SARF 100 - Review of freshwater treatments used in the Scottish freshwater rainbow trout aquaculture industry

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SARF 100 Review of freshwater treatments used in the Scottish freshwater rainbow trout aquaculture industry

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Executive Summary

Availability of effective treatments for control of infectious diseases is a critical requirement of the Scottish and wider UK rainbow trout industry. The purpose of this project was to: identify the key diseases that affect freshwater aquaculture operations in Scotland, particularly the trout sector, and determine their relative impact; to identify the main methods used to control these diseases; identify the potential consequences if any of the main control methods were to be withdrawn; and finally, to identify any new potential treatments that could be used instead, if any of the main treatments were to be withdrawn.

Producers, vets and health professionals surveyed confirmed that production was constrained by a limited group of common diseases that affected rainbow trout producers in England and Scotland. These included rainbow trout fry syndrome (RTFS) caused by the bacterium *Flavobacterium psychrophilum*, white spot disease caused by the endoparasite *Ichthyophonus multifiliis*, enteric redmouth disease (ERM) caused by the bacterium *Yersinia ruckeri*, proliferative kidney disease caused by the myxozoan parasite *Tetracapsuoidesa bryosalmonae*, red mark syndrome (RMS) and bacterial gill disease (BGD).

The main treatments available to control these conditions were limited, with florfenicol reportedly used by all producers to control RTFS, formalin used extensively to control white spot and a range of parasites and chloramine T to treat bacterial gill disease. ERM was mainly controlled by vaccination, particularly via dip vaccination of fry with the Relera dual antigen vaccine. Other licensed antibiotics (oxytetracycline, amoxicillin and oxolinic acid) were used to treat sporadic outbreaks of ERM, in fish where vaccine protection had waned, and furunculosis.

The major reliance of the industry on florfenicol and formalin was concerning. Firstly there were limited identified alternatives to control RTFS in the event of RTFS-causing strains of *F. psychrophilum* developing resistance to florfenicol. There is also pressure at an EU level to withdraw formalin from sale as a biocide. Possible alternatives to the use of formalin products purchased for biocidal applications were reviewed in the event of their withdrawal from sale. For control of white spot it may be possible to use a licensed product marketed in Spain for the control of parasites of turbot under the veterinary cascade. The bronopol containing medicine Pyceze is one identified alternative that may be used. Where systems can be engineered to allow its use, Salt (sodium chloride), either via low concentration continuous dosing for several days, or short duration high concentration flushes is also a potential treatment. Practical issues with regards either maintaining low concentrations of salt, or dealing with high concentration effluents, may limit the use of this treatment strategy though. Project staff also consulted with Danish producers who are trialing the use of peracetic acid. For control of some ectoparasites, particularly flukes (e.g. trichodina), praziquantel, either as a water-based or in feed treatment, may also be an option to explore. Review of the literature suggested that caprylic acid, green tea extract and epigallocatechin gallate (EGCG), Piscidin 2, quinine, Triclabendazole and potassium ferrate may all have some promise as alternative treatments. Selection of any alternative treatments should be guided by whether they are likely to be readily useable. In this regards, products that already have approval for use in food animal production, either as biocides, feed additives or as medicines should be preferred in the first instance.

Recommendations

- Undertake further controlled studies (laboratory and field based) on the effectiveness of peracetic acid for the control of white spot and other production diseases.
- Obtain further information on the margin of safety of peracetic acid at different temperatures via target animal safety studies, at both a farm and laboratory scale.
- Continue to support efforts to develop alternatives vaccines for the control of RTFS.
- Determine the effectiveness of alternative antibiotics to florfenicol to control RTFS infections caused by *Flavobacterium psychrophilum*.
- Explore practicalities of importing formalin-containing medicinal products licensed in other Ms for control of fish diseases for controlling white spot and other diseases.
- Investigate use of mechanical control measures to reduce the impact of white spot in rainbow trout production systems.
- In vivo trials are needed to follow up some of the potential alternative chemical treatments identified (e.g. caprylic acid, green tea extract and epigallocatechin gallate (EGCG), Piscidin 2, quinine, Triclabendazole and potassium ferrate).

Conclusions

The survey clearly demonstrates that the rainbow trout industry is heavily reliant on a very limited range of treatment options to control major production diseases.

Discussions with fish medicine producers and veterinarians also suggest that the freshwater stage of the Atlantic salmon industry is similarly reliant on a small range of similar treatments to those used in the trout industry. In particular, there is also heavy reliance on formalin to control white spot disease and Costia in some hatcheries, and similar reports that florfenicol is the only effective treatment for the control of *Flavobacterium psychrophilum*. They also report that formalin is used quite extensively to control saprolegniasis in vaccinated salmon smolts prior to seawater transfer.

These findings are collectively concerning as either the withdrawal of formalin from sale, or the development of resistance to florfenicol in *Flavobacterium psychrophilum,* could affect the viability of both industries.

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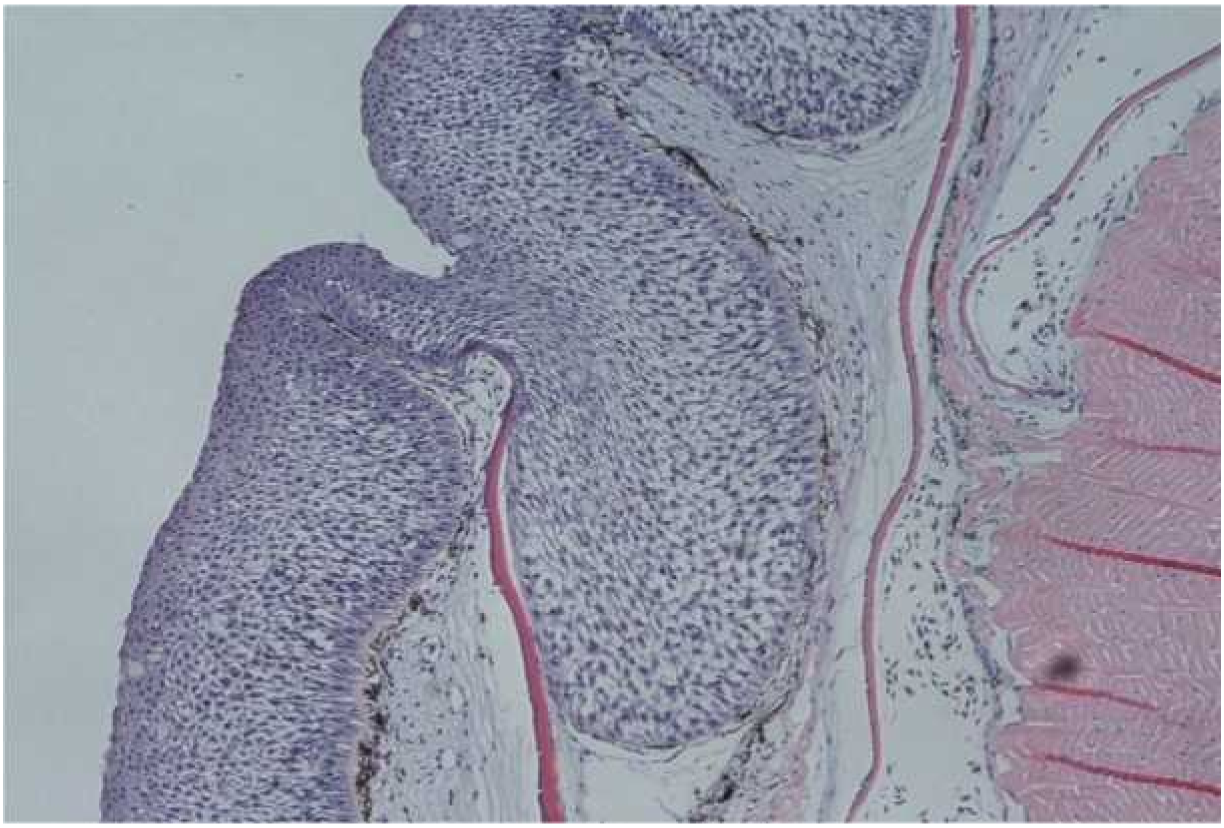

1 Introduction

Effective and safe chemical, pharmaceutical and vaccine treatments for the control of diseases are a critical requirement for aquaculture operators in both the freshwater and marine environments. Without access to such treatments disease can affect the economic sustainability of operations directly via mortalities, reduced growth rates and adverse effects on product quality. As diseases found in farmed fish can also potentially affect wild fish (e.g. sea lice (Boxaspen, 2006)), indirect effects on wild populations can adversely affect the environmental sustainability of the industry as well.

1.1 Aims

The purposes of this survey and review are to:

Identify the key diseases that affect freshwater aquaculture operations in Scotland, particularly the trout sector, and determine their relative impact

Identify the main methods used to control these diseases

Identify the potential consequences if any of the main control methods were to be withdrawn

Identify any new potential treatments that could be used instead, if any of the main treatments were to be withdrawn.

2 Materials and Methods

2.1 Treatments survey

A range of respondents who had knowledge of the trout industry in Scotland and the rest of the UK were surveyed for information on the types of diseases they were dealing with and what treatments they were using. An electronic Excel-based survey form was devised to elicit information on:

- The main diseases affecting producers
- The main treatments used to control these diseases
- The reported efficacy of these treatments
- Possible alternative treatments

Respondents were questioned either in person or via telephone. This also included project staff attending the aquaculture UK trade show in Aviemore Scotland in May 2014.

2.2 Identification of alternative treatments

Literature searches were conducted through the use of the scientific publications database, Scopus. Records between 1960 and present day were searched using keywords relevant to the control, treatment and management of the diseases where there were identified issues with regards availability of effective treatments. Internet search engines were used to identify grey literature associated with similar key word searches.

3 Overview of licensing and use of medicines and other chemicals in the freshwater industry

3.1 Medicines

Veterinary medicines used in fish are controlled under Directive 2001/82/EC as amended by Directive 2004/28/EC.

Article 1.2 of the Directive defines a "VMP" as:

- Any substance or combination of substances presented as having properties for treating or preventing disease in animals;
- or any substance or combination of substances that may be used in, or administered to, animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

With limited exceptions, any medicine needs to have a Marketing Authorisation before it can be used to treat farmed fish. In the UK, the Veterinary Medicines Directorate is the Government agency responsible for granting Marketing Authorisations (MAs) for veterinary medicines; and regulating the manufacture and distribution of veterinary medicinal products and animal feedingstuffs containing veterinary medicines and specified feed additives. It is also responsible for surveillance of Adverse Events (AE).

The processes for applying for an MA are outlined under Directive 2001/82/EC, as amended by Directive 2004/28/EC, but in essence, the manufacturer needs to demonstrate the product is of acceptable quality, effective and safe towards the user, consumer, target animal and the environment. When used in food producing species, such a farmed trout, it is also a requirement to demonstrate the product will not harm the consumer. A practical distinction can be drawn between the authorisation of pharmaceutical products, where there is a requirement to set maximum residue limits and appropriate withdrawal periods, and for the authorisation of immunological products (vaccines) where there is typically less of a requirement to demonstrate environmental and consumer safety (as they pose less relative risk to either). Exceptions would include where an immumnological product contained other substances, such as certain adjuvants where MRL may be required. In such cases, as per pharmaceuticals, a Phase I assessment will be required to determine if a phase II (which is a very comprehensive assessment) is required. (European Medicines Agency, 1997)(EMEA/CVMP/074/95FINAL).

COMMISSION REGULATION (EU) No 37/2010:

In selecting any alternative treatments that may be used to replace existing treatments, such as formalin (See Section 5), particular attention should be drawn to EU Regulation 37/2010 (EU, 2010). Any pharmacologically active substance that is to be applied to food producing fish, such as rainbow trout, must be listed as an allowed substance in Table 1 under EU Regulation 37/2010. This regulation repeals and replaces the earlier Council Regulation (EEC) No 2377/90 that previously listed substances for which an MRL has been established (Annex I), those for which an MRL does not need to be established (Annex II), those for which a provisional MRL has been set (Annex III) and those for which no MRL could be established because residues from that substance, at whatever limit, constitute a threat to human health. Under the new Regulation, substances previously listed under Annexes I-III are now collectively listed as allowed substances (Table 1) and those in Annex IV as prohibited substances (Table 2). Where a substance is not listed in either Table 1 or 2, detection of its residues in meat destined for human consumption would constitute an offence. A practical example here would include malachite green, although not listed in either Table 1 or Table 2, the UK authorities, through VMD, undertake surveillance to ensure farmed UK fish are free of malachite green, as well as levels of other drug residues that may exceed their permitted levels.

3.2 Biocides

Disinfectants are vital tools for effective farm biosecurity, used to inactivate potentially pathogenic micro-organisms on surfaces of equipment, tanks and clothing, or suspended in effluent. They are also used in the rainbow trout and salmon industries to disinfect gametes, principally ova. The industry typically uses a variety of different biocides and their continued availability remains important. In terms of information available to guide their use for aquaculture applications, the industry is largely reliant on marketing literature from the companies supplying the biocides.

