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Differential characterization of emerging skin diseases of rainbow trout – a standardized approach to capturing disease characteristics and development of case definitions

B Oidtmann[1], S E LaPatra[2,3], D Verner-Jeffreys[1], M Pond[1], E J Peeler[1], P A Noguera[4], D W Bruno[4], S St-Hilaire[5], C B Schubiger[2], K Snekvik[2], M Crumlish[6], D M Green[6], M Metselaar[6], H Rodger[7], H Schmidt-Posthaus[8], M Galeotti[9] and S W Feist[1]

1 Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, UK
2 Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA
3 Research Division, Clear Springs Foods Inc., Buhl, ID, USA
4 Marine Scotland Science, Aberdeen, UK
5 University of Prince Edward Island, Charlottetown, PE, Canada
6 Institute of Aquaculture, University of Stirling, Stirling, UK
7 Unit 7B, Vet-Aqua International, Oranmore, Co. Galway, Ireland
8 Centre for Fish and Wildlife Health, Institute of Animal Pathology, University of Bern, Bern, Switzerland
9 Department of Food Science, Faculty of Veterinary Medicine, Section Veterinary Pathology, University of Udine, Udine, Italy

Abstract

Farmed and wild salmonids are affected by a variety of skin conditions, some of which have significant economic and welfare implications. In many cases, the causes are not well understood, and one example is cold water strawberry disease of rainbow trout, also called red mark syndrome, which has been recorded in the UK since 2003. To date, there are no internationally agreed methods for describing these conditions, which has caused confusion for farmers and health professionals, who are often unclear as to whether they are dealing with a new or a previously described condition. This has resulted, inevitably, in delays to both accurate diagnosis and effective treatment regimes. Here, we provide a standardized methodology for the description of skin conditions of rainbow trout of uncertain aetiology. We demonstrate how the approach can be used to develop case definitions, using coldwater strawberry disease as an example.

Keywords: cold water strawberry disease, rainbow trout, red mark syndrome, skin diseases, US rash, US strawberry disease.

Introduction

There are several well-known disease conditions affecting the skin of salmonids and rainbow trout, (*Oncorhynchus mykiss* (Walbaum), in particular. These include furunculosis (*Aeromonas salmonicida*), columnaris disease (*Flavobacterium columnare*), bacterial coldwater disease (*Flavobacterium psychrophilum*) and saprolegniosis (*Saprolegnia* spp.). In these examples, other organs and tissues may also become infected, whilst infections with various ectoparasites, such as *Ichthyophthirius multifiliis* and *Ichthyobodo necator* predominantly affect the skin and gill epithelia. Skin conditions continue to emerge in farmed rainbow trout, and for several of these, a specific pathogen or

Correspondence *B Oidtmann, Centre for Environment, Fisheries & Aquaculture Science, Weymouth Laboratory, Barrack Road, Weymouth, Dorset DT4 8UB, UK (e-mail: birgit.oidtmann@cefas.co.uk)*

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aetiology has not been established. These include European warm water strawberry disease (WWSD), red mark syndrome (RMS) – in the UK also known as cold water strawberry disease (CWSD) (Ferguson *et al.* 2006; Verner-Jeffreys *et al.* 2008) – and a number of conditions found in North America, such as US strawberry disease, 'US rash', 'fungal berry' and 'cherry fin' (LaPatra *et al.* 1994; Bruno *et al.* 2007).

RMS first emerged in Great Britain in the winter of 2003/2004 (Verner-Jeffreys *et al.* 2006, 2008). The transmissible nature strongly suggests that a pathogenic agent is involved (Verner-Jeffreys *et al.* 2008). Both *Flavobacterium psychrophilum* and *Rickettsia*-like organisms (RLO) have been suggested as contributing to this condition; however, there is no conclusive evidence that either of these are aetiological agent(s) (Ferguson *et al.* 2006; Verner-Jeffreys *et al.* 2008; Metselaar *et al.* 2010). In North America, the aetiology of a condition similar to RMS, called US strawberry disease, is also unresolved, but a study by Lloyd *et al.* (2008) suggested the possible involvement of a *Rickettsia*-like organism.

These skin conditions can have a significant impact on the trout farming industry. For instance, the number of farms affected by RMS in the UK has risen from <5 in the winter of 2003/2004, to more than 80 farms in 2009 (Robert Hughes, pers. comm.). In terms of annual production, the condition affects more than 50% of the UK rainbow trout industry (Robert Hughes, pers. comm.). RMS causes substantial economical damage, because it affects rainbow trout as they approach market size, with rejection rates of up to 30% reported (B. Oidtmann, K. Adam, P. Noguera, J. Mewett, E. Peeler, M. Thrush & R.A. Reese, unpubl. data). The spread within the UK industry has been rapid, and efficient methods to manage the condition have yet to be developed. More recently, RMS has been identified in rainbow trout aquaculture in other European countries (Schmidt-Posthaus *et al.* 2009; Galeotti *et al.* 2011). Uncertainty regarding the nature of the aetiological agent makes diagnosis of the condition difficult, and therefore, obtaining reliable data on the epidemiology of the disease and investigating potential control methods remain challenging. There is consequently an urgent need to establish how RMS is transmitted and to determine what risk factors are associated with the condition.

When surveillance for a disease or syndrome or studies investigating risk factors of a disease are undertaken, a case definition is required. A case definition is a set of criteria used to classify an animal or epidemiological unit as a case of a disease (OIE 2009b). They are used for presumptive diagnosis in the field and confirmatory diagnosis, following laboratory examination. Case definitions are widely used in terrestrial animal health surveillance and have recently been added to the OIE diagnostic manuals for aquatic animal diseases (OIE 2009a). In diseases of known aetiology, demonstration of the aetiological agent is usually part of the confirmatory diagnosis. However, where the aetiological agent is unknown, or the disease is not caused by a pathogen, alternative case definitions can be developed. In terrestrial animals, case definitions have been used to establish the prevalence of certain conditions (such as obesity, respiratory disease or dystocia) in a given population (e.g. all dogs treated by a given set of small animal practices) (Mee 2008; Assié *et al.* 2009; Bland *et al.* 2009). Epidemiological studies undertaken to investigate the aetiology of bovine spongiform encephalopathy (BSE) used a case definition for clinical presentation (presumptive diagnosis) and histopathology (confirmatory diagnosis) (Wilesmith 1993). In aquatic animals, a case definition (based on pathology and haematology) was developed to investigate risk factors for the spread of infectious salmon anaemia (ISA), before the aetiological agent had been confirmed (Jarp & Karlsen 1997).

In this article, we outline a standardized methodology for describing skin conditions. Using this standardized approach, four similar skin conditions (RMS, WWSD, US rash and US strawberry disease) are described and compared. Based on the results of the comparison, we develop case definitions for field and laboratory examinations for RMS.

Descriptive methodology

A panel of generalized descriptors was developed to capture characteristics of the different skin diseases that are described in this study:

Epidemiology: Including (i) aetiology; (ii) host characteristics (i.e. species, size, age); (iii) whether the condition is known to be transmissible; (iv) its incubation period; (v) its prevalence (between ponds and within ponds of a farm); (vi) risk factors; (vii) geographic

distribution; (viii) environmental conditions under which the disease occurs.

Clinical signs: Including (i) fish behaviour; (ii) condition of fish (i.e. emaciated or good); (iii) whether the disease is associated with mortality.

Gross clinical presentation: A description of the skin lesion when the condition is at the height of clinical expression including, (i) localization of the skin lesions on the body; (ii) the pattern of spread of the lesions on the body; (iii) the nature of the skin disease (e.g. inflammatory or ulcerative); (iv) the description of the individual skin lesion [size, colour, shape, swelling (yes/no)]; (v) the presentation of the skin changes (where known) for the early stage of disease and after healing.

Histopathological features: (i) histopathological features of the skin and underlying tissue; (ii) description of inflammatory cell types; (iii) the involvement of other organs (if involved).

