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Changes in the whole body mineral concentration of the Rainbow trout (*Oncorchynchus mykiss* Walbaum) yolk-sac fry exposed to various combinations of aluminium and calcium concentrations in two different acidic water

Metin ÇALTA
Fırat Üniversitesi, Su Fakültesi, Temel Bilimler Bölümü, Elazığ-TURKEY

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Abstract: The effects of 12 combinations of pH (4.5 and 7.0), aluminium (4 and 8 µmol/L) and calcium (10 and 100 µmol/L) on survival and body mineral (Ca, Mg, K and Na) concentrations of rainbow trout (*Oncorhynchus mykiss* Walbaum) at the yolk-sac stage were investigated.

Mortality was low (< 17%) in most combinations and increased (> 27%) in the presence of aluminium and low pH (4.5).

Aluminium (4 and 8 µmol/L) impaired larval development, net uptake of calcium, potassium and sodium, and slightly increased the net loss of magnesium. It was found that these effects of aluminium were more severe at low calcium concentration (10 µmol/L) and low pH (4.5) than at high calcium concentration (100 µmol/L) and high pH (7.0). In the absence of aluminium, only the combination of low pH (4.5) and low calcium (10 µmol/L) significantly reduced body mineral concentrations.

Key Words: Aluminium, pH, calcium, rainbow trout, *Oncorhynchus mykiss*, body mineral concentration.

Farklı İki Asidik Su Ortamında Değişik Aliminyum ve Kalsiyum Kombinasyonlarına Maruz Bırakılan Yumurta Kesesi Evresindeki Gökkuşağı Alabalığı (*Oncorhynchus mykiss* Walbaum)' nın Vücudunda Mineral Konsantrasyonu Değişimi

Özet: Bu çalışmada, pH (7.0 ve 4.5), aluminium (0,4 ve 8 µmol/L) and kalsiyum (10 ve 100 µmol/L)'un 12 kombinasyonunun yumurta kesesi evresindeki gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum)'nın yaşamı ve vücut mineral (Ca, Mg, K and Na) konsantrasyonuna etkisi araştırıldı.

Ölüm oranı çoğu kombinasyonlarda %17'den daha düşük olup, pH (4.5) ve aluminyumun varlığında bu oran %27'nin üzerine çıktı.

Aliminyum (4 ve 8 µmol/L) larvaların gelişimini, kalsiyum, potasyum ve sodyum alımını yavaşlattı. Ayrıca azda olsa larvanın magnezym kaybını artırdı. Aliminyumun bu etkilerinin, pH(4.5) ve calcium (10 µmol/L)'da, pH (7.0) ve calcium (100 µmol/L)'da kine oranla daha şiddetli olduğu gözlendi.

Anahtar Sözcükler: Aliminyum, pH, kalsiyum, gökkuşağı alabalığı, *Oncorhynchus mykiss*, vucut mineral konsantrasyonu.

Introduction

The rainbow trout originally inhabited the Pacific coasts of North America. It was introduced Europe in 1875 and later to other continents. The rainbow trout is the most widespread member of the family Salmonidae in Europe. It occurs both in the wild and in trout farms. Its enormous adaptability to water conditions and its economical value have made it an important subject for scientific studies.

Recently, a decline in the trout population was observed in North America and Northern Europe. The factors usually implicated in the decline of fish populations are a combination of low pH at low water hardness and the presence of toxic trace metals such as aluminium (1).

It has been determined that the main target of aluminium is the fish gills which are the main site of ion exchange. Disturbed ion balance has been observed in salmonids exposed to aluminium at low pH and low calcium concentrations (2, 3).

The present study examined the effects of combinations of two pH values and two calcium concentrations on the calcium, magnesium, potassium and sodium content of yolk-sac rainbow trout in the absence and presence of aluminium frequently found in acidic waters.

Material and Methods

The experimentals animals (newly hatched rainbow trout) were obtained from Fırat University, Fisheries Faculty, Cip Fish Reproduction and Research Station. The experiment was conducted in small aquariums (20x15x10 cm) placed in a cold room (12-15 °C) in the Fisheries Faculty. The experimental media were prepared with deionized water by the addition of major ions in concentrations given in Table 1. Two aluminium concentaritons (4 and 8 µmol/L) were provided by the addition of $AlCl_3$. Two pH regimes were adjusted by the addition of 0.1N H_2SO_4 solution. All aquariums were aerated and dechlorinated before and during the experiments.

