Fish farm health evaluation:

interpretation of site mortality records

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BY

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Declaration

I declare that this thesis has been composed in its entirety by me. Except where specifically acknowledge, the work described in this thesis has been conducted by me and has not been submitted for any other degree.

Signature: _____

Signature of supervisor: _____

Date: _____

Abstract

In aquaculture worldwide, diseases are a significant constraint to economic expansion. The Scottish salmonid industry has experienced many cycles of development, with episodes of little or negative profitability caused by excess of production, and times of crisis due to different disease problems. In Scotland, the early implementation of regulation largely contributed to the control of infectious disease outbreaks. The recent Chilean outbreak of infectious salmon anaemia (ISA) illustrated the threats and the impacts of disease in the aquaculture industry and the importance of implementing good regulation and husbandry practices to reduce the impact of the spread of infectious disease.

Databases of site production data have an important role to play in the investigation and understanding of diseases. They store valuable data collected during the time of production, which are essential for the identification of potential health and production problems during the production cycle of farmed fish. Mortality records are one of the most important sources of information on a farm, especially if it includes the cause of death as deformities, predators and diseases. Any deviation from the expected levels of mortality may indicate production problems, infectious diseases, or inadequate welfare. The investigation of increased rates of mortality must include examining farm records, determining the influence of death rate on production and the potential risk factors of diseases in a farm.

This project demonstrated the importance of mortality records for setting industry standards of “expected” mortality losses and for investigating the value of recorded mortalities as a tool for aiding in surveillance and control of infectious diseases. It also

aimed to determine the utility of reported mortality in supporting and assisting management-strategy decisions at the farm and industry level.

In this project, we developed a baseline benchmark curve for expected mortality losses for Atlantic salmon in seawater. This novel approach constitutes a first attempt to establish a baseline curve for normal mortality, which allows detection of potential production problems based on deviations of mortality from the baseline curve of normal mortality. The results of this study also indicated that mortality levels may vary across production cycles, which can again be identified by using the baseline. We found that site was the factor with the highest contribution to variance in mortality. This site-to-site variation in mortality may have resulted from epidemics and environmental incidents, or other local event/effects. Temperature, and/or geographical area were also characteristics that contribute to variation in mortality.

The regulator, Marine Scotland Science, with the backing and support of the salmonid industry has suggested potential mortality thresholds as an indicator of presence of infectious diseases, which could be used as alerts for inspection by the official authority. In this study, high mortality rates on fish farms were investigated as an indicator of the presence of infectious disease. The analysis was performed using several analytical approaches: receiver operating characteristic (ROC) curve analysis, measures of sensitivity and specificity, and bootstrap methods. The study was performed by splitting the production cycle into small fish with mean weight below 750 g and large fish with mean weight over 750 g. In the small fish, the results did not suggest reported mortality as a strong indicator of the presence of infectious disease, which may be caused by the lack of records of infectious disease at this stage of the production cycle. In the larger

fish, high mortality rates were found to be a strong potential indicator of the presence of infectious diseases, including the suggested mortality threshold.

In a survey, the role of traditional diagnosis in the prevention and control of disease outbreaks was assessed. For that, key informant interviews were performed with open questions to the health or farm manager of several trout and Atlantic salmon farms and we also used the diagnostic reports of the Veterinary Diagnostic Services (VDS) from Stirling University to triangulate the data. We showed that disease diagnoses are of great importance for disease identification and control of actual diseases. Farmer's experience was also indicated as essential in the identification of the first signs of disease, which was principally through the daily monitoring of fish. This study suggested that disease diagnosis starts at the farm level with the daily monitoring of fish and the records of different parameters by the farmer, including mortality. Those records were showed to be vital to identify problems within the production.

This thesis illustrated a novel approach to investigate and interpret recorded mortality at the farm level. The results presented in this thesis indicated reported mortality as a vital on-farm tool for identification of diseases and production problems. This thesis suggested priority areas where further investigation is required.

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Chapter 1 General introduction

1.1. Global aquaculture development

Aquaculture is the fastest-growing animal-food-producing sector (FAO, 2008; FAO, 2010). On a global scale, aquaculture has the potential to meet the food supply demand for the increasing human population (Rana, 1997). Freshwater fish are the major group produced in aquaculture, followed by aquatic plants and molluscs (Figure 1-1). Marine fish, crustaceans and molluscs increased in production more recently.

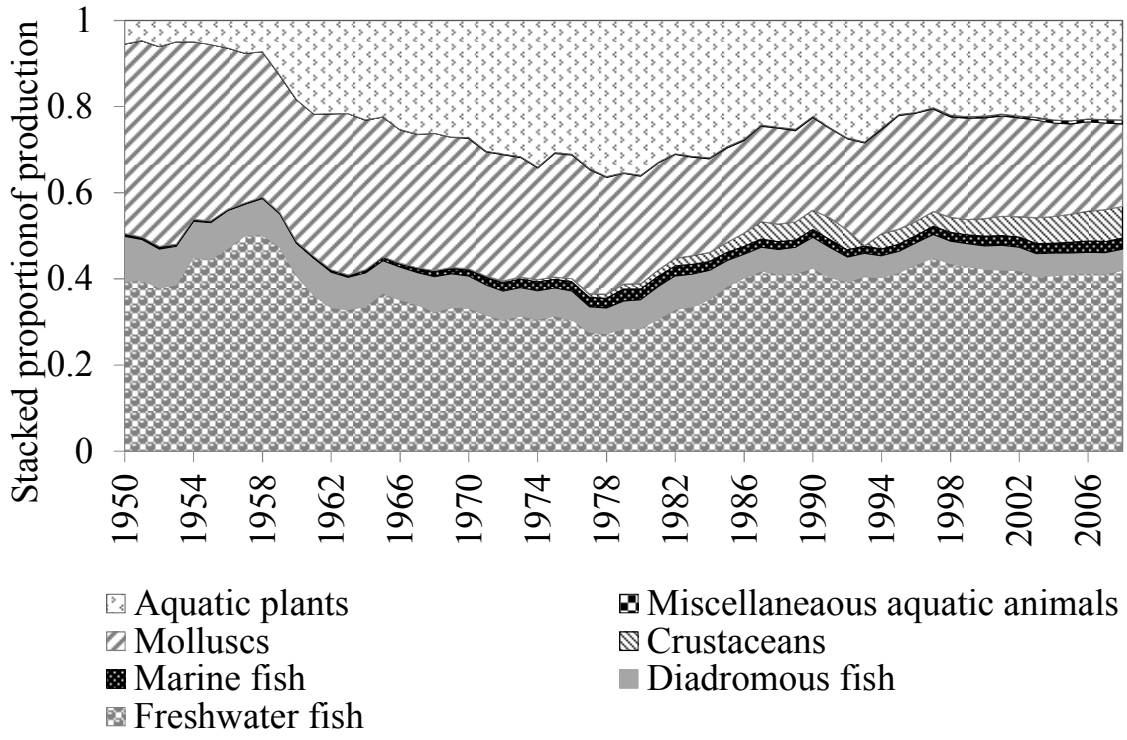

Figure 1-1 Proportional stacked bar chart of World aquaculture production groups (source: FAO 2010).

Europe is a small contributor to world fish production (4.5 %), though is the major producer of certain products (Ariel and Olesen, 2002, FAO, 2010). Within Europe, the main producers are European Union (EU) countries (FAO, 2010) and Norway from the non-EU countries. In EU, the production of fish from 1995 to 2000 had increased by 60

%, roughly 520 000 tonnes in 2000 (Ariel and Olesen, 2002), with a slight deceleration from 2000 to 2008 (FAO, 2010).

The EU countries produce 54 % of the global aquaculture production of trout (*Oncorhynchus mykiss* and *Salmo trutta*), 22 % of Atlantic salmon (*Salmo salar*), 99 % of European eel (*Anguilla anguilla*), 68 % of seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*), and 100 % of turbot (*Scophthalmus maximus*) production (MacAlister Elliott and Partners Ltd., 1999).

1.2. History of Scottish aquaculture

Fish farming was developed originally out of the intention to improve recreational fisheries through stocking (Williamson and Beveridge, 1994). In the 1800s a rapid advance in the understanding of fish biology, mainly from salmonids, allowed the setting up of salmon hatcheries in several rivers (Williamson and Beveridge, 1994) in Scotland. However, it was only in the 1960s that the industry expanded with the development of pelleted food (Read, 2008).

Rainbow trout (*Oncorhynchus mykiss*) first started to be produced for the table market in Scotland in the mid-1960s, using the well-established Danish industry as a model (Williamson and Beveridge, 1994). Salmon farming development began a few years later, with fast growth in the mid-1980s (Williamson and Beveridge, 1994, Henderson *et al*., 2004).

1.3. Scottish global salmonid production

Scotland is the largest producer of farmed Atlantic salmon in the EU and the third largest producer in the world, after Norway and Chile (Marine Scotland Science, 2009); recently Chilean production has declined substantially owing to disease problems

(Anonymous, 2009), notably outbreaks of infectious salmon anaemia (ISA) (Mardones *et al.*, 2009) and parasite introduction problems (e. g. sea lice) (Gustafson *et al.*, 2005, Nylund *et al.*, 1993, Vass, 2010). Within the UK, Scotland is the main source of Atlantic salmon and is responsible for 80 % of UK aquaculture production (Marine Scotland Science, 2009, Liu and Sumaila, 2008). In Scotland, Atlantic salmon is the dominant farmed fish. Scotland produced over 144 000 tonnes annually with an estimated farm-gate value of £412 million, while rainbow trout production was about 6 700 tonnes, 1 000 tonnes less than in 2008, with an estimated farm-gate value of £14.34 million in 2009 (Marine Scotland Science, 2009). In 2006, UK rainbow trout production was 88 % for the table market and 12 % for restocking (Tyson, 2008).

In Scotland, 85 % of salmon production is owned by Norwegian and Dutch companies (Ernest & Young, 2005), due to the recent trend of larger international aquaculture companies purchasing smaller companies in other areas of the world, such as in north west Europe, Canada and Chile (Ernest & Young, 2005).

1.4. Structure of the salmonid industry in Scotland

1.4.1. Atlantic salmon industry

Atlantic salmon (*Salmo salar*) is the most highly produced diadromous fish (FAO, 2010). The production of salmon has two main phases: the production of juvenile salmon in freshwater, and an on-growing phase in seawater. The *freshwater* phase starts with fertilised eggs and lasts until the moment of transfer to the sea as smolts with weight ranges from 30 to 100 g, depending on age. Smolt may have different classifications: S1 denotes that smolts are ready to go to sea in the spring of their second year, around 12 months after hatching. S½ smolts are ready after around 6 months from hatching as a result of temperature and/or photoperiod manipulation. The *seawater*

phase comprises the growth of smolts to market size of 3-5 kg. It occurs in cages, usually for a period ranging from 18 to 24 months. The fish that mature after one winter may be harvested at a smaller size (Scott, 2010) and are known as “grilse”. Production cycles are generally followed by a fallow period on the site to break the cycle of diseases and parasites (Scott, 2010).

1.4.2. Production in freshwater

In 2010, a review of the regulation of salmon farming in Scotland was made by the Institute of Aquaculture in Stirling (Scott, 2010). They found that in Scotland smolt production of Atlantic salmon in freshwater has ranged between 36 and 48 million smolts per annum from 2000 to 2009, showing a decline trend across these years, followed by a stabilisation in the most recent years (Marine Scotland Science, 2010a). In 2009, the production of Atlantic salmon smolts was close to 37 million, an increase by 0.4 million (1.1 %) compared with 2008 (Marine Scotland Science, 2010a). Of these, S1s were the dominant production, followed by S½s of the smolt production. In 2009, as in previous years, the production of smolts was more or less evenly distributed between freshwater cages and tanks/raceways, with the number of smolts put to sea (38.5 million) similar to the number of smolts produced. In 2009, the main areas of smolt production in freshwater were the North West, West and the Western Isles (Marine Scotland Science, 2010a) (Figure 1-2).

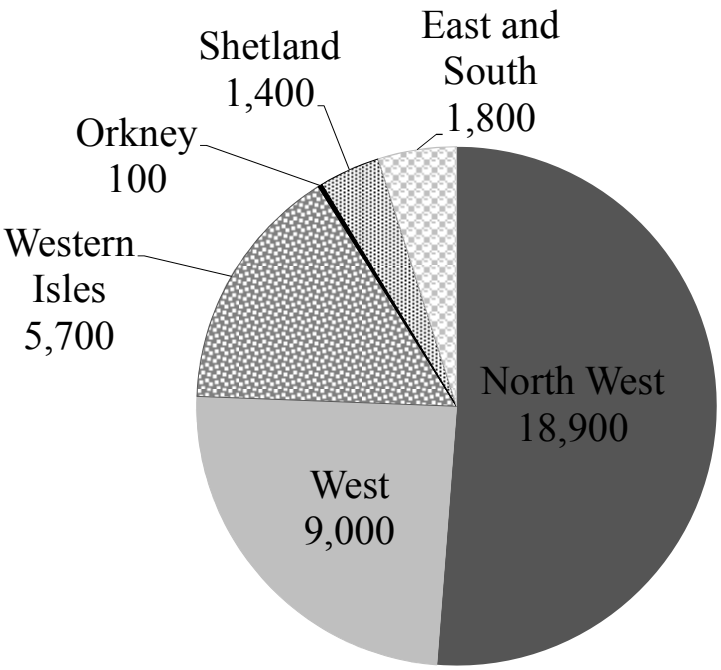

Figure 1-2 Production of smolt in freshwater (thousands) by production area in 2009.

1.4.3. Marine salmon production in Scotland

Marine Atlantic salmon production in Scotland has ranged between 129 and 170 thousand tonnes from 2000 to 2009 (Figure 1-3), with an estimated 150 thousand tonnes in 2010 (Marine Scotland Science, 2010a).

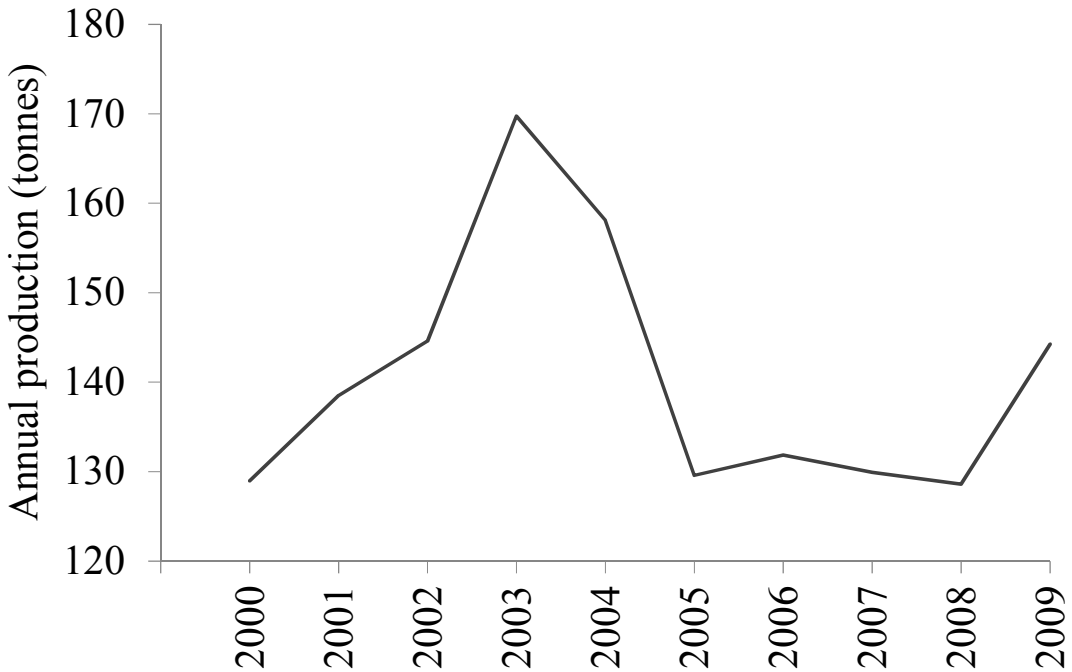

Figure 1-3 Annual production of Atlantic salmon (tonnes) during 2000-2009.

The vast majority of the production was from seawater cages with 254 active sites; only 88 tonnes of Atlantic salmon was produced in tanks in 2009. The average productivity per site harvested was 568 tonnes in 2009, 12 % more than 2008 (Marine Scotland Science, 2010a). In 2009, the number of sites producing below 500 tonnes of Atlantic salmon increased by 14, while those sites producing over 500 tonnes decreased by three. A total of 31 companies were registered, of which 9 accounted for over 95 % of salmon production.

1.4.4. The Scottish trout industry

The annual review of trout production performed by Marine Scotland Science (2010a), the official regulator in Scotland, stated that rainbow trout (*Oncorhynchus mykiss*) is by far the main trout species produced, although brown trout (*Salmo trutta*) and arctic charr (*Salvelinus alpinus*) are also farmed. Rainbow trout can be grown in freshwater and seawater, the majority produced in freshwater (Munro and Gregory, 2009, Marine Scotland Science, 2010a). The production of rainbow trout (*Oncorhynchus mykiss*) from 1996 to 2009, ranged from 5,000 to 7,000 tonnes (Marine Scotland Science, 2010a) (Figure 1-4), with an increase in the mass produced across the years (Figure 1-4). In 2009, rainbow trout production comprised 56 sites involving 27 companies (Marine Scotland Science, 2010a).

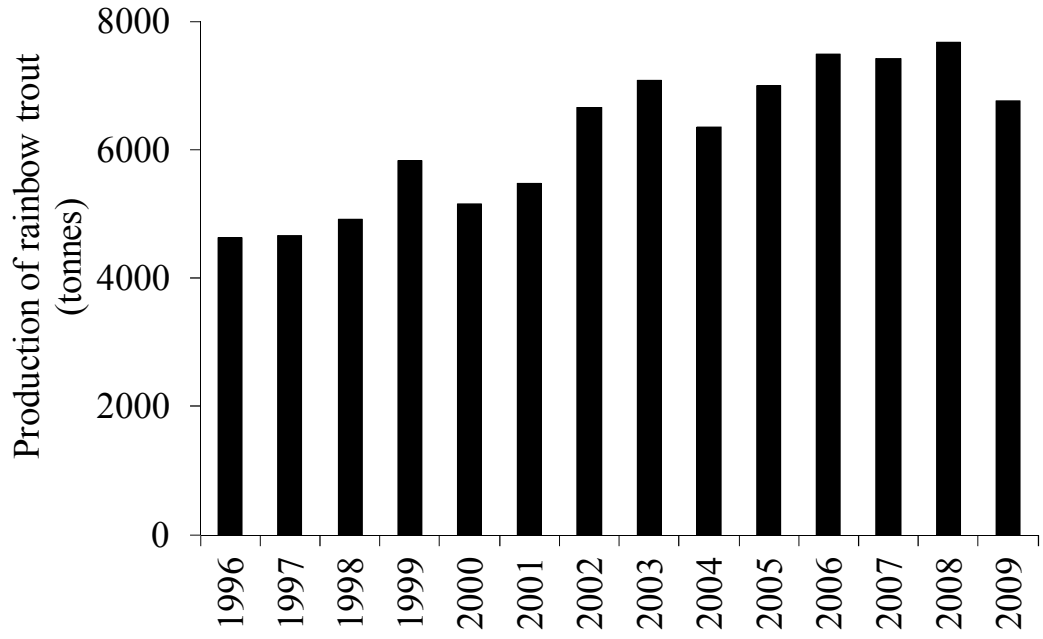

Figure 1-4 Total production (tonnes) of rainbow trout during 1996-2009.

In Scotland, according to MacIntyre (2008) and Marine Scotland Science (2010a), production is dominated by table market farms, followed by restocking farms and farms servicing both markets. In 2005, 31 farms produced for the table market, 16 farms for

restocking and 15 for both markets (MacIntyre, 2008). Fish produced for the table market are usually harvested at around 400 g (FAWC 1996), though rainbow trout produced for fillets in marine sites may be harvested at around 3 kg. There is considerable variability in the weight of fish leaving restocking farms, with many fish in the 500-800g range and infrequently fish are stocked at weight of 5 kg (MacIntyre, 2008).

Recently, in 2009, within the table market, the production of large size fish (> 900 g) increased and the small and medium size fish (< 450 g and 450-900 g) decreased, while in the restocking market, small fish production expanded and the medium and large size fish production fell (Marine Scotland Science, 2010a). The number of sites producing quantities of rainbow trout between 26 to 100 and over 200 tonnes also dropped, while sites producing quantities between 1 to 25 tonnes increased (Marine Scotland Science, 2010a). Brown and sea trout production fell by 112 tonnes in 2009 compared with 2008, mainly due to the reduction in fish produced in seawater for table market (Marine Scotland Science, 2010a).

1.5. Impact of diseases

The Scottish salmonid industry had a great development, with some problems of profitability in certain times caused by excess of production, and times of crisis due to different disease problems (Scott, 2010), such as emergence of sea lice (Pike and Wadsworth, 2000) and infectious pancreatic necrosis (IPN) (Hill, 1982). In Scotland from 1990 to 2007, the percentage of fish harvested reported after sea transfer was of 78 % (Marine Scotland Science, 2010a).

Diseases are one of the greatest challenges for the Scottish industry (MacIntyre, 2008, North *et al*., 2008, Read, 2008), being responsible for a large portion of financial losses

(Brun *et al*., 2003, Menzies *et al*., 2002, Read, 2008, Soares *et al*., 2011). In Ireland, gill disorders and pancreas disease were the leading causes of death in recent years in Atlantic salmon (Rodger, 2007). The structure of the salmonid industry also contributed to the spread of disease, due to the high number of movements on and off the sites, mainly in freshwater (Munro and Gregory, 2009, Werkman *et al*., 2011). The JGIW (Joint Government/Industry Working Group, 2000) recommended minimising movements between marine sites following ISA and this indeed has occurred, although reduction in number of movements between freshwater sites has not been achieved.

Diseases in fish often increase the mortality within the fish population to above normal levels (Wall, 2008), which has an economic impact because these fish cannot be sold for human consumption (Regulation (EC) No 1774/2002 2002). Diseases may also reduce market value of surviving fish (Hemmingsen and MacKenzie, 2001), if fish are rejected or downgraded at the processing plant (Del-Pozo, 2009) or exhibit slower growth at the production site (Speare *et al*., 1998, McLoughlin *et al*., 2003, Ramsay *et al*., 2004). Economic losses increase towards the end of the production cycle with higher expenditures incurred in terms of feed, time and husbandry (Brun *et al*., 2003, Del-Pozo, 2009, Soares *et al*., 2011). Diseases may affect fish welfare (Turnbull and Kadri, 2007, Ellis *et al*., 2011) and may also have important consequences in the environment by affecting wild populations (Murray and Peeler, 2005, Walker and Winton, 2010), for example through sea lice (Pike and Wadsworth, 2000, Anonymous, 2010).

In the UK, according to DEFRA and the farmer, the main diseases affecting the salmonid industry are infectious, including parasitic diseases (Table 1-1). Read (2008) stated that in UK trout species, whitespot commonly called “ich”, (*Ichthyophthirius*

multifiliis), proliferative kidney disease (PKD, *Tetracapsuloides bryosalmonae*), rainbow trout fry syndrome (RTFS, *Flavobacterium psychrophilum*), and enteric redmouth (ERM, *Yersinia ruckeri*), are the major causes of death, and together these cost the industry around £5 million a year. In Scotland, according to Marine Scotland Science (2010b), in salmon industry, pancreas disease (PD, salmonid alphavirus), is by far the largest cause of mortality by biomass. Cardiomyopathy syndrome (CMS) follows PD with biomass mortality close to 15 % (Marine Scotland Science, 2010b). Other infectious diseases, such as infectious pancreatic necrosis (IPN), salmon rickettsia (SRS) and sea lice (*Leophtheirus salmonis*), are responsible for over 9 % of the biomass mortality of salmon produced in Scotland (Marine Scotland Science, 2010b).

Emerging and notifiable diseases represent an important limitation to the growth of aquaculture and can result in severe financial losses (Murray and Peeler, 2005; Walker and Wilton, 2010). For instance, in Scottish salmonid farms, serious economic problems have been caused by the emergence of infectious salmon anaemia (ISA), (Murray, 2002; Stagg, 2002), red mark syndrome (RMS) (Ferguson *et al*., 2006; Verner-Jeffreys *et al*., 2008), sleeping disease (SD) (McLoughlin and Graham, 2007), and rainbow trout gastroenteritis (Barson, 2003). Brown (2000) defined an "emerging" disease as one that appeared in a new population or in a new geographical area for the first time or that was observed previously but has an increase in severity.

Table 1-1 Most important infectious diseases of salmonids in the UK (source: http://defra.gov.uk/).

UK infectious diseases	
Salmon	
Government view	**Farmer view**
Bacterial kidney disease (BKD)	Bacterial kidney disease (BKD)
Furunculosis	Cardiomyopathy syndrome (CMS)
Infectious pancreatic necrosis (IPN)	Furunculosis
	Infectious pancreatic necrosis (IPN)
	Salmon pancreas disease (PD)
	Sea and fresh water lice
Trout	
Enteric redmouth (ERM)	
Furunculosis	
Proliferative kidney disease (PKD)	
Rainbow trout fry syndrome (RTFS)	
White spot	

To control diseases, it is crucial to invest in research and in the cooperation among epidemiologists, fish health scientists, aquaculturists (Georgiadis *et al*., 2001) and economists (Wolf, 2005). In the UK, significant investments have been made to improve the knowledge of disease patterns and their risk factors. The monetary investment since 1999 in aquaculture research and development (R&D) has increased in the area of fish disease (James, 2006), being 56 % of the total commitment to aquaculture R&D from 1999 to 2006 (James, 2006).

1.6. Regulation of diseases in the Scottish industry

The United Kingdom has a long history of fish disease controls dating back to 1937, with the introduction of Diseases of Fish Act 1937. The Diseases of Fish Act 1937 requires the notification of the suspicion of the presence of certain diseases, known as notifiable diseases. Notifiable diseases are likely to have severe economic consequences for farmed and wild fish stock. It was introduced in response to several outbreaks in the rivers of England, Wales and Scotland from 1910 to the 1930s, which were attributed to the importation of infected live rainbow trout from Germany (Hill, 1996). In 1994, Diseases of Fish (control) Regulations (SI 1994 No 1447) was introduced, implementing disease control measures which are required under suspicion or confirmation of designated diseases. Other subsequent legislation includes Fish Health Regulations 1997 (SI 1997 No 1881), introduced in 1997, governing movement into the UK of live molluscs and live fish, their eggs and gametes, from zones within the EU not approved as free of certain diseases and the Aquaculture and Fisheries (Scotland) Act 2007, enforced in 2007, to regulate against the unauthorised introduction of fish to inland waters and for the control of the parasite *Gyrodactylus salaris*. Under this legislation, where a notifiable disease is suspected, such as infectious salmon anaemia (ISA) and infectious haematopoietic necrosis (IHN), the competent authority will undertake an investigation and apply controls to the affected area to minimise the risk of spreading. However, surveillance resources are necessarily limited, so their most efficient use is through risk-based surveillance whereby sampling is concentrated on sites that are most likely to be infected (Stark *et al*., 2006).

Additionally, the finfish aquaculture sector in Scotland is supported by a code of good practice (Scottish Finfish Aquaculture Working Group, 2010) providing fish-disease management standards in order to reduce the risk of spreading disease. These standards incorporate a set of measures to be implemented regardless of disease history (e.g. basic biosecurity measures and fallowing) and a set of measures to be implemented when suspicion and/or confirmation of diseases occurs, consisting of disease control measures such as movement controls or culling. The Scottish salmon farming sector is a good example of well-regulated industry, which is considered by the Scottish Salmon producers' Organisation (SSPO) (http://www.scotlandfoodanddrink.org/) to be the most tightly regulated aquaculture industry in the world, governed by over 50 regulations from ten different official bodies in the UK alone.

In the UK, a limited number of outbreaks of notifiable infectious fish diseases have occurred, and the few that have occurred, such as ISA (Murray *et al*., 2010), were confined within a certain farming area (Moran and Fofana, 2007). In the case of Scotland, the early implementation of regulations largely contributed to the control of an ISA outbreak in 1998 (McVicar, 2002) and again in 2008-2009 in Shetland (Murray *et al*., 2010). The recent Chilean outbreak of ISA (Henson, 2008; Mardones *et al*., 2009) illustrates the threats and the impacts of disease in the aquaculture industry and the importance of good regulation and husbandry practices to reduce the impact of spread of infectious disease.

1.6.1. Company fish health and stock management databases

Databases of site production have an important role to play in the investigation and understanding of diseases. They store valuable data for epidemiologists and to quantify production losses over time (Wolf, 2005, Dewey, 2008) and help facilitate development

of effective disease-control strategies (Menzies *et al*., 1996, Crockford *et al.*, 1999). Data records from multiple farms may be used to identify management factors that may decrease the production of animals, notably fish, or may trigger infectious disease outbreaks on an area basis (Jarp *et al*., 1994, Dewey, 2008). Farm records are also valuable resource-based measures of welfare and can be used to trace the history of fish on a farm and demonstrate that certain welfare standards were adhered to (North *et al*., 2008). Recording and maintaining accurate production data has the potential to usefully inform on the health status of farmed fish, to give a comprehensive overview of the current production or production trends and to be used as an advisory tool for farm managers (MacIntyre, 2008, Ellis *et al*., 2012, Soares *et al*., 2011).

The system of recording data on a farm may vary from a simple paper format to complex computer-based databases (Kelton *et al.*, 1997). It comprises of a wide range of information from the production records such as mortality (Frost *et al*., 1997, North *et al*., 2008), feed intake, water quality, biomass and disease treatments. In poultry (Frost *et al*., 2003; Stacey *et al*., 2004), pig (Parsons *et al*., 2007) and dairy cow (Frost *et al*., 1997) industries, systems for collecting real-time data have been developed for controlling growth, health and reproduction (Frost *et al*., 1997) and to support managers' decisions on husbandry practices. These systems also look for deviations between actual and expected production results (Frost *et al*., 1997), which are examined for statistical and economic significance (Frost *et al*., 1997). Systems for collecting real-time data provide real-time monitoring of animal production behaviour and health, allowing the implementation of necessary measures in a timely manner based on the most recent information. In the case of fish production, Ellis *et al*. (2012) suggested the removal and recording of the number of dead or dying fish from rearing systems as a real-time mortality metric system.

