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FLUORIDE EXPOSURE AND SELECTED CHARACTERISTICS OF EGGS AND BONES OF THE HERRING GULL (*LARUS ARGENTATUS*) AND THE COMMON GULL (*LARUS CANUS*)

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ABSTRACT: Fluorine concentrations were determined in the shell of 285 herring gull eggs (*Larus argentatus*) and 120 common gull eggs (*Larus canus*), collected May 1991 to 1993, from breeding colonies exposed to emissions from two Norwegian primary aluminum smelters located at Karmøy and Sunndal, and from unexposed reference localities in Eigersund, Sola, and Stavanger. Volume-index, shell thickness, thickness-index, and fertilization of the eggs also were monitored. In both species, the shell fluorine concentration was significantly increased in eggs collected at sites exposed to fluoride emissions. No effects on other egg characteristics were observed. In both exposed and unexposed sites, the last-laid egg in a clutch, normally containing three eggs, had the highest shell fluorine residue. Fluorine levels also were analyzed in femurs from 42 herring gulls, collected from Karmøy and Sola in May 1993. The relationship between sex and fluoride accumulation, and the relations between fluorine concentration in femurs of laying herring gulls and in the shell of their eggs, were evaluated. Bone morphology also was studied. Bone fluorine concentrations were raised significantly in emission-exposed female birds. Moreover, females from the exposed site had significantly higher fluorine residues than males. There was a positive correlation between fluorine levels in femurs of individual laying birds and those in the shells of their eggs. No changes in bone morphology due to fluoride exposure was found.

Key words: Fluorine, fluoride emissions, birds, Laridae, *Larus argentatus*, *Larus canus*, egg, bone.

INTRODUCTION

One of the main environmental concerns connected to aluminum production is the impact of fluoride emissions on local wildlife. Detrimental effects of chronic fluoride exposure have been extensively documented in domestic and wild animals (Shupe and Olson, 1983; Shupe et al., 1984). Yet, little is known about the potential hazard posed by fluoride to wild birds. Fluorine concentrations exceeding normal background levels have been documented in bones and eggs of wild birds living in the vicinity of aluminum smelters, including sparrows (species not given) (Macuch et al., 1969), great tits (*Parus major*) (van Toledo, 1978), and Canada geese (*Branta canadensis*) (Vikøren and Stuve, 1995). However, possible detrimental effects of fluoride were not examined in these studies, except for Vikøren and Stuve (1995) who found no effect on the circumference versus length ratio of tarsometatarsus in moderately fluoride exposed Canada geese. To our knowledge, there have been no field studies of wild birds to determine the relation between fluorine concentration in eggshells and other egg parameters. Yet, such relationships have been studied experimentally in captive American kestrels (*Falco sparverius*) (Bird and Massari, 1983) and in eastern screech-owls (*Otus asio*) (Hoffman et al., 1985; Pattee et al., 1988).

Herring gulls (*Larus argentatus*) and common gulls (*Larus canus*) breed in large colonies within the premises of two Norwegian aluminum smelters, Karmøy and Sunndal, respectively. Although both species are migratory and opportunistic omnivorous feeders (Haftorn, 1971), we evaluated their suitability as indicators of fluoride exposure because they nest within the premises of the smelters. Moreover, it had been reported locally that some common gull eggs with soft or wrinkled shell were found in the colony at Karmøy. The biology of the two gull species is well documented (Barth, 1967; Haftorn, 1971). However, no data on fluoride exposure has

been published, except for background fluorine levels in some gull species (Stewart et al., 1974; Turner et al., 1978).

Fluoride is attracted to mineralized tissues (Eanes, 1983). In eggs, Hahn and Guenter (1986) have shown that fluoride is mainly incorporated in the shell, only low concentrations being found in albumen and yolk. The shell fluorine concentration thus is regarded as the most suitable parameter for fluoride deposition in the egg. Moreover, Carrière et al. (1987) found in short term feeding experiments that the eggshell fluorine content is very sensitive to fluoride exposure. Wild birds normally produce a limited number of eggs in one clutch during the breeding season. Experimentally, the order in which the eggs are laid influences the shell fluorine residue (Bird and Massari, 1983; Pattee et al., 1988).