3.3 Disinfectant testing schemes

3.3.1 Defra disinfectant approvals

Defra has in place a statutory mechanism under The Diseases of Animals (Approved Disinfectants) (England) Order 2007 (www.legislation.gov.uk/uksi/2007/448). This allows veterinary disinfectants to be placed on an approved list for the control of different diseases if they demonstrate efficacy in laboratory testing and comply with the requirements of the Biocidal Products Regulation. The Animal Health and Veterinary Laboratories Agency (AHVLA) deliver the scheme on behalf of Defra (http://www.defra.gov.uk/ahvla-en/tests-and-services/disinfectant-approvals). Testing is done to demonstrate the effectiveness of dilutions of disinfectant against the following diseases:

- Foot and mouth disease

- Swine vesicular disease
- Diseases of poultry and the avian influenza and influenza of avian origin in mammals
- Tuberculosis
- General (testing is performed against Salmonella)

Effective dilutions are listed against the particular diseases.

3.3.2 Aquaculture disinfectant listing scheme

https://www.gov.uk/aquaculture-disinfectant-listing-scheme-apply-or-view#listed-disinfectants

There is also now a voluntary scheme running whereby manufacturers can demonstrate the effectiveness of their products against aquaculture-relevant bacterial and viral pathogens. Testing against the bacterial and viral pathogens follows modified CEN standards.

Table 1. Listed disinfectant products and their effective dilutions as demonstrated under the mandatory test conditions of 4°C ± 1°C test temperature, with a 30 minute ± 30 second contact time.

Product name	Product physical form	Company name	Company address and contact details	Bacterial diseases of aquaculture relevance* (Test Standard EN1656 Modified)	Viral diseases of aquaculture relevance* (Test Standard EN14675 Modified)
Aqua Des	Liquid	Aquatic Hygiene Ltd	Albyn House, Union St, Inverness, IV1 1QA; www.aquatic.as	1 to 200	1 to 200
FAM 30	Liquid	Evans Vanodine International PLC	Brierley Rd, Walton Summit, Preston, Lancashire, PR5 8AH; www.evansvanodine.co.uk	1 to 100	N/A
Vanoquat New Formulation	Liquid	Evans Vanodine International PLC	Brierley Rd, Walton Summit, Preston, Lancashire, PR5 8AH; www.evansvanodine.co.uk	1 to 100	N/A
Virasure® Aquatic	Pink powder	Fish Vet Group Ltd	22 Carsegate Road, Inverness, IV3 8EX; www.fishvet.co.uk	1 to 100	0.7 to 100

** Effective dilution for liquids expressed as 1 part product to x parts water, or for solids expressed as 1 gram product to x mls water. † The disinfectant does not need to be diluted.*

3.4 Use of biocides for disease control purposes

There are some chemicals that are marketed and available general purpose biocides that are used to control particular disease problems on farms. These include chloramine T and formalin. There is requirement for all products marketed as biocides to be registered through the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012). Most of the biocide chemicals used in aquaculture are registered with the BPR, with the exception of formalin (see Section Y).

3.5 Discharge consents

Release of chemicals from aquaculture facilities is regulated in the UK by SEPA in Scotland and the EA. In Scotland these are regulated under the Water Environment (Controlled Activities) (Scotland) Regulations 2011 (CAR). SEPA aims to protect the environment by limiting the amount of certain medicines that can be administered and discharged. Also of importance here are wider obligations under European legislation to protect the aquatic environment, in particular the Water Framework Directive (EU, 2000). Obviously this means that any aquaculture medicine that is likely to be used on a freshwater rainbow trout farm, particularly one that discharges to natural water courses, will have to demonstrate limited environmental impact if the appropriate discharge consents are to be granted. A practical example here would include copper sulphate. Although this chemical is widely available as a swimming pool treatment (algicide) and can be effective for the control of white spot and other diseases, its use in the rainbow trout industry has been discontinued as copper based products are deemed to present significant environmental risks under the WFD and other environmental regulations (6.2.9).

4 Rainbow trout production: Main diseases

4.1 Producers, veterinarians and health professionals surveyed

A total of 8 different producers were questioned as part of the survey. These covered three Scottish producers (one vertically integrated business that produced its own fry that it on grew in both freshwater and marine production units), one that predominantly grew juveniles for supply to on growers and another company that grew on trout, again mainly for large trout production in sea and freshwater loch cage systems. Total annual production from the two ongrowers questioned was in excess of 7000 tonnes per year and the juveniles supplier produced more than 2 million fingerlings per year.

This likely represents more than 90% of total Scottish production, based on recent farm survey information (http://www.scotland.gov.uk/Publications/2013/09/9210/3#tb1a).

Four English producers were also questioned, covering a major supplier of trout for the restocking (angling) trade and three other producers who mainly produced table trout. Total production represented by these producers was collectively approximately 2400 tonnes per year. This figure is equivalent to approximately 37% of annual total English production (http://www.cefas.defra.gov.uk/publications/finfishnews/FFN15.pdf). One of the producers also reared more than 4 million rainbow trout fry per year. A major egg and fry producer from Northern Ireland was also questioned.

Two veterinarians were surveyed, one from a major practice who works with a number of the major Scottish producers, and an independent veterinarian who works with a number of English and Welsh producers, including major Southern English table trout producers. A fish health expert who works for a major feed company, who has extensive knowledge of the industry across England and Wales, was also questioned.

4.2 Summary of main diseases identified in last 12 months by producers and health professionals

Table 2 Diseases recorded by producers within the last 12 months

Disease	Number of producers observing clinical disease	Life stages affected (range)	Main treatments used	Estimated impact (production costs) with present treatment options
Rainbow trout fry syndrome	8	1-50g (mainly smaller fish <10g)	Florfenicol (8/8)	< 1% (8/8)
Red Mark Syndrome	7	>100g	None (2/7) Ongrowing affected fish (5/7)	<1% (5/7), >5% (2/7)
White spot	6	2g-100g (mainly smaller fish)	Formalin (6/8)	<1% (5/8), 1-5% (3/8)
Bacterial gill disease	5	<2g- 100g (mainly fry)	Chloramine T (5/5)	<1% (4/5), 1-5% (1/5)
Other external parasites	6	All	Formalin (4/4)	<1% (4/4)
Enteric Redmouth Disease	4	>10g	Vaccinate fry (4/4*); oxolinic acid, oxytetracycline or amoxicillin	<1% (2/4), 1-5% (2/4)
Furunculosis	4	>100g	oxolinic acid, oxytetracycline or amoxicillin	<1% (4/4)
Saprolegnia	4	Eggs & broodstock	formalin	<1% (3/4), 1-5% (1/4)
Costia	4	>2g (mainly 2-100g)	Formalin (4/4)	1-5% (1/8)
PKD	4	>40g	Exposure programme (4/4)	>5% (2/4)
Gut fungus	2	<2g	Pyceze (2/2)	<1%
Flavobacterium branchophilium	2	<2g	Chloramine T	<1%
Strawberry disease	2	>100g	None	<1%
Puffy skin disease	1	>100g	None	<1%
BKD	1	>100g	None	<1%
Gill amoebae	1			<1%
RTGE	1	>100g	Withdraw feed/ salt diets	1-5% (1)
Eye disorders	1		None	<1%
Nodular bacterial disease	1	2-100g	None	<1%
sleeping disease	1	2-100g	None	<1%
cherry fin	1	>100g	None	1-5% (1)

**Note. All producers questioned vaccinated fry (or insisted that sourced fry for ongrowing were vaccinated) against ERM.*

As part of the survey, respondents were asked what diseases they have observed in the last 12 months, what stages they affected and their estimated present impact with available control options. Supplementary questions, covered in more detail in Section 5, covered the treatments used to control these diseases and likely impact if these were not available.

It was clear that all the operators and other respondents interviewed recognised that infectious diseases were a significant constraint on production and, for a number of these diseases, continued availability of effective treatments was highly important.

4.2.1 Rainbow trout fry syndrome (RTFS)

RTFS is caused by the Gram negative bacterial pathogen *Flavobacterium psychrophilum* (M. E. Barnes, 2011; L. Madsen, Møller, & Dalsgaard, 2005; Nematollahi, Decostere, Pasmans, & Haesebrouck, 2003). This pathogen also causes bacterial coldwater disease (BCWD) that typically affects older fish (Starliper, 2011).

Rainbow trout fry syndrome and BCWD are a significant problem affecting all the respondents surveyed (8/8; Table 2). Most batches of rainbow trout were treated at least once during every production cycle with florfenicol as per label (imposing significant treatment costs). Discussions with farmers and health professionals revealed that, where other antimicrobials had been used (oxytetracycline, oxolinic acid or amoxicillin) results had been mixed, with an often poor response. There is only limited recent data on the antimicrobial susceptibility of *Flavobacterium psychrophilum* isolates circulating in UK hatcheries.

4.2.2 Red Mark Syndrome

Red mark syndrome has become a major problem for producers since its emergence in the UK trout farming industry in 2006 (Verner-Jeffreys *et al*., 2008). RMS-affected fish present with unsightly external, raised, lesions on the flanks, dorsal and ventral surfaces (Oidtmann *et al*., 2013). The economic impact is severe in farmers that are not able to grade out and hold back fish (see below), as processors reject any affected fish, imposing significant costs on producers. The condition typically affects fish in excess of 100g as confirmed in this survey with all respondents reporting RMS in fish over this size. The disease is now well established across Great Britain, as evidenced by 7 of the 8 respondents surveyed reporting they had observed it within the last 12 months. Treatment options are limited, with no respondents now treating affected fish. Earlier, during the emergence of RMS, some farmers were treating the condition with antibiotics, particularly oxytetracycline (Verner-Jeffreys *et al*., 2008). Discussion with both farmers and fish health professionals noted that the approach is no longer favoured as the long withdrawal period for oxytetracycline (700 day-degrees) often meant that there was no real advantage in treatment. Either the lesions would have naturally resolved to some extent within the withdrawal period, or, the transitory protection afforded by treatment, resulted in lesion reoccurrence by the end of the withdrawal period. Where possible, farmers were holding fish back that had developed red mark syndrome as the condition spontaneously resolves and there is no reported impact on either survival or growth rate. This control option obviously lends itself better to producers rearing large trout for the table or restocking (greater than 1kg), as opposed to portion table trout producers (where harvest weight is typically 400g or so).

Strategies here could include instituting a pre-exposure programme analogous to that used for the management of PKD. Reports from farmers suggest that the fish typically do not show signs of the disease again after displaying symptoms.

Figure 1 Previously naive rainbow trout with Red Mark syndrome –associated external skin lesions, following cohabitation challenge with RMS-affected fish sourced from an RMS positive farm. (Verner-Jeffreys et al., 2008)

For 5/7 of the surveyed producers that had observed RMS (Table 2), reported impact was low, with expenses associated with dealing with the disease responsible for less than 1% of overall production costs. These producers all had options to manage the disease by holding back and grading out affected fish, either as they were producing fish for restocking (anglers typically pay a premium for larger fish) or they were table trout producers that were able to grow fish to > 1kg. For two of the 7 producers surveyed, who produce 4-500 g whole fish for the table market and could not manage the disease by grading out and holding back affected fish, the reported impact was very high (>5% of their production costs were attributed to RMS-associated costs).

4.2.3 White spot

Ichthyophthirius multifiliis or 'Ich' is a ciliate protistan parasite of freshwater fish, including rainbow trout. The parasite is tolerant to a wide range of environmental conditions and shows little host specificity. As a consequence the parasite is one of the most significant pathogens to affect cultured fish species, causing a condition known as 'white spot' or 'Ichthyophthiriasis' that leads to significant mortality, especially in small fish (Matthews, 2005).

The parasite has a direct life-cycle with no intermediate hosts, it does, however, have several distinct on and off host stages (Figure 1). The life-cycle begins with a free-swimming infective stage known as a theront, which can survive for 10 – 96 hours off a host depending on the temperature (less at high temperatures). They are approximately 0.15mm in size, phototactic and are propelled rapidly by their cilia. Upon contact with the host the theront burrows into the epidermis of the skin or gills and starts to develop into the parasitic trophont stage. This stage is still covered in cilia but has a large buccal cavity and can be easily identified through the presence of a large horseshoe shaped nucleus. The trophont sits within the epidermis, feeding on tissue loosened through the parasites movement. This stage grows to a size of 1.5mm and can be seen by the naked eye as white spots on the fish. As these spots appear to be on the surface of the skin, I. multifiliis is often referred to as an ectoparasite. However, the trophont does in fact sit protected within the epidermis and is therefore an endoparasite. Once the trophont reaches a suitable size it is able to exit its host to begin the next phase of the life-cycle known as the tomont stage. It is at this point that host mortality normally occurs. Although the trophont stage can clearly cause severe irritation to the host, the majority of damage occurs as the parasite exits, as the degree of open tissue damage to the skin and gill can cause severe osmoregulatory and respiratory shock, as well as allowing other pathogens to enter the fish through these open wounds. The smaller the fish, the more significant the damage is likely to be. At 14°C it takes approximately 10 to 22 days from infection by the theront to the tomont exiting.