1.6.2. Mortality records

Mortality records are one of the most important sources of information on a farm, especially if these comprise of the cause of death, include deformities, loss of fish through predators and diseases (MacIntyre, 2008, North *et al*., 2008). These records are essential to investigate patterns of mortalities across the production cycle, to benchmark expected losses from the input to the end of the production cycle and to set production goals (Dewey, 2008) at an industry level. Mortality records are also vital to set industry standards of the expected mortalities (Dewey, 2008). Mortality information is not only important in fish production but also in other animal industries, such as pigs (Chagnon *et al*., 1991, D'Allaire *et al*., 1991, Shankar *et al*., 2009), cattle (Loneragan *et al*., 2001, Thomsen and Houe, 2006) and poultry (Carver *et al*., 2000, Tabler *et al*., 2004, Aerni *et al*., 2005). Any deviation from the expected levels of mortality may indicate production problems (MacIntyre, 2008, North *et al*., 2008), infectious diseases, or inadequate welfare (Thomsen and Houe, 2006, North *et al*., 2008). Increased mortality may have a multifactorial background and several diseases may occur either simultaneously or sequentially (Anonymous, 2007, Ellis *et al*., 2012). The investigation of abnormal levels of mortality must therefore include examining farm records (Duran, 2001, McKenna and Dohoo, 2006), determining the impact of death on production profitability and the potential risk factors of diseases in a farm (Duran, 2001).

The records of mortality causes are identified and assigned by the farmer—with or without confirmation by laboratory diagnosis—to specific categories of causes of fish death (Ellis *et al*., 2012, Soares *et al*., 2011). This process has limitations due to the difficulty of splitting the *immediate* cause of death from the *underlying* cause of death (Aunsmo *et al*., 2008, Ellis *et al.,* 2012). For instance, Aunsmo *et al*. (2008) suggested

in a study that the mortality recorded as ulcers were the result of mechanical trauma and bacterial infection. The combination of physical trauma and bacteria was essential to cause the ulcer. The bacterial infection without the physical trauma would have not caused ulcers and therefore mortality.

Any system looking at the causes of death has the danger of producing bias as result of poor selection of the causal groups of fish death, in other words misclassification (Aunsmo *et al*., 2008) and underestimating mortality (Jarp *et al*., 1994). However, Aunsmo *et al*. (2008) stated in a pilot study in marine Atlantic salmon that the causes of fish death assigned within 24h had a confidence of 97 % even at low mortality levels, the specific causes were possible to be identified. The authors also stated that the histopathology performed on dead fish did not contribute significantly to the diagnosis of causes of fish death.

Surprisingly, there are few salmonid production studies that focus solely on mortality. Most of the studies performed have investigated mortalities associated with a specific disease. These studies may not give a wider picture of the real overall value of reported mortalities and the usefulness of these mortality figures to help identify potential production problems. To our knowledge, there are no agreed acceptable natural mortality figures within the salmonid industry. However, some weekly mortality thresholds are accepted as reference levels worthy of concern to increase the surveillance on site, for instance, 0.1 % by the site health or farm manager of salmon production and 0.5 % by the certifications scheme Freedom Foods (RSPCA, 2007). The aim of this study was to investigate and to interpret the meaning of mortality records at the site level and to determine the importance of mortality records for supporting and assisting management strategies at the farm and industry level within salmonid

production. This project was part-funded by Marine Scotland Science, therefore the project also aimed to show the importance of mortality records for setting industry standards of expected mortality losses and to investigate the value of recorded mortalities as a tool for aiding in the surveillance and the control of infectious diseases. To help achieve this, the study was structured into four main themes:

Chapter 2 had a main goal to produce a baseline benchmark of 'expected' losses in salmon farming from input of smolts until harvest time. This approach was a first attempt to set a standard curve of "expected" losses for marine salmon. This chapter also described the main causes of mortality losses across the production cycle.

Chapter 3 aimed to examine and describe in more detail the drivers for the mortalities to determine whether the potential mortality benchmarking data could be generalised.

Chapter 4 aimed to examine the usefulness of records of *abnormal* mortality in helping with the detection of infectious diseases using measures of sensitivity and specificity. This study aimed to investigate specific mortality thresholds to aid in the surveillance and control of infectious diseases which were mostly notifiable diseases. For that, the regulator, Marine Scotland, suggested examining a range of potential mortality thresholds which may then be used as surveillance alerts to trigger the inspection system. In this chapter, different statistical approaches were applied: receiver operating characteristic (ROC) curve analysis, sensitive and specificity measures, and bootstrap methods.

Chapter 5 aimed to describe the role of disease diagnosis, where diagnosis in this study was considered to include an investigation of the disease outbreak history, results of the pathology and pathogen identification at the farm level. The data were investigated to evaluate the influence of disease diagnosis within the health management system

employed and subsequent treatments applied for clinical disease outbreaks within salmonid systems.

The chapters in this thesis take the format of a series of draft manuscripts ready for publication. The contribution of Silvia Soares to all of chapters includes sampling, data collection, data analysis and writing. All the authors provided assistance and guidance with all aspects of the study including data analysis and commented on the writing of the entire thesis.

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Chapter 2 A baseline method for benchmarking mortality losses in Atlantic salmon (*Salmo salar*) production

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This chapter describes a baseline method for benchmarking "expected" daily mortalities of farmed salmon in seawater. The database comprises on-farm records from 2000 to 2006.

The main author, Silvia Soares, conducted all analytical work and developed the final benchmark. Dr. D. M. Green, Prof. J. F. Turnbull, Dr. M. Crumlish and Dr. A. G. Murray provided supervisory and editorial support throughout the whole study.

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2. A baseline method for benchmarking mortality losses in Atlantic salmon (*Salmo salar*) production

Authors: Silvia Soares, Darren M. Green, James F. Turnbull, Mags Crumlish, Alexander G. Murray

2.1. Abstract

A site production database provides a large diversity of information regarding fish health and stock-production outcome. Mortality records held in the site production database are indicators of fish health status and of great interest for studying fish health, such as patterns of diseases. Mortality records from a Scottish Atlantic salmon production database of one company were used to develop a method of benchmarking production losses due to mortality. The records concerned mortality loss numbers of Atlantic salmon in the seawater phase. The median, 10th and 90th percentiles of mortality were calculated for each week of production from 88 production cycles recorded in the database. The median, a measure that is not sensitive to extreme values, was used as the central line of comparison and the 10^{th} and 90^{th} percentile were used to delimit the range of a standard mortality curve. We presented a baseline benchmark for excepted mortality losses in marine Atlantic salmon. We compared mortality losses of different individual production cycles with the standard mortality curve and we highlighted the impact on the production costs and time consumed. We also showed the interannual variation in mortality time series and the variation in mortality of production cycles associated with three diseases (pancreas disease, cardiomyopathy syndrome and infectious pancreatic necrosis).

2.2. Introduction

Scotland is the largest producer of farmed salmon in the EU and the third largest producer in the world, after Norway and Chile (Marine Scotland Science, 2009); recently Chilean production has declined substantially owing to disease problems (Anonymous, 2009a), notably outbreaks of infectious salmon anaemia (ISA) (Mardones *et al.*, 2009) and parasite induction problems (e. g. sea lice) (Gustafson *et al.*, 2005, Nylund *et al.*, 1993, Vass, 2010). Within the UK, Scotland is the main source of salmon and is responsible for 80 % of the UK aquaculture production (Marine Scotland Science, 2009).

Farmed salmon, as with other cultivated species in aquaculture, face the problem of diseases. Diseases constitute a huge constraint to the development of aquaculture industry (FAO, 2007), and losses caused by various diseases represent a substantial proportion of loss costs in salmon industry (Menzies *et al.*, 2002; Brun *et al.*, 2003, Skall *et al.*, 2005). Therefore, disease control is crucial to the profitable production of any farmed species (Menzies *et al.*, 1996).

The systems for recoding data are diverse on a farm. It can be a simple paper format or a complex computer-based database (Kelton *et al.*, 1997). Presently, the majority of salmon producers use sophisticated IT software as a tool in production control and inventory accounting (Aunsmo *et al.*, 2008), with computerised record systems at the farm level to facilitate data collection. The introduction of these programs has been of great importance in facilitating the monitoring of health data, including cause-specific mortality (Aunsmo *et al.*, 2008). Fish weights, feed intake, fish movements, temperature and other environmental parameters and mortality numbers and biomass are examples of records found in fish production databases. This information can be used to inform

health status of farmed fish and to provide a comprehensive overview of the current production status and trends. The analysis of mortalities (patterns of losses and their causes) may provide a more detailed insight of a particular disease, such as disease risk factors and seasonality.

Hammel and Dohoo (2005), in a study to investigate and describe the mortality patterns attributed to infectious salmon anaemia virus (ISAV), reported that initial outbreaks of infectious salmon anaemia (ISA) were relatively low (median of < 7 % total mortality) when salmon were most likely naïve to ISAV with outbreaks exceeding 30 % of total cumulative mortality. In Scotland the prevalence of infectious pancreatic necrosis (IPN) in post-smolt Atlantic salmon increased from 1.2 % in 1990 to 12.5 % in 2002 and the mortality at sites with confirmed IPN varied between 0.03 % and 0.1 % per day in May and 0.5 % per day in July (Bruno, 2004). More recently, Mardones *et al.* (2009) found that roughly 20 % of farms at risk of ISAV in Chile became infected with the virus, with the incidence of ISA increasing slightly over time. Moreover, epidemiological studies of mortality in relation to site management practices may also be carried out to explain the effects of these practices on mortality rates in farmed Atlantic salmon (Wheatley *et al.*, 1995).

Mortality records have been used for the development of methods for benchmarking production mortality losses, in terms of numbers or biomass and are recognized as valuable tools for fish farmers (Anonymous, 2009b). For instance in trout (Anonymous, 2009b), dairy (Khade and Metlen, 1996), sheep (Geenty *et al.*, 2006) and pig (Davidson, 2005) industries.

Benchmarks may also be used to identify unusual patterns of mortality before serious loss has occurred, and thus allow management actions to pre-empt a problem. For

example, in poultry (Frost *et al.*, 2003, Stacey *et al.*, 2004), pig (Parsons *et al.*, 2007) and dairy cow (Frost *et al.*, 1997) industries, systems for collecting real-time data have been developed for controlling growth, health and reproduction (Frost *et al.*, 1997).

These systems are able to collect and analyse a huge variety of information of site production data, including mortality and production records (Frost *et al.*, 1997). They have the capability to monitor actual against expected production results and identify any deviations with statistical and economic significance (Frost *et al.*, 1997). Therefore, real-time data sources are of great value for monitoring growth, health and reproduction and to integrate benchmark approaches in health-management strategies by tracing and tracking deviations in salmon production. Benchmark approaches can also be a useful tool for research areas such as fish welfare, production, health and treatments and for informing governmental policies. Benchmark approaches may also be a valuable tool for analysis of costs and profitability of salmon production. An example of the value of benchmarks in the salmon industry is the study performed by the Canadian salmon industry (Anonymous, 2006), which compares the performance of the Canadian farmed salmon industry against the performance of the Norwegian and Chilean farmed salmon industries in the US market.

The aim of this work was to develop a baseline method for benchmarking "expected" daily mortalities of farmed salmon as an indicator of health status and to identify early production problems. The analysis also included the quantification of the main types of mortality causes across production cycles.

2.3. Material and methods

2.3.1. Data collection

The data were supplied from a single company and included over 60 million Atlantic salmon smolts that were moved into 82 marine production sites located on the western coast of Scotland (Kilburn, R.; Soares, S.; Murray; S., unpublished results). Production cycles between the years 2000 and 2006 were analysed, with only complete cycles of salmon production included. Production data for mortality causes, mortality losses, smolt input and harvest data were extracted from a BusinessObjects™ database. (Kilburn, R.; Soares, S.; Murray; S., unpublished results).

2.3.2. Mortality data

2.3.2.1. Daily mortality

Cage-level daily mortality was recorded as the number of dead fish retrieved by different methods, such as divers, hand hold baskets, lift-up collectors for dead fish removal and hand nets. When mortalities were not recorded daily, the daily count was calculated from the total mortalities divided by the number of days since the last count (Hammel and Dohoo, 2005; Aunsmo *et al.*, 2008). When the database contains entries of zero mortality, this was taken to imply the site was inspected but there were no dead fish to be collected on that day. However, absent records in the database were interpreted to mean that collection of dead fish by the farmer was not performed on those days.

Weekly averages of daily mortality on site were expressed in percentages. The mortalities over seven day periods were averaged to calculate mean daily mortality for the week. The day of transfer of the first fish onto the site was considered day zero. The

denominator used for calculating mortality was the count of fish on site as recorded in the database. Consequently, transfers into and out of the site during the production cycle were accounted for. Between-cage transfers were not a concern since the production cycle for the whole site was the study unit.

2.3.2.2. Cause of death

In the database, mortality was allocated by a code to one of the 52 pre-assigned causes by the farmers. We grouped these mortality causes into five categories (Table 2-1): *infectious disease*, *production*, *environment*, *predation* and *unknown* causes. The pre-assigned causes are written in italic every time each of them is referred to in the text. There was no description or information indicating how mortality codes were originally assigned in the site production database. However, events with unexpected mortality levels are usually investigated, and it is highly probable that the farmer's diagnosis is supported by veterinary laboratory tests in such cases. Infectious pancreatic necrosis (IPN) was distinguished with two codes: *suspected* and *confirmed* IPN outbreaks. Therefore, in this study, the positive weeks to IPN had assigned the code for confirmed outbreaks, while suspected ones were coded with suspected IPN. The remaining codes for diseases did not distinguish among *suspected* and *confirmed* outbreaks, except PD that was coded as suspected. We considered a week or a cycle positive to a disease whenever one of the codes was assigned.

Table 2-1 Mortality causes recorded grouped into five groups of mortality causes. The percentages (%) of each disease by total proportion of fish lost are represented as: * ≤ 0.5 %; ** 0.5 - 1 %; * ≥ 2 % (no infectious diseases had percentages within the interval 1 – 2 %).**

Unknown	Production	Infectious diseases	Environment
Blind	Accident loss	Bacterial kidney disease (BKD)**	Environmental
Decomposed	Caught in net	Cardiomyopathy (CMS)**	Jellyfish
Deformed jaw	Cull	Fungus*	Oxygen Starvation
Disappeared	Failed smolts	Infectious pancreatic necrosis (Confirmed-IPN)***	Plankton bloom
Event mortality	Jacks	Moritella*	Storm
Eye damage	Mature	Pasteurelosis*	
Fin rot	Net tear	Rickettsia (SRS)**	**Predation**
Gill damage	Normal	Sea lice*	Birds
Lesion	Parr	Suspected furunculosis*	Mink
Option missing	Precocious male	Suspected infectious pancreatic necrosis (Suspected-IPN)***	Seals
Other	Transfer	Suspected pancreas disease (PD)***	
Physical damage	Treatment kill		
Runts	Sample weighing		
Samples	Smolt transfer		
Unidentified	Suspected cannibalism		

2.3.3. Statistical analysis

The median, 10th and 90th percentile of daily mortality for each week of the production cycle were used to derive a benchmark curve of losses across all cycles. These percentiles were based on the distribution of daily mortality across all the production cycles for the given week since the production cycles commenced. The median, a measure that is not sensitive to extreme values, was used as the central line of comparison and the 10th and 90th percentile were used to delimit the range of a standard mortality curve. The first week of the cycle was considered the start point of the time series. Time zero was considered the day that fish was moved into the site. The other transfers, in and out of the site, were not a problem for the benchmark as the denominator used for calculating mortality was the count of fish on site as recorded in the database. Transfers between cages on a site also were not a constraint for the time series because the production cycle on the site was the unit of study.

2.4. Results

2.4.1. Database description

In this study, the production cycle was considered the study unit instead of the site. A production cycle is the time between input of fish onto a marine site and their removal for harvest. Mean cycle time was 89 weeks, but this varied from 54 to 124 weeks. Sites start their production cycles throughout of the year and as a result, the times on the benchmark curve for median mortality refer to a specific time after input not to time of year. Thus, the pens/cages belonging to the site were not individually considered as the cycle on site was the study unit. A total of 83 sites and 157 cycles in the database were recorded. Therefore, 157 cycles were considered instead of 83 sites (35 sites had one production cycle, 22 sites had two production cycles and 26 sites had three production cycles). Of the 157 cycles, only 88 were considered for the study. A total of 69 cycles excluded from the analysis, 31 production cycles were of halibut, four were from experimental units and so not appropriate for analysis of commercial salmon production cycles. An additional three cycles were from sites with continuous production that lacked discrete production cycles. Of the remaining 31 cycles excluded from the analysis, 25 had incomplete data (lack of mortality records at least during the first seven months after fish moved in, abnormal inputs of one or ten fishes and no records of input numbers and fish species), while six cycles had a cycle length of less than nine months.

2.4.2. Description of studied population

The 88 production cycles included in this study encompassed over 44 million Atlantic salmon in the marine stage between the years 2000 and 2006. Incomplete production cycles by the end of data collection in 2006 were not included in this study. The initial

range of fish weight at the site level was 45 – 100 g and the mean range of weight of fish harvested was 4.5 – 5 kg. The total mortality percentage from the beginning until the end of the production cycle of the population studied was 24 %.

In the studied database, the major cause for fish losses was *infectious disease* (31 %), followed by *production* (29 %), *environmental* (8 %) and *predation* (7 %) and finally the fish losses assigned to *unknown* causes were 26 % (Figure 2-1). The main causes of losses at the beginning of the production cycle were *infectious diseases* followed by production-related mortality. The mortality shows a decline trend over time, with a peak around week 70 caused by *infectious disease* causes (Figure 2-1). Losses due to the *unknown* group were observed in a continuous percentage throughout the weeks of production cycle (Figure 2-1), while predation and environmental causes were in small percentages along the weeks. *Environmental* factors caused a first peak of mortality around week 43 due to storm events, resulting in an extreme mortality event of 16 % for one of the production cycles. This represents 0.5 % of the total number of losses recorded. The second peak of mortality observed from week 47 to 50 was caused by plankton bloom (Figure 2-1).

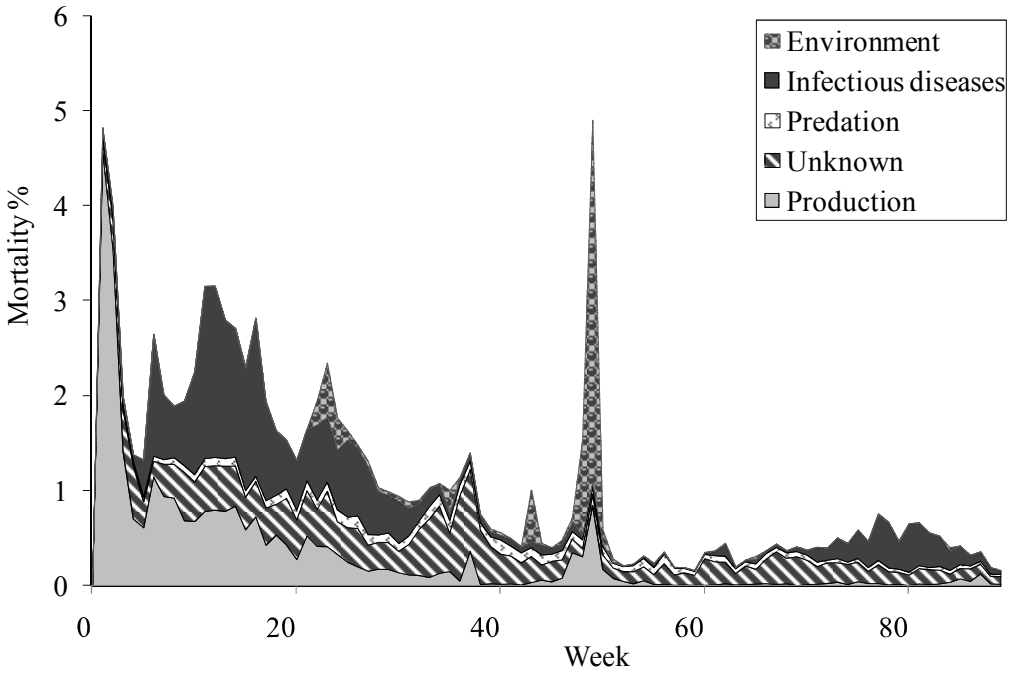

Figure 2-1 Percentage of total losses of all causes, across the weeks of production cycle of the population studied. The mortality causes were grouped in five categories (*environment*, *infectious diseases*, *predation*, *unknown* and *production*).

2.4.3. Mortality benchmarking curve

A standard mortality curve assessed variation in mortality rate between and within sites during the life-cycle of the population studied (Figure 2-2). This can be used as a benchmark in order to track any deviations in the daily mortality of fish on site across the weeks of production.

In the first weeks after stocking, a decline of weekly median of daily mortality was observed (Figure 2-2), followed by an increased trend until week 19 and a gradual decline from this week onwards. The peak of mortality observed in the first week of production (Figure 2-2) was caused by production factors, as seen in Figure 2-1, mainly

by transfer and failed smolts. After week 5, the mortality increased again until week 19 due to disease problems (Figure 2-1) attributed mostly to IPN recorded.

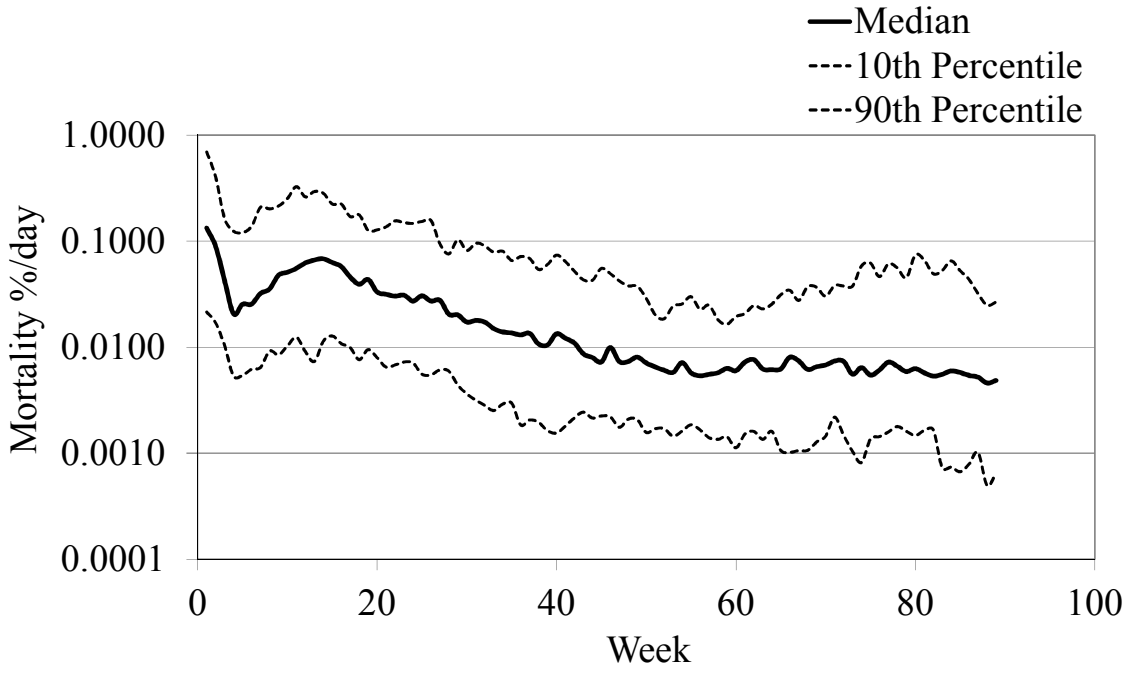

Figure 2-2 Standard mortality curve of daily mortality. 10th, 50th and 90th percentile of weekly mortality are shown, rescaled as daily mortality rates.

2.4.4. Benchmarking mortality of cycles

The weekly averages of daily mortality from four different production cycles were individually compared with the standard mortality curve superimposed (Figure 2-3). Graph (a) shows a production cycle that follows closely the standard mortality curve, (b) and (c) shows two production cycles with higher and lower mortalities and (d) a production cycle with a mortality increase towards the end of production.

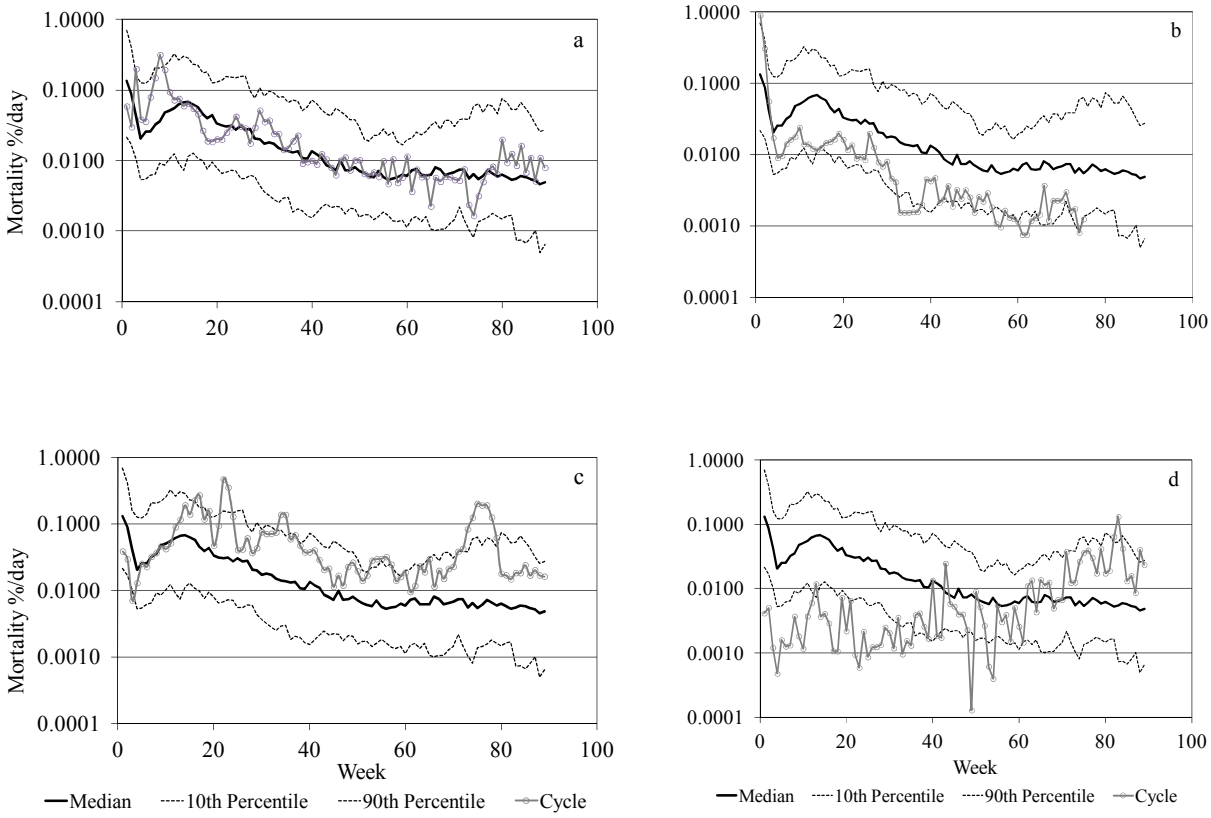

Figure 2-3 Benchmarking four different mortality time series with the standard mortality curve of the population studied. Production cycles show mortality close to the median (3a), persistently low mortality (3b), persistently high (3c) and low early, becoming high (3d).

2.4.5. Benchmarking interannual mortality variation

In this study, the year corresponds to year of the initial stocking and not the calendar year. Only cycles beginning in 2001, 2002, 2003 and 2004 were considered. Cycles with initial stocking in 2000 and 2005 were not included because there were too few cycles in these years to perform a statistically viable analysis. Although the database contains mortality records from the calendar year 2006, these were from cycles with initial stocking in 2005 and so there are no 2006 cycles. A benchmark analysis of the weekly median of daily mortality of 2001 to 2004 production cycles against the standard mortality curve showed that 2002 and 2004 cycle mortality followed closely the standard mortality curve. In 2001, the mortality had a peak of 15 % of the total

number of losses recorded in week 49 due to plankton blooming. This value did not show on the standard mortality curve because the median, which is not sensitive to extreme values, was used instead of the mean. This extreme mortality event does not compromise the benchmark reliability. Apart from week 49, 2001 had the lowest mortality levels across all the production cycles. Production cycles with initial stock in 2003 had the highest mortality levels after week 26. The main causes of mortality were *unknown* with the maximum of mortality percentage ranging between 1.0 % and 2.6 % of the total losses recorded and *infectious diseases* with a maximum mortality of 0.73 % of the total losses recorded.

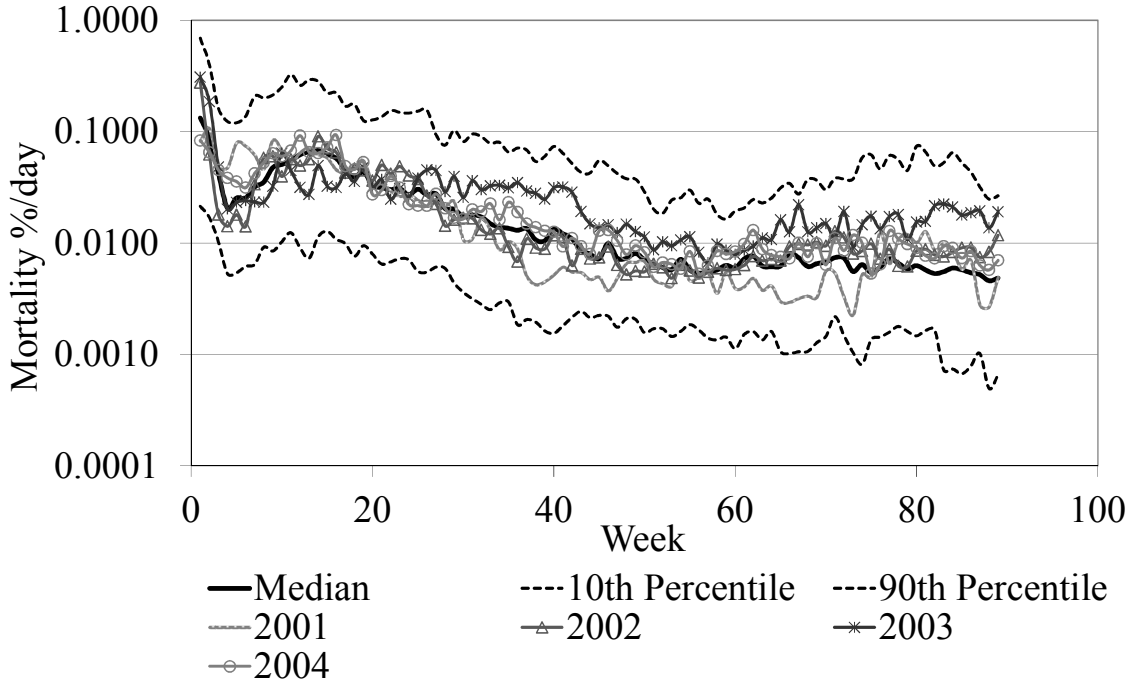

Figure 2-4 Benchmarking interannual variation in the weekly median of daily mortality.

