses, we calculated a 2 to 4-fold increase of Hg in eggs, among sites, resulting from the amount of Hg in insects in those boluses brought to nestlings. Because these food boluses were collected in late May and June, we do not know how much Hg was in insects foods when females were foraging in early April and early May during egg laying. If bioavailability of MeHg increases with the season, which our interclutch sample suggests (but see Burgess, 1997; Wenzel, 1996), the minimum values of magnification we have calculated for eggs may be low. Biomagnification values for carcasses (0.25 to 0.41) reflect the near total transfer (Burger & Gochfeld, 1992; Thompson & Furness, 1989; Furness,

Muirhead, & Woodburn 1986) of MeHg to growing feathers (9.3 to 18.4-fold increase of MeHg) where MeHg is strongly attached to disulphide bonds of keratin (Crewther, Fraser, Lennox, & Lindley 1965). Our tree swallow nestlings consistently transferred 78%–92% of the body burden of Hg to their feathers. We determined MeHg in total plumage because flight feathers alone (i.e., remiges and rectrices) do not correspond to dietary intake and may not adequately measure mercury content (Furness et al., 1986).

Spalding, Bjork, Powell, & Sundlof (1994) stressed how difficult it is to recognize Hg poisoning in wild birds, but correlated Hg > 6,000 ng/g in the liver with chronic disease and mortality of great white herons (*Ardea herodias occidentalis*) in Florida. Beyer, Spalding, and Morrison (1997) reported that 6,000 ng/g of Hg in liver of adult wading birds in Florida corresponded to 9,700 ng/g in feathers, an amount exceeding that reported (9,000 ng/g) as toxic in captive mallards (*Anas platyrhynchos*) (Heinz, 1979). In 1998–1999, the amount (ng) of mercury in feathers of 5 of 16 (31.2%) nestling tree swallows from Aunt Betty Pond ranged from 9,390 to 13,365.

4.3 Reproductive performance

Because females forage near nesting sites (St. Louis, Breebaart, & Barlow, 1990), mercury residues in tree swallow eggs and nestlings reflect site-specific contamination. St. Louis and Barlow (1993) in Ontario reported that egg hatchability was lower at acidified lakes compared with reference lakes with higher pH. Effects of Hg on avian reproduction have focused on hatchability of eggs and survival of offspring (Heinz, 1974, 1976; Fimreite, 1971). Fimreite (1971) reported on decreased hatchability of ring-necked pheasant (*Phasianus colchicus*) eggs when they contained 500–1,500 ng/g (wet wt.) of Hg. Heinz (1979) proposed that 800 ng/g (wet wt.) in a mallard (*Anas platyrhynchous*) egg was the toxic threshold for Hg. Others have adopted this criterion for field studies (Lonzarich, Harvey, & Takekawa, 1992). Although Heinz and Hoffman (2003) reported death of a mallard embryo containing ~740 ng/g of Hg, they stated that the threshold concentration of maternally deposited mercury at which embryotoxicity begins in mallards is ~1,000 ng/g (wet wt.). G.H. Heinz (personnal communication) provides a recent opinion on effects of injected MeHg chloride into viable tree swallow eggs:

> Based on laboratory studies in which MeHg has been injected into fertile tree swallow eggs and mallard eggs, it seems that embryos of tree swallows may be more sensitive to MeHg than are mallard embryos. The exact relationship between toxicity of injected MeHg and maternally deposited MeHg is not known, but it seems that the embryotoxic threshold of mercury in tree swallow eggs may be somewhat less than 1 ppm on a wet-weight basis.

If we apply the criterion that 800 to 1,000 ng/g (wet wt.) of Hg in an egg is embryotoxic to the most sensitive of tree swallow embryos, then some third eggs collected at Aunt Betty Pond, Grove Pond, and Plow Shop Pond exceeded this threshold. In 4 of 11 clutches that contained THg exceeding 885 to 1,313 ng/g in the third egg, individual eggs did not hatch when Hg was 916, 945, and 1,313 ng/g in the third egg collected, and two eggs did not hatch from a clutch when Hg was 1,138 ng/g in the third egg collected. These data suggest, however, that other eggs in these clutches, based on observed intraclutch variability, contained concentrations of Hg that exceeded the embryotoxic threshold, yet they hatched. Two eggs that failed to hatch at Hodgdon Pond contained 403 ng/g (644 ng) of Hg and 1,023 ng/g (1,534 ng) of Hg, but the cause of the deaths of these embryos is unknown. An infertile egg from Plow Shop Pond contained 154 ng/g (223 ng) of Hg, and a fertile egg with slight development from Seal Cove Pond contained 251 ng/g (427 ng) of Hg. Burger (2002) compiled data from 68 studies on mean concentrations (ng/g, wet wt.) of Hg in eggs of raptors, seabirds, and other fish-eating birds and reported that means ranged from 70 to 7,290 with a median of 340 ng/g of Hg. For perspective, means from all our sites, except Orono, exceeded the 340 ng/g median. Furthermore, 78% of individual eggs from Acadia NP and the Superfund site exceeded the median, whereas only 1 of 17 eggs exceeded the median at the terrestrial site in Orono.

