THE RESPONSE OF BROWN TROUT (SALMO TRutta L.) TO REPEATED CHALLENGE WITH INFECTIOUS SALMON ANAEMIA (ISA)

By A. NYLUND, S. ALEXANDERSEN, P. LIBIRI AND P. JAKOBSEN

Introduction
Infectious Salmon Anaemia (ISA) is a viral disease of farmed Atlantic salmon (Salmo salar L.) in Norwegian aqua culture (Thurud and Djupvik, 1988; Thurud, 1991; Evensen et al., 1991; Hjelmas et al., 1992; Olsen et al., 1992; Dannegj et al., 1993; Nylund et al., 1993; Hovland et al., 1994; Dannegj and Falt, 1994; Nylund et al., in press). ISA has only been diagnosed in Atlantic salmon after the fish has been transferred to sea or has been exposed to sea water in hatcheries.

It is unknown whether or not the agent is present in wild salmonids. However, studies of trout (Salmo trutta L.) have shown that natural populations of this species represent potential reservoirs for ISA (Nyland and Jakobsen, in press; Nylund et al., submitted). The virus was able to propagate in the trout, but no gross clinical signs of disease were observed. Even if the virus seem to be neutralized 45 days after challenge it was still present in sea trout 7 months after challenge. The sea trout had started to mature while kept on full sea water (34‰), and this stress could have initiated virus replication according to Nyland et al., submitted.

It is not known how the trout responds to repeated infection with ISA virus. The aim of this study was to see if naive trout responded different to ISA challenge compared trout that had been previously infected with ISA.

Materials and methods
The Atlantic salmon used in the experiment were supplied by a hatchery with no history of ISA or other infectious diseases, and taken into the laboratory in the fresh water phase. The brown trout (D+, I+) used were collected from Myrdalsvatnet 18 km south of Bergen. The fish were kept in 0.15 m³ tanks with UV-filtered running water (salinity = 8.5ppm) at a temperature of 9.5°C. The ISA virus (from ascites) was collected from Atlantic salmon during a natural outbreak in the disease in a fish farm outside Bergen. The ascites was passed through a 0.2 µm pore sized filter and diluted 1:5:1:0 with Hank’s Balanced Salt Solution (HBSS).

After two weeks of acclimatization the fish were divided into 4 groups. One group of trout (TISA) and one group of salmon (SISA) were challenged with an intraperitoneal (i.p.) injection of ISA infected filtered ascites. The two control groups TCON (trout) and SCON (salmon) received an i.p. injection of HBSS.

To test for the presence of ISA virus in the trout, tissue (liver and kidney) from groups TISA and TCON were collected 20 days after the start of the experiment. Thirty salmon in group S20 and S20C were each challenged with 0.2 ml of filtrate prepared from tissue of trout in group TISA and TCON, respectively. The two filtrates were prepared as follows; 0.3 grams of tissue were homogenized, filtered sterile (0.2 µm), and diluted in 8 ml of HBSS before injection in the salmon.

After 71 days the two trout groups (TISA and TCON) were each split into two new groups (IH and IP from group TISA and CH and IP from TCON, respectively). Groups IH and IP consisted each of 16 individuals and groups CH and IP of 18 individuals each. Specimens in group IH and CH received an i.p. injection of 0.1 ml of filtered (0.2 µm) ascites (containing ISA virus) di-
luted in HBSS (1:1), while the specimens in groups IP and CP received an i.p. of 0.1 ml filtered acetics (containing ISA virus) diluted in predilution saline (1:1). After 28 days the trout in groups CH, CP, IH and IP were killed and the livers taken out, sonicated, filtered (0.2 µm) and diluted 1:10 in HBSS. The livers from each group (IH, CH, IP and CP) were kept separate and 0.2 ml of the final solutions were I.p. injected into salmon in four different groups (SIIH, SCH, SIP and SCP). Each group containing 30 salmon. These salmon groups were kept in running sea water at 12°C.

Fish exposed to injection of the different solutions described above, were anaesthe-
tised with Benzocaine.

Fish developing any disease, moribund fish and dead fish were examined sampling the following: weight and length, haematocrit, registration of clinical signs of disease, and tissues for histological examination. Bacteriological tests were carried out by inoculation of kidney tissue on blood agar plates incubated at 20°C for six days, and on blood agar plates containing 2% NaCl incubated for six days at 15°C.

The work was carried out at the Department of Fisheries and Marine Biology and the Industrial Laboratory, HIB, Bergen, Norway.

Results

There were no mortalities in the two control groups (SICON and TICON) which had been given an i.p. injection of HBSS. All salmon smolts challenged with ISA infected acetics (group SIIA) died before day 30. The mortality in this group started at day 16. Two specimens died before day 30 in the trout group (TIIIA) challenged with ISA infected acetics. The trout specimens were dark pigmented and had pale gills and heart, yellow livers, a swollen spleen and an empty stomach and gut. Acetics were present in one individual.

There was no mortality in the control group SIIA, while all salmon in group SIIA died before day 30 with all clinical signs of ISA. There were no mortalities in the trout groups IH, CH, IP and CP during the experimental period of 28 days. The fish did not show any clinical signs of disease.

Discussion

The results from this experiment supports the view held by Nyland and Jakobsen (in press) that the ISA virus is able to propagate in sea trout without giving any gross clinical sign of disease. The results also indicate that the trout is able to mount an immune re-
to the ISA virus which enables it to handle a second infection faster than a pri-
mary infection. This suggests a specific reaction to the virus.