Occasional observations (observations not found regularly): (i) occurrence in fish outside of the usually affected size range; (ii) other species in which the disease can occasionally be seen; (iii) occurrence under atypical environmental conditions; (iv) occasional presentation of clinical disease or histological features.

Additional information: (i) presence of condition in wild populations; (ii) economical importance; (iii) whether the disease was known to respond to certain treatments or farm management practices.

Information for the various descriptors was obtained from published literature, non-peer-reviewed reports, unpublished studies, the personal experience of fish farmers elicited during farm visits and workshops, and experience of other scientists presented at scientific meetings. However, most of the information comes from the authors' knowledge and experience.

Results

Disease descriptions

Table 1 provides the full suite of criteria considered relevant to describe the skin conditions addressed. These include about 35 attributes consistently associated with the diseases. In addition, further attributes that are observed only occasionally or which add additional information are provided (Table 2). The information presented is based on experience gained from around 40 cases (each case representing a separate farm, usually with multiple fish showing signs of the disease) of RMS in England, Scotland and Wales; six cases of RMS in Switzerland, one case in Austria, and five cases in Italy; seven cases of WWSD in England, 20 cases of US SD and four cases of US rash (both in the USA; multiple outbreaks of US SD and US rash have been intensively investigated at four farms over a 20-year period; LaPatra unpublished).

The following section provides a brief summary of the main features of the conditions with reference to the clinical and histological appearance of each. However, full details are provided in Tables 1 and 2.

Red mark syndrome (= cold water strawberry disease)

Red mark syndrome is observed in farmed rainbow trout of generally more than 100 g (Bruno *et al.* 2007; Verner-Jeffreys *et al.* 2008). It is characterized by the appearance of bright red, usually raised, non-ulcerative lesions between 5 mm to several cm in diameter, usually on the flank of the fish (Fig. 1a, b) (Ferguson *et al.* 2006; Verner-Jeffreys *et al.* 2008; Schmidt-Posthaus *et al.* 2009). Affected fish are generally in good condition and show normal behaviour. The onset of the disease is normally observed at water temperatures below 15 °C (Ferguson *et al.* 2006; Verner-Jeffreys *et al.* 2008), and clinical disease regresses as temperatures rise above 16 °C. In severely affected farms, all units holding fish of more than 100 g can be affected. However, in most cases, <50% of all units are affected (Oidtmann, B, Adam, K, Noguera, P, Mewett, J, Peeler, E, Thrush, M, Reese, R A unpubl. data). Prevalence within an infected unit or pond can reach up to 90%, but more frequently between 10% and 30% (Oidtmann, B, Adam, K, Noguera, P, Mewett, J, Peeler, E, Thrush, M, Reese, R A unpubl. data). The disease appears to be transmissible; however, a specific pathogen remains to be conclusively associated with the condition (Ferguson *et al.* 2006; Verner-Jeffreys *et al.* 2006, 2008; Metselaar *et al.* 2010). Histologically, severe lymphohistiocytic dermatitis is reported with scale resorption (Ferguson *et al.* 2006; Noguera 2008; Verner-Jeffreys *et al.* 2008; Schmidt-Posthaus *et al.* 2009). In comparison with the dermal lesions, the epidermis is unaffected or only mildly affected, showing mild epidermal hyperplasia and exocytosis, mainly

Table 1 Attributes of red mark syndrome (RMS, also termed cold water strawberry disease), warm water strawberry disease (WWSD), US rash and US strawberry disease. (a) Epidemiology; (b): Clinical signs and gross clinical presentation; (c) histopathological features. Attributes presented describe the typical presentation of the conditions. Attributes that assist in discriminating between the conditions are shaded in grey. The information presented is mainly based on the experience and knowledge of the co-authors. Where the information has also previously been published, references are provided

	Red mark syndrome (RMS)	Warm water strawberry disease Europe (WWSD)	US rash	US strawberry disease
(a) EPIDEMIOLOGY				
Aetiology	Currently unknown, infectious aetiology (1–4); involvement of *Flavobacterium psychrophilum* (3) and *Rickettsia*-like organism (5, 6) has been suggested	Currently unknown	Currently unknown	Currently unknown (7), *Rickettisia*-like organisms (8), bacteria (*Aeromonas salmonicida*), chlamydial agents, and three different viruses (9) have been implicated
Species affected	Rainbow trout (1–4, 6)	Rainbow trout	Rainbow trout (10)	Rainbow trout (farmed) (7, 10)
Age/size of fish affected	Mostly reported in fish over 100 g (2, 4, 9)	Only in large close to market size fish	<800 g and <14 months (<6000°days)	>100 g, >8 months (7)
Freshwater/marine	Freshwater (1–4)	Freshwater	Freshwater	Freshwater (7)
Spread of disease within units	Yes (1, 2)	Currently unknown	Currently unknown	Currently unknown
Spread of disease within farm	Yes (1, 2), but inconsistent. Depends on farm layout/water connectivity	Prevalence within a farm appears to progress with the re-use of water	Yes, but inconsistent. Depends on farm layout/water connectivity	Yes, but inconsistent. Depends on farm layout/water connectivity
Incubation period	Long incubation period (>500 day degrees) (2)	Unknown	Unknown	Unknown
Prevalence farm level (units within a farm)	Varies depending on farm layout and management practices; from a single to all units on a farm can be affected; usually below 50%	30–50%	1–25%; varies depending on farm layout, management practices and age of standing crop	Varies depending on farm layout, management practices and age of standing crop
Prevalence fish level (within a raceway/pond)	5–80% (usually 10–30%) (Oidtmann, B, Adam, K, Noguera, P, Mewett, J, Peeler, E, Thrush, M, Reese, R A unpubl. data); 5–50% (4)	5–50%	1–50%	1–50% (usually 2–15%)
Currently known geographic spread	Scotland, England, Wales (2, 3, 11), Switzerland, Austria (4), Italy (6) and Serbia (12). Similar conditions also recorded in France (13), Spain (3) Germany (A. and J.Tautenhahn pers. comm.) Finland (9)	Only one case observed in Scotland and six cases mainly in southern England	USA	USA (7), Canada, Chile
Country and year of first observation	England, 2001 (Cefas, unpubl. data)	Since 1998, England	USA, 1990s	USA, 1960s (14)
Temperature range	<16 °C (1); <15 °C (3, 4) (2); Since 2007, temperature range has broadened to <17–18 °C	>14 °C (2)	<15 °C	<15 °C Independent of season (no variation due to constant temperature of the spring water)
Season	Variable between years (temperature related); more common over winter months (UK)	Summer months (UK)	Spontaneously occurs throughout the year	Spontaneously occurs throughout the year; highest in late winter/early spring (7)