Mean percent of eggs that hatched (88.6%–94.3%) was not different among sites (P = 0.65) but was higher than that for tree swallows exposed to Hg near a reservoir in Ontario (73.0%–88.0%) (Gerrard & St. Louis, 2001). Blancher and McNicol (1988) reported that 396 of 439 (90.2%) tree swallow eggs intact

through incubation hatched near Sudbury, Ontario, whereas lower hatching success was reported in Massachusetts (71.6%, Mason, 1968; 83.6%, Low, 1934), and at Long Point, Ontario (73.7%; Holroyd, 1983). Among seven tree swallow populations, egg hatchability averaged 86.9% for 10,107 eggs from nests in which at least one egg hatched (Robertson, Stutchbury, & Cohen, 1992).

Almost all nestlings fledged except for single nestlings in a few broods and the entire brood for one nest at Hodgdon Pond in 1999. This brood seemed sickly, failed to gain weight as expected, and had one of the higher THg burdens (79.3 ng/g) in the carcass. Mean percent (88.9–100) of nestlings fledged was not different among sites, except for slightly lower success at Orono where raccoons (*Procyon lotor*) affected three broods one year. Average fledging success in Ontario ranged from 66.7% to 98.6% (Gerrard & St. Louis, 2001). Fledging success averaged 83.1% for 21,130 hatchlings from 18 populations in the eastern part of the breeding range (Robertson et al., 1992).

Pollutants in eggs within a clutch may be uniform, increase, or decrease with laying sequence (Becker, 1992). We collected the third egg laid from each clutch at Maine sites and the first three eggs laid at the Ayer, MA site to standardize collections. In 3 of 6 sets of sequentially laid eggs, the second egg laid contained 26 to 52% more Hg than the previous egg, but for two other sets the second egg laid contained 19 to 36% less Hg, while Hg in one set of eggs did not change. These differences in amount of Hg in sequential eggs suggest daily variability either in species of insects captured (McCarty, 1997; Cohen & Dymerski, 1986; Johnson & Lambardo, 2000) or in amounts of MeHg in insects captured (Hall et al., 1998) or a combination of both. The interclutch variability we noted for two sets of clutches may reflect seasonal change in Hg-contaminated insect prey, whereas more Hg was in the May 20 clutch (156%) and June 11 clutch (149%) compared with the May 7 clutch at Grove Pond. The reverse was true for the two clutches at Aunt Betty Pond with a 31% decrease in Hg from first to second clutch. Finally, egg mass of tree swallows increases with egg sequence, especially for the most common clutch size of six eggs (Wiggins, 1990). Egg mass increased with egg laying order (F_5 = 3.7, P < 0.003) in our study of 66 clutches, and this may have relevance to amounts of Hg in eggs other than third eggs that we collected.

4.4 Mercury related to sampling sites

Amount of THg in food boluses and tree swallow tissues is explained partly by characteristics of the sampling sites. Residues were highest at aquatic sites and lowest at the suburban old-field site in Orono, ME. Tree swallows undoubtedly foraged over the nearby Penobscot River where whole fish collected in 1984 contained mercury (i.e., 330 ng/g, wet wt. in smallmouth bass [*M. dolomieu*] and 140–180 ng/g in white suckers [*Catostomus commersoni*]) (Schmitt & Brumbaugh, 1990), but we observed that many tree swallows foraged daily over fields near nest boxes. Food boluses collected from tree swallows in Ontario foraging over a field contained 44% aquatic diptera (Nematocera) and other non-aquatic taxa, whereas those birds foraging over a sewage lagoon contained 90% aquatic diptera (Quinney & Ankney, 1985). In an upland old-field in Michigan diets of nestling tree swallows contained fewer (1.1%–6.7%) aquatic orders such as Odonata and Ephemeroptera (Johnson & Lombardo, 2000).

