

# Emerging skin disorders of rainbow trout in the UK: Puffy skin transmission trials establishment

Cano I, Verner-Jeffreys D, van Aerle R, Rimmer G, Paley R, Feist SW

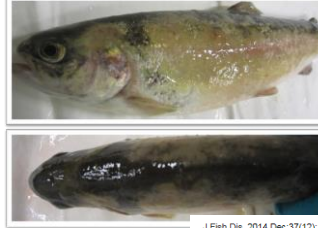
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## Introduction



- > In 2002 a new skin condition in farmed rainbow trout in England: puffy skin disease (PSD)
- > The disease persisted on affected farms
- > Occurrence of new cases increased from 2006
- > 2012 diagnosed in rainbow trout fisheries
- > Not observed in brown trout

J Fish Dis. 2014 Dec;37(12):1021-9. doi: 10.1111/jfd.12241. Epub 2014 Apr 10.  
**Acute dermatitis in farmed trout: an emerging disease.**  
Peeler EJ<sup>1</sup>, Ryder D, Thrush MA, Mewett J, Hulland J, Feist SW

J Fish Dis. 2014 Nov 14. doi: 10.1111/jfd.12306. [Epub ahead of print]  
**Puffy skin disease (PSD) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a case definition.**  
Maddocks CE<sup>1</sup>, Nolan ET, Feist SW, Crumlish M, Richards RH, Williams CF  
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## Aim

To test if PSD has an infectious aetiology

## Objectives

- > To study PS transmission to naive fish by direct cohabitation with PS affected fish
  - > To isolate and characterize the PS agent



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## Cohabitation challenge design



- > Fish held at 15 °C for 49 days after initiation of the direct cohabitation challenge in 300 L tanks.
- > Weight fish 300 - 500g
- > Ratio Naive-Trojans 2:1 (first trial)  
3:1 (second trial)
- > Naive fish VIE tagged



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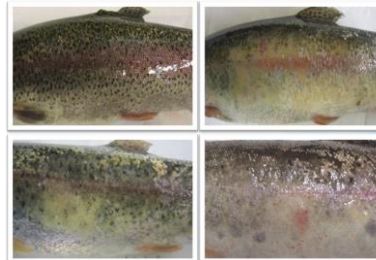
## Experimental design of transmission experiments.

Trial	Time	Source of naive fish	Source of cohabitant (Trojan) fish	Experimental design	Sampling
1 (proof of concept)	Winter 2013	Fish reared in house from eggs (diploid)	Site A	Single tank <ul style="list-style-type: none"> <li>• 15 fish from site A mixed with 20 naive fish in a single tank.</li> <li>• No negative control group.</li> </ul>	Trojan fish sampled prior to start of challenge. Naive fish sampled at 14, 23, 28 and 41 dpc
2	Summer 2014	Site C. No history of PSD on farm. Fish held for weeks at CWL prior to start of experiment	Site A and Site B	Three tanks. <ul style="list-style-type: none"> <li>• Tank 1: 39 naive fish from site C mixed with x fish from Site A.</li> <li>• Tank 2: 39 naive fish from site C mixed with x fish from Site B.</li> <li>• Tank 3: negative control group, fish from site C held for the duration of the experiment</li> </ul>	Trojan fish sampled prior to start of challenge. Naive fish sampled at 14, 21, 35, 49 dpc

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## 1<sup>st</sup> transmission trial- gross pathology

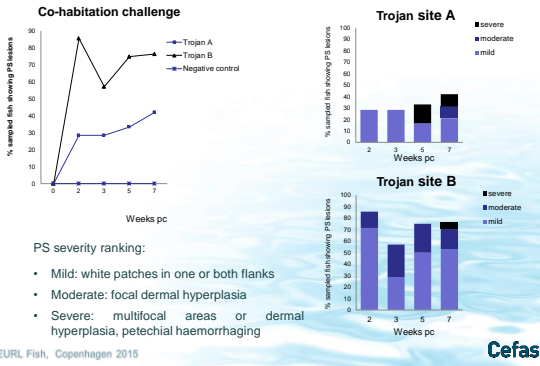


- Severe mucus production observed after 10 days pc
- Irregular swimming - flashing - observed during the challenge
- Some raised scales and loss
- Development of white patches on flanks after 14 days pc, diffuse petechial haemorrhaging in severe lesions
- Skin oedematous and spongiosis appearance observed after 23 days pc, skin erosion

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## 2<sup>nd</sup> transmission trial- gross pathology



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## Sample analysis: skin scrapes



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## Histopathological changes observed naïve fish sampled during first transmission experiment

Changes	19	23	28	41
<b>Raised scales</b>	NAD 3/3	2/3	4/4	1/10
<b>Scale pocket oedema</b>	NAD 3/3	NAD 3/3	NAD 3/3	8/10
<b>Epidermis oedema, dermatitis</b>	NAD 3/3	1/3	1/4	4/10
<b>Epithelial erosion, sloughing</b>	NAD 3/3	2/3	4/4	8/10
<b>Heart epicarditis</b>	NAD 3/3	NAD 3/3	NAD 3/3	3/10
<b>Peritonitis</b>	NAD 3/3	1/3	NAD 3/3	NAD 3/3
<b>Inflammation</b>	NAD 3/3	1/3	NAD 3/3	NAD 3/3
<b>Others</b>	NAD 3/3	NAD 3/3	NAD 3/3	Renal Haemorrhaging and focal necrosis 1/10 Spleen focal haemorrhaging, inflammation and some cellular necrosis 2/10

