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ALUMINIUM ACCUMULATION ON EGGS AND HATCHING SYSTEMS DURING THE INCUBATION PERIOD - AN IMPORTANT CAUSE OF VARIABLE HATCHING SUCCESS ?

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ABSTRACT.

Groups of rainbow trout eggs were incubated in duplicate in trays with PVC screens and original aluminium screens. During incubation aluminium bound to sediments was deposited on eggs and hatching systems.

By adding acid the pH of the inlet water to a PVC hatching system with sediments from the hatchery water in it, was experimentaly lowered. Buffering of the outlet water was observed from pH 5.0 and lower, and at pH 4.5 and lower an increase of aluminium concentration in outlet water was measured.

If a variable pH of the hatchery water is to be expected, the aluminiumrich sediments deposited on eggs and hatching system during incubation may represent a threat to the survival of eggs and fry.

INTRODUCTION.

Acid precipitation is a serous environmental problem for Atlantic salmon (Salmo salar) and rainbow trout (Salmo gairdneri) hatcheries in southern and south western Norway. Several hatcheries have reported both acute and longterm mortalities of eggs and fry exceeding 50 %. It is suspected that in many events low pH and high concentrations of aluminium in the inlet water are the main reasons for this situation (Ulgenes, unpublished).

Rainbow trout and Atlantic salmon are ranked as the most sensitive species towards acidification (Overrein et al., 1981). The youngest stages (eggs, fry and fingerlings) seem to be the most sensitive to several environmental pollution factors(NAS, 1972). This is also true for low pH (Mount, 1973, NAS, 1972) and most probably also for high concentrations of aluminium. Combined with low pH the high aluminium concentrations occuring in many river systems have aggravated the recruitment problems for salmonid fish, and several populations are extinct in southern Norway (Muniz and Leivestad, 1980).
At pH 6.0 there probably is very little effect on fish of the aluminium concentrations occuring in natural waters (Muniz and Leivestad, 1980). Concequently adjustment of pH in the intake water of hatcheries is neccesary for most fish farmers in southern Norway. Nevertheless, depending on adjustment method used, pH in the hatchery water does fluctuate to some degree (Hansen et al., 1984).

Apart from aluminium dissolved in the intake water some fishfarmers have claimed that use of aluminium screens in the hatching trays are detrimental to eggs and fry (Solberg,1982). Based on this theory many Norwegian fish farmers have replaced the original aluminium screens with PVC plastic screens in the hatching trays.

The aim of this work was to investigate if any accumulation

of aluminium on eggs and hatching trays could be registered, and whether there was a difference in the accumulation using aluminium screens or PVC plastic screens in the hatching trays during incubation.
Further it was investigated if any elution of aluminium could be measured as a function of pH in the inlet water to the hatching system.

MATERIAL AND METHODS.

Hatching system and eggs.

Two parallel hatching troughs A and B, each with seven trays (Ewos no. 2001 and 2003) were used. In trough A the trays were modified by replacing the original aluminium screens with PVC plastic screens. In trough B all the original aluminium screens were used.
Water from the Matre river (total hardness 1.5 - 2.5 ppm as CaO, conductivity 10 - 15 umho , pH adjusted to 5.9 - 6.2 with NaOH) was supplied at a flow rate of ca. 10 l/min. through each trough. The same water quality was used in both troughs, and the water depth was adjusted to 10 cm.

Seven newly fertilized groups of rainbow trout eggs from separate broodfish were incubated. Each egg group was divided, one half was put in trough A, while the other half was put in trough B. The parallels were incubated in tays the same distance from the inlet. The eggs were treated three times a week with malachite green to reduce fungal growth.

Water sampling

Several times during the incubation period water was collected from the inlet and outlet of each trough in acid washed polyethylene bottles.

At the eyed stage the eggs in each tray were washed in 2 litres of water and trays were washed in 10 litres. Aliquots of this water were taken for chemical analysis.

Sample treatment and chemical analysis.

Aliquots of the water samples were passed trough a cation exchanger (Dowex 50, 50-100 mesh, Fluka AG) prepared with Na+. The fraction passing through the column is believed to be nonlabile mainly organic bound aluminium. The inorganic monomeric forms are estimated from the difference between this fraction and the total acid reactive aluminium (Skogheim & Rosseland, 1984, Driscoll et al., 1980).
Aliquots of the water samples from the washed eggs and trays were filtered through a Watman GF-C filter to remove particles from the solution, leaving the total acid reactive aluminium not bound to solids. The nonlabile forms of aluminium in the filtered water was measured after passing the sample through the cation exchanger as described above. Unfiltered samples and blank were homogenized with an Ultra Torax Omnimixer and cooked at 120Æ C with sulphuric acid to dissolve aluminium bound to solids. Cooked samples were diluted 1:10 before analysis.

