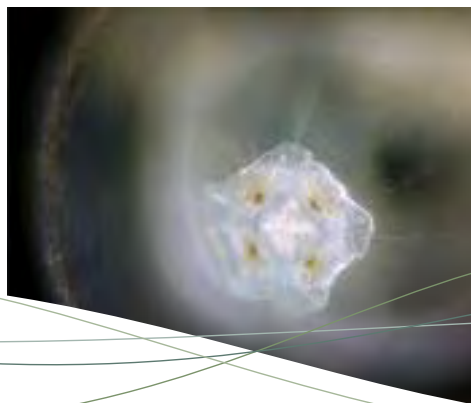


Marine Research Sub-Programme
(NDP 2007-'13) Series



GILPAT - An Investigation into Gill Pathologies in Marine Reared Finfish

Project-based Award



Lead Partner: Marine Institute



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Marine Research Sub-Programme 2007-2013

Project-based Award

GILPAT: An Investigation into Gill Pathologies in Marine Reared Finfish

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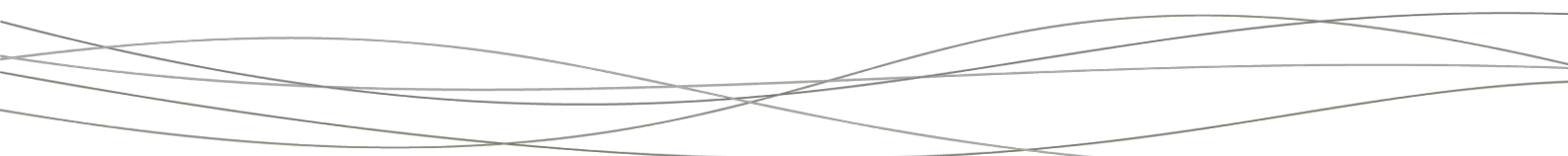
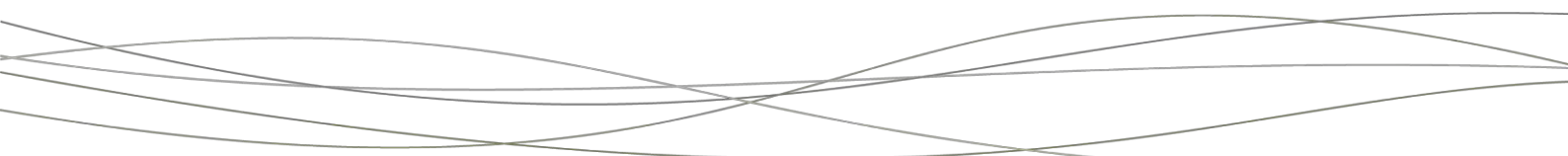
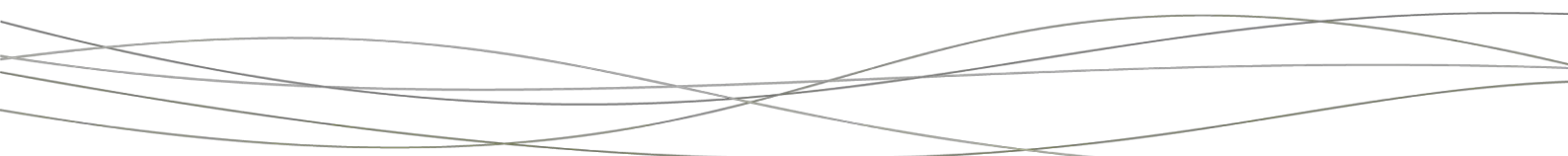


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EXECUTIVE SUMMARY

The role of jellyfish as causative agents of gill disorders and mortalities in marine-farmed finfish had received very little attention until the advent of the GILPAT project. Without knowledge of the abundance, distribution, and seasonality of the detrimental jellyfish species around aquaculture sites an understanding of such interactions cannot be developed. There are numerous species of jellyfish throughout our coastal waters capable of forming high density blooms, however their potential to cause gill damage remains largely unknown. It was the aim of this project (GILPAT) to begin an investigation into the jellyfish species found around the coast of Ireland and their potential impact on the finfish aquaculture industry. Therefore extended targeted monitoring of the health of marine-farmed fish and jellyfish populations, in combination with the development of controlled experimental studies, were performed to gain a more comprehensive understanding of mechanisms behind jellyfish-induced gill disorders. In addition to this a range of molecular diagnostic methods were developed and used to screen farm samples for potential pathogens which have been linked to the development of gill disease in other countries.

This project has resulted in 14 peer-reviewed publications (either published, in press or accepted) and a PhD thesis. Work from GILPAT has been presented at a range of international meetings including those organised by the European Parliament, European Association of Fish Pathologists, and the Fish Veterinary Society. A number of training workshops were also held for industry at the Marine Institute and the National Maritime College of Ireland.

Literature reviews and epidemiological investigations

A review of the literature highlighted the large knowledge gaps which exist at present. Gill disease is a multi-factorial condition occurring due to the interactions of particular environmental conditions, the presence of phyto/zooplankton blooms, and pathogen (viral, bacterial or parasitic) involvement. The Marine Institute's phytoplankton monitoring programme provides a lot of information on the occurrence and distribution of these species, however there is no current programme available for zooplankton/jellyfish species. There is also little known about the prevalence and role played in gill disease by pathogens such as the bacterium *Tenacibaculum maritimum*, and the parasite *Desmozoon lepeophtherii*. Epitheliocystis also appears to be linked to gill disease, but it has yet to be established whether this is a primary or secondary factor.

An epidemiological investigation highlighted gill disease as a significant cause of mortality, it resulted in fish welfare challenges and gave rise to loss of growth in the majority of the marine

salmon farms during the study period. Results indicated that mortalities due to gill disorders were lower in the salmon S0s compared to the S1s¹. Results also indicated that sites positive for the presence of epitheliocystis had significantly higher mortalities due to gill disease when compared to negative sites. There were no statistically significant differences in gill disorder losses when net washing (using high pressure underwater cleaners) was compared to net changing although the authors feel that this is an area which needs to be investigated further.

Zooplankton monitoring

The role of gelatinous zooplankton in the gill disorders of marine-farmed salmon was investigated using both field sampling and controlled challenge experiments. To aid these investigations, a semi-quantitative gill scoring methodology was developed over a longitudinal study to enable gill damage to be rated in terms of extent and severity. Over the longitudinal study, the highest observed gill scores coincided with an intrusion of the scyphozoan jellyfish *Pelagia noctiluca* into one particular farm. Small hydrozoan jellyfish (the siphonophore *Muggiaea atlantica* and the hydromedusa *Solmaris corona*) were linked to clinically significant gill damage at densities in orders of magnitude lower than for previously reported problems. The abundance of small hydrozoan jellyfish was also significantly correlated with the average daily fish mortality (with a lag of three to four days) indicating the potential for such abundances to cause background level mortalities.

Experimental challenge trials

Through the use of controlled experimental challenge trials, the role of two cnidarian agents in causing gill disorders of marine-farmed salmon was investigated. The potential for gill damage caused by the common jellyfish *Aurelia aurita* may have been previously underestimated and understudied due to the benign nature of its sting to humans. However, following exposure to macerated *A. aurita*, it was possible to explicitly show for the first time that Atlantic salmon post-smolts suffered significant and persistent gill damage. Through the use of histological sampling over a time-series from initial exposure, the pathogenesis of the gill damage could be tracked and was shown to increase in severity and extent up to 38 hours after the jellyfish were removed. This lag in the maximum gill damage observed may similarly relate to the lag in mortality that was observed in the monitoring programme, three to four days after the peak in jellyfish abundance. Even three weeks from the start of the challenge, fish had longstanding gill damage. No mortalities were observed, although it should be considered that the fish were in controlled conditions (with UV sterilisation of the water) where the likelihood of secondary bacterial infections was minimal.

¹ Salmon smolts put to sea in autumn/winter are referred to as S0 smolts while those put to sea in spring are referred to as S1 smolts.

In the second experimental challenge trial, the significant and rapid growth of biofouling hydroids was quantified and the potential for damage caused by these organisms was investigated for the first time. The results show that the hydroids have a significant impact on the health of the gills after exposure. These studies have implications for management practices, such as *in situ* net washing, which will require further investigations.

Molecular diagnostic methods

During this project, sensitive molecular diagnostic methods were developed for five potential pathogens which have been associated with gill disease in other countries: Atlantic salmon paramyxovirus; *Neoparamoeba perurans*; *Tenacibaculum maritimum*; *Candidatus Piscichlamydia salmonis*; and *Desmozoon lepeophtherii*. The Atlantic salmon paramyxovirus was not found in any samples tested. *N. perurans* was detected in fish with amoebic gill disease and levels increased in fish with more severe pathology. The bacterium *T. maritimum* appears to be commonly found in fish gill tissues, however high levels of the bacteria were associated with gill disease. The bacteria were also found in some species of jellyfish, suggesting that they may act as vectors. The occurrence of epitheliocystis, caused by the bacterium *Piscichlamydia salmonis*, has also been associated with gill disease and increased loads of the bacteria on gill tissue occurred in cases with severe gill pathology.

