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Effects of Dietary Fluoride on Reproduction in Eastern Screech-Owls

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Abstract. Sixty-six eastern screech-owls (*Otus asio*) were paired and randomly assigned to dietary treatment groups of 0, 40, or 200 ppm (mg/kg) fluoride (as sodium fluoride) in November 1981. Hatching success was adversely affected at the 200 ppm (mg/kg) level, suggesting potential detrimental impacts to wild populations exposed to fluoride pollution. Eggshell thickness was unaffected. Although fluoride concentrations were elevated in bone and eggshells, large variations among individuals were observed as well as a trend for eggshell residues to increase with sequence of laying. Females had higher residues of fluoride in bone than males. Although fluoride levels in bone and eggshells are useful indicators of exposure, the variability in residues among individuals makes residue data from field collections of limited usefulness in assessing hazards in wild birds.

Fluoride is ubiquitous in the environment; it is estimated to constitute between 0.06% and 0.09% by weight of the upper layers of the lithosphere (Koritnig 1951; Leech 1956). Fluoride is also an environmental pollutant, originating from such anthropogenic sources as steel, aluminum, ceramics, and phosphate fertilizer production as well as coal combustion (Smith and Hodge 1979). The best known examples of fluoride pollution are associated with aluminum production (NRC 1971). Problems associated with fluoride pollution occur in many localities and may become more wide-spread in the future if not stringently controlled.

Little is known about the effects of fluoride on wild birds. Chronic fluoride toxicosis has been frequently observed in cattle and deer (Shupe *et al.* 1963; Krook and Maylin 1979); fluorotic lesions in permanent dentition are the most obvious signs. Structural bone changes also occur in response to ingestion of elevated levels for prolonged periods (Shupe and Alther 1966; Shupe *et al.* 1963). Yu and Driver (1978) suggested that poultry tolerate higher levels of fluoride exposure than sheep and cattle and found 150 ppm to cause detectable physiological and biochemical changes. Guenter (1979) found that 200 ppm enhanced egg production and feed efficiency in chickens. Merkley and Sexton (1982) reported no deleterious effects when 100 ppm was added to the drinking water of chickens, whereas van Toledo and Combs (1984) found decreases in feed consumption, egg production, and eggshell characteristics (breaking strength, weight, thickness) at 900–1,200 ppm of fluoride.

In wild birds, Newman (1977) suggested that house martin (*Delichon urbica*) nesting densities were lower adjacent to an aluminum smelter in Czechoslovakia due to fluoride emissions. Van Toledo (1978) reported that a crippled tawny owl (*Strix aluco*) with elevated fluoride levels and the discovery of two unsuccessful tawny owl nests with indented eggs were associated with aluminum smelter emissions. Since insectivorous and carnivorous birds have apparently been affected by fluorides, eastern screech-owls, who eat a variety of small prey including mice and insects, were selected to test the effects of dietary fluoride on residue accumulation and reproduction. Hoffman *et al.* (1985) reported potential teratogenic effects,

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growth, and blood chemistry in 1- and 7-day-old owls from this study.

Materials and Methods

Sixty-six eastern screech-owls from a captive colony at the Patuxent Wildlife Research Center, Laurel, Maryland were paired and randomly assigned to outdoor pens on 25 November 1981 (Pattee 1984). Each pair of owls was fed 100 g/day of a commercial bird of prey diet; the diet was supplemented with Vionate[2] (1%), calcium phosphate (0.5%), and the antibiotic, nf 180® (0.0055%:N-[5-nitro-2-furfurylidene]-3-aminol-2-oxazolidone). Diets were mixed in a Hobart® vertical cutter mixer and three treatment levels were prepared by the incorporation of sodium fluoride: control (no fluoride added); 40 ppm (mg/kg) fluoride; 200 ppm (mg/kg) fluoride. Eleven pairs were randomly assigned to each treatment level; treated diets were started on 30 November 1981. Uneaten food was removed daily and the amount uneaten estimated. Birds were maintained on the treated diets for 5–6 months until each clutch hatched or failed. Adults and young were either sacrificed immediately after hatch or were placed on untreated food and sacrificed when the youngest nestling in each brood was 7 days old (Hoffman *et al.* 1985).