2.4.6. Benchmarking three infectious diseases over weeks of production

The mortality time series from cycles suspected to be positive to *PD* and *cardiomyopathy syndrome (CMS)* were represented (Figure 2-5). An increase in mortality was observed in both *PD*- and *CMS*-positive cycles throughout the latter part

of the cycle (Figure 2-5), with *CMS*-positive cycles having a slightly higher mortality in the latter part of the cycle when compared with the *PD*-positive cycles. Both *PD*- and *CMS*-positive cycles showed an increased mortality in the first four to six months. The similarity in timing of the two mortality causes is not surprising because both diseases have similar clinical signs and the only way to differentiate CMS from PD is through histology description of the lesions.

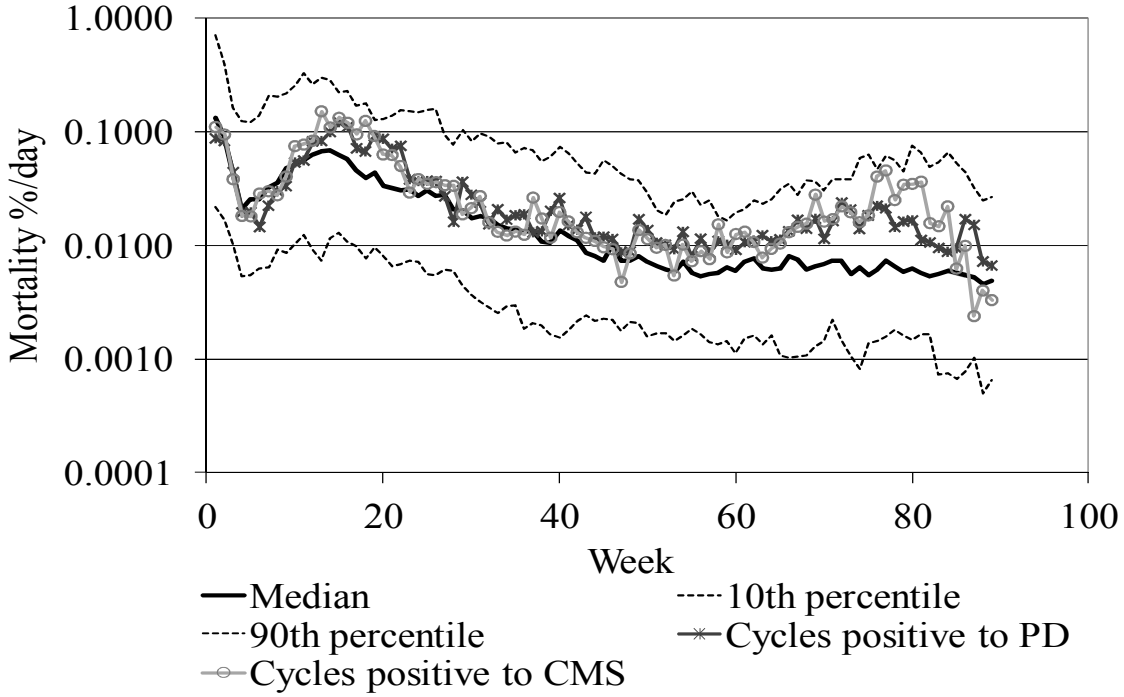

Figure 2-5 Benchmarking PD and CMS daily mortality time series with the standard mortality curve over 89 weeks.

In *IPN*-positive cycles, a slightly higher mortality percentage is observed in the first 40 to 50 weeks, while in the *IPN*-negative cycles the mortality losses were slightly lower than the standard mortality curve (Figure 2-6).

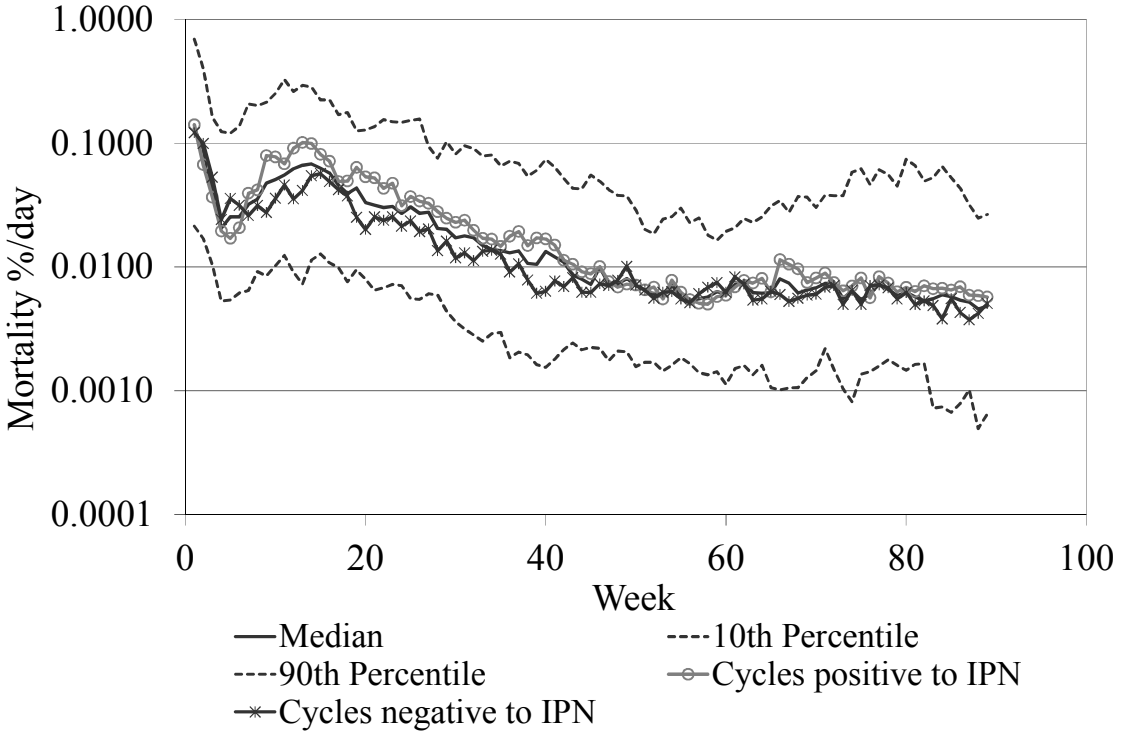

Figure 2-6 Benchmarking IPN daily mortality time series with the standard mortality curve over 89 weeks.

2.5. Discussion

The aim of this study was to develop a baseline method to benchmark "expected" mortality losses in salmon production at seawater stage. For that, a database from 2000 to 2006 was analysed. Variations on the median of the standard mortality curve of the population studied caused by *IPN*, *PD* and *CMS* and the interannaual variation among the years were investigated.

In this study, only 56 % of the cycles were used. Forty four percent of cycles held in the database were not included in the analysis due to the data pertaining to non-salmon species, experimental sites or sites with continuous production (24 %). These sites were considered not representative of commercial sites of farmed Atlantic salmon and therefore not relevant to the analysis. The remaining 20 % of cycles were excluded to avoid biased results caused by either missing data or data errors. Other difficulty

concerns the missing data and the zeros recorded in the database. There was no information indicating the difference between them. Therefore, we assumed that the days with missing records meant that dead fish were not collected on that day, while the days with a zero recorded meant that the dead fish collection resulted in zero on that day. There was no information available concerning the criteria of disease identification used to assign the mortality codes in the site production database, with no differentiation between *suspected* and *confirmed* diseases conditions. The only exception was for infectious pancreatic necrosis, with two mortality codes to differentiate suspected from confirmed outbreaks and pancreas disease, which was classified as *suspected*. Furthermore, it was suspected that great majority of causes were assigned by the farmer at the farm level without laboratory confirmation. Incorrect identification of diseases can result in incorrect entries in the database, leading to biased results.

The data were restricted to a single company in the west coast of Scotland, limiting the application of the benchmark to the industry level. However, this benchmark approach can be used within any company to track deviations in the production between sites (units) or group of sites. The possibility of benchmarking losses during the cycle or at the end will give the opportunity to allow the farmer to monitor the economic impact of mortality losses, for instance costs due to feed input and time invested. The economic impact of losses increases towards the end of the production cycle, when the expenditures incurred in terms of feed, input and husbandry are higher (Brun *et al*., 2003). Production cycles with initial stocking in 2001 and 2003 had different economic impacts, with mortality losses below the standard mortality curve after week 35 for 2001 cycles in comparison with 2003 that show mortality levels above the standard

mortality curve toward the end of the cycle, when there is a higher fish farming investment and commercial value.

This study would benefit from an economic impact analysis of mortalities in the production as seen in the study of mortality rate of calves and its effects on three levels of production (feed, milk and cheese) made by Khades and Metlen (1996) in the dairy industry. Likewise, studies regarding the direct cost incurred by a disease may also be performed as described by Menzies *et al.* (2002) and Brun *et al.*, (2003) for cataracts and cardiomyopathy syndrome problems in Atlantic salmon production. The mortality information can also be used to identify the main causes of losses during the production cycles, diseases patterns and drivers allowing a better understanding of disease outbreaks, as seen in the study made by Mardones *et al.* (2009) regarding the Chilean outbreak of ISAV. The definition of baseline mortality rates may allow companies and official regulators to identify situations in which intervention is required for, e.g. official inspection may be triggered if mortality exceeds a specific level dependent on production cycle stage. The baseline may also support epidemiologists in the detection of emerging diseases, anticipating potential future problems and allowing the implementation of prevention and control management strategies against disease outbreaks as seen for instance in the U.S.A. and Canada through the development of the surveillance plan for viral hemorrhagic virus (VHSV) IVb (Anonymous, 2010).

This study represents a first step towards the development of benchmark approach for mortality losses, with a wider value if extended to industry level in the future. A benchmark approach for the industry allows the assessment of plans for control of fish diseases and production-management practices and the identification of early production problems (Anonymous, 2009b).

2.6. Acknowledgements

This research was supported by Marine Scotland Science.

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Chapter 3 Factors affecting variation in mortality in marine Atlantic salmon (*Salmo salar* L.)

Authors: Soares, S., Murray, A.G., Crumlish, M., Turnbull, J.F., Green, D.M.

This chapter examined the drivers for mortality to determine whether the potential mortality benchmarking data can be generalised. It investigated which factors (e. g. temperature, age, and site) were associated with the variation in mortality during the marine-phase cycle of Atlantic salmon production. The site production database was the same used in Chapter 2. The daily mortalities calculated in Chapter 2 were used in this chapter for the basis of this analysis.

The main author, Silvia Soares, conducted all analytical work and developed the final report. Dr. A. G. Murray, Dr. M. Crumlish, Prof. J. F. Turnbull and Dr. D. M. Green provided supervisory and editorial support throughout the whole study.

3. Factors affecting variation in mortality in marine Atlantic salmon (*Salmo salar* L.)

Authors: Silvia Soares, Darren M. Green, James F. Turnbull, Mags Crumlish, Alexander G. Murray

3.1. Abstract

Diseases pose an important constraint to economic expansion of aquaculture; they are dependent on the complex interacting factors of pathogen, environment, and host, and the causes of death can be related to nutritional, environmental and genetic factors of the host or infectious agents, such as microbial pathogens. Databases of site production have an important role to play in the investigation and understanding of diseases, since they store valuable amounts of disease and management data. We examined the drivers for the mortalities from a single site production database, which represented one third of Scottish farmed salmon in 2005, to determine whether the potential mortality benchmarking data could be generalised. We show that mortality records at the farm level have an important role and meaning for studying mortality losses and for identification of management problems in production. We found that mortalities varied across the months of the year and with the time of year of initial stocking. Production cycles started in the third quarter of the year had the highest mortality overall. Furthermore, we found site-to-site variation in mortality may be caused by either random occurrence of epidemics and environmental events, or local effects.

3.2. Introduction

Aquaculture is the fastest growing food-producing sector in the world and an important industry in Scotland. Within the UK, Scotland is the main source of salmon and it is responsible for 80 % of UK aquaculture production. Furthermore, Scotland is the largest

producer of farmed salmon in the EU (Marine Scotland Science, 2009), producing over 144,000 tonnes in 2009 (Marine Scotland Science, 2010). In 2009 freshwater production of smolts increased by 1.1 % to 36.9 million, with over half (62.5 %) being first-year smolts (S1) and the remainder being half-year smolts (S1/2) (37.5 %); and seawater production biomass increased by 12 % (Marine Scotland Science, 2010).

Diseases pose an important constraint to economic expansion of aquaculture (Bondad-Reantaso *et al*., 2005, Subasignhe, 2005, Murray and Peeler 2005). Diseases in farmed fish can cause mortality, inadequate growth and poor food conversion, increased production costs and interrupted production schedules (Hedrick, 1998). All these reduce the total profitability of the companies and industry.

Disease outbreaks are caused by several factors. Diseases require the presence of the pathogen, combined with the optimal environmental conditions for the disease and a susceptible host (Snieszko, 1974, Hedrick, 1998). Prevention is a key element in the control of disease establishment (Wagner *et al*., 2002). Early and precise diagnosis, efficient prevention measures and accurate epidemiological surveys can be the key to minimize the impact of pathologies in fish culture. In order to contribute to more efficient disease control in fish populations, it is necessary to have a good level of understanding of the various factors predisposing to or causing diseases in farmed fish, as well an understanding of the association between potential risk factors and the presence of specific diseases (Menzies *et al*., 1996). Cooperation among epidemiologists, fish health scientists, aquaculturists (Georgiadis *et al*., 2001) and economists (Wolf, 2005) is crucial for developing aquaculture sustainability.

In the production of marine salmon, site production databases are kept for management purposes and may vary from a simple paper-based system to complex computerised

databases (Kelton *et al.*, 1997). A wide range of information is recorded in these databases, which often includes water temperature, stock origin, age, feed intake and mortality. These databases have an important role to play in the investigation and understanding of diseases, since they store valuable data for epidemiologists and they allow quantification of production losses over time (Wolf, 2005). Furthermore these data can facilitate development of effective disease control strategies (Menzies *et al*., 1996, Crockford *et al.*, 1999) through epidemiology.

One of the most important variables recorded at the farm level is fish mortality rate (MacIntyre, 2008, Anonymous, 2009; Soares *et al*., 2011), which may include the cause of death, such as environmental problems, predators and/or disease (MacIntyre, 2008, North *et al*., 2008). Mortality levels vary across production cycles with the presence of diseases. Different diseases may lead to different levels of mortality, with some highly virulent diseases registering no mortality cases in some years. Mass mortality can also be associated with environmental causes, such as seasonal factors or storms (Pillay and Kutty, 2005, Soares *et al*., 2011). Mortality records are also essential to investigate patterns of mortalities across the production cycle, to benchmark expected losses from the input to the end of the production and to set and work towards attaining production goals (Dewey, 2008, Soares *et al*., 2011).In this study, we build on this analysis to examine the causes and explanatory factors for mortality, to determine whether this mortality benchmark can be generalised, or whether it is dominated by site-specific and unpredictable effects. We investigated which risk factors (such as temperature, age, or site) were associated with variation in mortality during the marine phase of Atlantic salmon production. These risk factors were selected based on previous studies, where they were found to be associated with disease (e. g. infectious pancreatic necrosis (IPN) and furunculosis) and mortality in Atlantic salmon (Jarp *et al*., 1994, Wheatley *et al.*,

1995, Jarp and Karlsen, 1997, Murray *et al*., 2004). For instance, the risk of IPN outbreaks is associated with geographical location of site and age of fish transfer (Jarp *et al*., 1994). Our study used a site production database from a single company, which represented one third of Scottish farmed salmon in 2005 (Ernest & Young, 2005). A general linear model was applied to identify and quantify any patterns identified within the mortality records.

3.3. Material and methods

3.3.1. Data collection

This analysis used a site production database provided from a single company. This database encompassed over 60 million Atlantic salmon smolts that were moved into 82 marine production sites located on the western coast of Scotland (Soares *et al*., 2011). The study was restricted the period from 2000 to 2006, which only included complete cycles of salmon production. We extracted from a BusinessObjectsTM database (Soares *et al*., 2011) production data concerning mortality causes, mortality losses, smolt input and harvest data.

3.3.2. Definition of production cycle

In this study, the production cycle was the study unit, rather than the site. A production cycle is defined as the time between input of fish into a marine site and their removal for harvest, and one site may host multiple production cycles over time. The length of the production cycle varied from 54 to 124 weeks after which the sites were fallowed. Sites start their production cycles throughout the year. In this analysis, there were 88 production cycles of Atlantic salmon in the marine stage between the years 2000 and

2006. Production cycles with continuous stocking and that were not complete at the end of the period of recording (2006) were not included in this study (Soares *et al*., 2011).

3.4. Mortality data

3.4.1. Daily mortality

The number of dead fish recovered by different methods (e. g. divers, hand hold baskets, lift-up collectors for dead fish removal and hand nets) was recorded as cage-level daily mortality. On the days on which mortality was not recorded, the daily count was calculated from the total mortalities divided by the number of days since the last count (Hammel and Dohoo, 2005; Aunsmo *et al.*, 2008). The entries of zero mortality on the database were taken to indicate that during the inspection of site no dead fish was collected on that day. However, records not present in the database were understood to mean that collection of dead fish by the farmer was not performed on those days.

Weekly averages of daily mortality on site were expressed in percentages. We averaged the mortalities over a period of seven days to calculate mean daily mortality for the week. The weekly median of daily mortality on the site was also expressed in percentages and used as a central line of comparison with the standard curve of mortality (Soares *et al*., 2011). The count of fish on site as recorded in the database was the denominator used for mortality calculation, which accounted for transfers into and out of the site during the production cycle. Production cycle was considered the study unit and therefore between-cage transfers were not a concern.

3.3.2. Cause of death

The causes of death in the site production database were categorized in five categories: (Table 3-1): *infectious disease*, *production*, *environment*, *predation* and *unknown*

causes. A total of 52 pre-assigned mortality causes were identified in the analysis through a mortality code. However, there is no information concerning the process in place to assign a specific mortality code. In this analysis, a week of a production cycle was considered positive (negative) for a mortality cause when the mortality cause was (not) recorded during that week. Events with abnormal mortality levels are usually investigated and it is highly likely that the farmer's diagnosis is supported by veterinary laboratory tests. For infectious pancreatic necrosis (IPN), two mortality codes were given to distinguish between *suspected* and *confirmed* outbreaks of IPN. In this paper, an IPN-positive week is one with mortalities attributed to *confirmed* IPN. Pancreatic disease (PD) was only coded as *suspected.* The remaining codes for diseases did not distinguish between *suspected* and *confirmed* outbreaks.

Table 3-1 Mortality causes recorded grouped into five groups of mortality causes. The percentages (%) of each disease by total proportion of fish lost are represented as: * ≤ 0.5 %; ** 0.5 - 1 %; * ≥ 2 % (no infectious diseases had percentages within the interval 1 - 2 %). The numbers beside the category headings and mortality causes are the number of production cycles that fell into each category and mortality cause.**

Unknown (88)	**Production (88)**	**Infectious diseases (80)**	**Environment (27)**
Blind (4)	Accident loss (0)	Bacterial kidney disease (BKD) (7)**	Environmental (2)
Decomposed (58)	Caught in net (4)	Cardiomyopathy (CMS) (8)**	Jellyfish (5)
Deformed jaw (18)	Cull (20)	Fungus (9)*	Oxygen Starvation (10)
Disappeared (2)	Failed smolts (62)	Infectious pancreatic necrosis (Confirmed-IPN) (46)***	Plankton bloom (12)
Event mortality (12)	Jacks (13)	Moritella (0)*	Storm (11)
Eye damage (10)	Mature (28)	Pasteurelosis (5)*	
Fin rot (49)	Net tear (2)	Rickettsia (SRS) (4)**	**Predation (82)**
Gill damage (14)	Normal (61)	Sea lice (19)*	Birds (60)
Lesion (61)	Parr (42)	Suspected furunculosis (1)*	Mink (1)
Option missing (13)	Precocious male (4)	Suspected infectious pancreatic necrosis (Suspected-IPN) (69)***	Seals (80)
Other (48)	Transfer (70)	Suspected pancreas disease (PD) (18)***	
Physical damage (74)	Treatment kill (32)		
Runts (85)	Sample weighing (10)		
Samples (54)	Smolt transfer (20)		
Unidentified (15)	Suspected cannibalism (0)		

3.4.3. Production cycles grouped by quarters of initial stocking

Production cycles were grouped by the farm manager into quarters according to the month that the production cycle started, named Q1 to Q4. These quarters do not rigorously follow month boundaries, with Q1 ranging from January to early March; Q2, March until the end of June; Q3, July until the end of September, and Q4 from October to the end of December. For example, any production cycle that started at the end of the year (mid to late December) was included in the Q1 period because the farmers were confident that none of the management activities before the end of December had a significant effect on growth. Similar flexibility was also applied for the other quarterly periods.

3.5. Statistical analysis

General linear model in the form of analysis of covariance (ANCOVA) models were used to investigate the relationship between mortality and explanatory variables (see appendix 1). ANCOVA was performed in Minitab statistical software version 15. There was a large quantity of data and therefore many associations would be statistically significant due to statistical power regardless of any biologically meaningful effect sizes. Therefore, the sequential sum of squares (Seq. SS) and eta-square (η^2) were the measures used to report the variance in mortality explained by the factors and covariates and p-values were not considered. The Seq. SS is the reduction in the error sum squares as each term is fitted, in the specified order, while η^2 describes the proportion of variance explained (in the dependent variable, mortality) by a factor while controlling for the other factors already fitted in the model. η^2 is influenced by the size of the sample. η^2 values range from zero to one: higher values indicate the term explains more of the variability within the dependent variable. η^2 is calculated as:

η^2 = SSfactor / SStotal, where SSfactor is the sum of squares of the factor and SStotal is the total sum of squares.

We selected from the database several factors, including calendar year, calendar month (actual month of the year), calendar week (1-52 weeks, actual week of the year), age at sea, temperature, feed intake (feed per unit biomass at that time) and site, to investigate potential management- and environment-related factors and their potential contribution to variation in mortality. The mortality time series was converted to weekly averages of daily mortality on a site. However, below, all mortality is given units of per day. To investigate the time-scale of mortality events, lagged mortality was calculated using a one week lag interval, corresponding to the actual mortality in the previous week.

Mortality was recorded as a proportion and then transformed using a logarithmic transformation. In each analysis, mortality was the dependent variable. Temperature and feed intake were continuous variables, calendar year, month, week and age at sea were discrete variables and site was a categorical variable.

3.6. Results

3.6.1. Variation in mortality with seasonal temperature averages

The year 2001 showed lowest mortalities across the year with the exception of October, which reached a mean mortality of 0.3 % day^{-1} (Figure 3-1). This peak of mortality was caused by a plankton bloom that affected several sites. The highest variation in mortality across the year was observed in 2003 and a similar mortality pattern was observed in 2004. The year 2002 did not have high mortality across the year but the period of May to July had the highest mortality values. The main peak of mortality occurred in October across all years after the temperatures reached the highest peak in July/August (Figure 3-1).

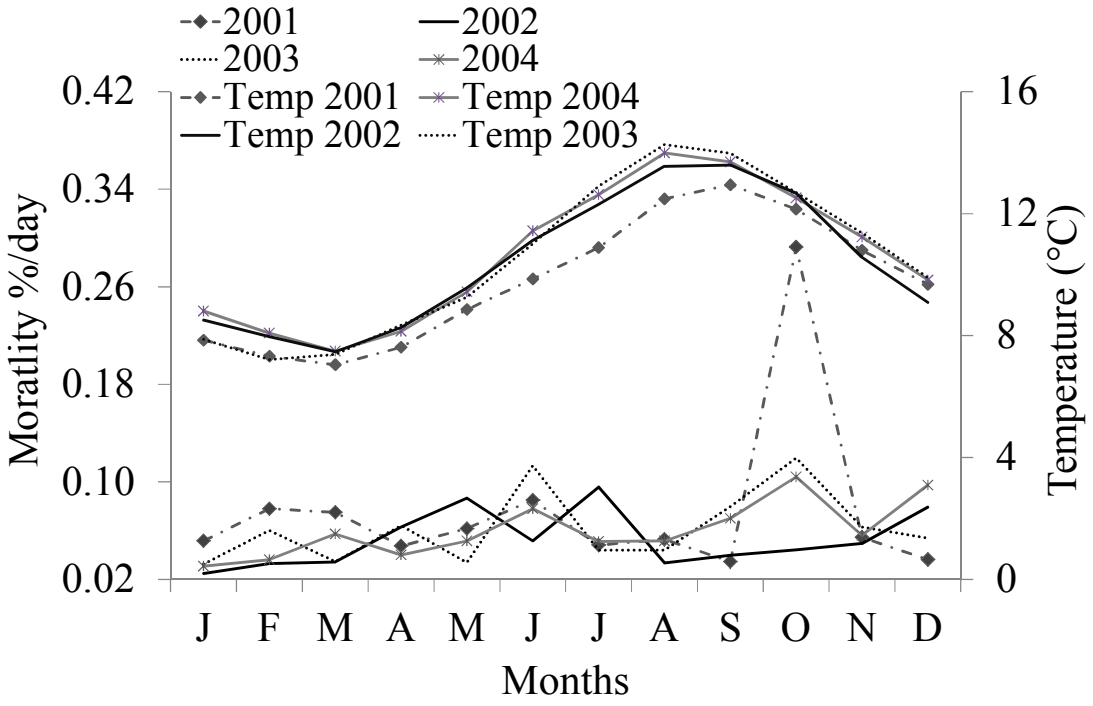

Figure 3-1 Weekly mean of daily mortality of salmon production of different years across calendar months, with mean monthly temperatures (upper set of curves).

We calculated the medians of the weekly mortality averages across all sites (below, weekly median of daily mortality). In production cycles with initial stocking in autumn and winter the median mortality were generally lower, while the median mortality of production cycles with initial stocking in spring and summer were generally increased. These production cycles showed the same pattern when compared with the standard curve of mortality (Soares *et al*., 2011). The increased mortality observed in production cycles started in spring and summer coincided with the increase of mean water temperatures at this time of the year (Figure 3-2).

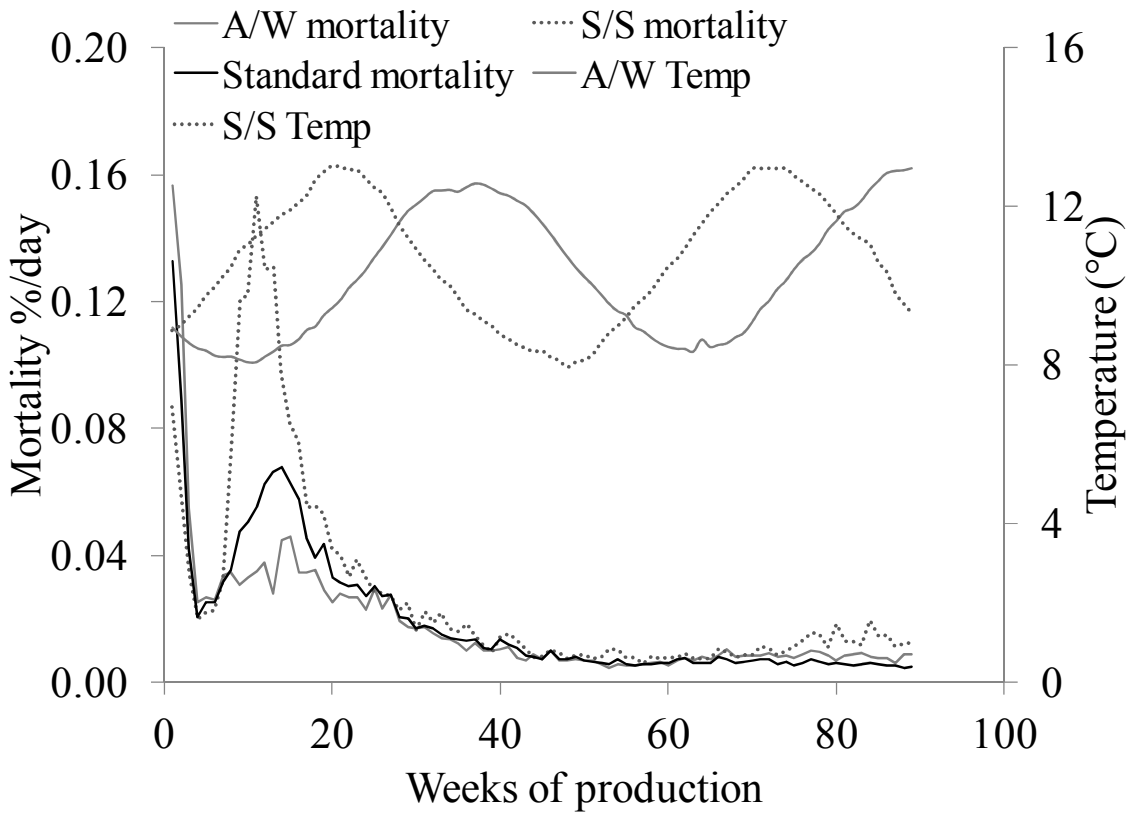

Figure 3-2 Weekly median of daily mortality versus week of production cycle, for cycles initially stocked in autumn/winter (A/W) and spring/summer (S/S) time, alongside mean temperatures (upper set of curves) and the overall median of expected mortality losses.

3.6.2. Variation in mortality across the production cycle

Production cycles showed generally increased mean mortality at the start and at the end of the cycle (Figure 3-3). This was thought to be due to fish losses post-transfer handling and through infectious diseases, including PD and cardiomyopathy (CMS). This contrasts with median mortality which was low at the end of production cycles (Soares *et al*. 2011). Mortality were also observed during the production cycle due to storms (Soares *et al*., 2011). The mortality peaked in week 43 (0.21 % day^{-1}) and in week 49 (0.37 % day^{-1}) with fish mean weight in those weeks of 1.5 kg and 1.8 kg. These mortality peaks were caused by storms and a plankton bloom, which caused very high mortality on a few sites that had been stocked at the same time.

