The effect of aqueous aluminium on fish ectoparasites

Cand.scient. thesis in Zoology

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Preface and acknowledgements

This cand. Scient. thesis was carried out at the University of Oslo during 2000 - 2003. I wanted to contribute to the research on the topic: does acidic aqueous aluminium have any toxic effect on ectoparasites infecting freshwater fish. My experiments were performed in the fish holding department and the fish with parasites were sampled nearby Oslo.

I would like to thank Professor Leif Asbjørn Vøllestad for excellent supervision during these years. Associated professor Antonio B. S. Poléo also deserves many thanks as my co-supervisor, particularly in commenting on my language and structure of the manuscript. Their office doors are always open and my questions were always immediately answered even though they were engulfed in their own work. Fil. mag. Lars Flodmark is appreciated for his constructive criticism and valuable suggestions on changes of manuscript. His comments were always positive.

May time at the university would not have been the same without my fellow students, they have given my inspiration and motivation to keep working. I had many meaningful constructive discussions and social gatherings at “the fish lab”. Many tips and tricks were learned over a cup of coffee.

I would like to thank my family for backing me up all the way. To all my friends thank you for keeping up with me all these study years.

Oslo, June 2003

Ruben Alexander Pettersen

Note: Illustration picture of G. derjavini on the first side was taken with a SEM. The fish skin with parasites was fixed in 3.5% glutaraldehyde in 0.1 M cacodylate buffer for 24 h. The section was rinsed three times in 15.2% sucrose in 0.1 M cacodylate buffer. After rinsing another three times with buffered sucrose the samples was dehydrated through ethanol, critical point dried and sputter-coated with gold-paladium, before being examined with a SEM.
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Abstract

The present study focuses on the effect of acidic water and aqueous aluminium on different species of ectoparasites infecting freshwater fish species. Three common fish species with a natural infection of ectoparasites were taken in to the laboratory and exposed to acidic Al-rich water (pH 5.8), acidic Al-poor water (pH 5.8) and near neutral water as a control (pH 6.3). The difference in total aluminium between the acidic Al-rich water, and the acidic Al-poor and control water was approximately 125 $\mu$g · L$^{-1}$. Species tested was *Gyrodactylus derjavini* on brown trout (*Salmo trutta*), *G. macronychus* on minnow (*Phoxinus phoxinus*), and glochidia of duck mussels (*Anodonta anatina*) on perch (*Perca fluviatilis*). Individual fish infections were monitored. Free living little fish louse (*Argulus foliaceus*) was also exposed to the various water qualities. All parasite species suffered substantial mortality when exposed to the acidic aluminium-rich water. The most pronounced effect was the total elimination of *G. derjavini* after 3 days in the acidic Al-rich water. The mechanism behind the toxic effect of aluminium on parasites is, however, still unknown. The results show that aqueous aluminium can limit ectoparasite infections, without affecting the host. Thus, treatment with aqueous aluminium may constitute an effective ectoparasite disinfection agent in the future.
Introduction

Freshwater acidification is recognised as an important environmental problem in the northern hemisphere (Galloway, 2001). During the last century Norwegian lakes and rivers have been severely affected by acidification (Henriksen & Brakke, 1988; Henriksen et al., 1989; Henriksen & Hesthagen, 1993). The acidification of soil water systems results in a mobilisation of aluminium from the edaphic to the aquatic environment (Cronan & Schofield, 1979; Gensemer & Playle, 1999). Aqueous aluminium is recognised as the principal toxicant killing fish in acidified surface waters (reviewed by Gensemer & Playle 1999). Not only fish, but also freshwater invertebrates are affected by acidification (Sutcliffe & Hildrew, 1989; Herrmann, 1990; Raddum & Fjellheim, 1994). Recently, it was documented that acidic water and aluminium most probably influence the interaction between parasites and their hosts (Soleng et al., 1999). Soleng et al. (1999) showed that aqueous aluminium eliminated the monogenean ectoparasite Gyrodactylus salaris Malmberg 1957 from infected Atlantic salmon (Salmo salar L.). Interestingly, the Atlantic salmon has been shown to be among the most sensitive freshwater fish species to aluminium (Grande et al., 1978; Poléo et al., 1997). Yet, no fish died due to the Al-exposure in the experiments preformed by Soleng et al. (1999). As far as I know the effect of aluminium on ectoparasites has not been studied, apart from the study performed by Soleng et al. (1999). Ectoparasites in general are restricted by abiotic factors in the surrounding macroenvironment, which influence their population dynamics, dispersal and distribution (Malmberg, 1957; Malmberg, 1970; Chubb, 1977). It is therefore possible that aluminium may have an influence on ectoparasites in general. Thus, in the present study the effect of aluminium on four different ectoparasites has been investigated.
Ectoparasite ecology

The four ectoparasites in this study are representatives of quite different groups of organisms, two monogeneans *G. derjavini* Mikailov 1975, *G. macronychus* Malmberg, 1957, one bivalve, the duck mussel (*Anodonta anatina* L.), and one crustacean, the little fish louse (*Argulus foliaceus* L.).