Birds were weighed at the start of the experiment (November 30), on 1 February, and at the time of sacrifice. Nest boxes were monitored until egg laying commenced. Once laying started, boxes were checked daily at 0900 and eggs numbered sequentially. Five to 7 days after the last egg was laid, the third egg was removed and stored at 5°C until processed. An estimated hatch date was determined based on when the last egg laid would hatch and birds were left undisturbed until 2–3 days prior to this date. At this time, boxes were again checked daily and the status of each egg determined. Unhatched eggs were removed when it became apparent that they would not hatch. Eggs were opened, their contents examined for evidence of embryonic development, and the shells preserved (Pattee 1984) for thickness measurements (Ratcliffe 1967) and determination of fluoride content.

The shells from all third eggs were analyzed for fluorides as well as those of an additional 19 unhatched eggs from clutches in which at least two eggs failed to hatch. Due to their medullary bone content, the femurs from 15 randomly selected pairs (five per treatment) were also analyzed for fluorides. Food samples collected during the study were pooled by treatment and analyzed for fluoride, then pooled into a single sample and analyzed for nutrient content.

Eggshells and bone were analyzed for fluoride as outlined by Singer and Armstrong (1968) with the following modifications. Fifty mg of ash were dissolved with 2 ml of 3N nitric acid; the pH was adjusted to 1.5 using 3N sodium hydroxide, then to a final pH of 5.4 with a 15% sodium acetate solution. Sample volume was adjusted to 5 ml, then 5 ml of TISAB buffer solution (Orion instrument manual 1977—EDTA substituted for CDTA) was added. Fluoride measurements were made with an Orion Model 96-09 combination electrode in conjunction with an Orion 901 research ionanalyzer. Recovery data from spiked chicken eggshells averaged 93% with a lower limit of reportable fluoride of 0.1 ppm (mgd/kg). Feed samples were analyzed for fluoride content and nutrient content by Hazleton Laboratories America, Inc. (3301 Kinsman Blvd, Madison, WI 53707). Analyses according to the Association of Offical Analytical Chemists (1980) were as follows: fluorine, methods 25.049–25.055; moisture, method 16.233; fat, method 7.058; ash, method 14.006; crude fiber, method 7.066; protein, method 2.057; calcium, methods 2.109–.113 and 7.091–.095; phosphorus, methods 2.019–.025, 7.120–.123, 11.032–.034. Other nutrients analyzed included carbohydrates (Watt and Merril 1963; pp 164) and calories (Watt and Merril 1963; pp 159–160).

[2] Reference to trade names does not imply endorsement by the U.S. Fish and Wildlife Service