The mean proportion of MeHg to THg depends on the feeding behaviors of invertebrates, increasing from 35% to 50% in detritivores-grazers (dipterans, ephemeropterans, trichopterans), but is 70%–95% in predators (heteropterans, coleopterans, odonates) (Tremblay et al., 1996). Previous work during 1994–1996 in ANP revealed that Hg (mean ± SD, ng/g, dry wt.) in dragonfly larvae (Anisoptera) from Hodgdon Pond (THg = 448 ± 174; MeHg = 71 ± 37%) was greater (P = 0.034) than in Seal Cove Pond (THg = 318 ± 130; MeHg = 57 ± 23%) (Burgess, 1997; Bank et al., 2005). Furthermore, THg in dragonfly larvae was higher (P = 0.002) in Hodgdon Pond than in Seal Cove Pond in spring, but not in summer. Total mercury in dragonfly larvae within Hodgdon Pond was greater (P = 0.041) in spring than in summer, but just the opposite for Seal Cove Pond (Burgess, 1997). THg in composite insect samples of Coleoptera, Hemiptera, Odonata, Megaloptera, Trichoptera, and Ephemeroptera from unspecified aquatic sites in Maine averaged 300 ng/g, dry wt. (range, 200–600) and in Maryland it averaged 300 ng/g (range 100–800) (Albers & Camardese, 1993). Samples of emerging insects at two Wisconsin marshes of Lake Michigan averaged 13 and 56 ng/g (wet wt.); samples obtained earlier in spring were higher in mercury than later samples (Wenzel, 1996). Mercury

in mayflies in the Upper Mississippi River ranged from 44 to 177 ng/g (dry wt.) with amounts greater in males than females (Dukerschein, Weiner, Rada, & Steingraeber, 1992). Most insects inhabit pond sediments as larvae, thus they are exposed to and accumulate mercury while in sediments where inorganic Hg is transformed to MeHg during microbial activity (Jackson, 1988).

Higher THg residues in tree swallow tissues from the ANP ponds can be associated with chemical and physical features (i.e., higher water temperature, lower pH, anoxic hypolimnion layer, high humic content reflected in color or DOC, lower dissolved oxygen, and a higher ratio of watershed area to wetland area) of wetlands. These variables influence methylation of organic mercury, thereby increasing its bioavailability. Aunt Betty Pond has the shallowest basin with slightly higher temperatures than Hodgdon Pond, but temperature is twice as high as in Seal Cove Pond where mercury residues in tissues were lowest. Higher water temperatures stimulate microbial activity and MeHg production (Winfrey & Rudd, 1990), but Burgess (1997) found that profundal zone and surface water in Hodgdon Pond did not warm sooner than in Seal Cove Pond and profundal zone water remained 3–4 °C higher throughout summer. This should have resulted in more MeHg in Hodgdon Pond sediments than in Seal Cove Pond, but no difference was detected. The ~2-meter-deep water in Aunt Betty Pond evidently warms faster, which may enhance MeHg production. In 2002, amounts of THg (mean ± SE, ng/g, wet wt) in golden shiner (*Notemigonus crysoleucas*) from Aunt Betty Pond averaged 341 ± 57 ranging to 672.8 ng/g; THg correlated with fish length (r = 0.88, P = 0.001, n = 9). THg in mummichog (*Fundulus heteroclitus*) averaged 301 ± 17 ranging to 337 ng/g (T.A. Haines, unpublished data).

Anoxic hypolimnetic water below the thermocline also may be the site for increased methylation (Watras et al., 1995), although methylation is not limited to this part of the water column. Burgess (1997) reported that anoxic water developed in Hodgdon Pond in July and was completely anoxic by August at ~5 m, whereas the anoxic zone developed later and at ~10 m in Seal Cove Pond. We lack data about anoxic water in Aunt Betty Pond. Although pH was lowest in Aunt Betty Pond compared with Hodgdon Pond and Seal Cove Pond, pH did not increase Hg in fish at the latter two ponds (Burgess, 1997), but pH can affect Hg distribution (Rada, Powell, & Wiener, 1993; Driscoll et al., 1995). Water color and dissolved organic carbon (DOC), which explained most variation in THg and MeHg in surface water of Wisconsin lakes (Watras et al., 1995), have been recorded as highest in Aunt Betty Pond, followed by Hodgdon Pond and Seal Cove Pond (Table I). In Atlantic Canada, THg concentrations increased significantly with total organic carbon (TOC) (Clair, Burgess, Brun, & Léger, 1988). Jackson (1988) concluded that rate of MeHg production in water or sediment interface in reservoirs is primarily a function of environmental variables, and that influx of terrestrial organic matter related to flooding that substantially stimulates MeHg production.

The ratio of watershed area to wetland area, which may affect amounts of mercury in run-off into a wetland (Lee & Iverfeldt, 1991), also may affect differences in amounts of mercury in wetland biota. The ratios for Aunt Betty Pond (22:1) and Hodgdon Pond (28:1) (Table I) were 3–4 times greater than that of Seal Cove Pond (ratio 8:1), which had lower mean amounts of THg in swallow tissues.