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## 2<sup>nd</sup> challenge- Histology

Skin	NAD	Mild scales and raised scales	Epithelial erosion and sloughing	Stomatitis	Epithelial oedema	Subcutaneous oedema	Inflammation with infiltrating cells	Focal dermatitis	Dermis (haemorrhaging)	Necrosis	Epithelial hyperplasia	Rodlet Cells	Scale hyperplasia	PMS	Ichthyobodo necator	Ichthyophthirius multifiliis							
																	Negative group	Naive fish at arrival	Control fish at 2 wpc	Control fish at 3 wpc	Control fish at 7 wpc	Naive fish cohob Trojan A	2 wpc
[Detailed histology data table with color-coded cells]																							

## 2<sup>nd</sup> challenge- Histology

Gill	NAD	Epithelium lifting	Proliferation Leucocytes	Proliferation eosin	Luminal bacteria	Epithelium necrosis	Oedema	Hyperplasia	Necrosis	Inflammation (Intraepithelial)	LUMN	Ichthyobodo necator	Ichthyophthirius multifiliis											
														Negative group	Naive fish at arrival	Control fish at 2 wpc	Control fish at 3 wpc	Control fish at 7 wpc	Naive fish cohob Trojan A	2 wpc	3 wpc	5 wpc	7 wpc	Naive fish cohob Trojan B
[Detailed histology data table with color-coded cells]																								

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## 2<sup>nd</sup> challenge- Histology internal organs: NAD

Intestine	NAD	Mild	Moderate	Severe	Necrosis	PMS	NAD	Mild	Moderate	Severe	Necrosis	PMS	Heart	NAD	Mild	Moderate	Severe							
																		Negative group	Naive fish at arrival	Control fish at 2 wpc	Control fish at 3 wpc	Control fish at 7 wpc	Naive fish cohob Trojan A	2 wpc
[Detailed histology data table with color-coded cells]																								

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## Metagenomics sequencing of Puffy Skin condition

1 <sup>st</sup> trial	Fish group	PS symptoms	DNA	RNA	rRNA depleted RNA
	Naive no. 11 <sup>1</sup>	severe			x
	Naive no. 11 <sup>1</sup>	non affected			x
	Naive no. 15 <sup>1</sup>	severe			x
	Naive no. 15 <sup>1</sup>	non-affected			x
	Naive no. 25	severe	x	x	
	TA <sup>2</sup>	severe	x	x	
	TA	severe	x	x	
2 <sup>nd</sup> trial					
	Naive no. 27	Negative control	x	x	
	Naive no. 28	Negative control	x	x	
	Naive no. 40 cohah TA	mild	x	x	
	Naive no. 42 cohah TB <sup>3</sup>	mild	x	x	
	TB no. 71	severe	x	x	
	TA no. 79	severe	x	x	
	Naive no. 85 cohah TA	severe	x	x	
	Naive no. 92 cohah TB	severe	x	x	
	Naive no. 98 cohah TB	moderate	x	x	
	Naive no. 148 cohah TA	severe	x	x	

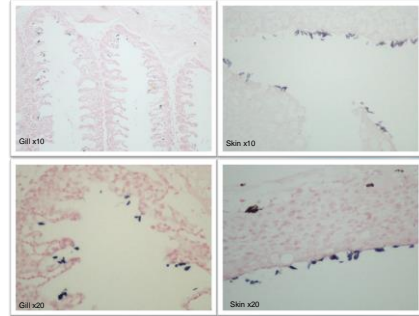
### Human Micro platform

From the first trial, a total of 42,201,893 sequence reads were produced and analysed. Assembled transcripts derived from skin tissue samples were annotated using Metaxa and resulted in the identification of several different species, including a number of fish pathogens including *Saprolegnia parasitica*, *I. multifiliis*, *Ophryotrocha carolinis*, and *I. necator*.

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## ISH *Ichthyobodo necator* in puffy skin fish



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## PSD is a transmissible disease

To develop and recommend effective control strategies to minimise the impact of this emerging condition in UK farmed rainbow trout

It is transmitted only by a direct contact between fish?

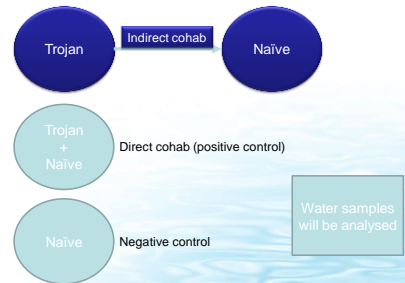
Can it be transmitted by water flow?

3<sup>rd</sup> challenge: Indirect cohabitation challenge

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## 3<sup>rd</sup> infection trial: indirect cohabitation challenge



Planned for June-July 2015

Results for the EAFF conference!

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## Acknowledgments

Microbiology team  
Aquatic Pests and Pathogens (APP) group

Histology lab

EF

Cell culture lab

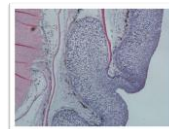
Defra project FB002A



Department  
for Environment  
Food & Rural Affairs

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Thanks for your attention



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