Effects of aqueous aluminium chloride and zinc chloride on survival of the gill parasitizing monogenean *Pseudodactylogyrus anguillae* from European eel *Anguilla anguilla*

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**Abstract**
The gill parasitizing monogeneans *Pseudodactylogyrus anguillae* and *P. bini* are pathogens which represent a major threat to eel farming. Legislation and selection of anthelmintic resistance make parasite eradication using conventional anthelmintics difficult and emphasize the needs for development of alternative control methods. Conditioning of fish tank water with aluminium chloride (up to 25 mg/l) have been demonstrated to have a negative impact on the infection level of *P. anguillae*. Zinc chloride (up to 25 mg/l) has a weaker effect. However, the impact of these metals on the parasites is pH-dependent. Lowering of pH to below 6 increases the effect of both Al and Zn whereas the effect of these substances at pH 7.5 is insignificant. Addition of small amounts of especially aluminium to recirculated eel farm water may prove to be an interesting way to prevent pseudodactylogyrosis. The environmental importance of these findings are discussed.

**Introduction**
Eel farming operations in recirculation systems have suffered severely from infections by the ectoparasitic flatworms *Pseudodactylogyrus anguillae* and *P. bini* (Buchmann, 1997). Formerly formaldehyde was the only effective control agent used by eel farmers. However, the advent of mebendazole water bath treatments (1 mg/l) changed the situation significantly for a number of years because this drug was found effective against all stages of the parasite’s life cycle (Szekely & Molnar, 1987, Buchmann & Bjerregaard, 1990). The risk of development of anthelmintic resistance was noted by Buchmann et al. (1992) following artificial selection of drug tolerating parasite strains in laboratory experiments. Nonetheless serious problems with drug efficacy were recorded within a few years posing a threat to European eel farming operations (Waller & Buchmann, 2001). This led to a renewed increase of formaldehyde use in eel farms, a practice which is undesirable due environmental concern and to the health compromising action of this chemical. Therefore, the eel farming industry is in a need for alternative and sustainable control methods which will not interfere with legislation, will pose no occupational health risks to eel farmers and are environmentally safe.

Biological control based on parasite egg predation by turbellarians and oncomiracidium predation by copepods were suggested by
Buchmann (1988, 1997). However, management of such delicate ecosystems in fish tanks is admittedly difficult and less labor intensive techniques are preferred by eel farmers. Recent studies have indicated that aqueous aluminium eliminates another pathogenic monogenean *Gyrodactylus salaris* parasitizing the skin of Atlantic salmon (Soleng et al., 1999). Likewise, zinc has a toxic effect on the digenean cercariae of *Parorchis acanthus* (Morley et al., 2001) and *Cryptocotyle lingua* (Cross et al., 2001). The findings that flatworms are vulnerable to low concentrations of aluminium and zinc raised the possibility that the use of water conditioning with related compounds could prevent pseudodactylogyrosis. Therefore the present study was initiated. It elucidates the effects of aluminium chloride and zinc chloride in fish tank water on adult *P. anguillae* on gills of the European eel.

**Materials and methods**

**Parasites**

Larger eels (approx. 200 g body weight) infected with 10-30 specimens of *P. anguillae* were obtained from a commercial eel farm and stocked in four fish tanks (tank volume 200 l) each with 100 l freshwater and 10 cm gravel at bottom. Eels burrowed into the gravel and deposited parasite eggs which hatched and released oncomiracidia to the tank water. This larva containing water was used for experimental infection of smaller eels (see below).

**Eels**

Uninfected eels, mean body length cm (SD): 12.1 (1.6), mean body weight g (SD): 1.9 (0.7), were obtained from a commercial eel farm and brought to the laboratory for infection.

**Water**

Municipal water with a hardness of 390 mg/l CaCO₃, pH 7.2-7.5 and temperature 20 ºC was used to establish the parasite population and infect smaller eels. Water was aerated and recycled by internal Eheim filters and daily monitoring showed no nitrite (Merckoquant 1.10022, Merck). Nitrate concentrations were below 25 mg/l (Merckoquant 1.10020, Merck) and ammonia levels were below 2.5 mg/l (Merckoquant 1.14723, Merck). During experimentation in smaller aquaria hydro-chloric acid (HCl) was used for pH adjustment to various levels.