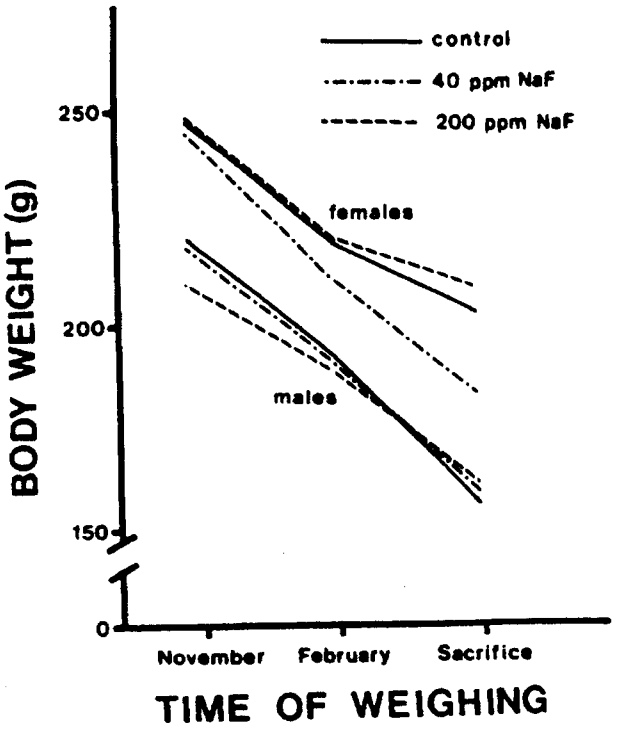

Fig. 1. Body weights of male and female eastern screech-owls fed fluoride between November 1981 and May 1982

Data concerning food consumption, body weights, clutch size, number of fertile eggs, number of eggs hatched, residues in eggshells, shell thickness, and thickness index were compared by one-way ANOVA ($P \leq 0.05$). The percentage of fertile eggs laid and the percentage of eggs incubated that hatched were arcsin transformed to equalize variance before one-way ANOVA was conducted. Retransformed means are reported herein. Fluoride residues in bone were compared by two-way ANOVA (treatment and sex). Residue values of fluoride in eggshells were converted to common logarithms to correct for the skewed distribution of values before comparison by one-way ANOVA. When significant treatment differences were detected, Duncans's Multiple Range Test ($P \leq 0.05$) was used to separate means. Correlation coefficients were used to examine relationships among residue levels in bone, eggshells, and number of fertile eggs.

Results

Food consumption of any given pair varied throughout the study period but monthly means were similar for different treatment groups. Pairs

Table 1. Reproductive success of eastern screech-owls fed fluoride

Treatment	N	Eggs/Pair (±SE)	Fertility: Number/Clutch (±SE)	Fertility: % [b]	Hatching success[a]: Young/Clutch (±SE)	Hatching success[a]: %
Control	11	5.6 ± 0.24A[c]	5.5 ± 0.46A	99.9A	4.2 ± 0.42A	96.4A
40 ppm	10	5.2 ± 0.26A	4.4 ± 0.48AB	91.5A	3.2 ± 0.44AB	79.9A
200 ppm	11	5.1 ± 0.24A	3.9 ± 0.46B	82.0A	2.6 ± 0.42B	64.6A

[a] One egg removed from each clutch: these eggs excluded from hatching success data
[b] Percentages retransformed from arcsine transformed data analyses
[c] Means with different letters significantly different ($P < 0.05$)

consumed 1,300–1,700 g/month, about 50% of the food provided. Males were lighter than females and body weights of both sexes declined throughout the study (Figure 1), a normal occurrence for this species (Wiemeyer In Press). Within sex, there were no differences between treatment groups except for the females fed 40 ppm who were significantly lighter at the end of the study than the females from the control or 200 ppm groups.

Of the 33 pairs of birds, all females laid eggs except one in the 40 ppm treatment group where the male died 11 March. Nesting chronology with respect to initiation of egg laying, initiation of incubation, hatching date, or intervals between any of these events was similar among treatment groups. No significant differences occurred among treatments with regard to clutch size, percent fertility, or percentage of eggs hatched of those incubated (Table 1). Reproductive success in the 200 ppm NaF group, as measured by the number of young produced per clutch (Table 1), was significantly lower than controls. Pairs receiving 200 ppm NaF produced significantly fewer fertile eggs than controls although no significant differences ($P = 0.072$) occurred between controls and F-treatments in percentage of eggs that were fertile.

Eggshell thickness and the thickness indices were not significantly different among treatments, whereas eggshell fluoride concentrations increased with increasing dietary fluoride (Table 2). Results from a number of haphazardly collected eggshells (Table 3) suggest a trend for residues to increase with egg order within the clutch. At the 200 ppm dietary level, about a 3-fold increase occurred within clutches. Femur fluoride concentrations also increased with increasing dietary fluoride (Table 4) in both sexes. However, there were no significant differences between treatment groups. Females exhibited significantly higher levels than males within the 40 ppm and 200 ppm treatment groups. Fluoride concentrations in femurs of females were significantly correlated ($r = 0.51$, $N = 15$) with those in the shell of the third egg but were not correlated with number of fertile eggs laid ($r = -0.50$, $N = 15$). Fluoride concentrations in the shell of the third egg were not significantly correlated with the number of fertile eggs in that clutch ($r = -0.34$, $N = 31$) nor was the fluoride concentration in the femurs of the males correlated with the number of fertile eggs in their clutch ($r = -0.08$, $N = 15$).

