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Effects of dietary aluminum sulphate on reproductive success and growth of Ringed Turtle-Doves (*Streptopelia risoria*)

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Elevated levels of aluminum have been reported in surface waters and in certain aquatic organisms including aquatic insects. Toxic effects have been demonstrated in fish and it has been suggested that aluminum affects avian reproduction. In this study Ringed Turtle-Doves were assigned to a control group fed a diet reduced in calcium and phosphorus and to a treated group fed the same diet supplemented with 0.1% aluminum. A 4-month feeding period with aluminum sulphate did not result in any effect on egg production, fertility, or hatchability. Egg permeability was decreased initially but subsequently recovered to a normal level. Dietary aluminum sulphate did not affect plasma calcium, phosphorus, or magnesium of adults nor did it affect the pattern of growth and the final weight of chicks feeding on the same diet. Bone aluminum levels of breeding females averaged twice those of the controls ($P<0.05$). The pattern of accumulation between the two sexes was significantly different ($P<0.05$). Growth of juvenile Ringed Turtle-Doves was not affected by dietary levels of aluminum sulphate up to 1500 ppm, although aluminum tended to accumulate in the bones of the sternum. No significant influence of aluminum on reproduction or growth, at levels similar to those likely to occur in the environment, could be found in the course of our laboratory experiment.

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Les eaux de ruissellement et certains organismes aquations, y compris des insectes, contiennent des concentrations importantes d'aluminium. L'aluminium produit des effets toxiques chez les poissons et certains travaux suggèrent qu'il a aussi des effets délétères sur la reproduction des oiseaux. Au cours de cette étude, des tourterelles à collier ont été assignées à un groupe d'oiseaux témoins soumis à un régime réduit en calcium et en phosphore, ou alors à un groupe expérimental soumis à un régime identique, mais additionné d'aluminium (0,1%). Après 4 mois d'exposition à un régime additionné de sulfate d'aluminium, aucun effet sur la production d'oeufs, la fertilité ou le taux d'éclosion n'a été noté. Chez les oiseaux du groupe traité, la perméabilité de la coquille de l'oeuf a d'abord diminué, mais a ensuite rejoint la valeur enregistrée chez le groupe témoin. La présence de sulfate d'aluminium dans le régime n'a pas affecté les concentrations de calcium, de phosphore et de magnésium dans le plasma des adultes et n'a pas affecté non plus la forme de la courbe de croissance ou la masse ateinte par les oisillons soumis au même régime. Le concentrations d'aluminium contenues dans les os des femelles reproductrices atteignaient en moyenne deux fois la valeur enregistrée chez les femelles du groupe témoin ($P<0,05$). Les mâles et les femelles accumulent l'aluminium de façons significativement différentes ($P<0,05$). Le sulphate d'aluminium dans le régime, même à une concentration de 1500 ppm, reste sans effet sur la croissance des oisillons de tourterelles, bien qu'il y ait accumulation d'aluminium dans les os du sternum. Même à des concentrations expérimentales semblables aux concentrations naturelles, l'aluminium ne semble pas avoir d'influence significative sur la reproduction ou la croissance.

Introduction

Increased aluminum concentrations in surface waters resulting from acid precipitation has stimulated interest in the effects of this element on aquatic biota. Toxicity of aluminum to fish has been well documented (Muniz and Leivestad 1980; Spry *et al.* 1981; Baker and Schofield 1982). Reports of reproductive impairment of wild birds nesting in the vicinity of an acidified lake in Sweden has been attributed to aluminum intoxication (Nyholm and Myhrberg 1977; Nyholm 1981). Medullary bones from pied flycatchers (*Ficedula hypoleuca*) that produced eggs with defective shells showed the presence of aluminum while those from normal-laying birds did not. Subsequently this was attributed to elevated dietary aluminum from insect prey (Nyholm 1981).

