**Aeromonas salmonicida** infected fish transfer disease to healthy fish via water

**Research Article**

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Received 23 January 2012; Accepted 25 May 2012

**Abstract:** Experimental studies of infection transmission via water from infected to healthy fish were conducted. The dark-brown bacterial colonies typical for *Aeromonas salmonicida* on tryptone soya agar (TSA) have been isolated and counted (from $3.0 \pm 0.6 \times 10^2$ to $3.5 \pm 0.5 \times 10^5$ c.f.u. g$^{-1}$) from the internal organs of naturally infected (NI) and experimentally infected (EI) perch and sea trout. No significant differences in dark-brown bacterial counts were detected between EI perch and EI sea trout. The assessment and comparison of the alterations of the biological parameters of EI European perch and sea trout with bacterium *Aeromonas salmonicida* subsp. *salmonicida* with naturally infected perch were conducted. No mortality was recorded in groups of EI perch and sea trout. Whereas, the mortality of NI perch (collected from the main sites of outbreak of disease) was observed from the second day of the experiments. Changes in morphophysiological parameters of EI perch and sea trout were similar. Different alterations in blood cell parameters of EI fish were observed, and the most noticeable was the decrease ($P \leq 0.01$) in white blood cell count (WBC) of EI perch and sea trout. Based on these results it can be deduced that there is infection transmission of bacterium *A. salmonicida* from European perch via water to other fish species.

**Keywords:** Aeromonas salmonicida subsp. salmonicida • Infection • Transfer • European perch • Sea trout

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1. Introduction

Mass mortalities in European perch (*Perca fluviatilis* L.) occurred in North Lithuanian Rivers (Lėvuo, Mūša and Nevėžis) in 2008 and reached a maximum in mid-autumn at a water temperature of 6°C. Pathogenic *Aeromonas salmonicida* subsp. *salmonicida* were identified as a possible causative agent of fish disease - septicemia, which usually resulted in high mortality rates [1]. The bacterial isolates were identified by sequence analysis of the 16S rRNA gene [1]. Whilst European perch (*Perca fluviatilis* L.) are commonly widespread in European water bodies, data available on this disease in wild perch are scarce. Epizootic of sub-acute furunculosis caused by *Aeromonas salmonicida* was described among perch in a drainage channel in 1972 [2]. A similar accident was followed by high mortality of perch as recorded in West Ukraine, Dnestr River, and Dnestr water reservoir in October-November 2010 (Khudyi O., personal communications). Outbreaks of disease in natural water bodies might cause severe declines in wild fish populations; however, main triggers inducing the disease outbreak and the high mortality remain unknown.

The present investigation of the experimental infection is a continuation of our previous study [1], and referred to the transmission and impact of infection with *Aeromonas salmonicida* subsp. *salmonicida* of European perch and sea trout. Infection transmission to healthy perch and sea trout in this study was conducted via water from infected perch collected in the known main site of disease outbreak. Numerous experimental studies have examined the transmission of the *A. salmonicida* via water by bath immersion [3], or intraperitoneal and intramuscular injections at different bacterial doses of different farm-reared fish [4-7]. However, until this study, there were no references to infection transmission via water from infected wild fish to healthy fish of different species. Laboratory-controlled investigation of the transmission of infection from wild fish should provide new information towards the
validation of disease occurrence in wild and farm-reared fish and the general health condition of infected fish of different species.

Gills, skin, and wounds are likely to be the main routes of entry for *A. salmonicida* [6,8]. It has been shown that particulate bacterial and viral agents are readily absorbed via the gills [9]. Bacteria can also enter the host by the fins [10], and can cross the gastrointestinal lining in three different ways. In undamaged tissue, bacteria can translocate by transcellular or paracellular routes [11]. The presence of infection can be diagnosed via changes in the morphophysiological, physiological, microbiological parameters of the fish. Quantitative aspects such as survival, growth, weight-length relationship, condition factor, and tissue-somatic indices of fishes are important tools in the study of fish biology [12]. The condition factor reflects information on the physiological status of fish [13]. Haematological parameters (red and white blood indices) and microbiological (bacterial colonies count) endpoints are indicative in diagnosing and evaluating the functional status of fish [14,15]. They are considered to be a reliable approach in the assessment of the affect of pathogens on fish [9].

