

# Cold Water Strawberry Disease of rainbow trout (*Oncorhynchus mykiss* Walbaum)

## Introduction

Cold Water Strawberry Disease (CWSD), otherwise known as Red Mark Syndrome (RMS), is a disease of rainbow trout, characterised by the appearance of multiple ulcerated skin swellings, of varying intensity, on the flanks of affected fish (Ferguson *et al.* 2006; Verner-Jeffreys *et al.*, 2006). The condition was first noted in Scotland. By 2005, it was found on 12 sites, all of which were sites receiving live fish from the hatchery. In early 2005, the condition was diagnosed for the first time in fish farmed in England. Farmers in both countries report that the disease is prevalent at low temperatures (less than 15°C). Early signs can include scale loss, prior to the emergence of the characteristic external lesions, and there are no systemic signs of infection (i.e. no effect on appetite, growth or mortality). The condition causes severe economic losses to farmers both in treatment costs and in that affected fish are down-graded at harvest. The results of investigations of affected farms in England and Wales and subsequent multidisciplinary studies to determine an aetiological agent are reported. As a recent study has implicated the rainbow trout bacterial pathogen *Flavobacterium psychrophilum* as potentially being linked to the condition (Ferguson *et al.* 2006), particular effort was made to identify whether this, or a closely related organism, was associated with diseased fish.



Figure 1

## Materials and Methods

### Outbreak investigations

Epidemiological outbreak investigations of farms in England and Wales, suffering from CWSD, were carried out. Two of the farms investigated, Farm A and Farm B, were also used as a source of fish for transmission experiments. At each visit, 10 fish with CWSD were examined and samples taken for diagnostic testing (bacteriology, mycology, virology, histopathology, parasitology, biochemistry). Farm records and fish movements were also discussed with the fish farmer.

### Transmission trials

Cohabitation trials were carried out to investigate whether CWSD has an infectious aetiology. Indirect: 15 CWSD-affected fish were held on one side of a mesh screen with 40 naïve fish on the other. Direct: naïve fish in direct contact with CWSD-affected fish. The effect of water quality was examined by using a surface-draining standpipe to allow accumulation of feed and faeces.

### Bacteriology

Representative colonies of dominant bacteria, isolated on solid media from affected fish, were identified by a combination of phenotypic testing methods, and 16S rRNA sequence analysis (Pond *et al.* 2006). All colonies resembling *Flavobacterium* sp. (orange-yellow pigmented colonies), along with DNA extracted from a selection of skin/muscle tissue samples, were subjected to nested *F. psychrophilum* PCR (Ferguson *et al.* 2006). Partial 16S rRNA genes were directly amplified from DNA templates prepared from lesion and negative control skin samples from the cohabitation trial, cloned into *E. coli* and analysed, as previously described (Pond *et al.* 2006).

### Virology

Samples from skin, or kidney, brain and spleen were inoculated onto fat head minnow (FHM), bluegill fin (BF), epithelial papilloma carp (EPC), chinook salmon embryo (CHSE), salmon head kidney (SHK), rainbow trout gonad (RTG) and tilapia ovary (TO) cell lines, under a range of incubation conditions, and checked for cytopathic effect.

## Results

### Outbreak investigations

- The initial spread of CWSD was associated with movement of live fish from Scotland to sites in England (including Farm A).
- The condition subsequently spread to other production sites linked to the initially infected farms.
- Within a farm, spread from affected stocks to previously unaffected naïve fish was demonstrated.
- There was another outbreak of CWSD in a farm in Wales (Farm B) in 2005 with likely spread to other farms supplied by Farm B.
- Prevalence was variable (between 5-70% of fish in a stock affected).
- Farmers report that treatment of affected fish with antibiotics controls the development of the disease, which may indicate a bacterial aetiology.



Figure 2. Lesions on fish exposed to Farm B CWSD affected fish at termination of infection trial.