Engineering review

This report looked at the threat of harmful jellyfish blooms to salmon aquaculture and a number of possible mitigation measures were described and discussed. Cessation of feeding, oxygenation of cages, and forcing salmon deeper in cages are methods that are being or have been used by salmon farm operators in order to defend against jellyfish blooms. While there is logical reasoning behind the use of each of these methods, no evidence of the effectiveness of these techniques has been found. It is recommended that control studies for each of these techniques be undertaken to assess their effectiveness.

In terms of mitigation technologies that have not been used previously, air bubble curtains may be the most promising. Air bubble curtains have long been suggested to prevent jellyfish blooms entering salmon cages but there is little evidence of their effectiveness. Small scale trials using water filled ping pong balls to stimulate jellyfish have indicated that bubble curtains may be effective in pushing jellyfish to the surface where they would accumulate at a float and collected using a suction pump. The energy required to run a bubble curtain may limit its use, especially where there is a strong current as costs may be prohibitive.

Closed containment systems are still not cost competitive with conventional salmon cages. The main reason for the costs of closed containment aquaculture being so high are the energy

costs associated with pumping water. This needs to be reduced in order for it to become competitive. Concepts where renewable energies are used to supply aquaculture energy requirements should be developed and the economics of combining the two industries studied.

Removal of biofouling is a labour and capital intensive task for the aquaculture industry. Current methods of dealing with fouling in Ireland are replacing nets for cleaning and *in situ* cleaning with power washers. Replacing nets has the effect of causing stress on the salmon while power washing results in fragments of fouling drifting in to the cages where they may cause gill problems in the salmon. Research on anti-fouling coatings which are suitable for use on organic salmon farms should be undertaken. Field trials should also be undertaken on the combined water jet and vacuum system which would prevent removed fouling from entering the salmon cages.

Recommendations for future areas of work

This project has identified two general areas where more research is required: 1) early warning systems consisting of a combination of zooplankton/jellyfish monitoring and investigations into pathogens; and 2) developing mitigation measures.

Zooplankton/jellyfish monitoring: as no official monitoring programme exists, the training of people involved in the aquaculture industry to sample and identify zooplankton species will be necessary. Developing models to track and predict the movement of jellyfish is an area which needs to be developed as well as studies identifying which particular species are harmful to fish.

Pathogen investigations: the roles of the various suspect pathogens in gill disease need to be fully elucidated. It is also important to determine how these pathogens are transmitted to the fish and what the risk factors for gill disease are. Once the roles have been determined, research into the development of vaccines, therapeutants or special diets should be carried out.

Mitigation measures: the effectiveness of various strategies such as the use of bubble curtains, flocculating agents, and the use of anti-foulants need to be investigated. Alternative cage design is also an area which could prove useful in mitigating the effects of harmful jellyfish blooms.

A NOTE FROM THE AUTHORS

As with all research in biology, the study of living organisms is a dynamic process, constantly changing. So it is with the study of fish diseases in aquaculture, new diseases emerge, old diseases are managed and, in this case, a known disease re-emerges with a significant impact. We are talking of course about amoebic gill disease (AGD), which during the time of this project was not a major disease in Ireland, but in the short time since this project finished has emerged as the most significant issue for aquaculture today. Not only in Ireland but also in Scotland, and AGD has the potential to cause significant problems in Norway. Despite the fact that this project was primarily focussed on the interaction of farmed salmon with zooplankton and jellyfish, a molecular diagnostic tool for the detection of *Neoparamoeba perurans*, the causative agent of AGD, was developed and used on samples from the field (Chapter 3).

Although the *GILPAT* project has finished, research on gill pathologies will continue. Through the results reported from this project and the collaborations made, gill pathologies have become a major area in aquaculture which is attracting more and more interest. This has resulted in the setting up of the *Gill Health Initiative*, an international voluntary forum involving the aquaculture industry, pharmaceutical companies, government agencies, and research institutes, with the aim of bringing together all the stakeholders to stimulate discussions on developing practical management solutions and stimulating research.

I. INTRODUCTION

I.1. Background

The Irish finfish sector was worth an estimated €58.4 million in 2007, with three species (Atlantic salmon, rainbow trout, and Atlantic cod) currently reared on marine sites (Anon. 2008). Atlantic salmon dominate both the volume produced and the value of the total harvest, comprising 88% of the total volume and harvest value of the industry in 2007. Sea reared trout made up 5% of the total volume and 3% of the value (Anon. 2008). Therefore the finfish industry in Ireland is dependent on the performance of the salmon sector. With losses on marine salmon sites in the region of 20%, this level of loss is of concern to the Irish industry from both an economic and welfare point of view. The average mortality of Atlantic salmon reared at sea, due specifically to gill pathologies, for the three year period of 2003-05 was estimated to be 12% (Rodger 2007). Prior to the GILPAT project, the actual causative agents of gill pathologies in Irish salmon had yet to be identified, although it was believed to be a multifactorial condition involving environmental parameters and biological species such as phytoplankton and zooplankton blooms, while the role of pathogens such as parasites, bacteria, and viruses was unknown.

The gill tissues of teleost fish are in intimate contact with the surrounding environment and therefore are vulnerable to water borne irritants, environmental changes, and parasitic infestations. During the marine stage of salmon production gill disorders have been a sporadic historical challenge in all regions where the species is cultivated, ranging from amoebic gill disease in Australia (Clark & Nowak 1999) to proliferative gill inflammation in Norway (Kvellingstad et al. 2005).

From the 1980's when aquaculture developed in Ireland, a syndrome termed 'Summer Syndrome' caused serious mortalities in farmed salmon. Typical mortalities averaged between 2-10%, but reached 20% on some sites. Early investigations suggested that solar ultraviolet radiation may be a factor in the syndrome (McArdle & Bullock 1987). O Connor (2002) described symptoms of the syndrome included bleeding gills, 'burn' marks on the sides of fish, and high numbers of the protozoan species *Trichodina* on the gill of fish. Although an in-depth study in Kilkieran Bay, Co. Galway in 2000 (Anon. 2000) did not determine the cause of summer syndrome, a number of areas such as increased zooplankton swarms were highlighted. A possible zooplankton/jellyfish bloom was implicated as a potential cause of the severe mortalities which occurred in a number of salmon farms in Donegal in 2003 (Cronin et al.

2004). Since 2003, gill disorders have emerged as the most serious cause of mortality in Irish farmed salmon (Rodger 2007). Average mortalities in marine farms due to gill disorders during the period 2003-2005 have been 12%, ranging from 1-79%. All marine stages of production are affected, however preliminary investigations showed that the younger, post-smolt stage salmon appear to be more severely affected in terms of mortality and that the majority of gill problems occur in the summer or autumn months with S1 smolts (Rodger 2007). In 2005 a protracted bloom of *Karenia mikimotoi* resulted in mortalities of both farmed and wild fish along the west coast of Ireland (Silke et al. 2005).

The aetiology of gill disorders are believed to be multifactorial and can originate with one primary insult which then allows secondary opportunistic pathogens to infect, resulting in epithelial damage and respiratory distress for the host fish. As the infectious agents remain undefined, medication and vaccination are not feasible and preventive measures taken against gill pathologies have proven insufficient. This study aimed to clarify the role of potentially harmful water borne organisms and pathogens which should then lead to strategies or methods to reduce the impact or exposure on aquaculture sites.

1.2. Possible Causes of Gill Pathologies

The possible causes of gill pathologies have been grouped into a number of broad categories (Rodger 2007). The two major categories of significance to Ireland are 1) phytoplankton blooms and 2) zooplankton blooms. Two other categories include 3) gill parasites and 4) bacterial and viral pathogens, of which there is limited information available in Ireland and their role in the development of gill pathologies is unknown.

1.2.1. Phytoplankton blooms

Algal blooms can give rise to gill pathologies through direct physical damage e.g. *Chaetoceros* sp., where the diatom skeletons or silicaceous spicules cause irritation of the gill epithelia (Kent et al. 1995; Treasurer et al. 2003). In addition, the production of toxins from species such as *Karenia mikimotoi* can cause high mortalities (Silke et al. 2005; Mitchell & Rodger 2007) and through deoxygenation following phytoplankton die-off (Richardson 1997).

1.2.2. Zooplankton blooms

Harmful zooplankton swarms such as siphonophores (pelagic hydrozoans e.g. *Apolemia uvaria*, *Muggiaea atlantica*), scyphozoans (jellyfish e.g. *Cyanea capillata*, *Pelagia noctiluca*), and hydrozoans (e.g. *Solmaris corona*) can cause direct damage to the gill tissue through the effects of their

nematocysts. Many zooplankton and jellyfish species have been implicated in fish kills including the siphonophores *A. uvaria* (Båmstedt et al. 1998) and *M. atlantica* (Hellberg et al. 2003), the scyphozoans *C. capillata* (Bruno & Poppe 1996) and *P. noctiluca* (Doyle et al. 2008), and the hydrozoan *S. corona* (Purcell et al. 2007).