Relative to other metals, the toxicity of dietary aluminum is low (Sorenson *et al.* 1974). In birds, the principal toxic effect seems to be the disturbance of phosphorus metabolism. Aluminum binds with phosphorus in the alimentary canal and forms insoluble compounds which cannot be absorbed (Ondreicka *et al.* 1971). When the dietary calcium level is low, the parathyroid gland increases the production of calcium-binding proteins resulting in an enhanced absorption of calcium in the gut (DeGrazia 1971). However these proteins also bind with metals including lead (Shields and Mitchell 1941; Six and Goyer 1970) and cadmium (Washko and Cousins 1977). It has also been shown that the absorption of many metals including zinc and cadmium is enhanced when the level of vitamin D is high and when the parathyroid activity is increased (e.g. during egg laying) (Worker and Magicovsky 1961*a*, 1961*b*). Mayor *et al.* (1977) found a similar relationship between the level of parathyroid hormone and the gastrointestinal absorption of aluminum in the rat. Consequently the aluminum level necessary to cause an effect may be lower when calcium and phosphorus in the diet are low and the demand for these elements is high.

The purpose of this experiment was to study the effects of a long-term exposure to dietary aluminum on reproduction and growth of Ringed Turtle-Doves (*Streptopelia risoria*) fed a diet low in calcium and phosphorus. The aluminum levels were based on the available data for aluminum concentrations in some of the potential prey organisms of insectivorous birds. In Sweden, Nyholm (1982) found levels of about 70 to 1230 ppm Al (dry weight basis) in emerging insects collected from an acidified lake. Hall and Likens (1981) reported levels of 840 ± 140 ppm of aluminum in aquatic insects surviving in an artificially acidified stream (pH = 4). In the present experiment we used the level of dietary aluminum of 1000 ppm, which seems to be close to the maximum levels found in prey items of

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wild birds living in acidified habitats. We set the calcium level to 0.9% and phosphorus to 0.5% based on a series of preliminary experiments. These levels are lower than those found in commercial breeding diets and closer to the levels likely to occur in surface waters in acid-sensitive areas. Data reported since this study was completed indicate that Ca and P levels in insects may in fact be much lower than these values and that aluminum concentrations may exceed those of calcium and phosphorus (Sadler and Lynam 1985).

Materials and methods

Ringed Turtle-Doves with known breeding performance were kept in a light- and temperature-controlled room where the photoperiod was adjusted to 14 h of light and 10 h of darkness and the mean daily temperature and humidity were maintained at 22 ± 0.52°C and 35.1 ± 10.7%, respectively. All pairs were successful breeders at the beginning of the experiment. Breeding cages with wired mesh floors measured 70 × 60 × 30 cm.

In August 1982, the pairs were placed on a commercial diet of Purina pigeon chow supplemented with oystershell. Initial egg weight, moisture loss, hatchability, and fledging success were recorded for both eggs of each clutch. The reproductive cycle of the pairs was then synchronized by removal of eggs. Once all the pairs were at late incubation, the eggs were removed once more and the treatment was started. The diets used in both the adult breeding study and the juvenile growth study were prepared by ICN-Nutritional Bio-Chemicals (Cleveland, OH, U.S.A. 44128, diet No. 904603). In January 1983, the pairs were randomly assigned to one of two dietary regimes: (*i*) control group, 20 pairs received the ICN-Nutritional diet with a reduced calcium and phosphorus level (0.9% Ca, 0.5% P); (*ii*) treated group, 20 additional pairs were assigned to the same diet as the control group but with 0.1% aluminum added as $Al_2(SO_4)_3 \cdot 18H_2O$.

Aluminum sulphate was chosen as it is the most common form of aluminum in acidified waters (LaZerte 1984) and soluble aluminum compounds have been reported to be more toxic to growing chicks than insoluble forms (Storer and Nelson 1968).

Both control and treated groups were divided into two equal subgroups of 10 pairs each to study, in undisturbed conditions, the breeding productivity in one subgroup while the egg permeability and the growth of young were measured in the other subgroup.

Breeding productivity study

Breeding birds were disturbed as little as possible and eggs were measured and weighed only once right after laying. Fertility was determined on the basis of apparent embryonic development when the eggs were candled at day 4. The eggs were incubated until hatching and the nestlings were removed and sacrificed at day 21 following weaning by parents.

Egg permeability and growth study

The breeding pairs were inspected two times a day, in the early afternoon and again in the evening. The eggs from the first, third, and fifth clutches were incubated by the adults and weighed every 2nd day until hatching. The fraction of initial egg weight lost per day over a 5-day period was calculated as an index of egg permeability. This period was chosen because after 5 days, small cracks were found to appear and the eggs became coated in faeces, an occurence which likely could affect egg weight measurements.

