## **Brief report**

# Lead and delta-aminolevulinic acid dehydratase in blood of Common Eiders (*Somateria mollissima*) from the Finnish archipelago

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#### 1. Introduction

Since the mid-1980s, breeding populations of Common Eiders have declined in the eastern Finnish archipelago of the Baltic Sea (Hario 1998, Hario 2000). Causes of mortality previously described in Common Eiders, including predation by gulls and parasitism (Mendenhall & Milne 1985, Persson 1974), may account for some of the losses while others, such as avian cholera (Christensen et al. 1997), have not been reported in Finland. Low densities and the lack of large size classes of blue mussels (Mytilus edulis) in the eastern archipelago have raised the issue of food availability as a contributor to the eider decline, although results of one study indicated that clutch size and body mass of females at the end of incubation were not directly related to differences in blue mussel biomass (Hario et al. 1999). However, the authors of a recent study that focused on serum biochemistry parameters in incubating Common Eiders suggested that nutrition and disease may play a role in eider population dynamics in the Baltic Sea (Hollmén *et al.* 2001).

The effects of contaminants, such as lead, also should be considered as potential factors in the eider population decline. Lead exposure in birds can cause mortality or chronic sublethal effects on nutritional status and possibly disease susceptibility through its impact on physiology and immunocompetence (Rocke & Samuel 1991, Scheuhammer & Norris 1996). The ingestion of spent lead shot has traditionally been recognized as an important cause of lead poisoning in waterfowl (Scheuhammer & Norris 1996). Although lead shot was banned for waterfowl hunting in Finland in 1996 and in several nearby states in the early to mid-1990s (Fawcett and van Vessem

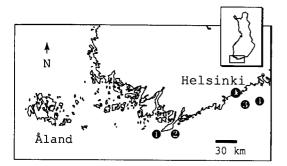


Fig. 1. Locations in the Finnish archipelago where blood samples were collected from incubating Common Eider females. 1 = Hanko, 2 = Tvärminne, 3 = Söderskär, and 4 = Långören.

1995), research in the United States has shown that lead shot may remain available for some time after its use for waterfowl hunting has been prohibited (Hohman *et al.* 1995, Rocke *et al.* 1997). In addition to the ingestion of spent lead shot, waterfowl may be exposed to lead resulting from its emission into the environment from a variety of other anthropogenic sources, including mining and smelting, industrial discharges, and sewage effluent (Pain 1995). Some of these anthropogenic inputs have contributed to concentrations of lead in sediments of the Baltic Proper that are several times higher than the levels that would be expected from natural loading (Jansson & Dahlberg 1999).

Lead poisoning and elevated lead concentrations in tissues have been reported in Common Eiders (Somateria mollissima) sampled in the Finnish archipelago between 1994 and 1998 (Hollmén et al. 1998, Franson et al. 2000a). A piece of ingested lead shot was found in one of the poisoned eiders, but the source of lead exposure was not determined in several other carcasses or in live eiders with high concentrations of lead in their blood. In a recent study of contaminants in Common Eiders, Franson et al. (2000b) reported evidence suggesting increased exposure to lead from west to east in the Finnish archipelago. In the current study, we measured the concentration of lead (Pb) and the activity of delta-aminolevulinic acid dehydratase (ALAD), an enzyme inhibited by lead (Pain 1995), in the blood of Common Eiders nesting in the Finnish archipelago. We compared the results among geographic locations, testing the hypothesis that Common Eiders nesting in the eastern archipelago exhibit greater evidence of lead exposure than eiders nesting farther west.

#### 2. Material and methods

Blood samples were collected by jugular venipuncture from incubating female eiders captured on their nests in May and June of 1999 and 2000. We collected blood from 25 eiders nesting at Hanko (59°50′N, 22°50′E) and Långören (60°09′N, 25°37′E), 24 at Tvärminne (59°50′N, 23°15′E), and 27 at Söderskär (60°06 N, 25°25 E) (Fig. 1). Blood was placed in heparinized plastic tubes and stored at -20 °C and -80 °C for analysis of lead and ALAD, respectively. Lead concentrations in blood were determined by graphite furnace atomic absorption spectrophotometry according to DeStefano et al. (1991) and are expressed as parts per billion (ppb) on a wet weight basis. The lower limit of detection was 20 ppb wet weight and the mean (SD) recovery of lead from spiked blood samples was 101 (4.6)% of the known concentrations. The activity of ALAD was measured colorimetrically (Burch & Siegel 1971). One unit of enzyme activity is defined as an increase in absorbance at 555 nm of 0.100, with a 1.0 cm light path, per ml of erythrocytes per hour, at 38 °C.