Table 2. Mean shell thickness, thickness index, and geometric mean fluoride concentrations (dry weight) of eastern screech-owl eggshells from birds fed fluoride

Treatment	N	Shell thickness (mm ± SD)	Thickness index (±SD)	ppm Fluoride
Control	11	0.250 ± 0.017	1.277 ± 0.079	6.4A[a]
40 ppm	10	0.230 ± 0.018	1.186 ± 0.087	53.3B
200 ppm	11	0.240 ± 0.022	1.212 ± 0.122	87.2B

[a] Means with different letters were significantly different ($P < 0.05$); all others were not different

Wet weight concentrations of fluorides in the diet were 56.5 ppm and 232 ppm versus the predicted 40 ppm and 200 ppm, 10–20% higher than expected due to fluoride in the control diet (27.2 ppm). Nutrient content (Table 5) data are provided to confirm the adequacy of the diet and may be compared to those reported by Pattee (1984). The calcium/phosphorus ratio was 1.7/1.0.

Discussion

The addition of 200 ppm fluoride to the diet of eastern screech-owls significantly reduced reproductive success as indicated by the number of young produced. Success intermediate between controls and the 200 ppm group was noted at 40 ppm. The reduction in number of young produced in the 200 ppm group appeared attributable to a combination of factors which, by themselves, were

Table 3. Fluoride content (ppm, dry weight) of eggshells of eastern screech-owls from clutches in which two or more eggs failed to hatch

Egg Laying Sequence	Pen number and treatment						
	664 (Control)	655A (40 ppm)	674 (40 ppm)	635 (200 ppm)	661 (200 ppm)	672 (200 ppm)	679 (200 ppm)
1	7.7	26	12	—[a]	72	30	74
2	8.6	32	13	—	140	42	74
3	6.6	97	17	30	140	72	200
4	—	88	—	74	—	86	220
5	—	—	—	86	210	130	—

[a] Dash indicates that egg hatched and shell was unavailable

Table 4. Fluoride levels (ppm, dry weight) in femurs of eastern screech-owls fed fluoride

Treatment	N	Sex	ppm ± SD
Control	5	Male	964 ± 565
Control	5	Female	1,040 ± 536
40 ppm	5	Male	724 ± 122
40 ppm	5	Female	1,600 ± 552
200 ppm	5	Male	1,134 ± 259
200 ppm	5	Female	1,720 ± 286

Table 5. Nutrient content of the diet given eastern screech-owls November 1981 to June 1982

Moisture (%)	56.2
Protein (%)	20.5
Fat (%)	9.7
Ash (%)	6.0
Crude fiber (%)	3.4
Carbohydrates (%)	4.2
Calories (per 100 g)	186.0
Calcium (%)	1.74
Phosphorus (%)	1.05

not significant. For example, 65–68% of the reduction could be attributed to a decrease in egg fertility. Our findings coincide with the results of van Toledo and Combs (1984), who found differences in egg production of chickens fed fluoride. However, our results conflict with those of Bird and Massari (1983), who found an increase in the fertility of American kestrels (*Falco sparverius*) fed fluoride. Their kestrel data appear suspect because their control birds exhibited fertility rates of only 35–41% compared to 85–95% in our study. Van Toledo and Combs (1984) found major differences between two strains of white leghorn hens and there may be even greater interspecific differences. A reduction of 40% in the number of young produced, as found in the 200 ppm group of our study, could have long-term impacts on the success and survival of wild bird populations. This reduction in reproductive success might explain the extirpation of wild house martins reported by Newman (1977). Certainly differences in sensitivity to fluorides exist.