Table 1 *(Continued)*

	Red mark syndrome (RMS)	Warm water strawberry disease Europe (WWSD)	US rash	US strawberry disease
(b) CLINICAL SIGNS AND GROSS CLINICAL PRESENTATION				
CLINICAL SIGNS				
Fish behaviour	Normal	Normal	Normal	Normal (7)
Condition of fish	Affected fish are generally in good condition	Affected fish are generally in good condition	Affected fish are generally in good condition	Affected fish are generally in good condition (7)
Condition associated with mortalities	No (1, 4)	No	No	No (7)
GROSS CLINICAL PRESENTATION				
Affected area of body	Preferentially flanks and ventrum between pectoral fins. Head and fins not usually affected (2)	Mainly ventrum, occasionally flanks. Head and fins not usually affected.	Preferentially ventral surface; can also appear on side. Head and fins not usually affected.	Preferentially flanks, with rare cases on ventrum, fins not usually affected (8)
Pattern and spread of lesions on body	Often limited to a few focal or multi-focal lesions (2, 3), usually not well circumscribed	Diffuse, large, not circumscribed lesion. Multifocal	Diffuse, not circumscribed. Multifocal	Often limited to a few focal or multi-focal lesions, usually well circumscribed (7)
DESCRIPTION OF INDIVIDUAL LESIONS				
Lesions at height of clinical expression				
Appearance/ colour	Lesions bright red in colour (2, 4). Size and number of lesions increased compared to early stage (2)	Localised pinprick haemorrhagic lesions develop within pale regions. Size and number of lesions increased compared to early stage.	Localised pinprick haemorrhagic lesions develop within pale regions	Active lesions bright red (7, 8)
Diameter	Lesions from 5 mm to several centimetres in diameter (2–4)	Lesions from 5 mm to several centimetres in diameter	Extremely variable; diffuse pinprick haemorrhaging	Lesions from 2 mm to several centimetres in diameter (7)
Shape	Mixed. Often elongate/ovoid (dorsoventral)	Mixed. Some are small irregular-shaped areas, some circular, some elongated	Extremely variable; diffuse pinprick haemorrhaging	Can be elongate/oval shape
Scale loss	Yes (1–4)	No	No	Yes (8)
Ulcerative?	Usually no (3). Ulceration can occur due to secondary infections	Usually no	No	Usually no. Ulceration can occur due to secondary infections
Lesions raised?	Yes (2–4)	Usually no	No	Yes (8)
Appearance of lesion at an early stage	Opaque raised patches; underlying pigmentation pattern still visible, not well circumscribed	Small petechiae, skin surface smooth, not raised; appearance similar to fully developed lesions, only smaller	Currently unknown	Raised, small round foci with red centre characterized by slight lifting of scales (8)
Appearance of lesion on healing	Pale yellow/grey patches, reducing redness	Currently unknown	Currently unknown	Slightly raised, poorly circumscribed, pale pink to yellow to tan

Table 1 *(Continued)*

	Red mark syndrome (RMS)	Warm water strawberry disease Europe (WWSD)	US rash	US strawberry disease
(c) HISTO PATHOLOGICAL FEATURES *Lesions at height of clinical expression*				
Epidermis	Epidermis largely unaffected (2) possibly some lymphocytic infiltrate; epithelium may be lost towards the centre of the lesion.	Necrosis. Mild lymphocytic infiltration	Cell degeneration, sloughing of cells, necrosis, and cell loss, no blood	Epidermis generally unaffected, possibly some lymphocytic infiltrate
Dermis	Severe inflammatory response, especially around scale pockets (2–4), but also generally throughout the dermis; haemorrhages	Mild lymphocytic infiltration; areas of haemorrhage, especially around scales, congestion of blood vessels	Mild lymphocytic infiltration, congested blood vessels, loose blood cells around scales, lining of scale pocket with necrosis	Severe inflammatory cell infiltrate, predominantly small mononuclear cells resembling lymphocytes, congested blood vessels, loose blood cells around scales
Hypodermis	May be affected (3); inflammation, can extend into underlying adipose and muscle tissues and peritoneal serosa.	Lymphocytic infiltration may occur	Lymphocytic infiltration may occur and can extend into underlying adipose and muscle tissue	May be affected (7); inflammatory response can extend into underlying adipose and muscle tissue (7)
Scales affected	Lifting and resorption of scales with osteoclast involvement may occur (1–3). Scale pockets frequently affected by a severe inflammatory reaction	No	No	Lifting and sloughing of scales
Involvement of cell types	Predominantly lymphocytic inflammation (2, 3); neutrophil cell involvement around sites of scale resorption (3). Plasma cells prominent in the hypodermis	Lymphocytic inflammation	Lymphocytic inflammation	Predominately lymphocytic inflammation initially with progressively increasing numbers of neutrophils and macrophages later in disease (8)
Layers of skin affected by inflammatory response	Mainly dermis (2, 3), but epidermis and hypodermis may also be affected	Epidermis and dermis	Epidermis and dermis	Mainly dermis, but epidermis and hypodermis may also be affected
Oedema	Yes, around scale pockets (2–4) as well as in stratum spongiosum associated with vasodilation	No	Yes, in dermis	Yes, around scale pockets as well as in stratum spongiosum (8)
Appearance of lesion at an early stage	Epidermis intact, inflammatory cells (predominantly lymphocytes) in scale pockets and below the stratum compactum in the dermis (2) and scattered in the hypodermis	Epidermis with lymphocytic infiltrate; dermis and hypodermal layer unaffected		Epidermis intact. Lymphocytic dermatitis at junction of stratum compactum and stratum spinosum and along base of scale pocket

References: (1) Verner-Jeffreys *et al.* 2006; (2) Verner-Jeffreys *et al.* 2008; (3) Ferguson *et al.* 2006; (4) Schmidt-Posthaus *et al.* 2009; (5) Metselaar *et al.* 2010; (6) Galeotti *et al.* 2011; (7) Olson *et al.* 1985; (8) Lloyd *et al.* 2008; (9) Bruno *et al.* (2007); (10) LaPatra *et al.* 1994; (11) Noguera (2008); (12) Radosavljevic *et al.* 2009; (13) Fleury *et al.* 1985; (14) Erickson, 1969; (15) RMS Meeting 2009; (16) Oman, 1990; (17) St-Hilaire & Jeffery 2004.

Table 2 Occasional observations associated with red mark syndrome (RMS), warm water strawberry disease, US rash and US strawberry disease and additional information. The information presented is mainly based on the experience and knowledge of the co-authors. Where the information has also previously been published, references are provided

	Red mark syndrome (RMS)	Warm water strawberry disease Europe (WWSD)	US rash	US strawberry disease
Occasional observations				
EPIDEMIOLOGY				
Age/size of fish affected	Has been observed in rainbow trout from 20 g upwards	May be present in smaller fish (i.e. $\geq$100 g)		
Prevalence	May reach 100% of units on farm; within unit prevalence of up to 90%			May reach 80% (7)
Other species that may be affected	Brown trout (15)			Cutthroat trout and whitefish (16)
Freshwater/ saltwater	Persistence of condition in salt water (anecdotal)			
Temperature range	Observed at temperatures up to 20 °C			May occur at temperatures up to 21 °C (16)
GROSS CLINICAL PRESENTATION				
Ulceration	In severe cases ulceration may occur (3) (likely involvement of secondary pathogens)			In severe cases ulceration may occur (likely involvement of secondary pathogens)
HISTO PATHOLOGICAL FEATURES				
Layers of skin affected by inflammatory response	In severe cases, inflammation can extend deep into the musculature (2)	In severe cases, inflammation can extend deep into the musculature	No inflammation in the musculature	In severe cases, inflammation can extend deep into the musculature (7)
Other tissues	Acute necrotizing myocarditis (3), mostly in smaller fish (9). Inflammation of intestinal muscle and connective tissues (2) Splenic congestion and perivascular lymphocyte infiltrate. Peritonitis with adherence between the abdominal wall and the intestinal wall			Lymphocytic epicarditis that ranges from multifocal to perivascular
Necrosis	In severe cases, necrosis in connective tissues immediately below the epidermis and between the stratum compactum and underlying adipose tissue and myofibrils (2);			In severe cases, dermal necrosis associated with remnant scale pockets with rare small randomly located foci of necrosis within the hypodermal and dermal inflammation

Table 2 *(Continued)*

	Red mark syndrome (RMS)	Warm water strawberry disease Europe (WWSD)	US rash	US strawberry disease
Additional information				
Disease affecting value of fish	Yes, both for table market and restocking (1, 2)	Yes, both for table market (17) and restocking	Yes	Yes (7)
Responsiveness to management practices/ treatments	Yes: improves under antibiotic treatment (e.g. oxytetracycline, oxolinic acid, florfenicol), and sodium percarbonate (4), but equally it resolves alone (1, 2)	Yes: Vitamin C, Response-type diets as a prophylactic, antibiotic treatment (e.g. oxytetracycline), chloramine T (in France)	No	Responds to antibiotics (oxytetracylin (7), but not Romet®). Lower prevalence in fish that are fed feeds made by expansion/extrusion composed of high grade proteins.
Condition resolves alone	Yes (1, 2)			
Condition found in wild fish	Condition has been observed in restocked fish	No	Currently unknown	Yes