1.2.3. Gill parasites

Amoebic gill disease (AGD), caused by the protozoan *Neoparamoeba* sp., is a significant disease of salmonid aquaculture in Australia (Clark & Nowak 1999). AGD was first reported in Ireland in 1995 (Rodger & McArdle 1996) resulting in significant losses on the sites affected (Palmer et al. 1997). In Ireland, protozoans (*Ichthyobodo*-like flagellates, trichodinid ciliates) and adverse environmental conditions have been shown to play a role in the onset of AGD, although no causative organism could be identified at the time (Bermingham & Mulcahy 2004; 2006). It has been shown in recent years that a newly identified species, *Neoparamoeba perurans* causes AGD in Australia (Young et al. 2008) and Norway (Steinum et al. 2008). It was also shown that this species is present in Ireland however its role, if any, in gill pathologies is as yet unknown (Young et al. 2008)².

1.2.4. Bacterial & viral pathogens

Bacteria such as *Tenacibaculum maritimum* and a Chlamydia-like bacteria *Piscichlamydia salmonis* have been associated with gill disease. However their role has yet to be established and they may just act as secondary pathogens (Draghi et al. 2004). In Norway, the Atlantic salmon paramyxovirus (ASPV) has been detected in farms affected by proliferative gill inflammation and may be a contributing factor to the disease (Kvellestad et al. 2005). No information is available on the prevalence of similar pathogens in Ireland, although there have been observations and indications that bacterial pathogens are involved in some sites (Rodger 2007).

1.3. Project Overview

The aims of the GILPAT project were to take a multidisciplinary approach in order to further understand the underlying causes of gill disease in Irish farmed fish. A specific aim was to establish a pilot zooplankton monitoring programme and use training workshops to enable fish farmers to upskill in areas such as zooplankton sampling and basic identification of the main zooplankton/jellyfish species common to Irish waters. Complimenting this was the

² Since the finish of this project, AGD has emerged as a significant disease of farmed salmon in Ireland and Scotland stimulating new projects into this parasites with the aim of providing information on how to treat and control the disease.

development of a number of molecular diagnostic methods for the detection of potential pathogens suspected of being involved in the development of the condition. Together with a comprehensive literature review, epidemiological study, and experimental challenge studies, the project aimed to bring all these elements together with the objective of outlining potential mitigation measures and identifying areas for future research.

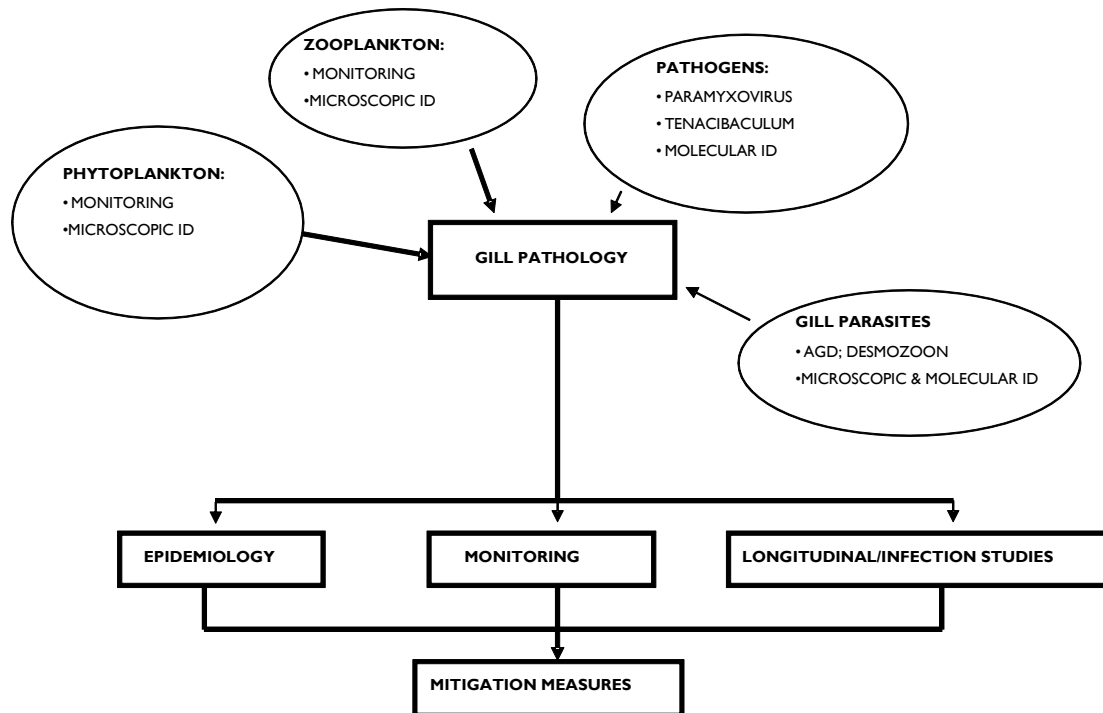


Figure 1.1: An overview of the GILPAT project illustrating the factors responsible for the development of gill pathologies.

1.4. Training

Throughout the project, training workshops for fish farm personnel were held annually at the Marine Institute (in conjunction with the *AquaPlan* project). The workshops consisted of a mixture of lectures, practicals, and discussions. An overview was provided on gill diseases with a description of the pathology, potential causative agents, and the current disease situations in Ireland. Practical training was provided on the correct procedures for sampling and identifying the most common zooplankton species. An identification training manual was also developed during the project and provided for each participant. Additional training was provided on phytoplankton monitoring and identification as well as sea lice monitoring and identification.

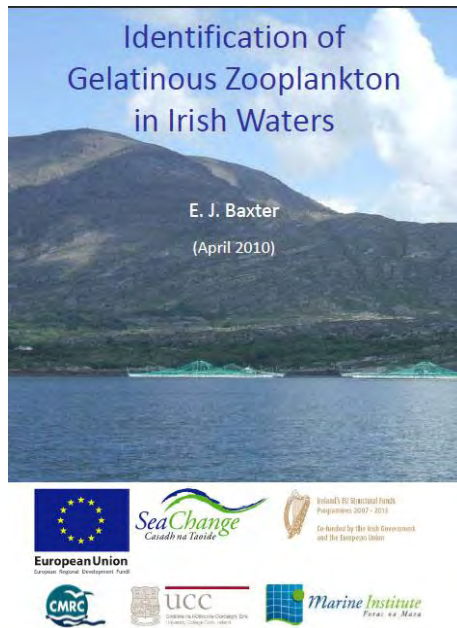


Figure 1.2: Identification manual prepared for the GILPAT workshops. Copies are available from any member of the project consortium or can be download from the Marine Institute website (www.marine.ie).

2. EPIDEMIOLOGY

2.1. Literature Reviews

2.1.1. Non-infectious gill disorders

Gill disorders present a significant challenge in salmonid farming regions throughout the world. This review of gill disorders and diseases of marine fish is focused on the non-infectious causes of gill disease in marine stage salmonids and these are grouped into harmful algae, such as *Karenia mikimotoi*, harmful zooplankton such as *Pelagia noctiluca*, other environmental challenges such as pollutants, as well as nutritional and genetic or congenital causes. This review has been published in full, (Rodger et al. 2011a), and a brief overview is presented in this section.

Harmful Algae

Reports of algal blooms affecting finfish or salmonid culture are numerous, and details from a number of these can be found in Rodger et al. (2011a). Black et al. (1991) proposed four main mechanisms by which fish mortalities can be caused by harmful algal blooms: (1) physical damage e.g. *Chaetoceros* sp., *Skeletonema* sp.; (2) asphyxiation due to oxygen depletion; (3) gas-bubble trauma due to oxygen super-saturation caused by algal photosynthesis; and (4) ichthyotoxin damage e.g. *Karenia mikimotoi*.

Harmful Zooplankton

The increase of reports in the literature on harmful zooplankton blooms are reflecting mounting concerns that the marine ecosystem food chain is changing with a trend towards dominance of jellyfish. Coastal development, pollution, and overfishing are impacting on the populations of natural predators of jellyfish e.g. loggerhead turtles and also competitors such as tuna, sardines, and anchovies (Purcell 2005). Apart from the direct loss due to mortalities in aquaculture, the implications of zooplankton swarms are far-reaching. A considerable cost is incurred during retrieval and disposal of dead fish, zooplankton swarms increase stress in affected fish populations and the incidence of disease and secondary bacterial infections. Many gelatinous zooplankton possess epidermal cells known as cnidocytes containing stinging structures (nematocysts) which can damage fish gills and skin. The nematocysts discharge when triggered and a filament penetrates the prey delivering protein toxins which paralyse and damage cells. In addition, the physical presence of high numbers of gelatinous zooplankton may in some cases give rise to physical clogging of gills and reduced oxygen levels, particularly in species which do not have nematocysts.

Medicines & Remedies

Remedies used for the control of infectious disease have in some cases been associated with damage to the gills of the fish treated, e.g. formalin, hydrogen peroxide, and chloramine-T. Formalin treatments are commonly used on freshwater sites for treatment of external diseases caused by parasites, bacteria, and fungi. Hydrogen peroxide is used as a bath treatment for external parasites such as sea lice and chloramine-T is used as a treatment against bacterial gill disease and ectoparasites. To reduce the potential impact on the gill tissue, the manufacturers' instructions should always be followed when administering remedies.