**Experimental infection of small eels**

Uninfected eels were placed in small net-cages (mesh size 1 mm) which were submerged in oncomiracidium containing water (Buchmann et al., 1992). The parasite larvae entered the net-cages and infected eels through a period of one week after which eels were removed to separate aquaria for 14 days to allow parasite maturation. The infected eels were then used for experimentation two to three weeks post-infection.

**Metals**

Stock solutions of Aluminium chloride (Sigma A 3017) and Zinc chloride (Sigma A 3017) were prepared in municipal water from which appropriate volumes were added to experimental aquaria. Experimental solutions were 0, 0.1 mg/l, 1.0 mg/l, 10 mg/l and 25 mg/l at pH 5, 6 and 7.5.

**Experimental design**

Groups of eels, each comprising 4 small infected specimens, were immersed into 4 l aerated water (aquarium volume 19 l) with different concentrations of aluminium chloride
or zinc chloride at different pH which was adjusted continuously. Following 72 h exposure eels were examined for infection and the infection level was compared with control fish kept at the same pH (Tables 1 and 2). Experiments with aluminium at pH 5 and 7.5 was conducted in duplicate.

**Parasite counts**

The number of parasites infecting eels were determined by carefully dissecting all gill arches using a pair of micro-scissors under the dissection microscope. Abundance (mean number of parasites per host including both infected and un-infected) was calculated according to Bush et al. (1997) and differences of means were compared statistically using the Mann-Whitney U-test with a probability level of 0.05. When no significant difference was found between duplicate groups these were combined and tested together.

**Results**

The number of parasites per eel varied considerably as indicated by the high standard deviation producing an over-dispersed distribution with variance to mean ratios exceeding unity. All concentrations of aluminium chloride and zinc chloride (0.1, 1.0, 10, 25 mg/l) showed no significant effect at pH 7.5 and pH 6. However, at pH 5 a clear impact on the number of parasites was found following 72 h exposure to aluminium chloride in solutions with concentrations of 10 and 25 mg/l (Tables 1 and 2). A clear but non-significant trend to a lowered infection level after zinc chloride exposure (10 and 25 mg/l) was seen. No adverse effect on experimental eels were recorded during the experiments with any of the applied concentrations of metals.

**Discussion**

Pseudodactylogyrosis has been the most devastating disease in eel farms for years (Buchmann, 1997). It is caused by heavy infections by the gill parasitizing congeners *Pseudodactylogyrus anguillae* and *P. bini*. Formerly the use of mebendazole (1 mg/l) was the preferred control method in eel farms. This drug replaced the use of formaldehyde which was used in the 1980’s. However, following the advent of MBZ-resistance in eel farms (Waller & Buchmann, 2001) and legislation prohibiting use of un-registered anthelmintics the need for alternative control methods have been strongly emphasized. Possible biological control based on predation of eggs and oncomiracidia by turbellarians and copepods was indicated by Buchmann (1988, 1997) but management of such delicate ecosystems in

<table>
<thead>
<tr>
<th>Conc. AlCl₃</th>
<th>pH 7.5 (a)</th>
<th>pH 7.5 (b)</th>
<th>pH 6</th>
<th>pH 5 (a)</th>
<th>pH 5 (b)</th>
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<tbody>
<tr>
<td>0 (Control)</td>
<td>24.5 (20.5)</td>
<td>10.3 (7.4)</td>
<td>19.3 (15.8)</td>
<td>24.5 (27.3)</td>
<td>10.8 (3.6)</td>
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<td>0.1 mg/l</td>
<td>12.5 (24.3)</td>
<td>5.0 (3.4)</td>
<td>9.3 (12.9)</td>
<td>17.0 (3.9)</td>
<td>18.3 (24.2)</td>
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<tr>
<td>1.0 mg/l</td>
<td>19.5 (37.7)</td>
<td>10.5 (9.8)</td>
<td>16.8 (14.5)</td>
<td>4.5 (2.9)</td>
<td>27.5 (30.8)</td>
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<tr>
<td>10 mg/l</td>
<td>4.8 (5.0)</td>
<td>9.5 (10.0)</td>
<td>5.0 (3.4)</td>
<td>10.0 (12.0)**</td>
<td>0.5 (0.6)*, **</td>
</tr>
<tr>
<td>25 mg/l</td>
<td>14.3 (10.0)</td>
<td>18.0 (19.1)</td>
<td>1.5 (2.4)</td>
<td>6.5 (13.0)**</td>
<td>0.3 (0.5)*, **</td>
</tr>
</tbody>
</table>