*G. derjavini* and *G. macronychus*

The former of these monogeneans prefers brown trout (*S. trutta* L.) as a host, whereas the latter is found only on cyprinids (Malmberg, 1957; Mo, 1993). The species in the family Gyrodactylidae are viviparous, fast-growing, 0.5-mm-sized, with mainly a parthenogenetic reproduction (Malmberg, 1957). The gyrodactylids are attached to the skin of their hosts by a disk full of hooks (opisthaptor). If these animals detach from their hosts, they die within a few days if unable to reattach to a host (Lester & Adams, 1974; Scott & Anderson, 1984).

Duck mussel

The duck mussel belongs to the bivalve subclass Palaeoheterodonta. An important feature of this species is that it has a parasitic early life stage called Glochidium, which is produced in large numbers by the adult. This larva looks like a miniscule mussel equipped with two hooks. In autumn, the larva clamps on to its host by closing the valves and becomes encysted in the skin or the fins. The duck mussel glochidium may infect any fish species within its habitat. In spring, the larva has developed into a tiny copy of the adult mussel. At this stage it leaves the host to settle into the substratum as a free living mussel. In contrast to gyrodactylids, the glochidium is immobile when attached to the host.
Little fish louse

The little fish louse, which previously was included among the parasitic copepoda, is now belonging to the crustacean subclass Branchiura. In contrast to other crustaceans, the little fish louse develops directly to an adult, without a nauplius stage. Adults of this species are 10 mm long. As for the duck mussel, the little fish louse can live on any fish species present. It is an excellent swimmer, and has a high activity level when searching for a host. The adult females have to leave the host to deposit their eggs on stones and plants. The little fish louse pierces the skin and sucks blood from its host, resulting in cutaneous haemorrhage. Severe infections may cause anaemia and death in fish infected by the little louse. In aquaculture, hazardous chemicals such as lindane, potassium permanganate and trichlorfon are widely used as pesticides against the little fish louse (Huet & Timmermans, 1994). Thus, there is a strong incentive to develop new treatments against this parasite.

Aluminium in the environment

Aluminium is the third most common element in the earth’s crust, where aluminosilicates such as feldspars predominate and constitute the primary aluminium sources. Weathering and erosion of such minerals create aluminium products that eventually disintegrate completely to their basic components. When acidic precipitation percolates into soil with scanty buffer capacity, it increase the solubility of aluminium compounds, which are then washed into rivers and lakes (Cronan & Schofield, 1979; Seip et al., 1989). Aqueous aluminium is present on many various forms, dependent on water pH, temperature, and the presence of inorganic and organic ligands (Hem & Roberson, 1967 Bloom, 1979; Wada & Wada, 1980; Lydersen et al., 1990). The various Al-forms differ in toxicity, where low molecular cationic species are considered to be the most toxic forms (Poléo, 1995; Gensemer & Playle, 1999).
Aim of the study

The objective of the present study was to answer the question whether aluminium has an effect on other ectoparasites than *G. salaris*. Moreover to contribute to the understanding of the importance of water quality for ectoparasites infecting freshwater fish species. In the experiments, fish infected by the four parasites in question was exposed to acidic Al-containing water. The concentration of aqueous aluminium was kept well below the level know to be acute toxic to fish (Poléo *et al.*, 1997). The results from this study might have relevance for the development of new parasiticides against freshwater ectoparasites.
Materials and methods

Experimental animals

Fish with natural parasite infections were obtained from several locations close to Oslo (Tab. 1). In September 2000, brown trout with *G. derjavini* were collected by electro fishing in River Sandvikselva. In March 2001, perch (*Perca fluviatilis* L.) with duck mussel glochidia were collected by angling in the Lake Nærevann. In June 2001, minnow (*Phoxinus phoxinus* L.) with *G. macronychus* were collected by electro fishing in River Sørkedalselva, and various fish infected with the little fish louse were collected by shore seine in Lake Lyseren. Before the experiments started, all fish with attached parasites were acclimated in activated-charcoal filtered Oslo city tap water for 24 h (appendix 1.). The little fish lice, however, were removed from the hosts and transformed to floating containers without any acclimation prior to the exposures.

Fish infected by parasites were individually marked. Perch and brown trout were marked by Alcian blue applied with a cannula on various positions of the belly skin. The minnow, which were too small for marking with Alcian blue, were marked by fin clipping.