The tomont is a short phase in the life-cycle, upon exit this stage spirals downwards to the bed of the system, settling on a suitable firm substrate. The parasite then produces a coat and encysts, firmly adhered to the substrate. This process takes up to 5 hours. Once encysted the parasite undergoes binary fission, dividing to produce hundreds of tomites within the cyst. Once division is complete the cyst ruptures and infective theronts are released into the water to begin the life-cycle again. The time taken from encysting to release is dependent on environmental factors, of which temperature and pH are probably the most important. At the parasites optimal pH of 7 development takes between 19 and 129 hours at 24 and 4°C, respectively. Studies by (Taylor & Shinn, 2004) show that at 22°C these timings can approximately double if the pH is reduced to 6 or increased to 8.

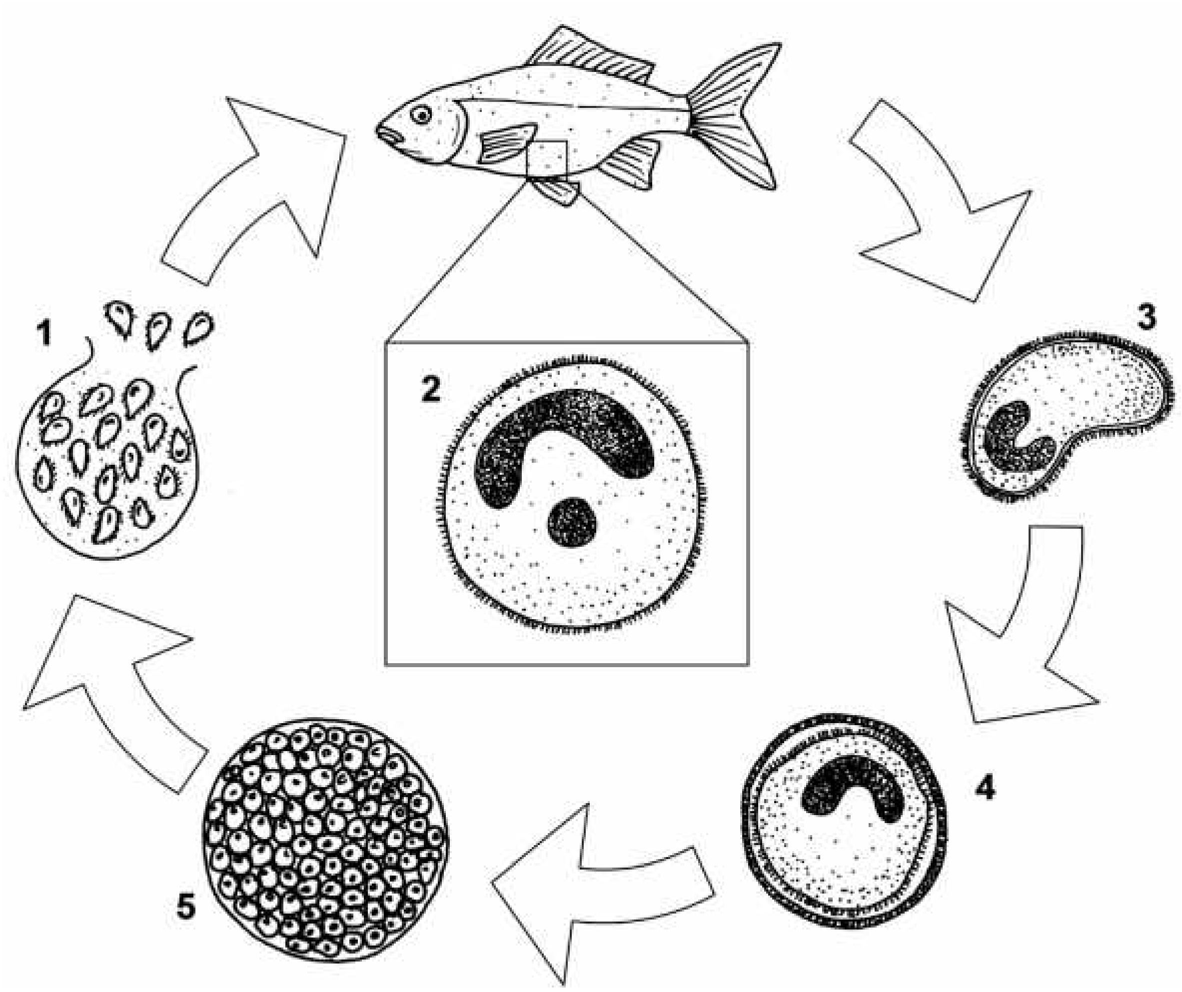

Figure 2 Life-cycle of Ichthyophthirius multifiliis. 1) Infective theronts released from cyst. 2) Parasitic trophont stage. 3) Exiting tomont. 4) Cyst. 5) Dividing tomites within cyst.

I. multifiliis is a serious problem in hobbyist aquaria, it is also a major problem to aquaculture where it causes massive mortality and subsequent financial losses. For many years control of *I. multifiliis* in these systems centred on the use of bath treatments containing a formalin and malachite green combination. Such treatments were perceived to be effective at controlling both the infective theronts and the parasitic trophont. However, due to concerns over its potential carcinogenic, mutagenic and teratogenic properties the use of malachite green in food fish was banned throughout the EU in 2000. Additionally there are health concerns associated with long-term user exposure to formalin, and this compound has been shown to have significantly reduced efficacy when used without malachite green. Although the use of these compounds for control of *I. multifiliis* in hobbyists aquaria is still permitted, it seems likely that in the not too distant future they may be withdrawn from sale and alternative yet effective treatments will be required.

White spot was identified as one the major disease problems in the survey. Six of the eight producers questioned had observed white spot in their systems (Table 2) within the last 12 months and all treated with formalin when the parasite was observed. Three of the producers reported that the costs associated with white spot (control measures and direct mortality/morbidity) were equivalent to between 1-5% of their total annual production costs. The remainder said that, with the current treatment options, costs were less than 1% of their total annual production costs.

4.2.4 Bacterial gill disease (BGD)

BGD is a condition that affects, as the name suggests, the gills of rainbow trout and other freshwater species (Starliper & Schill, 2012). Left untreated the condition can be devastating to producers. The aetiology is complex , although bacteria such as *Flavobacterium branchophilum* are commonly implicated (Good, Davidson, Wiens, Welch, & Summerfelt, 2014).

Bacterial gill disease was reported by 5 of the 8 producers surveyed (Table 2). As discussed above, left untreated, the impact can be severe. However, four of the five producers that were affected by the disease said that impact is relatively minor as the condition responds well to prompt and early intervention via chloramine T treatments. Producers, vets and the health professional consulted all said that standard treatment for bacterial gill problems was application of chloramine T. One producer reported that losses due to BGD and its control equated to 1-5% of their annual costs.

4.2.5 Enteric redmouth disease (ERM)

All the respondents identified Enteric Redmouth Disease, caused by the Gram negative bacterial pathogen *Yesinia ruckeri* (Wheeler *et al*., 2009)(A. C. Barnes, 2011; Tobback, Decostere, Hermans, Haesebrouck, & Chiers, 2007) as a potential problem on their farms, although only four out of eight questioned had seen clinical ERM in the last 12 months. In unprotected stock, ERM can cause high mortalities, as well as affecting the quality of the fish and growth (A. C. Barnes, 2011). The survey also identified that dealing with diseased fish takes up considerable staff time, adding further costs. Fortunately, fish can be vaccinated against ERM and all the respondents either routinely vaccinate fish themselves when they are fry (<2g), or insist that they have been vaccinated prior to reception from another site. In response to concerns about the possible development of vaccine-evading emergent strains of *Yersinia ruckeri* (Arias *et al*., 2007)(Wheeler *et al*., 2009)(Welch *et al*., 2011), many farmers are also reportedly using the Relera vaccine (http://www.msd-animal-health.co.uk/Products_Public/Aquavac_Relera/Product_data_sheet.aspx) that is based on both biotype 1 and biotype 2 serotype O1 strains. There was some variation in how the vaccine is administered, with all farmers using dip vaccinatied fry but some also injection vaccinating and/or oral boosting larger fish to get a longer lasting immune response over the grow out period.

Of the 8 producers questioned, six listed it as one of their present major disease concerns (Table 2). The vaccines are reportedly effective in providing protection for fry and juveniles, however if protection is not then boosted, then ERM can cause problems in larger fish. Those that had disease problems all treated with antibiotics when clinical disease was observed. This was confirmed by the vets and health professional questioned who suggested that Branzil (oxolinic acid) was an effective treatment, as well as other antibiotic treatments, including oxytetracycline and amoxicillin. Where possible the vets questioned relied on sensitivity test results to determine which antibiotic to treat ERM infections. Three of the 8 producers (who were all ongrowers) reported it had an impact equivalent to between 1-5% of their annual production costs, with the remainder suggesting costs associated with ERM disease and its control being less than 1% of their annual production costs. Discussions with farmers affected by ERM in their larger ongrowing fish revealed that they would prefer to boost protection by oral or additional dip vaccinations, but the additional costs were typically considered excessive. One farmer questioned estimated that the present cost of dip vaccinating fry equated to approximately 3p per fish. Doubling this cost to include an additional dip or oral vaccine application when the fish are 50g or so was likely to be prohibitive.

4.2.6 Furunculosis

Furunculosis caused by the bacterium *Aeromonas salmonicida* subsp. *salmonicida* (Bernoth, 1997)is major problem in unvaccinated Atlantic salmon and brown trout. It is less of a concern in rainbow trout that appear to be more naturally ressitynt to this pathogen than members of the Salmo genus.

However in fish that are immunoocompromised or reared at high temperatures furunculosis outbreaks can take place. Four of the eight producers and all the fish vets and the health professional surveyed had noted furunculous in their systems in the last 12 months. Treatment options were typically to medicate with an antimicrobial (Branzil™, Florocol™, Aquatet™ or Vetremox™ . Ideally prescribing was based on sensitivity data and there were some reports from the vet and health professional working with southern English producers that drug resistant strains *of A. salmonicida* were an issue in the industry. Of the antimicrobials sued, the vets and health professional expressed a preference for using oxolinic acid where possible.

4.2.7 Saprolegniasis

Saprolegniasis caused by the oomycete *Saprolegnia parasitica* causes disease in eggs and immunocompromised juvenile and adult salmonids, including rainbow trout (van den Berg, McLaggan, Diéguez-Uribeondo, & van West, 2013; van West, 2006).

Clinical saprolegnia infection was reported by 4 of the surveyed producers over the previous 12 months (Table 2). The reported impact of saprolegnia was relatively limited for most of the producers surveyed. Unless fish are immunocompromised, there is limited requirement to treat fish with available treatments (typically formalin or Pyceze were reportedly used when needed). A major fry producer surveyed did report that saprolegnia infection of eggs and broodstock was a significant problem for them. They typically used formalin treatment of eggs combined with egg picking to remove infected eggs and prevent infection spreading to other eggs. The effectiveness of dead egg 'picking' is shown by recent work that has demonstrated experimentally that the spread of saprolegnia infection in Atlantic salmon eggs requires an infection focus represented by dead eggs or debris (Thoen, Evensen, & Skaar, 2011).

4.2.8 Costia

Costia is caused by Ichthyobodo necator, an ectoparasitic flagellate that infects the skin and gills of a range of fish species, including rainbow trout (Todal *et al*., 2004).

Infection by costia was reported by 4 of the 8 producers surveyed in the last 12 months (Table 2). Affected farmers said they saw it in both fry and juveniles (2g- 100g). Similar to impact by whitespot, farmers affected generally reported that the condition was readily controllable as long as the intervention was made quickly. As with white spot and other external and skin parasites, standard treatment was 200-250 ppm formalin bath or flush treatments. If left untreated the impact could be high, causing significant mortalities. One producer reported that costia control and other associated costs was from 1-5% of their annual production. Other producers reported that costia-associated costs were less than 1% of their annual production, with the present available control strategies.

4.2.9 Other ectoparasites

Most respondents reported sporadic infections by a combination of microcotyle, trichodia and or other external parasites. As with costia, farmers typically treated as soon as any of these parasites were observed. In most cases, farmers did not differentiate any of these parasites and saw limited need to as all these conditions respond well to formalin treatment. In most cases treatment involved formalin baths or dips. In general, as with costia, response to this treatment regime is reported as very good with limited recurrence as long as fish are treated shortly after first detection.