Elution experiment.

A hatching system made exclusively of PVC plastic in which sedimentation of particles and humus had been going on for two months was used. The system was supplied with water from the Matre river at a constant temperature of 11.5ÆC and flowrate 2.6 l/min.
The pH of the inlet water was adjusted from 6.2 down to 3.5

by adding 0.1 M sulphuric acid through a tubing pump. The difference between pH levels was 0.5 pH-units and each level was kept for 1/2 hour.
The pH of supply water and outlet water was measured three times at each pH level. Simultaneously watersamples from the inlet and outlet were taken for analysis of total acid reactive and nonlabile aluminium.

Analysis of aluminium.

Aluminium was analysed with the pyrocatecol violet method (NS 4747, 1980) by a slightly modified automated technique described by Henriksen (1975). Absorbance at 582 nm was measured in a Spectronic 2000 spectrophotometer equipped with a 10 mm thermoelectric flowcell. Standards from 20 to 280 ug Al/l were used.

Statistical analysis.

The results were analysed statistically by using students t-test at a 5% significance level ($p = 0.05$)

RESULTS.

During the incubation period the concentration of aluminium in inlet water to trough A was 79 +- 4 ug/l and in outlet water 77 +- 4 ug/l. In trough B the values were 74 +- 2 ug/l and 73 +- 1 ug/l respectively. In the inlet water to trough A a mean of 53 % of the total acid reactive aluminium was in nonlabile forms. The nonlabile forms of aluminium in outlet water from trough A were on average 58 % of the total. In trough B the same comparisions showed average values of 48 % in the inlet water and 52 % in the outlet water. The differences in aluminium concentrations in inlet and outlet water were not significant.

Table 1 shows the aluminium concentration of the filtered water used for washing eyed eggs and hatching trays. There was no significant difference in either total acid reactive or nonlabile aluminium after washing eggs wich had been incubated on PVC screens (trough A) compared to those incubated on aluminium screens (trough B). The same was true when comparing the aluminium concentrations after washing trays with aluminium screens and PVC screens.

Table 2 shows the total amount (in mg) of aluminium bound to the particles and humus sedimented on eggs and trays. There was a somewhat higher deposition of aluminium on the hatching trays than on the eggs in both troughs A and B. Accumulation of particle bound aluminium was not significantly higher on eggs incubated on aluminium screens than on eggs incubated on PVC screens. The same result was obtained by comparing the amount of aluminium deposited on trays with aluminium screens with that deposited on trays with PVC screens.

Table 3 shows the estimated concentrations of aluminium in the water volume of one hatching tray (16 litres) if all the particle bound aluminium was dissolved simultaneously. Comparing the values in system A and B there would have been no significant differences in concentration.

Figure 1 shows the data from pH-adjustment of the inlet water to the PVC hatching system. The pH of the inlet water started at pH 6.2 with no addition of sulphuric acid. At this pH-level no buffering in the system was observed. Adjusting the pH down to 5.5 and lower at intervals of 0.5 pH-units, the system buffered the water from pH 5.0 and below. This is shown by the upper curve in Figure 1A. The difference in pH was from 0.4 to 0.1 units, with the greatest difference between pH 5.0 to 4.0. At pH 3.5 the buffering had decreased.

Given in Figure 1 are also the differences in aluminium concentrations (total and nonlabile) between inlet and outlet water. The data showed that from pH 4.5 and below there was a large increase in the labile forms of aluminium while there were only small differences in the nonlabile forms. The amount of dissolved labile aluminium also seemed to reflect the pH level, as indicated by the stepwise increase in aluminium concentrations with decreasing pH.

DISCUSSION.

During the period of incubation the concentration of aluminium in the water supplied to the hatching system can not be considered as very high (70 - 80 ug/l) as there was little runoff and relatively high pH in the river water. During periods of heavy rainfall the pH of the river decreases and total concentrations of aluminium may exceed 300 ug/l (Ulgenes , unpubl.) This trend is also found in other river systems (Henriksen et al. 1984). Since there were no significant differences in the aluminium concentrations as measured from inlet and outlet water during incubation it is not possible to calculate any acumulation from these data.