Eutrophication & Pollution

Coastal eutrophication encourages the production of phytoplankton and ultimately leads to phyto and zooplankton blooms. Factors involved in this enrichment of nutrients include sewage, agriculture runoff, and discharge as well as industrial effluent and discharge. In addition to their role in plankton enrichment of the marine ecosystem these factors also play a direct role in gill disease and mortalities in farmed finfish. Damage to gill filaments or the osmoregulatory cells on the filaments decreases the ability of the fish to respire and to maintain an osmotic balance.

Nutritional Factors

It has been shown that a number of nutritional deficiencies can lead to gill damage in farmed fish including deficiencies in vitamin C, vitamin K, biotin, and pantothenic acid. The increasing cost of producing feed for the aquaculture industry based on fish meal has led to the use of alternative ingredients; however care should be taken to avoid the introduction of deficiencies into the diet when alternative feeds are used.

Genetic & Congenital Factors

Shortening of the opercular cover of gills results in the exposure of the gill filaments to the external environment. The problem was believed to be genetic as it was associated with particular strains, however environmental conditions during egg incubation and first feeding are now considered important as the condition may be related to elevated temperatures during the egg incubation period.

2.1.2. Infectious gill disorders

There are numerous aetiological agents considered to be involved in, or responsible for, gill disease with a range of clinical and pathological presentations. The major infectious agents associated with gill disease in marine salmonid farming are reviewed here under the headings

of (1) parasites, (2) viruses, and (3) bacteria. For full details on the review of infectious gill disease see Mitchell & Rodger (2011).

Parasites

The most significant disease caused by gill parasites is amoebic gill disease (AGD) and has been reported in all the major salmonid producing countries (North America, New Zealand, Australia, Ireland, Scotland, France, Norway, Spain, and Chile). Although the aetiological agent of AGD was assumed to be a member of the genus *Neoparamoeba*, it was not until a recent study by Young et al. (2008) that the new species *N. perurans* was described as the predominant agent of AGD epizootics around the world. Trichodinids are important protozoan parasites of both freshwater and marine fish where they can cause significant pathology of the skin and gills. Species such as *Trichodina* sp. and *Ichthyobodo* (= *Costia*) sp. are commonly found associated with gill problems. The microsporidian parasite, *Desmozoon lepeophtherii*, was recently reported in Atlantic salmon and has been associated with proliferative gill inflammation (PGI) in Norway (Steinum et al. 2010). It is not known how significant this parasite is as it has also been found in fish with no obvious clinical signs of disease.

Viruses

Only two viruses to date have been associated with gill disease in farmed fish, these are the Atlantic salmon paramyxovirus (ASPV) and salmonid gill pox virus (SGPV). ASPV has been found to be associated with PGI but it has not yet been determined that it is the primary cause of disease. Similarly, SGPV has also been associated with gill disease but its significance as a gill pathogen remains to be determined.

Bacteria

Tenacibaculosis, a bacterial infection of the gills, is caused by the Gram-negative, filamentous bacterium *Tenacibaculum maritimum*. It was recently reported that high numbers of the bacteria were found on a jellyfish *Phialella quadrata* suggesting that jellyfish and/or zooplankton may act as vectors of bacterial pathogens (Ferguson et al. 2010). *Aeromonas salmonicida*, the causative agent of furunculosis is generally a septicaemic condition, however colonies of the bacteria can be found in the gill lamellae (McArdle et al. 1986). Epitheliocystis is another bacterial condition that has been reported to affect the gills of over fifty freshwater and marine fish species. The disease is caused by a group of intracellular Gram-negative bacteria belonging to the phylum *Chlamydiae*. The two main species found in salmonid fish are *Piscichlamydia salmonis* and

Clavochlamydia salmonicola, however their role in the development and progression of gill disease has yet to be determined (Steinum et al. 2010).

Summary

Development of early warning systems and the employment of oceanographic tracking models would be advantageous to predict deleterious coastal blooms. Such advance warning would allow for more time to put a chosen management strategy in place (Table 2.1). Predictive models and mitigation techniques employed to minimise the harmful effects of algal blooms are insufficiently advanced or developed to ensure the complete safety of aquaculture animals. In addition there is a significant lack of information and studies on the potential synergisms between the infectious agents mentioned above and their interactions with environmental parameters and natural phenomena such as phytoplankton blooms and zooplankton swarms (Table 2.2).

Table 2.1: Selected non-infectious gill disease conditions with research priorities (from Rodger et al. 2011a).

	Risk Factors	Potential Control	Priorities
Phytoplankton blooms	Geographical location Environmental conditions	Routine monitoring Oxygenation/aeration Protective pen enclosures Submersion of pens	Benefit or otherwise of aeration Is gas supersaturation triggered in some blooms? Dietary modifications Use of flocculating agents
Zooplankton swarms	Geographical location Environmental conditions	Routine monitoring Oxygenation/aeration Protective pen enclosures Submersion of pens Use of bubble curtains Towing pens out of bloom	Development of predictive models Early warning systems Relationship with pathogens Significance of biofouling organisms Challenge trials with suspect organisms Improved mitigation of impact of swarms

Table 2.2: Selected infectious gill disease conditions with research priorities (from Mitchell & Rodger 2011).

	Risk Factors	Control	Priorities
PGI	Epitheliocystis Season (summer/autumn) SI post-smolts	Unknown	Epidemiology of PGI/epitheliocystis/viral diseases Isolation of Chlamydia and its potential role What triggers proliferative response in gills?
Tenacibaculosis	Primary insult to gill tissue Elevated water temperatures High salinities Stress/elevated ammonia	Stable temperature Reduce salinity Antibiotic treatment	Transmission route/reservoirs Agents causing initial gill damage Vaccine development
AGD	Elevated temperature/salinity	Improve pen environment Freshwater baths Fallowing	Vaccine development Oral therapy Interaction with other gill pathogens

2.2. Epidemiology

2.2.1. Introduction

In the marine stage farming of Atlantic salmon (*Salmo salar*) gill disorders have been a sporadic historical challenge in all regions where the species is cultivated (Munday et al. 1990, Bruno & Poppe 1996). However, in Ireland between the years 2003 to 2005 gill disorders emerged as the most serious cause of mortality in the marine environment (Rodger & Mitchell 2005). The mean mortalities in marine farms due to gill disorders during the three year period (2003 to 2005) were 12% with a range from 1% to 79%. All stages of fish from post-smolts to harvest sized fish were affected although younger, smaller life stage salmon were more severely affected in terms of mortality (Rodger 2007). The majority of the gill disorders occur in the summer or autumn months. A similar pattern has been reported in Norway where the multifactorial condition proliferative gill inflammation (PGI) affected as many as 150 farms in 2003 (Kvellestad et al. 2005). In addition to the direct mortalities, loss of fish growth and the additional costs of mortality removal and disposal give rise to serious economic challenges for

affected farms. As part of the GILPAT project, a survey was undertaken in 2009 and 2010 to investigate the risk factors associated with gill disease in finfish farms in Ireland, and to assist in the identification of possible means to reduce the impact of this condition.

2.2.2. Materials & methods

Survey Population

The population surveyed in this study was commercially reared finfish reared in sites along the west coast of Ireland during 2008. A total of seventeen populations of finfish were studied of which thirteen were Atlantic salmon, three were rainbow trout (*Oncorhynchus mykiss*), and one was Atlantic cod (*Gadus morhua*). One Atlantic salmon site was located in the north west region, nine were in the mid west, and three in the south west. One site of rainbow trout was located in each of the three regions and the cod site was located in the mid west. Eleven of the thirteen sites had mortalities as a result of gill disorder, ten salmon sites and one rainbow trout. All sites were stocked with young fish either in the autumn/winter of 2007 (S0) or spring of 2008 (S1).

Data Collection

Data was collected using a detailed questionnaire for each site. The questionnaire consisted of open ended and closed questions covering a number of areas of management. Information on the stock was collected including fish number, fish weight at time of gill disorder, stocking density, strain of fish, and smolt type. Weekly stock sheets detailing numbers, biomass, average weight, mortality, strain, origin, and feed for each pen were also obtained. Information on the management of the sites included whether fallowing was employed, whether the fish were moved during their life cycle in the sea, and what method of net cleaning was used. Information on gill disorder and other diseases was also collected including the time of the episode, percentage mortality to the disorder, what other diseases affected the stock, and the percentage mortality caused by them. Information on any vaccines used, lice levels, and loss of growth as a result of the gill disorder were also collected. To help with the interpretation of the questionnaires and to insure data retrieved was as accurate as possible, there was extensive liaising between the consulting veterinarian and the farmers.

Diagnosis of Gill Disorder

Each farm was sampled by Vet-Aqua International several times throughout 2008 and a detailed picture of the status of the stocks was therefore available. At each sampling the fish were clinically examined and samples taken for histopathology. In sites which were positive for

gill disorder, histopathology revealed fusion, hyperplasia, and necrosis of the gill lamellae at the start of the gill disorder episode which subsequently gave rise to high mortalities in the stocks.