*Table 1. Effects of aluminium chloride at different pH. Abundance (mean number of parasites in infected and un-infected eels) (SD: standard deviation) following 72 h exposure at 20 C. *: significant different from control group (p<0.05). **: Significant different when duplicate groups were combined.*
The present studies have shown that aqueous solutions of especially aluminium chloride and to a lower degree zinc chloride in concentrations at 25 mg/l have a negative impact on adult specimens of *Pseudodactylogyrus anguillae* provided that pH is kept at pH 5. Low pH alone is not sufficient to affect the gill parasites which was seen from high infection in control eels kept at different pH. Therefore, a pH at 5 seems to be relevant for these studies. The pH dependent action of aluminium was also registered by Soleng et al (1999) working with *Gyrodactylus salaris* on salmon. The finding that presence of metals, like aluminium and zinc, in fish tank water is associated with impaired survival of parasites has both ecological importance for natural waters and for management of infectious diseases in aquacultural enterprises. Metal pollution including aluminium and zinc in our natural waters may affect indigenous parasite communities. This was in fact suggested by Poulin (1992) reviewing the importance for fish parasites of contaminants such as metals. In contrast, recirculated eel farming operations, which are considered environmentally safe due to their minimal and controlled outlet to the environment, may benefit from these findings for control of gill parasitic infections. Any addition of metals to the fish tank units can be recovered again and should not pose any environmental problems. However, possible pathological influences caused by low pH and aluminium on the Atlantic salmon were indicated by Berntssen et al. (1997) and the effect of zinc on fish epidermis was studied by Hemalatha & Banerjee (1997). Therefore, it should be noted that any adverse effects of metals and low pH on the farmed European eel should be investigated. Further, in this study it was not determined if juvenile

<table>
<thead>
<tr>
<th>Concentration of ZnCl₂</th>
<th>pH 7.5</th>
<th>pH 6</th>
<th>pH 5</th>
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<tbody>
<tr>
<td>0 (Control)</td>
<td>26.5 (31.8)</td>
<td>13.3 (15.1)</td>
<td>5.0 (5.7)</td>
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<tr>
<td>0.1 mg/l</td>
<td>25.3 (20.2)</td>
<td>15.3 (17.7)</td>
<td>6.3 (5.3)</td>
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<tr>
<td>1.0 mg/l</td>
<td>35 (33.4)</td>
<td>5.8 (7.5)</td>
<td>20 (23.1)</td>
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<tr>
<td>10 mg/l</td>
<td>9.5 (4.7)</td>
<td>26 (35.4)</td>
<td>3.5 (5.7)</td>
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<tr>
<td>25 mg/l</td>
<td>10.3 (3.1)</td>
<td>10.8 (8.2)</td>
<td>1.3 (1.3)</td>
</tr>
</tbody>
</table>

Table 2. Effects of zinc chloride at different pH. Abundance (mean number of parasites in infected and uninfected eels) (SD: standard deviation) following 72 h exposure at 20°C.
and larval stages of *Pseudodactylogyrus angiullae* respond to the two chemicals. Therefore, further studies should elucidate the effects of these metals on oviposition, egg development, hatching, oncomiracidium infectivity and maturation of juvenile parasites on eels. Likewise, it should be accentuated that the present observations were conducted under laboratory conditions. Large scale experiments should be performed before any implementation of these control methods are considered.

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**References**