Wright *et al.* (1978) reported fluoride levels (dry weight) in small mammals from a fluoride polluted environment ranging from 12.6 ppm (muscle) to 4,387 ppm (femur). Andrews *et al.* (1982) found in a fluoride-contaminated grassland fluoride levels (dry weight) in invertebrates ranging from 321–3,204 ppm and in small mammals from 27.6 ppm (muscle) to 1,283 ppm (femur) with whole body levels of 332 ppm in *Microtis agrestis* and 1,063 ppm in *Sorex araneus*. Although the availability of fluoride stored in bone, muscle, and other tissues is unknown, the levels of fluoride utilized in this study appear to fall within the range of environmental samples from polluted areas.

The thin eggshells found in wild tawny owl nests by van Toledo (1978) and attributed to fluoride could not be confirmed by this study, even though our eggshell residues approached or exceeded those found in tawny owl eggshells. Nor could we confirm the trend towards thicker eggshells reported by Bird and Massari (1983). Again, their kestrel data must be qualified, because the treated birds had significantly thicker shells than the controls before treatment; shell thickness of birds on the fluoride diet increased only 0.004–0.006 mm.

Fluoride concentrations in tissues and shells were variable, although a trend towards higher residues at higher treatments was apparent. We also noted the same trend reported by Bird and Massari (1983) for fluoride levels in eggshells to increase in successively laid eggs. Eggshell fluoride levels for controls were similar to those from uncontaminated locations (van Toledo 1978; Seel 1983), whereas eggshell concentrations from our 200 ppm treat

ment were comparable to those found in Great tits (*Parus major*) and tawny owls from contaminated environments (van Toledo 1978). The data suggest that eggshell fluoride residues have limited usefulness in interpreting impacts on reproduction. Not only does egg order affect residue concentration in eggshells, the variability between individuals serves to further confound treatment differences. Some birds from high and low treatment levels had similar residues. This is also evident in the bone fluoride levels and their relationship to productivity. This variability suggests different uptake, excretion, and/or deposition rates among individual birds; part of this may be tied to calcium uptake as evidenced by the differences between males and females on the same diets. This is especially noteworthy because females dumped fluoride into their eggshells yet still had higher bone fluoride levels than males. Whether this is also tied to bone volume, as reported by van Toledo and Combs (1984), was not investigated.

Bone fluoride levels are of limited usefulness because of the variability in residue levels. However, they are of comparative value. Bird and Massari (1983) reported lower bone levels than this study at comparable or higher dosages; this appears to be related to their shorter treatment time and their analysis of only the diaphyses. Seel and Thomson (1984) determined fluoride concentrations in femurs of predatory birds in the British Isles and found mean levels greater than 1,500 ppm (dry weight) in 4 of 12 species and levels greater than 500 ppm in 8 of 12 species. It should be noted that the controls in our study had femur fluoride levels exceeding 900 ppm, possibly reflecting the 27 ppm (wet weight) fluoride in the diet. The value of bone fluoride concentrations is rather like that of bone lead concentrations (Pattee 1984)—useful as an indicator of exposure but not specific enough to be useful in assessing hazard.

The information presented in this paper and by Hoffman *et al.* (1985) indicates that fluoride ingestion may have a detrimental effect on reproductive success of eastern screech-owls. The levels of sodium fluoride used in our study yielded bone and eggshell residues which are comparable to those found in wild populations of birds. However, the data also suggest that individual variability seriously hampers the usefulness of fluoride residue data in evaluating field situations. The available literature also suggests major species differences in fluoride accumulation and, perhaps, their response to elevated levels. Based on the results of the present study, a closer scrutiny of the problem in the field in concert with additional laboratory work appears justified, since fluoride ingestion at environmentally realistic levels may induce moderate to severe impacts on recruitment in some avian populations.

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