**Table. 1:** Overview of the experiments and the experimental animals used.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Parasites</th>
<th>Host Species</th>
<th>Length(cm)</th>
<th>Weight(g)</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. September 2000</td>
<td><em>Gyrodactylus derjavini</em></td>
<td>Brown trout n = 30</td>
<td>(10.3 - 14.3)</td>
<td>(8.1 - 29.4)</td>
<td>N 59°53.8 E 10°30.3</td>
</tr>
<tr>
<td>2. September 2000</td>
<td><em>G. derjavini</em></td>
<td>Brown trout n = 30</td>
<td>(8.2 - 11.4)</td>
<td>(5.7 - 15.3)</td>
<td>N 59°53.8 E 10°30.3</td>
</tr>
<tr>
<td>3. March 2001</td>
<td>Glochidium of Duck mussel <em>(Anodonta anatina)</em></td>
<td>Perch n = 30</td>
<td>(9.3 - 12.9)</td>
<td>(5.8 - 27.5)</td>
<td>N 59°44.9 E 9°7.5</td>
</tr>
<tr>
<td>4. June 2001</td>
<td><em>Argulus foliaceus</em></td>
<td>Minnow n = 45</td>
<td>(6.3 – 8.0)</td>
<td>(1.0 – 3.0)</td>
<td>N 59°59.2 E 10°36.8</td>
</tr>
<tr>
<td>5. June 2001</td>
<td><em>G. macronychus</em></td>
<td>Minnow n = 45</td>
<td>(6.3 – 8.0)</td>
<td>(1.0 – 3.0)</td>
<td>N 59°59.2 E 10°36.8</td>
</tr>
</tbody>
</table>
Experimental set-up

The experiments were performed in the fish holding department at the University of Oslo. For the exposures, a continuous flow-through system was constructed (Fig. 1). Fish were exposed to three different water qualities in parallel: acidic Al-rich water, acidic Al-poor water and control water. The acidic Al-rich water was prepared by adding an acidic stock solution of HNO$_3$ and Al(NO$_3$)$_3$·9H$_2$O, to the department water, to obtain a nominal pH of 5.8 and 200 µg Al · L$^{-1}$. The acidic Al-poor water was prepared by the addition of HNO$_3$ only (pH 5.8). Untreated department water (pH 6.3, 100 µg Al · L$^{-1}$) acted as control.

Figure 1. Schematic presentation of the experimental set up. The acidic aluminium stock solution (Al(HNO$_3$)$_3$·9H$_2$O + HNO$_3$) and acid (HNO$_3$) solutions were added to the tap water using peristaltic pump (pp).
After addition of the acidic aluminium stock solution, the acidic Al-rich water was led into a large mixing tank (ca 1000 L) for ageing. The ageing time was approximately 16.5 h. The aged Al-rich water was pumped into an exposure tank of 80 L. Similarly, HNO₃ was added to the department water as it was led into a mixing tank of 60 L (ageing time approximately 1 h). The acidic Al-poor water was led from the mixing tank to another exposure tank of 80 L. The department water was also led directly into a third exposure tank for the control (Fig. 1). The water flow rate into each exposure tank was approximately 1 L · min⁻¹, providing at least 7.2 L of water · g fish⁻¹ · day⁻¹, as recommended by Sprague (1973). Water temperature was kept constant during each experiment (see Tab. 3).

For the exposures of the little fish louse small floating containers were used (Fig. 2). Four containers were floating freely within each exposure tank, ensuring that the animals were exposed to the same conditions within each tank.

Figure. 2. Schematic presentation of floating containers used in experiments 4 with the little fish louse.
Experimental protocol

The study was performed as 5 separate experiments (Tab. 1), lasting between 3 and 18 days. Each experiment was started by placing the infected fish into each exposure tank, 10 fish (brown trout or perch) in each tank in experiment 1-3 and 15 fish (minnow) in experiment 5. In experiment 4, 10 uninfected trout were placed within each exposure tank to control for a possible toxic effect of the exposure to the fish. In experiment 1, the acidic Al-poor group was excluded from the experiment because 6 out of 10 fish escaped from the exposure tank. The fish were not fed during the experiments.

Daily, water temperature was measured directly in the exposure tanks, while pH and conductivity were measured in water samples taken from the tanks. A water sample for analysis of total aluminium (Alr) was also taken from each tank every day. In addition, a water sample for aluminium fractionation was taken at the start, in the middle and at the end of each experiment from each tank. The exposure tanks were checked each day for dead fish. Water and chemical flow rates into all tanks were also controlled daily. In experiment 1, 2 and 5, the number of parasites was counted repeatedly on individual fish, approximately every 24 h. In experiment 3, the number of parasites was counted every 24 h in the beginning of the exposures, and then after 3 days and after one week. The reason for longer intervals in this experiment was that the perch seemed to be affected by the anesthetica used. In experiment 4, mortality of the little fish louse within each floating container was monitored every day. The experiments were terminated when all parasites were absent or dead in the acidic Al-rich water, except in experiment 3 which was terminated when no change in the number of parasites was observed.
Counting of parasites and analytical techniques