For statistical analysis, we assigned a concentration of 10 ppb (one-half of the lower limit of detection) to the five blood samples in which no lead was detected. Based on the Kolmogorov-Smirnov test (Sokal & Rohlf 1995), the ALAD data were normally distributed, but blood lead data required log transformation to obtain a normal distribution. We compared blood lead and ALAD values among locations with analysis of variance (SAS Institute 1990). We also calculated combined means for lead and ALAD from the western study sites (Hanko and Tvärminne) and the eastern sites (Söderskär and Långören) and compared them with two-tailed t-tests, using the appropriate P value according to the results of Levene's test for homogeneity of variances. We report back-transformed means (geometric means) and 95% confidence intervals for the blood lead data, and arithmetic means and 95% confidence intervals for ALAD results. To evaluate the relationship between ALAD activity and the lead concentration in the blood, we tested four combinations of raw and log transformed data (ALAD vs. blood lead, ALAD vs. ln blood lead, ln ALAD vs. blood lead, and ln vs. ln blood lead) with linear regression (SAS Institute 1990). We selected the model with the highest R<sup>2</sup> as the one that best fits the data.

#### 3. Results

Detectable residues of lead were found in 96 of the 101 blood samples. Analysis of variance revealed no significant location effect in the comparison of lead concentrations (P = 0.093) or ALAD activities (P = 0.236) in the blood of ei-

Table 1. Geometric mean lead concentrations (ppb wet weight) and arithmetic mean delta-aminolevulinic acid dehydratase (ALAD) activities, with 95% confidence intervals, in blood of nesting Common Eiders (Somateria mollissima) in the Finnish archipelago.

Location (n)	Lead	ALAD <sup>1)</sup>
Hanko (25)		
Mean (95% CI) Min–Max	52.4 (38.3, 71.6) ND <sup>2)</sup> –190	111 (95.1, 128) 53–203
Tvärminne (24)	E0.7 (40.0. 70.6)	101 (114 140)
Mean (95% CI) Min–Max	58.7 (43.8, 78.6) ND-250	131 (114, 149) 43–199
Söderskär (27)		
Mean (95% CI) Min–Max	86.9 (62.6, 121) 20–1480	139 (117, 160) 5–260
Långören (25)		
Mean (95% CI) Min–Max West <sup>3)</sup> (49)	79.4 (53.1, 119) ND-680	122 (96.1, 147) 12–251
Mean (95% CI)	55.4 (45.0, 68.1)	121 (109, 133)
Min-Max East <sup>4)</sup> (52)	ND-250	43–203
Mean (95% CI) Min-Max	83.3 <sup>5)</sup> (64.9, 107) ND-1480	131 (114, 147) 5–260

One unit of enzyme activity is defined as an increase in absorbance at 555 nm of 0.100, with a 1.0 cm light path, per ml of erythrocytes per hour, at 38 °C.

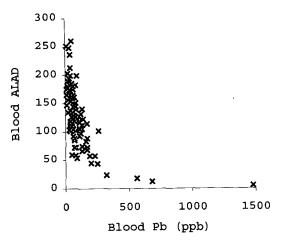


Fig. 2. Relationship between delta-aminolevulinic acid dehydratase (ALAD) activity and lead concentration (ppb wet weight) in the blood of Common Eiders nesting in the Finnish archipelago. The regression model that best fits the data was: In ALAD = 5.02-0.003 blood Pb; R² = 0.68, P < 0.001, n = 101. One unit of ALAD activity is defined as an increase in absorbance at 555 nm of 0.100, with a 1.0 cm light path, per ml of erythrocytes per hour, at 38 °C.

ders among the four locations. However, when the data from the two western study sites were combined and compared with the combined results from the eastern sites, the mean concentration of lead in the blood of eiders was significantly (two-tailed t-test, P = 0.014) greater in the east (Table 1). One of the Common Eiders from Söderskär had a much higher concentration of lead in its blood (1480 ppb) than the others. When data for that bird was removed from the analysis, the combined mean blood lead concentration of the two eastern sites (78.6 ppb; 95% CI = 62.7 ppb, 98.7 ppb) remained significantly different (twotailed t-test, P = 0.024) from the combined mean of the western sites (55.4 ppb; 95% CI = 45.0 ppb, 68.1 ppb). The mean activity of ALAD in eider blood was not significantly different between the eastern and western sites. The ALAD activity in the Common Eider with the greatest concentration (1480 ppb) of lead in its blood was almost totally inhibited (Fig. 2). The linear regression model that best fit the data for the relationship between ALAD activity and lead concentration was:  $\ln ALAD = 5.02 - 0.003 \text{ blood Pb}; R^2 = 0.68,$ P < 0.001, n = 101. The linear regression model was similar when the eider with 1480 ppb lead in

<sup>&</sup>lt;sup>2)</sup> Not detected (< 20 ppb). <sup>3)</sup> West = Hanko and Tvärminne combined.

