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The Effects of Toxic Aluminium and Low pH on Gill Development of Rainbow Trout (*Oncorhynchus mykiss*, Walbaum) Larvae.

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Abstract: In this study, the gill development of rainbow trout (*Oncorhynchus mykiss* Walbaum) larvae exposed to toxic aluminium (200 μg/L), those exposed low pH (4.5) alone and those exposed to both for 30 days were investigated under scanning electron microscopy. General appearance of gills, filament and lamella lengths and the number of lamellae per filament were determined in order to evaluate the effects of these toxicants on gill development. In addition, the death rates and general conditions of larvae were observed.

In larvae exposed to low pH alone and in those exposed to aluminium alone, a slight thickness in the apical part of the filaments, shorter lamellae and filament lengths and reduced numbers of lamellae per filament were found. But these findings statistically were not significant ($P>0.05$) compared with control.

In larvae exposed to the combined effects of low pH and aluminium, shortened and thickened filaments, fusion of filaments and lamellae and low numbers of lamellae per filament were determined. Filament and lamella lengths and the numbers of lamellae per filament in this larva group were significantly lower ($P<0.001$) than those in the control group, and the mortality rate was quite high (80%). In addition, intensive mucous secretions were observed.

Key Words: *Oncorhynchus mykiss*, rainbow trout, pH, aluminium, gill development.

Toksik Aluminyum ve Düşük pH'ın Gökkuşağı Alabalığı (*Oncorhynchus mykiss* Walbaum) Larvasının Solungaç Gelişimine Etkileri

Özet: Bu çalışmada, yalnız toksik aliminyum (200 μg/L), yalnız düşük pH (4.5) ve bunların kombine etkisine maruz bırakılan gökkuşağı alabalığı (*Oncorhynchus mykiss*) larvasının solungaç gelişimi skan elektron mikroskobu altında incelendi. Solungaçların genel görünümü, filament ve lamella uzunluğu ve her bir filamentteki lamella sayısı belirlenerek solungaç gelişiminin etkilenme düzeyi ortaya konulmaya çalışıldı. Ayrıca ölüm ve larvaların genel durumuda gözlendi.

Yalnız düşük pH ve yalnız aliminyum'a maruz bırakılan larvaların solungaçları normal bir gelişme göstermekle birlikte, filament uçlarında hafif bir kalınlaşma gözlendi. Ayrıca istatistiksel olarak önemli olmamakla birlikte ($P>0.05$) kontrole göre daha düşük sayıda lamella, daha kısa filament ve lamella uzunluğu bulunmuştur.

Aliminyum ve düşük pH kombinesine maruz bırakılan larvalarda filamentler tamamen kalınlanmış, kısalmış ve birbirlerine yapışık durumdadırlar. Lamellalar sadece filamentlerin alt kısımlarında mevcut olup, filamentlere yapışık durumdadırlar. Filament ve lamella uzunluğu, her bir filamentteki lamella sayısı kontrol grubuna göre istatistiksel olarak önemli derecede ($P<0.001$) düşük bulunmuştur. Bu gruptaki larvalarda ölüm oranı oldukça yüksek (% 80) olarak saptanmıştır. Ayrıca aşırı mukoz salgısı belirlendi.

Anahtar Sözcükler: *Oncorhynchus mykiss*, gökkuşağı alabalığı, pH, aliminyum, solungaç gelişimi.

Introduction

In aquatic ecosystems, the levels of toxic metals have increased either directly, as a result of atmospheric deposition and waste-water discharge and runoff (e.g. Pb, Hg, Cd, Cu and Zn), or indirectly, through increased solubilization and mobilization from sediments owing to acidification (e.g. Al and Fe) (1). Metal toxicity to fresh water organisms, particularly during environmental acidification, has been reviewed by Spry *et al*. (1981) (2) and McDonald *et al*. (1989) (3).