4.2.10 Proliferative kidney disease

Proliferative kidney disease is caused by the myxozoan parasite *Tetracapsuoidesa bryosalmonae* (S. W. Feist, Longshaw, Canning, & Okamura, 2001; Stephen W Feist & Longshaw, 2006). The infective stage of the parasite is released by bryozoans, especially *Fredericella sultana,* mainly during the spring and summer months. Clinical signs in the fish host become apparent within approximately six weeks and the disease has a severe effect on renal function in particular. However, the parasite becomes systemic and several organs show significant pathological changes. In the UK, the parasite in rainbow trout rarely produces mature spore stages which would be infective to the bryozoans host.

Four out of 8 producers reported clinical PKD in their systems in the last 12 months. For those that had PKD on their farms the impact was very severe. All these ongrowers had to ensure fish were exposed to PKD when they were introduced as fingerlings in the mid to late summer to ensure they had sufficient immunity to withstand PKD challenge the following spring/summer when they were exposed again. The impact is both that affected fish can suffer severe mortalities but also that it constrains production schedules as the producers need to synchronise introductions of fry with the exposure program. PKD impact is variable depending on whether farms are exposed to PKD through sourcing water from rivers where the bryozoans host the disease. With the banning of malachite green as a treatment, no other treatment method, other than the exposure programme, is available to control PKD.

4.2.11 Strawberry disease

Strawberry disease is another skin disease of rainbow trout. It has been differentiated from RMS on the basis of both histopathological presentation and that it is typically observed at higher temperatures than RMS (Oidtmann *et al*., 2013). Only one producer reported SD in the last 12 months and its impact does not appear to be high.

4.2.12 Puffy skin disease

Puffy skin disease is severe dermatitis that has emerged in UK farmed rainbow production in 2002. Since 2006 cases have increased markedly (Peeler *et al*., 2014). Clinical signs include white or grey skin patches, which become raised and red with excessive mucous production and scale loss. Fish are inappetant and lose condition. Histologically, the key feature is epithelial hyperplasia (Figure 4). Initial evidence suggests the condition is infectious, based on epidemiological analyses *(*Peeler *et al.*, 2014) and tank-based transmission studies recently completed at the Cefas Weymouth Laboratory (Cano Cejas *et al.* unpublished data)

Figure 3 Rainbow trout with severe puffy skin disease symptoms

4.2.13 Infectious pancreatic necrosis (IPN)

IPNV is a an aquatic birnavirus (Hill, 2006) that affects salmonid fish, including rainbow trout and Atlantic salmon. IPN was not reported from any of the rainbow trout farms surveyed and its reported impact was limited. Typically it only affects smaller fish and hatcheries tend to avoid the disease by practicing good biosecurity. Cefas routinely isolates IPNV from UK farmed rainbow trout, under its diagnostics and research programmes (Keith Way, unpublished data) suggesting the virus is endemic within the ongrowing sector, but has limited impact.

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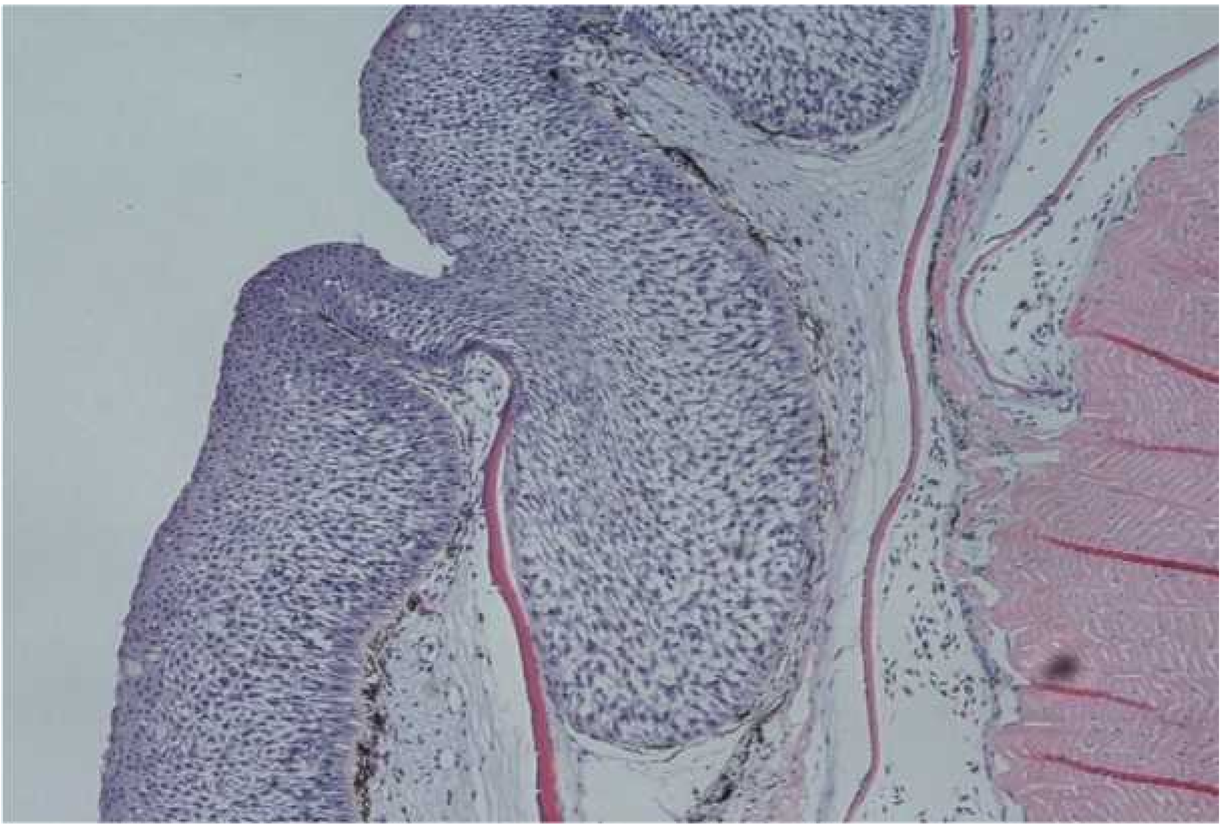

Figure 4 Histopathological features of PSD. Transverse section of skin showing progressive dermal hyperplasia of the epithelium with spongioform appearance.

4.2.14 Bacterial Kidney Disease (BKD)

Bacterial kidney disease, caused by the bacterium *Renibacterium salmoninarum* (Wiens, 2012)(Brynildsrud *et al*., 2014), was reported from one of the producers surveyed. *Renibacterium salmoninarum* is a Gram-positive slow-growing facultative intracellular pathogen. BKD is a chronic, progressive granulomatous infection that can cause significant mortalities and morbidity in susceptible salmonid species (Wiens, 2012).

Figure 5 Rainbow trout infected with BKD. Note swollen kidney.

In the single producer that had observed BKD (Table 2), impact was reportedly low (<1% of equivalent annual production costs). In part this may be related to the typical grow out systems which typically force fish to market weight relatively quickly, limiting the opportunity for a slow growing chronic condition like *R. salmoninarum* to have much in the way of impact. It is also likely that the controls that have been put in place to manage BKD have been effective, minimising transfer of *R. salmoninarum* between farms, even if there is possible sporadic reintroduction from wild reservoirs and elsewhere.

At the present time the impact of the disease is relatively limited. Only one producer reported the condition and both vets questioned have, to date, seen limited numbers of cases. However as the condition can cause severe effects (emaciation and ultimately mortality, coupled with severe lesions resulting in rejection by processors) there is concern that the disease may continue to spread and have severe impact on the industry. A further concern is that there is, to date, no recognised treatment. The causative agent has also not, so far, been identified.

4.2.15 Cherry fin

Cherry fin disease is a gross inflammation involving primarily the pectoral fins which, to date has only been observed on a single site from one of the producers surveyed. Cherry fin is most noticeable in market sized fish (450-550g) but early stage lesions can be observed in smaller fish. The aetiology of the condition is unknown (Jakeman & Feist, 2013).

Figure 6 Rainbow trout with cherry fin symptoms

4.2.16 Rainbow trout gastroenteritis (RTGE)

RTGE is a gastro intestinal disease of rainbow trout (Jorge Del-Pozo, Crumlish, Ferguson, Green, & Turnbull, 2010; Jorge Del-Pozo, Crumlish, Ferguson, & Turnbull, 2009). Available evidence suggests it primarily affects major rainbow trout producers in the UK. There is debate on the likely causes of RTGE,(J. Del-Pozo, Turnbull, Ferguson, & Crumlish, 2010) with some suggestions that a segmented filamentous bacterium could be implicated, although the evidence is still equivocal. Only one of the producers reported major problems with RTGE. The best control method appears to be withdrawal of food as soon as RTGE is detected. There were also reports that application of salt-supplemented and other specialised diets can help limit RTGE impact. For those farms that had had RTGE, the impact could be quite considerable unless there is early intervention.

4.3 Reported most important diseases

Respondents were asked to rank up to 5 of the most important diseases that affected them. Based on this scoring system, as can be seen in Figure 7, the most important disease, in terms of likely overall impact at the present time with available control methods, was RTFS, followed by ERM, white spot, PKD, RMS and BGD.

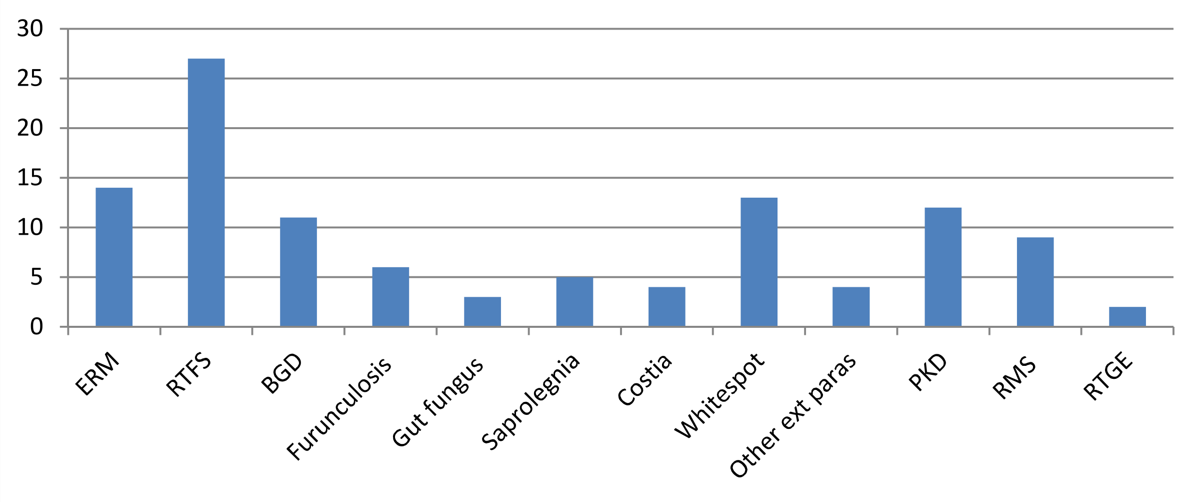

Figure 7. Most important disease issues affecting producers. The 8 respondents were asked to rank up to 5 of the main diseases affecting their systems, with the ranked diseases scored from 1-5, with 5 being the most important disease affecting them. The total sum of scores for each disease is recorded (theoretical maximum score possible = 40).

4.4 Estimated impact of diseases without main control strategies

Respondents were asked to estimate the likely impact of the diseases they determined to be of most importance if they were not able to treat or control using their preferred treatments.

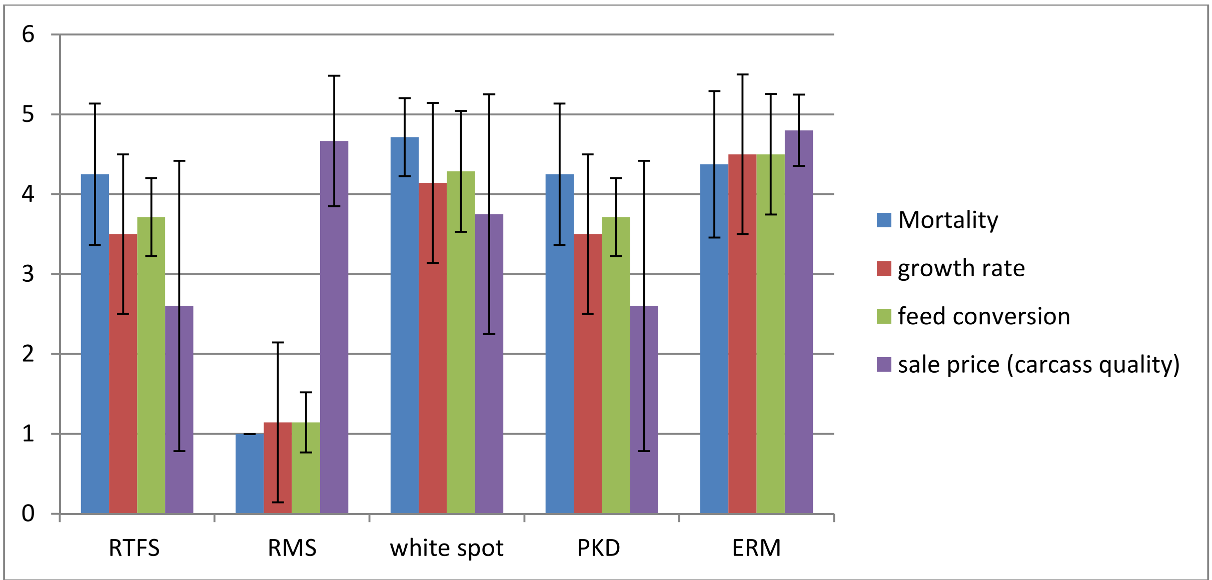

Figure 8 Estimated impact of the 5 main diseases identified as a concern to producers and health professional, if the main available control strategies were not available. Respondents were asked to provide an estimate of impact from 1 (no impact) to 5 (very severe/devastating impact) if the control measures were not available on mortality, growth rate, feed conversion and sale price. Responses are drawn from those producers and health professionals that identified the diseases as a particular concern.