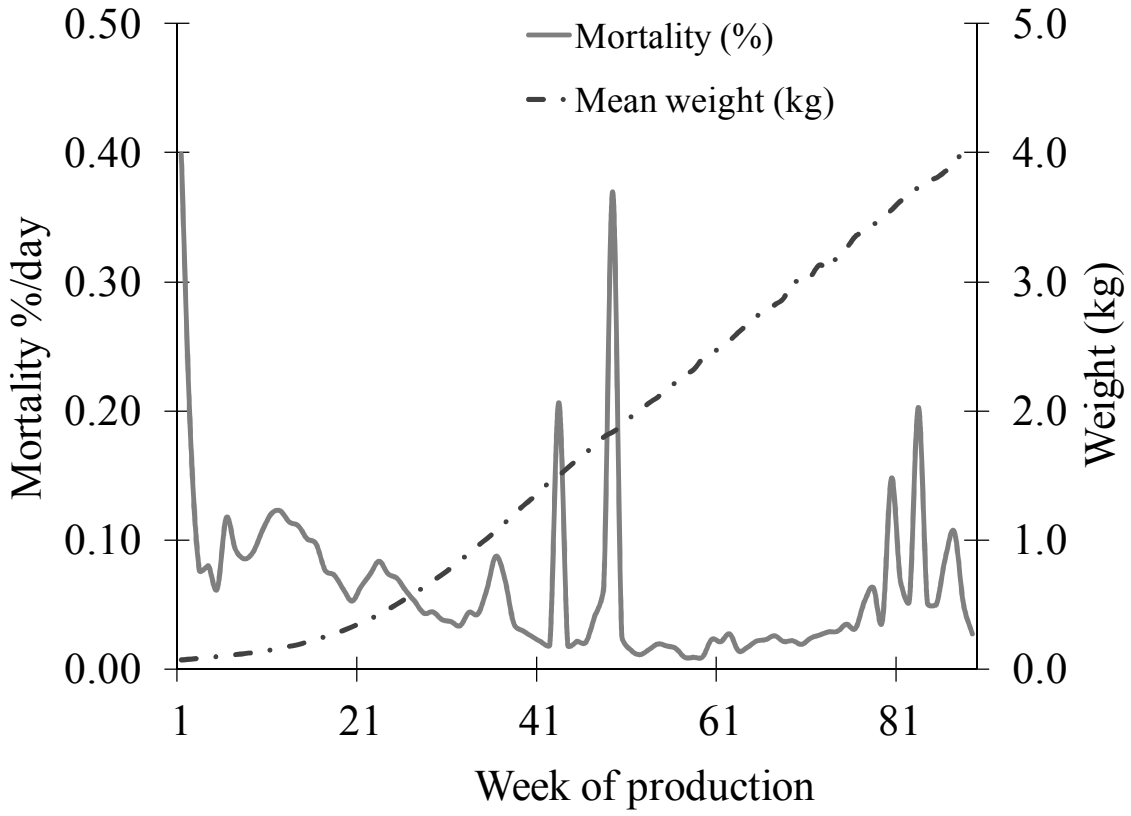

Figure 3-3 Weekly mean of daily mortality versus week of production cycle alongside the fish mean weight.

3.6.3. Variation in mmortality *versus* initial stocking quarter

Mortality plotted against temperature across the year showed a bimodal behaviour, with highest mortalities in at higher temperatures (Figure 3-4b). Production cycles started in Q2 and Q3 showed the highest mortality percentages associated with temperatures ranging between 9°C and 13.3°C, with a mortality peak at 13°C (Figure 3-4b). In contrast, Q4 production cycles had the lowest overall percentage mortality across the year followed by Q1 (Figure 3-4a). Q2 showed an increase in mortality from week 21 until week 31 with a peak in week 28 (0.04 % day^{-1}), (Figure 3-4a), which coincided with the highest average temperatures of the year (Figure 3-4b).

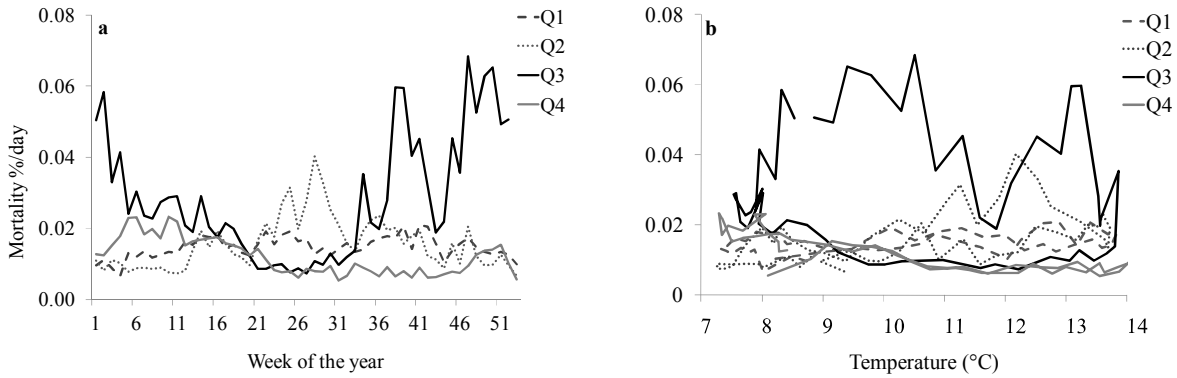

Figure 3-4 Weekly median of daily mortality against (a) weeks of the year and (b) mean temperature across the year with production cycle grouped by quarter of initial stocking.

Q1 had the lowest dispersion of weekly median of daily mortality (0.02 % - 0.007 %, interquartile range - 0.004 %) and Q3 had the highest dispersion (0.07 % - 0.007 %, interquartile range - 0.03 %), (Table 3-2).

Table 3-2 Maximum, minimum and interquartile range values of weekly median of daily mortality for the week of production cycle grouped by quarter of initial stocking.

%	Q1	Q2	Q3	Q4
Maximum	0.021	0.040	0.068	0.023
Minimum	0.007	0.007	0.007	0.005
Interquartile range	0.004	0.01	0.027	0.008

The weekly median of daily mortality of production cycles grouped by quarters of initial stocking was compared with the "benchmark" standard mortality curve, defined as the median daily mortality for weeks of production cycle from the site production database (Soares *et al*., 2011). This benchmark aims to identify unusual mortality patterns in the time series (Figure 3-5). Production cycles started in Q3 showed a higher level of mortality across the production cycle, with two mortality peaks in the first part (5 - 40 weeks) and in the last weeks (68 - end) of the production cycles. For production cycles started in the remaining quarters (Q1, Q2 and Q4), the mortality curve followed

more closely the standard mortality curve. Q1 cycles had the lower mortality levels in the first part (until week 30) of the production cycles and Q4 cycles (after week 30) in the last part, when compared with the standard mortality curve. Mortality showed a peak from week 5 to 15 for production cycles started in Q2. The noise in the later part of the time series observed in Q3 is the result of the low number of production cycles (7) in this group. The other groups, included more production cycles: 25 for Q1, 29 for Q2 and 27 for Q4.

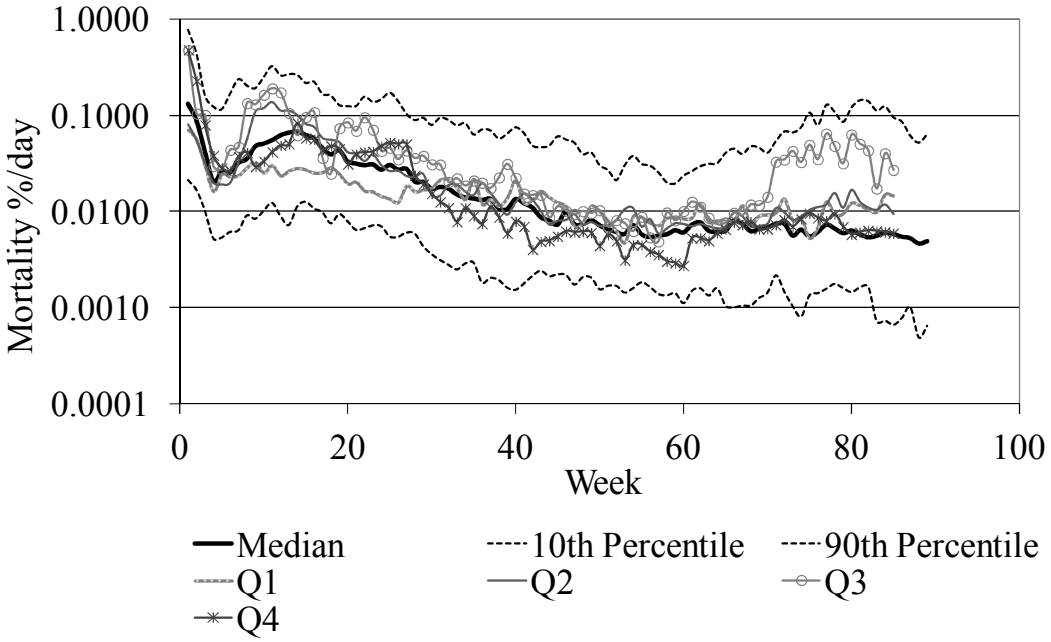

Figure 3-5 Mortality time series for production cycles grouped by quarter of initial stocking (Q1, Q2, Q3 and Q4), compared with the standard mortality curve (Soares *et al.*, 2011).

3.6.4. Variation of mortality and its drivers

The variation in mortality that could be accounted for by each one of the individual covariates was generally low across all covariates (η^2 < 10.1 %) with the exception of site (η^2 = 17.6 %) and sea age (η^2 = 10.1 %), which demonstrated a higher contribution to the variation in mortality (Figure 3-6). Age itself is related to different life periods of

fish, different fish sizes, and varying susceptibility to particular diseases and different sensitivity to environmental change.

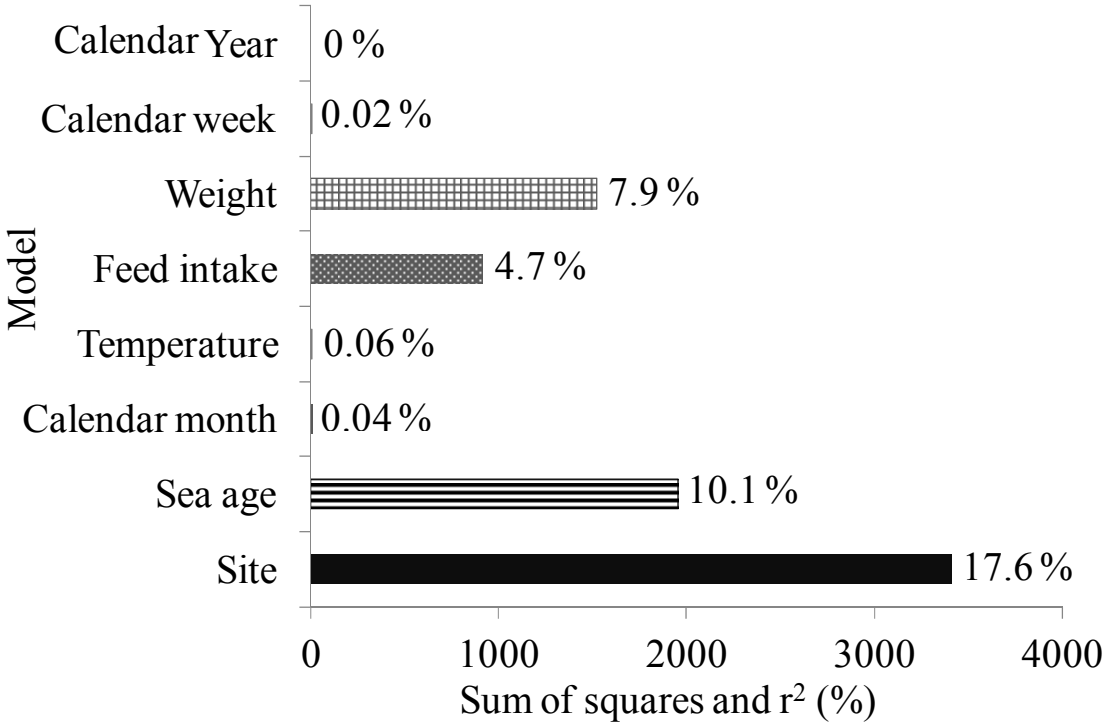

Figure 3-6 Analysis of covariance for mortality data: Total sums of squares (bars) and r-squares models (percentages) for univariate analysis of several covariates.

Site was combined with other covariates in a multivariate model, including calendar year, calendar month calendar week, weight, feed intake, temperature and sea age (Figure 3-7) to investigate confounding effects between site and other covariates when related to mortality (Figure 3-7). Site was entered into the model either before (Figure 3-7a) or after (Figure 3-7b) the other covariate(s) to test for confounding. Overall, site showed a high contribution to the variation in mortality. In all models, regardless of the order of entry of model terms, where site was included, it was the largest contributor to variance in mortality (Figure 3-7). In all models where age was included, the age accounted for 10 to 12 % of the variation in mortality (Figure 3-7a and Figure 3-7b).

Age and site were relatively independent, as might be expected given all sites hosted complete production cycles.

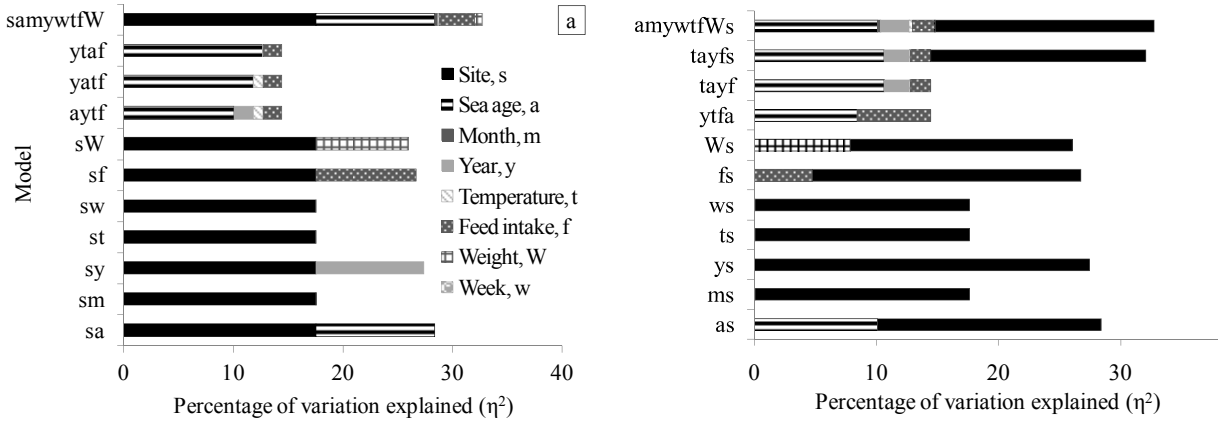

Figure 3-7 Values of η^2 (percentage of the variance in mortality explained by terms) for different models of variance in mortality. Variables included: site (s) sea age (a), month (m), year (y), temperature (t), feed intake (f), weight (W), week (w). Terms are entered into each model in the order (left to right) they appear on the graph bars. Week (w), month (m) and year (y) are calendar time.

The one-week lag term was combined with other variables—calendar year (y), calendar week (W), weight (w), feed intake (f), temperature (t), calendar month (m) and age (a)—to investigate the effects of serial correlation and potential confounding effects in our earlier results (Figure 3-8). For each one of the models, the one-week lag term was entered in the model as either the first (Figure 3-8a) or last term (Figure 3-8b). Mortality of the previous week is highly correlated with the mortality of the week in question (Figure 3-8; $\eta^2 = 71$ %). The one-week lag term contributed substantially to variation in mortality in all two-predictor the models examined, where it was combined with one of temperature, weight, year, week and month, irrespective of the order of the model terms. In the models with all the remaining predictor variables, the one-week lag term only had a slight decrease in its η^2 value and still made a substantial contribution to

variation in mortality. This means that the mortality of the previous week is highly correlated with the week in question.

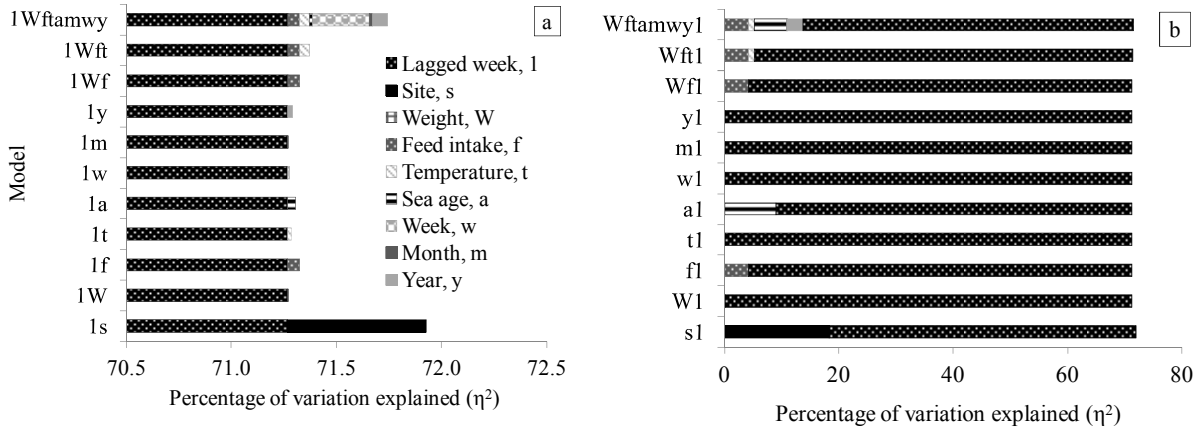

Figure 3-8 Values of η^2 (percentage of the variance in mortality explained by terms) for different models of variance in mortality. Variables included: lagged week (1), site (s), sea age (a), month (m), year (y), temperature (t), feed intake (f), weight (W) and week (w). Terms are entered into each model in the order (left to right) they appear on the graph bars. Lagged week is one-week lag term, week (w), month (m) and year (y) are calendar time.

3.7. Discussion

The aim of this study was to investigate the variation observed in mortalities encountered during the marine production cycle of Atlantic salmon. Reported mortalities were regressed against a set of explanatory variables (e. g. temperature, age, site and feed intake).

This analysis was beneficial in identifying the variables that contribute to variation in mortality and that can lead to fluctuations of *normal* mortality. Similar studies to understand patterns of mortality and their causes have been performed in pigs (Chagnon *et al*., 1991, Shankar *et al*., 2009) and poultry (Carver *et al*., 2000, Tabler *et al*., 2004) industry. As stated by Soares *et al*. (2011), fluctuations in mortality rates, their causes and explanatory factors can be identified by a benchmarking analysis helping individual farms or the industry to identify specific problems in production, and therefore to make efforts to overcome those weakness. This analysis also had some limitations due to the fact that it was restricted to a single company in the west coast of Scotland which may cause some bias. However, given the resources available and the commercial sensitivity of such data a census of the whole industry would prove impractical. The methods employed in this study may also be used within a company as a tool to investigate the drivers of variation in mortality for its own production. At the industry level, this analysis may be used as a preliminary study to identify and quantify patterns in mortality, which were overlaid by fluctuations due to infectious diseases and specific environmental events.

In this study, production cycles from Q3 had the highest mortalities, (Figure 3-4 and Figure 3-5). This high mortality may be because smolt transfer occurred during the period of increasing water temperature, when fish are more susceptible to outbreaks of

diseases such as PD (Crockford *et al.*, 1999). The small number of production cycles (7) observed in Q3 was the result of a health-management decision by the company to avoid smolt transfers at this time of the year. For this very reason, the small number of cycles resulted in the less smooth benchmark curve for cycles beginning in Q3.

Site, year, feed intake and the one-week lag term were associated with the variance in mortality (Figure 3-7 and Figure 3-8). The site-to-site variation in mortality may be caused by the unpredictable occurrence of epidemics and environmental events, such as storms or plankton bloom (Soares *et al*., 2011), in certain sites and not in others. Higher levels of temperatures occurred in certain years, for example 2003, which also contributed to the increase of the prevalence of certain infectious diseases as suggested in previous studies (Lannan *et al*., 1992, Crockford *et al.*, 1999 and Cusak *et al*., 2002), including IPN and PD, therefore increased mortality (Soares *et al*., 2011). Differences in management practices between years and among production cycles on the same site may also be a cause of mortality variability (Wheatley *et al*., 1995, Crockford *et al*., 1999). The site location and/or geographical area can be also a risk factor for certain disease outbreaks, for example IPN outbreaks (Jarp *et al*, 1994), or predispose sites to specific environmental problems, such as plankton blooming and storms (Pillay and Kutty, 2005, Soares *et al*., 2011). Site-specific variables that may influence the variation of mortality may have not been fully captured in the other data fields (e.g. temperature or season) leading to a large residual effect associated with site.

The one-week lag term was included in order to capture the correlation between the mortality of the previous week and the week in question. The high correlation between mortality in sequential weeks decreased over time, with a significant drop after the

second week. This suggests that the majority of mortality events were quite short lived and therefore the correlation in time drops quickly.

Feed intake was also associated with on-site variation in mortality. However, the variation associated with feed intake should be considered more as a consequence than as a cause since diseased fish tend to reduce their feed intake caused by the loss of appetite and fasting strategy (Damsgård *et al*., 1998, Pirhonen *et al*., 2003, Ramsay *et al.*, 2004). Additionally, in winter time, fish also reduce feed intake due to the lower temperatures (Elliot, 1991, Koskela *et al*., 1997).

3.7.1. Conclusions

This study allowed the identification of several possible factors, such as site, temperature and age at sea, that may contribute for fluctuations in mortality rates. It showed that variables such as site and temperature may contribute to variance in mortality which can be a risk factor for certain infectious diseases. This variation in mortality can be identified by the use of benchmark analysis (Soares *et al*., 2011), the aim of which is to quantify possible problems of production either in the industry or at the farm or company level, against which levels of unusual mortality can be noted.

A wider database would be of great benefit and would allow the identification of other combinations of factors and to resolve more complex factor interactions. It would also allow a better understanding of the challenges faced by Scottish salmon industry and support the development of industry-level benchmarks that can help both the commercial sector and regulators in the prevention and control of fish diseases.

3.8. Acknowledgments

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Chapter 4 Evaluating abnormal mortality as an indicator of disease presence in the Atlantic salmon industry using the receiver operating characteristic (ROC)

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This chapter describes an attempt to explore the effectiveness of reported mortality at detecting infectious disease based on different mortality cut-off values. The daily mortalities calculated in Chapter 2 were used as a base for calculating the weekly mortality.

The main author, Silvia Soares, conducted all analytical work, developed the final model and wrote this manuscript. Dr. A. G. Murray, Dr. M. Crumlish, Prof., J. F. Turnbull and Dr. D. M. Green provided supervisory and editorial support throughout the whole study. Filipa Soares also provided the assistance to implement the ROC analysis.

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4. Evaluating abnormal mortality as an indicator of disease presence in the Atlantic salmon industry using the receiver operating characteristic (ROC).

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4.1. Abstract

Aquaculture faces many threats, including diseases, of which some are notifiable under current UK regulation, *e.g.* infectious salmon anaemia (ISA) and infectious haematopoietic necrosis (IHN). Abnormal mortality is one possible indicator of the presence of infectious disease on a site that may be used, by the regulator, as a surveillance alert that allows them to identify possible notifiable diseases and to activate measures of control to reduce the risk of spreading those diseases. Therefore, mortality records at the farm level may be a useful indicator for regulatory surveillance purposes in order to identify potential disease outbreaks. In the UK, regulators and producers have discussed abnormal rates of mortality that may be considered as a trigger to notify the official regulator. In our study, the receiver operating characteristic (ROC) approach was used on mortality data from production cycles of a site production database of marine Atlantic salmon belonging to a single company. The usefulness of these data in helping detection of infectious diseases was determined using measures of sensitivity and specificity. For fish under 750 g, the abnormal rates of mortality did not provide a strong indication of the presence of disease. The area under the curve ($0 \leq AUC \leq 1$) values were generally low with the exception of cardiomyopathy syndrome (CMS) that showed $AUC = 0.77$ for weekly mortality and $AUC = 0.73$ for five-week rolling mortality. However, abnormal levels of mortality for fish with weight over 750 g provided a strong indication of the presence of disease with the exception of both

suspected and *confirmed* IPN. The probabilities of triggering official notification were low since mortality events over the percentages proposed happened infrequently. The most efficient trigger will be for weekly mortality (1 %) for fish with weight over 750 g since abnormal mortalities in such large fish are more likely to be associated with the presence of disease.

4.2. Background

The control of diseases is essential to the profitable production of any farmed species (Menzies *et al*., 1996). In the UK, legislation was first implemented to prevent the introduction and spread of serious fish diseases under the Diseases of Fish Act 1937, which introduced the legal requirement to notify the competent authority of suspicion or presence of certain diseases in fish (McVicar, 2002). Additionally, the finfish aquaculture sector in Scotland is supported by a code of good practice (Anonymous, 2010) that provides guidelines to reduce the risk of spreading disease. The guidelines from the code of good practice aim to prevent spread of infection by providing standards for management of fish disease. These standards incorporate a set of measures to be implemented regardless of disease history (e.g. basic biosecurity measures and fallowing) and a set of measures to be implemented when suspicion and/or confirmation of diseases occurs, consisting of disease control measures such as movement controls or culling. The code of good practice in conjunction with the legislation of Diseases of Fish (control) Regulations (SI 1994 No 1447), introduced in 1994, implemented measures of disease control that are required when suspicion or confirmation of a disease outbreak occurs. The Fish Health Regulations 1997 (SI 1997 No 1881) were introduced in 1997 to control the movement of live molluscs and live fish, their eggs and gametes as well as certain dead fish into the UK from elsewhere in the EU. The

Aquaculture and Fisheries (Scotland) Act 2007 was introduced in 2007 to regulate against the unauthorised introduction of fish to inland waters and for the control of *Gyrodactylus salaris*.

As part of these legislations, the regulator requires notification to the official services of the suspicion of certain diseases—notifiable diseases—such as infectious salmon anaemia (ISA) and infectious haematopoietic necrosis (IHN), in order to carry out surveillance. However, surveillance resources are necessarily limited, so their most efficient use is through risk-based surveillance whereby sampling is concentrated on sites that are most likely to be infected (Stark *et al*., 2006). The recent Chilean outbreak of ISA (Henson, 2008; Mardone *et al*., 2009; Vass, 2010) illustrates the threats and the impacts of disease in the aquaculture industry and the importance of a good regulation and husbandry practices to reduce the impact of spread of infectious disease. In Scotland, the early implementation of regulations largely contributed to the control of an ISA outbreak in 1998 (McVicar, 2002) and again in 2008-2009 in Shetland (Murray *et al*., 2010). In 2008-2009 during the ISA outbreak, Marine Scotland used farm-level mortality as an indicator of disease. Abnormal mortality rates alerted the Marine Scotland Science Fish Health inspectors to the area affected by ISA in 2008, and sampling based on this mortality allowed rapid detection that confined the disease to a small area of south-east Shetland (Murray *et al*. 2010).

The presence of abnormal mortality rates on a site is one possible indicator of disease. Different diseases may lead to different levels of mortality. Mass mortality can also be related with non-pathogen driven causes including natural causes such as storms (Pillay and Kutty, 2005, Soares *et al.*, 2011). Nevertheless, farm-level mortality records are a potential indicator that may be used to trigger surveillance and allow the official

authority responsible for fish health in Scotland, Marine Scotland, to control and study the frequency, the spread and the disease patterns within farmed fish populations.

Currently, the Scottish Government is planning to introduce statutory reporting of abnormal mortality as a possible measure to combat disease threats more efficiently and mitigate the impact of a serious disease. Potential mortality threshold values have been discussed with the industry and cut-off values selected by the regulator Marine Scotland in consultation with the industry. These thresholds are considered to be of value to identify when abnormal mortalities have occurred which could then be used for inspection alerts. The introduction of mortality thresholds may allow a rapid detection of the presence of notifiable diseases and activation of measures of appropriate disease control, where required. The optimal abnormal mortality threshold used to trigger surveillance is a trade-off between fewer missed true positive tests at the expense of more false alerts. An increased number of false alerts is an important factor in overall surveillance system cost.

The aim of this study was to explore how effective reported mortality would be at detecting the presence of outbreaks of infectious disease based on different mortality cut-off values and then to extrapolate further to allow for rapid detection of notifiable diseases. Since limited mortality information is available for notifiable diseases, production cycles from a site production database without notifiable diseases were used to analyse mortality patterns for infectious diseases and to support the identification of adequate mortality surveillance thresholds. Abnormal mortality percentages of 1.5 % for weekly mortality and 6 % for five-week rolling mortality for fish with average weight under 750 g and 1 % for weekly mortality and 4 % for five-week rolling mortality for fish with average weight over 750 g were considered in this analysis as

potential thresholds for official regulators to be notified. In this study, the usefulness of mortality recorded at the farm level for aiding detection of infectious disease was assessed using measures of sensitivity and specificity, i.e., the probability that exceeding the cut-off rate of mortality is associated with the presence of disease (sensitivity) and mortality below the cut-off is associated with absence of disease (specificity).

4.3. Receiver operating characteristic (ROC)

In our study, the receiver operating characteristic method (ROC) was applied on mortality data from Atlantic salmon in seawater from a single company, which represented one third of total Scottish farmed salmon production in 2005 (Anonymous, 2005). For that, we used measures of sensitivity and specificity for each test across a variety of possible test thresholds. For such a test (see appendix 2-Table App 2-1):

Sensitivity = True positive / (True positive + False negative)

Specificity = True negative / (True negative + False positive)

In many cases, the result of a diagnostic test is derived from a continuous measurement or test score, such as binding or reaction rate, and when the score exceeds a fixed reference value, called the threshold or cut-off value, the test is said to be positive (Schulzer, 1994). Once each test score is classified either positive or negative based on the cut-off value, the true positive and negative can be identified. A “condition” positive is considered “true” positive based on the cut-off value positive with a true disease status and a “condition” negative is considered “true” negative based on the cut-off value negative with a non-disease status. Sensitivity is then derived as the percentage of all true positive tests from the total of cases with disease, while specificity is derived as

the percentage of all true negative tests from the total of cases with absence of disease. Sensitivity and specificity depend on the cut-off value used to define positive and negative test results (Obuchowski, 2003). Each point on the ROC chart is derived by using different cut-off values and the ROC curve is built from the set of all possible cut-off values (Obuchowski, 2003). The accuracy of the positive and negative classification of a diagnostic test, which can be termed true disease status, is estimated by standard ROC methods (Zou *et al*., 2007). The true disease status is named as Gold Standard (Zou *et al*., 2007). A gold standard is needed for identification of specificity and sensitivity of a test because any test can give incorrect results.