To count the monogeneans and duck mussel larvae, individual fish were firmly taken out from each exposure tank with a landing net, anaesthetised and placed on a black tray (21×16×6 cm) under a stereomicroscope. Chlorobutanol (0.3 g · L⁻¹) was used as anaestheticum for brown trout and minnow, while metomidate hydrochloride (5-7 ppm) was used for perch. The transparent living parasites were easily observed under the stereomicroscope and counted. Living gyrodactylids cannot be identified with certainty. Therefore, fish with gyrodactylids were fixed in 96% ethanol. The parasites were then removed, put in 70% ethanol to increase pliability before treatment with ammonium-picrate-glycerin (Malmberg, 1957) prior to species identification. Fifteen parasites from each of fifteen individual fish were examined. Individuals of the little fish louse that did not move were also studied under the stereomicroscope, to check if they were dead. Fish louse that still did not show any movements were judged to be dead. All equipment used for counting parasites was disinfected between counts with Bufodil®.

Water temperature was measured to the nearest 0.1°C by an ordinary mercury thermometer. Water pH was measured using a Radiometer PHM 80 pH-meter with a Radiometer GK 2401 C combined glass reference electrode. Three pH measurements were taken from each water sample. The pH readings were taken to the nearest 0.05 pH unit when the pH-meter drifted less than 1.0 unit · min⁻¹. The standard deviation of the measured pH in each sample was calculated to be ± 0.01 pH units. Observed pH-values were converted to [H⁺], and the mean ± S.D. was calculated and then converted back to pH-values. Water conductivity was measured by a Radiometer CMD 80 conductivity meter. Readings were taken when three consecutive measurements varied less than 0.1 µS · cm⁻¹.
Aqueous aluminium was fractionated (Tab. 2.) by a HQ-MIBK (hydro-quinolin-metyl-isobutyl-keton) extraction technique (Barnes, 1975) combined with a cation exchange procedure (Driscoll, 1984) according to a protocol described by Poléo et al. (1997). The amount of total monomeric Al-species (Alₐ) was determined by direct extraction of water samples (Driscoll, 1984). Water samples were also run through a column of Amberlite IR-120 as cation exchange resin. As recommended by Driscoll, the cation exchange flow-rate was 3.8 mL min⁻¹ bed-volume. The resin was prepared by displacing some of the exchangeable hydrogen ions with sodium ions. When an eluate with an ionic strength comparable to that of the water samples being analysed passed through the exchanger, the pH of the effluent was similar to the pH of the water sample. A volume of 80 mL 10⁻⁴ M NaCl was used for conditioning the resin between runs of samples. For preconditioning purposes, 80 mL of water sample were passed through the column, before another 80 mL of eluate were collected for extraction. The aluminium present in the eluate was defined as organic monomeric aluminium (Alₒ). The amount of inorganic monomeric aluminium (Alᵢ) was calculated as Alₐ-Alₒ. Total aluminium (Alᵣ) was determined by HQ-MIBK-extraction of water samples acidified to pH 1.0 with 65% HNO₃ and stored for at least 24 h. The concentration of aluminium was measured in the extracts by a Shimadzu UV-1201 spectrophotometer at 395 nm (Tikhonov, 1973; Bloom et al., 1979). Absorbance was also measured at 600 nm to correct for iron interference (Sullivan & Seip, 1986). The standard deviation of the HQ-MIBK-extraction method is assumed to be less than 1% of the mean (Sullivan & Seip, 1986). Detection limit for the method is 13 µg Al · L⁻¹ according to Vogt et al. (1994).
Table 2: Definitions of the various aluminium fractions obtained by the Barns-Driscoll method (Barnes, 1975; Driscoll, 1984).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Total monomeric aluminium, determined by extraction of an untreated water sample.</td>
</tr>
<tr>
<td>Alo</td>
<td>Organic monomeric aluminium, determined by extraction of a cation-exchanged water sample.</td>
</tr>
<tr>
<td>Alr</td>
<td>Total aluminium, determined by extraction of a water sample acidified to pH 1.0, after 24 h.</td>
</tr>
<tr>
<td>Ali</td>
<td>Inorganic monomeric aluminium, calculated as Ala - Alo.</td>
</tr>
</tbody>
</table>

Statistical analyses

Survival of parasites was analysed by a nonparametric proportional hazards model. This model is a special semiparametric regression model to examine the effect of explanatory variables on survival times. $\lambda(t)$ gives the likelihood for a given individual to survive to time $t + 1$, given the individual has survived to time $t$. When all covariates $Z$ are zero, the proportional hazard can be expressed as $\lambda_0(t)$. The regression parameters ($\beta$) associated with the explanatory variables and their standard errors are estimated using the maximum likelihood method. It assumes that the hazard for all values of $Z$ can be written as:

$$\lambda(t; Z) = \lambda_0(t) \cdot e^{\beta Z}$$  \hspace{1cm} (1)