Our objectives were to determine the fluorine concentration in eggshells of herring gulls and common gulls exposed to fluoride emissions from aluminum smelters, and to evaluate the use of this parameter as an indicator of local fluoride exposure; determine the relationship between egg-order and shell fluorine residues; determine the relation between the bone fluorine concentration in female herring gulls and the eggshell fluorine level; test for sex differences in bone fluoride deposition; and identify possible detrimental effects of fluoride exposure on eggs and bones of the gulls.

MATERIALS AND METHODS

We tested 285 herring gull eggs, 120 common gull eggs, and 42 herring gulls. Normally, herring gull and common gull clutches contain three eggs (a, b, and c), with the first laid usually designated the a egg being. The last laid egg (c) was identified due to diverging color pattern, size, and shape (Barth, 1967).

On 19 May 1991 and 2 and 13 May 1992, pairs of herring gull eggs containing the a or b (a/b) and the c eggs, were randomly collected from a colony within a distance of 500 m from the aluminum smelter in Karmøy (59°18′N, 05°19′E). For reference data, eggs were collected in 17 May 1991 and 13 May 1992 from a colony in Eigersund (58°27′N, 05°46′E), an area unaffected by aluminum smelter emissions. On 14 and 15 May 1993, herring gulls from Karmøy were shot while brooding, and collected together with all three eggs (a, b, and c) in the clutch. Due to unfavorable topographic conditions for shooting brooding birds at the primary reference site at Eigersund, herring gulls and eggs for reference data were collected from two colonies in the uncontaminated area of Sola (58°55′N, 05°30′E and 58°53′N, 05°26′E), 14 to 20 May 1993.

Pairs of common gull eggs (a/b and c) were collected from the Karmøy colony on 2 June 1992. On 25 May 1993, eggs were collected from a common gull colony situated within 500 m of the Sunndal aluminum smelter (62°40′N, 08°32′E). Reference material were collected on 22 May 1992 from a common gull colony at Flataskjer (58°57′N, 05°50′E) in Stavanger.

The length (L) and the width (W) (long and short axes, respectively) of the eggs were measured with sliding calipers to an accuracy of 0.5 mm. The eggs were opened and examined for evidence of fertilization and embryonic development. The duration of incubation was not known. The volume of the eggs was estimated according to the equation for volume-index = $(\pi/6)LW^2k$, k = 0.971, as described by Barth (1967). The inner membranes of the eggs were removed from the shell using running tap water and a small brush. The shells were left to dry at 18 to 24 C for several weeks and the shell thickness then measured at the blunt end using a spring-loaded micrometer (0-25, Helios, Niedernhall, Germany) with an accuracy of 0.01 mm. The mean of three measurements was recorded as the shell thickness. Additionally, the shell weights were registered for herring gull eggs collected in 1993, and the thickness-index (shell weight/LW) was calculated as described by Ratcliffe (1967).

All herring gulls shot were necropsied shortly after death. Sex and body weight were recorded, and the right femur was fixed in 10% formalin for histological preparation. Following decalcification of the femurs in 14% hydrochloric acid, 5 μm thick paraffin-embedded sections were prepared, stained with hematoxylin and eosin, and examined microscopically. The left femur from each gull was dissected, cleaned of soft tissue, and kept frozen at −18 C until submission for fluorine analysis. The contents of the gizzards were identified using a wild M5A stereomicroscope with magnification up to 50× (Wild Heerbrugg Ltd., Heerbrugg, Switzerland).

The fluorine analyses were performed at the laboratory of Hydro Aluminium in Årdal, Norway. Eggshells and femurs were reduced to ash

TABLE 1. Shell fluorine (F) concentrations in successively laid herring gull eggs (a/b and c) collected during 1991, 1992, and 1993 at an aluminum reduction plant, Karmøy, Norway, and at reference sites not exposed to industrial fluoride emissions.