The chicks from clutch 1 were raised by the adults until day 21 when the chicks were removed to other cages of the same size. Every 7 days from day 1 to day 63, the chicks were weighed and the tarsus length was measured. At day 63 they were sacrificed and the carcasses stored at −40°C for subsequent chemical analysis. Chicks from clutches 3 and 5 were left in the adult cages until weaning at approximately day 21, when they were removed and sacrificed.

From the eggs laid in the first 20 days of the treatment period, one eggshell was selected from each of the control and treated groups and examined using a GOL 730 superprobe scanning electron microscope interfaced with a Kontron image analyser SEM-IPS. Ten sections selected at random on the eggshells were analysed for the number of pores, which were counted and averaged over the surface area examined. Comparisons between the two means were performed using a *t*-test.

Adult birds from control and treated groups were sacrificed 12 h after the laying of the first egg of the eighth clutch. Selected tissues (kidneys, humeri, and brain) were removed and stored frozen until aluminum assays were performed. Blood was removed by heart puncture, using a heparinized syringe. Blood samples were then transferred to 3-mL plastic acid-washed test tubes. After centrifugation, plasma was stored in 0.5-mL acid-washed plastic storage tubes. Plasma calcium levels were determined on an autoanalyser using a Sigma diagnostic kit (No. 586) which is based on the *o*-cresolphthalein reaction (Gitelman 1967). Total phosphorus was also measured by an autoanalyser using the Pierce Rapid Stat phosphorus kit, which is based on the formation of a molybdenum complex (Henry 1964).

An additional experiment to study the growth of juvenile Ringed Turtle-Doves exposed to dietary aluminum was run with a total of thirty-one 21-day-old postweaning chicks selected from the (undosed) breeding colony stock. These were divided into four groups. The control group (N=12 chicks; 7 ♀, 5 ♂) was fed a purified diet No. 904603 (ICN-Montreal, P.Q., Canada) modified to contain 0.6% calcium and 0.3% phosphorus. The remaining groups were given the same diet with 500 ppm (1 ♀, 5 ♂), 1000 ppm (5 ♀, 2 ♂), and 1500 ppm (5 ♀, 1 ♂) aluminum, respectively, mixed as $Al_2(SO_4)_3 \cdot 18H_2O$. The birds were weighed within 0.1 g precision every 7 days between day 0 and 63. On day 63, the birds were sacrificed and the bodies were stored at −40°C. The brains, kidneys, and sternum were subsequently removed from the carcasses and the humeri, radius, and ulnae and the bones of the manus of both wings were pooled with the femurae, tibiae, ulnae, peronae, and the bones of both feet for each individual bird for aluminum analysis.

Aluminum analyses of tissues were performed by the Laboratory Services and Applied Research Branch of the Ontario Ministry of Environment (Rexdale, Ontario) using flame atomic absorption. Bones, kidney, and brain tissues were digested in a mixture of perchloric and nitric acids. The samples were heated at 120°C for 3 to 4 h, at 150°C for an additional 3 to 4 h, and then at 180°C until less than 1 mL of clear, colorless digestate remained. The samples were cooled and brought to 25 mL with double-distilled water.

Statistical analysis of data was done using the SAS catalog of programs (SAS Institute 1982). Moisture loss expressed as a percentage of the initial egg weight was analysed using a mixed analysis of variance model. Egg production, hatchability, and fledging success were analysed through permutation tests (Crump and Howe 1979) where the nest was taken as the treatment unit. The observations from the last predosing clutch were compared with the observations for the first clutch during dosing using a paired permutation test. Plasma calcium, phosphorus, and magnesium were analysed by a two-way ANOVA to determine the effect of treatment and sex. Bone aluminum content was first analysed by a similar two-way ANOVA but the finding of a significant ($P<0.05$) interaction between treatment and sex made it necessary to assess the simple effect of each of these two factors separately using a one-way ANOVA.

The growth of all chicks in both experiments was analysed by best fitting the Gompertz equation to the observed weight increase using the nonlinear least squares program of the SAS package of programs. A two-way ANOVA was used to determine the effect of treatment, sex, and sex–treatment interaction on the asymptotic weight (Y_{inf}) and the constant rate of relative growth rate (A), which are two components with stable statistical estimates of the Gompertz equation (Schnute 1981).