References: (1) Verner-Jeffreys *et al.* 2006; (2) Verner-Jeffreys *et al.* 2008; (3) Ferguson *et al.* 2006; (4) Schmidt-Posthaus *et al.* 2009; (7) Olson *et al.* 1985; (9) Bruno *et al.* (2007); (15) RMS Meeting 2009; (16) Oman, 1990; (17) St-Hilaire & Jeffery 2004.

of lymphocytes (Fig. 2a–d) (Noguera 2008; Verner-Jeffreys *et al.* 2008; Schmidt-Posthaus *et al.* 2009). The limited epidermal involvement in comparison with the dermis is considered a key characteristic of the condition, distinguishing it from other skin conditions. The disease first occurred in winter 2003/2004 in Great Britain and by 2009 more than 80 farms in Scotland, England and Wales had become affected (R. Hughes, personal communication). Subsequently, there have been reports of RMS from Switzerland, Austria and Italy (Schmidt-Posthaus *et al.* 2009; Galeotti *et al.* 2011). A similar condition has also been reported in France (Fleury, Vuillaume & Sochon 1985), Spain (Planas *et al.* 1993), Germany (A. and J.Tautenhahn pers. comm.), Finland (Bruno *et al.* 2007) and Serbia (Radosavljevic *et al.* 2009).

Warm water strawberry disease

Warm water strawberry disease is a skin condition observed in near market size (300–400 g) rainbow trout (St-Hilaire & Jeffery 2004). The condition has been reported from Scotland and England since 1998 where seven farms are known to have become affected. The skin lesions are usually located on the ventrum of affected fish, are non-ulcerative and usually not raised (Fig. 1c). They are characterized by localized pinprick haemorrhages, which develop within pale regions of between 5 mm and several centimetres in diameter. Affected fish are generally in good condition and show normal behaviour. The onset of the disease is observed at water temperatures above 14 °C (Verner-Jeffreys *et al.* 2008). Prevalence within an infected unit or pond can reach up to 50%. Within an affected farm, 30–50% of units are often affected. In contrast to RMS, the epidermis is affected, becoming mildly infiltrated with lymphocytes, with some necrosis (Fig. 2e,f). Whilst an inflammatory response can be seen in epidermis and dermis, it is less pronounced in the dermis compared with RMS. Scales remain unaffected. The aetiology of this condition is currently unknown (St-Hilaire & Jeffery 2004).

US rash

US rash is observed in near market size rainbow trout. Similar to WWSD, pinprick haemorrhage develops within regions of pale skin, 5 mm to several centimetres in diameter. The ventral body

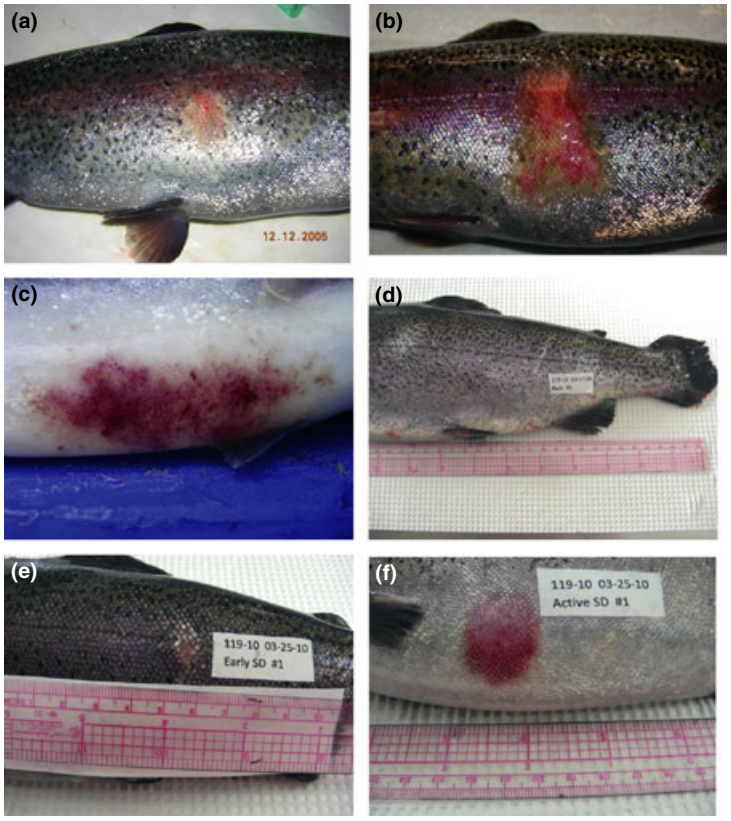


Figure 1 Gross appearance of red mark syndrome (a, b), warm water strawberry disease (WWSD) (c), US rash (d) and US strawberry disease (e, f) in rainbow trout. (a) Early red mark syndrome (RMS) lesion showing a pale focal region with indistinct borders, minor haemorrhaging and scale loss. (b) Advanced RMS lesion exhibiting scale loss and extensive haemorrhaging. (c) WWSD: diffuse, large, non circumscribed lesion on ventrum; pinprick haemorrhagic lesions. (d) US rash: small discrete foci of petechial haemorrhaging on ventral surface. (e) Early US SD lesion on flank: pale focal region of raised scales. (f) US SD lesion at height of clinical expression: Large well-circumscribed bright red lesion with scale loss.

surface is usually affected; however, lesions may also appear on the flank (Fig. 1d). The non-ulcerative lesions are not raised and affected fish remain in good condition and show normal behaviour. Scales are unaffected. The disease is observed on farms where water temperatures are below 15 °C. As water temperatures in affected farms remain fairly constant (spring water supply), it is unknown whether the disease would resolve at higher temperatures. Prevalence within an affected unit can range from 1% to 50%. The number of units affected in a farm varies depending on farm layout, management practices and age of stock. Sloughing of cells can be seen in the epidermis, whilst lymphocytic infiltration is found in the dermis (Fig. 2g,h). The aetiology is currently unresolved. The condition has been reported from rainbow trout farms in the USA (LaPatra *et al.* 1994 and S.E. LaPatra, unpubl. data).

US strawberry disease

US strawberry disease is found in farmed rainbow trout (Olson *et al.* 1985). Affected fish usually weigh over 100 g. Lesions are commonly located on the flanks, are bright red in colour and range from 2 mm to several cm in diameter (Fig. 1e,f) (Olson *et al.* 1985; Lloyd *et al.* 2008). The lesions are usually non-ulcerative and raised with lifting and sloughing of scales. Affected fish are generally in good condition and show normal behaviour. The disease is observed on farms where water temperatures are below 15 °C. As water temperatures on affected farms remain fairly constant (spring water supply), it is unknown whether the disease would resolve at higher temperatures. Prevalence within an infected unit or pond can reach up to 50% (but tends to be around 2–15%). The number of units affected in a farm varies depending on farm layout,