While sensitivity and specificity are measures of accuracy, predictive values are measures of performance (Schulzer, 1994). The predictive value of a test is a measure of how often the test result (positive or negative) is correct, i.e. the proportion of all positive tests that are true positives is the positive predictive value (PPV) and the proportion of all negative tests that are true negatives is the negative predictive value (NPV) (Zweig and Campbell, 1993; Schulzer, 1994). For such a test (see appendix 2-Table App 2-1):

Positive predictive value = True positive / (True positive + False positive)

Negative predictive value = True negative / (True negative + False negative)

In this study, the PPV and NPV are dependent on disease prevalence in the studied population. They are affected by the prevalence differently: the PPV increases with increasing prevalence, while NPV decreases (Zweig and Campbell, 1993; Schulzer, 1994).

The ROC methodology provides an opportunity of identifying an optimum reporting cut-off value by identifying the point on the curve at which the sum of sensitivity and specificity is maximized (Zweig and Campbell, 1993). An ROC curve is a graphical representation of the sensitivity (true positive rate (TPR)) as the *y* coordinates versus 1 − specificity (the true negative rate (TNR)) as the *x* coordinates (Park *et al*., 2004) of a diagnostic test across a variety of possible test thresholds. A good model performance (Figure 4-1) is characterised by a curve that maximizes the sensitivity for low values of 1 − specificity, where the ROC curve passes close to the upper left corner of the plot (Robertson *et al.*, 1983; Schulzer, 1994). The diagonal line $y = x$ (Fawcett, 2006) is the ROC curve corresponding to an uninformative test that is no better than a random guess (see Figure 4-1). The area under the curve (AUC) is a global (i.e. based on all possible cut-off values) summary statistic of diagnostic accuracy (Greiner *et al.*, 2000). The possible range of the AUC is from zero to one. The uninformative test gives 0.5, and below 0.5 means worse performance than random chance (which may imply the test has value as a negative test).

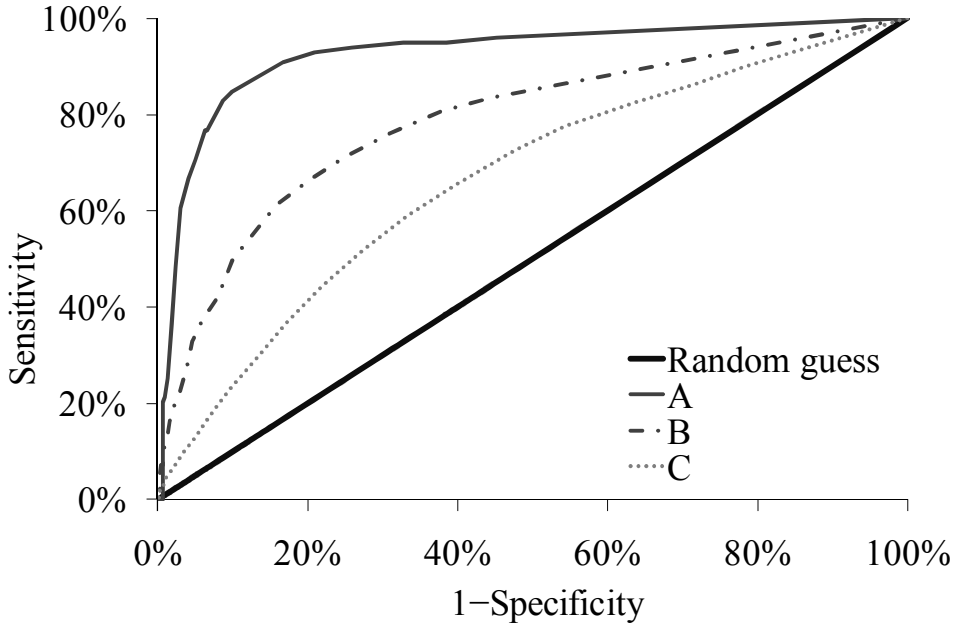

Figure 4-1 Three ROC curves with different AUC values. A, indicates high accuracy with AUC over 0.9. B and C are ROC curves of tests with some ability to distinguish between subjects with and without disease, with test B (0.8) more useful than test C (0.65). Random guess with AUC of 0.5.

4.4. Material and methods

4.4.1. Data collection

The data were supplied by a single company and included over 60 million Atlantic salmon smolts that were moved into 82 marine production sites located on the western coast of Scotland (Soares *et al.*, 2011). Only complete production cycles of salmon production between the years 2000 and 2006 were analysed. Production data for mortality causes, and mortality losses were extracted from a BusinessObjectsTM database (Soares *et al.*, 2011).

The study unit was the production cycle and it was defined as the period of time between the transfer of the first fish onto the site and their removal for harvest. Production cycles start on sites across the year and their length raged between 54 and

124 weeks. A total of 88 production cycles of Atlantic salmon in the marine stage between the years 2000 and 2006 were used (Soares *et al*., 2011).

4.4.2. Daily mortality

Daily mortality at cage level was entered as the number of dead fish collected by divers, hand-held baskets for dead fish removal, lift-up collectors and hand nets. For days with no mortality records, the daily count was calculated from the total mortalities divided by the number of days since the last count (Hammel *et al*., 2005; Ansumo *et al*., 2008). Two different types of information were found in the database: entries with zero and absent records for some of the days. The first was taken to indicate that the site had been inspected by the farmer and no dead fish was found, while the second were taken as indicating no inspection had occurred on that day.

4.4.3. Weekly mortality

Each production cycle was split by suggestion of the regulator and the industry into two periods according to mean fish weight: under or above 750 g, dividing production cycles into two different periods usually affected by different infectious diseases and with different economic impacts. In fish with an average weight under 750 g, the first six weeks of post initial stocking were not included due to transfers being a potential cause of high mortality.

The weekly mortality was the sum of daily percentage mortality over seven days (Soares *et al*., 2011). The five-weeks rolling mortality was derived as the rolling sum of the last five weeks of weekly mortality. The day of transfer of the first fish onto the site was considered day zero. The denominator used for calculating percentage mortality was the count of fish on site as recorded in the database, which took into account

transfers into and out of the site during the production cycle. Between-cage transfers were not a concern since the production cycle was the study unit.

4.4.4. Cause of death

Mortality in the site production database was attributed by the farmer to one of 52 pre-assigned mortality causes. To simplify, we re-grouped mortality causes with records in the database into five categories (Table 4-1): *infectious disease*, *production*, *environment*, *predation* and *unknown* causes. Under this analysis, the week of the production cycle was considered as positive to a mortality cause when the mortality cause was recorded in the database in that week and negative when the mortality cause was not recorded in the database in that week. The mortality causes were identified in the database through a mortality code. There were no metadata detailing how mortality causes were originally assigned on-farm. However, events with abnormal mortality rates are usually investigated and it is highly likely that the farmer's diagnosis is supported by veterinary or laboratory based diagnosis in such cases. For infectious pancreatic necrosis (IPN), two mortality codes were given to distinguish between *suspected* and *confirmed* outbreaks of IPN. In this paper, an IPN-positive week is one with mortalities attributed to *confirmed* IPN. Pancreatic disease (PD) was only coded as *suspected.* The remaining codes for diseases did not distinguish between *suspected* and *confirmed* outbreaks.

Table 4-1 Mortality causes recorded in the on-farm database grouped into five groups of mortality causes. The percentages (%) of each disease by total number of fish lost are represented as: * ≤ 0.5 %; ** 0.5 - 1 %; * ≥ 2 % (1 - 2 %, there were no infectious diseases with percentages within this interval).**

Unknown	Production	Infectious diseases	Environment
Blind	Accident loss	Bacterial kidney disease (BKD)**	Environmental
Decomposed	Caught in net	Cardiomyopathy (CMS)**	Jellyfish
Deformed jaw	Cull	Fungus*	Oxygen Starvation
Disappeared	Failed smolts	Infectious pancreatic necrosis (Confirmed-IPN)***	Plankton bloom
Event mortality	Jacks	Moritella*	Storm
Eye damage	Mature	Pasteurelosis*	
Fin rot	Net tear	Rickettsia (SRS)**	**Predation**
Gill damage	Normal	Sea lice*	Birds
Lesion	Parr	Suspected furunculosis*	Mink
Option missing	Precocious male	Suspected infectious pancreatic necrosis (Suspected-IPN)***	Seals
Other	Transfer	Suspected pancreas disease (PD)***	
Physical damage	Treatment kill		
Runts	Sample weighing		
Samples	Smolt transfer		
Unidentified	Suspected cannibalism		

To study the value of abnormal mortality as an indicator of presence of infectious diseases, a range of mortality cut-off values were considered; only the most relevant cut-off values described in Table 4-2 are studied in detail as potential thresholds to be used to generate regulatory surveillance alerts.

Table 4-2 Percentage mortality thresholds suggested by the regulator, Marine Scotland Science, to generate regulatory alerts in salmon production seawater phase.

Production cycle average weight	Weekly mortality	Five-week rolling mortality
Under 750 g	1.5%	6.0%
Above 750 g	1.0%	4.0%

4.4.5. Receiver operating characteristic curves (ROC)

A range (34) of mortality cut-off values were chosen based on a distribution of power of 10 across of 0.0 to 7.9. Additionally, the percentage mortality cut-off value of 1.5 %,

4 % and 6 % were also included because they were considered relevant for the purpose of this study and were not part of the cut-off values generated.

4.4.5.1. Diagnostic Test and Predictive Model

In this study, there was no *"*gold standard*"* test because mortality causes were not necessarily confirmed by veterinary or laboratory diagnostic testing. In order to derive the ROC curve, a week of production cycle was classified as either "test" positive or negative depending upon whether weekly mortality was above or below the cut-off value. A week was classified as "condition" positive if a specific disease cause or group of causes occurred during that week. Sensitivity and specificity were then derived by cross-tabulation the true/false "test" data against the true/false "condition" data.

4.4.5.2. Applying the receiver operating characteristic (ROC) method

A bootstrap method was used to calculate ROC curve confidence intervals (Henderson, 2005). The bootstrap resampling was performed using a Visual Basic macro. One thousand samples of the mortality data were drawn with replacement from the original data, with bootstrap sampling at the level of the production cycle. Each bootstrap sample therefore had the same number of production cycles as the original dataset, but not necessarily the same number of week records. The number of week records depends on the length of the production cycle. For each one of the samples, the ROC curve and the respective AUC were calculated. The 95 % confidence intervals were identified from the percentiles of the distribution of values obtained. This method was only applied to three ROC curves: *confirmed* IPN records, PD records and for *infectious diseases* category. The two diseases, *confirmed* IPN and PD were chosen because they were relevant for the industry (Table 4-1). Additionally, the bootstrap method was used to derive the ROC curve based on 45 samples for the weekly mortality cut-off values of

0.05 %, 0.1 % and 0.5 % for graphical representation of the variability of the AUC measures for *confirmed* IPN, PD and *infectious diseases* category. The mortality cut-off values of 0.1 % and 0.5 % weekly mortality were chosen as these are applied by farmers (0.1 %) as an indicator for increased surveillance at the site or the threshold level suggested by the certification scheme (0.5 %) (Anonymous, 2007). The cut-off value at 0.05 % was chosen to provide an extra point to represent the ROC curve graphically as the sensitivity and specificity had high values at this cut-off. The mortality cut-off values of 1.0 %, 1.5 %, 4.0 % and 6.0 % were not plotted because the sensitivity values in those points were close to zero.

4.5. Results

4.5.1. Weekly and five-week rolling mortality for fish above and below 750g mean weight

In fish with mean weight under 750 g, few production cycles had weekly mortality over 1.5 % for the weekly mortality (Figure 4-2a) or over 6 % for the five-week rolling mortality (Figure 4-2b). In fish with mean weight over 750 g, production cycles with weekly mortality over 1.0 % (Figure4-2c) or over 4 % for five-week rolling mortality (Figure 4-2d) were few in number. However, the triggers occurred more frequently for 1.5 %, weekly mortality, and 6 %, five-week rolling mortality, for fish with average weight under 750 g when compared with 1 % cut-off for weekly mortality and 4 %, five-week rolling mortality, for fish average weight over 750 g.

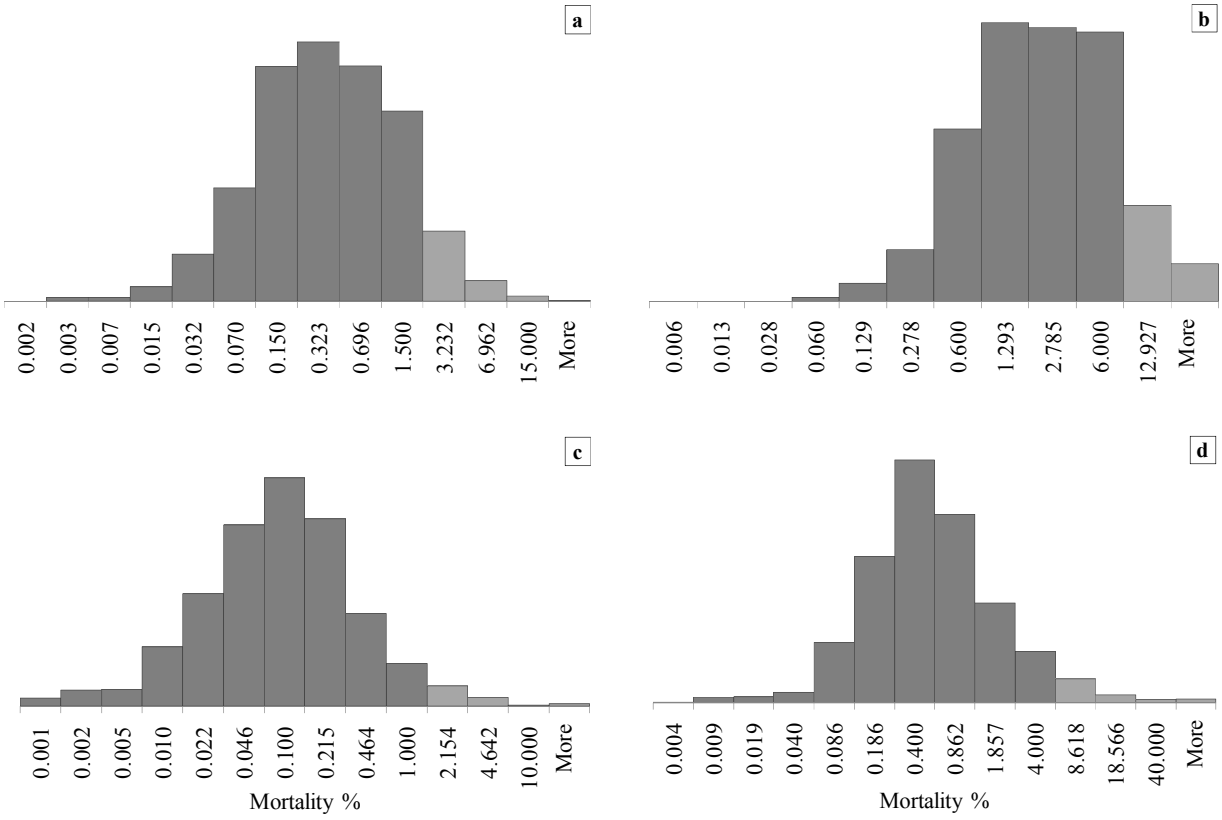

Figure 4-2 Histogram to indicate the frequency of mortality above cut-off levels (1.5 %, weekly mortality, and 6 %, five-week rolling mortality, for fish below 750 g; 1 %, weekly mortality, and 4 %, five-week rolling mortality, for fish above 750 g). a) and b) represent mortality percentages above 1.5 %, weekly mortality, and 6 %, five-week rolling mortality, for fish below the mean weight 750 g. c) and d) represent mortality percentages above 1 %, weekly mortality, and 4 %, five-week rolling mortality, for fish over 750 g mean weight. The values of x-axis indicate the maximum value of the bin.

4.5.2. Receiver operating characteristic (ROC) curves mortality as a test for infectious disease

The weekly mortality for fish with average weight under 750 g did not provide a strong indication of the presence of infectious disease (Figure 4-3). The AUC (Table 4-3) values were generally low, ranging from 0.50 to 0.66 with the exception of *confirmed* IPN (0.68), *infectious disease* (0.70) and cardiomyopathy syndrome (CMS) (0.77) (Table 4-3). A similar situation can be observed for the five-week rolling weekly

mortality, with values ranging between 0.50 and 0.65, with the exception of CMS (0.73), *confirmed* IPN (0.71) and *infectious disease* (0.73) (Table 4-3). There were very few occurrences of CMS for small fish, therefore the AUC was based on few data points, i.e. two records, in one week of two production cycles. The ROC curve showed no ability to discriminate for sea lice and fungus with AUC values of 0.50 and 0.57 for the weekly mortality and 0.57 and 0.56 for the five-week rolling mortality. The weekly mortality recorded for fish with average weight over 750 g provided a strong indication of presence of infectious diseases (Figure 4-3b). The AUC values were generally high for weekly mortality for most of the infectious diseases recorded, ranging from 0.75 to 0.91, with exception of *suspected* IPN (0.49) and *confirmed* IPN (0.65) (Table 4-3). Similar results were observed for five-week rolling mortality, with AUC ranging between 0.75 and 0.92 for all the diseases with exception of *suspected* IPN (0.45) and *confirmed* IPN (0.68) (Table 4-3).

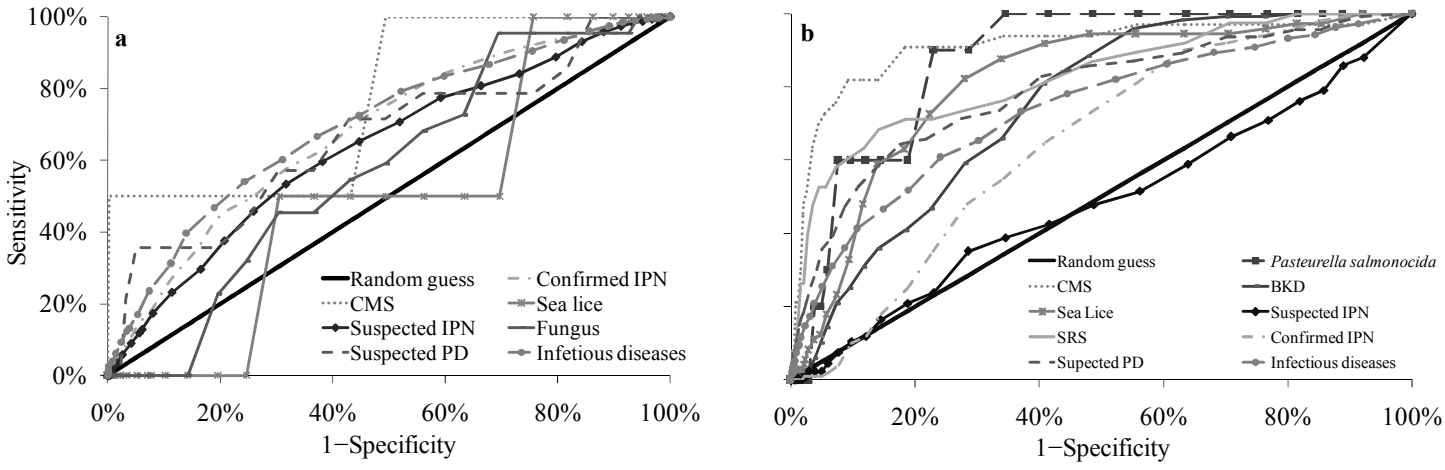

Figure 4-3 ROC curve mortality based on weekly mortality for infectious disease recorded in an site production database divided according to average fish weights (under 750 g and above 750 g). a) mean fish weight under 750 g, b) mean fish weight over 750 g (for abbreviations, see Table 4-1).

Table 4-3 AUC values for ROC curves for weekly and five-week rolling mortality for fish with average weight under 750g and over 750g for all the infectious diseases recorded. 95 % confidence intervals (in parentheses) of confirmed IPN, suspected PD and *infectious diseases* were calculated based on bootstrap estimates (for abbreviations, see Table 4-1).

Diseases	**AUC**	
Under 750 g	**Weekly mortality**	**Five-week rolling mortality**
Sea lice	0.50	0.57
Fungus	0.57	0.56
Suspected IPN	0.64	0.64
Suspected PD	0.66 (0.41 - 0.81)	0.65 (0.39 - 0.81)
Confirmed IPN	0.68 (0.62 - 0.73)	0.71 (0.64 - 0.76)
Infectious diseases	0.70 (0.64 - 0.75)	0.73 (0.67 - 0.78)
CMS	0.77	0.73
Over 750 g	**Weekly mortality**	**Five-week rolling mortality**
Suspected IPN	0.49	0.45
Confirmed IPN	0.65 (0.60 - 0.73)	0.68 (0.62 - 0.78)
Infectious diseases	0.74 (0.64 - 0.82)	0.77 (0.65 - 0.85)
BKD	0.75	0.75
Suspected PD	0.79 (0.61 - 0.92)	0.80 (0.62 - 0.93)
Sea lice	0.81	0.82
SRS	0.83	0.87
Pasteurella salmonocida	0.87	0.92
CMS	0.91	0.89

A bootstrap method was applied for estimation of the confidence in sensitivity and specificity. A confidence interval that includes AUC equal to 50 % corresponds to uninformative test. This bootstrap was based on the weekly and five-week rolling mortality (Figure 4-4) for fish with average weight under 750 g (Figure 4-4a and Figure 4-4b) and above 750 g (Figure 4-4c and Figure 4-4d). The sensitivity and the specificity show low levels of confidence for *infectious diseases* and *confirmed* IPN based on the weekly and five-week rolling mortality, with the exception of pancreas disease, irrespective of the fish average weight. PD showed some variation amongst bootstrap samples for each mortality cut-off value, mainly in the sensitivity. The variation

observed in PD was due to the presence of fluctuation in mortality within production cycles with the *suspected* PD as the mortality code, possibly as the result of the incorrect identification of the infectious disease, which caused variation in prevalence of PD across the production cycles, even though the prevalence was low. The correlation between sensitivity and specificity was analysed for each one of the cases represented in Figure 4-4. The sensitivity and the specificity did not show any correlation (maximum value: $R^2 < 0.55$) across all cut-off values, meaning that sensitivity and specificity were independent of each other and confidence intervals of both can be sensibly stated independently of each other.

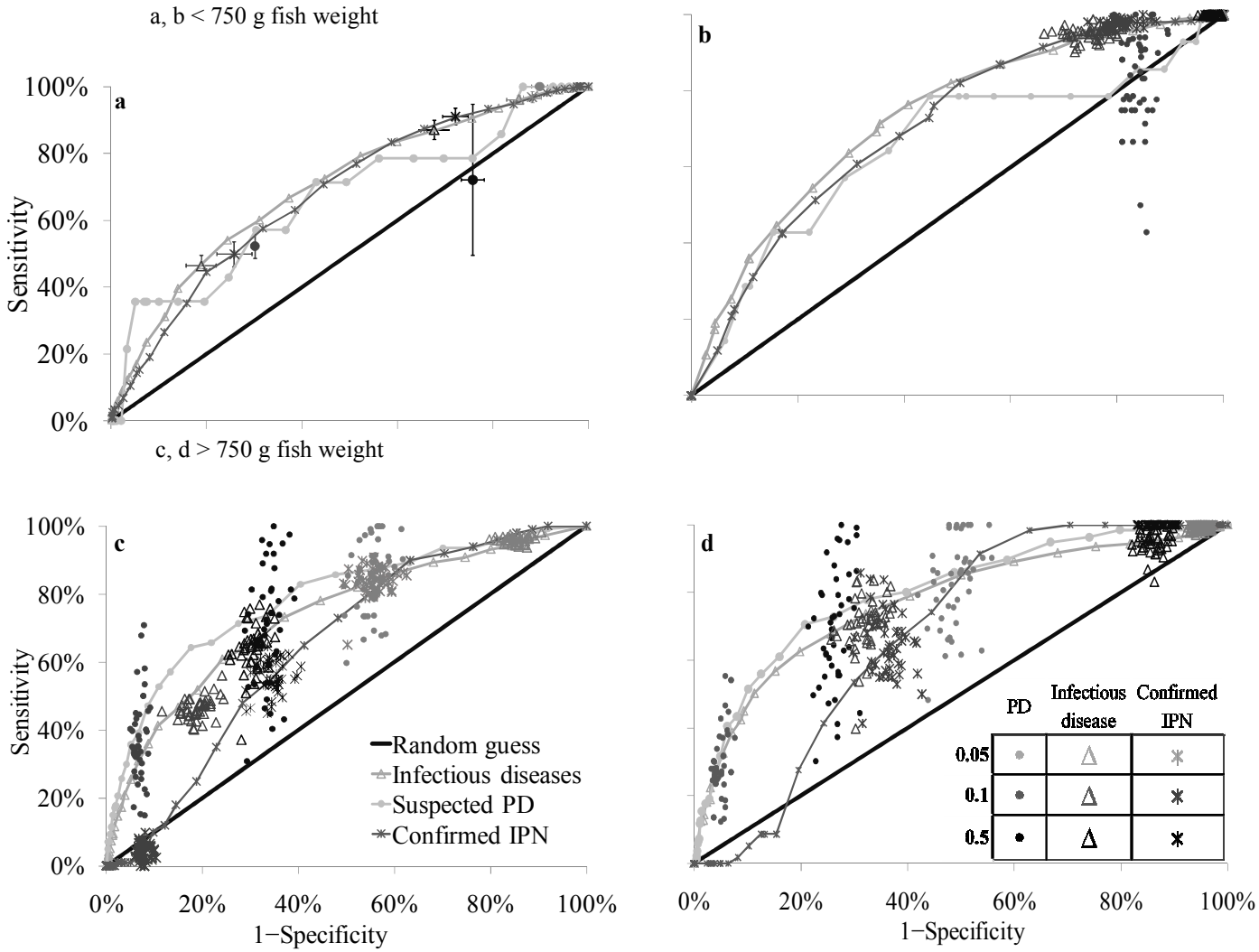

Figure 4-4 ROC Bootstrap estimates for three cut-off values: 0.05 %, 0.1 %, and 0.5 %. The analysis was divided according to average fish weights (under 750 g and above 750 g). a) and b) the variation of the sensitivity and 1− specificity of three cut-off values belonging to the ROC curve of weekly and five-week rolling mortality. a) represents an example of the variation of bootstrap iterations based on the confidence intervals (95 %). c) and d) the variation of the sensitivity and 1− specificity of three cut-off values for weekly and five-week rolling mortality (for abbreviations, see Table 4-1).

4.5.3. The utility of mortality level in disease detection

Sensitivity was low; while specificity was over 88.8 % for all cut-off values (Table 4-4) for fish with an average weight under 750 g. For fish with average weight over 750 g, similar results can be observed with the sensitivity generally low and specificity values over 95.1 % for all cut-off (Table 4-4). All the PPV were below 70 % for fish with average weight under 750 g (Table 4-5), with the exception of the *infectious diseases* category with a PPV of 71.1 % for weekly mortality and 1.5 % cut-off value and PPV of 78.0 % for five-week rolling mortality and cut-off value of 6.0 %. The categories *suspected* IPN, *suspected* PD, CMS, sea lice and fungus had few records of mortality, therefore the prevalence was zero or close to zero, resulting in low PPV values.

For fish with average weight under 750 g, the NPV ranged between 77 % and 100 % for the weekly mortality and 76 % and 99.9 % for the five-weeks rolling mortality, with the exception of the *infectious diseases* category with low values for weekly mortality cut-off values of 0.5 % (68.1 %) and 1.5 % (60.8 %) and a low value for five-week rolling mortality for the 6 % cut-off (58.5 %). For fish with average weight over 750 g, the PPV were low across all the infectious diseases for weekly and five-week rolling mortality, with highest values for the *infectious diseases* category (> 40 %) for weekly mortality for cut-off values of 0.5 % and 1 % and five-week rolling mortality for the cut-off value of 4 %. The NPV values were all above 80 % across all the infectious diseases recorded with similar values for cut-off values of 0.1 % (93.3 % - 100 %), 0.5 % (89.8 % - 99.9 %) and 1.0 % (88.8 % - 99.8 %), weekly mortality, and for cut-off values of 4 % (90.1 % - 99.8 %), five-week rolling mortality (Table 4-5).

Table 4-4 Sensitivity and specificity at the 1.5 % cut-off value for weekly mortality, and 6 % for five-week rolling mortality for fish with average weight under 750 g and 1 % for weekly mortality and 4 % for five-week rolling mortality for fish with average weight over 750 g (see appendix 2-Table App 2-2 & Table App 2-3, for abbreviations, see Table 4-1).

Under 750 g				
Cut off (%)	**1.5**		**6**	
Disease	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Confirmed IPN	15.2	94.1	22.6	91.9
Suspected IPN	12.8	93.9	17.1	90.9
Suspected PD	35.7	92.5	28.6	89.0
CMS	50.0	92.3	50.0	88.9
Sea lice	0	92.3	0	88.9
Fungus	0	92.2	0	88.8
Infectious diseases	13.1	96.2	19.0	95.5
Over 750 g				
Cut off (%)	**1**		**4**	
Disease	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Confirmed IPN	1.0	96.1	0	95.1
Suspected IPN	2.2	96.1	0	96.1
Suspected PD	25.7	96.8	27.2	95.9
CMS	63.6	96.6	54.5	95.5
Sea lice	11.0	96.3	13.7	95.3
BKD	5.8	96.2	10.3	95.3
SRS	47.4	96.5	60.5	95.6
Pasteurella salmonocida	20.0	96.2	20.0	95.2
Infectious diseases	14.7	97.7	18.8	97.0

Table 4-5 Positive and negative predictive values (PPV and NPV) for weekly and five-week rolling mortality for fish with average weight under 750 g and above 750 g. The PPV and NPV values above 70 % are highlighted in bold (see appendix 2-Table App 2-2 & Table App 2-3, for abbreviations, see Table 4-1).