Data from the gyrodactylid experiments were analysed using repeated measures ANOVA (r.m. ANOVA), where the fish were considered as replicates, and number of living parasites was the response variable. Due to the continuous parthenogenetic reproduction in gyrodactylids it was impossible to keep track of individual parasite, therefore the proportional hazard analysis was inappropriate. The statistical analyses were conducted using JMP 4.0 and EXCEL 2000/XP.
Results

Water chemistry

Water temperature varied between 10 and 12°C during most of the experimental period (Tab. 3). In March, when experiment 3 was performed, temperature was between 7 and 9°C. Due to different ageing time of the waters used (Tab. 3) in the experiments, there was a small difference in temperature (0.5 - 1.0°C) between the tanks. In both the acidic Al-rich and Al-poor waters, pH was stable (5.8) throughout all experiments (Tab. 3). In the control water, pH was also stable throughout the experimental period (mean ± S.D., 6.3 ± 0.1. n = 122). Water conductivity of the various test waters varied between 20 and 32 µS cm⁻¹ during the experiments (Tab. 3).

Table 3: Water temperature, conductivity and pH (mean ± S.D.) in the various test media used in the experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test medium</th>
<th>Temperature(°C)</th>
<th>Conductivity (µS cm⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acidic Al-rich</td>
<td>11.3 ± 0.3</td>
<td>31.6 ± 2.0</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Acidic Al-poor</td>
<td>10.5 ± 0.0</td>
<td>31.8 ± 3.1</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10.0 ± 0.0</td>
<td>30.5 ± 2.4</td>
<td>6.3 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>Acidic Al-rich</td>
<td>12.0 ± 0.0</td>
<td>29.2 ± 1.2</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Acidic Al-poor</td>
<td>12.0 ± 0.0</td>
<td>30.0 ± 1.1</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11.8 ± 0.5</td>
<td>27.3 ± 1.3</td>
<td>6.3 ± 0.0</td>
</tr>
<tr>
<td>3</td>
<td>Acidic Al-rich</td>
<td>8.8 ± 0.3</td>
<td>26.9 ± 2.5</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Acidic Al-poor</td>
<td>8.8 ± 0.2</td>
<td>24.4 ± 2.0</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.1 ± 0.4</td>
<td>22.3 ± 2.1</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>Acidic Al-rich</td>
<td>12.0 ± 0.0</td>
<td>25.6 ± 0.1</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Acidic Al-poor</td>
<td>11.0 ± 0.0</td>
<td>25.6 ± 2.1</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10.0 ± 0.0</td>
<td>23.4 ± 0.9</td>
<td>6.3 ± 0.0</td>
</tr>
<tr>
<td>5</td>
<td>Acidic Al-rich</td>
<td>12.9 ± 0.2</td>
<td>27.2 ± 2.0</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Acidic Al-poor</td>
<td>12.0 ± 0.0</td>
<td>25.9 ± 0.7</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11.5 ± 0.2</td>
<td>25.3 ± 1.1</td>
<td>6.3 ± 0.0</td>
</tr>
</tbody>
</table>

Number of observation in experiment 1, 2, 4, 5: temp (n = 11), cond (n = 33), pH (n = 33). Experiment 3; temp (n = 20), cond (n = 60), pH (n = 60).
In the acidic Al-rich water the total concentration of aluminium (Alr) was between 200 and 260 µg · L⁻¹, and varied somewhat in the experiments (Fig. 3). The difference in Alr between the acidic Al-rich water and the control water, however, was relatively constant within each experiment, and throughout the experimental period, i.e. 125 ± 9 µg · L⁻¹ (mean ± S.D). Thus, the variation in Alr was mainly due to variations in the background concentration in the department water, which varied between 75 and 125 µg · L⁻¹. The concentration of Alr was approximately similar in the acidic Al-poor and control water. There were no significant increase or decrease in Alr-concentration in the experiments (linear regressions, lowest p = 0.27).