As can be seen, white spot disease, RTFS, ERM and PKD were all predicted to cause devastating (business viability threatening) losses through greatly increased mortality, reduced growth rate and carcass quality (hence sale price). All of the producers determined that not treating RMS had negligible impact on mortality, growth rate or feed conversion but there was significant impact on sale price, due to processors rejecting affected fish. For those producers unable to manage the disease by holding fish back and ongrowing them, estimated impact on sale price was very high due to likely rejection by processors.

5 Main treatments

5.1 Florfenicol

The florfenicol containing product Florocol™ is the treatment of choice to control rainbow trout fry syndrome, caused by the bacterium *Flavobacterium psychrophilum*, in rainbow trout hatcheries in Scotland and England. Respondents generally reported the treatment is effective for control of RTFS in fry and juveniles. All respondents were using florfenicol as recommended per label (in feed at 10 mg kg/bw for 10 days). Although florfenicol is licensed for use in Atlantic salmon, it can be used to treat trout under the veterinary cascade (http://www.vmd.defra.gov.uk/pdf/vmgn/VMGNote13.pdf) as there is no effective alternative medicine. When treating trout, a minimum 500 degree day withdrawal period needs to be applied. This is practically of limited concern as the disease typically affects small fry and juvenile fish.

5.2 Other antimicrobials: oxytetracycline, amoxicillin and oxolinic acid

Other licensed antimicrobials are mainly used in the industry to treat septicaemic Gram negative bacterial infections, such as furunculosis and enteric redmouth disease (Table 3. Use is typically guided by review of antimicrobial sensitivity data. Veterinarians and health professionals generally expressed a preference for oxolinic acid, despite the requirement for a Special Imports Certificate from the VMD (https://www.vmd.defra.gov.uk/sis/default.aspx/).

5.3 Pyceze Ē

Use of Pyceze™ to control saprolegnia infections in rainbow trout hatchery and production systems was very limited Table 3). In contrast, Atlantic salmon hatcheries reportedly use Pyceze™ quite widely, particularly for the control of post-vaccination saprolegniasis. Respondents cited cost as an issue, and also questioned whether Pyceze™ was an effective control for the fungal infections affecting rainbow trout farmers.

Table 3 List of treatments currently used by producers and vets/health professional questioned

Treatments	Regulatory status	Diseases used to control (life stages)	Number of producers (x/8) and health professionals questioned (x/3) that used or recommended treatment	Dosing , duration and number of treatments	Reported effectiveness of treatment
Formalin (from wholesalers)	Marketed as a biocide but not listed under BPR	White spot (fry-100g juvelines), costia(fry-100g juvelines), other external parasites (fry-100g juveniles) , Saprolegnia (eggs and broodstocK)	8/8 3/3	200-300 ppm 30-60 min	>90% Effective (8/8 producers) >90% effective (1 of two vets) 70-90% effective (1 x vet and 1 x health professional)
Chloramine T (many products)	Biocide	Bacterial gill disease/ 'gill health' treatment	7/8 3/3	2-25 ppm 30-60 min	70-90% effective (6/7 producers) 30-70% effective (1/7 producers; 3/3 vets and health professionals
Benzalkonium chloride (Bac 50 and other products)	Biocide	Bacterial gill disease/ 'gill health' treatment	3/8 3/3	2-6mg/l for 30-60 min (most commonly 2 mg/l)	70-90% effective (3/3 producers; 2 x vets) 30-70% effective
Salt (sodium chloride)	Allowed substance (listed in EU Regulation 37/2010 Table 1)	White spot 'Health' (osmotic) support RTGE (in feed)	1/8 3/3	Variable : 1-10 ppt continuous for 1-10 days 1%-2% short flush (30-60 min)	Not enough responses to determine effectiveness for different conditions 'treated'

Treatments	Regulatory status	Diseases used to control (life stages)	Number of producers (x/8) and health professionals questioned (x/3) that used or recommended treatment	Dosing , duration and number of treatments	Reported effectiveness of treatment
Florfenicol (Florocol™)	UK Licensed veterinary medicine for use in salmon for control of furunculosis	RTFS (all producers), ERM & Furunculosis (limited use)	8/8 3/3	10 mg/kg bw daily for 10 days	>90% Effective (8/8 producers) >90% effective (1 of two vets) 30-70% effective (1 x vet and 1 x health professional)
Oxolinic acid (Branzil Vet™)	POM Not licensed in UK. Licensed veterinary medicine for control of trout diseases in other European MS (Denmark; listed in EU Regulation 37/2010 Table 1)	ERM, furunculosis	2/8 2/3	10 mg/kg bw daily for 10 days	70-90% effective (1 vet and 1 health professional surveyed)
Oxytetracycline (Aquatet™)	POM UK Licensed veterinary medicine for use in furunculosis due to *Aeromonas salmonicida* and columnaris disease in Atlantic salmon, and furunculosis and enteric redmouth disease in Rainbow trout.	ERM, furunculosis	2/8 2/3	75mg/kg bw daily for 10 days	70-90% effective (1 vet and 1 health professional surveyed)

Treatments	Regulatory status	Diseases used to control (life stages)	Number of producers (x/8) and health professionals questioned (x/3) that used or recommended treatment	Dosing , duration and number of treatments	Reported effectiveness of treatment
Bronopol (Pyceze™)	POM UK Licensed veterinary medicine for use in salmon and trout	Saprolegnia in eggs and broodstock; gut fungus	0/8 2/8	Eggs: 50 mg bronopol/litre for 30 minutes daily until hatch Fish: 20 mg/l daily 30 min n bath, up to 10 days	>90 % effective (gut fungus; vet (n=1) and Scottish producers who had used it (n=2)
Amoxicillin (Vetremox™)	POM UK Licensed veterinary medicine for use in salmon for control of furunculosis	Furunculosis/ other Gram negative septicaemias . Ocassionally for RTFS	1/8 2/3	80 mg kg/bw day for 10 days	Occasionally prescribed/. Recommended by a vet and health professional. 30-70% effective Not reported as effective as other antibiotics generally 30-70%

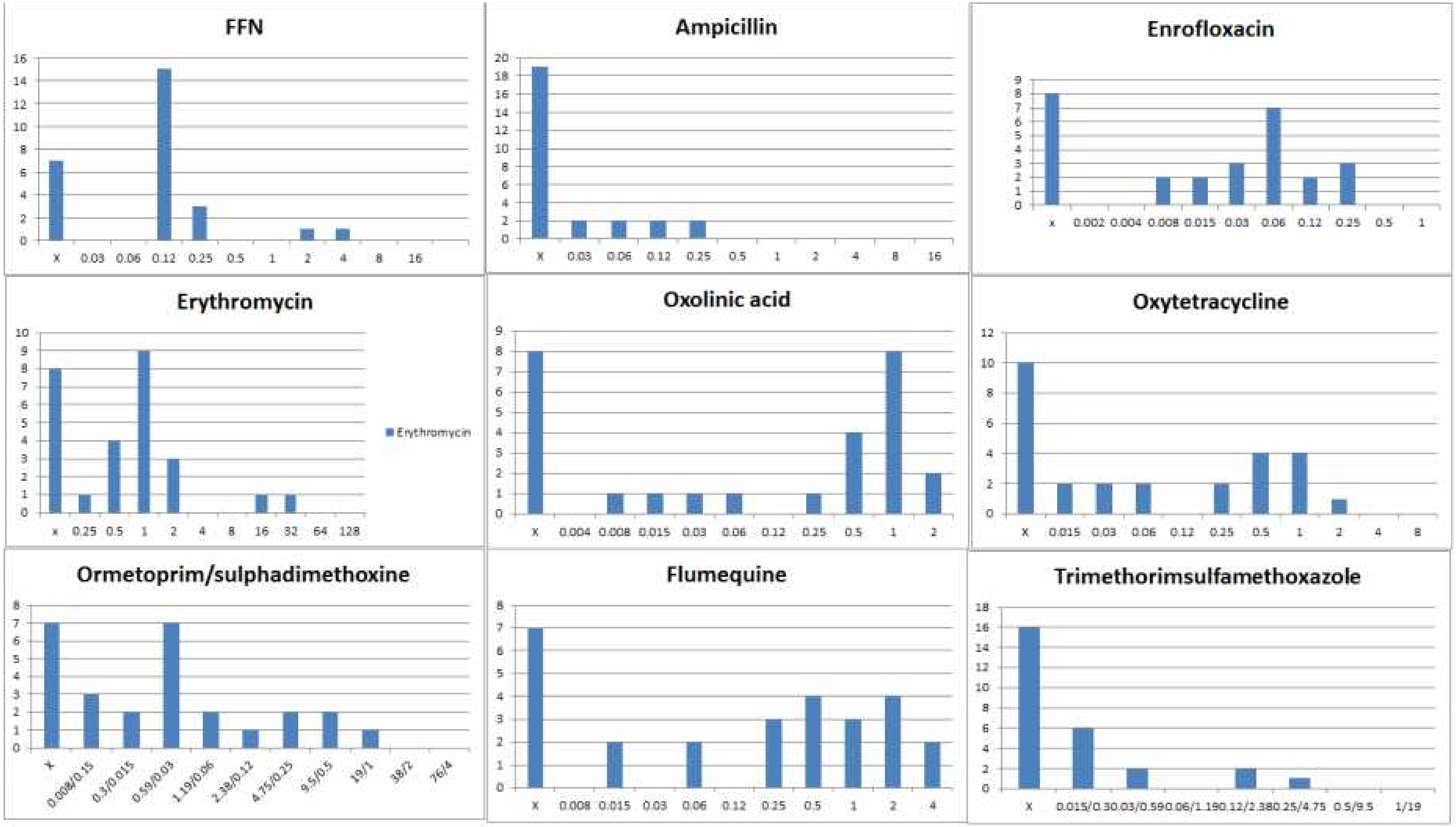

Figure 9 Minimum inhibitory concentrations (mg/l) of nine antimicrobials determined for 25 *F. psychrophilum* isolates recovered from UK rainbow trout and salmon using a broth microdilution testing method from (Chadwick, 2012). For each antimicrobial tested, the vertical Y axis indicates the numbers of isolates that had a particular MIC, as indicated in the X axis. Isolates in the column marked 'X' on the horizontal axis had MIC levels below the lowest concentration tested.

5.4 Formalin

Formalin is an aqueous solution of 37-40% formaldehyde gas and 6-13% (12%) methane (that prevents polymerization and the formation of paraldehyde, which is toxic to fish. Formalin was widely used by all the respondents to control an important range of disease problems. These included white spot, bacterial gill disease and a range of other ectoparasites (particularly costia, microcotyle and flukes such as trichodina).Reasons for its use were its reported high efficacy against the main diseases indicated, coupled with its cheap relative cost and its relatively high margin of safety. The high margin of safety is reflected in the wide range of dosing and application rates quoted by the respondents. Formalin is effective both as a bath or dip treatment for fry and older fish, but is also a good egg treatment for control of saprolegnia. One respondent reported that when he treated eggs with formalin they were visibly cleaner after the treatment and were reportedly more easily transported. This was in contrast to treatment with others chemicals (eg Pyceze), where the eggs were reportedly quite 'sticky' after treatment.

5.4.1 Likely continued availability of formalin

Formalin is typically sourced from wholesalers as a biocide and its use is not directed by veterinarians (it is not a licensed medicine). There are concerns over the likely continued availability of formalin. Although its potential environmental risks are considered to be limited (US FDA, 1995), a number of studies have raised concerns over its reported safety to workers. This has culminated in the production of a report by the US National Toxicology program that states formalin is 'known to be a carcinogen' based on sufficient evidence of carcinogenicity from studies in humans and supporting data on mechanisms of carcinogenesis (National Toxicology Prgram, 2011). The BPR has exclusion criteria (Article 5. 1a) that prohibit the authorisation of active substances 'which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, carcinogen category 1A or 1B'. However, there is some latitude in the regulations that allows a product containing an active substance to be approved that are referred to in Article 5. 1 (Article 5.2) where:

- The risk to humans, animals or the environment from exposure to the active substance in a biocidal product, under realistic worst case conditions of use, is negligible, in particular where the product is used in closed systems or under other conditions which aim at excluding contact with humans and release into the environment
- It is shown by evidence that the active substance is essential to prevent or control a serious danger to human health, animal health or the environment.