Diseases	Positive predictive values (PPV) (%)				Negative predictive values (NPV) (%)			
Mortality threshold %	**0.1**	**0.5**	**1.5**	**6.0**	**0.1**	**0.5**	**1.5**	**6.0**
Under 750 g								
Confirmed IPN	22.9	31.3	37.9	42.0	**92.7**	**86.2**	**82.4**	**82.1**
Suspected IPN	26.0	35.0	39.1	39.5	**84.6**	**81.7**	**77.9**	**76.0**
Suspected PD	0.7	1.2	3.0	2.0	**99.4**	**99.6**	**99.6**	**99.4**
CMS	0.1	0.1	0.6	0.5	**100**	**99.9**	**100**	**99.9**
Sea lice	0.1	0.1	0	0	**100**	**99.9**	**99.9**	**99.9**
Fungus	1.3	1.5	0	0	**99.8**	**99.0**	**98.9**	**98.7**
Infectious diseases	47.7	63.8	**71.0**	**78.0**	**77.3**	68.1	60.8	58.5
Mortality threshold %	**0.1**	**0.5**	**1.0**	**4.0**	**0.1**	**0.5**	**1.0**	**4.0**
Over 750 g								
Confirmed IPN	3.2	1.1	0.5	0	**98.6**	**97.9**	**97.9**	**98.7**
Suspected IPN	3.1	2.7	1.6	0	**97.4**	**97.3**	**97.2**	**98.1**
Suspected PD	6.0	14.6	19.0	16.8	**98.8**	**98.1**	**97.8**	**97.7**
CMS	1.8	6.6	11.1	8	**99.9**	**99.8**	**99.7**	**99.7**
Sea lice	3.8	4.5	4.2	4.5	**99.7**	**98.8**	**98.6**	**98.5**
BKD	4.6	6.6	3.7	5.5	**98.7**	**97.9**	**97.6**	**97.6**
SRS	1.7	5.8	9.5	10.5	**99.7**	**99.6**	**99.6**	**99.7**
Pasteurella salmonocida	0.6	1.6	1.1	0.9	**100.0**	**99.9**	**99.8**	**99.8**
Infectious diseases	23.8	41.9	48.1	45.0	**93.3**	**89.8**	**88.8**	**90.1**

4.6. Discussion

In Scotland, farmers in aquaculture are obliged by the code of good practice for Scottish finfish aquaculture to remove, count, record, and identify the cause of fish death whenever possible (Anonymous, 2010). In this study, the dataset of site records analysed had the identification of the cause of fish mortality already provided by the farmers. This may have been with or without confirmation provided through laboratory diagnosis. In some cases, laboratory diagnoses may have been used but this was not universally applied throughout the dataset. In addition, the experience and the ability of

farmers to identify diseases may also influence the attribution of the possible mortality causes. The system of recording and assigning specific mortality causes may bring concerns regarding possible bias as the result of wrong selection of the cause groups, assigning the wrong cause of death (Aunsmo *et al*., 2008) and mortality underestimation (Jarp *et al*., 1994). However, in a pilot study, Aunsmo *et al*. (2008) stated that the causes of fish death assigned by investigators within an interval of 24 h were ascertained with a confidence of 97 %. Another difficulty observed in this study was regarding the difference between *suspected* and *confirmed* IPN. There was not enough information provided concerning the criteria applied by the farmers to allow this to be differentiated with confidence.

One diagnosis listed in the database is that of mortality due to *Pasteurella salmonicida*, in fact this may have been due to *P. skyensis*. However *P. skyensis* was only named formally in 2002 (Birbek et al. 2002) and, while it is listed in the database (for one occasion involving 10 fish) it is possible that cases are listed as *P. salmonicida*.

In this study, the first six weeks of production cycles with an average fish weight under 750 g were excluded because after fish transfer into the farm, it is likely to have an increase in mortality caused by the stress (Jarp *et al*., 1994), potentially confusing the analysis. Therefore, those production cycles with disease records in the first six weeks were also not considered. The analysis is necessarily biased towards the last part of the production cycles, fish with average weight over 750 g, as there were more data pertaining to the second – longer – part of the production cycle. This implied that for most infectious disease, longer production cycles are more likely to be positive just because of their length. Moreover, some diseases such as CMS are much more likely to cause mortality in a later part of the production cycle (Rodger and Turnbull, 2000; Brun

et al., 2003; Soares *et al*., 2011). Some infectious diseases such as suspected furunculosis, fungus and bacterial kidney disease were not included in this analysis either in the first or in the second part of the production cycle because there were not enough positive records for a viable analysis.

Receiver operating characteristic (ROC) curves have a wide application within a range of different disciplines including fish mortalities as shown in the present study. However, care must be taken in the use and interpretation of the ROC values as stand-alone. Ideally a gold standard should be applied which in this study may have included laboratory-based confirmation of the aetiology for the recorded fish mortalities within the dataset. However, laboratory confirmation may not always be practical or economic, and the lack of the gold-standard test should not diminish the potential value of the ROC information, as has been shown for other disciplines (Faraone and Tsuang, 1994; Hui and Zhou, 1998 and Rodríguez-Cortés *et al*., 2010).

The low ROC accuracy levels shown in fish with average weight under 750 g may be the result of a lack of records of infectious diseases at this stage of salmon life. For smaller fish, mortality as an indicator of presence of infectious disease does not appear to be a strong indicator where other causes of high mortality are likely to occur and with limited utility as a tool for aiding risk-based surveillance in small fish. On the other hand, larger fish (> 750 g) had a stronger association between mortality and infectious diseases. The most robust diagnosis was *infectious diseases*, this is because different individual diseases give similar signals of increased mortality and may occur simultaneously and so confound each other. The overall *infectious diseases* category is of most interest to Fish Health Inspection (FHI), since this can target inspection and officially identify the specific disease(s). According to the ROC curve suggested by

Swets (1988), the values of AUC indicated that abnormal mortality, including the cut-off values of 1 % for weekly mortality and 4 % for five-week rolling mortality, at this stage of the production cycle, were reasonably good indicators of the presence of an infectious disease with exception of *suspected* IPN, fin rot and *confirmed* IPN. This agrees with the observed baseline mortality for sites affected by *confirmed* IPN or *suspected* IPN, as it was found that these differed little from the baseline mortality for all sites, while diseases such as PD and CMS were associated with abnormal levels of mortality with respect to the mortality baseline (Soares *et al*., 2011). An additional factor may be that farmers increase their monitoring and observations of their fish stocks towards the end of the production cycle as mortalities at this stage can be very expensive. Abnormal mortality is thus a strong indicator of potential presence of infectious disease for a population of larger fish and therefore, it may be a useful tool to assist with farm level risk-based surveillance.

Predictive values vary across populations with different infectious disease prevalence (Shiu *et al.*, 2008). The low values of PPV, with the exception of *infectious diseases* at cut-off 1.5 % and 6 %, and high values of NPV observed in fish with average weight under 750 g may be the result of the relatively rare presence of any individual infectious diseases and even infectious disease as whole, when compared with the many other forms of mortality considered in this study. Thus, high mortality and disease (true positive) is likely to be rare, when compared with high mortality without disease (false positive) and low mortality without disease (true negative) will be quite common, while low mortality with disease will be rare, even if the disease can occur without causing high mortality levels. This would indicate that high mortality may suggest presence of infectious disease that is worth investigating for confirmation.

Some of the diseases might be severe and consequently have an impact on the salmon production as in the case of infectious salmon anaemia (ISA) disease (Henson, 2008; Mardone *et al*., 2009) and PD (Aunsmo *et al*., 2010). In the database analysed in this study there were no occurrences of ISA. In the case of ISA, outbreaks are generally associated with high mortality; therefore highly likely to trigger surveillance based on the proposed mortality cut-offs. Under UK legislation, ISA is a notifiable disease and therefore the official regulators have to be notified and take samples to confirm the suspicion associated with the high mortalities (Murray *et al*., 2010). Additionally, some other infectious diseases may be rarely recorded because the farmer may not have identified the cause, meaning that mortality codes erratically or inappropriately used will confound the analysis. In this analysis different infectious diseases may occur at the same time, or infectious diseases, such as *suspected* PD and *suspected* IPN, can have identical gross clinical signs and can therefore be easily confused and recorded incorrectly. This may lead to false situations, resulting in unnecessary inspections. Generally, high mortality rates are not ascribed to *suspected* IPN, but to *confirmed* IPN, therefore *suspected* IPN only gets ascribed to low mortality rates and the AUC value will be worse or lower than the random chance prediction.

This study provides a basis for further analysis including a set of cut-off values that provide a strong indication of the presence of infectious diseases and therefore, may be relevant for regulatory surveillance purposes. An interesting further study would be to compare the disease mortality patterns associated with noticeable disease outbreaks, for instance ISA outbreaks, with the mortality patterns observed during regular monitoring and how long would have taken to generate alerts and activate regulatory surveillance. Faraone and Tsuang (1994), Hui and Zhou (1998) and Rodríguez-Cortés *et al*. (2010) discussed the problem of the lack of a gold standard test and the impossibility of having

it in certain situations. The limited information does not allow investigation of the biases introduced by the lack of effective gold standard in this model. This study would benefit from an analysis on the lack of a gold standard. Another interesting study as suggested by Aunsmo *et al*. (2008) would be to investigate the accuracy and the reliability of assigning causes by the farmers.

This study showed that *abnormal* mortality in large fish (>750 g), including cut-off values of 1 % for weekly mortality and for 4 % for five-week rolling mortality, provided a strong indication of the presence of infectious diseases. Consequently, the cut-off values suggested in large fish (>750 g) may be used as trigger point cut-offs to generate alerts and activate regulatory surveillance by Marine Scotland Science Fish Health Inspectors. The mortality rates exceeding the suggested cut-offs were found to be infrequent. This would benefit the application of this new strategy as Fish Health inspectors would not be notified too often and needless inspections would be avoided. This would also suggest that application of such a strategy could result in a low number of false positive surveillance alerts. An increased number of false alerts are an important factor within the overall system cost. The trade-off between cost, time and false alarm rates will have to be considered by the regulator in the design of any surveillance system. This study would benefit from a cost-benefit analysis to assess the economic impact of mortality threshold alerts as indicative of presence of infectious disease in the system of Marine Scotland Science Fish Health Inspectors.

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4.8. References

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Chapter 5 The role of disease diagnosis within farmed salmonid health practices in Scotland

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This chapter describes the main role of disease diagnosis on the identification of diseases and production problems at the farm level. This chapter also describes the role of disease diagnosis on the implementation of measures for prevention and control of diseases. We also investigated the value of on-farm records, including recorded mortality, for the daily running of salmonid production. This study was performed by using key informant interviews with open questions to the health or farm manager of several salmonid farms and using the diagnostic reports of Veterinary Diagnostic Services (VDS) from Stirling University, which provided veterinary health consultancy and expertise to the trout farms interviewed.

The main author, Silvia Soares, conducted all the practical and analytical work and wrote the final manuscript. Dr. D. M. Green, Dr. A. G. Murray, Prof. J. F. Turnbull and Dr. M. Crumlish provided supervisory and editorial support throughout the whole study.

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5. The role of disease diagnosis within farmed salmonid health practices in Scotland

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5.1. Abstract

In aquaculture, diseases are a significant constraint to economic expansion. Our current understanding is that accurate and timely diagnosis is essential for effective control and eradication of any diseases. Disease diagnosis at the farm level is important as a first screening of possible causes of mortality and morbidity and to support the identification of efficient measures to prevent and control disease outbreaks. In this study, we aimed to describe the role of disease diagnosis at the farm level and identify the influence in the health management and treatment of diseases within salmonid systems in Scotland. It was shown that in the Scottish slamonid industry the primary diagnosis included assessment based on traditional laboratory tests, clinical signs and the history of the stock and farm; all of which were considered valuable tools by the health and farm manager for management-strategy decisions and population-based health control. We found that in both case studies, the site health and farm manager played a key role in the identification of first signs of disease by actively observing the fish closely for any modification in behaviour, including a decrease in feeding response and increase in morbidity/mortalities, thus allowing early identification of a potential health problem. Our results demonstrate that farm-level experience of different disease conditions remains highly valuable for the day-to-day running of the production site. Therefore, we consider that primary veterinary diagnoses still have a role to play in the Scottish salmonid industry as part of health management practice.

5.2. Introduction

Aquaculture is the fastest growing food-producing sector in the world (FAO, 2010) and an important sector in Scotland (Marine Scotland Science, 2009). In aquaculture worldwide, diseases are a significant constraint to economic expansion (FAO, 2000, Bondad-Reantaso *et al.*, 2005, Subasinghe, 2005). The outbreak of infectious salmon anaemia, which occurred in Chile (FAO, 2010, Mardones *et al*., 2009) demonstrated the rapid impact that an infectious disease outbreak can have on the sustainability of an established industry. These impacts not only result in fish losses but also increase production costs, lead to refusal of fish importations and if significant enough can cause job losses (Henson, 2008). It is therefore essential to determine applicable measures for disease treatment and prevention within the industry, which ideally can be implemented in a cost-efficient manner and may become an integral part of the health-management practice of fish farming (Wagner *et al*., 2002). Disease monitoring and diagnosis at the farm level are an integral part of any health-management strategy (Rowland *et al*. 2007). They are vital as a first screening of possible causes of mortality and morbidity in fish. They support the identification of efficient measures to prevent and control disease outbreaks (Bondad-Reantaso *et al*., 2001, Read *et al*., 2007, Rowland *et al*., 2007).

5.2.1. Methods for disease diagnosis

Gross observations of fish on-farm, such as behaviour and mortality, post-mortem necropsy and histopathology are the primary methods for diagnosis of fish and shellfish diseases. This is often combined with direct culture of pathogens (FAO, 2000a) which are then processed and used as confirmation of the aetiological agent of the disease. These methods are considered as first steps for identifying an infectious disease and are

essential tools for disease diagnosis. Some diseases, such as external parasitic infections, can be diagnosed on-site with the use of suitable equipment including a microscope and a dissecting kit (FAO, 2007, Read *et al*., 2007). A trained person, such as the farm-health manager, a pathologist or a veterinarian, should perform these diagnoses (Bondad-Reantaso *et al*., 2001, FAO, 2007, Rowland *et al*., 2007, Shirley *et al*., 2011). The diagnosis of a disease at the farm level can also be assisted by laboratory methods to confirm the identification of the infectious pathogen (Shirley *et al*., 2011). A rapid on-site diagnosis of a disease by a trained person allows the immediate application of chemotherapy or remedial measures to control or eradicate the disease (Pillay and Kutty, 2005) and therefore to minimise losses (Rowland *et al*., 2007).

In a laboratory, the identification of a disease can be provided from pathology. The performance and the interpretation of results of the pathology of the clinical material may take some time even in human medicine (McGladdery, 2000). To overcome this delay, the health and farm manager often uses remedial action based on presumptive diagnoses, resulting from observations of gross pathology or behavioural changes (Pillay and Kutty, 2005, Rowland *et al*., 2007). In aquatic disease diagnosis, this is most effective within a well-defined history of diseases or outbreaks (McGladdery, 2000). In the case of emerging diseases in new farmed species, or appearing at a location for the first time, diagnostic methods may not be able to identify the pathogen of a disease for prolonged periods or may misdiagnose the disease (McGladdery, 2000), as was the case of the first outbreak of infectious salmon anaemia (ISA) in 1996 in Canada, that was first described as haemorrhagic kidney syndrome (HKS) (Byrne *et al*., 1998).

5.2.2. Importance of laboratory tests

Laboratory tests are a fundamental part of any veterinary practice (McKenna and Dohoo, 2006). They can be applied at an individual level, as is the case for terrestrial animals, or at a population level, for fish and poultry. The purpose of the tests conducted will depend on the nature of the health problem as it may include investigating clinical disease outbreaks and infectious processes (Greiner and Gardner, 2000, Georgiadis *et al*., 2001, Mckenna and Dohoo, 2006) but may also include health monitoring and screening for the presence of specific pathogens.

The use of a veterinary laboratory for identification of disease or production problems, even if only intermittently, can provide limited information on pathogen presence at a farm level (FAO, 2001). Pathogen identification tests support animal health certification processes, providing information on the presence of pathogens in an animal population, essential for control of pathogen transfer (FAO, 2000b), and test for the presence of certain pathogens prior to international trade of live fish (Greiner, 2006). Pathogen identification tests are also widely used to certify that aquatic products may be sourced from pathogen-free regions or countries or to certify that broodstock or fry/postlarvae are free of a specific pathogen (SPF). They are also used to certify high-health (HH) stocks (FAO, 2007) and to confirm the aetiological agent present in a clinical disease case (Geriner, 2006). Due to the wide range of applications of varied pathogen identification tests, great care must be given to the interpretation of laboratory results or health certification.

Laboratory tests can always give incorrect results (McKenna and Dohoo, 2006); even validated tests can give incorrect results if they are not gold standard. Validation of tests gives them a measure of reliability and thus appropriate levels of uncertainty in

interpretation of results. Laboratory tests are not always gold standard for both sensitivity and specificity but many tests may be close to an accepted gold standard for specificity. In aquaculture, a gold standard test can include a combination of laboratory methods. For example, with infectious salmon anaemia (ISA), a combination of tests is the agreed criteria for pathogen identification and confirmation (Anonymous, 2010a) and in systemic bacterial diseases, the diagnosis results from the culture of bacterium combined with macroscopic and histological observations consistent with disease (Colquhoun and Duodu, 2011). In international trade, for instance, the culture of cell lines is considered the gold standard for screening fish stocks free of presence of viral haemorrhagic septicaemia (VHS) virus (OIE, 2009, Garver *et al*., 2011).

Limited resources and the lack of a "gold standard" are typical constraints (Geriner, 2006) for validation in any field of diagnosis. In an attempt to overcome this problem, diagnostic laboratories occasionally develop "in-house" assays for use in response to a specific need, without being necessarily validated and standardised out with the laboratory providing the service (Anonymous, 2003).

5.2.3. Stock management databases

Secondary data on previous disease outbreaks on the affected farms are valuable information sources to assist with the diagnosis (McKenna and Dohoo, 2006). For that, reliable on-farm records, such as mortality and analysis of those records (Eysker and Ploeger, 2000, Rowland *et al*., 2007) play an important role to help identify the problem in a timely manner (Bondad-Reantaso *et al*., 2001, Rowland *et al*., 2007, North *et al*., 2008). Ideally, every aquaculture company should have at least one trained person and basic facilities to undertake regular health and environmental monitoring at the farm level (Pillay and Kutty, 2005, FAO, 2007, Rowland *et al*., 2007).

In this study, the aim was to investigate and describe the role and the importance of data records (e. g. mortality records) as a tool for management decisions and health control on production sites. We also aimed to identify the value of the health and farm manager's experience in the identification of clinical signs of diseases at the farm level and in prevention of diseases. We described the role and the importance of methods for disease diagnosis, such as disease outbreak history, histopathology and pathogen recovery, in the health-management strategies, treatment and control of diseases within salmonid systems in Scotland. For that we used a recognised diagnostic service to approach the production sites and thus ensure compliance with this primary data collection exercise. Data were gathered from two sources a) through an informant interview performed with producers of Atlantic salmon and trout (primary data) and b) from the reports of a veterinary diagnostic laboratory from 2000 to 2007 (secondary data). The approach used allowed two case studies to be produced where Case Study 1 focused on the farmed Scottish trout industry and Case Study 2 concentrated on the Scottish salmon industry.

5.3. Materials and Methods

5.3.1. Study population

Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) production systems were included in this study. In total, 15 trout farms and 11 Atlantic salmon farms from six different companies in Scotland were contacted by phone and e-mail. Of the six companies, three companies produced both rainbow and brown trout and the other three produced Atlantic salmon. All Atlantic salmon was produced for the table market, while the trout production included those producing fish

for different commercial targets: table market, restocking and hatchery operators, which sell eggs or fry fish.

5.3.2. Farm visits

Primary data were collected by personal interviews with the site health or farm manager during prearranged farm visits. The informant interviews were conducted from March 2009 until March 2010. A total of 26 farms were contacted by phone and e-mail to arrange a visit. Interviews were restricted to Scottish farms. The companies contacted were important producers of trout and salmon production in the Scottish industry. Two farms and two companies did not wish to participate in the study, therefore were not included. The site health or farm manager of the farms was visited once for a key informant interview using an open questionnaire covering environmental, biological and stock production areas, including management and disease-prevention practices. During the process of data collection, the interviews also covered the system for recording mortality, water temperature, feeding and other variables (see appendix 3-App 3.1).

5.3.3. Data collected from laboratory reports

Secondary data were provided by Veterinary Diagnostic Services (VDS) from Stirling University, which provided veterinary health consultancy and expertise to the trout farms covered in the primary data collection. Their Laboratory Information Management System (LIMS) uniquely identifies the clinical samples submitted (case records, see appendix 3-App 3.1 & 3.2) to the laboratory and records the laboratory procedures undertaken and all the diagnosis and health-check reports (see appendix 3App 3.3) returned to the client. The laboratory and health-check reports pertaining to the farms in the primary data, generated from 2000 to 2007 were extracted from the LIMS system. These data were used as an independent benchmark to compare with the

data gathered in the key informant interviews. For some samples recorded in the LIMS, the diagnostic report was not available (categorised as missing report).

No secondary data were available for farmed Atlantic salmon, therefore the triangulation of data gathered in the key informant interviews against laboratory reports was not performed in this study. Veterinary Diagnostic Services (VDS) from Stirling University did not provide consultancy and expertise to the visited farms producing Atlantic salmon.

The laboratory report results were grouped into four categories according to causes of problems identified: *infectious disease*, *other* (Table 5-1), *non-conclusive* (which included descriptive diagnosis with undetermined aetiology) and *no evidence of disease* (which included all results that did not identify any evidences of infectious diseases).

Table 5-1 Causes of health problems listed in laboratory report results from Veterinary Diagnostic Services (VDS) at Stirling University for rainbow and brown trout production.

Infectious diseases identified	Non-infectious problems identified
Bacterial disease problem	Dermatitis
Bacterial gill disease	Incubation problems or stripping
Bacterial kidney disease (BKD)	Over inflation of the swimbladder
Enteric redmouth disease (ERM)	Physical trauma
Fungal peritonitis	Vaccine peritonitis
Furunculosis	
Infectious pancreatic necrosis (IPN) carriers	
Parasites	
Rainbow trout gastroenteritis (RTGE)	
Rainbow trout fry syndrome (RTFS)	
Red mark syndrome (RMS)	
Sleeping disease (SD)	

5.4. Results

5.4.1. Response rate

Of the 26 farms contacted, 24 were available for an informant interview, representing a compliance rate of 92 %. The farms participating in this study were 13 out of 56 Scottish trout sites in production and for Atlantic salmon, four out of 105 of freshwater sites and seven out of 254 of the marine sites currently in production (Marine Scotland Science, 2010). The sites visited and interviewed were producers of trout and salmon from the largest trout and salmon farming companies in Scotland.

5.4.2. Case study 1 (trout production)

5.4.2.1. Primary data characterization

The main species in trout production was rainbow trout (*Oncorhyncus mykiss*) in ponds, raceways and cages. Five of the 13 trout farms also produced brown trout (*Salmo trutta*) in ponds and raceways.

From the 13 farms visited, seven were specialized in producing fish solely for the table market and two only produced for the restocking market. The remaining four farms were trout farms and produced fish for both table and restocking (Table 5-2). From the 13 trout farms visited, four farms also had hatchery facilities. Of these four, two were hatchery production only and the other two also carried out research trials. Some farms with hatchery operations supplied more than one table farm or restocking farm.

Table 5-2 Numbers and percentages of type of trout production by farms visited.

	Trout	
	Farms	%
Table market	7	54
Restocking	2	15
Table/Restocking	4	31

5.4.2.2. Management strategy

In more than half of the trout farms interviewed, the site health or farm manager indicated that they did not have an all-in, all-out fallowing plan in place. They reported that the farm site was fallowed in sections due to a permanent animal presence within the farm site. All production stages from eggs to adult trout in ponds were present in the farm site. Only two farm sites had a fallowing plan of all-in and all-out due to being produced in cages in a loch. Two farmers did not reply to this question.

For the majority of the farms, the site health or farm manager reported a vaccination plan was implemented to prevent the onset of enteric redmouth disease (ERM) using a monovalent ERM vaccine. Fish are first vaccinated at approximately 5 g weight and then a booster is administered 6 months later. This vaccine was administered in nine farms out of the 13 farms visited, although four did not administer the booster. As reported by the site health and farm manager, this was because their fish stocks had been vaccinated prior to coming on-site or the fish would be moved off-site before the 6-month booster time.

The farm manager of one of the rainbow trout farms reported problems of sleeping disease (SD) and stated a health-management strategy was in place to avoid outbreaks of SD. The strategy included either delaying the transfer into the site at certain times of the year (spring/summer time) or only stocking fish with a mean weight above 45 g to 100 g.

The majority of trout farms did not implement certification schemes for product quality. From the 13 farms, only two farms followed a certification scheme: Freedom Food certification. All of them followed the code of good practice "A code of good practice for Scottish finfish aquaculture" (Anonymous, 2010), with the exception of the restocking farm.

5.4.2.3 Main disease problems

The main infectious diseases reported by the site health or farm manager were parasitic infections (n=11), followed by rainbow trout fry syndrome (RTFS) (5). Bacterial kidney disease (BKD) and SD were also reported three times. Red mark syndrome (RMS), ERM, gill disease, infectious pancreatic necrosis (IPN) and rainbow trout gastroenteritis

(RTGE) were also reported but to a lesser extent (Figure 5-1). One of the farms had a disease outbreak of BKD and SD occurring simultaneously.

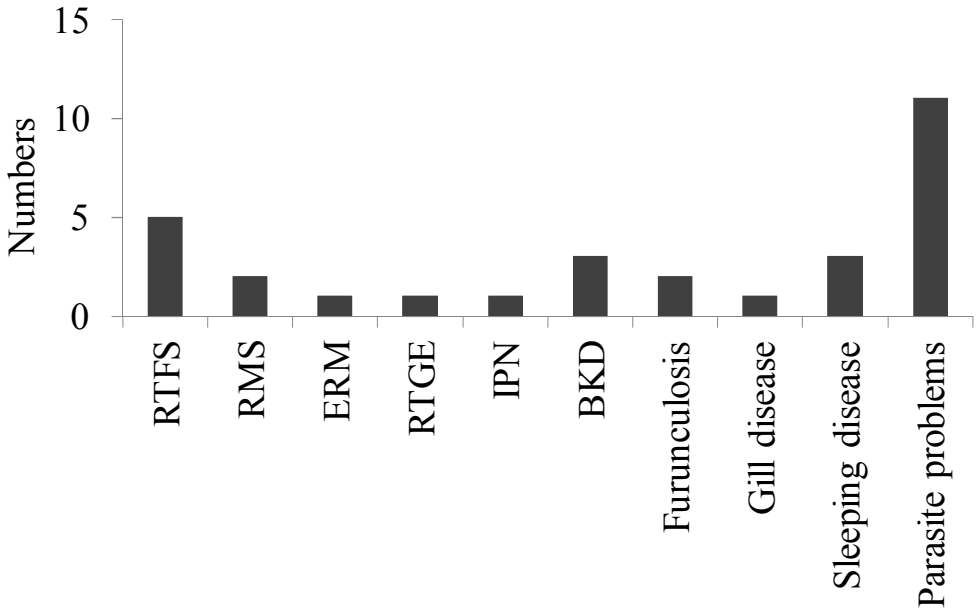

Figure 5-1 Main infectious disease problems listed in the key informant interview by the site health or farm manager in rainbow and brown trout production (for abbreviations, see Table 5-1).

5.4.2.4. Secondary data characterization

A total of 19 % of laboratory reports were missing because those reports were not found archived and therefore not retrieved. 2001 was the only year without missing reports. 2000, 2003, 2004 and 2007 were the years with higher number of missing laboratory reports (Figure 5-2).

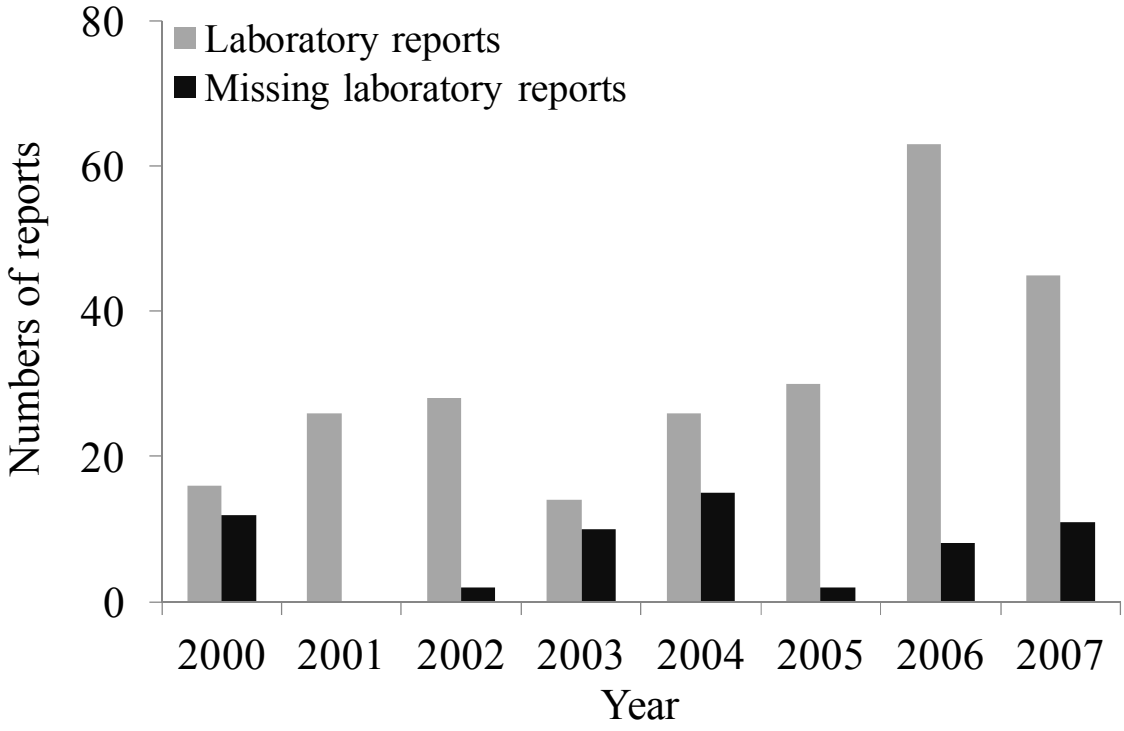

Figure 5-2 Number of actual and missing (absent) clinical laboratory reports for trout samples from 2000 to 2007 in Veterinary Diagnostic Services.