Table 1. Nominal concentrations of major ions in experimental media (µmol/L).

Ions		
Ca^{+2}	10	100
K^{+}	5	5
Mg^{+2}	20	20
Na^{+}	50	50
Cl^{-}	60	150
NO^{-}_{3}	5	5
SO_4^{-2}	45	65

At the beginning of the experiments, 10 newly hatched larvae were killed and treated as described below for the determination of dry weight and the whole body mineral content, and the results were compared with those obtained at the end of the experiments. A total of 50 newly hatched larvae were transfered to 12 aquariums containing different experimental media (Table 2).

During the experiments, all aquariums were checked daily and any dead animals were removed. At the same time, pH, conductivity and temperature measurements were taken in all aquariums. At 3 day intervals, water samples were taken from each aquarium for analysis of total aluminium and calcium. The concentrations of total aluminium and calcium were determined with an atomic absorption spectrophotometer (Perkin-Elmer model 370).

Experiments continued the yolk-sac phase of the larvae (approximaetely 30 days). After absorption of yolk by the control animals, 10 of the surriving larvae were removed from each aquarium and they were killed in anaesthetic solution (Sandoz, MS 222) and rinsed twice deionized water. In order to determine the dry weight and the whole body mineral content in dry weight, the animals were transferred individually into 4 ml preweighed vials. They were dried in an oven (60-65 °C) for 24 h and then transferred to a low pressure desiccator for several days until constant mass was obtained. After weighing for dry weight; the larvae were digested in HNO_3 16 µmol/L for 24 h at room temperature, and then the digests were diluted with $LaCl_3$ in deionized water to give [La]=1% w/v. Ca, K, Mg and Na concentrations were determined in the whole body of the larvae with an atomic absorption spectrochotometer. A blank was prepared for comparison, following the same procedure used for the digests.

The results were expressed as mean ± standard error. Statistical analyses were performed by using Analysis of Variance (ANOVA) and Kruskal-Wallis Test.

Table 2. Water quality data for experimental media. Mean ± standard error (n=30;*n=15).

Aquarium	pH		Total aluminium (µmol/L)		Calcium (µmol/L)		Temperature (°C)	Conductivity (µS/cm)
No	Nominal	Actual	Nominal	Actual*	Nominal	Actual*	Actual	Actual
1			0	0	10	9±0.4	13±1.5	17.6±0.62
2			0	0	100	92±6	13±2.0	38.8±1.2
3	7.0	6.7-7.4	4	3.65±0.20	10	8±0.6	14±1.0	18.0±0.40
4			4	3.82±0.18	100	90±5	13±1.5	37.6±0.9
5			8	7.60±0.51	10	8±0.5	14±1.0	16.8±0.54
6			8	7.45±0.60	100	93±4	13±2.0	39.2±1.1
7			0	0	10	9±0.6	14±1.5	17.4±0.33
8			0	0	100	95±4	13±2.0	38.5±0.8
9	4.5	4.2-4.6	4	3.78±0.17	10	9±0.4	13.±1.5	17.6±0.40
10			4	3.92±0.18	100	93±6	14±1.0	37.8±1.3
11			8	7.68±0.42	10	8±0.7	13±2.0	16.7±0.28
12			8	7.70±0.52	100	91±5	13±1.5	39.0±0.8

Results

Water Quality

Nominal and actual quality data for the experimental media are given in Table 2. The actual concentrations of Al and Ca were slightly lower than the nominal values. The actual pH values in all aquariums were quite close to the nominal values during the experiments.

Mortality, Behaviour and Appearance of the Larvae

At the end of the experiments, mortality was less than 17 % in all animals except in those exposed to combinations of aluminium (8 µmol/L) and pH (4.5 and 7.0) at low calcium (10 µmol/L) (mortality higher than 27%).

In these absence of aluminium, larvae had nearly absorbed the yolk after 30 days. They were also very active and reached the swim-up stage. However, in the presence of aluminium, larvae still possessed substantial yolk reserves as a result of retardation of the resorption of yolk. They were also less active and never reached the swim-up stage, especially at low pH (4.5) and low calcium (10 µmol/L).

Dry Weight and Whole Body Mineral Content of the Larvae

In general, calcium, potassium and sodium concentrations in the larvae were higher at the end of the experiment (especially in the control) than at the beginning (see Figures 1, 3 and 4), while magnesium concentration remained approximately constant or slightly lower (see Figure 2).