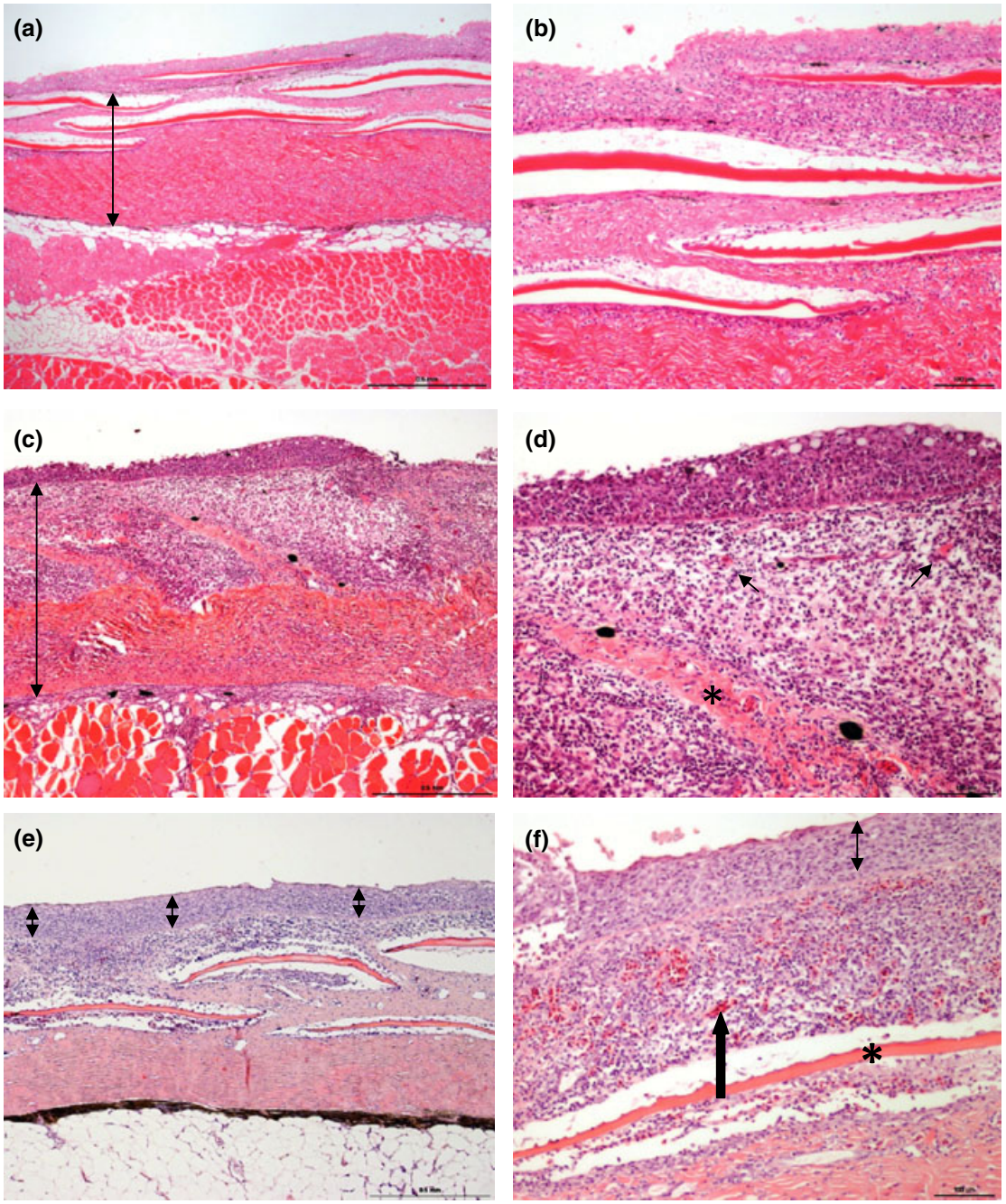
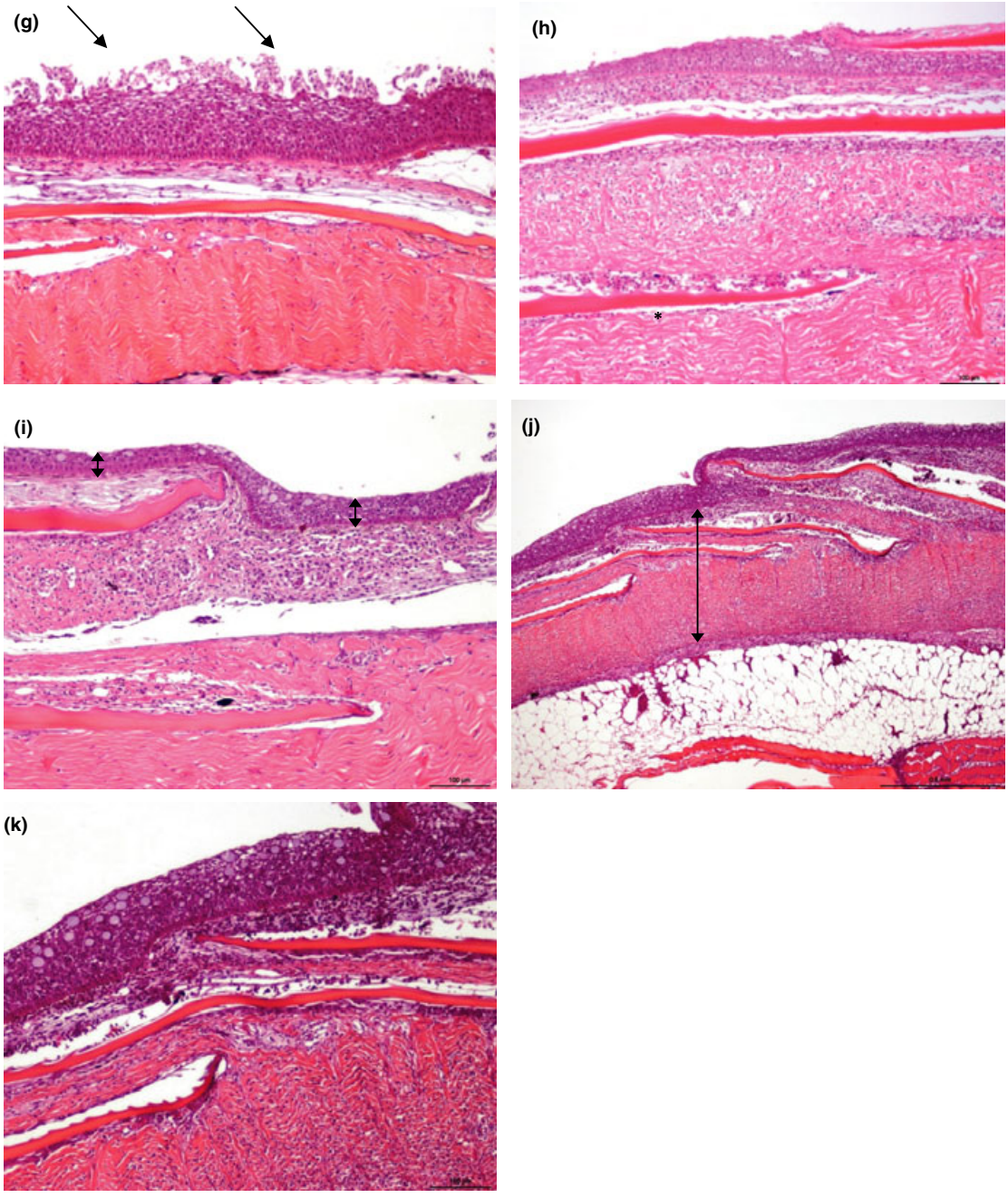


Figure 2 Histopathology of red mark syndrome (a–d), warm water strawberry disease (e, f), US rash (g, h) and US strawberry disease (i–k) in rainbow trout. (a) Low power view of a section from a case similar to that shown in Fig. 1a. Mild lymphocytic infiltration of the dermis. Bar = 500 μm, H&E. (b) High power view from (a) showing mild lymphocytic infiltration of the dermis. Scales intact. Bar = 100 μm, H&E. (c) Low power view of a section from a case similar to that shown in Fig. 1b. Marked inflammation of the dermis (extent of dermis indicated by double headed arrows) is evident, extending through the stratum compactum and into the underlying adipose and muscle tissue. Note the loss of scales. Loss of epithelium is seen to the right of the figure representing the region to the centre of lesion. Bar = 500 μm, H&E. (d) High power view from (c) showing marked lymphocytic infiltration of the dermis with red blood cell involvement (arrow) and oedema. Strands of connective tissue (*) in the location of the scale pockets remain. Bar = 100 μm, H&E. (e) Section showing moderate lymphocytic infiltration of epidermis (extent of epidermis



indicated by double headed arrow) and lymphocytic infiltration surrounding the scale pockets; epithelium intact. Bar = 500 μm, H&E. (f) High power, different fish. Moderate lymphocytic infiltration of epidermis (double headed arrow), sub-epidermal region around a scale (*) with moderate lymphocytic infiltration and red blood cell involvement (arrow). Bar = 100 μm, H&E. (g) Section showing necrosis and desquamation of the superficial layers of the epithelium (arrow). Bar = 500 μm, H&E. (h) High power, different fish. View of the region around a scale (*) showing mild to moderate lymphocytic infiltration. Bar = 100 μm, H&E. (i) Section through an early US SD lesion showing intact epithelium (double headed arrows); moderate lymphocytic infiltration in the upper region of the dermis and minimal involvement of the stratum compactum. Bar = 100 μm, H&E. (j) active US SD lesion showing marked lymphocytic infiltration of the dermis (extent of dermis indicated by double headed arrows). Bar = 500 μm, H&E. (k) High power view of j. Severe inflammatory response in the dermis and loose blood cells around scales. Bar = 100 μm, H&E.

management practices and age of the stock. Similar to RMS, histological features are a pronounced infiltration of the dermis with mononuclear cells resembling lymphocytes, whilst the epidermis is largely unaffected (Fig. 2i–k). The aetiology is currently unresolved (Olson *et al.* 1985; Lloyd *et al.* 2008).