Data Analysis

The data were summarised, entered, and sorted into different categories using Microsoft Excel. As the majority of the populations were Atlantic salmon, analysis was carried out on the variable factors associated with these sites. The following categories of data were compiled for the thirteen Atlantic salmon sites:

Table 2.3: Variables examined for association for the Atlantic salmon populations in the survey.

Variable Factor	
1	Regional Location
2	Salmon Smolt Type (S0 or S1)
3	Strain of Salmon
4	Vaccine Type
5	Size at Input
6	Length of Fallow Period
7	Movement of Salmon to Another Site
8	Input Number of Salmon per Site
9	Occurrence of Epitheliocystis
10	Occurrence of Pancreas Disease
11	Net Washing Method
12	Distance to Nearest farm
13	Stocking Density
14	Feed Type

2.2.3. Results

The study population comprised a total of 5,768,934 fish in seventeen separate sites. Of these 5,308,934 were Atlantic salmon in thirteen sites, 420,000 rainbow trout in three sites, and 40,000 cod in one site.

Table 2.4: Summary data of the numbers and regional distribution of the seventeen study populations.

Species	North West	Mid West	South West	Total
Atlantic salmon	821,920	3,379,724	1,107,920	5,308,934
Rainbow trout	100,000	200,000	120,000	420,000
Atlantic cod	-	40,000	-	40,000
Total	921,920	3,619,724	1,227,920	5,768,934

The overall mortality to gill disorder for the seventeen sites combined was 13.0%. Ten of the thirteen Atlantic salmon populations were affected by gill disorder and one of the three rainbow trout sites. In the affected Atlantic salmon populations mortality ranged from 8% to 54%. Affected fish presented with similar pathologies at all sites: multifocal hyperplasia; fusion; and necrosis of the gills. The progression of the condition was broadly similar on all affected farms, characterised by a rapid rise in mortalities shortly after the initial occurrence. Table 2.5 presents summary data for the percentage mortality, relative to the input number, for the three regions and three species.

Table 2.5: Summary data showing percentage mortality to gill disorder (as a % of input number) for each of the species in the different regions.

Species	North West	Mid West	South West	Total
Atlantic salmon	0%	17.5%	13.8%	14%
Rainbow trout	0%	0%	5%	1.4%
Atlantic cod	-	0%	-	0%
Total	0%	16.3%	13%	13%

2.2.4. Results by variable (Atlantic Salmon)

Regional Location

There were 821,920 salmon in one site in the north west region, 3,379,724 salmon in nine sites in the mid west, and 1,107,920 salmon in three sites in the south west. The mean percentage mortality to gill disorder of the population in the north west was zero, the mid west was 20.7% (SD±14.5), and the south west was 18.0% (SD±31.2). There was no significant difference in gill disorder mortalities between the mid west and south west regions (P=0.90).

Salmon Smolt Type

A total of 2,561,267 S0 Atlantic salmon smolts were put to sea in eight sites between October and December 2007. 2,747,667 S1 smolts were put to sea in five sites between February and April 2008. The mean percentage mortality to gill disorder of the S0 sites was 17.6% (SD±17.7) and the S1 population was 19.8% (SD±21.2). There was no significant difference in gill disorder mortalities between the S1 and S0 populations ($P=0.85$). Looking only at sites affected by gill disorder, the mean percentage mortality of the S0 sites was 23.5% (SD±16.5) and the S1 population was 24.7% (SD±20.8). There was no significant difference in gill disorder mortalities for affected sites between the S1 and S0 populations ($P=0.92$).

Strain of salmon

There were three strains of Atlantic salmon in the study population: 3,501,321 fish of strain X; 1,074,512 fish of strain Y; and 733,101 of strain Z. Strain X and strain Y occurred on six sites and strain Z on four sites. Strain Y and Z were both present in two sites and X and Z were both present in one site but they were all in separate, identifiable pens. The mean percentage mortality to gill disorder of the strain X populations was 7.7% (SD±10.9), strain Y was 16.2% (SD±14.3), and strain Z was 47.2% (SD±16.1).

Table 2.6: Percentage mortality of Atlantic salmon to gill disorder for each site and smolt strain.

X	0%	8%	28%	10%	0%	0%
Y	10%	22%	8%	16%	41%	0%
Z	26%	45%	64%	54%	-	-

There was no significant difference in gill disorder mortalities between the strain X and strain Y populations ($P=0.27$). There was a significant difference in gill disorder mortalities between the strain X and strain Z populations ($P=0.007$). There was a significant difference in gill disorder mortalities between the strain Y and strain Z populations ($P=0.02$). Mortality to gill disorder was substantially higher in the population of strain Z salmon.

Looking only at sites affected by gill disorder the mean percentage mortality of the strain X populations was 15.3% (SD±11.0), strain Y was 19.4% (SD±13.2), and strain Z was 47.2% (SD±16.1). There was no significant difference in gill disorder mortalities between the strain X and strain Y populations ($P=0.66$). There was a significant difference in gill disorder mortalities between the strain X and strain Z populations ($P=0.02$), and also between the strain Y and strain Z populations ($P=0.03$; Figure 2.1).

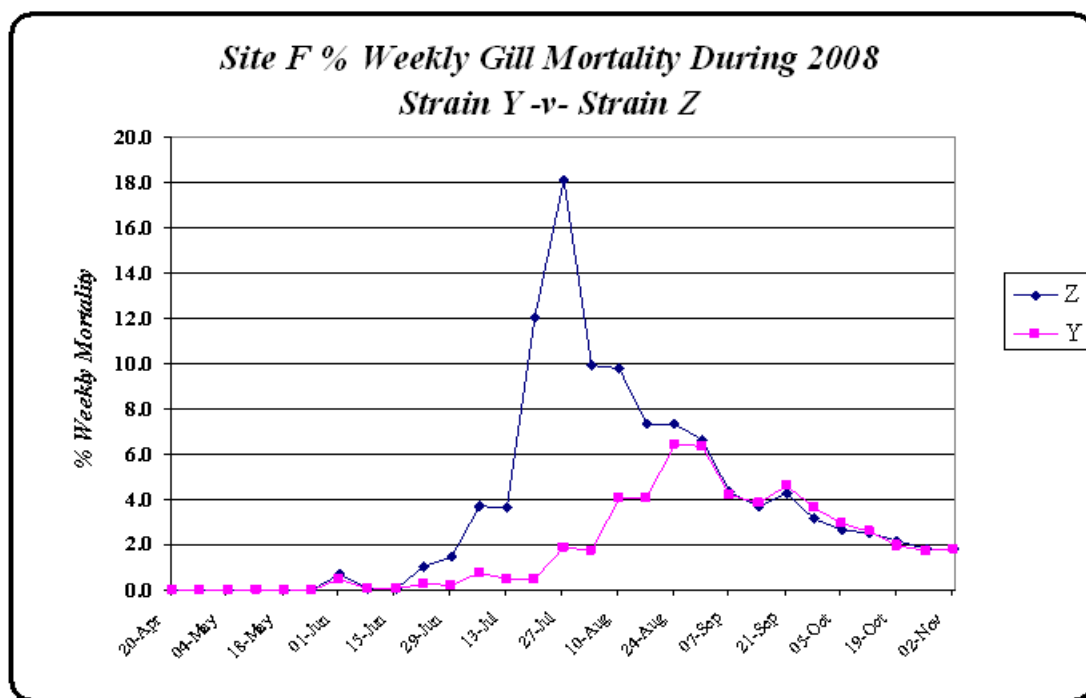


Figure 2.1: Percentage weekly mortality of Atlantic salmon due to gill disorder at site F for strain Y and strain Z.

Vaccination Type

There were 2,744,938 Atlantic salmon vaccinated with type X vaccine, 1,774,908 salmon with type Y vaccine, and 789,088 unvaccinated fish in the study population. Seven sites had salmon vaccinated with type X, three sites with type Y, and three sites with unvaccinated fish. The mean percentage mortality to gill disorder of the type X vaccinated sites was 18.6% (SD±18.5), type Y was 12.0% (SD±14.2), and unvaccinated was 24.7% (SD±25.4). There was no significant difference in gill disorder mortalities between the type X vaccinated and type Y vaccinated populations (P=0.57). There was no significant difference in gill disorder mortalities between the type X vaccinated and the unvaccinated populations (P=0.73). There was no significant difference in gill disorder mortalities between the type Y vaccinated and the unvaccinated populations (P=0.51).

Looking only at sites affected by gill disorder the mean percentage mortality of the type X vaccinated sites was 26.0% (SD±16.5), type Y was 18.0% (SD±14.1), and unvaccinated was 24.7% (SD±25.4). There was no significant difference in gill disorder mortalities between the type X vaccinated and type Y vaccinated populations (P=0.58). There was no significant difference in gill disorder mortalities between the type X vaccinated and the unvaccinated

populations ($P=0.94$). There was no significant difference in gill disorder mortalities between the type Y vaccinated and the unvaccinated populations ($P=0.73$).