During the incubation of eggs there was a conciderable sedimentation of particles and fungal growth in the system.
As shown in Tables 2 and 3 there was a conciderable accumulation of aluminium boumd to sediments. The data showed no significant difference between trough A (with PVC screens in the trays) and trough B (with aluminium screens in the trays) concerning total amount of aluminium sedimented in the systems. It therefore seems to be no difference in waterborne or sedimented aluminium using either aluminium screens or PVC screens. A suggestion derived from this conclusion is that the type of material used in the hatching trays - aluminium or plastic -does not have any influence on the concentration of sedimented or free aluminium. This suggestion is based on very few data and further investigation is needed to finally confirm this. However the finding was expected because aluminium is pacified with the formation of an oxide on the surface which can withstand most chemical attacks from the environment (Mahan ,1975).

During incubation of salmon and trout eggs the usual practice is to minimalize disturbance of the eggs from fertilizing to the eyed egg stage. This means that with water temperature around 6 C there is a period of approximately one month with sedimentation of particles and humus on the eggs and hatching systems. The amount of particles in hatchery water is dependent upon runoff in the river and filter techniques used for intake water. Further, despite using malachite gereen as anti fungus, there is also some fungal growth on dead eggs and this may act as an effective filter for particles in the water. It is well known that aluminium and other metals are catched up by organic substrates such as humus (Gjessing, 1982), which is also suggested by these data. It was somewhat surprising, however, to registrate the massive amount of aluminium deposited concidering the low concentrations of aluminium in the hatchery water during incubation .

Figure 1 shows the tremendous increase in the concentration of aluminium in outlet water after adjusting pH with acid. The concentration increase was most visible from pH 4.5 and lower while the buffering of water started at pH 5.5.
The reason for the marked buffering from pH 5.0, the strong elution below pH 4,5 and no conciderable increase in the aluminium concentration in the outlet water above pH 4.5, may be somewhat difficult to explain. A theory may be that the sediments mainly contain organic detritus and humic substances. This material consists of partly degraded polymers (carbohydrates an proteins) and consequently a lot of more or less free carboxylgroups are formed which effectively bind cations in the water. Thus aluminium in form of a mixture of hydroxides may be bound to the negative groups. When lowering the pH, the increase in the concentration of H+ ions may be followed by an exchange of H+ ions with metal ions at the negative sites making the corresponding carboxyl acids. In this way the sediments may act as a "cation exchanger" (Gjessing, 1982). Further the observed buffering from pH 5.0 may be caused by the eqilibrium

$$Al(OH)_2^{+} + H^{+} = Al(OH)^{++} + H_2O \quad (I)$$

$$Al(OH)^{++} + H^{+} = Al^{+++} + H_2O \quad (II)$$

The formation of water in this buffering process will remove H^+ ions. The formation of two or three positive charges on the aluminium ion may result in a stronger binding to the sediments. The net result from this can be a buffering effect in the upper investigated pH area. In the lower pH area it may be a replacement of aluminium ions with hydrogen ions, resulting in the observed increase of labile aluminium concentration in the outlet water. Recalling that the pH of hatchery water may fluctuate to some degree, despite of pH adjustment, it may be speculated that the aluminium bound to sediments represent a threat to the survival of eggs and fry during incubation.

As a conclusion the data showed no difference between PVC and aluminium screens in the trays as measured by waterborne or sedimented aluminium, indicating that the aluminium screens have no effect on waterborne aluminium. There was a conciderable accumulation of aluminium bound to the sediments on the eggs and hatching facilities but the accumulation process was not detectable by measurements of inlet an outlet water during incubation. At low pH of the intake water, aluminium bound to sediments on eggs and trays may be dissolved. This may under certain rearing conditions represent a threat to eggs and fry in the incubation system and therefore contribute to the variable hatching success as seen in commercial hatcheries.

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Table 1. **Acid soluble and nonlabile aluminium (ug/l) in filtered water from washing eyed eggs and trays. Averages +/- 95% confidence interval.**

Material washed	total acid reactive Al	nonlabile Al
Eggs, trough A	143 +/- 49	99 +/- 36
Eggs, trough B	137 +/- 54	86 +/- 29
Trays in trough A (PVC - screens)	107 +/- 2	55 +/- 2
Trays in trough B (AL - screens)	111 +/- 8	57 +/- 3

Table 2. Total amount of aluminium (mg) bound to sediments deposited on eggs and trays during the incubation from fertilzation to the eyed egg stage. Averages +/- 95 % confidence interval.