5.4.2 Use of formalin containing medicinal products in other EU Member States and internationally

As described above, formalin-containing products used in the UK rainbow trout industry are typically marketed for use as biocides. At the present time, there is no product with formalin as its main active ingredient that is marketed for use as a veterinary medicinal product in the UK. There is a product, Aquacen (http://www.cenavisa.net/acuicultura), that has an MA from the Spanish authorities (2127 ESP) for the control of disease in turbot, *Psetta maxima*, caused by ectoparasite *Philasteridis dicentrachi*. A veterinarian may be able to prescribe under the cascade a veterinary medicinal product (VMP) not authorised in the UK, but authorised in another Member State (MS) for use, in this case any food producing animal species, in accordance with an import certificate issued by the VMD (https://www.vmd.defra.gov.uk/sis/sic-application.aspx). This would be to avoid causing unacceptable suffering, where there is no suitable veterinary medicine authorised in the UK to treat a condition, or veterinary medicine authorised in the UK for use in another animal species or for a different condition in the same species.

http://www.vmd.defra.gov.uk/pdf/vmgn/vmgnote13.pdf

Of less direct relevance, there are also three formalin containing (37% by weight of formaldehyde gas in water) licensed products available in the US to treat ectoparasites in freshwater fish species, including rainbow trout. Information on these products and their approvals can be found on the FDA website.
http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/ucm132954.htm

In summary, all three approvals cover use of these products in the US for the control of (a) external protozoa (Chilodonella spp., Costia spp., Epistylis spp., Ichthyophthirius spp., Scyphidia spp. and Trichodina spp.), and the monogenetic trematode parasites (Cleidodiscus spp., Dactylogyrus spp., and Gyrodactylus spp.) on all finfish, (b) fungi of the family Saprolegniaceae on all finfish eggs and(c) external protozoan parasites (Bodo spp., Epistylis spp., and Zoothamnium spp.) on penaeid shrimp.

For actual treatment of external parasites, the labels recommend using 1 hour maximum exposures to up to 170 mg/l for treatments over 50°F (=) and up to 250 mg/L for treatments less than 50°F . Important safety and efficacy data used to support the licensing of these formalin products was obtained under the US Aquatic Animal Drug Approval Partnership (AADAP) Program http://www.fws.gov/fisheries/aadap/home.htm.

5.5 Chloramine T

The chlorine releasing biocide Chloramine T (Tosylchloramide or N-chloro tosylamide) is widely used by farmers to control a range of conditions, in particular bacterial gill disease. Chloramine T is typically applied as a bath treatment of 4-25 ppt for 30-60 min. There is data demonstrating it is highly effective *in vitro* against bacteria at concentrations greater than 0.1% (Verner – Jeffreys *et al*., 2009). The product was less effective against tested viral pathogens, with greater than 3% concentrations required to inactivate infectious pancreatic necrosis virus (IPNV). In the UK a range of biocides containing chloramine T are marketed and can be easily obtained from Agricultural suppliers

Chloramine T is registered under the biocidal products regulations and there is unlikely to be any immediate threat to its continued availability to aquaculture producers for biocidal applications. A Chloramine T product is also marketed by Vetark Professional (GMP Registration SAM0009 and WDA number 2014166), for use in aquarium fish species for control of Myxobacteria and other waterborne pathogenic bacteria, Ichthyobodo (Costia), and white spot, under the VMD small animals exemption scheme (http://www.vmd.defra.gov.uk/pdf/vmgn/VMGNote12.pdf).

5.6 Benzalkonium chloride

Benzalkonium chloride is a quaternary ammonium compound (a nitrogenous mixture of alkylbenzyldimethylammonium chlorides of various even-numbered alkyl chain lengths). It is typically sold and used for biocidal applications, having activity against bacteria and viruses. A number of the producers surveyed used benzalkonium chloride, typically at about 2 mg/l for up to 30-60 min. The health professional and vets questioned confirmed it is widely used through the industry, particularly as an alternative treatment for the control of bacterial gill disease. It was noted that, as it can be toxic at higher concentrations than the 2-3 mg/l typically applied, it had to be used carefully.

5.7 Vaccines

The use of vaccines on freshwater farms was largely limited to use of commercial ERM vaccines and some use of dip control against furunculosis on some sites. There was reported use of autogenous rainbow trout fry syndrome vaccines (based on *Flavobacterium psychrophilum* isolates recovered from the premises), although reports on their effectiveness were mixed.

Hatcheries rearing rainbow trout for seawater transfer were also vaccinating their fish with furunculous and *Vibrio anguilarum* vaccines prior to seawater transfer.

6 Alternative treatments

6.1 Alternatives to florfenicol for treatment of RTFS

6.1.1 Vaccines

Unfortunately there is no readily available vaccine to prevent RTFS. Farmers that have used them have reported variable results with autologous vaccines. Much more work is required to identify effective vaccines able to provide protection against the range of *F. psychrophilum* (and other Flavobactericeae) causing disease problems in farmed rainbow trout and freshwater phase salmon. Reported issues constraining effective vaccine development are, firstly, a lack of complete understanding on the antigenic properties of the range of Fp isolates circulating within the industry. Secondly, the disease can affect very small fish that have not obtained full immunocompetance, thus presenting a further challenge to effective vaccination. Further information is needed if effective vaccines, that provide a full protection against the types of *F. psychrophilum* isolates, are to be developed.

6.1.2 Other antibiotics

Respondents reported that the efficacy of alternative antibiotic treatments (oxytetracycline and oxolinic acid) was questionable. Work is needed to determine the sensitivities of the strains of *F. psychrophilum* circulating in UK farmed rainbow trout. Initial data from a survey of 26 recent *F. psychrophilum* isolates suggests that UK isolates display a non-wild type phenotype (reduced sensitivity) to oxolinic acid and oxytetracycline, correlating with a lower reported field efficacy of these treatments (Figure 9). The limited range of isolates tested did appear to be sensitive to ampicillin. It is highly likely that such isolates would also be sensitive to amoxicillin (another beta lactam antibiotic closely reacted to ampicillin). Amoxicillin is an available antibiotic with the same regulatory status as florfenicol (licensed for the treatment of Atlantic salmon, but can be used under the cascade in rainbow trout, if there is no effective rainbow trout-licensed product available). Further work is needed to confirm whether amoxicillin may represent a viable alternative antimicrobial treatment for RTFS to florfenicol.

6.1.3 Other treatments

Other alternatives to antibiotics and vaccines for control of RTFS are being investigated. There have been some promising preliminary studies involving use of phage therapy (Lone Madsen, Bertelsen, Dalsgaard, & Middelboe, 2013). However these studies are still, only at a very preliminary stage.

6.2 Alternatives to formalin for treatment of white spot

The main use of formalin identified in the survey was for control of white spot disease (5.4). However, with the regulatory uncertainty currently surrounding the uses of formalin, urgent alternatives are now required to control this important disease.

There has been a considerable body of work internationally to identify alternatives to control white spot in food producing species, such as rainbow trout. This was originally driven by the search for alternatives to malachite green, a previously used effective treatment for control of all the life stages of *I. multifillis* (Alderman, 1985) that is now banned. This work has continued, driven both by concerns as to the toxicity of formalin to operators, and also because, unlike malachite green, formalin at 200-250 ppm is only able to inactivate the free living stages of *I. multifiliis*.

Available data on the effectiveness of drug and non drug interventions in the treatment of white spot have been thoroughly reviewed recently (Picón-Camacho, Marcos-Lopez, Bron, & Shinn, 2012). Picón-Camacho *et al* provide a detailed list of 116 different compounds used to control *I. multifiliis* under laboratory or field conditions. The treatments listed below are those, relatively limited in number, identified by Picón-Camacho et al. (2012) and Taylor (Annex 1) as having some potential as effective alternative treatments of white spot to formaldehyde. Their likely practicality for use in UK farmed rainbow trout production systems is considered.

6.2.1 Salt

Salt (sodium chloride) is a readily available cost-effective chemical that is generally recognised as safe for application to fish, and has very limited consumer or operator safety issues. Some farmers and health professionals already increase the salinity of their system to combat certain issues. Although none of the producers questioned in this survey used salt to specifically control white spot (all preferring to use formalin at the present time), it is understood that it is widely used in other countries for this purpose, being the second most widely used treatment after formalin (Picón-Camacho et al., 2012). A number of authors have demonstrated that salt at varying concentrations is an effective white spot control option (reviewed by Picon *et al*. 2012). These are briefly summarised below

6.2.2 Salt: continuous dosing

Shinn *et al*. showed that, *in vitro*, application of 5-10g/l NaCl for 24h resulted in >95% reduction of theronts (free swimming stage) while 2.5 g/l was partially effective (approximately 50% mortality). However, trophonts of *I. multifiliis* were relatively resistant to even high concentrations of NaCl with no mortality observed at up to 15g/l for up to 10 h. At 20g/l for 10 h, all theronts were killed but this is a very high concentration of salt to maintain in any normal freshwater system.

There have also been a range of studies where fish were exposed to concentrations of salt from 1-5g/l for much longer periods (7-45 days), with often encouraging results (Picón-Camacho *et al*., 2012).

6.2.3 Salt: short duration dosing

There are also some studies where fish are exposed to much higher concentrations of salt for shorter periods of time. Although not particularly effective against the trophonts, theronts may well be sensitive to short applications of greater than 20g/l for up to 60 min. In terms of effectiveness, these results compare favourably to the effectiveness of therapeutic concentrations of formalin (200-250ppm).

6.2.4 Issues with regards use of salt to control white spot

There are some important issues which would need to be resolved that may prevent wide scale adoption of salt as an alternative white spot treatment. Firstly, most systems are not readily adaptable for applications of salt (either maintenance of levels greater than 2g/l for extended periods or high concentrations (>20g/l) for short periods, followed by rapid flushing). Secondly, there may be issues with regards agreed SEPA and EA discharge consents, as release of high salinity water from a point source farm discharge into a freshwater river system could pose some environmental risks. However, remediation via initial discharge of the high salinity raw farm effluent into a reed bed/wetland area on the farm may mitigate such potentially adverse impacts.

6.2.5 Benzalkonium chloride

Benzalkonium chloride was identified by both vets and the health professional surveyed as a treatment used on occasion for control of bacterial gill disease. However it is unlikely to be particularly effective for the control of white spot disease at concentrations that are not toxic to the fish, so it is not recommended for further investigation.

6.2.6 Bronopol

A bronopol-containing medicine, Pyceze™, is marketed in the UK by Novartis for

- the prevention of growth of fungal infections (Saprolegnia spp) in the face of suspected or known challenge in farmed Atlantic salmon eggs and rainbow trout eggs.
- prevention or reduction of fungal infections (Saprolegnia spp) in farmed Atlantic salmon and rainbow trout kept in fresh water.

Few of the rainbow trout farmers used bronopol-containing medicines in their operations for control of saprolegnia or other diseases. Where saprolegnia was a problem for egg producers, farms typically picked affected eggs and/or treated with formalin. Pyceze™ may have a role for the control of white spot though. Promising work has been done by Shin and co workers (A. P. Shinn et al., 2012) that demonstrates that bronopol concentrations of 20 mg/l were effective *in vitro* for the control of *I. multifiliis* protomonts. They also showed that long, low dose (1 mg/l) exposure to bronopol was also efficacious against theronts. Survival after 12 h was 29% (c.f. 100% in control parasites), and <1% after

24 h exposure (c.f. 74% in control parasites). Theronts surviving these exposures demonstrated reduced infection success compared to control theronts. None of the farmers or health professional interviewed had used or prescribed Pyceze for the control of white spot to this time so reports of field effectiveness are otherwise limited.

6.2.7 Potassium ferrate

Potassium ferrate (K_2FeO_4) has been identified as a potentially effective white spot treatment in a recent review (Picón-Camacho *et al*., 2012). It is a strong oxidising agent which has non toxic break down products (FeIII and oxygen). Recent work has shown that 4.8 mg/l potassium ferrate (VI) for 2 hours was very effective *in vitro* in killing theronts and, when applied continuously over 3 days *in vivo*, caused an 80% measured reduction in the numbers of trophonts on the test fish. A dose of 19.2 mg/l for 3 days resulted in complete clearance of infection in treated gold fish. It has been identified as a chemical for use in waste water treatment applications, due to its reported high stability, strong oxidising power and limited environmental impact. However, to this author's knowledge, no potassium ferrate product is marketed for use as a biocide in the EU at the present time and it is not listed as an allowed substance under EU Regulation No 37/2010 Table 1. This, practically, means it cannot be purchased, or used, at the present time for control of white spot infections or biocidal applications. There may also be some stability issues with such a highly reactive compound.