The aim of the present study was to investigate experimentally if *Aeromonas salmonicida* infections can be transferred via water from infected European perch to wild healthy perch and to healthy fish of different species (sea trout); to evaluate the alterations in the biological (microbiological, morphophysiological, physiological) parameters of experimentally infected perch and sea trout, and to compare with the same indices of healthy perch and sea trout and of naturally infected perch.

### 2. Experimental Procedures

#### 2.1 Fish sampling

Live perch (*Perca fluviatilis* L.) (naturally infected – NI group) were collected from the Nevėžis River (the main site of disease outbreak and of the high mortality of the perch), and from the Savenes River outflowing from Kazimieravos Lake, where no mortality of perch was recorded (healthy, control fish group). Fish were caught using gill nets with a mesh size 12 mm and transported in aerated tanks to the Institute of Ecology of Nature Research Centre (Vilnius) and kept alive in aerated tanks to the Institute of Ecology of Nature Research Centre (Vilnius) and kept alive in aerated tanks to the Institute of Ecology of Nature Research Centre (Vilnius) and kept alive in aerated tanks to the Institute of Ecology of Nature Research Centre (Vilnius) and kept alive in aerated tanks to the Institute of Ecology of Nature Research Centre (Vilnius) and kept alive in aerated tanks to the Institute of Ecology of Nature Research Centre (Vilnius) until examination. Sea trout (*Salmo trutta trutta*) were reared in laboratory and kept separately in aquarium. Sea trout had no history of *A. salmonicida* infection and *A. salmonicida* bacteria were not found in liver and kidney of healthy, control perch. No pollutants in the water of Nevėžis and Savenes Rivers were determined (Report of Hydrometeorology Service of Lithuania, unpublished data, 2008). No parasitic and viral infections were detected in experimental fish (Report of Lithuanian National Food and Veterinary Risk Assessment Institute, http://www.Balsas.lt/naujiena/222156).

All tests were performed using artesian water of high quality. Average hardness of water was ~284 mg/l as CaCO$_3$, alkalinity was 244 mg/l as HCO$_3^-$, mean pH was 8.0, temperature was ~6–8°C, and oxygen concentration ranged from 8 to 10 mg/l.

The approval of the animal use in the experiments was given by the Ministry of Environment of Lithuania 2009-04-10 No 9F09-21.

#### 2.2 Experimental design

Experimental procedures are shown schematically (Figure 1). After the acclimation period experimental fish (EI) were transferred from holding tanks and placed into 80-l constantly aerated aquaria. The first group of healthy perch were placed into two aquaria (aquarium 1, 2), which served as controls (N=20). Infection assays were conducted using the second group of control perch placed into two other aquaria (aquarium 3, 4) (N=14) (experimentally infected – EI) to which the group of marked perch collected from the main site of disease outbreak (naturally infected – NI) (N=14) were transferred. The third group of fish (two aquaria – 5, 6) consisted of the perch collected from the main site of disease outbreak (N=20). The fourth group – healthy sea trout was kept in two aquaria (aquarium 7, 8) as controls (N=20). The fifth group of control sea trout was also placed into two aquaria (experimentally infected – EI) (N=14) and infected perch (naturally infected – NI) (aquarium 9, 10) were gently transferred to these aquaria. Fish were fed a dry commercial feed (DANA-FEED, Denmark) daily. All fish were sampled during days 5–8 taking both control and infected fish on each day.

#### 2.3 Morphological, morphophysiological and haematological examination

The physiological state of the fish was assessed by use of biological parameters. Morphological parameters studied were: mean body weight (Q) 29.62±2.0 g and mean length (L) 14.5±0.3 cm of control, 29.5±2.2 g and 14.2±0.4 cm of NI, 32.8±3.0 g and 14.3±0.4 cm of EI (M±SEM) of perch; 11.0±2.6 g and 10.8±0.2 cm of control, 12.6±2.0 g and 10.2±0.4 cm EI (M±SEM) of sea trout. Weight of spleen, gills, liver, heart (g), and weight of fish without viscera (g) were measured. Morphophysiological parameters evaluated including the: condition factor (CF) and tissue-somatic index (TSI). CF was calculated using the formula [13]:

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\text{Condition factor} = \left[ \frac{\text{the weight of the fish (g) x the length of fish (cm)}}{100} \right]
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