Eggs, trough A	3,74 +/- 1,54
Eggs, trough B	3,08 +/- 1,54
Trays from trough A	5,42 +/- 2,26
Trays from trough B	5,00 +/- 0,46

Table 3. Estimated aluminium concentrations (ug/l) if all aluminium bound to sediments on eggs and trays was simultaneously dissolved in the water volume of the trays (16 litres). Averages +/- 95% confidence interval.

Water in trough A (PVC screens)	592 +/- 251
Water in trough B (Al - screens)	549 +/- 74

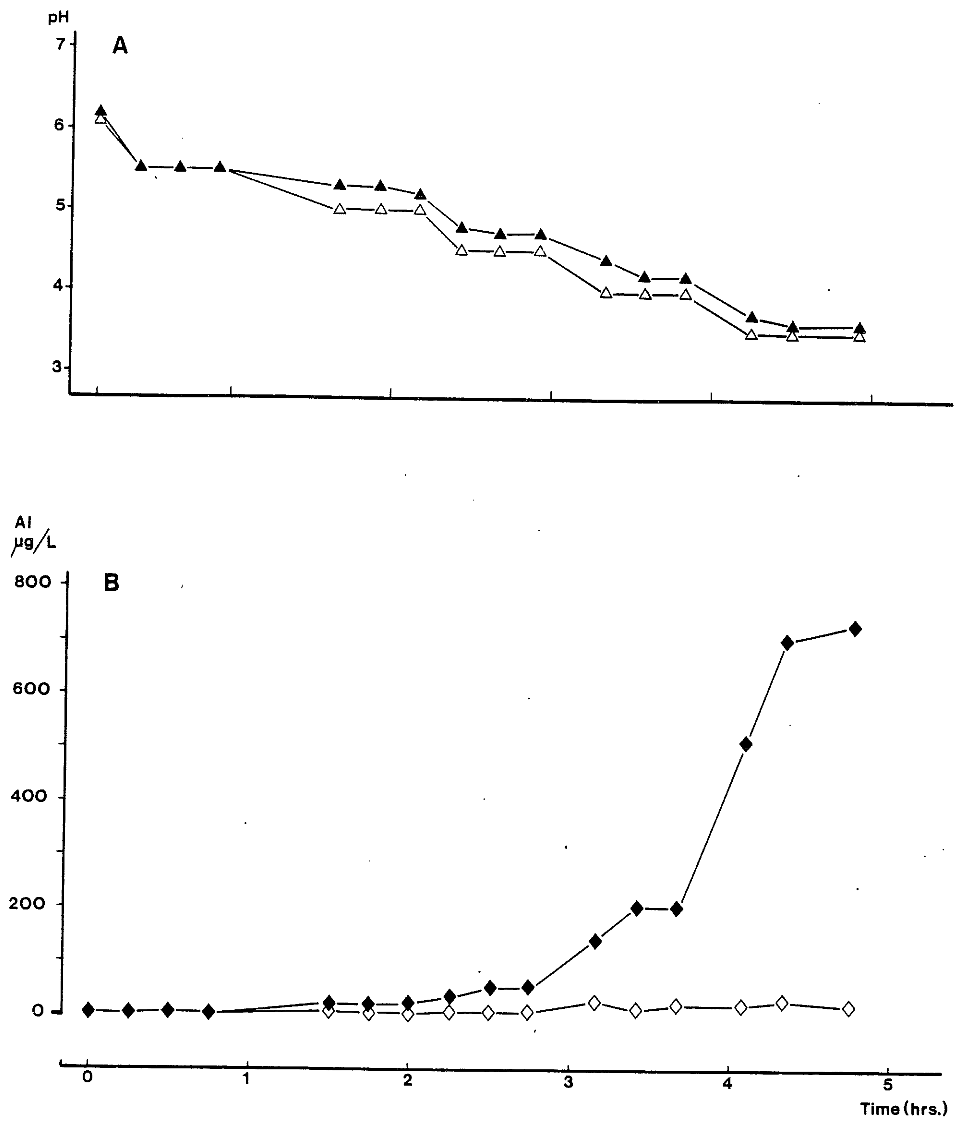

Figure 1. The eluation experiment in a PVC hatching system.

A: The pH of inlet water △———△

and outlet water ▲———▲

B: The difference in aluminium concentration from inlet to outlet water.

◇———◇ = nonlabile aluminium

◆———◆ = total acid reactive aluminium