Results

With the exception of a slight decline in moisture loss followed by a recovery, none of the parameters of reproduction were significantly affected by the aluminum treatment. Mean weight of males and females were not significantly ($P>0.05$) different in the two groups. In the control group the initial mean

TABLE 1. Reproductive success of Ringed Turtle-Doves for each of the first, third, and fifth clutches laid during an aluminum dosing experiment

	Interval to next laying (days)*	Egg production†	Fertility‡	Hatchability§	Fledging success‖
First clutch					
Control	8.00±4.72 (20)¶	40	35/40	22/35	15/22
Treated	7.55±1.64 (20)	40	39/40	20/39	18/20
Third clutch					
Control	10.75±5.28 (20)	39	31/39	19/31	14/19
Treated	11.15±5.32 (20)	39	35/39	19/35	17/19
Fifth clutch					
Control	10.22±4.80 (18)	36	32/36	17/30	—
Treated	9.70±3.40 (20)	40	38/40	21/34	—

*Time between removal of eggs or chicks to the laying of the next egg.
†Total no. of eggs produced.
‡No. of fertile eggs/no. of eggs laid.
§No. of hatched eggs/total no. of fertile eggs.
‖No. of young surviving to subadult stage/no. hatched.
¶Mean ± SD (no. of pairs).

TABLE 2. Mean daily weight loss* of eggs expressed as a percentage of initial egg weight during the administration of experimental diets

	Clutch 1	Clutch 3	Clutch 5
Control			
Egg 1	1.255±0.523(2)†	1.116±0.186(5)	1.212±0.556(3)
Egg 2	0.977±0.180(8)	1.215±0.148(4)	1.116±0.169(5)
Treated			
Egg 1	0.860±0.110(6)	1.094±0.200(3)	1.065±0.198(7)
Egg 2	1.609±0.173(7)	1.163±0.267(4)	1.141±0.187(6)

*Mean daily weight loss = $\frac{\text{(initial wt.)} - \text{(egg wt. at day 5)}}{\text{(initial wt.)} \times \text{(no. of days)}} \times 100$.
†Mean ± SD (no. of eggs).

TABLE 3. Aluminum content (μg/g on a dry weight basis) in the diaphyses of femurae, in kidneys, and in brain of adult Ringed Turtle-Doves

	Males	Females
Control		
Femurae	7.87±2.51 (6)*	7.42±2.14 (6)
Kidneys	0.75(1)	0.97(1)
Brain	ND†	ND
Treated		
Femurae	6.75±1.66 (6)	15.87±10.30 (6)
Kidneys	0.92±0.13 (5)	1.92±0.47 (4)
Brain	ND	ND

*Mean ± SD (no. above the detection limit out of six replicates).
†Not detectable.

weights were 156.3 ± 13.70 and 158.5 ± 10.53 g for males and females, respectively, and the final weights were 161.5 ± 11.84 and 162.0 ± 0.60 g, respectively. In the treated group the initial mean weights were 168.3 ± 7.80 and 165.5 ± 13.21 g for males and females, respectively, and the final weights were 172.3 ± 10.53 and 163.6 ± 11.90 g, respectively.

The mean interval to next laying was not affected by treatment and no trend was observed in either treatment group. Breeding success was virtually identical between the control and treated groups (Table 1).

During the treatment a significant trend over time ($P<0.05$) was found for moisture loss (Table 2) and initial egg weight for both control and treated groups. In the treated group, immediately following the initiation of treatment, a weak but significant decrease ($P<0.05$) in the rate of moisture loss was observed for the first egg but not for the second one. This effect was transient, however, and moisture loss for the first eggs from the third clutch was similar in both the treated and control groups.

Examination of an eggshell from each treatment group using the scanning electron microscope did not reveal any differences in the shell surface. The pore density was 40.5 ± 19.8 per 1000 μm^2 for the shell from the control group and 51.6 ± 22.6 per 1000 μm^2 in the shell from the treated group. The surface area of the pores was also not significantly different between the two eggshells examined. The pores of the eggshells covered 1.3 ± 0.7 and 1.6 ± 0.9% of the surface area of the control and treated shell samples, respectively. Based on these preliminary findings additional work did not appear to be justified.