McDonald and Wood (1993) reported that the effects of Cu, Al and Cr are directed mostly towards disturbing the Na^+ and Cl^- balance, whereas Cd, Zn and Mn are directed mainly towards disrupting Ca^{2+} balance (1). In spite of some limitations in the available data, one common theme that emerges is the central importance of ionoregulatory disturbances in the toxic mechanisms of the metals. The main target of the metals is the gill, which is the main site of several vital functions: gas exchange, ion exchange, acid-base regulation and nitrogenous excretion. In an extensive review of the

literature, Mallatt (1985) summarised the main structural damage to the gills by aquatic irritants, including some of the toxic metals, as follows: uplifting of the lamellar epithelium from the underlying tissue, necrosis of chloride cells and pavement cells, swelling by increased intercellular spaces, rupture of the epithelium and fusion of lamellae. This damage is often accompanied by hyperplasia and hypertrophy of lamellar cells (4). This physical damage can clearly explain the increases in ionic permeability and reductions in transport function commonly caused by toxic metals.

Aluminium, which is the world's most common metal, is found in almost all rocks, in surface waters, and in living organisms. The concentration of aluminium in most fresh waters is usually very low, generally < 1 mg 1^{-1}, but in water with low pH (around 5.5 mg l^{-1}) and low ionic strength, dissolved aluminium can reach concentrations which are toxic to aquatic organisms. Howells *et al*. (1990) reviewed the toxic effects of aluminium to aquatic organisms (5).

Many morphometric and physiological studies indicate that elevated aluminium at low pH causes damage to the gills. Daye and Garside (1976) reported the lifting of outer epithelium from the pillar cells on the lamellae, as well as some hypertrophy of filament cells (6). Fathead minnows chronically exposed to pH 5.5-5.0 showed increased numbers of chloride cells (7). Chevalier *et al*. (1985) found increased numbers of chloride cells in brook trout, in addition to hyperplasia of the filaments (8). Conklin *et al*. (1992) observed both a significant increase and rapid degeneration of chloride cell populations in the gills of larval brook trout exposed to soft, acid water. In addition, filament length, lamellar length and the number of lamellae per filament were decreased (9). Jagoe *et al*. (1987) also reported similar gill anomalies in Atlantic salmon alevins exposed to 75 μg Al l^{-1} at pH 5.5 (10).

Although when aluminium is added to acid exposure, histological damage in fish gills is similar to that reported for acid exposure alone, the effects are often more severe. Changes described with Al/acid exposure include extensive necrosis and degeneration of gill tissue (11, 12, 13). Karlsson-Norrgren *et al*. (1986b) also reported lamellar swelling (11). Similar damage has been observed after exposure to other metals, such as zinc (14, 15), cadmium (16), copper (17) and chromium (18).

Most studies on the effects of pH and toxic metals have been done in media with low calcium concentrations. There is a lack of information on the effect of toxicants in hard water. Therefore, the present study investigates of low pH and toxic aluminium under high calcium condition.

Material and Methods

Experimental Animals and Procedure

Eyed eggs of rainbow trout were obtained from a commercial fish farm (Trent Fish Culture Ltd., Mercaston, Derbyshire UK). They were incubated in a fiber glass aquarium with stainless steel mesh at a temperature of 10 ± 1.0 °C, in an artifical photoperiod (12 h light: 12 h dark). After hatching, the larvae were divided into four groups, each group having three replicates exposed to three different media (see Table 1 for experimental medium) in a fiber glass aquarium until the swim-up stage. Control groups were provided for comparison.

Temperature, conductivity, dissolved oxygen and pH were measured in all aquariums once a day. Any dead animal was removed and noted. Water samples for analysis of aluminium, calcium, magnesium, potassium and sodium were taken from all chambers at intervals. Water samples for analysis of aluminium were preserved by addition of HCl (B.C.H. "AnalaR") to yield a concentration of 0.1 mol 1^{-1}. Water samples for analysis of ions were preserved by addition of HCl (B.D.H.

Table 1. Water Quality Data for Experimental Medium (Mean ± SD. a_n=90; b_n=30).