Size at Input

There were 2,383,200 salmon put to sea at an average weight of $<76\text{g}$ and 2,925,734 fish put to sea at an average weight of $>76\text{g}$ in the study population. There were seven sites with an input average of $<76\text{g}$ fish and six sites with an input average of $>76\text{g}$ in the study population. The mean percentage mortality to gill disorder of the $<76\text{g}$ sites was 10.0% ($SD\pm 11.3$) and $>76\text{g}$ was 28.3% ($SD\pm 20.7$). There was no significant difference in gill disorder mortalities between the $<76\text{g}$ and $>76\text{g}$ populations ($P=0.09$). Looking only at sites affected by gill disorder the mean percentage mortality of the $<76\text{g}$ sites was 17.5% ($SD\pm 9.0$) and $>76\text{g}$ was 28.3% ($SD\pm 20.7$). There was no significant difference in gill disorder mortalities between the $<76\text{g}$ and $>76\text{g}$ populations ($P=0.29$).

Length of Fallow Period

A total of 2,031,310 salmon smolts were put to sea in five sites which had a fallow period of >1 year and 3,277,624 were put to sea in eight sites which had a fallow period of <1 year. The mean percentage mortality to gill disorder of the >1 year sites was 15.0% ($SD\pm 23.3$) and the <1 year population was 20.6% ($SD\pm 15.8$). There was no significant difference in gill disorder mortalities between the >1 year and <1 year populations ($P=0.65$). Looking only at sites affected by gill disorder the mean percentage mortality of the >1 year sites was 37.5% ($SD\pm 21.9$) and the <1 year population was 20.6% ($SD\pm 15.8$). There was no significant difference in gill disorder mortalities between the >1 year and the <1 year populations ($P=0.49$).

Movement of Salmon to Another Site

Within the study population, eight sites carried out transfers of fish (3,179,307 fish) and five sites (2,129,627 fish) did not. The mean percentage mortality to gill disorder of the transferred pens was 22.0% ($SD\pm 14.9$) and in the pens of fish where there was no transfer it was 12.8% ($SD\pm 23.4$). There was no significant difference in gill disorder mortalities between the moved and static populations ($P=0.46$). Looking only at sites affected by gill disorder the mean percentage mortality of the transferred pens was 22.0% ($SD\pm 14.9$) and the pens of fish not transferred was 32.0% ($SD\pm 31.1$). There was no significant difference in gill disorder mortalities between the moved and static populations ($P=0.73$).

Input Number of Salmon per Site

There were 4,064,086 salmon put to sea in sites with an input number >300,000 and 1,244,848 salmon put to sea in sites with an input number <300,000 in the study population. Seven sites had an input >300,000 and six sites had an input <300,000 in the study population. The mean percentage mortality to gill disorder of the sites with input >300,000 was 13.3% (SD±11.7) and <300,000 was 24.5% (SD±23.5). There was no significant difference in gill disorder mortalities between the sites with inputs of >300,000 and <300,000 (P=0.32). Looking only at sites affected by gill disorder the mean percentage mortality of the sites with input >300,000 was 18.6% (SD±9.1) and <300,000 was 29.4% (SD±22.6). There was no significant difference in gill disorder mortalities between the sites with inputs of >300,000 and <300,000 (P=0.37).

Occurrence of Epitheliocystis

There were 2,916,036 salmon in sites where epitheliocystis was found to be present and 2,392,898 salmon in sites where it was not found in the study population. There were seven sites that were positive and six sites which were negative in the study population. The mean percentage mortality to gill disorder of the positive pens was 28.3% (SD±18.9) and the negative pens was 7.0% (SD±8.8; Table 2.7). There was a significant difference in gill disorder mortalities between the sites positive for epitheliocystis and those that were negative (P=0.026). Mortality to gill disorder was substantially higher in the pens of fish which were positive for epitheliocystis. Looking only at sites affected by gill disorder the mean percentage mortality of the positive sites was 28.3% (SD±18.9) and the negative pens was 14.0% (SD±6.9). There was no significant difference in gill disorder mortalities between the pens positive for epitheliocystis and those that were negative (P=0.12).

Table 2.7: Percentage mortality of Atlantic salmon to gill disorder for sites positive or negative for epitheliocystis.

Positive	8%	28%	26%	8%	21%	53%	54%
Negative	0%	10%	10%	22%	0%	0%	-

Occurrence of Pancreas Disease

There were 4,060,378 salmon that were in sites where PD was found to be present and 1,248,556 salmon in sites where it was not found in the study population. There were nine sites that were positive and four sites that were negative in the study population. The mean percentage mortality to gill disorder of the positive sites was 14.8% (SD±9.7) and the negative sites was 26.7% (SD±30.9; Table 2.8). There was no significant difference in gill disorder mortalities between the sites positive for PD and those that were negative (P=0.50). Looking only at sites affected by gill disorder the mean percentage mortality of the positive sites was

16.6% (SD±8.5) and the negative sites was 53.5% (SD±0.7). There was a significant difference in gill disorder mortalities between the sites positive for PD and those that were negative (P=0.000006). Mortality to gill disorder was substantially higher in the salmon sites which were negative for PD.

Table 2.8: Percentage mortality of Atlantic salmon to gill disorder for sites positive or negative for pancreas disease.

Positive	0%	8%	28%	10%	10%	26%	22%	8%	21%
Negative	53%	54%	0%	0%	-	-	-	-	-

Net Washing Method

There were 1,923,018 salmon that were in sites that used the Idema net washing method and 3,385,916 on sites where the traditional method of changing nets was used in the study population. Six sites carried out net washing using the Idema and seven sites did not. The mean percentage mortality to gill disorder of the Idema cleaned sites was 21.7% (SD±18.2) and the non Idema pens was 15.3% (SD±19.0). There was no significant difference in gill disorder mortalities between the Idema cleaned sites and where it was not used (P=0.55). Looking only at sites affected by gill disorder the mean percentage mortality of the Idema cleaned sites was 26.0% (SD±16.5) and the non Idema pens was 21.4% (SD±19.5). There was no significant difference in gill disorder mortalities between the Idema cleaned sites and where it was not used (P=0.70).

Distance to Nearest Farm

There were 3,038,041 salmon that were on sites that were <5 miles from the nearest farm and 2,270,893 salmon that were on sites ≥5 miles from the nearest farm in the study population. Seven sites were <5 miles and six were ≥5 miles from the nearest farm. The mean percentage mortality to gill disorder of the sites <5 miles was 17.6% (SD±8.7) and the pens ≥5 miles was 19.5% (SD±26.2). There was no significant difference in gill disorder mortalities between the sites that were <5 miles to the nearest farm and the sites which were ≥5 miles from the nearest farm (P=0.87). Looking only at sites affected by gill disorder the mean percentage mortality of the sites <5 miles was 17.6% (SD±8.7) and the sites ≥5 miles was 39.0% (SD±25.1). There was no significant difference in gill disorder mortalities between the sites that were <5 miles and those which were ≥5 miles from the nearest farm (P=0.29).

Stocking Density

There were 1,950,035 salmon that were at a mean stocking density of <5 fish/m³ at the time of the outbreak of gill disorder and 1,715,923 salmon that were at a mean stocking density of >5

fish/m³ in the study population. There were seven sites where the stocking density at the time of the outbreak was <5 fish/m³ three sites at >5 fish/m³ in the study population. The mean percentage mortality to gill disorder of the sites with <5 fish/m³ was 21.7% (SD±15.7) and the sites with >5 fish/m³ was 29.3% (SD±23.1). There was no significant difference in gill disorder mortalities between the sites at <5 or >5 fish/m³ at the time of the outbreak (P=0.64).

There were 2,708,376 salmon that were at a mean stocking density of ≤2 kg/m³ at the time of the outbreak of gill disorder and 957,582 fish that were at a mean stocking density of >2 kg/m³ in the study population. Six sites had a stocking density of ≤2 kg/m³ and four sites had a stocking density of >2 kg/m³ in the study population. The mean percentage mortality to gill disorder of the sites with ≤2 kg/m³ was 24.3% (SD±16.2) and the sites with >2 kg/m³ was 23.5% (SD±21.2). There was no significant difference in gill disorder mortalities between the sites that were stocked at ≤2 and >2 kg/m³ (P=0.95).

Feed Type

There were three types of feed given to the Atlantic salmon in the study population: 3,509,556 salmon were given type X; 1,296,524 were given type Y; and 502,854 were given type Z. Eleven sites fed type X, three sites fed type Y, and two sites fed type Z. Three sites fed type X and type Z on the same site but to different, identifiable pens. The mean percentage mortality to gill disorder of salmon fed type X was 20.0% (SD±19.6), type Y was 2.7% (SD±4.6) and type Z was 10.0% (SD±0).

Table 2.9: Percentage mortality of Atlantic salmon to gill disorder for populations fed three different feed types.