Plasma calcium, phosphorus, and magnesium levels were not significantly affected by treatment ($P>0.05$). In both control and treated groups, females had calcium and magnesium levels significantly higher ($P<0.005$ and $P<0.01$, respectively) than males. Femurae of the females of the treated group (Table 3) contained more than double the aluminum content of male femurae. The significant ($P<0.045$) sex–treatment interaction reflects the different pattern of aluminum accumulation between females and males. The simple effect of treatment was significant ($P<0.01$) in females only. Aluminum levels in brain samples were all below the detection limits, which varied between 0.5 and 1.2 ppm depending on sample weight. In the treated group, the mean aluminum levels in kidneys were 1.92 ppm for females and 0.92 ppm for males. In the control group, kidney samples were mostly below the detection limit; however, one male was found to have 0.75 ppm and one female 0.97 ppm of aluminum in the kidney.

Growth rates of young of clutch 1, monitored in the course of the breeding productivity study (from day 0 to 63) showed no significant effects ($P>0.05$) resulting from treatment, sex, or the treatment–sex interaction as measured by the asymptotic weight (control group: 146.3 ± 12.1 g; treated group: 154.1 ± 13.5 g).

TABLE 4. Calcium, phosphorus, and aluminum levels (μg/g on a dry weight basis) in bones of growing Ringed Turtle-Doves administered four dietary levels of aluminum sulphate

	Sternum	Pooled leg and wing bones
Control		
Calcium	169 000 ± 17 700(12)*	137 000 ± 16 000(12)
Phosphorus	79 800 ± 8 700(12)	64 900 ± 7 500(12)
Aluminum	9.9 ± 3.3(12)	6.6 ± 1.7(12)
500 ppm		
Calcium	158 000 ± 21 000(6)	141 000 ± 9 300(6)
Phosphorus	75 700 ± 9 300(6)	67 700 ± 7 500(6)
Aluminum	10.6 ± 4.2(6)	7.0 ± 1.6(6)
1000 ppm		
Calcium	160 000 ± 29 000(7)	129 000 ± 12 500(7)
Phosphorus	76 600 ± 13 200(7)	62 000 ± 5 300(7)
Aluminum	11.3 ± 3.9(7)	6.2 ± 1.4(7)
1500 ppm		
Calcium	182 000 ± 23 000(5)	143 000 ± 17 700(5)
Phosphorus	86 400 ± 10 600(5)	69 000 ± 8 500(5)
Aluminum	15.9 ± 6.7(6)	6.7 ± 1.6(5)

*Mean ± SD (*N*).

Nor was the relative growth rate significantly ($P>0.005$) affected by aluminum treatment (control group: 0.1279 ± 0.0221; treated group: 0.1341 ± 0.0245). There was also no effect on these parameters in juvenile birds raised from day 21 to 63 on 0, 500, 1000, and 1500 ppm aluminum.

In the tissues of the juvenile birds, calcium and phosphorus levels in bones (Table 4) were not significantly affected by treatment. The Ca:P ratio in bone was 2.1:1 for each of the four treatment groups. Aluminum in the pooled leg and wing bones was not significantly affected by treatment ($P>0.05$) but a significant tendency ($P<0.01$) was found for the bones of the sternum to accumulate dietary aluminum.

Actual levels of aluminum in the diet were measured and found to be within 10% of projected levels.

Discussion

In our study, aluminum accumulation was not associated with any change in the reproductive parameters. Hatchability was not affected and no malformations were found. The higher bone aluminum levels in females of the treated group compared with females of the control group and males of both groups indicate that aluminum was absorbed during egg formation. N. E. Nyholm[1] also found elevated levels of aluminum in bones of captive Japanese quail (*Coturnix coturnix japonica*) fed aluminum citrate. As in our experiment, these higher bone aluminum levels were not associated with any reproductive failure.

Since most of the doves in the treated group had kidney aluminum levels above the detection limit, while those in the control group did not, it is likely that accumulation also occurred in this organ. Rats fed dietary aluminum have been found to accumulate aluminum in bones (Berlyne *et al.* 1972; Thurston *et al.* 1972; Mayor *et al.* 1977) and to a lesser extent in brain, muscle, and kidneys. Valdivia *et al.* (1978), working with growing steers, also reported aluminum deposition in several organs including liver, kidney, brain, and bones.