Whole body calcium content declined significantly ($P<0.001$) with increasing aluminium concentration at all combinations of pH and low calcium concentration (10 µmol/L). The effect of aluminium was less severe ($P<0.01$) at the higher calcium concentration (100 µmol/L) (see Figure 1). There was little difference in the effect of aluminium between the two pH regimes. In the absence of aluminium, body calcium content was significantly ($P<0.001$) lower at pH 4.5 at both calcium concentrations.

Whole body magnesium content declined as aluminium concentration increased (Figure 2). The analysis of variance indicated that the effect of aluminium was significant ($P<0.001$). However, there were no significant effects of pH or calcium concentration.

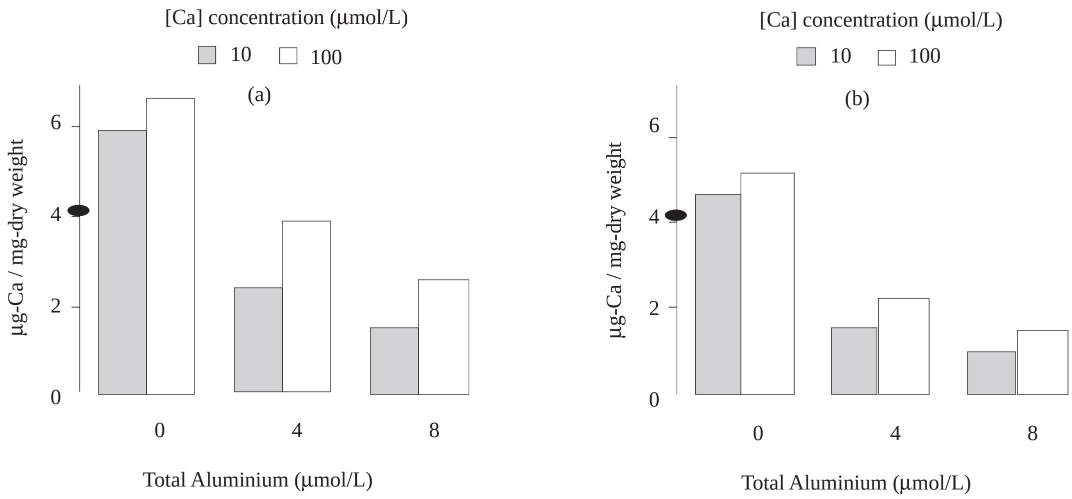

Figure 1. Whole body calcium concentration of the larvae at the end of the experiment. (a) pH 7.0; (b) pH 4.5. ⬬ whole body Ca concentration of the newly hatched yolk-sac fry

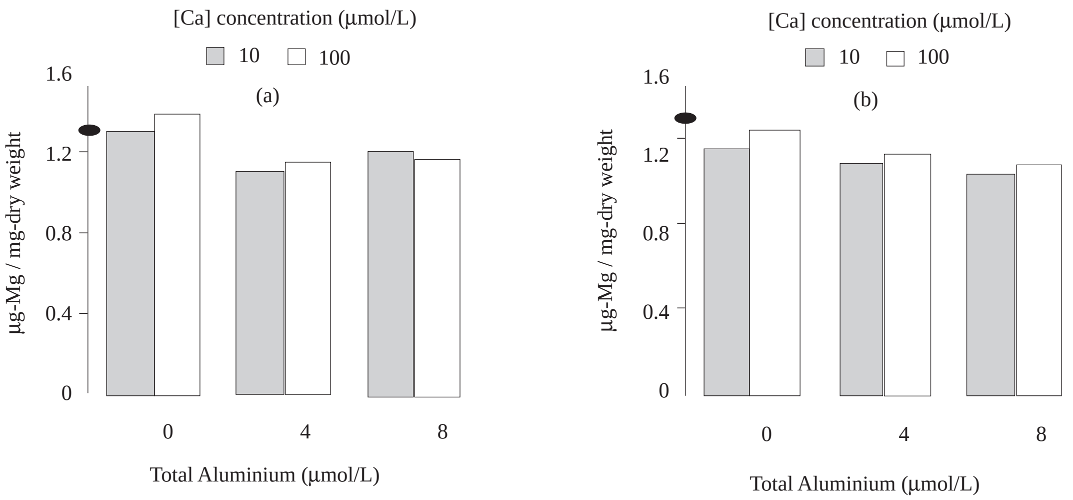

Figure 2. Whole body magnesium concentration of the larvae at the end of the experiment. (a) pH 7.0; (b) pH 4.5. ⬬ whole body Ma concentration of the newly hatched yolk-sac fry