Locality	Egg category	Number of eggs	Fluorine concentration (ppm F in ash)				
			Mean	SD	95% Confidence interval	Median	Range
Karmøy	a/b eggs	78[a]	120[d,f]	54	108–132	108	54-376
	c eggs	62	195[d,g]	80	175–215	180	74-545
	All eggs	140	153[h]	76	140–166	139	54-545
Reference	a/b eggs	81[b]	83[e,f]	61	70–96	70	20-306
	c eggs	61	143[e,g]	61	127–159	133	47-301
	All eggs	145[c]	109[h]	68	98–120	92	20-306

[a] In 16 of 62 clutches, both the a and b eggs were included (1993 material).
[b] In 20 of 61 clutches, both the a and b eggs were included (1993 material).
[c] Three eggs in one clutch could not be categorized as a, b, or c.
[d,e,f,g,h] Means with same superscript were significantly different ($P < 0.001$).

at 700 C for a minimum of 8 hr. The fluorine (F) concentrations in ash samples were determined by a solid-state fluoride-selective electrode (Sintalyzer-system) as described by Nagy and Keul (1978). Two parallel ash samples from each specimen were analyzed, and the mean recorded as the fluorine concentration. Results were expressed as ppm F (mg F/kg) of ashed eggshell or bone.

The data from the two reference localities for herring gull, Eigersund and Sola, were pooled and employed in the statistical analyses as one unit (reference group). The Student procedure was used for construction of 95% confidence intervals for the means (Altman, 1991). The localities were compared using a two sample *t*-test (Altman, 1991). Within localities, differences between a/b and c eggs were investigated using a paired *t*-test (Altman, 1991). In the clutches with both the a and b eggs (herring gulls in 1993), the data used for statistical analyses were based on the mean of the a and b eggs. Correlation analysis was carried out using the Pearson model (Altman, 1991). We used $\alpha = 0.05$ as the level of statistical significance.

RESULTS

Shell fluorine concentrations in herring gull eggs from Karmøy were significantly higher than in the reference group, both when comparing all eggs and when comparing a/b eggs and c eggs separately (Table 1). Within each group, c eggs had significantly higher shell fluorine levels than a/b eggs.

There were significant differences in common gull eggshell fluorine concentration between the three localities (Table 2). Shells from Sunndal had the highest mean fluorine level, followed by Karmøy. Comparing shell fluorine concentrations in a/b eggs and c eggs separately, significant differences between localities were found, except for a/b eggs between the reference group and Karmøy, and for c eggs between Sunndal and Karmøy. Within localities, fluorine levels in c eggs were significantly higher than in the a/b eggs. However, in both species the relative difference between the shell fluorine content in c and a/b eggs was no greater in exposed sites compared with the unexposed.

There were no significant differences in the volume-index of herring gull eggs between exposed and unexposed sites (Table 3). Within each group, the a/b eggs had a significantly higher volume-index than the c eggs. Corresponding results were also found for common gull eggs (Table 4). No correlation between shell fluorine concentration and thickness was found in either of the gull species. Furthermore, no differences in shell thickness, either between or within localities, were observed (Tables 3 and 4). There were no significant difference in the thickness-index of herring gull eggs collected in 1993 between or within localities (data not shown). In all groups of

TABLE 2. Shell fluorine (F) concentrations in successively laid common gull eggs (a/b and c) collected during 1992 and 1993 at the aluminum reduction plants at Karmøy and Sunndal, Norway, and at a reference site not exposed to industrial fluoride emissions.

Locality	Egg category	Number of eggs	Fluorine concentration (ppm F in ash)				
			Mean	SD	95% Confidence interval	Median	Range
Sunndal	a/b eggs	20	185[a,d,e]	66	154–216	174	91–341
	c eggs	20	212[a,f]	64	182–242	205	126–369
	All eggs	40	199[h,i]	66	178–220	194	91–369
Karmøy	a/b eggs	20	137[b,e]	51	114–161	125	64–237
	c eggs	20	175[b,g]	73	141–209	153	99–358
	All eggs	40	156[i,j]	65	136–177	140	64–358
Reference	a/b eggs	20	85[c,d]	34	69–101	85	34–148
	c eggs	20	128[c,f,g]	50	105–151	127	45–233
	All eggs	40	106[h,i]	48	91–122	102	34–233

[a,b,c,d,e,f,g,h,i,j] The means with same superscript were significantly different with the following probabilities; [a,b] $P < 0.05$, [c,e,i] $P \leq 0.01$, [d,f,g,h,j] $P \leq 0.001$.

eggs, both from herring gulls and common gulls, 95% or more of the eggs were fertilized, and the embryos had reached different stages of development.