6.2.8 Praziquantal

Under the small animals exemption scheme, in the UK, VETARK Professional market a product containing 50% praziquantal for control of treatment of skin and gill flukes as well as tapeworms in ornamental fish species. This water soluble product is designed to be administered by immersion at a rate of 4 grams of product (2 grams of praziquantel) per 1000 litres of pond or tank water. Discussion with veterinarians suggests it is at least, if not probably more, effective than using formalin for this purpose. There may also be scope to prescribe praziquantal for use in food producing species, such as rainbow trout, under the cascade, as it is an allowed substance in another food producing species (Ovidae) under EU Regulation No 37/2010 Table 1b, with no MRL set. It is likely to be a more expensive treatment option than using formalin purchased from a biocide wholesaler, but it is not known how it would compare in terms of cost effectiveness to using a licensed formalin containing product.

6.2.9 Copper sulphate

Copper sulphate has been used in the past to control, white spot and other diseases (Darwish, Bebak, & Schrader, 2012; Griffin & Mitchell, 2007; Tieman & Goodwin, 2001; Williams & Wootten, 1981). Although it is still available to use an algicide for swimming pools and other applications, its use under the Biocidal Products Regulations is under scrutiny, with special derogations issued under those regulations for use of copper-containing products for specialised applications. Concerns over the potential toxicity of copper products being discharged into the environment have also resulted in environmental quality Standards being set for copper at the UK (national) level. It is also listed under the Water Framework Directive (EU, 2000) Annex VIII. For these reasons, use of copper sulphate is not likely to be a viable alternative treatment option.

6.2.10 Peracetic acid

Peracetic acid is being recommended as an alternative to formalin for control of whitespot and other parasites in a range of countries, particularly in Denmark. There is evidence from the peer-reviewed literature that peracetic acid has the potential to control parasite infections, including infective *I. multifiliis* theronts and newly settled reproductive tomonts at PAA levels below 1.0 mg/l PAA (Pedersen *et al*. 2013). The results of field and *in vitro* studies investigating the effectiveness of peracetic acid for the control of ectoparasitic disease problems are summarised by Pedersen *et al*. 2013, to which the reader is referred.

PAA is typically applied as a bath treatment to systems. Peracetic acid-containing products are available for purchase as biocides from a number of suppliers. However, reports of PAA effectiveness in Danish and other farms are mixed, with some producers suggesting it is highly effective and a good replacement to the use of formalin, with others reporting only low efficacy. This is likely related to the low half life of peracetic acid in typical aquaculture systems, particularly where levels of organic loading are high (e.g. application of PAA in earth pond systems and in outdoor recirculating systems with low water exchange and without adequate solids removal). Pendersen *et al* demonstrate experimentally how increasing organic matter content significantly facilitated PAA decay, with half lives of less than a few minutes demonstrated (Pedersen, Meinelt, & Straus, 2013). This is likely why applications of peracetic acid were less effective in earth ponds than in concrete raceways (Rintamäki-Kinnunen, Rahkonen, Mykrä, & Valtonen, 2005; Rintamäki-Kinnunen, Rahkonen, Mannermaa-Keränen, et al., 2005).

Although effective against the free living stages of *I multifiliis*, PAA is not particularly effective against the settled tomont stages. Thus, the control of this non synchronous multi-life stage parasite requires repetitive PAA applications. As the safety margins for use of peracetic acid are relatively low, care also has to be taken to ensure the effective treatment concentrations of PAA in the systems are maintained below levels toxic to the fish. Current practice among some Danish fish farmers is the use of prophylactic application of PAA 3 times a week with an expected nominal concentration of 2.5–3.0 mg/l ((Pedersen *et al*., 2013).

A recommendation from Danish researchers interviewed is that better systems are needed to measure PAA consumption in treated systems, so application rates can be better managed. The Danish producers interviewed as part of this project now routinely use peracetic acid in control of white spot in their system, typically applying it in combination with formalin at the present time.

There are also reports that Scottish salmon smolt producers are experimenting with peracetic acid for the control of saprolegnia infections. Again, they report mixed results and could benefit from better information on how PAA can be best applied.

In summary, PAA is very likely to be a useful treatment for the control of both white spot, ectoparasites and saprolegnia. However, compared to formalin, its use needs to be very carefully tailored to the aquaculture system concerned. Particular attention needs to be paid to the possible effects of organic loading that may significantly affect efficacy by reducing the half life of the active chemicals. Advice from Danish producers with the most experience of applying peracetic acid is to gradually increase the levels used in a new system to ensure any adverse effects are minimised.

6.2.11 Hydrogen peroxide

Hydrogen peroxide, H_2O_2 is a strong oxidising agent that is used in the healthcare setting for cleaning wounds and other applications. Similar to peracetic acid, it reacts to produce water and oxygen, thus its break down products are non toxic. Hydrogen peroxide is used to control ectoparasites, particularly sealice in the marine salmon industry (Aaen, Aunsmo, & Horsberg, 2014; Bruno & Raynard, 1994; McAndrew, Sommerville, Wootten, & Bron, 1998; Treasurer & Grant, 1997). There is now an authorised product for control of sea lice containing hydrogen peroxide (Paramove; Solvay). In the US there are also hydrogen peroxide products authorised for use for control of fungi on all stages of fish under their FDA-approved new animal drug application with the trade name 35% PEROX-AID. Hydrogen peroxide may be considered for control of other external parasites as an alternative to formalin, although its effectiveness against white spot is not reported as particularly high. It may also be a viable alternative to chloramine T for control of bacterial gill disease caused by *Flavobacterium branchophilium* and other organisms, based on the USDA approvals for PEROX-AID (Table 4 FDA-approved dosages for 35% PEROX-AID® (35% weight/weight hydrogen peroxide).

Table 4 FDA-approved dosages for 35% PEROX-AID® (35% weight/weight hydrogen peroxide)

Fish Species and Life Stage	Target Disease Organism	Dosage Rate	Duration (min)	Frequency
All freshwater-reared cold- and coolwater finfish eggs	*Saprolegnia*	500–1000 mg/L	15	Once per day or on alternate days until hatch
All freshwater-reared warmwater finfish eggs	*Saprolegnia*	750–1000 mg/L	15	Once per day or on alternate days until hatch
Freshwater-reared salmonids	Bacterial gill disease (*F. branchiophilum*)	100 mg/L in continuous flow or static bath	30	Once every other day for three treatments
Freshwater-reared salmonids	Bacterial gill disease (*F.branchiophilum*)	50–100 mg/L in continuous flow or static bath	60	Once every other day for three treatments
Freshwater-reared coolwater finfish fingerlings and adults	Columnaris (*F.columnare*)	50–75 mg/L in continuous flow or static bath	60	Once every other day for three treatments
Freshwater-reared coolwater finfish fry	Columnaris (*F.columnare*)	50 mg/L in continuous flow or static bath	60	Once every other day for three treatments
Channel catfish fingerlings and adults	Columnaris (*F. columnare*)	50–75 mg/L in continuous flow or static bath	60	Once every other day for three treatments
Channel catfish fry	Columnaris (*F.columnare*)	50 mg/L in continuous flow or static bath	60	Once every other day for three treatments

6.2.12 Chloramine T

As described earlier, the organic chlorine compound chloramine T is already widely used within the industry to control bacterial gill disease. There are some reports that it may also be effective for the control of white spot. Shinn and co-workers showed it was effective *in vitro* against both protomont and theront stages of I multifiliis (A. P. Shinn, Wootten, Somerville, & Conway, 2001)(Picón-Camacho et al., 2012). *In vivo*, however, it had to be applied at high concentrations (e.g. 100 mg/l for 30 min over 10 days) to be effective (Picón-Camacho et al., 2012). Continuous repeated dosing with high doses of chloramine T is likely to have toxic effects on the gill epithelia and may have other toxic effects (Picón-Camacho et al., 2012). For instance (Powell & Harris, 2004) showed that the average lethal time (LT50) for a dose of 50 mg/l was 167 min in freshwater Atlantic salmon juveniles. Histopathological changes have been observed in *I. punctatus* exposed to 80 mg/l chloramine T for 3 h. (Picón-Camacho *et a*l., 2012) recommend that future work should explore the efficacy of using 30 min baths of Chloramine T, at varying time between treatments intervals, from 30 to 80 mg/l over the full course of the parasite life cycle (e.g. 10 days at typical UK ambient temperatures).

As well as chemicals identified by Picón-Camacho *et al*., 2012, further chemicals were also short-listed for further review, based on previously conducted literature reviews by Cefas staff: Caprylic acid, green tea extract, piscidin 2, quinine and triclabendazole. Patent searches conducted for all five of these chemicals identified patents for each, relating to their use in the treatment of pathogens. The existence of such patents may require further investigation should these chemicals be demonstrated to have efficacy against *I. multifiliis* infections.

6.2.13 Caprylic acid

Caprylic acid is a medium chained fatty-acid also known as octanoic acid. It is naturally occurring, found in coconut oil and known to have antifungal properties. It is only slightly soluble in water. Several medium chain fatty-acids have been shown to have anti-parasitic properties, for example dodecanoic acid is effective at reducing the burden of infections with the human pathogen Giardia duodenalis(Rayan, Stenzel, & McDonnell, 2005)). Caprylic acid has been tested against several fish parasites (Fajer-Ávila, Velásquez-Medina, & Betancourt-Lozano, 2007)(Hirazawa, Ohtaka, & Hata, 2000)(Hirazawa, Oshima, Mitsuboshi, & Hata, 2001), including *I. multifiliis* and its marine equivalent Cryptocaryon irritans(Hirazawa, Oshima, Hara, Mitsuboshi, & Hata, 2001; Hirazawa, Oshima, & Hata, 2001).

N. Taylor *et al.* (unpublished data) demonstrated a 64% reduction in the number of trophonts infecting fish treated for 8 days post infection with a blend of caprylic acid and citrus extract top coated onto a commercial feed and fed at a dose of 10mg/kg B.W./day. This work also suggested that this treatment had the potential to reduce the parasite burden if used as a prophylactic treatment fed prior to infection. Unfortunately the final results were inconclusive due to variability in the initial infection levels between treatment and control tanks. Taylor *et al.* also found that this formulation was not suitable for use as a bath treatment due to its lack of solubility in water.

Hirazawa *et al.* (2001b, c) tested the efficacy of caprylic acid against *C. irritans* as both a bath and an in-feed treatment. As a bath treatment, a 1mM solution was found to kill 100% of the infective theronts. No indication was given as to whether a suitable solvent was used to make the caprylic acid soluble in water, nor was it stated whether such a carrier was used independently against the control theronts. As an in-feed treatment, doses of 37.5 and 75 mg/kg B.W./day led to a significant reduction in trophont numbers at 17°C. A dose of 75 mg/kg B.W./day gave a significant reduction at 24°C. The data from these trials appeared reliable; however, the in-feed treatments were started 5 days prior to infection, so it is not clear whether prophylactic treatment is required to control the parasite or whether treatment post infection is sufficient.

Caprylic acid appears to be efficacious against a number of different aquatic parasites. Its effectiveness as a bath treatment is, however, called into question due to its lack of solubility in water.

This compound does appear to have potential as an in-feed treatment and appears to have a wide efficacious range and safety margins for use in fish. In vivo trials are recommended to establish the effectiveness of caprylic acid on its own against *I. multifiliis* and whether it is necessary to have a period of prophylactic treatment in order for it to be effective. Furthermore a suitable, non-harmful carrier would need to be identified.

6.2.14 Green tea extract and epigallocatechin gallate (EGCG)

The antiparasitic effects of green tea (Camellia sinensis) extract and many of its component parts have been the subject of much study in recent years. Green tea extract and its various component parts have proven their efficacy in the control of helminth (Molan, Sivakumaran, Spencer, & Meagher, 2004) coccidian (Jang et al., 2007) and protistan (Güida et al., 2007; Paveto et al., 2004; Suzuki, Misaka, & Sakai, 2006) parasites as an in-feed additive or as a bath treatment. Of the components of green tea extract, epigallocatechin gallate (EGCG) appears to be the most active in terms of its antiparasitic properties. This component has also been the subject of significant safety testing (Isbrucker, Bausch, Edwards, & Wolz, 2006; Isbrucker, Edwards, Wolz, Davidovich, & Bausch, 2006a, 2006b)which has demonstrated no adverse effects at doses in excess of 500mg/kg/day in rodents or mammals.

Only one peer-reviewed scientific study appears to have been conducted to look at the efficacy of green tea based diets in controlling parasites. This was conducted by Jang *et al.* (2007) on a coccidian parasite of chickens, Eimeria maxima. In this study, standard feed was supplemented with either 0.5 or 2% green tea extract and fed to chickens for two weeks prior to infection. The results showed a significant reduction in parasite oocysts being shed at both doses, and suggests that green tea extract had a prophylactic effect.