Parameters	Control	Low pH (LpH)	Aluminium (Al)	LpH+Al
[a]Temperature, °C	10±1.5	10±1.8	10±1.4	10±2.0
[a]Conductivity, mS/cm	625±10	688±8	634±9	685±15
[a]D. oxygen, mg/L	9.5±1.2	9.2±1.7	8.8±1.7	10±2.0
[a]pH	7.14±0.05	4.45±0.07	7.23±0.04	4.56±0.07
[b]Al, mg/L	0	0	194±11	209±13
[b]Na. mg/L	16.28±0.37	18.12±0.41	15.89±0.35	20.32±0.53
[b]K, mg/L	3.29±0.16	3.55±0.20	3.32±0.13	4.06±0.15
[b]Ca, mg/L	53.80±1.08	54.22±1.1	53.56±0.09	54.355±1.3
[b]Mg, mg/L	9.38±0.29	9.45±0.32	9.13±0.30	9.39±0.25

"Analar"), ca. 5.0 mol 1^{-1}, to yield a final concentration of 0.05 mol 1^{-1}. Aluminium concentrations of preserved water samples were measured by absorptiometry (Pye Unicam, SP500 spectrophotometer). The concentrations of calcium, sodium, cadmium and aluminium were measured by atomic absorption spectrophotometry (Pye Unicam, model SP9 combined with SP9 computer).

At the end of the experiment, five surviving fish were sampled from each group. First left arch of each animals was dissected and prepared for scanning electron microscopy (SEM) by the following technique.

SEM Technique

Gill samples were fixed for 2 hours in 5% glutaraldehyde buffered with 0.1M phosphate and postfixed for 1.5 hours in phosphate-buffered 1% osmium tetroxide. After washing with distilled water for 1 hour, specimens were dehydrated through a graded ethanol series and dried by Plaron E3000 critical point dryer using liquid CO_2 as transitional fluid. Dried specimens were stacked on a stub with double-sided tape and then coated with a thin layer of gold in a sputter coating unit (Polaron E5100). Coated specimens were observed in a Jeol (JSM-840) scanning electron microscope and photographed with a Polaroid camera.

Filament and lamella lengths, and the number of lamellae per filament of observed specimens were measured (Figure 1). Mean values (± standard deviations) for filament and lamella lengths, and for the number of lamellae per filament were calculated. The results from each treatment were compared with control results by using Student's *t*-test.

Results

Water Quality

Water quality data for experimental media are presented in Table 1. There was no significant difference in temperature and dissolved oxygen between groups. pH was rather close to nominal value. Conductivities in low pH and low pH+Aluminium combination media were higher than in control and aluminium.

Aluminium concentration was very close to nominal values (200 µg/L). However, the aluminium concentration in the LpH+Al medium was slightly higher than that in Al medium (see Table 1). This could be caused by the dissolvent effect of low pH on aluminium. Na, K, Ca concentrations in the low pH medium and the low pH+Al medium were higher than in the control and aluminium media. There was no considerable change for Mg between groups.

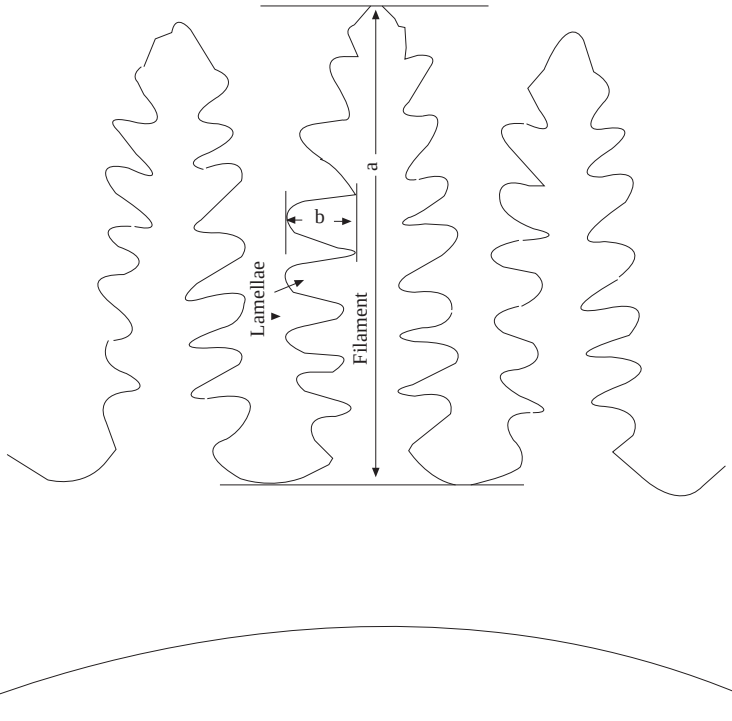


Figure 1. Diagram of measurement of filament length (a) and lamella length (b).