Individual femur fluorine concentrations among herring gulls varied considerably (Table 5). Bone fluorine levels in breeding female birds were significantly higher in Karmøy compared with the reference locality. Furthermore, females from Karmøy had significantly higher mean fluorine concentrations than the males from the same colony. A significant positive correlation ($r = 0.71$, $n = 27$, $P < 0.01$) was found between fluorine concentrations in the femurs of herring gull females and the corresponding c eggshells. This correlation increased when comparing data from Karmøy only ($r = 0.92$, $n = 14$, $P < 0.01$).

On histological examination, there were varying amounts of medullary bone in femurs of the females. No histopathological lesions related to fluoride exposure were observed in femurs. The gizzard contents in herring gulls were basically of marine origin, consisting mainly of mollusks, crabs, fish, and small amounts of plant material; there was no difference by sex.

DISCUSSION

Significantly higher shell fluorine content occurred in gull eggs collected from colonies within the premises of the two aluminum smelters, compared to eggs from colonies not exposed to industrial fluoride emissions. The mean fluorine levels were 40 to 90% higher in exposed areas than in reference areas. Using great tits as indicators, van Toledo (1978) found a 3.5-fold increase in shell fluorine levels in eggs collected in the vicinity of an aluminum smelter compared to background levels. Thus, shell fluorine concentration seems to be a suitable parameter for monitoring local fluoride exposure on wild birds, including the migratory gull species examined in our study.

We found no differences in egg volume, shell thickness, and percent fertilized eggs, between eggs collected near the aluminum smelters and those collected in reference areas. Nor was bone morphology altered in herring gulls collected from Karmøy. Guenter (1979) found that shell thickness was reduced when pullets (*Gallus domesticus*) were fed 200, 400 or 800 ppm F in the ration for 112 days, however no effect was seen on fertility or hatchability, and egg size was not substantial reduced until birds were fed 1000 ppm F in the ration. Hoffman et al. (1985) found a significant reduction of 6% in eggvolume when eastern screech owls were fed 40 and 200 ppm

TABLE 3. Volume-index and shell thickness (mm) in successively laid herring gull eggs (a/b and c) collected during 1991, 1992, and 1993 at an aluminum reduction plant, Karmøy, Norway, and at reference sites not exposed to industrial fluoride emissions.

		Volume-index				Shell thickness			
Locality	Egg category	Number of eggs	Mean	SD	Range	Number of eggs	Mean	SD	Range
Karmøy	a/b eggs	75[a]	87.8[d]	8.13	63.9–104.3	78	0.289	0.025	0.230–0.367
	c eggs	60	81.5[d]	7.38	60.0–95.3	62	0.288	0.032	0.143–0.347
	All eggs	137[b]	85.1	8.45	60.0–104.3	140	0.288	0.028	0.143–0.367
Reference	a/b eggs	81[c]	89.7[e]	6.02	78.5–105.9	81	0.286	0.021	0.247–0.350
	c eggs	61	83.8[e]	6.93	67.8–100.5	61	0.283	0.022	0.240–0.333
	All eggs	142[b]	87.2	7.03	67.8–105.9	145	0.284	0.022	0.228–0.350

[a] In 15 of 60 clutches, both the a and the b eggs were included.
[b] Three eggs were partly crushed.
[c] In 20 of 61 clutches, both the a and the b eggs were included.
[d,e] The means with same superscript were significantly different ($P < 0.0001$).

F in the ration for 5 to 6 mo. This feeding regime gave 7.3 and 12.6 times higher shell fluorine concentrations in the 40 and 200 ppm F groups, respectively, than in the control group, not fed fluoride (Pattee et al., 1988). No effect on shell thickness was demonstrated (Pattee et al., 1988). Thus, the moderate increase in shell fluorine concentrations found in our study are evidence that the fluoride exposure posed on the gulls was far from the magnitude necessary to induce toxic effects on the egg characteristics studied.