Green tea or its components have been tested against several different endoparasites. Paveto *et al*. (2004) found doses as low as 0.12pM of EGCG added to blood infected with Trypanosoma cruzi caused over 50% lysis of the parasite. Guida *et al*. (2007) produced similar results in *in vitro* trials but also demonstrated through *in vivo* trials that the survival of infected mice could be increased from 11% to 60% through daily intraperitoneal treatment with EGCG at a dose of 0.8mg/kg/day.

Bath treatments with EGCG have been shown to immobilise the infective larvae of the sheep nematodes Teladorsagia circumcincta and Teladorsagia colubriformis at doses above 100ug/ml (Molan *et al*., 2004). However, the most relevant research that gives insight as to the potential of green tea extract to control *I. multifillis* was by Suzuki *et al*. (2006) who studied the efficacy of green tea extract and EGCG on the protozoan ectoparasite of fish, *Ichthyobodo necator* infecting fry of chum and masu salmon. *In vivo* bath challenge experiments were conducted for both green tea extract and EGCG at both low dose long exposure, and high dose short exposure.

Doses of green tea extract up to 0.03% were found to induce little mortality in fish if used for up to 30 minutes (long) exposure. Doses of 2.7% for up to 5 minutes (short) exposure were also shown to cause little mortality. Long bath (30 to 60 minutes), low dose treatments of 0.03% caused a reduction in parasite burden of between 86.5 and 96.9%. High doses of 0.3% or greater used as a short bath for between 1 and 10 minutes were very efficacious reducing the parasite burden by between 88.1 and 100%. Baths of EGCG were also effective in treating the parasite with long baths of 0.0042% for 60mins reducing the parasite burden by 78.3% and 96.9% and short baths of 0.126% for 5 minutes reducing the burden by 83.5% and 100%. No toxicity data was given for EGCG and the upper safe limit is unknown. Furthermore, it is not clear whether long-term exposure to green tea extract or to EGCG is detrimental to host survival and this needs further investigation.

This is potentially one of the most exciting of the compounds discussed in this report, as it has the potential to be used as an in-feed treatment, in-feed prophylactic or bath treatment. As a bath treatment it is interesting as it has potential to be used as either a low dose, long bath or a high dose, short bath which makes it adaptable to different types of aquarium set-up.

The first step with this compound should be to test the efficacy of bath treatments in vitro of both green tea extract and EGCG against free-living stages of *I. multifiliis*. If efficacy is demonstrated at doses safe for fish, in vivo bath and in-feed trials are recommended.

6.2.15 Piscidin 2

Piscidins are a group of antimicrobial/antibiotic polypeptide produced by fish (Noga & Silphaduang, 2003). Piscidin production occurs predominantly in mast cells in fish of the suborder Percoidei (Silphaduang, Colorni, & Noga, 2006). Of the piscidins, piscidin 2 is regarded as being the most active and broad ranging in terms of its antimicrobial properties. Piscidin 2 was demonstrated by (Colorni, Ullal, Heinisch, & Noga, 2008) to reduce the survival of four aquatic parasites in *in vitro* trials. This included *I. multifiliis*, for which a dose of 6.3µg/ml was sufficient to kill all theronts within 10 minutes.

However, as piscidin 2 appears to have broad-spectrum antimicrobial properties, obtaining an authorisation for its use in the control of white spot in open aquaculture systems would likely be very challenging (as the potential environmental impact may be high). Furthermore, the likely high cost of obtaining these peptides would also be prohibitive. For these reasons, it is recommended that no further work is conducted on Piscidin 2 unless a cost effective method of mass production becomes available.

6.2.16 Quinine

Quinine is an alkaloid extract from the bark of the South American tree, *Cinchona officianalis* L. that has long been used as an antimalarial (Budavari, O'Neil, Smith, Henckelman. 1989). The efficacy of quinine at killing free-living aquatic protozoa has been demonstrated (Moreno-Garrido & Canavate, 2001). Currently there appear to be no licensed veterinary applications for quinine in the UK (Anon 2008), however, there are many grey sources advocating its use against aquatic protozoa affecting aquarium fish both in books and on the Internet.

Quinine is available in both soluble and insoluble forms. Many are degraded through exposure to light. Grey literature suggests that bath treatments in solutions of quinine salts are effective at controlling *I. multifiliis* infections and other aquatic protozoa, but to date no peer-reviewed research has been published (to the authors knowledge). Van Duijn (1956) suggests quinine hydrochloride at a solution of 1g per 30L is most effective, but that pH can influence its efficacy. Slightly acid waters were stated as being optimal. At doses higher than this damage to aquatic plants can occur. The salt solution should be added over a period of 1.5days. Treatment should last for 1 to 2 weeks. Van Duijn (1956) also states that long-term exposure to quinine can effect to fertility of fish, but provides no information on the research that resulted in this conclusion.

Much of the Internet based grey literature advocates the use of quinine sulphate over quinine hydrochloride, suggesting that it is less toxic to fish. A range of doses and durations of treatment are recommended on these websites, however, again no trial data was presented to show how effective the treatment is compared with untreated systems. Nor is there data showing the toxicity of either quinine hydrochloride or quinine sulphate to fish. Information from the Internet does suggest quinine is toxic to many invertebrates, especially gastropod molluscs. It is recommended that the use of carbon filters, UV sterilisation and protein skimmers be stopped during treatment with quinine, as these may prevent the compound being efficacious (presumably by removal or deactivation).

The only peer-reviewed studies of quinine being used in bath form against an aquatic protozoan parasite are that conducted by Iglesias *et al*. (2002) and Panko *et al*. 2008. Iglesias *et al*. (2002) found through *in vitro* trials that 100ppm bath of quinine sulphate killed the ciliated protistan fish parasite Philasterides dicentrarchi within 24 hours. No work was conducted to establish whether this dose was safe for use with fish. Panko *et al*. (2008) demonstrated that a concentration of 50µg/mL (0.13 mM) quinine hydrochloride significantly decreased the viability of six geographic isolates of *Perkinsus marinus* from oysters after three hours in vitro. Although the compound effectively decreased *P. marinus* viability, all concentrations tested decreased oyster haemocyte viability, and concentrations required to kill the parasite were lethal to infected oysters. Given the information available through fish keeping forums it seems likely that fish would be far more tolerant of treatment with quinine salts than molluscs.

Quinine has been demonstrated to have efficacy against I. multifiliis when used as an in-feed treatment. Schmahl, Schmidt and Ritter (1996) demonstrated that such treatments had significant pathological effects on the parasitic trophont stage and that long-term exposure at the doses used had no obvious toxicological effects on the fish tested. Although peer reviewed, this publication provides the reader with little information with which to make conclusions as to the most effective treatment regime. The paper does not state the form of quinine used, but just states it was a non-water-soluble formulation. Although a dose of 5g/kg feed was used, the fish were fed *ad libitum* making it difficult to determine the dose the fish actually received. The study suggests that in-feed treatment has potential as an effective treatment against *I. multifiliis*, but research is required to determine dosage and the best form of quinine to include in the feed, as water-soluble forms of quinine may not be suitable unless they can be encapsulated to prevent exposure to the aquarium water.

Quinine appears to have great potential in the control of *I. multifiliis* (and other aquatic protozoa). There is a need to formally test the efficacy of both quinine sulphate and quinine hydrochloride as a bath treatment against each stage in the life-cycle of the parasite. Preliminary *in vitro* trials to establish the minimum dose required to kill free-living stages of the parasite would prove a valuable starting point. If efficacious, the next step would be to conduct trials to determine toxicity to a range of common aquarium fish. An ideal compound for development would have high toxicity to the parasite and low toxicity to the fish, allowing a wide margin of safety in the applied dosage.

6.2.16.1 Triclabendazole

This is a benzimidazole derivative marketed under the trade name “Fasinax” for the control of the helminth liver fluke, Fasicola hepatica (Budavari, 2006) (Budavari, O’Neil, Smith, Henckelman. 1989).

The compound is thought to act on the parasite by affecting microtubial production. Triclabendazole has been tested against three aquatic ecto/endo-parasites: *Pseudodactylogyrus* spp., *Ichthyobodo necator* and *I. multifiliis*. (Buchmann & Bjerregaard, 1990) found that Triclabendazole has little or no efficacy as a bath treatment against *Pseudodactylogyrus* spp. at doses as high as 10mg/L.

Used as an in-feed treatment Triclabendazole appears to be efficacious in the control of to protozoan parasites *I. necator* and *I. multifiliis* (Luzardo, nez-Mazagatos, Santamarina, Otero-Espinar, & Blanco, 2003; Tojo & Santamarina, 1998)(Tojo & Santamarina 1998 and Luzardo-Alvarez et al. 2003). Tojo & Santamarina (1998) found that a dosage of 40g/kg feed at 2% body mass per day successfully removed all parasites from the sampled fish. A substantial reduction in the parasite burden was also noted in fish only treated for 5 days. No obvious toxic effects were observed in the rainbow trout hosts used at either dose. Luzardo-Alvarez *et al*. (2003) conducted a very comprehensive study that tested complexes of Triclabendazole and α-cyclodextrin in-feed against *I. multifiliis* infections in rainbow trout. They tested a range of different molar ratios from 1:1 to 1:3 of Triclabendazole: α-cyclodextrin at doses of 10 and 20g Triclabendazole/kg feed per day fed at a rate of 2% body mass per day for 10 days. Non-complexed Triclabendazole feed was also tested at a rate of 20 g/kg feed per day fed at a rate of 2% body mass per day for 10 days. The study showed Triclabendazole on its own did not reduce the parasite burden, however, the 1:3 complex led to a significant reduction of the parasite at both the 10 and 20g doses with no obvious harmful effects to the fish. This 1:3 complex was also shown to increase palatability and prevent the drug leaching into the water before pellets could be eaten.

The work conducted by Luzardo-Alvarez *et al*. (2003) is very comprehensive and suggested that complexed Triclabendazole can reduce the burden of *I. multifiliis* by between 43 and 58% at doses 10 and 20g/kg feed, respectively. This work did not, however, compare survival rates of the fish. It is recommended that a 1:3 Triclabendazole: α -cyclodextrin complex is taken forward for further in-feed trials to determine the maximum safe dose for ornamental fish species and the minimum efficacious dose required to reduce mortality caused by the parasite.

Research conducted by Buchmann & Bjerregaard (1990) suggested that Triclabendazole may not work well as a bath treatment and it is therefore recommended that bath challenge experiments are not attempted.

6.2.17 Mechanical and other management methods

As well as alternative chemical based control methods to formalin, for control of white spot and other diseases, there is also scope to considerably reduce the impact of these diseases by changes to management and other practices. In particular, white spot impact can be significantly lowered by reducing the stocking densities and temperatures at which fish are reared. Minimising live fish movements between sites can also reduce the incidence of diseases. Shinn and co-workers (Shinn, Picon-Camacho, Bawden, & Taylor, 2009) also demonstrated that white spot impact could be significantly reduced by employing a mechanical system developed to remove cysts from commercial trout raceways. This consisted of two parts: a specially designed suction head connected to a pump that is used to vacuum the bottom of hatchery raceways, and a low-adhesion polymer raceway lining. The mechanical system led to a significant reduction in the abundance of the parasite in test raceways. Additionally, fish survival was significantly higher in test raceways over control, with a mean of 84.5% of the stock surviving in the test raceways compared with only 70.6% in the controls by trial end.

7 Summary

The survey clearly demonstrates that the rainbow trout industry is heavily reliant on a very limited range of treatment options to control major production diseases.

Discussions with fish medicine producers and veterinarians also suggest that the freshwater stage of the Atlantic salmon industry is similarly reliant on a small range of similar treatments to those used in the trout industry. In particular, there is also heavy reliance on formalin to control white spot disease and costia in some hatcheries and similar reports that florfenicol is the only effective treatment for the control of *Flavobacterium psychrophilum*. They also report that formalin is used quite extensively to control saprolegniasis in vaccinated salmon smolts prior to seawater transfer.

These findings are collectively concerning as either the withdrawal of formalin from sale, or the development of resistance to florfenicol, in *Flavobacterium psychrophilum* could affect the viability of both industries.

7.1 Recommendations

1. Undertake further controlled studies (laboratory and field based) on the effectiveness of peracetic acid for the control of white spot and other production diseases.
2. Obtain further information on the margin of safety of peracetic acid at different temperatures via target animal safety studies, at both a farm and laboratory scale.
3. Continue to support efforts to develop alternative vaccines for the control of RTFS.
4. Determine the effectiveness of alternative antibiotics to florfenicol to control RTFS infections caused by *Flavobacterium psychrophilum*.
5. Explore practicalities of importing formalin-containing medicinal products licensed in other Ms for control of fish diseases for controlling white spot and other diseases.
6. Investigate use of mechanical control measures to reduce the impact of white spot in rainbow trout production systems.
7. *In vivo* trials are needed to follow up some of the potential alternative chemical treatments identified (e.g. caprylic acid, green tea extract and epigallocatechin gallate (EGCG), Piscidin 2, quinine, Triclabendazole and potassium ferrate).

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