In experiment 1 and 2, the fraction of monomeric aluminium (Alo) in the acidic Al-rich water constituted approximately 75% of Alr. In the other three experiments (3-5), the Alo-fraction constituted approximately 65% of Alr. In addition to this difference concerning the Alo-fraction, the acidic Al-rich water in experiment 1 and 2 also had a lower fraction of Al1 compared to the other three experiments, and consequently a higher Al1-fraction (Fig. 4). In experiment 1 and 2, the Al1-concentration in the acidic Al-rich water was 78 ± 10 µg · L⁻¹, while it was 42 ± 6 µg · L⁻¹ in average in the three other experiments. The amounts of the various Al-fractions in the control water varied somewhat throughout the experimental period, but the Al1-fraction was always relatively low (< 25 µg · L⁻¹). In the acidic Al-poor water, the various Al-fractions corresponded well with those in the control water (Fig. 4).
Figure 3. Concentrations of total aluminium (Alr) in the various experiments, (●) control water; (▼) acidic Al-poor water; (○) acidic Al-rich water (n = 1 for each point).
Figure 4. The concentrations (mean ± S.D., n = 3) of aluminium fractions in the various experiments. Experiment 1 and 2 are shown together because it was a short time interval between them, and Al-fractionations were performed only three times during these two experiments.
Parasite infection

Experiment 1 and 2: *G. derjavini* on brown trout

In experiment 1, the infection of *G. derjavini* decreased rapidly in fish exposed to the acidic Al-rich water (r.m. ANOVA F$_{4,15} = 5.08$, p < 0.001; Fig. 5). The number of parasites on individual fish declined from 92 ± 22 (mean ± S.E.) to 11 ± 6 during the first 36 h, and after 60 h exposure there were only one fish with two individuals of the parasite left. Finally, after 84 h of Al-exposure the infection was totally eliminated. Parasite densities remained unchanged, in the control (Fig. 5, total mean: 55 ± 23).

Figure. 5. Number of *G. derjavini* (mean ±S.E., n = 10) on brown trout exposed to untreated control water at pH 6.3 (▼) or acidified Al-enriched water at pH 5.8 (●).
Also in experiment 2, the infection of *G. derjavini* decreased rapidly in fish exposed to the acidic Al-rich water but not in the Al-poor or the control (r.m. ANOVA, F$_{6,50}$ = 8.45, p < 0.001; Fig. 6). The number of parasites on individual fish declined from 156 ± 31 (mean ± S.E.) to 60 ± 16 during the first 24 h, and then to 6 ± 3 during the next 24 h. Finally, after 72 h of Al-exposure the infection was totally eliminated. There was also a small but non-significant decline in the number of parasites on individual fish in the exposure to acidic Al-poor water, from 77 ± 16 to 49 ± 17. Similarly, there was no significant change in parasite densities in the control, which were approximately 55 parasites per fish.

**Figure. 6.** Number of *G. derjavini* (mean ± S.E., n = 10) on brown trout exposed to untreated control water at pH 6.3 (▼); acidified Al-enriched water at pH 5.8 (●); acidified Al-poor water at 5.8 (○).
Experiment 3: Glochidium of duck mussel on perch
The density of duck mussel glochidia decreased with time, and the rate of decrease differed among treatments (likelihood ratio test, $\chi^2 = 116.5 \ p < 0.0001$; Fig 7.). In the acidic Al-rich water, density decreased to about 50% of the initial density within approximately 60 h. Then density continued to decrease, but to a slowly. At the end of the experiment, the density of glochidia was $13 \pm 2$ (mean ± S.E.). In the exposure to acidic Al-poor water, there was also a decrease in parasite density, from $39 \pm 8$ to $30 \pm 6$, as well as in the control, from $47 \pm 13$ to $43 \pm 13$. The Proportional Hazard model gave a risk ratio of 1.25 ($\pm 0.05$; ± 95% confidence limits) for acidic Al-rich water compared to acidic Al-poor water, while the corresponding ratio for control vs. acidic Al-poor water was 0.86 ($\pm 0.03$). One fish died in the acidic Al-poor water and one in the control.

![Figure 7](image.png)

**Figure. 7.** Number of duck mussel glochidium the (mean ± S.E. n = 10) on perch exposed to untreated control water at pH 6.3 (▼); acidified Al-enriched water at pH 5.8 (●); acidified Al-poor water at pH 5.8 (○).
Experiment 4: Free-living little fish louse
In this experiment, all treatments resulted in relatively high but significantly different death rates (likelihood ratio test, $\chi^2 = 70.8 \ p < 0.0001$; Fig. 8). The highest death rate was observed in fish louse exposed to acidic Al-rich water, and all lice were dead after 96 h. In the acidic Al-poor water all lice were dead after 148 h, while at that time 50% was dead in the control. The Proportional Hazard model gave a risk ratio in acidic Al-rich water compared to acidic Al-poor water of 1.79 (upper and lower 95 % C.L: 1.50, 2.12), and in the acidic control vs. Al-poor the corresponding ratio was 0.57 (0.50, 0.67).