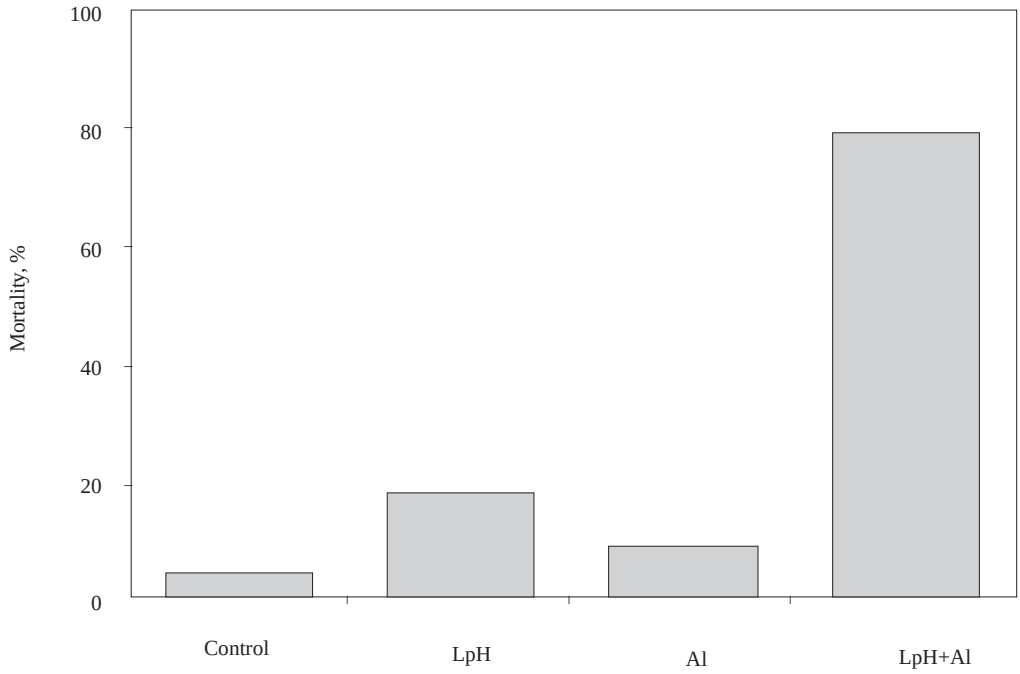


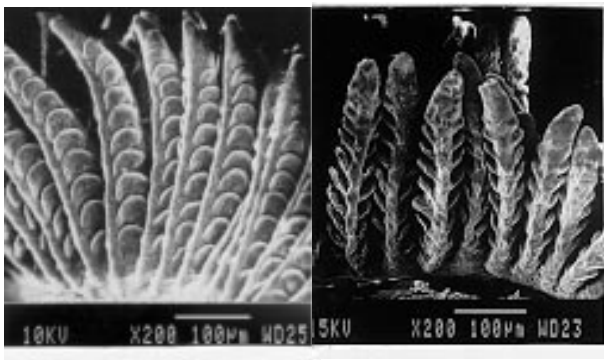
Figure 2. Mortality percentage of control, low pH, Al and low pH+Al-exposed larvae over the 30-day experiment period.