<sup>&</sup>lt;sup>4)</sup> East = Söderskär and Långören combined.

<sup>&</sup>lt;sup>5)</sup> Significantly different from West mean (two-tailed t-test, P = 0.014).

its blood was excluded from the data:  $\ln ALAD = 5.14 - 0.004$  blood Pb;  $R^2 = 0.66$ , P < 0.001, n = 100.

#### 4. Discussion

We found greater concentrations of lead in the blood of Common Eiders in two nesting areas (Söderskär and Långören) in the eastern part of the Finnish archipelago than in eiders nesting in two areas (Hanko and Tvärminne) farther west, which agrees with an earlier report from the same region (Franson et al. 2000b). The greatest concentration of lead (1480 ppb) was found in the blood of an eider nesting in the east. Although that individual had enough lead in its blood to be considered evidence of acute toxicity, the mean concentrations of lead that we found in the blood of eiders were below levels considered toxic (Pain 1996). However, the ALAD activity in the blood was substantially inhibited by increasing lead concentrations, indicating that lead had a physiological effect on the eiders.

Because mean blood lead concentrations in eiders were relatively low, it is unlikely that the major source of lead exposure in the areas we sampled was the ingestion of spent lead shot. It seems more probable that industrial or other anthropogenic sources of lead were responsible. Irrespective of the source(s) of lead, our data suggest that eiders nesting in the eastern region of the Finnish archipelago are likely to exhibit evidence of greater exposure to lead than eiders nesting in the west. Lead exposure should be considered as one of the potential factors in the declines of eider populations that have been observed in the eastern Finnish archipelago, including Söderskär and Långören (Hario 1998, Hario 2000), because lead exposure has been associated with reduced survival in other species of ducks (Samuel et al. 1992, Hohman et al. 1995). Poorer survival of ducks exposed to lead could be related to the sublethal effects of lead exposure reported in waterfowl, such as adverse hematologic impacts, reduced body condition, and immunosuppression (Pain 1989, Hohman et al. 1990, Rocke & Samuel 1991).

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### Selostus: Suomen saaristossa pesivien haahkojen (Somateria mollissima) veren lyijy- ja delta-aminolevuliinihappodehydrataasipitoisuuksista

Keräsimme verinäytteitä yhteensä 101 hautovasta haahkasta neljältä Suomen saariston pesimäalueelta vuosina 1999 ja 2000. Näytteistä määritettiin lyijypitoisuus ja delta-aminolevuliinihappodehydrataasi (ALAD) -entsyymin aktiviteetti, jonka tunnetaan alenevan lyijyn vaikutuksesta. Lyijypitoisuudet vaihtelivat välillä < 20 ppb ja 1480 ppb (tuorepainosta), ja keskimääräiset pitoisuudet olivat korkeammat itäisillä tutkimusalueilla (Söderskär ja Långören) verrattuna läntisiin (Hanko ja Tvärminne). Lyijyn todettiin alentavan veren ALAD-entsyymin aktiviteettia, mutta vaikutukset olivat samanlaiset kaikilla tutkimusalueilla. Korkeimmat keskimääräiset pitoisuudet todettiin kannanlaskun alueilla keskisellä ja itäisellä Suomenlahdella, mutta lyijyn vaikutuksia alueiden pesimäkantoihin ei tunneta.

#### References

Burch, H. B. & Siegel, A. L. 1971: Improved method for measurement of delta-aminolevulinic acid dehydratase activity of human erythrocytes. — Clin. Chem. 17: 1038–1041.

Christensen, T. K., Bregnballe, T., Andersen, T. H. & Dietz, H. H. 1997: Outbreak of pasteurellosis among wintering and breeding Common Eiders Somateria mollissima in Denmark. — Wildl. Biol. 3: 125–128.

DeStefano, S., Brand, C. J., Rusch, D. H., Finley, D. L. & Gillespie, M. M. 1991: Lead exposure in Canada Geese of the eastern prairie population. — Wildl. Soc. Bull. 19: 23–32.