Whole body potassium content declined significantly ($P<0.001$) with increasing aluminium concentration at all combinations of pH and low calcium concentration (10 µmol/L). The effect of aluminium was less severe ($P<0.01$) at the higher calcium concentration (100 µmol/L) (Figure 3). There was little difference in the effect of aluminium between the two pH regimes. In the absence of aluminium, there was no significant difference attributable to pH or to calcium concentrations.

Whole body sodium content declined significantly ($P<0.001$) as the aluminium concentration increased (Figure 4). Analysis of variance of the results from treatmens without aluminium indicated that the effect of pH was less significant ($P<0.01$). Body sodium content was lower at the lower calcium concentration (10 µmol/L) at each combination of pH and aluminium concentration. This effect was more obvious at lower pH (4.5).

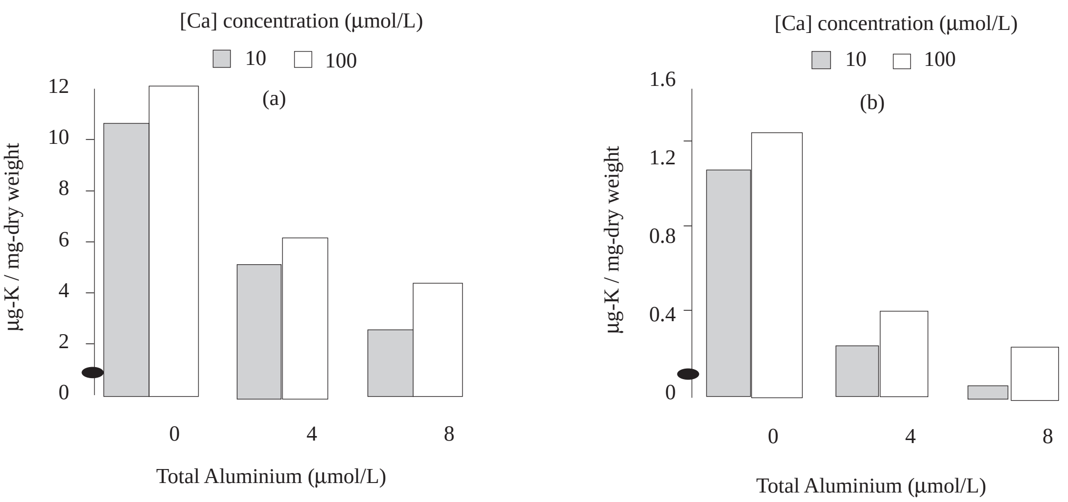

Figure 3. Whole body potassium concentration of the larvae at the end of the experiment. (a) pH 7.0; (b) pH 4.5. ⬬ whole body K concentration of the newly hatched yolk-sac fry