Mortality, Behaviour and Physical Appearance of Larvae

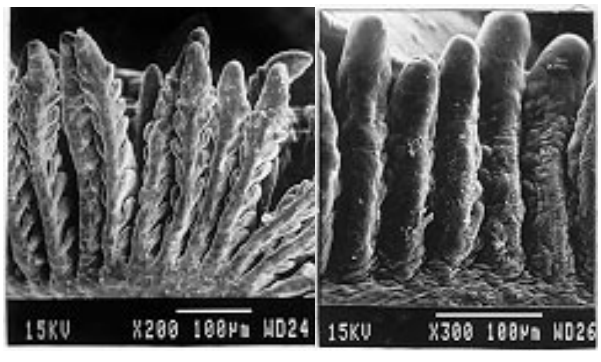
The mortality percentages of the animals are given in Figure 2. Control animals and animals exposed to aluminium alone started active swimming on day 28. Animals exposed to low pH and those exposed to low pH+Al showed decreased larval activity and abnormal swimming behaviour, such as spiral swimming. At the end of the experiment, the control and Al groups had no visible trace of yolk remaining, while the low pH and low pH+Al groups still had yolk remaining.

Gill Development

Over the 30 days of exposure, qualitative differences , in gill development in control, low pH, Al and low pH+Al-exposed larvae are presented in Figure 3. The gills of the control larvae appeared normal, exhibiting features characteristic of development for salmonids (19, 20) such as straight filaments carrying equally-spaced lamellae and relatively long and well-developed filaments with an ordered array of lamellae (Figure 3A). In constant, filaments of low pH and Al-exposed larvae exhibited fusion between neighboring lamellae in the apical part of the filaments (Figures 3B, C). Filaments of low pH+Al-exposed larvae exhibited a random unordered array of lamellae, and many incidents of apparent lamellar fusions (Figure 3D).



(A) (B)



(C) (D)

Figure 3. Micropcopic appearance of gill of control (A), low pH (B), Al (C) and low pH+Al (D) exposed larvae on day 30 of exposure