After a primary infection with ISA trout seem to neutralize the virus within 45 days (Nyland et al., submitted). However, it has also been demonstrated that the virus is pre-
sent in trout 7 months after challenge. In the latter case the trout had survived for a while kept on full sea water and Nyland et al., suggested that this stress could have initiated virus replication. The result pre-
seated in this paper supports this view.

There is a significant difference in virus titre after 28 days in trout from group IH and IP according to the mortality in the two salmon groups (SIIH and SIP) challenged with tissue from the two trout groups. The trout group (IP) that were given predi-
one acetics at the same time as the second injection of ISA virus, do not seem to han-
dle the infection as good as the trout group (IH) just given a second injection of ISA virus. However, injection of predilution acetic acid does not seem to have a significant influence the ability of the trout (group CH and CP) to handle the primary infection with ISA virus according to the mortality in the salmon groups SCH and SCP.

The results from this and previous experi-
ments with trout and ISA suggest that the trout may constitute a natural reservoir for the virus. The possibility that stressed ISA-infected trout may represent a hazard for salmon is also indicated. This could be a problem for fish farmers in areas with ISA and sea trout, and a problem for national populations of salmon which means the sea trout in estuaries during maturation. It has been shown that sea trout may migrate more than 50 km from their natal rivers (Berg and Berg, 1987).

Table 1. Mortality in the four salmon groups 40 days after challenge. Hct = mean haematocrit value for survivors in the groups, S.D. = standard deviation

<table>
<thead>
<tr>
<th>Code</th>
<th>N</th>
<th>Dead</th>
<th>Hct</th>
<th>S.D.</th>
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</thead>
<tbody>
<tr>
<td>SCH</td>
<td>30</td>
<td>14</td>
<td>37.8</td>
<td>±5.3</td>
</tr>
<tr>
<td>SCP</td>
<td>30</td>
<td>14</td>
<td>39.9</td>
<td>±4.3</td>
</tr>
<tr>
<td>SH</td>
<td>30</td>
<td>2</td>
<td>45.8</td>
<td>±5.2</td>
</tr>
<tr>
<td>SIP</td>
<td>30</td>
<td>27</td>
<td>51.3</td>
<td>±6.7</td>
</tr>
</tbody>
</table>

![Figure 1: Cumulative mortality in the 4 salmon groups (SIIH, SCH, SIP, and SCP) challenged with liver filtrate from 4 brown trout groups (IH, CH, IP and CP). Temperature 12°C.](image-url)
MORTALITY OF CAPTIVE ARAPAIMA GIGAS (OSTEOGLOSSIDAE) HEAVILY INFECTED WITH THE GILL MONOGENEAN DAWESTREMA CYCLOANCISTRIUM.

BY K. BUCHMANN1, A. UDAR7, S. MELLEROAARD1

The freshwater teleost Arapaima gigas (Osteoglossidae) with the local name pirarucu has a limited distribution in the Northern South America inhabiting the river systems of Amazonas, Orinoco, Essequibo and Rupununi. This ostegoosid is considered an endangered species and international trade of the fish is restricted. A number of parasitic helminths have been recorded from this fish host and the gill monogenean Dawestrema cycloancistrum, D. cycloucancistroides and D. punctatum (Ancyrocephalidae) from Arapaima gigas were recently treated by Kreisky et al. 1985. So far no reports of epidemics and host mortalities associated with Dawestrema infections in the natural environment are available. However, some monogeneans are able to reproduce vigorously in confined water bodies and produce infections of epidemic proportions (Nigrelli & Breder 1934, Paperna 1963, 1986, Proctor 1963, Molnár 1971, Bauer et al. 1973, Bachmann et al. 1987, Thoney & Hargis 1991, Bakke & MacKenzie 1993) with resulting host mortality. The present report elucidates the reproductive potential of Dawestrema cycloancistrum in a confined environment and describes a case of fatal Dawestrema infections of captive Arapaima gigas in a public aquarium.

A total of 8 specimens of Arapaima gigas (6 with approximate weights of 500 g, length 40 cm, 2 with approximate weights of 2500 g, length 63 cm) died ultimo November, primo December 1993 in the public aquarium (Danmarks Alvaturm) located in Charlottenlund, Denmark. Two of the fishes had been confiscated (July 1993) by the local authorities in Hong Kong and later transported to Danmarks Alvaturm. Here the fishes were fed sandeels and expressed high growth rates and showed no signs of morbidity. In October 1993 two of these fish were transferred to a 6000 litre aquarium containing six hatchery reared specimens of Arapaima gigas newly imported from Brazil. By the end of November all the fishes turned lethargic and succumbed within two weeks. The fishes were subjected to a pathological examination and found to be heavily infected with several hundreds of gill monogeneans per host. The gill tissue was severely affected by the infection showing extended necrotic areas, inflammatory reactions and exteriorized secondary lamellae. Parasite specimens were fixed in 10% formalin or 70% ethanol and later mounted in glycerine gelatin or ammonium picrate for identification.

The gill monogeneans recovered all belonged to the genus Dawestrema. According to the morphology and measurements exclusively the species D. cycloucancistrum was found. The two pairs of anchors with connecting bars, the body of the egg, the cirrus and the anterior end of the platylemnith are shown in Figure 1 A-D. Illegal capture and transportation of endangered species may be hazardous for various fish species. However, a new dimension adds to these problems. Mortalities due to monogenean infections of an endangered species as the osteoglossid Arapaima gigas could show problematic in hatcheries aiming at producing fry or fingerlings for restocking. Such aquaculture facilities have recently been established in Brazil in order to meet the high demands for this commercially as well as ecologically important fish.