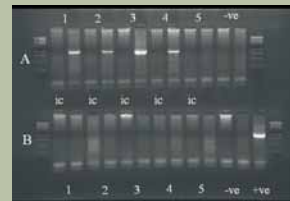


Figure 3. *F. psychrophilum* PCR of lesion material from 5 Farm A and 5 Farm B rainbow trout used in the cohabitation trials. ic = internal control sample (unaffected area of skin and muscle); -ve = skin and muscle from a control population of unaffected rainbow trout; +ve = *F. psychrophilum* NCIMB 1947; 100bp ladder.

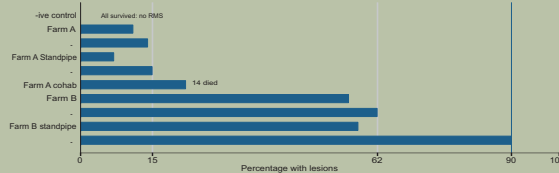


Figure 4. Cohabitation trials: Farm A or Farm B fish as the cohabitant source, with or without a standpipe; Farm A cohabs, direct cohabitation challenge; -ive control, 40 naïve stock fish only. Fish were held at 10°C in freshwater and examined for the presence of lesions after 110 days.

### Diagnostic bacteriology, virology and mycology of field samples and laboratory infected material

- No bacterial agent consistently isolated.
- No fungal agents isolated.
- Lesion samples from trial infected fish and cultured bacteria not positive for *F. psychrophilum* by PCR.
- Lesion samples from 4/5 Farm A infected fish, used as infection source in trial, tested positive for *F. psychrophilum*.
- Lesion samples from 0/5 Farm B infected fish, used as infection source in trial, tested negative for *F. psychrophilum*.
- No virus cultured.

### Culture independent analysis of cohabitation trial lesion material for 16S rRNA genes of bacterial origin

- Over 300 cloned partial 16S rRNA genes were analysed, from which at least 8 distinct phylotypes were recovered:
  - Alpha proteobacterium-like, *Methylobacterium*-like, *Bradyrhizobium* sp., *Sphingobium yanoikuyae*-like, Beta proteobacterium-like, *Acidovorax*-like, *Pseudomonas fluorescens*-like and *Bacillus* sp.
- All the main phylotypes were also recovered from negative control material.

## Histology

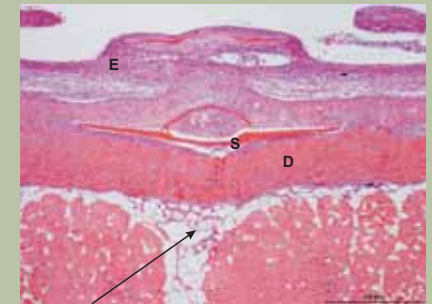


Figure 5. Typical transverse section through epidermis (E) and dermis (D) of a moderate CWSD lesion. Note lymphocytic infiltration into the connective tissue of the dermis and extending into the underlying adipose and muscle tissue (arrow). S = scale.

- Epidermis generally unaffected, possibly some lymphocytic infiltration.
- Lifting and dissolution of scales with osteoclast involvement.
- Predominantly lymphocytic inflammation, focal to multifocal lesions, initially in the connective tissue of the dermis and extending into the underlying adipose and muscle tissue.

## Conclusions

- CWSD was apparently transmitted from affected to naïve fish in the field and in the laboratory, demonstrating it has an infectious aetiology.
- CWSD has a long latency: greater than 50 days when fish are held at 10°C.
- Differences in the rate of transmission of condition between fish in contact with Farm A as opposed to Farm B were observed. It is not clear whether this was related to differences in virulence of the responsible agent or due to the interfering effects of observed concurrent infections in the Farm A-affected fish.
- Environmental factors (water quality) did not noticeably affect transmission rate in the laboratory.
- F. psychrophilum* DNA was not consistently isolated from field or laboratory infected fish, lessening the likelihood that it, or a closely related organism is the responsible aetiological agent.

## Further work required

- Risk to other farmed and wild fish needs to be established.
- Aetiological agent needs to be identified.
- Prevalence and spread of disease needs to be better understood.
- Suitable control methods need to be identified (as an alternative to continued application of antibiotics).
- Case definition needs to be established.

## Acknowledgments

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## References

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