4.5 Mercury in tree swallows at other sites

For our study, THg (ng/g, wet wt) in tree swallow eggs averaged 259 to 493 and ranged from 97 to 1,313 among Maine sites, and averaged 574 to 615 and ranged from 231 to 1,075 for Massachusetts sites. In contrast, in Wyoming, Custer et al. (2001) reported a geometric mean of 0.30 (μg/g, dry wt.) for seven eggs, which converts to ~60 ng/g, wet wt. Mercury in tree swallow eggs from the Upper Hudson River ranged from non-detected to 95 ng/g wet wt. (Secord & McCarty, 1997). In the Great Lakes-St. Lawrence River Basin, THg ranged from 43 to 79 ng/g (wet wt.) in eggs (Bishop, Koster, Chek, Hussell, & Jock, 1995). After converting from dry wt. to wet wt. basis (dry wt. value × 0.20), approximate MeHg mean concentration per year ranged from 43 to 104 ng/g in northwestern Ontario (Gerrard & St. Louis, 2001).

Total mercury (ng/g, wet wt.) in our defeathered nestlings averaged 36 to70 and ranged from 17 to105 among Maine sites, and at Massachusetts sites THg averaged 57 to 59, and ranged from 35 to 121 ng/g. After converting from dry wt. to wet wt. basis (dry wt. value × 0.32), approximate MeHg mean concentrations per year in nestlings was 22 to 60 ng/g in Ontario (Gerrard & St. Louis, 2001). Total mercury

(geometric mean) in a four-bird carcass composite sample (minus bill and lower legs) at Tintamarre National Wildlife Area, NB was 71 ng/g, wet wt. and in a five-bird composite sample from River Herbert, NB, THg was 29 ng/g, wet wt. (N. Burgess, personnal communication).

In feathers of our tree swallows THg (ng/g, wet wt.) in Maine averaged 1,566 to 3,447 and ranged from 1,051 to 4,950 among sites, and in Massachusetts THg averaged 2,177 to 2,461 among sites and ranged from 1,317 or 3,900. In northwestern Ontario MeHg (ng/g, dry wt.) mean concentrations per year in feathers averaged from 796 to 1,340 in tree swallows from the experimental reservoir and 604 to 924 in tree swallows from reference lakes (Gerrard & St. Louis, 2001).

Mercury concentrations increase from east to west in blood and feathers of the common loon (*Gavia immer*) (Beauchamp et al., 1998; Evers, Kaplan, Meyer, & Reaman, 1998). Amounts of Hg in tree swallow tissues also seemed greater in the Northeast compared with concentrations from the Midwest. Availability of Hg in the Northeast is increased by effects of impoundments (Gerrard & St. Louis, 2001), local sources of Hg discharge (e.g., Holtrachem, Orrington, ME; Courtemanch, Hopeck, & Ostrowski, 1997), and the uniquely contaminated Superfund site in MA that we studied.

5 Conclusions

Toxic mercury is being transferred through insect foods from aquatic environments to eggs and fledglings of breeding tree swallows at Acadia National Park and at a Superfund site in Ayer, MA. Although eggs were contaminated by mercury, embryotoxicity of mercury was minimal, except for individual eggs that contained 800 –1,000 ng/g of THg. Fledging success of hatchlings was essentially unaffected, which reflects the transfer of most (81%–92%) of the body burden of ingested mercury to growing feathers where Hg is strongly bound to the protein keratin. Residues of THg in eggs of tree swallows in other parts of its range are low compared to those in tree swallows at our study sites. These findings support the view that biota in the northeastern states are especially exposed to toxic mercury because of atmospheric deposition of inorganic mercury and hydrological conditions conducive to converting inorganic mercury to a biologically active form, MeHg. Because most of the mercury that nestlings ingest is rapidly deposited in feathers and retained in them after the blood supply atrophies as feathers mature, less Hg remains in the body to affect physiological processes. The high Hg burdens in whole bodies (carcass plus feathers) of tree swallows; however, are available for transfer to local species of hawks, owls, and non-raptorial species that eat tree swallows (Robertson et al., 1992), including the peregrine falcon (*Falco peregrinus*) that nests at Acadia National Park. Other predator species may be exposed to Acadia's mercury-laden tree swallows in southern wintering areas because contaminated hatchings do not complete molt of feathers (Stutchbury & Rohwer, 1990) until after hatchlings migrate to Florida, Cuba, and Honduras (Butler, 1988). Regionally accrued burdens of Hg in tree swallows may be transported to areas far from sites of Hg deposition.

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