Practical measurements to combat *Flavobacterium psychrophilum* on a rainbow trout farm

Stephan Hofer

Hofer Forellen GmbH, Stuttgarterstrasse 66, 78727 Oberndorf, Germany

Correspondence: info@hofer-forellen.de

Abstract

Flavobacterium psycrophilum infections are a major cause for severe problems in Rainbow trout fry production (*Oncorhynchus mykiss*). Treatment with antibiotics is not satisfactory to control the problem. Management and husbandry steps can help to reduce the problem. Measures include broodstock rearing, egg disinfection, site and material design, cleaning and disinfection, surface disinfection procedures during fry rearing, immunostimulants, as well as staff training and management. Nevertheless trout farmers need further assistance to combat the problem. Vaccination, the use of bacteriophages or improvements in fry nutrition might be the solution to the problem.

This paper is a report on experiences gained on an operational trout farm. It is not based on specific experiments.

Hofer Forellen GmbH has a long trout farming history since 1896. All our trout production is from own broodstock. The two farming sites are classified as category 1 sites according to the European fish health directive (88/2006(EC)). In addition the sites are free of other infectious diseases like IPN Virus or *Yesinia ruckeri*. Nevertheless we have a long history of problems with *Flavobacterium psychrophilum* [1] causing RTFS. At the beginning we did experience elevated mortalities in 1 to 2 g fry. The fish could easily be treated with antibiotics. Usually no more mortality occurred in fish larger than 2 g.

Over the following 10 years the problem became increasingly worse. First outbreaks occurred at first feeding, multiple outbreaks occurred, trout up to 50 g were affected and resistance to antibiotics emerged. When comparing our two sites with broodstock, we did realise that the impact of *F. psychrophilum* was worse at the site where the hatchery water has to be re-used in the broodstock tanks after leaving the hatchery. At the other broodstock and hatchery site there is enough spring water to keep the water flow between the broodstock tanks and the hatchery separate, resulting in reduced RTFS outbreaks in fry.

After seeking external advice we have taken several measures to reduce the impact of *F. psychrophilum*:

a. Equipment disinfection

The hatchery is cleaned with an alkaline detergent followed by disinfection with a peroxide based disinfectant followed by a two week drying period between batches. Everything which can be

removed is put into a heat chamber at 60°C for at least 2 hours. Those measures were only really successful after the concrete tanks in the hatchery were coated with polyurea in order to close all pores of the concrete. Before coating the chemical disinfection was probably not capable to reach all pathogens attached to the concrete.

b. Egg disinfection

The eggs are disinfected at incubation time before entering the hatchery according to the following protocol. The disinfection method was adapted form methods published in [2], but iodine was replaced with hydrogen peroxide, since we believe that iodine is not efficient for the elimination of *F. psychrophilum.*

1) rinsing eggs in isotonic water
2) fertilisation with artificial activation solution (5 min)
3) rinsing with isotonic water + 1 ml 40% H2O2 / l
4) disinfection in isotonic water + 1 ml 40% H2O2 / l
5) rinsing, hardening and incubation in normal spring water

Just before hatching the eggs are disinfected a second time according to the following procedure:

1) very precise egg picking
2) disinfection in spring water + 1 ml H2O2 /l (15 min)
3) transfer eggs to the cleaned and disinfected hatchery building

c. Good hygiene practice

The work at the farm is organised in a manner that hatchery work is done at the beginning of the working day before doing any other work on the trout farm. The hatchery is only entered with special clothing and boots for the hatchery. Hands are being disinfected with alcohol before entering the building and disposable gloves are worn. In addition the number of people working in the hatchery is reduced to a minimum.

d. Water and tank disinfection when the hatchery is in use

Because antibiotic treatments have to be repeated we do suspect that biofilms of pathogens act as a reservoir for infection [3]. This hypothesis was supported when the impact of *F. psychrophilum* was reduced after coating the concrete surfaces with polyurea plastic. Daily flushing of the tanks with peracetic and peroctanoic acid (Incimaxx Aquatic, Ecolab) did reduce the deposits on the tank surface. We did try continuous ozonation since this was successful in minnow culture [4] of the hatchery water with some little residue of ozone in the hatchery tanks, but with no success.

e. Promote immunisation

Addition of immunostimulants like Ergosan (Intervet) does appear to have a beneficial effect. Nevertheless it is difficult to control the effect of imunostimulants on a fish farm since all feed manufacturers claim to add such substances to their feed. This makes it difficult to control the effect. Prophylactic antibiotic treatment before signs of clinical disease appear is of no use to

prevent outbreaks of clinical disease. It appears that the fish need to be exposed to the pathogen in order to be able to produce immunity. This gives hope to combat the problem by vaccination. We have tried a commercial vaccine (Pharmaq) and believe to have had a weak beneficial effect.

Since the introduction of the control measures we reduced the incidents of RTFS outbreaks. In consequence we have reduced the use of antibiotics. We have managed to produce trout with no use of antibiotics at all. Nevertheless there is a high risk the trout will get ill when they are transferred to the ongrowing sites.

Even though we have introduced several measures at the same time, making it impossible to validate the effect of each individual measure, we believe the following measures have a positive effect to protect trout from *F. psychrophilum* infection:

- Egg disinfection with hydrogen peroxide in order to reduce transmission from parent to fry
- Cleaning and disinfection between batches with oxidising disinfectants or heat
- Smooth surfaces instead of concrete
- Separate water supplies and outflows for broodstock and hatchery
- Improved staff organisation and workforce hygiene

With a lower degree of certainty we do believe the following measures have a positive effect, too:

- Water and tank surface disinfection during operation time of the hatchery
- Use of immunostimulants in the feed
- Vaccination

Egg disinfection with iodine is not efficient to prevent transmission of *F. psychrophilum*. Poor rearing conditions for broodstock make the resulting fry more susceptible for *F. psychrophilum* infection. Re-using hatchery water to rear broodstock has a negative effect, too. Continuous ozonation of hatchery water does not appear to prevent *F. psychrophilium* infection. Maybe continuous exposure to ozone does cause permanent stress or irritation to the gills simplifying the rout of entry for infection. Nevertheless repeated treatments with antibiotics are not satisfactory, too due to the risk of resistance and reduced performance of the fish.

From a fish farmers point of view it remains an open question whether there is true vertical transmission of *F. psychrophilum*. The answer to this question is important for determining the best control strategy. Does it make sense to vaccinate or treat broodfish with antibiotics prior to stripping? The importance of milt as a rout of infection during fertilisation is not clear. Trout farmers have an urgent need for treatments other than antibiotic therapy, such as vaccination or the use of baceriophages.

Acknowledgements

I would like to thank OECD for their support. We have already benefited from the participation at the conference.

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