X	0%	8%	28%	26%	22%	8%	21%	53%	54%	0%	0%
Y	0%	8%	0%	-	-	-	-	-	-	-	-
Z	10%	10%	-	-	-	-	-	-	-	-	-

There was a significant difference in gill disorder mortalities between those populations fed type X and those fed type Y feed (P=0.02). There was no significant difference in gill disorder mortalities between those populations fed type X and those fed type Z feed (P=0.12). There was no significant difference in gill disorder mortalities between those populations fed type Y and those fed type Z feed (P=0.11). There was a significant difference in gill disorder mortalities between those populations fed type X and those fed other types of feed (P=0.04). Mortality to gill disorder was substantially higher in the sites where the fish were fed type X feed.

Looking only at sites affected by gill disorder the mean percentage mortality of the salmon fed type X was 27.5% (SD±17.7) and other feed types combined was 9.3% (SD±1.2). There was a significant difference in gill disorder mortalities between those populations fed type X and those fed other types of feed (P=0.02). Mortality to gill disorder was substantially higher in the sites where the fish which were fed type X feed.

2.2.5. Conclusions

Gill disease was a significant cause of mortality, caused fish welfare challenges, and gave rise to loss of growth in the majority of the marine salmon farms during the study period. Although the study showed that there were no statistically significant differences in gill disorder losses when net washing (using high pressure underwater cleaners) was compared to net changing, it was felt by the authors that the net washing results were compounded by the debris produced. A washing method which also removes the debris should result in lower mortalities from gill disorders (see section 4.3.1). There was also a strong genetic susceptibility to gill disease, with one strain of salmon in particular showing significantly higher mortality rates when compared to the other two strains used during the study period. Results also indicated that sites positive for the presence of epitheliocystis had significantly higher mortalities due to gill disease when compared to epitheliocystis negative sites, suggesting that this disease may exacerbate the effects of gill pathologies. Results also indicated that sites which were negative for pancreas disease had significantly higher gill pathology mortalities, although this finding may also be due to the small sample size (see Table 2.8).

Further research and investigation into gill disease should include:

- Net cleaning/changing methods which reduce the risk of gill damage (including further investigations into the role hydroids and other fouling organisms may have on gill tissues)
- Genetic selection for resistance to gill disorders/pathologies
- The role of epitheliocystis in gill health and the means to reduce its impact.

2.3. Descriptive Epidemiology from Four Sites

A retrospective study of four marine salmon farms was undertaken to give descriptive epidemiological findings of gill disease. The clinical and pathological findings were described and

for a full report of the results see Rodger et al. (2011b). The study showed that in some of these sites there was an initial insult to the gills, probably due to small harmful gelatinous zooplankton, which coincided with tenacibaculosis and then eventual parasitism (Figs 2.2 & 2.3). The epidemiological aspects of the gill disease in the farms were that the condition was associated with the summer months in all sites, there was apparent spread from pen to pen in at least two of the four sites, and there was a very strong genetic or strain susceptibility to the condition. The significance of the findings were that many of the components of this gill disease in marine-stage salmon bear similarities to respiratory disease, specifically pneumonia, in intensively reared land animals, where the cause may be one or multiple pathogens, or physical or chemical agents. It is the interaction of these agents with the host animal and environmental or management conditions which determine the clinical outcome.



Figure 2.2. Atlantic salmon from a marine farm, with severe gill necrosis evident as white patches with eroded filaments and exposure of gill filament cartilage.

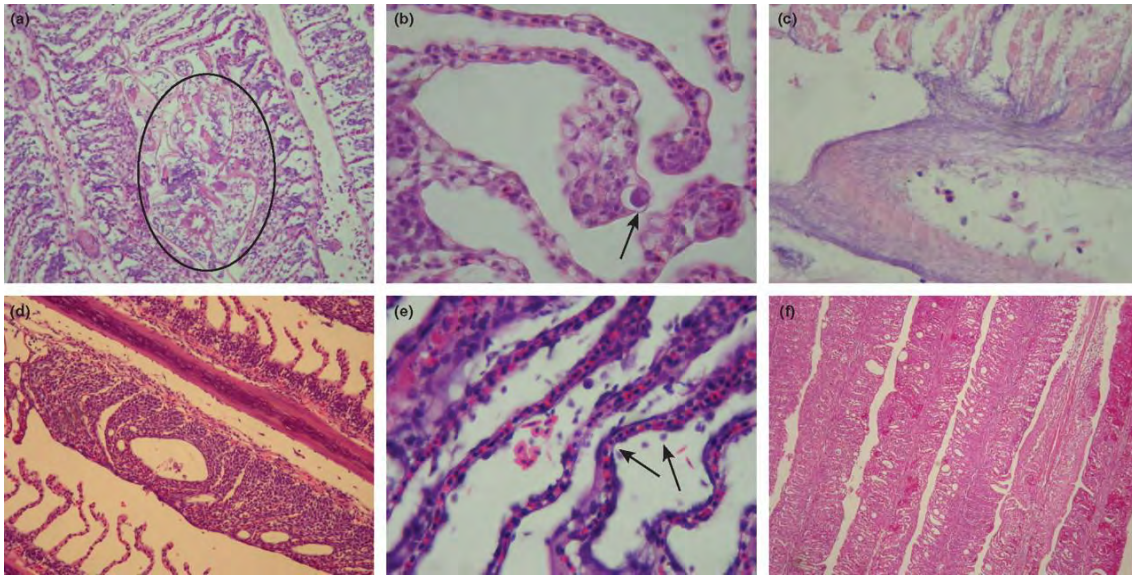


Figure 2.3. Histopathology of Atlantic salmon gills sampled from four marine farms in Ireland during 2008. (a) Epithelial necrosis and lifting with haemorrhage associated with an unidentified small zooplankton (jellyfish) between filaments (circled). Haematoxylin and eosin (H&E). × 400. (b) Epitheliocystis colony (arrow). H&E. × 400. (c) Filamentous *Tenacibaculum* sp. mats on gill lamellae. H&E. × 400. (d) Focal hyperplasia and fusion of gill lamellae with lacunae formation. H&E. × 400. (e) Marine costia (*Ichthyobodo* sp.) infestation (arrows) with epithelial necrosis and haemorrhage. H&E. × 400. (f) Severe diffuse hyperplasia and fusion of gill lamellae with haemorrhage and lacunae formation. H&E. × 200

3. PILOT MONITORING PROGRAMME

3.1. Zooplankton Monitoring

3.1.1. Background

The negative interactions between gelatinous zooplankton (primarily cnidarians, although sometimes ctenophores) and fish in aquaculture appears to be an increasing problem through the intensification of aquaculture operations in many coastal areas worldwide. Over the last three decades there have been numerous fish kill incidents or health problems in marine-farmed fish that have been associated with gelatinous zooplankton (Purcell et al, 2007). The majority of problems have occurred with marine-farmed salmonids in northwest Europe; nevertheless aquaculture operations in other regions such as Asia, North America, and Australia have also been affected (Yasuda 1988; Willcox et al. 2008; Rodger et al. 2011b). Although large-scale gelatinous zooplankton blooms can sometimes be the obvious cause (in the case of conspicuous scyphomedusae), problems may also be associated with small or near-invisible species and a lack of sampling and investigation around these events can obscure the cause (Cronin et al. 2004). Furthermore, whilst mass mortality events often get reported in the literature and the media, associated lower level health problems such as gill disorders are rarely reported despite the scale of the problems in some regions (Rodger 2007). Gelatinous agents that have been previously known or implicated in fish kill events and gill disorders include hydromedusae, siphonophores, scyphozoans, ctenophores, and hydroids. The incidents involving each group are outlined below.

Hydromedusae

A bloom of *Solmaris corona* (small oceanic narcomedusa) was implicated as the causative agent in the mortality of ~900,000 salmon at Scottish aquaculture sites in August/September 1997 (Båmstedt et al. 1998); and again in 2002 when there were around 650,000 mortalities in two days (Rodger et al. 2011a). The neritic leptomedusa *Phialella quadrata* has also been implicated in fish kill events and as a vector of bacterial gill disease. In the 1980's 1,500 salmon died at farms on the Shetland Isles, Scotland. Histopathological examination revealed severe epithelial stripping and necrosis of the lamellae, amongst other damage, and some of the fish were reported to have up to 40 *P. quadrata* in their stomach contents (Bruno & Ellis 1985). Recent research has also identified *P. quadrata* as a potential vector for the bacterial pathogen *Tenacibaculum maritimum*. These filamentous bacteria were found on both the manubrium of *P. quadrata* and on the gills of salmon from a Scottish salmon farm (Ferguson et al. 2010). However, although there was evidence of bacteria on both the jellyfish and the fish gills, based

on the data available, this study does not present enough information to confirm jellyfish as vectors of bacterial disease.

Siphonophores

The mass occurrence of the physonect, *Apoemia uvaria* (up to several metres in length but fragile) on the west coast of Norway in the winter of 1997/1998 caused severe lesions and the mortality of farmed fish. Fish were stung on the skin, eyes, and gills, with those blinded having the highest mortality (Båmstedt et al. 1998). The smaller, more neritic calyphoran *Muggiaea atlantica* has also caused problems in Norway and Ireland. In August 2002, a bloom of *M. atlantica* with maximum densities of 13,000 individuals m⁻³ caused the death of >100,000 salmon in Norway, again with stings to the skin and gills (Fosså et al. 2003). Furthermore, the mortality of over 1,000,000 farmed salmon and trout on the west coast of Ireland in September 2003 is also thought to be attributable to *M. atlantica*, although a lack of investigation at the time of the event (before and after) cannot confirm this suspicion entirely (Cronin et al. 2004).