Figure 8. Number of surviving little fish louse (mean ± S.E., of 4 replicates n = 40) exposed to untreated control water at pH 6.3 (▼); acidified Al-enriched water at pH 5.8 (●); acidified Al-poor water at 5.8 (○).
Experiment 5: *G. macronychus* on minnow

In this experiment, the infection of *G. macronychus* decreased fast in fish exposed to the acidic Al-rich water but not in the Al-poor or the control (r. m. ANOVA, $F_{16,70} = 4.41, p < 0.001$); Fig. 9. After 43 h of exposure the *G. macronychus* density was reduced from $210 \pm 20$ to $88 \pm 9$ (mean ± S.E.), and after 187 h the infection was eliminated.

![Graph showing the number of *G. macronychus* on minnow exposed to different water conditions over time.](image)

**Figure. 9.** Number of *G. macronychus* (mean ± S.E., $n = 10$) on minnow exposed to untreated control water at pH 6.3 (▼); acidified Al-enriched water at pH 5.8 (●); acidified Al-poor water at 5.8 (○).


**Discussion**

**The effect of aluminium and acidity on ectoparasites**

The present study shows that aqueous aluminium has a strong impact on freshwater fish ectoparasites under the conditions tested. The four parasite species tested all showed increased mortality when exposed to 200-260 µg Al ⋅ L\(^{-1}\) at pH 5.8. The gyrodactylids were eliminated and the little fish louse died, while the duck mussel glochidia density decreased. The elimination of gyrodactylids was in accordance with previous observations of *G. salaris* exposed to aluminium (Soleng *et al.*, 1999; Grimsmo, 2000).

Aluminium seems to have a general toxic effect on freshwater ectoparasites under acidic conditions, but the sensitivity to the challenge differs among the various parasites. The elimination of *G. derjavini* was more rapid than the elimination of *G. macronychus* under low pH and high aluminium conditions. Studies on *G. salaris* infected Atlantic salmon showed that this gyrodactylid has a similar sensitivity to aluminium as *G. derjavini*, and thus seems to be more sensitive than *G. macronychus* (Soleng *et al.*, 1999; Grimsmo 2000). It is important to note, however, that three gyrodactylids mentioned above have different hosts. On the other hand, in the exposure of *G. derjavini* and *G. salaris* (Grimsmo 2000), the concentration of inorganic monomeric aluminium (Al\(_i\)) was approximately 80 µg ⋅ L\(^{-1}\), compared to 42 µg ⋅ L\(^{-1}\) in the exposure of *G. macronychus*. Therefore, it is reason to believe that *G. macronychus* would have responded more rapidly and similar to the two other gyrodactylids if the Al\(_i\)-concentration was raised to 80 µg ⋅ L\(^{-1}\). It is generally known that the toxicity of aqueous aluminium is dependent mainly on the concentration of the Al\(_i\)-fraction (reviewed by...
Gensemer & Playle 1999). It should also be noted that the initial infection of *G. macronychus* was higher compared to the initial infection of *G. derjavini* and *G. salaris* (Grimsmo 2000). Overall, bearing in mind the host and infection density differences, it seems that the genus *Gyrodactylus* has a more or less similar and high sensitivity to aqueous aluminium in acidic water.

Not only gyrodactylids, but also parasites within other phyla respond negatively to acidic water or aluminium. The present study showed that the density of duck mussel glochidia decreased and little fish louse died when exposed to acidic Al-rich water. Others have in field studies found that parasite species richness was significantly higher in yellow eels (*Anguilla rostrata*) from rivers with pH above 5.4 compared to more acidic rivers (Cone *et al.*, 1993; Marcogliese & Cone, 1996). Similarly, Halmetoja *et al.* (2000) reported that the mean number of metazoan parasite specimens on perch was markedly lower at pH 5.3 - 5.9 compared to pH 6.4. Interestingly, one of the metazoans studied by Halmetoja *et al.* (2000) was the glochidia of the swan mussel (*A. piscinalis*), a close relative to the duck mussel. They reported a complete absence of swan mussel glochidia at pH below 5.9. In the present study, however, the glochidia of the duck mussel showed a rapid initial decrease in density, but were not completely eliminated when exposed to aluminium at pH 5.8. One reason why the glochidia were not eliminated completely in the present study might simply be that the duration of the exposure was to short. On the other hand, the complete absence of swan mussel glochidia in acidified waters may indicate that other life history stages of the mussel are more sensitive to low pH and aluminium than glochidia.

Even though many authors do not refer to aluminium, the effects of acidification are most probably due to aqueous aluminium alone or in combination with low pH in the most acidic
waters, since acidified freshwater systems are characterised by elevated concentrations of aqueous aluminium (Gensemer & Playle 1999). In the present study, acidity alone (pH 5.8) had a clear effect on the little fish louse. The acidic Al-poor water contained small amounts of aluminium, but the concentrations of the various Al-fractions were more or less similar to the concentrations found in the control water (pH 6.3). The only significant difference between the acidic Al-poor and control water was the pH. When exposed to the combination of acidity and aluminium, however, the effect on the fish louse was stronger. Accordingly, it has been reported that acidic Al-poor water (pH 5.0) eliminated *G. salaris* from infected salmon, while an addition of aluminium increased the rate of elimination (Soleng *et al*., 1999). In that study, however, no effect on *G. salaris* of acidity alone was observed at pH 5.8 (Soleng *et al*., 1999).