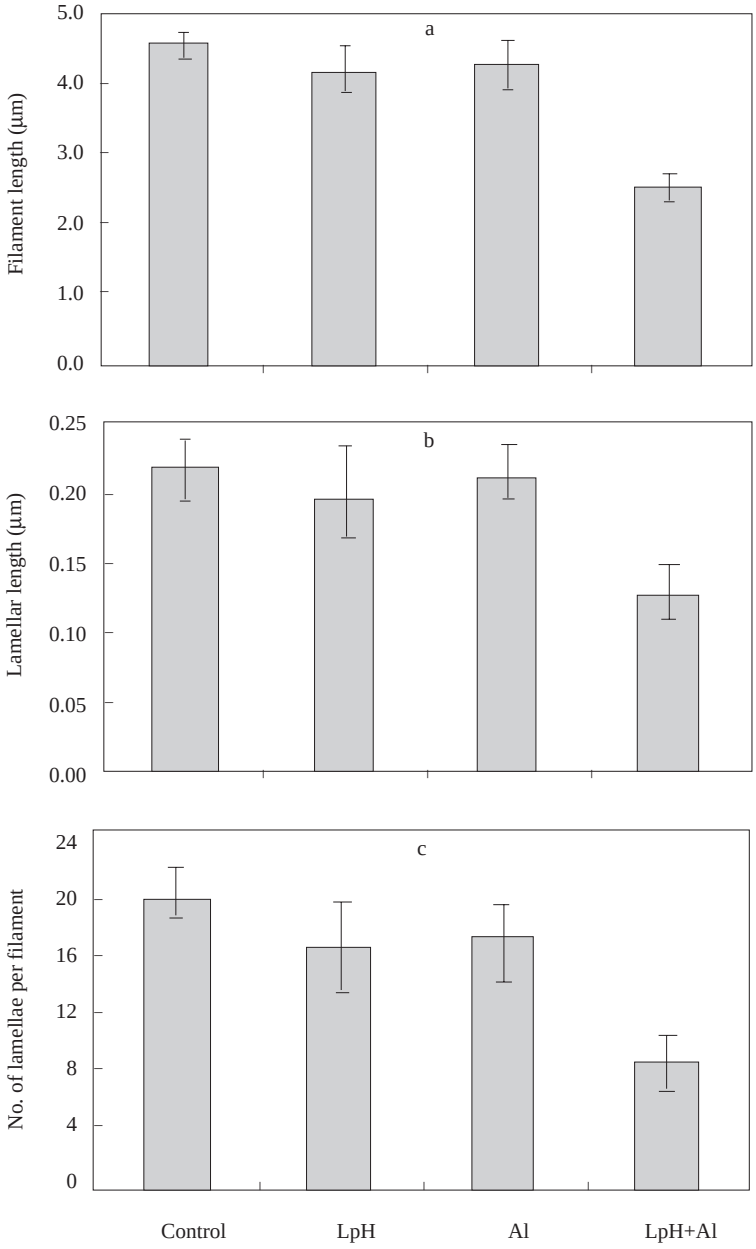


Figure 4. Filament lengths (a), lamella lengths (b) and number of lamellae per filament (c) in larval rainbow trout exposed to low pH (LpH) alone, in those exposed to aluminium (Al) alone, and those exposed to both (LpH+Al) during the early development stage (Mean±SD, n=5).

Quantitative measurements of gills in larvae are presented in Figure 4. Statistical analysis (Student *t*-test) showed that gill development of low pH and Al-exposed larvae had no significant difference in comparison with that of control larvae ($p > 0.05$). However, the combined effect of low pH and Al on gill development was statistically significant ($p<0.001$) compared with control.

Discussion

In the present study, it was found that a combination of low pH and elevated aluminium in fresh water is toxic to fish. The gills of larvae from control chambers had straight filaments and equally spaced lamellae on the filaments, characteristics of normal salmonid gills (19, 20).

At the end of the experiment, filament and lamellae lengths, and the number of lamellae per filament, in the gills of animals exposed to low pH (4.5) and aluminium (200 µg 1^{-1}) appeared to be slightly lower than those of control animals, but not significantly. Over the 30 days of combined exposure to low pH and aluminium, the lengths of filaments and lamellae and the number of lamellae per

filament decreased significantly. In addition, the gills of larvae exposed to low pH alone and those exposed to Al alone showed fusion between neighboring lamellae in the apical part of filaments. In contrast, the gills of low pH+Al-exposed larvae showed a severe fusion of lamellae and filaments and abnormally large amounts of mucus. Jagoe et al. (1987) reported similar gill anomalies in Atlantic salmon alevins exposed to a combination of low pH (5.5) and aluminium (75 μg 1^{-1}) (10). They also observed no anomalies in larvae exposed to low pH (4.5-6.5) alone. In contrast, Conklin et al. (1992) found similar anomalies in brook trout larvae exposed only to pH (5.25) alone (9). The difference may be due to the calcium content of water. Since calcium concentration of exposure water in Conklin et al.'s study (1992) (9) was three times lower than that determined by Jagoe et al. (1987) (10) and ten times lower than that of the present study.

Intensive mucus production (21), loss of microridges (16) and large non-tissue spaces (11, 15) have been reported in the gills of fish exposed to high concentration of various metals. Mueller *et al*. (1991) concluded that as a result of lamellar fusion, the lamellar surface area may be reduced by as much as 75 % (22). This would impair ion uptake and oxygen delivery to the tissues. Enlargement of non-tissue space could result in inadequate gas exchange, and, consequently, in a reduced diffusion capacity. These structural lesions of the gills could contribute to an increase in permeability. Increased gill permeability to cations could be the most important factor in the ion loss. It has been shown that under the normal conditions the permeability of the epithelial cell is determined by the characteristics of the cellular membranes and of the tight junctions that interconnect the epithelial cells (23). Calcium levels in the plasma are very important for the control of membrane permeability. Hunn (1984) suggested that the permeability of the cellular membranes to water and ions is determined by the amount of calcium bound to the negatively charged groups of the membranes (24). The loss of bound calcium from the membranes and the tight junctions of fish gills caused by trace metals could lead to increased permeability, and consequently an increase in efflux of ions.

In conclusion, it is generally accepted by most scientists working on this subject that the main target of aluminium is the gills of fish. Death of animals is due mostly to impaired gill functions such as respiration and ion exchange. Intensive production of mucus caused by the combined effect of low pH and Al in the present study suggests that high mortality should be attributed to respiratory failure rather than to impaired ion regulation.

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