The weak but significant ($P<0.05$) decrease in moisture loss of eggs of the treated group cannot be readily explained. The examination of an eggshell from each of the treatment groups with a scanning electron microscope indicated that the number of pores and the pore areas were similar. Since Ar *et al.* (1974) and Paganelli (1980) have shown that the net flow of water through the shell is dependent on the pore length (shell thickness) and the pore area, we are unable to explain the observed decrease of permeability.

The levels of plasma calcium and phosphorus in breeding doves were not affected by the aluminum treatment. Higher levels of calcium and phosphorus in the plasma of females were expected since they all had an egg in the oviduct when they were sacrificed. Elevated calcium levels are normal in laying female birds (Simkiss 1961). In our experiment, females from the treated group had plasma calcium levels slightly lower than females of the control group. We have no further data indicating that the metabolism of calcium had been affected by treatment with aluminum.

Lower plasma phosphorus levels caused by dietary aluminum have been reported in poultry species (Deobald and Elvehjem 1935; Storer and Nelson 1968). Lipstein and Hurwitz (1981) reported similar effects in growing chicks fed a diet naturally rich in aluminum. According to Street (1942) a drop of circulating phosphorus is likely to occur only when the concentration of soluble aluminum equals the concentration of available phosphorus. In our study the concentration of phosphorus, although limited, was still five times the concentration of aluminum. This might explain the lack of effect of aluminum in this experiment.

Our results indicate that dietary aluminum does not affect the final weight nor the growth rate since in neither the breeding experiment nor the juvenile growth study were any effects on these parameters seen.

Aluminum given as aluminum sulphate and hydroxide at levels between 2000 and 25 000 ppm to 1-day-old chicks caused a decrease of growth, a high mortality after 3 weeks (Deobald and Elvehjem 1935; Storer and Nelson 1968), and a syndrome of wing and leg weakness (Steinborn *et al.* 1957). In all of these studies the level of phosphorus was lower than the level of aluminum, which was not the case in our study. This probably explains the lack of effect resulting from aluminum. The growing doves fed the four levels of aluminum tended to accumulate aluminum in the sternum although it did not significantly ($P>0.05$) affect the calcium and phosphorus content. The sternum contained much higher levels of calcium than the pooled leg and wing bones. Vozar (1964) found that dietary aluminum caused accumulation without bone demineralization in rats. The variability in the amounts of aluminum in the different bones in the growing doves may indicate that aluminum was only beginning to accumulate, as it was found mostly in bones of high phosphorus content. This may be due to the tendency of aluminum to form stable aluminum phosphates (Nofre *et al.* 1963). It has also been reported that aluminum tends to displace calcium from the bone during long-term exposure (Boyce *et al.* 1982; Ott *et al.* 1982). In this study, a longer exposure to dietary aluminum at the higher level (1500 ppm) would possibly have revealed some osteoid demineralization.

The interesting feature of this experiment was the overall lack of effect found on reproduction and health of breeding female

[1]N. E. Nyholm, A. Gohansson, and J. Paulsson. Tissue concentrations of aluminum in female and male Japanese quail (*Coturnix coturnix japonica*) with peroral intake of aluminum citrate. In preparation.

doves. The treated groups, as shown by their increased bone aluminum levels, did absorb aluminum from the diet. Our results nevertheless do not support the hypothesis of Nyholm (1981) that dietary aluminum can decrease apatite deposition and adversely affect the mobilization of the calcium necessary for eggshell formation. The difference between our laboratory experiments and the field-based studies could well be due to species to species variation in response to aluminum stress or to differences caused by the exact form of aluminum binding in the diet.

It is also possible that the lack of effect observed in this study was due to the amounts of calcium and phosphorus available in the experimental diets. While it was intended to reduce the availability of these essential minerals relative to aluminum in the diet, the lack of base-line data for mineral requirements of most wild birds made it difficult to determine conditions that would be deficient. Hence Ca and P may not have been low enough in relation to aluminum to cause the problems reported in other studies.

Many areas subjected to acidification and mobilization of aluminum are areas where the calcium and phosphorus levels in surface waters are also low. Under these conditions, it is possible that the availability of aluminum could exceed those of the essential minerals in prey organisms and thereby cause reproductive impairment in avian predators.

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