Fawcett, D. & van Vessem, J. (eds.), 1995: Lead poisoning in waterfowl: international update report 1995. JNCC Report No. 252. — Joint Nature Conservation Com-

- mittee, Peterborough.
- Franson, J. C., Hollmén, T., Poppenga, R. H., Hario, M. & Kilpi, M. 2000a: Metals and trace elements in tissues of Common Eiders (Somateria mollissima) from the Finnish archipelago. Ornis Fennica 77: 57–63.
- Franson, J. C., Hollmén, T., Poppenga, R. H., Hario, M., Kilpi, M. & Smith, M. R. 2000b: Selected trace elements and organochlorines: some findings in blood and eggs of nesting Common Eiders (Somateria mollissima) from Finland. — Environ. Toxicol. Chem. 19: 1340–1347.
- Hario, M. 1998: Recent trends and research results for four archipelago bird species Common Eider, Velvet Scoter, Herring Gull and Lesser Black-backed Gull. In: Solonen, T. & Lammi, E (eds.), The Yearbook of the Linnut Magazine 1997: 12–24. Birdlife Finland, Kuopio, Finland.
- Hario, M. 2000: The archipelago birds census in 1999: Recent trends of Common Eider, alcids and sea terns in Finland. — In: Solonen, T. & Lammi, E (eds.), The Yearbook of the Linnut Magazine 1999: 40–50. Birdlife Finland, Kuopio, Finland.
- Hario, M., Hollmén, T., Kilpi, M., Lehtonen, J. T., Mustonen, O., Westerbom, M. & Öst, M. 1999: Riittääkö haahkalle ravintoa Suomenlahdella? (Summary: Are northern Baltic eiders food-limited?). — Suomen Riista 45: 34–43.
- Hohman, W. L., Moore, J. L. & Franson, J. C. 1995: Winter survival of immature Canvasbacks in inland Louisiana. — J. Wildl. Manage. 59: 384–392.
- Hohman, W. L., Pritchert, R. D., Pace, R. M. III,
  Woolington, D. W. & Helm, R. 1990: Influence of ingested lead on body mass of wintering Canvasbacks.
  J. Wildl. Manage. 54: 211–215.
- Hollmén, T., Franson, J. C., Poppenga, R. H., Hario, M. & Kilpi, M. 1998: Lead poisoning and trace elements in Common Eiders Somateria mollissima from Finland.
   — Wildl. Biol. 4: 193–203.
- Hollmén, T., Franson, J. C., Hario, M., Sankari, S., Kilpi, M. & Lindström, K. 2001: Use of serum biochemistry

- to evaluate nutritional status and health of incubating Common Eiders (Somateria mollissima) in Finland.

   Physiol. Biochem. Zool. 74: 333–342.
- Jansson, B. & Dahlberg, K. 1999: The environmental status of the Baltic Sea in the 1940s, today, and in the future. Ambio 28: 312–319.
- Mendenhall, V. M. & Milne, H. 1985: Factors affecting duckling survival of eiders Somateria mollissima in northeast Scotland. — Ibis 127: 148–158.
- Pain, D. J. 1989: Haematological parameters as predictors of blood lead and indicators of lead poisoning in the Black Duck (Anas rubripes). — Environ. Pollut. 60: 67–81.
- Pain, D. J. 1995: Lead in the environment. In: Hoffman, D. J., Rattner, B. A., Burton, G. A., Jr. & Cairns, J., Jr. (eds.), Handbook of ecotoxicology: 356–391. Lewis Publishers, Boca Raton.
- Pain, D. J. 1996: Lead in waterfowl. In: Beyer, W. N., Heinz, G. H. & Redmon-Norwood, A. W. (eds.), Environmental contaminants in wildlife: interpreting tissue concentrations: 251–264. CRC Press, Boca Raton.
- Persson, L. 1974: Endoparasitism causing heavy mortality in eider ducks in Sweden. — XIth International Congress of Game Biologists, Stockholm: 255–258. National Swedish Environment Protection Board, Stockholm.
- Rocke, T. E., Brand, C. J. & Mensik, J. G. 1997: Site-specific lead exposure from lead pellet ingestion in sentinel Mallards. J. Wildl. Manage. 61: 228-234.
- Rocke, T. E. & Samuel, M. D. 1991: Effects of lead shot ingestion on selected cells of the Mallard immune system. — J. Wildl. Dis. 27: 1–9.
- Samuel, M. D., Bowers, E. F. & Franson, J. C. 1992: Lead exposure and recovery rates of Black Ducks banded in Tennessee. — J. Wildl. Dis. 28: 555–561.
- SAS Institute, 1990: SAS/STAT user's guide, version 6.
   SAS Institute, Cary, NC.
- Scheuhammer, A. M. & Norris, S. L. 1996: The ecotoxicology of lead shot and lead fishing weights. Ecotoxicol. 5: 279–295.