In summary, the effects of aluminium on the various parasites in the present study confirm the importance of water quality in general, and acidification in particular, for the distribution and population dynamics of ectoparasites in freshwater environments.

**Aluminium and its mode of action**

It has been discussed whether aluminium has a direct effect on ectoparasites, or an indirect effect through the host (Grimsmo, 2000). In fish, Al-toxicity is evidently caused by the binding and accumulation on the gill surfaces (Poleo 1995; Gensemer & Playle 1999). In the same way, aluminium might interact directly with the surface of the parasites. Larsen *et al.* (2002), however, found that brown trout experimentally infected with an intestinal nematode (*Anisakis* sp.) had a lower susceptibility for *G. derjavini*. This indicates that the activation of the immune system by *Anisakis* sp. had a general effect on parasites. In the same way, aluminium might also trigger an immune response in the fish, which in turn indirectly affects parasites. In the present study, it was evident that aluminium had a direct effect on the little
fish louse. Since the fish louse were not attached to fish in the experiment, it is not possible to eliminate a possible effect of aluminium through the host. Atlantic salmon pre-exposed to aluminium, however, showed no increase in susceptibility when infected with *G. salaris* compared to control fish (Grimsmo, 2000). Therefore, it still remains to investigate future if aluminium can have an indirect effect on ectoparasites, through their hosts.

The toxicity of aqueous aluminium depends on the Al-forms present in solution. It is generally accepted that inorganic monomeric aluminium (**Al**<sub>i</sub>) is the principle toxicant in acidified waters (Gensemer & Playle, 1999). In experiment 1 and 2 the **Al**<sub>i</sub> concentration was $78 \pm 10 \mu g \cdot L^{-1}$ and the parasite elimination was faster than in experiment 5, where the **Al**<sub>i</sub> concentration was $42 \pm 6 \mu g \cdot L^{-1}$. This is in accordance to the results obtained by Grimsmo (2000), and support the argument that **Al**<sub>i</sub> is the decisive factor for the Al-toxicity in the various studies on parasites in acidic water, regardless of a direct or indirect effect.

**Aluminium as a possible parasiticide**

Several methods have been applied in order to find a proper method to control and eliminate ectoparasite infections in freshwater fish. The main strategy involves chemical treatment of the infected fish. Many of the various chemicals used have environmental side effects. Either by killing whole fish communities, as is the case for the piscicide rotenone frequently used to treat Norwegian salmon rivers against *G. salaris* (Haukebø *et al.*, 2000), or by accumulation of heavy metals or organic compounds in the environment, as most parasiticides are based on such chemicals. Aluminium is the most abundant metal in the earth crust, and hence present already in most freshwater systems. Using aluminium involve a transient presence of toxic forms, which subsequently precipitate out of solution. The present study shows clearly that aluminium is able to eliminate ectoparasites without killing the fish hosts. This is in
accordance to previous studies on the effect of aluminium on *G. salaris* infected Atlantic salmon (Soleng *et al*., 1999; Grimsmo 2000). On the other hand, elevated levels of aqueous aluminium are responsible for the decline in freshwater fish communities in acidified waters. The relatively large difference in sensitivity between the ectoparasites and the fish species studied in the present and previous studies, therefore allows limited time exposures that kills or eliminates the parasites without killing the fish. Added to this, is the fact that the effects of aqueous aluminium in fish are reversible (Hytterød *et al*., 2001). It has been shown that the most Al-sensitive freshwater fish species, the Atlantic salmon, recovers rapidly after an exposure to aluminium (Hytterød *et al*., 2001). It should also be mentioned that the concentrations of aluminium used in the present study are well below the concentrations found in many limed rivers (DN, 2002). Thus, it is reason to believe that the amount of aluminium added to a water system in a possible future treatment against ectoparasites is of negligible environmental significance.

The present study provides results which may prove useful for parasitologists interested in pursuing further research in this area, and may also be used as a framework for applying new treatment methods in fish farming or even for treating natural freshwater systems. A possible aluminium treatment indicated in the present study may be developed further to an effective disinfection method against ectoparasites. The key to success is to understand the mechanism by which aluminium and other metals affect the parasites and their hosts.
References


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Appendix 1

General composition of Oslo City tap water (mean ± S.D, n = 18).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean ± S.D</th>
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<tr>
<td>Na⁺</td>
<td>mg·L⁻¹</td>
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<td>K⁺</td>
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<tr>
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<tr>
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Analysed by Norwegian Institute of Water Research.