Differences between the diseases

Most attributes of the diseases are either identical or too similar to be used for discriminating the four conditions. However, displaying the attributes of all conditions side by side as shown in Table 1 assists in identification of those attributes that differ. The key epidemiological and clinical features which distinguish the diseases are as follows: (i) temperature range, (ii) affected area of body, (iii) pattern and spread of lesions on body and (iv) appearance of the individual lesions. Amongst the histopathological features, the diseases differ on (v) the layers of skin predominantly affected by inflammatory response and (vi) whether or not the scales are affected.

Identifying these differences is particularly important where two conditions appear in the same geographic area. To illustrate this, we summarize the differences between CWSD and WWSD, which both occur in the UK:

Epidemiology: CWSD occurs preferably at a lower temperature (<16 °C) compared with WWSD (>14 °C).
Gross appearance: whereas CWSD lesions are characteristically located on the flanks and ventrum, are confluent (Fig. 1a,b) and raised, WWSD lesions are predominantly located on the ventrum, have a pinprick haemorrhagic appearance (Fig. 1c), and are not raised. Scale loss is frequently associated with CWSD lesions but absent in WWSD lesions.
Histopathology: In WWSD, the main layer of skin affected by inflammatory host response is the epithelium, whereas in CWSD, it is the dermis. Scale resorption is frequently observed in CWSD, but is not found in WWSD.

When comparing CWSD and US strawberry disease (US SD) – two diseases occurring in different geographic locations – these are found to be so similar that they may be the same disease. Furthermore, US rash and European WWSD are similar to each other, but clearly differ from CWSD and US SD using the attributes mentioned above. Despite the high similarity between US rash and European WWSD, it is not certain whether the two conditions can be regarded as the same, because clinical signs and temperature range differ. To illustrate how the information presented in Table 1 can be used to guide the diagnosis as to which skin condition is present, a diagnostic tree was developed. It focuses on the characteristics that were found suitable to discriminate the conditions presented in the current study (Fig. 3).

Development of a case definition for RMS

Data presented in Table 1 were analysed to identify key attributes that characterize the disease. Based upon the outcomes of the comparison, we developed case definitions for RMS (suspect case and confirmed case, Tables 3 and 4).

The criteria for a suspect case can be identified at the pond side (species affected, fish size, macroscopic appearance of lesions, location and size of lesions). If the defined attributes are met, the fish (and therefore the pond and farm) would be considered a suspect case, samples from those fish taken and submitted for histological analysis to assess whether criteria for a confirmed case are met. Variations of case definitions are presented (Tables 3 and 4). Criteria for choosing a narrower or wider case definition are discussed below.

Discussion

Panel of descriptors

A panel of descriptors was developed to capture characteristics of similar skin diseases of unknown aetiology in rainbow trout. Describing the different skin conditions using these attributes greatly facilitated identifying the differences between them, which to date have been difficult to discriminate. Published studies on rainbow trout skin conditions have addressed some attributes, but omitted others, which can make it difficult for other researchers or fish disease specialists to identify whether a condition they observe is the same as those previously published. The list of attributes provided in the current study should capture all relevant aspects required to fully characterize a skin condition of unknown aetiology and can also be used to determine whether a condition occurring in a new location is likely to be an already recognized condition. Some judgement may be required, for example, when a condition is observed in a new species.

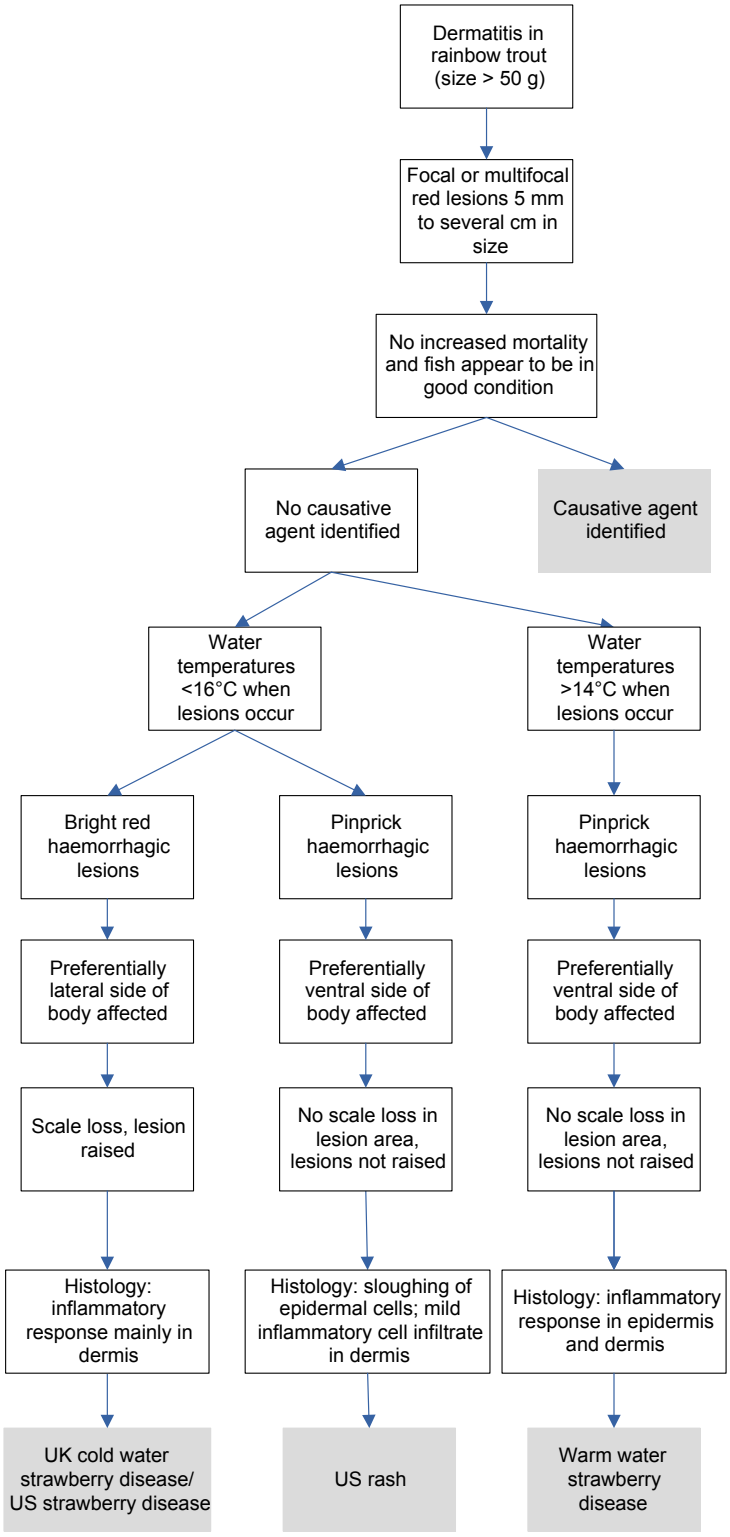


Figure 3 Diagnostic tree for cold water strawberry disease/red mark syndrome, warm water strawberry disease, US cold water strawberry disease and US rash