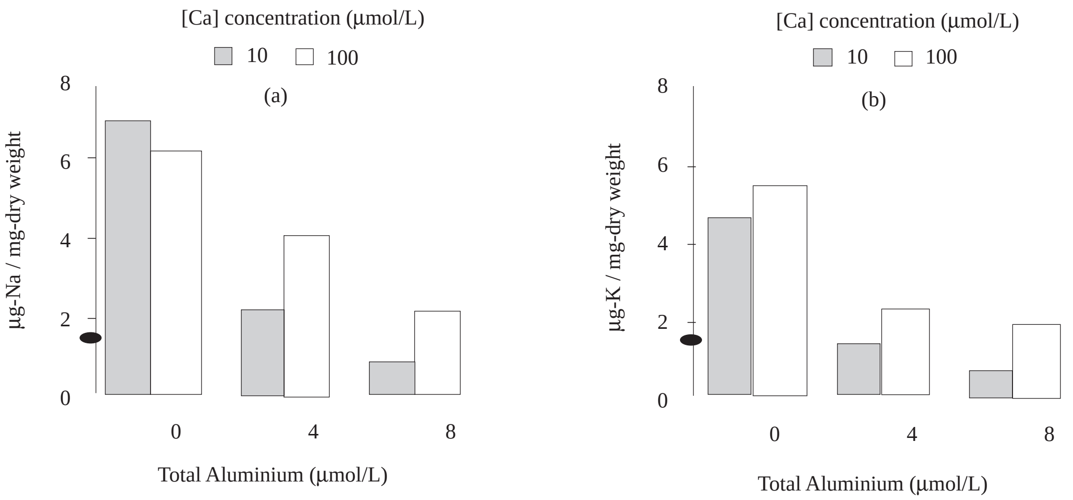

Figure 4. Whole body sodium concentration of the larvae at the end of the experiment. (a) pH 7.0; (b) pH 4.5. ⬬ whole body Na concentration of the newly hatched yolk-sac fry

Discussion

The recently hatched brown trout fry in this study supplemented their calcium, potassium and sodium content by net uptake from the water, whereas there was no net magnesium uptake. A relationship was observed between whole body calcium content and the net uptake of calcium, potassium and sodium (but not magnesium) in Atlantic salmon, *Salmo salar*, alevins (4) and in brown trout alevins (5, 6).

The results of the present study showed that the effects of low pH alone were less than the effects of aluminium and pH combinations, whereas a number of reports of laboratory experiments with salmonid alevins indicated severe effect at pH levels relevant to the present study (7-10).

It was observed that water calcium concentration plays an important role in the effects of pH and aluminium. The deleterious effects of combinations of pH and aluminium increased with decreasing water calcium level. In previous reports, impaired skeletal calcium deposition (10) and impaired otolith growth (11) were found in salmonids at low pH and low calcium levels.

The form in which aluminium is present in water is highly pH-dependent. Its solubility decreases to a minimum at around pH 5.5-6.0 and increases towards both extremes of the pH scale. Soluble, monomeric, cationic species, e.g. Al^{+3}, $Al(OH)^{+2}$ and $Al(OH)_2^{+}$, are formed at pH levels lower than about 5.5, and soluble aluminate species, e.g. $Al(OH)_4^{-}$, predominate at pH levels greater than about pH 5.5. Between pH 5.5 and 6.0 most aluminium is present in the form of insoluble $Al(OH)_3$ (12, 13).

Aluminium toxicity is generally regarded as an important factor in the decline of fish population in soft acidic water. Toxicity is modified by pH and tends to be greater at lower calcium concentrations (14). The deleterious effects of aluminium observed as mortality, impaired potassium, sodium and calcium uptake in the present study were all ameliorated by high calcium concentration (100 compared with 10 μmol/L) and the retarded gross development and impaired uptake of potassium were ameliorated by high pH (7.0) compared with 4.5. The slight increase in the net loss of magnesium caused by exposure to aluminium was not significantly modified either by calcium concentration or by pH.

High mortality (> 27%) occurred only at aluminium concentration greater than 4 μmol/L, in the low calcium concentration (10 μmol/L). Lower concentrations of aluminium are lethal to older juvenile brown trout (15) and rainbow trout, *Salmo gairdneri* (3). However, yolk-sac fry are less sensitive to aluminium than older animals (14, 16). Neville (3) concluded that the cause of death in aluminium exposed rainbow trout was predominantly disturbance of the sodium and chloride balance below pH 5 and hypoxia at pH 6.1, with a transition between two mechanisms arount pH 5.0-5.5. Active uptake of sodium from the medium is inhibited by aluminium in yearling brown trout below pH 5.0 (17). At this pH, aluminium is predominantly in the form of monomeric cations, and these cations are presumably responsible for the impairment of the sodium and chloride balance. This sort of toxicity is similar to that of hydrogen ions alone at very low pH, in which death probably results from circulation failure brought about by ionoregulatory disturbances (18). Net sodium uptake in the present study was more severely impaired at pH 4.5 than at pH 7.0. Net uptake of calcium was impaired by aluminium, whereas active uptake of calcium by brown trout fingerlings is not inhibited under similar conditions in short-term experiments (19).

The aluminium-induced increase in the loss of magnesium suggests a general increase in membrane permeability, but this disturbance was not exacerbated at low calcium concentration: calcium is important for the control of membrane permeability.

Whatever the true mechanism of aluminium toxicity on fish is, it is clear from the present study that aluminium can have a deleterious effect on the development of larval fish under conditions representative of soft acidic waters experiencing fish population decline.

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