Company 1, which had nine farms, submitted the greater amount of clinical material for laboratory analysis over the study period, followed by Company 2, which had only two farms and Company 3, which was a restocking company with only one farm (Figure 5-3). The average number of clinical samples submitted to the laboratory per farm during the study period was similar for both company 1 and 2. Company 3 submitted the lowest average number of clinical samples (n=9), mostly likely due to the fact that this company only submitted samples from a single site and did not have any hatchery operator within the site (Figure 5-3).

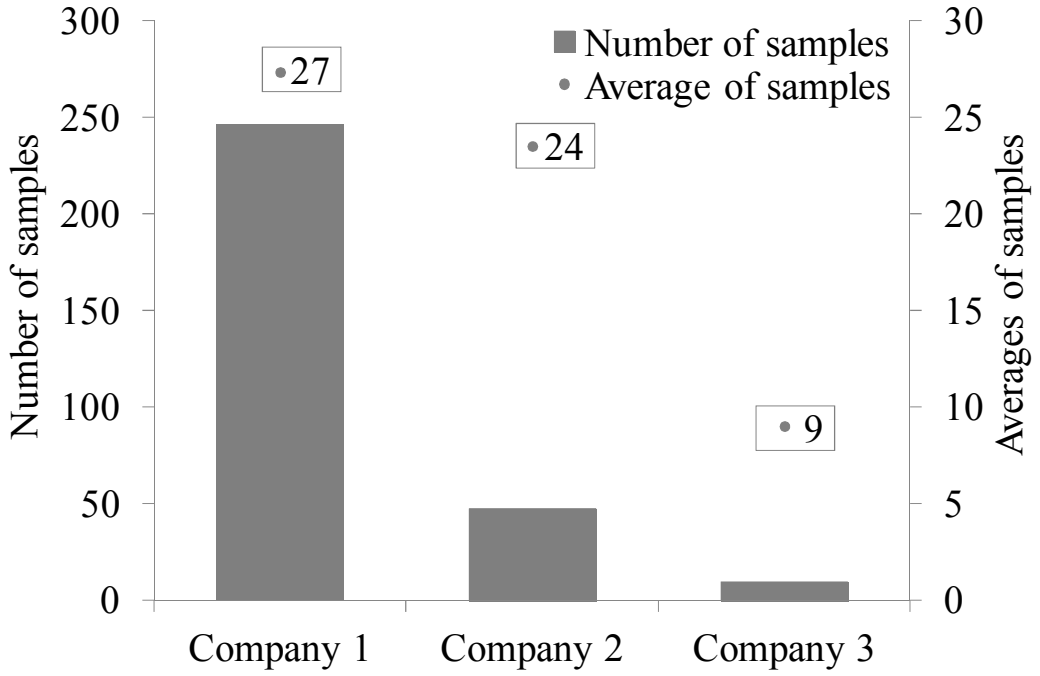

Figure 5-3 Number of clinical samples submitted to the laboratory per company and average number of clinical samples per farm within company during the study period.

5.4.2.5 Results of laboratory reports

The samples submitted to the laboratory by the site health or farm manager during the study period were mainly for histopathology (64 %), followed by bacteriology (30 %), parastiology (4 %) and virology (2 %) (Figure 5-4a). A similar trend was observed across all years in this study period (2000-2007) (Figure 5-4b). There was an increase in the number of suspected bacterial and parasitological samples submitted between 2000 and 2002. The smallest number of samples provided throughout the study period (Figure 5-4a) were for virology detection where in 2000, 2002, 2006 and 2007 these correspond to specific requests for health check and detection of IPN and SD (Figure 5-4b).

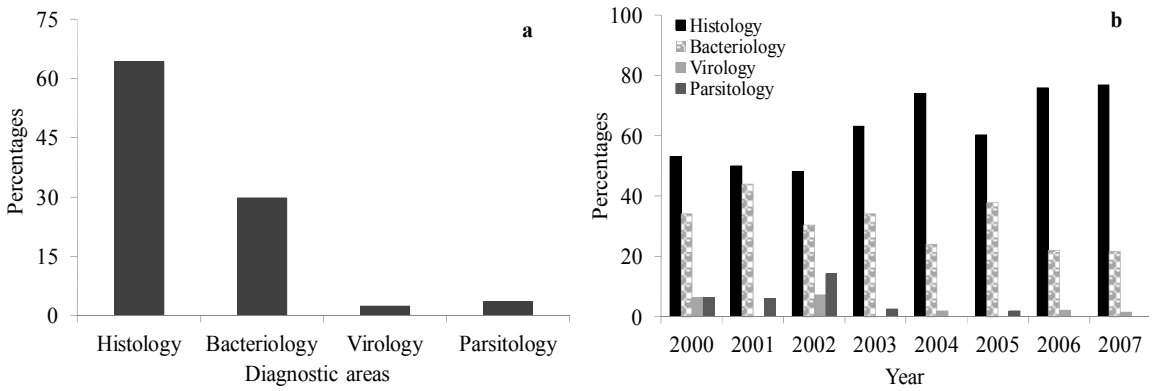

Figure 5-4 Types of diagnostic areas requested in a diagnostic laboratory for farmed fish. Percentages of the main diagnostic areas requested a) overall and b) across time.

As seen in Figure 5-5, there is similar pattern among samples submitted by the trout producing companies. However, Company 3 submitted the same proportion of samples for histology and bacteriology and provided fewer samples for parasitology compared with the other companies (Figure 5-5).

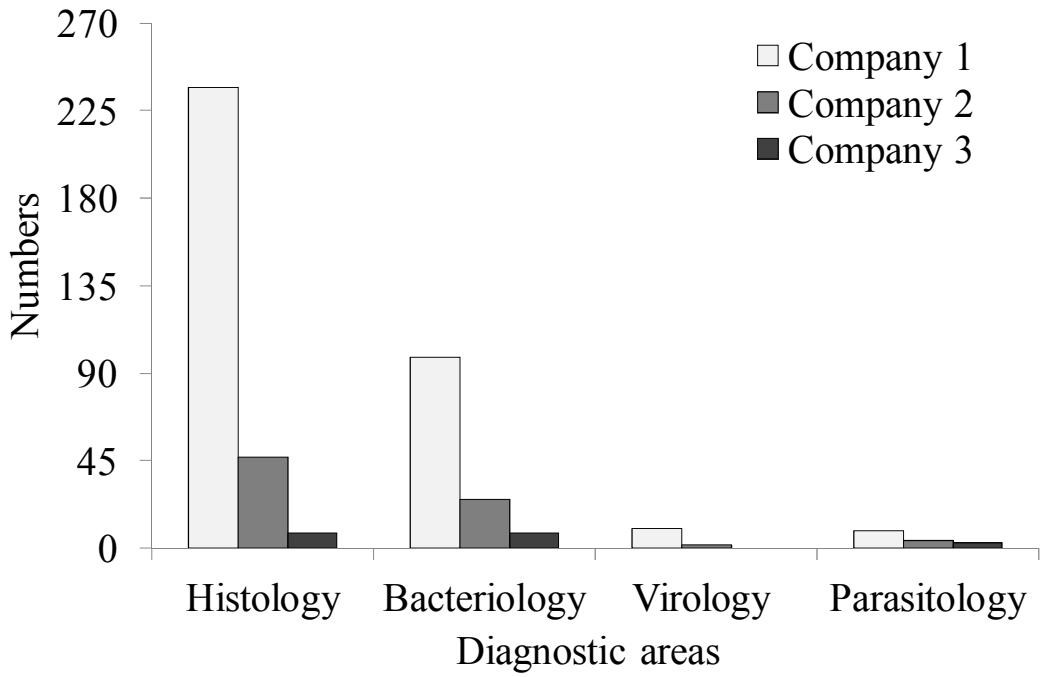

Figure 5-5 Types of diagnostic areas requested by each company.

The majority of samples sent to the laboratory by the site health or farm manager had descriptive information concerning the suspected disease problem. For a few samples submitted for analysis, the site health or farm manager did not identify the suspected cause of the mortality and morbidity. In 68 out of 202 submitted samples to the laboratory, we could not identify the farmer suspected cause of disease based on the laboratory reports (Figure 5-6a). Those reports did not have the farm's suspicion written on the report. A similar trend was observed across all the years (Figure 5-6b).

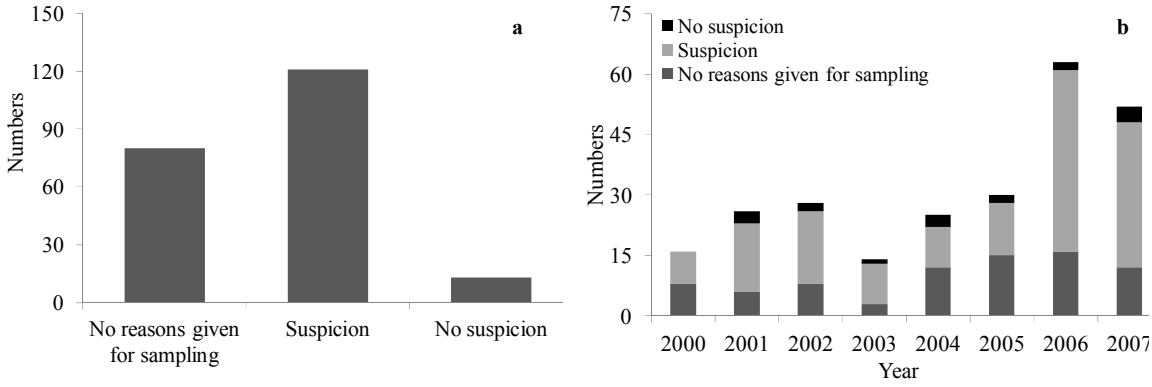

Figure 5-6 Number of samples submitted to the laboratory by site health or farm manager a) with suspicion, no suspicion and no reasons given for sampling and b) with suspicion, no suspicion and not identified across the years.

In this study, the majority of fish samples submitted by the site health or farm manager were due to suspicion of RTFS, followed by general health check analysis of fish (Figure 5-7a). A similar pattern was observed across the years (Figure 5-7b). Year 2001, 2002, 2004, 2006 and 2007 had a higher number of samples submitted for RTFS diagnosis, with a peak in 2006 (14 out of 69). Diseases such as enteritis, ERM, SD, RTGE and RMS/strawberry disease varied in frequency among years, although all with small numbers (Figure 5-7b).

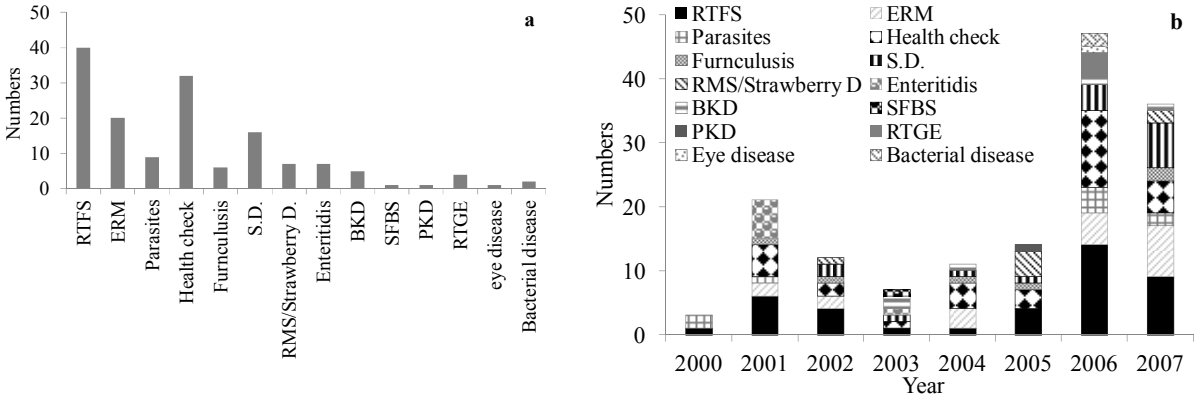

Figure 5-7 Number of reasons addressed by the site health or farm manager to send samples to the laboratory. a) reasons in overall and b) across the years (for abbreviations, see Table 5-1).

The results obtained from the laboratory reports of the samples analysed were grouped in four categories: *infectious diseases*, *no evidence of disease, non-conclusive* and *other pathologies* (Figure 5-8). *Infectious diseases* was the main group reported, followed by *no evidence of disease* and then *non-conclusive* and *other pathologies* (Figure 5-8a). As expected with samples submitted for laboratory test, the group of *infectious diseases* had the highest numbers of reports followed by *no evidence of disease, non-conclusive* and *other pathologies* (Figure 5-8b). The numbers of reports of *no evidence of diseases* decreased when the results of health checks were not considered. In this case the *non-conclusive* reports were the second highest number. From the laboratory tests performed, more than one pathogen was identified in 12 % of the samples submitted, even though the presence of more than one pathogen did not mean that were all actively causing disease. The year 2006 showed the highest percentage (5.5 %) of cases with the presence of more than one pathogen.

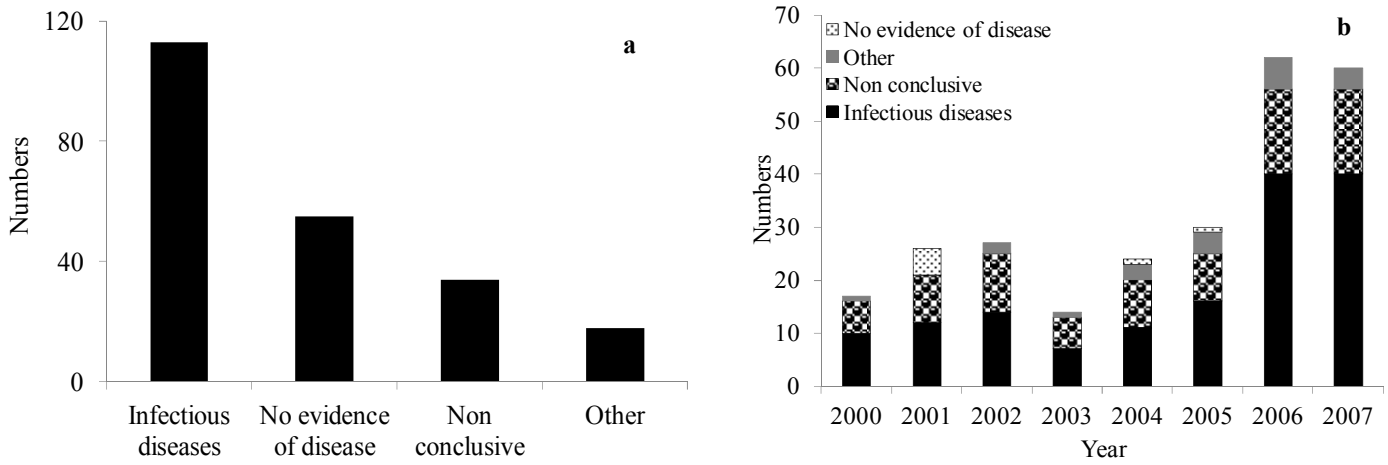

Figure 5-8 Category groups of results of laboratory reports from the samples submitted by the health and farm manager. a) main diagnosis groups in overall and b) across the years.

In total, *infectious diseases*, followed by *no evidence of disease* were the main groups diagnosed in company 1. The high numbers of *no evidence of disease* was caused by the need to perform routine health checks to comply with legislation and the agreement with the veterinary service. *Other pathologies* and *non-conclusive* group were less frequent (Figure 5-9). In company 2, the main diagnosis was *non-conclusive* due to the presence of a condition of unknown aetiology, with similar numbers in the remaining groups. In company 3, *infectious diseases* was the main group identified. This company only had case records at laboratory when there was a health problem at the farm (Figure 5-9).

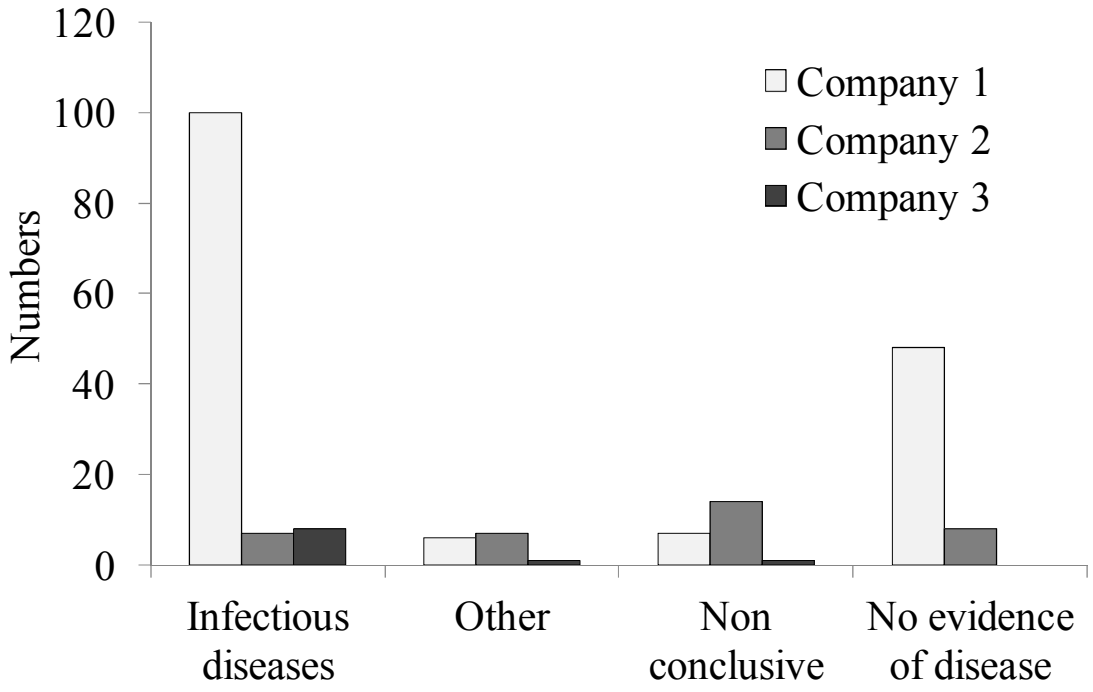

Figure 5-9 Category groups of results from laboratory reports by company.

5.4.2.6. Primary against secondary data

Rainbow trout fry syndrome was the major disease problem identified in the laboratory reports followed by RMS, furunculosis and parasite problems in the trout samples. In the key informant interviews, the main disease reported by the site health or farm manager was parasite problems, followed by RTFS (Figure 5-10). Parasite problems, RTFS, ERM and furunculosis were the diseases with highest difference between primary and secondary data. The interviewees reported that the majority of times parasites were diagnosed at the farm level without the need of laboratory diagnosis. The other infectious diseases, RTFS, ERM and furunculosis were mainly reported in the laboratory records. At the time of the key informant interview, in most of the farms visited, the site health or farm manager did not report those infectious diseases because they were not currently a disease problem. We also identified that when a disease problem occurred at the farm, the site health or farm manager's suspicion was usually of

a well-known infectious disease (e. g. RTFS and ERM). In these cases, the laboratory test was used for confirmation of the disease outbreak and the majority of the results from that laboratory were in agreement with the suspicion of the health or farm manager.

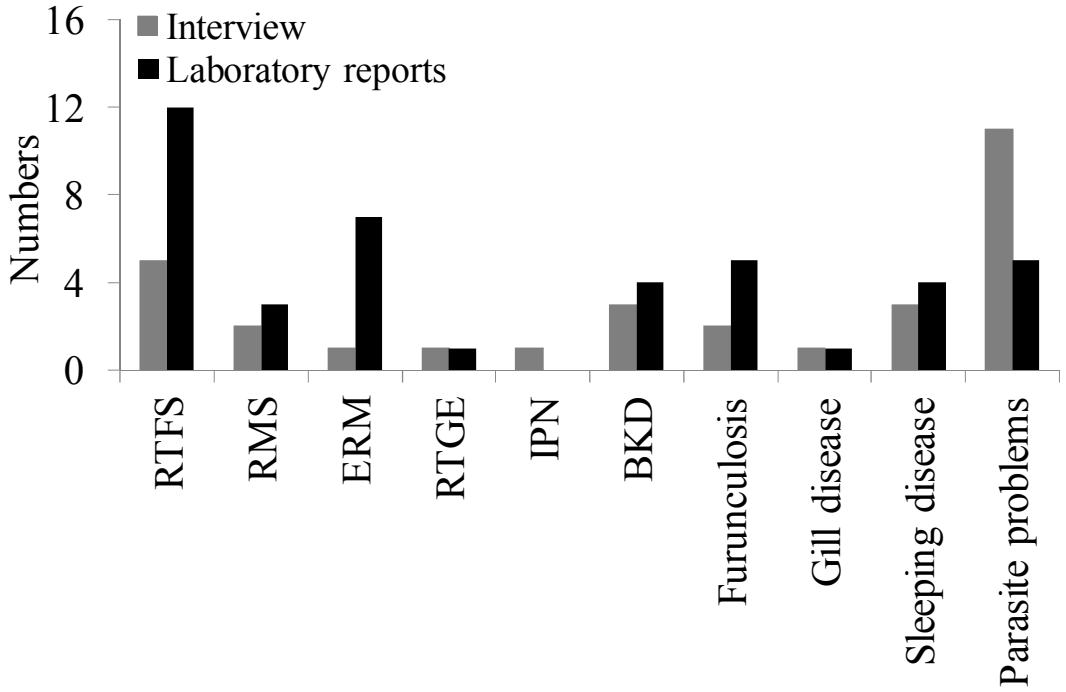

Figure 5-10 Causes of infectious disease problems listed in the key informant interview and in the laboratory report results from the Veterinary Diagnostic Services (VDS) at Stirling University for rainbow and brown trout production (for abbreviations, see Table 5-1).

5.4.3 Case study 2 (salmon production)

5.4.3.1. Primary data characterization

In this study, both freshwater and seawater phases of salmon production were included and sites of both types visited for primary data collection. Some of the freshwater sites also had hatchery facilities. In two companies visited, only a visit to one marine site was authorised. One of the hatchery operators of trout also produced salmon fry.

5.4.3.2 Management strategy

In salmon production, all the health managers reported that the marine sites visited had a fallowing plan and practiced the all-in, all-out system, while in freshwater, all sites fallowed only sectors of the farm and there were always eggs or fry present on the farm. The health managers also reported that in marine sites, they try to synchronise the fallowing period of those sites in a certain geographical area with the other companies present in that area.

All health managers reported that Atlantic salmon were vaccinated against furunculosis and infectious pancreas necrosis (IPN) in the freshwater phase, before moving to seawater. In one of the freshwater salmon farms, the health manager reported that they also vaccinated against ERM, when demanded by the client. None of the fish were vaccinated in the seawater production systems.

All the salmon companies had a health manager responsible for monitoring fish health either in the freshwater, seawater or both production phases. For salmon in the seawater stage, two companies had a health plan for monitoring PD covering all their farm production sites. One of the companies sacrificed fish on a monthly basis to collect organs for histopathology and tested serology samples from 3 to 12 fish per time for detection of PD. If diagnosed positive or with suspected PD, the management approach was to reduce handling of the fish and to feed the stock with a PD-adapted diet. Other preventive measures observed in one of the companies was related with the broodstock, where females and males were tested for IPN and eggs and milt were also tested for IPN at the time of stripping.

Certification schemes of product quality for market were commonly followed amongst the salmon farms visited in this study. All the companies were certified under Freedom

Food certification and a code of good practice "A code of good practices for Scottish finfish aquaculture" (Anonymous, 2010). In two of the three companies, salmon production was also compliant with certification scheme "Label Rouge" standards.

5.4.3.3 Main disease problems

The main health problems reported by the site health or farm manager during the informant interviews in freshwater salmon farms were parasitic infections, followed by IPN, fungus and fin rot. Rainbow trout fry syndrome and gill disease were also observed but to a lesser extent (Figure 5-11).

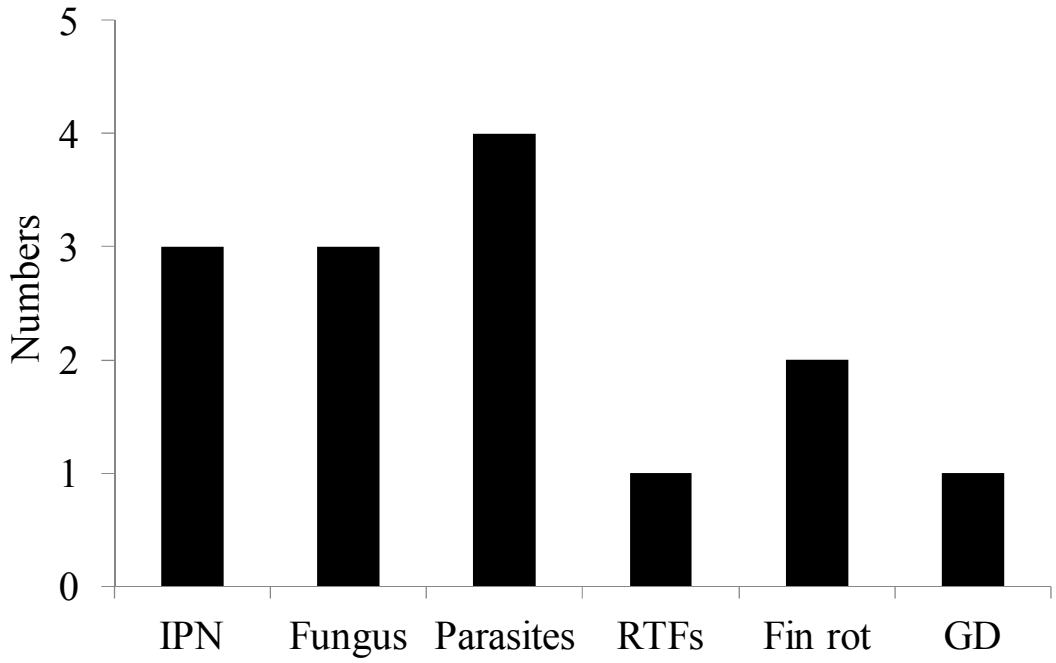

Figure 5-11 Causes of infectious disease problems listed in the key informant interview for Atlantic salmon in freshwater phase. GD, gill disease (for abbreviations, see Table 5-1).

In salmon production in seawater phase, the health manager reported sea lice and PD as the main disease problems. Gill diseases and IPN were also reported in salmon production but in a fewer cases (Figure 5-12).

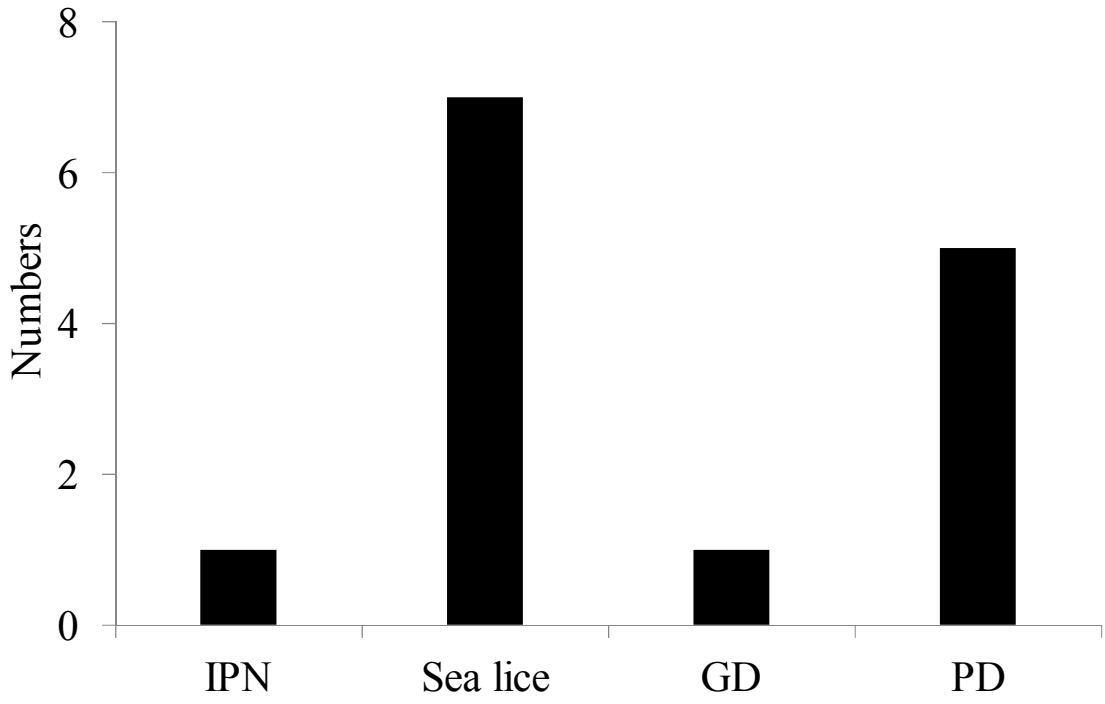

Figure 5-12 Causes of infectious disease problems listed in the key informant interview for Atlantic salmon in seawater phase. GD, gill disease; PD, pancreas disease (for abbreviations, see Table 5-1).

5.5. Discussion

This paper has presented two case studies describing the role of disease diagnosis at the farm level in salmon and trout production sites and their strategic role in the health-management decisions and treatment of diseases within Scottish salmonid aquaculture.

Although both case studies covered a relatively small number of Scottish farms, the farms interviewed belong to the largest production companies of farmed trout and salmon in Scotland and so were thought to be representative of the industry. One of the trout and three salmon companies interviewed are major producers in Scotland. Seventeen of the farms visited belonged to two single companies and therefore followed a single management strategy implemented by the company, which might bias our

characterization of the trout and salmon production at the Scottish level. Nowadays in Scotland, production is quite standardised since the industry is tightly regulated and surrounded by code of conduct and code of practice, such as "A code of good practice for Scottish finfish aquaculture" (CoGP) (Anonymous, 2010b), and quality assurance schemes with attendant inspections and accreditations, with which producers and companies have to comply (Read, 2008, Scott, 2010). This increases the ability to accurately represent the target population even with a small sample size.

5.5.1. Case study 1

One of the trout companies only produced for restocking and therefore it did not have to strictly follow the regulations concerning records of fish movement (Munro and Gregory, 2009), compared with those producing for the table market. This company also did not have a consultancy agreement with any veterinary diagnostic service. The other two trout companies represented large and medium size producers of trout for the table market and for the table and the restocking market.

In the UK, according to MacIntyre (2008), only 80 % of the restocking companies and both table and restocking farms reported that they kept mortality records, which are required by law (Registration of Fish Farming and Shellfish Farming Businesses Order 1985 as amended). In our study, all farms complied with the legislation requirements.