Table 3 Alternative case definitions for a suspect case of red mark syndrome (RMS) (based on field observations)

Study unit	Case definition
Individual fish *(option 1, narrowest)*	A rainbow trout (>100 g body weight, kept in freshwater at <16 °C) with skin lesions which are i) on its flanks, ii) focal to multifocal, iii) bright red and iv) 5 mm to several cm in diameter
Individual fish *(option 2)*	A rainbow trout (>50 g body weight and kept in freshwater at <16 °C) with skin lesions which are i) on its flanks, ii) focal to multifocal, iii) bright red and iv) 5 mm to several cm in diameter
Individual fish *(option 3, widest)*	A rainbow trout (>50 g body weight and kept in freshwater) with skin lesions which are (i) on its flanks, (ii) focal to multifocal, (iii) bright red and (iv) 5 mm to several cm in diameter
Pond/unit	A pond/unit with one or more fish meeting the case definition at fish level. Affected fish are generally in good condition, show normal behaviour, and there is no increased mortality observed in the pond/unit
Farm	A farm with one or more ponds/units meeting the definition for a suspect case for a pond/unit

Increasingly restrictive

Table 4 Case definitions for a confirmed case of red mark syndrome (RMS) (based on laboratory examination)

Study unit	Case definition
Individual fish (option 1, narrow definition)	A fish meeting the case definition of a suspect case and that under histopathological examination shows a pronounced dermal lymphohistiocytic response *with scale resorption*. The epidermis is largely unaffected
Individual fish (option 2, wide definition)	A fish meeting the case definition of a suspect case and that under histopathological examination shows a pronounced dermal lymphohistiocytic inflammatory response with the epidermis largely unaffected
Pond/unit	A pond/unit with one or more fish meeting the definition for a confirmed case in an individual fish
Farm	A farm with one or more ponds meeting the definition for a confirmed case for a pond/unit

When applying the approach to other skin conditions, further attributes may need to be captured; however, the attributes outlined here (Table 1) should be considered a suitable starting point.

A disease that would have benefited from the present approach is epizootic ulcerative syndrome (EUS). Researchers around the globe remained unaware that the same disease had occurred in different geographic locations. In 1971, a disease called mycotic granulomatosis (MG) was described from Japan (Egusa & Masuda 1971) and in the 1980s red spot disease (RSD) from Australia (Callinan, Fraser & Virgona 1989) and ulcerative mycosis (UM) in the USA (Dykstra *et al.* 1986). It took another 10–15 years until these conditions were recognized as the same disease (Lilley & Roberts 1997; Blazer *et al.* 2002; Baldock *et al.* 2005). The long delay was largely due to uncertainty about the pathogen involved in causing the disease. A further difficulty was that the disease had appeared in different parts of the world and affected different species. The discovery and isolation of the causative agent *Aphanomyces invadans* resulted in the confirmation that this was a globally important disease, and it was subsequently listed by the OIE (World Organization for Animal Health).

Apart from the skin conditions described in this article, other skin conditions of unknown aetiology are known in rainbow trout (e.g. cherry fin, fungal berry (USA), and puffy skin disease (UK) (Bruno *et al.* 2007). Further studies on these would be useful to provide comprehensive descriptions for comparison with those presented here. In this study, we focused on a single host species; it remains to be explored if the method will work in recognizing a single condition in a range of species. With time, further knowledge about the diseases may become available (e.g. an aetiological agent may be identified), and the individual categories may need updating. Furthermore, the presentation of the disease may change over time. In the case of RMS, this already appears to be the case: RMS had previously only been described below 16 °C, but fish farmers now report that the disease occasionally appears at temperatures above 16 °C (RMS Meeting 2009).

Comparison of different skin diseases

In the absence of a known aetiological agent, other criteria, including histopathological features, were applied to discriminate the four skin conditions described in this article. The available literature was reviewed and critically reassessed using the experience of the co-authors. In a few cases, this meant that the information presented in Table 1 deviates from published information. For example, here, ulceration is considered to be a possible complication of the original lesion of RMS, whereas RMS was previously described as being characterized by ulcerative skin lesions (Verner-Jeffreys *et al.* 2006).

The comparison of RMS (CWSD) and US strawberry disease revealed that the diseases are very similar, and no feature was found that would clearly separate the two conditions. Consequently, we conclude that RMS and US strawberry disease are the same condition.

Warm water strawberry disease and US rash are also very similar. Features that differ between the two conditions (WWSD and US rash) are the temperature range in which the conditions occur and presence or absence of oedema in the dermis. It may be that WWSD and US rash are even more similar than described, but different environmental conditions (constant cool water temperatures due to spring water supply in the US farms where US rash occurs vs. periods of water temperatures above 14 °C in the UK) in the geographic regions where the conditions occur lead to the small differences. Studies where US rash affected fish are held at higher temperatures (above 15 °C) could provide clarification.

Case definition

In this article, we demonstrate how case definitions for presumptive and confirmed diagnosis of a condition of unknown aetiology can be developed.

Application of a case definition for field use allows an investigator to decide on site whether a given farm, unit or fish meets the suspect case definition criteria. Secondly, the case definition for confirmatory diagnosis is applied where the study design (e.g. a survey) includes analysis of samples taken from farms, ponds and fish. In the laboratory, further diagnostic tests can be employed to confirm or refute the presumptive diagnosis. For diseases where an aetiological agent is known, additional diagnostic tests would be culture, histology, PCR and sequencing. In diseases where an aetiological agent has not been identified, alternative methods of confirming diagnosis are required. Case definitions are particularly important for diseases of unknown aetiology.

When choosing attributes to be included in a case definition, it is important to understand which attributes are observed consistently and which only occasionally. Narrowing down a suspect case definition will usually lead to fewer fish or farms being incorrectly identified as suspect cases (increased specificity); however, the chance of not detecting a fish (unit or farm) affected by the disease would increase (decreased sensitivity). How broad or narrow a case definition should be set depends on a number of factors, including its intended application. Where it is pivotal to prevent the spread of a given transmissible disease (which RMS appears to be) from affected to unaffected farms, a broad case definition for a suspect case would be more suitable. Similarly, if a study was undertaken to investigate the progression of RMS/CWSD throughout a prolonged period within a farm, the temperature criterion could be widened or dropped from the presumptive diagnosis to ensure that as far as possible *all* individual fish or ponds affected by RMS/CWSD are captured at the first stage (sampling on site); their status can be confirmed based on histopathology. On the other hand, if the main objective is to study farms that are very likely to have the disease (e.g. to investigate treatment methods on affected farms), a more specific case definition would be chosen to identify farms suitable for study. These aspects need to be carefully considered when deciding on a case definition for a planned study.

Apart from presenting variations in defining a suspect case, we also provide two variations of case definitions for a confirmed case (based on histology; Table 4). From the experience of the co-authors, scale resorption is not present in every single fish displaying the described macroscopic skin lesions, whilst the pronounced lymphohistiocytic response is. This is explained by the fish being sampled at various stages of the disease, and therefore, not all the histopathological features may be represented; the sampled fish may have developed scale resorption had they been sampled

at a later stage. Using a broader case definition would mean that such earlier stages of disease would be identified as cases – with the trade-off of possibly reduced specificity (i.e. other conditions may incorrectly be identified as cases). Once an aetiological agent is identified and suitable diagnostic tests available, a different case definition which would require the detection of the respective pathogen for a confirmed case would be likely to be used in future studies.

If one compared a case definition with a diagnostic test, the variations described above essentially result in an increase or decrease in the test sensitivity or specificity and the pros and cons would need to be considered carefully.

Conclusions

We provide a standardized methodology for describing skin conditions. This will enable other researchers to describe new skin conditions in a consistent manner, facilitating their comparison. We also show how a case definition can be developed allowing surveillance for diseases to be undertaken, even if the aetiological agent or cause is unknown. Case definitions are needed for epidemiological studies of disease risk factors such as case-control studies. Such studies are likely to assist in narrowing the likely cause of such diseases and possibly identify management or biosecurity procedures. Conditions of uncertain aetiology can have a devastating effect on the aquaculture industry. It is therefore important that surveillance for such conditions and studies into risk factors for their occurrence can be undertaken even if the aetiology is not resolved.

