

# Hydrogen peroxide as pre-treatment stressor in experimental immersion challenge of rainbow trout (*Oncorhynchus mykiss*) fry with *Flavobacterium psychrophilum*

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## Abstract

*Flavobacterium psychrophilum* is the causative agent of rainbow trout fry syndrome (RTFS), also called bacterial cold water disease (BCWD) in USA. Since being discovered in USA in the 1940s, the disease has spread to aquacultures worldwide [1]. Mortality rates as high as 90% have been reported in fry, although the mortality rate in Danish outbreaks average around 30–40%. Rainbow trout (*Oncorhynchus mykiss*) is the major species in Danish aquaculture and various rainbow trout related products, including eyed eggs and roe for export, amount to 40 % of the Danish fishery sector. This is equivalent to about 900 million DKK, making rainbow trout fry health an important issue [2].

Presently no commercial vaccines are available and followingly RTFS is controlled with antibiotics. Because the *F. psychrophilum* is most serious in fry, an immersion vaccine and a model for testing it is very desirable.

Many approaches to experimental challenges have been carried out. Although injection-based experimental challenges are reproducible this approach is unsuitable for some types of investigations, since the mechanical barriers of the fish are bypassed in the delivery of the pathogen. Furthermore, the many difficulties producing mortality in immersion and co-habitation challenge models have highlighted the limited pathogenicity of *F. psychrophilum* without application of stress or scarification [3]. In addition, experiments, which were successful in producing mortality, have been hard to reproduce [4].

Various chemicals are used in aquaculture, including formalin, which has been used as a pre-treatment stressor ahead of immersion challenge resulting in a mortality rate over 50% [5]. Formalin is to be phased out of Danish aquaculture by 2014 due to human health risks and hydrogen peroxide has been proposed as a possible substitute and has already been used for several purposes in Denmark for a number of years. In two studies regarding *Tenacibaculum maritimum* and *Flavobacterium columnare* infections in respectively turbot (*Scophthalmus maximus*) and channel catfish (*Ictalurus punctatus*), hydrogen peroxide was shown to accelerate the diseases [6,7], making it an evident candidate for a stressor. Furthermore, several studies uncovering the adverse effects of hydrogen peroxide in rainbow trout have been published over the past 15 years [8–13]. Varying recommendations for the prophylactic use of hydrogen peroxide against external pathogens are given in the literature, since many aspects must be taken into consideration. These include the size of the fish, starting concentration of hydrogen peroxide, duration of treatment and the water temperature while treating.

The aim of this study was to establish a reproducible method for immersion challenge of rainbow trout fry to be used in investigations concerning immune response and vaccine testing. First, various concentrations of H<sub>2</sub>O<sub>2</sub> were tested before being combined with immersion exposure to the virulent *F. psychrophilum* isolate 950106-1/1, which was used for all infections.

In conclusion, pre-treatment with H<sub>2</sub>O<sub>2</sub> elevated the cumulative mortality following immersion challenge with *F. psychrophilum*, although variation between replicates was very high. The results from the experiments are still being processed and are consequently not included.

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