The higher shell fluorine level in common gull eggs from Sunndal compared to Karmøy probably was related to the higher level of emission in Sunndal, particularly with respect to the gaseous emissions. In Sunndal, the mean monthly emission of gaseous fluoride between March and May 1993 (year of sampling) was 6.5 kg F/hr, whereas the corresponding figure at Karmøy in 1992 was 2.4 kg F/hr (Anonymous, 1994). The mean total fluoride (gaseous and particulate) emission during these periods was 11.7 and 9.3 kg F/hr, respectively (Anonymous, 1994). Generally, birds are likely to be exposed to fluorides through ingestion of polluted feed and drinking water, and through inhalation. In gulls, the

TABLE 4. Volume-index and shell thickness (mm) in successively laid common gull eggs (a/b and c) collected during 1992 and 1993 at Karmøy and Sunndal aluminum reduction plants, Norway, and at a reference site not exposed to industrial fluoride emissions.

		Volume-index				Shell thickness			
Locality	Egg category	Number of eggs	Mean	SD	Range	Number of eggs	Mean	SD	Range
Sunndal	a/b eggs	20	49.0[b]	5.03	38.2–60.0	20	0.215	0.016	0.190–0.253
	c eggs	20	46.4[b]	4.10	38.9–54.7	20	0.208	0.016	0.190–0.253
	All eggs	40	47.7	4.72	38.2–60.0	40	0.212	0.016	0.190–0.253
Karmøy	a/b eggs	20	47.9[c]	4.17	41.0–55.5	20	0.215	0.016	0.177–0.240
	c eggs	20	43.9[c]	3.61	34.3–49.3	20	0.212	0.015	0.190–0.237
	All eggs	40	45.9	4.34	34.3–55.5	40	0.213	0.015	0.177–0.240
Reference	a/b eggs	20	48.3[d]	4.37	42.3–57.3	20	0.212	0.015	0.180–0.247
	c eggs	19[a]	44.5[d]	3.77	38.7–52.0	20	0.214	0.014	0.187–0.237
	All eggs	39	46.5	4.49	38.7–57.3	40	0.213	0.014	0.180–0.247

[a] One c egg was partly crushed.
[b,c,d] The means with same superscript were significantly different ($P \leq 0.001$).

TABLE 5. Bone fluorine (F) concentrations in female and male herring gulls collected in 1993 at Karmøy aluminum reduction plant, Norway, and at a reference site not exposed to industrial fluoride emissions.

			Fluorine concentration in femur (ppm F in ash)				
Locality	Sex	Number of birds	Mean	SD	95% Confidence interval	Median	Range
Karmøy	Female	14	3,800[a,b]	929	3,264–4,336	3,615	2,808–6,185
	Male	7	2,605[b]	483	2,158–3,052	2,512	2,118–3,608
Reference	Female	13	3,022[a]	656	2,626–3,418	3,191	1,759–3,906
	Male	8	3,013	545	2,557–3,469	3,107	2,143–3,714

[a,b] The means with same superscript were significantly different with the following probabilities; [a] $P = 0.02$, [b] $P < 0.01$.

relative importance of each of these sources is difficult to assess. They probably are less exposed through contaminated feed than herbivorous species. Yet, inhalation of fluorides may be a significant route of absorption, because we often observed gulls fly close to and over the aluminum smelters, where air concentrations of fluorides are highest.

The gulls were subject to highest fluoride exposure during the breeding season, when living near or on the aluminum smelter sites in Karmøy and Sunndal. Considering the migration behavior of these two species (Haftorn, 1971), we assumed they were exposed to fluoride emissions for an average of 5 to 6 mo each yr, from March or April until August or September. Considering the time of egg-laying (Haftorn, 1971), 2 to 3 mo of exposure preceded egg-laying. In female birds, medullary bone is formed 1 to 2 wk before egg production starts (Simkiss, 1975). Fluoride absorbed by females during this period will be accumulated mainly in the developing medullary bone, since freshly ingested fluoride concentrates preferentially in developing bone undergoing active mineralization (Eanes, 1983). The extent of bone mineral mobilization during shell formation is dependent on the gastrointestinal absorption of calcium (Sturkie and Mueller, 1976). Thus, we believe that the fluoride exposure of female gulls during medullary bone formation and egg production is decisive for the eggshell fluorine residue. However, redistribution of fluoride deposited in other parts of the skeleton may also contribute to the shell fluorine content.