A recognised limitation of the analysis performed in this study is the restriction of the secondary data to only one laboratory and three companies, resulting in a small representation of the industry that may introduce some bias. However, one of the companies in this study is the major Scottish trout producer with several farms under their management, which may also introduce some bias in this study but also provides

confidence in the data collected that may be is representative of the Scottish sector. The size of the companies (large, medium and small) and the market outlet for their product may require different health management strategies and different levels of investment in terms of disease diagnosis. For instance, production for restocking and marketing has different aims (leisure industry and food market) and therefore different requirements in terms of health-management strategies.

We found differences between the diseases reported by the site health or farm manager during the interviews, when compared with the laboratory diagnostic secondary data. These differences may have been due to either different reporting periods as the key informant interviews were performed in 2009 whereas the secondary health data covered 2000-2007 or result from previous acquired knowledge and experience of the site health or farm manager site (Eysker and Ploeger, 2000, Rowland *et al*., 2007, Read, 2008). The experience of site health or farm manager may have allowed prompt recognition of particular disease conditions, leading to implementation of control measures without the need to submit clinical material to the laboratory. For instance, two health or farm managers indicated the existence of health-management strategies to minimise the effects of SD, by taking into consideration the age and time of the year when transferring fish (Graham *et al*., 2007). In addition, the interviewees reported the existence of contracts with veterinarians for regular visits to monitor the fish stocked who are able to identify clinical signs and diagnose certain diseases at the farm level (Eysker and Ploeger, 2000, FAO, 2007, OIE, 2011). Presumptive diagnoses can be performed on site (Bondad-Reantaso *et al*., 2001, Read *et al*., 2007, Read, 2008) thus reducing the need to send samples to the laboratory.

The interviewees also indicated they use vaccination as a measure to control and prevent outbreaks of certain infectious diseases as in the case of ERM. Vaccination plans also diminish the need for laboratory services and are considered by site health or farm manager as a pivotal measure to control many animal diseases, including in fish production (Anonymous, 2003, Shirley *et al*. 2010).

In this study, new and emerging diseases were reported which included RTGE. During the period from 2000 to 2007, the diagnosis of RTGE showed differences between the samples submitted to the laboratory with suspected RTGE and confirmed diagnosis. Those differences may be caused by companies and farmers being aware of a new disease problem which led to a higher number of samples submitted for RTGE screening. The laboratory reports diagnosed RTGE in 2002, 2004 and 2006, which are in agreement with Branson (2003) regarding the first identification of RTGE in Scotland. This showed that farmers are still willing to use a service if there is a new or emerging disease condition. This is very positive for the relationship between the site health or farm manager and veterinary laboratory but also shows that laboratory help for disease diagnosis and pathogen identification is a valuable tool even for experienced farmers. Differences in laboratory report results and key informant interviews may also be caused by different data periods being covered for each data source.

Although RTFS has been a problem for the trout sector for numerous years without a commercial vaccine, there is still a need for clinical samples to be sent to a laboratory for positive diagnosis and subsequent antibiotic sensitivity testing to screen for appropriate therapeutic control (Silverstein *et al*., 2009). In our results, the laboratory reports showed high numbers of identification of the agent for RTFS and ERM, followed by furunculosis and parasitic problems. These results are in agreement with

Read (2008) and DEFRA, in the UK. They stated that the major causes of pathogen-driven mortality in farmed trout are currently whitespot (ectoparasite), PKD, RTFS, ERM and furunculosis (Read, 2008, http://defra.gov.uk). Whitespot was frequently reported during the primary data collection, although the laboratory reports do not identify this disease with the same level of frequency. Whitespot is a well-known ectoparasitic disease and farmers are able to identify the clinical signs easily at the farm level (Read *et al*., 2007), in contrast with PKD which is also a well-known disease condition but only identified in the laboratory reports. At the time of informant interviews, PKD may have not been a significant problem and therefore not reported by the site health or farm manager.

In 34 % of the laboratory reports, it was not possible to identify the suspected cause that triggered the need for sample submission. The identification of the suspected cause was difficult due to the lack of information written on the reports. In some cases, the farmer contacted the veterinarian either by phone or by e-mail and the underlying cause was not recorded in the Laboratory Diagnostic Services Information Management System (LIMS). Therefore, a good laboratory management system is vital to store the information provided by the client through time, keep a trace record of the samples and the integrity of the laboratory data.

The submission of clinical samples to the laboratory may be the result of emerging diseases, increases or changes in legislation and market requirements for the final product. Other factors may include that disease can be cyclical leading to an increase or decrease of samples submitted depending on the spread and disease outbreaks. In this study, the increase submission of clinical samples to the laboratory in 2006 from the largest fish farm Company (Company 1) was because of a combination of an increased

demand for general fish health checks and suspicion of RTFS outbreaks. This variation may be due to market expansion to other countries or to new markets in the UK. Both of which require different health screening methods (Council Directive 2006/88/EC), including pathogen-free stock or routine health check for the farm or company prior to movement of fish within UK. In England and Wales, fish production sites, subject to Section-30 health checks from the Environmental Agency which controls aquatic animal health and movements, are obliged to make a health check before introducing fish to open waters where water can flow from one body of water to another (Environment Agency, 2011). This Section 30 obliges all fish moving from Scotland to England or Wales to open waters to have health certification prior to live fish movement. Scotland does not follow Section-30 health checks. In Scotland, the majority of diagnostic laboratories can currently provide disease diagnosis and pathogen screening services.

Histopathology was by far the main laboratory method used in this study. This was not surprising since this laboratory-based technique is the only one that can provide the actual diagnosis; all the other techniques are confirmative tests. Not only does histopathology give the actual disease diagnosis but it can also provide the suspected aetiological agent or the husbandry or environmental factors causing the health problem experienced (Bondad-Reantaso *et al*., 2001). Other laboratory tests are important for pathogen identification of infectious disease problems and are frequently used for the various health checks already described.

5.5.2. Case study 2

The most common diseases mentioned by the interviewees in the salmon freshwater production stage were parasites, fungus, IPN and fin rot. In the seawater stage the most

common diseases were sea lice, followed by PD. Both diseases are currently a big problem for marine salmon production (Johnson *et al*., 2004, Aunsmo *et al*., 2010, http:// defra.gov.uk). DEFRA also identified cardiomyopathy syndrome (CMS) as an important infectious disease in marine salmon. In the key informant interviews, the interviewees did not report this disease in any of the sites visited, which given the importance of this condition, was surprising. This may be explained as this disease has similar clinical signs to PD. The only way to differentiate CMS from PD is through histological characterization of the lesions. Therefore, if a site health or farm manager suspected PD rather than CMS they may not have seen the need to submit clinical material to the diagnostic service. Of course, it may also be that the sites visited did not have this problem at that time.

In this study, the health or farm managers indicated that disease outbreaks in the salmon farms are closely monitored. For instance, the site health or farm managers reported the existence of tight surveillance systems due to concerns of PD infections and cost of fish losses. The suspicion of this disease in the farm triggers preventive measures, such as reducing stress of grading and moving and reduction in feeding (McLoughlin *et al*. 2003, Graham *et al*., 2007). In addition to those preventive measures, samples may be sent to the laboratory to confirm the presence of the suspected disease.

5.5.3. Rainbow trout and salmon production similarities

Munro and Gregory (2009) stated that smolt producers for salmon and on-growing sites for trout have some similarities in terms of structure and health monitoring. This was also found in the current study. Smolt sites for salmon and on-growing sites are intermediate types of sites where young fish are moved onto site and held until they reach a size or a condition appropriate to be transferred (Munro and Gregory, 2009).

The differences in production strategies between the trout and salmon industries are largely due to species requirements (Munro and Gregory, 2009). Rainbow trout are produced in seawater and showed similarities with marine sites of Atlantic salmon, which have few or no movements off-site. In the case of sea trout, there are no movements off-site after transfer to the sea cages (Munro and Gregory, 2009) until harvesting.

5.5.4. Certification

In this study, certification schemes were not commonly applied amongst the trout farms visited. Only one trout company had Freedom Food certification. The majority of trout farms only followed the code of good practice, "A code of good practice for Scottish finfish aquaculture", with the exception of the restocking farm that did not follow any. The great majority of farms visited belonged to one single company that might not have applied for a certification scheme, since the implementation of certification schemes costs money to farms and companies and the market for certified aquaculture products is still a niche market (FAO, 2007). On the other hand, all salmon farms belonged to companies with certification schemes implemented. Some of the companies had more than one certification scheme which is presumably to allow them to reach different market niches. Food Certification International (FCI) stated that roughly 95 % of the total production in Scotland is quality assured under one of the range of FCI-certified product certification schemes or codes of good practice (Food Certification International, 2011). These certification schemes have specifications and standards that influence the policy and health-management strategies of the certified companies and farms.

5.5.5. Conclusion

Both case studies show the key role played by the site health or farm managers in the identification of the first signs of disease by actively watching the fish closely for any modification in fish behaviour and increase in mortalities, allowing an early diagnosis of a potential health problem (Bondad-Reantaso *et al*., 2001, Rowland *et al*., 2007, Read, 2008). The early identification of possible causes of diseases determines the policy and health-management strategies adopted by the company. Laboratory methods are a primary tool to either identify or confirm diseases, even though the test results may not be conclusive as observed in 13 % of the trout reports in this study. Histology is by far the main method used for diagnosis; the other methods are used to confirm the pathogen presence. This study showed that in both salmon and trout farming there is still a role for the conventional methods of disease diagnosis as well as the more advanced methods of pathogen identification.

In the Scottish trout and salmon industries, disease diagnosis, which includes not only laboratory methods, such as histology, but also the clinical signs and the history of the stock and farm experience, was shown to have great value for management decisions and health control. The extension of these case studies to a larger population that comprises a wider number of Scottish fish-producing companies, would allow a breakdown of the industry by size, activity and other relevant factors that influence health-management strategies of the companies. The analysis of laboratory results based on a wider dataset would allow a wider understanding of the importance of the disease diagnoses at industry level and to understand the major diseases affecting the industry.

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Chapter 6 General discussion

6.1. Summary

This final chapter summarizes the main findings of the previous chapters and indicates some future areas of study.

This project was sponsored by the University of Stirling and Marine Scotland Science, which provided the database analysed. The practical work was based on reported mortality of a single site production database (**chapter 2, 3 and 4**), which belonged to a single company for operations based in Scotland, and data collected by myself (**chapter 5**) by interviewing the site or health manager of trout and salmon farms.

This work concerned the investigation and interpretation of the meaning of mortality records at the site level. The main aim of this project was to investigate and explore the value of mortality records to support and assist management strategies at the farm and industry level. The thesis also aimed to illustrate the importance of mortality records for setting industry standards of expected mortality losses and to assess the value of recorded mortalities as a tool for aiding in surveillance and control of infectious diseases. This project also described the role of disease diagnosis in management decisions and health control. This included investigation of the role of farmers' experience on identification of diseases and production problems. The importance of on-farm records on disease diagnosis was also studied.

6.2. General discussion

Site production databases have an important role to play in the investigation and understanding of the spread and outbreaks of diseases. Such data are archives of

information collected during the time of production, which are essential for the identification of potential health problems during the production cycle of livestock (Eysker and Ploeger, 2000, McKenna and Dohoo, 2006) and farmed fish (North *et al*., 2008, Ellis *et al*., 2012). One of the main types of data recorded in site production databases is mortality (MacIntyre, 2008, North *et al*., 2008, Ellis *et al*., 2012). Mortality among farmed fish constitutes a problem both in terms of financial losses (lost value of dead fish, decreased production and extra labour) and compromised animal welfare (suffering before death). To our knowledge, records of fish mortality has been included in many studies associated with infectious diseases (Jarp *et al*., 1994, Crockford *et al*., 1999, Guy *et al*., 2006, Mardones *et al*., 2009), but not as the primary study point to understand the overall meaning of mortality. Only Aunsmo *et al*. (2008) performed a study to develop methods to quantify causes and investigate patterns of mortality after salmon transfer to the sea. This thesis aims to give an insight into the importance and the usefulness of databases of fish production sites, focusing on interpretation of mortality records at the farm level, based on a site production database from a single company and in a key informant interview performed to the health or farm manager of salmonid productions in Scotland. The key informant interview comprised the investigation of the role of farmers' experience on the identification of disease and production problems.

6.2.1 Reported mortality

Mortality records with the cause of death identified, including deformities, predators and disease (North *et al*., 2008), are pivotal for investigation of patterns of mortalities across the production cycle, to benchmark expected losses from the input to the end of the production and to produce and work towards attaining production goals (Dewey,

2008, Soares *et al*., 2011). **Chapter 2** found that reported mortality is a valuable tool to identify unusual losses experienced on a salmon fish farm. According to Thomsen and Houe, (2006), North *et al*. (2008) and Ellis *et al*. (2012), a change in daily mortality may be an indicator of welfare and health problems. Those deviations in mortality can be identified by the use of a benchmark standard for mortality losses. **Chapter 2** produced a baseline benchmark for expected mortality losses of marine salmon, which constituted a first attempt to create a baseline of *normal* mortality in marine Atlantic salmon. This novel approach can be used to detect possible production problems. Potential anomalies may be detected based on deviations of mortality from the benchmark of "expected" mortality. The identification of the main causes of fish death across the weeks of production and in different stages of fish growth is one of the usefulness of reported mortality.

Mortality rates may vary across production cycles. This variation may be caused by several factors identified by a benchmark analysis. **Chapter 3** investigated the drivers that cause variation in mortality during production cycles. The results in **chapter 2** identified that the majority of mortality were associated with actual outbreaks of diseases, specific environmental events including storms and critical periods of production such as transfers (Ellis *et al*., 2012, Soares *et al*., 2011). All of which can cause fluctuations in mortality (**chapter 2 & 3**). **Chapter 3** found that variation in mortality was highly related to site. This site-to-site variation in mortality may have been influenced by the occurrence of epidemics and environmental events, or local effects. The results in **chapter 3** found that temperature, site and/or geographical area are characteristics that may contribute to variation in mortality. Those characteristics also are risk factors for certain disease outbreaks including IPN and PD (Jarp *et al*., 1994, Lannan *et al*., 1992, Crockford *et al*., 1999, Cusak *et al*., 2002). Wheatley *et al*.

(1995) and Crockford *et al*. (1999) suggested that management practices may be a potential source of variation in mortality, which may contribute to site-to-site variation. Although the Scottish aquaculture industry is ruled and guided by tight regulation, including the code of conduct, those variations in management practices may occur at site level as shown in **chapter 3 & 5**.

6.2.2. Surveillance and monitoring

The variation in the expected levels of mortality is a good indicator of the health status of fish (**chapter 2 & 3**) and therefore high levels of mortality may indicate disease and production problems related to poor health (**chapter 2, 3 & 5**). The Scottish Government is considering the introduction of statutory reporting of abnormal mortality levels as a possible measure to combat disease threats more efficiently thus mitigate the impact of a serious infectious disease outbreak. It was postulated by the Scottish Regulatory authorities that abnormal mortality levels on fish farms could be a useful indicator of potential infectious disease. To investigate this, the regulator with the full backing and support of the salmonid industry suggested potential mortality thresholds to be analysed in order to identify the adequate mortality threshold level that could be used as inspection alerts by the official authority. **Chapter 4** explored the meaning of high levels of reported mortality, including specific mortality thresholds as an indicator of the presence of infectious diseases. The study was performed by splitting the production cycle into small fish with mean weight below 750 g and large fish with mean weight over 750 g. In the small fish the results did not show reported mortality as a strong indicator of the presence of infectious disease which may be due to the lack of records of infectious disease at this stage of Atlantic salmon life (**chapter 4**). In the larger fish, however, high mortality levels were found to be a strong potential indicator of the

presence of infectious diseases. Therefore, in larger fish, high levels of mortality, including the suggested mortality threshold are a useful tool to use in farm level risk-based surveillance (**chapter 4**) although high mortality may occur from non-infectious sources.

6.2.3. Disease diagnosis

Chapter 5 described the role of diagnosis in the prevention and control of disease outbreaks. For that, we performed a key informant interview with open questions to the health or farm manager of several trout and Atlantic salmon farms and we also used the diagnostic reports of the Veterinary Diagnostic Services (VDS) from Stirling University to triangulate the data. In **chapter 5** we found that disease diagnoses are of great importance for diagnosis and control of actual diseases. The study indicated that disease diagnosis starts at the farm level with the daily monitoring of fish and the records of different parameters by the farmer, including mortality (**chapter 2 & 3**). The results of **chapter 5** indicated that on-farm records continue to play a vital role in disease diagnoses because they archive valuable data, including mortality numbers, which may be analysed (**chapter 2 & 3**) to identify problems within the production (Eysker and Ploeger, 2000, McKenna and Dohoo, 2006). Farmer's experience was indicated in **chapter 5** as pivotal in the identification of the first signs of disease or potential health problems (Read *et al*., 2007, Rowland *et al*., 2007) which was principally through the daily observation and monitoring of their fish. The experience of a farmer and the awareness of the economic impact of losses, mainly diseases (Menzie *et al*., 2002, Brun *et al*., 2003, Skall *et al*., 2005, Ellis *et al*., 2012), in the production profitability are vital in the day-to-day running of a salmonid production. The results in **chapter 5** also indicated that the early identification of any health problems, including infectious

diseases, by the farmer allowed an early implementation of management strategies and controls to mitigate future losses. In **chapter 5**, the results suggested that the confirmation or disease diagnosis by laboratory methods will allow the implementation of mitigation measures at the population level. The results in **chapter 5** also showed that laboratory methods, such histology, are the primary tool to either identify or confirm diseases. Histopathology was the main method used for the diagnosis of the health problem experienced.

6.3. Conclusions

The following outputs can be drawn from the results presented in this thesis:

- Benchmark analyses of mortality records allow the investigation of unusual losses and therefore enhance the control and prevention of eventual problems, such as production and health.

- Mortality has some drivers, such as temperature, site and geographical area that may contribute to mortality variation across the production cycle.

- Mortality records are a potential tool for triggering alerts of infectious disease problems in larger fish at the farm level and therefore it may be a useful tool to assist with farm level risk-based surveillance.

- Mortality records are an important component of the primary diagnosis and valuable tool for the management decisions and health control at farm level.

- Farmers' experience can be very effective for detecting early indications of diseases.

6.4. Final considerations

This thesis aimed to understand the potential of on-farm records, mainly mortality records. In this study, we showed that mortality records at the farm level have a key role for the control and monitoring of infectious diseases. The comprehension of mortality records has a great value for supporting management decisions and health control strategies either to the producer or to the health authorities.

In this thesis, there is scope for further studies in this field. The analysis performed in this study only took consideration of one single site production database of Atlantic salmon in seawater. This database comprised a large amount of data from a single company, which is one of the largest companies of salmon production from Scotland. One of the problems faced in **chapter 4** was the lack of a "gold standard" for the assigned causes of dead fish. As Aunsmo *et al*. (2008) suggested the investigation of the accuracy and reliability of assigned causes would be of great interest. Future research is needed in this area using a wider database, which would allow representation of the industry by size, activity and other relevant factors that may influence the health-management strategies of the farms and companies. An extension of this study to freshwater stage would also be of great benefit for controlling mortality losses. Other future relevant research would be the study of variation in mortality biomass and the impact of biomass losses in different stages of marine salmon production.

This thesis represents the first attempt in aquaculture to investigate and interpret recorded mortality at the farm level as a primary focus. This novel approach provides tools that can be used by the Atlantic salmon industry or in any other fish farmed species for controlling and preventing losses caused by the presence of diseases and

other production problems. This study also constitutes a strong foundation for further research concerning the value of reported mortality in aquaculture.

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Appendix 1 (Chapter 3)

Table 7 App 1-1 Analysis of variance model for mortality. F ratios and P values were calculated using adjusted sums of squares (Adj. SS). Sequential sums of squares (Seq. SS) are also shown, with terms included in the model in the order they are presented in the table. Mortality data recorded as percentage were subjected to logarithmic transformation for statistical analysis.

	df	Seq SS	Adj SS	Adj MS	F	P
Site ID	87	3416	3563	41	23	≤0.001
Sea age	1	2105	2105	2105	1205	≤0.001
Error	7964	13914	13914	2		
Total	8052	19434.8				

Goodness of fit statistics:

Root mean square error = 1.3 %

r^2 = 28.4 %

Adjusted r^2 = 27.6 %

Model covariates (s.e):

Constant: −3.30 (0.0294) t= −112 P<0.001

Table 7 App 1-2 Analysis of variance model for mortality. F ratios and P values were calculated using adjusted sums of squares (Adj. SS). Sequential sums of squares (Seq. SS) are also shown, with terms included in the model in the order they are presented in the table. Mortality data recorded as percentage were subjected to logarithmic transformation for statistical analysis.

	df	Seq SS	Adj SS	Adj MS	F	P
Sea age	1	1958	2105	2105	1205	≤0.001
Site ID	87	3563	3563	41	23	≤0.001
Error	7964	13914	13914	2		
Total	8052	19434.8				

Goodness of fit statistics:

Root mean square error = 1.3 %

r^2 = 28.4 %

Adjusted r^2 = 27.6 %

Model covariates (s.e):

Constant**:** −3.30 (0.0294) t= −112 P<0.001

Table 7 App 1-3 Analysis of variance model for mortality. F ratios and P values were calculated using adjusted sums of squares (Adj. SS). Sequential sums of squares (Seq. SS) are also shown, with terms included in the model in the order they are presented in the table. Mortality data recorded as percentage were subjected to logarithmic transformation for statistical analysis. Lagged week is one-week lag term.

	df	Seq SS	Adj SS	Adj MS	F	P
Lagged week	1	13398	11815	11815	17453	≤0.001
Weight	1	4	1	1	2	≤0.183
Feed Intake	1	7	7	7	11	≤0.001
Error	7961	5390	5390	1		
Total	7964	18799.4				

Goodness of fit statistics:

Root mean square error = 0.8 %

r^2= 71.3 %

Adjusted r^2 = 71.3 %

Model covariates (s.e):

Constant: −0.65 (0.0280) t= −23 P<0.001

Table 7 App 1-4 Analysis of variance model for mortality. F ratios and P values were calculated using adjusted sums of squares (Adj. SS). Sequential sums of squares (Seq. SS) are also shown, with terms included in the model in the order they are presented in the table. Mortality data recorded as percentage were subjected to logarithmic transformation for statistical analysis. Lagged week is one-week lag term.

	df	Seq SS	Adj SS	Adj MS	F	P
Weight	1	1343	1	1	2	≤0.183
Feed Intake	1	252	7	7	11	≤0.001
Lagged week	1	11815	11815	11815	17453	≤0.001
Error	7961	5390	5390	1		
Total	7964	18799.4				

Goodness of fit statistics:

Root mean square error = 0.8 %

r^2 = 71.3 %

Adjusted r^2 = 71.3 %

Model covariates (s.e):

Constant: −0.65 (0.0280) t= −23 P<0.001

Appendix 2 (Chapter 4)

Table 8 App 2-1 Contingency table that cross-tabulates actual numbers of reported weekly data of mortality for presence/absence of reported condition against no mortality reported.

	Reported condition		
Criteria	**Reported infectious diseased**	**No mortality reported**	**Total**
Below	FN	TN	TN+FN = test negatives
Above	TP	FP	FP+TP = test positives
Total	FN+TP = diseased	TN+FP = Nondiseased	TN+FN+FP+TP = total sample size

Sensitivity (SE) = True positive / (True positive + False negative)

Specificity (SP) = True negative / (True negative + False positive)

Positive predictive value (PPV) = True positive / (True positive + False positive)

Negative predictive value (NPV) = True negative / (True negative + False negative)

Table 8 App 2-2 Contingency table that cross-tabulates actual numbers of reported weekly data of mortality for presence/absence of all infectious diseases against no mortality reported for 1.5 % and 6 % cut-off for fish under 750 g.

Fish < 750 g							
Criteria (1.5 %)	**Condition reported**		**Total**	**Criteria (6 %)**	**Condition reported**		**Total**
	All infectious diseases	**No mortality reported**			**All infectious diseases**	**No mortality reported**	
Below	795	1232	2027	**Below**	680	959	1639
Above	120	49	169	**Above**	160	45	205
Total	915	1281	2196	**Total**	840	1004	1844

SE = 120 / (120 + 795) = 0.13

SP = 1232 / (1232 + 49) = 0.96

PPV = 120 / (120 + 49) = 0.71

NPV = 1232 / (1232 + 795) = 0.61

SE = 160 / (160 + 680) = 0.19

SP = 959 / (959 + 45) = 0.96

PPV = 160 / (160 + 45) = 0.78

NPV = 959 / (959 + 680) = 0.59

Table 8 App 2-3 Contingency table that cross-tabulates actual numbers of reported weekly data of mortality for presence/absence of all infectious diseases against no mortality reported for 1 % and 4 % cut-off for fish above 750 g.

Fish > 750 g							
Criteria (1 %)	**Condition reported**		**Total**	**Criteria (4 %)**	**True Condition**		**Total**
	All infectious diseases	**No mortality reported**			**All infectious diseases**	**No mortality reported**	
Below	529	4192	4721	**Below**	428	3910	4338
Above	91	98	189	**Above**	99	121	220
Total	620	4290	4910	**Total**	527	4031	4558

SE = 91 / (91 + 529) =0.15

SP = 4192 / (4192 + 98) = 0.98

PPV = 91/ (91 + 98) = 0.48

NPV = 4192/ (4192 + 529) = 0.89

SE = 99 / (99 + 428) = 0.19

SP = 3910 / (3910 + 121) = 0.97

PPV = 99 / (99 + 121) = 0.45

NPV = 3910 / (3910 + 428) = 0.90

Appendix 3 (Chapter 5)

App 3.1 Key informant interview questionnaire

This was a key informant questionnaire so the questions were designed following the natural flow of the conversation between the person being interviewed and the person conducting the interview. For each question the time given for reply was an average of 10 to 15 min. The information was recorded on paper sheet by the person conducting the interview. The interviewer also used some external validations points (e. g. feeding method, type of culture operation and fish species stocked), while the questionnaire was performed. The questionnaire has been separated into the different sections which represented the key questions required for each of the sections of interested per site. The questions not replied or not known were recorded as such.

Questionnaire:

SECTION 1.Stock:

Q1. What fish species do have stocked?

Q2. How many fish do you have stocked?

Q3. How many fish in average do you harvest per year?

Q4. What is the average fish weight at stock?

Q5. What is the average weight at harvest time?

Q6. What is the stocking density average?

Q7. When fish increase size, are they moved to different tanks/cages?

Q8. What is the age of fish (e. g. fry, smolt) at stocking time?

Q9. What is the age of fish (trout/salmon) at transfer?

Q10. Where do you buy your fish, in international or national companies?

Q11. How many companies supply you fish?

Q12. Are fish from different sources stocked in the same tanks/cages or separately stocked by source?

SECTION 2. Site:

Q1. What kind of market (restocking, table market or both) do you produce fish for?

Q2. Is the site an organic or non-organic production?

Q3. What type of facilities (cage, raceways, tanks or ponds) are the fish (trout/salmon) grown?

Q4. Which type of culture operation (e. g. hatchery and grow-out) is this site?

Q5. How often are fish moved in and out of a site?

Q6. How many production cycles do you have on site?

Q7. Could you explain me how you transfer fish within the site?

SECTION 3. Feeding

Q1. Which is the main method (automatic or manual) of delivering feed to fish?

Q.2. How many times a day do you feed the fish?

Q.3. Which is the percentage of feed given to fish per day?

Q.4. Which feed mill company do you use?

SECTION 4. Health & Welfare

Q1. Which plan do you have for fallowing?

Q2. Are the site totally/partially fallowed between stocks?

Q3. What plan do you have for cleaning and disinfection of the site?

Q4. What are the measures for predator control?

Q5. What is the vaccination strategy to prevent diseases?

Q6. What type of diseases do you vaccinate fish against?

Q7. Which is the type of vaccines do you administer to fish?

Q8. What is the age of fish vaccination?

Q9. Are fish revaccinated?

SECTION 5. Record keeping

Q1. Which kind of records do have on site (e. g. mortalities, water quality parameters, medicines, etc.)?

Q2. How often are the records done (on a daily base or weekly base)?

Q3. How long do you keep the records?

App 3.2 Case records at Veterinary Diagnostic Services

Laboratory number	Entrance date	Site	Test				Observation
			Histopathology	Bacteriology	Virology	Parasitology	
R020010	_____	_____	√	√		√	_____
R020037	_____	_____	√			√	Live alevins + in formalin
R020061	_____	_____	√				_____
R020072	_____	_____	√	√		√	_____
R020099	_____	_____	√	√			_____
R020153	_____	_____	√	√			Smears
R020171	_____	_____	√	√			Smears
R020199	_____	_____	√	√			_____
R020261	_____	_____	√	√			Smears
R020287	_____	_____	√	√			Visit
R020330	_____	_____	√	√		√	Visit, Strawberry disease deaths
R020366	_____	_____	√	√		√	Visit, Strawberry disease
R020381	_____	_____	√		√		Visit, Sleeping disease
R020406	_____	_____	√	√			Visit
R020457	_____	_____	√	√		√	Live fish RT
R020433	_____	_____	√			√	Visit

Veterinary Diagnostic Services, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK

App 3.3 Sample submission form at Veterinary Diagnostic Services

Institute of Aquaculture – Histopathology

Owner: _____ date: _____ Reference: _____

Material: _____ Pathologist: _____ Site: _____ Fixed: [ ] Unfixed: [ ]

Examination required:

General: [ ] Health certification: [ ] Other: [ ] GLP study: [ ] GLP Study number: [ ]

Details:

FOR LABORATORY USE ONLY

Cassetted					Tech				
Processed					Tech				
Cut					Tech				
Stained					Tech				
Slides checked					Tech				

Details	C	T	Chk	Details	C	T	Chk	Details	C	T	Chk
A				J				S			
B				K				T			
C				L				U			
D				M				V			
E				N				W			
F				O				X			
G				P				Y			
H				Q				Z			
I				R							

C = Number of cassettes Chk = Slide checked against Block

T = Number of tissues TOTAL NO. CASSETTE: _____

Histo/bookform RUNNING TOTAL: _____

App 3.4 Case report at Veterinary Diagnostic Services

Case Record: _____ Date: _____

Client: _____

Report:

Yes, these fish do indeed all have severe lesions compatible with IPN, as evidenced by severe exocrine necrosis.

There were no other significant (concurrent) lesions.

Veterinary Date

_____ _____

_____

Veterinary Diagnostic Services, Institute of Aquaculture, Stirling University, Stirling FK9 4LA, UK