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This article is dedicated to the memory of Edward Branson, who died prematurely in November 2006. Edward was probably the first veterinarian in the UK to have recognized red mark syndrome as a condition different from UK warm water strawberry disease. He realized the importance of the condition from an early stage and initiated the first workshop on the disease in the UK providing the impetus for these ongoing studies. We would also like to thank all fish farmers and fish health professionals who provided information. This work was funded by Defra projects F1166 and FB001, and FB002.

References

Assié S., Bareille N., Beaudeau F. & Seegers H. (2009) Management- and housing-related risk factors of respiratory disorders in non-weaned French Charolais calves. *Preventive Veterinary Medicine* **91**, 218–225.

Baldock F.C., Blazer V., Callinan R.B., Hatai K., Karunasagar I., Mohan C.V. & Bondad-Reantaso M.G. (2005) Outcomes of a short expert consultation on epizootic ulcerative syndrome (EUS): re-examination of causal factors, case definition and nomenclature. In: *Diseases in Asian Aquaculture 5: Proceedings of the Fifth Symposium on Diseases in Asian Aquaculture*, November 24-28, 2002, Queensland, Australia (ed. by P.J. Walker, R.G. Lester & M.G. Bondad-Reantaso), pp. 555–585.

Bland I.M., Guthrie-Jones A., Taylor R.D. & Hill J. (2009) Dog obesity: owner attitudes and behaviour. *Preventive Veterinary Medicine* **92**, 333–340.

Blazer V.S., Lilley J.H., Schill W.B., Kiryu Y., Densmore C.L., Panyawachira V. & Chinabut S. (2002) *Aphanomyces invadans* in Atlantic menhaden along the East Coast of the United States. *Journal of Aquatic Animal Health* **14**, 1–10.

Bruno D., Crumlish M., Lapatra S., Noguera P. & Verner-Jeffreys D. (2007) Workshop on salmonid skin diseases. *European Association of Fish Pathologists 13th International Conference on Fish and Shellfish Diseases, Grado, Italy.*

Callinan R.B., Fraser G.C. & Virgona J.L. (1989) Pathology of red spot disease in sea mullet, *Mugil cephalus* L., from eastern Australia. *Journal of Fish Diseases* **12**, 467–479.

Dykstra M.J., Noga E.J., Levine J.F. & Moye D.W. (1986) Characterization of the *Aphanomyces* species involved with ulcerative mycosis (UM) in menhaden. *Mycologia* **74**, 664–672.

Egusa S. & Masuda N. (1971) A new fungal disease of *Plecoglossus altivelis*. *Fish Pathology* **6**, 41–46.

Erickson D. (1969) An investigation on strawberry disease in trout. *American Fishes and U.S. Trout News*, pp. 26.

Ferguson H.W., Girons A., Rizgalla G., Lapatra S., Branson E.J., Mackenzie K., Davies M., Collins R.O., Diab A. & Crumlish M. (2006) Strawberry disease in rainbow trout in Scotland: pathology and association with *Flavobacterium psychrophilum*. *The Veterinary Record* **158**, 630–632.

Fleury H.J.A., Vuillaume A. & Sochon E. (1985) Isolation of an adeno-like virus from two cases of strawberry disease in rainbow trout. *Annales de l'Institut Pasteur. Virology* **136**, 223–228.

Galeotti M., Giavenni R., Volpatti D., Beraldo P. & Feist S.W. (2011) Red mark syndrome/cold water strawberry disease: emergence in Italy and histopathological investigations. In: *15th International Conference of the European Association of Fish Pathologists, Split, Croatia.*

Jarp J. & Karlsen E. (1997) Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **28**, 79–86.

LaPatra S.E., Groff J.M., Lauda K.A., Munn B.& Jones G.R. (1994) Dermatitides in cultured rainbow trout. In: *International Symposium on Aquatic Animal Health*, September 4–8, 1994. Seattle, WA.

Lilley J.H. & Roberts R.J. (1997) Pathogenicity and culture studies comparing the *Aphanomyces* involved in epizootic ulcerative syndrome (EUS) with other similar fungi. *Journal of Fish Diseases* **20**, 135–144.

Lloyd S.J., LaPatra S.E., Snekvik K.R., St-Hilaire S., Cain K.D. & Call D.R. (2008) Strawberry disease lesions in rainbow trout from southern Idaho are associated with DNA from a Rickettsia-like organism. *Diseases of Aquatic Organisms* **82**, 111–118.

Mee J.F. (2008) Prevalence and risk factors for dystocia in dairy cattle: a review. *Veterinary Journal* **176**, 93–101.

Metselaar M., Thompson K.D., Gratacap R.M.L., Kik M.J.L., Lapatra S.E., Lloyd S.J., Call D.R., Smith P.D. & Adams A. (2010) Association of red-mark syndrome with a Rickettsia-like organism and its connection with strawberry disease in the USA. *Journal of Fish Diseases* **33**, 849–858.

Noguera P. (2008) Red mark syndrome. *Fish Farmer* **31**, 38.

OIE (2009a) Manual of diagnostic tests for aquatic animals. http://www.oie.int/Eng/normes/fmanual/A_summry.htm. (Accessed April 2010).

OIE (2009b) Terrestrial animal health code, chapter 1.4. Animal Health Surveillance. http://www.oie.int/doc/ged/D6514.PDF (Accessed September 2012).

Olson D.P., Beleau M.H., Busch R.A., Roberts S. & Krieger R.I. (1985) Strawberry disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **8**, 103–111.

Oman E. (1990) *Strawberry Disease in Salmonids*. MSc thesis, University of Idaho, USA, pp. 52.

Planas E., Ortega C., Muzquiz J.L., Docando J., Alonso J.L., Sanz F., Ramos P. & Arnal M.C. (1993) Description of a case of strawberry disease in Spain: aetiological, clinical and epidemiological study. In: *4. Natl. Congr. on Aquaculture, Vilanova de Arousa, Galicia (Spain), 21–24 Sep 1993* (ed. by A. Cervino, A. Landin, A. De Coo, A. Guerra, M. Torre & E. Planas), pp. 503–507. Centro de investigaciones marinas, Pontevedra (Spain).

Radosavljevic V., Jeremic S., Cirkovic M. & Milosevic N. (2009) Emergence of red mark syndrome in rainbow trout (*Onchorynchus mykiss*) in Serbia. In: *14th European Association of Fish Pathologists International Conference*, Sep. 14–19, 2009, Prague, Czech Republic.

RMS Meeting (2009) RMS meeting, Bewleys Hotel, Manchester Airport, 1st October 2009.

Schmidt-Posthaus H., Bergmann W., Knüsel R., Heistinger H. & Licek E. (2009) Appearance of red mark syndrome/cold water strawberry disease in Switzerland and Austria. *Diseases of Aquatic Organisms* **88**, 65–68.

St-Hilaire S. & Jeffery K. (2004) Strawberry disease in rainbow trout. *Trout News* **37**, 24.

Verner-Jeffreys D., Algoet M., Feist S., Bateman K., Peeler E. & Branson E. (2006) Studies on red mark syndrome. *Finfish News* **1**, 19–22.

Verner-Jeffreys D.W., Pond M.J., Peeler E.J., Rimmer G.S.E., Oidtmann B., Way K., Mewett J., Jeffrey K., Bateman K., Reese R.A. & Feist S.W. (2008) Emergence of cold water strawberry disease of rainbow trout *Oncorhynchus mykiss* in England and Wales: outbreak investigations and transmission studies. *Diseases of Aquatic Organisms* **79**, 207–218.

Wilesmith J.W. (1993) BSE: epidemiological approaches, trials and tribulations. *Preventive Veterinary Medicine* **18**, 33–42.

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