We observed that the last-laid egg in a clutch contained the highest shell fluorine concentration. This agrees with experimental findings in captive American kestrels (Bird and Massari, 1983), and in eastern screech owls (Pattee et al., 1988). The mechanism underlying the increase in fluorine residues in eggs with increasing order of laying is not known. In herring gulls, the brooding behavior is induced when the first egg is laid (Haftorn, 1971), and the onset of incubation during the laying period is responsible for the smaller c egg (Parsons, 1972). Onset of brooding is affected by prolactin which decreases the production of gonad-stimulating hormones (Parsons, 1976). As a result, ovarian follicles degenerate and secretion of estrogen is depressed (Parsons, 1976). It is possible that these hormonal changes associated with brooding may alter intestinal calcium absorption or medullary bone resorption during the laying period in herring gulls. If intestinal absorption of calcium is reduced when the shell of the last-laid egg is formed, more calcium has to be mobilized from the skeleton (Sturkie and Mueller, 1976). Such mobilization then would also release any deposited fluoride, with higher plasma fluorine levels; in turn this would increase shell fluorine residues in the c egg.

The positive correlation between the fluorine concentration in femurs of herring

gull females and the corresponding shell of the last-laid egg, was stronger in our study (r = 0.71) than that reported for eastern screech owls by Pattee et al. (1988) (r = 0.51, n = 15). In our study, the correlation between fluorine levels in femurs and eggshells was more marked when using data from exposed birds only, and it might have become even stronger if it was possible to relate the shell fluorine level in the c egg to the bone fluorine level just prior to egg production.

A significant sex difference in bone fluorine level was found in herring gulls from Karmøy, but not in birds from the reference locality (Table 5). These results correspond with the findings of Pattee et al. (1988). Experimentally, Michel et al. (1984) found no difference in bone fluoride deposition between male and inactive female domestic chicks (*Gallus domesticus*); however, increased fluoride deposition was found in egg-producing female birds. Thus, absorption of excessive amounts of fluoride by females in association with egg production, seems to give rise to a sex difference in bone fluorine concentration. Divergent results on the influence of sex on fluoride accumulation in birds have been reported (Seel et al., 1986; Pattee et al., 1988; Henny and Burke, 1990); however, the importance of the reproductive status of the females has not been taken into account in some of these studies.

Comparing contaminated and uncontaminated localities, we found significantly higher bone fluorine levels in exposed versus unexposed females. This difference was not evident in males. The extent of the fluoride exposure in the vicinity of the Karmøy aluminum smelter probably was not sufficiently great to cause bone fluorine concentrations to rise beyond background levels in males. Yet, the age of the birds was unknown. Thus, age might have been a confounding factor, if the males from the exposed site generally were younger than those from the reference area.

Background bone fluorine levels have not previously been studied in herring gulls, but figures were given for red-billed gulls (*Larus novaehollandiae scopulinus*) and black-backed gulls (*Larus dominicanus*) in two separate studies from New Zealand (Stewart et al., 1974; Turner et al., 1978). The mean fluorine level in femur of herring gulls (3019 ppm F, n = 21) was lower than found in tibia of red-billed gulls (4,003 ppm F, n = 16; 5,402 ppm F, n = 31) and higher than in tibia of black-backed gulls (1,907 ppm F, n = 16; 1,489 ppm F; n = 33). This might be due to different dietary exposure to fluoride, and differences in age and sex distribution of the birds included in the studies. The background fluorine levels in gulls exceed those reported in several species of Passeriformes, Ardeidae, Strigiformes and Falconiformes (Stewart et al., 1974; Seel and Thompson, 1984).

Based on our results, we propose that the fluorine concentration in eggshells of gulls is a useful parameter for monitoring local exposure to industrially-emitted fluorides. The shell fluorine residue, particularly in the last-laid egg, seemed very sensitive to fluoride exposure. Moreover, a correlation between fluorine concentrations in the skeleton of females and their eggshells occurred in herring gulls. No detrimental effects caused by fluoride were registered in our study, indicating exposure below the toxic threshold. Thus, the current fluoride emissions from the smelters in Karmøy and Sunndal seemed not to affect health and reproduction of the two gull species studied. Historical data describing the situation during periods with higher fluoride emissions are not available.

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