Scyphomedusae

The lion's mane jellyfish, *Cyanea capillata*, has been implicated in causing thousands of mortalities at fish farms in Scotland on more than one occasion (Bruno & Ellis 1985; Purcell et al. 2007), as well as problematic irregular invasions in Norway (Båmstedt et al. 1998). Although medusae of the common jellyfish (*Aurelia* spp.) are often perceived as quite harmless, there have been occasional reports of problematic interactions between these jellyfish and fish in aquaculture. Ephyrae and small medusae of *A. aurita* were thought to have caused huge losses in Norway and Scotland in the mid-1990's when small individuals were inhaled by farmed fish, stinging the gills and causing suffocation (Båmstedt et al. 1998). *A. aurita* were also the suspected agents of mass mortalities of farmed salmon in Tasmania and fish in Asia (Yasuda 1988; Willcox et al. 2008). Nevertheless, there is limited information on the actual effects of these cosmopolitan jellyfish and their potential impact on the finfish aquaculture industry as their blooms are often overlooked and causal links to fish mortalities may go unnoticed. Blooms of the mauve stinger, *Pelagia noctiluca*, have been noted to irritate the gills of salmon and trout in France (Merceron et al. 1995) and caused mass mortality of 250,000 harvest-sized Atlantic salmon fish in Northern Ireland (Doyle et al. 2008). This species has also caused recurrent problems in Scotland and Asia (Purcell et al. 2007; Hay & Murray 2008). These large-scale events caused by conspicuous species such as *P. noctiluca* have been a cause for much concern, and are responsible for highlighting the negative interactions between jellyfish and fish in aquaculture.

Ctenophores

There has only been a single reported incident of a ctenophore causing the death of farmed fish; a bloom of the lobate ctenophore *Bolinopsis infundibulum* was thought to have caused the death of farmed fish through direct suffocation (clogging of the gills) in Norway in 1986 (Båmstedt et al. 1998).

Hydroids

Although not strictly plankton, the hydroid stage of hydrozoans could also pose a potential impact on the health of finfish in aquaculture. These sessile animals also possess nematocysts and are known to rapidly and extensively foul aquaculture structures (pontoons and netting) (Carl et al. 2010). As yet it is not known whether hydroids have an impact on farmed fish health and if they play a role in gill disorders (Rodger et al. 2011a). Some hydroid species, such as *Ectopleura larynx* (syn. *Tubularia larynx*), have become the dominant fouling organisms of the fish farming industry in Norway causing problems due to a reduction in water flow and quality (Guenther et al. 2009; Carl et al. 2010). This species is also common and abundant in many European coastal waters (Browne 1897; Boero & Fresi 1986; Östman et al. 1995; Galea 2007) and could potentially pose health risks to farmed fish. This risk was briefly mentioned in an examination of clubbing and gill necrosis syndrome in Atlantic salmon in Tasmania (Clark et al. 1997). However, other than a passing statement, this area of jellyfish/aquaculture science has been neglected thus far and hence necessitates further investigation.

The aims of the Pilot Monitoring Programme were to improve the understanding of the aetiology and pathogenesis of jellyfish-inflicted gill disorders in marine-farmed salmon through the use of monitoring, methodological developments, and experimental challenge trials. Additionally, the collation of knowledge through monitoring was vital to provide feedback to the industry on the detection of potentially harmful species and management advice for the mitigation of gill disease in finfish aquaculture. This project addressed several gaps in the knowledge of the distribution, abundance, and seasonality of gelatinous zooplankton communities around Irish finfish farms. Furthermore, the interaction of gelatinous zooplankton with finfish in aquaculture and their role in the gill disorders of marine-farmed fish was investigated. Due to the multifactorial nature of gill disorders, a range of environmental and biological parameters were investigated over the course of the study.

3.1.2. Findings of the monitoring programme

The objectives of the zooplankton monitoring programme were to (1) identify the role of small hydrozoan jellyfish as agents of gill disorders, (2) investigate inter-site variations in jellyfish and gill disorders at salmon farms, and (3) investigate inter-annual variations in jellyfish populations in order to elucidate the peak risk period for gill disorders and the potential of long-term monitoring for gelatinous zooplankton. In order to fully investigate the causative agents of gill disorders, samples of phytoplankton and histological screening of fish tissues were necessary to assess the presence, or otherwise, of all potential causative agents of gill disorders and mortality. Two marine salmon farms located on the west coast of Ireland (Bantry Bay and Clifden Bay) were monitored for zooplankton and phytoplankton as well as temperature and fish health over the course of one year. Samples were taken fortnightly from March to October inclusive and then monthly thereafter. In the following year, zooplankton monitoring continued at the Bantry Bay site to investigate inter-annual variations in jellyfish abundance³.

From a total of 300 samples taken over the two years, 33 species/genera of gelatinous zooplankton representing six taxa were identified. All species/taxa were more frequently recorded and abundant in Bantry Bay compared to Clifden Bay (with the exception of *Oikopleura* spp. and *Obelia* spp.). Jellyfish were highly abundant in Bantry Bay, peaking from August to November, and three species previously implicated in mass mortality events of farmed salmonids were identified at this site (*M. atlantica*, *P. quadrata*, and *S. corona*) (see Fig. 3.1).

³ Full details on the methodology and findings can be found in Baxter *et al.* (2011a)

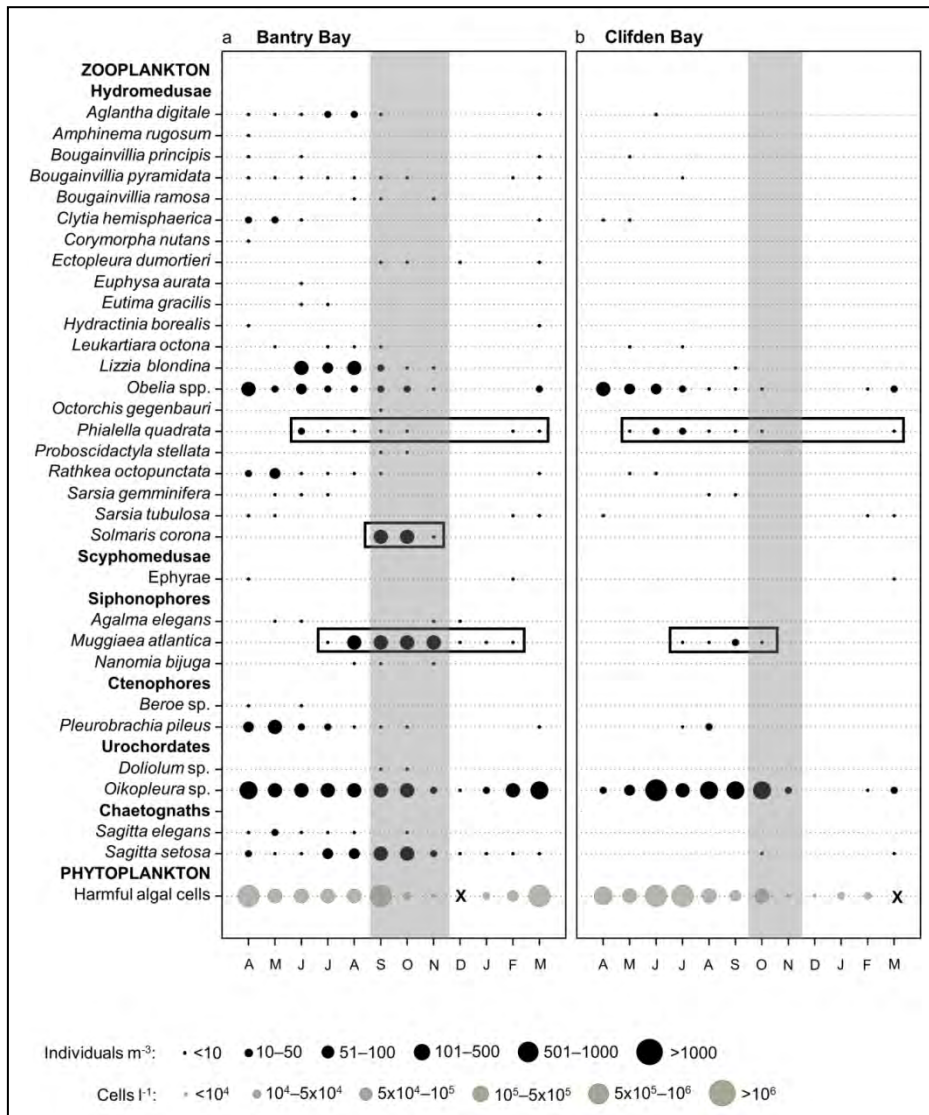


Figure 3.1: Seasonal abundance of gelatinous zooplankton in Bantry Bay and Clifden Bay (taken from Baxter et al. 2011a)

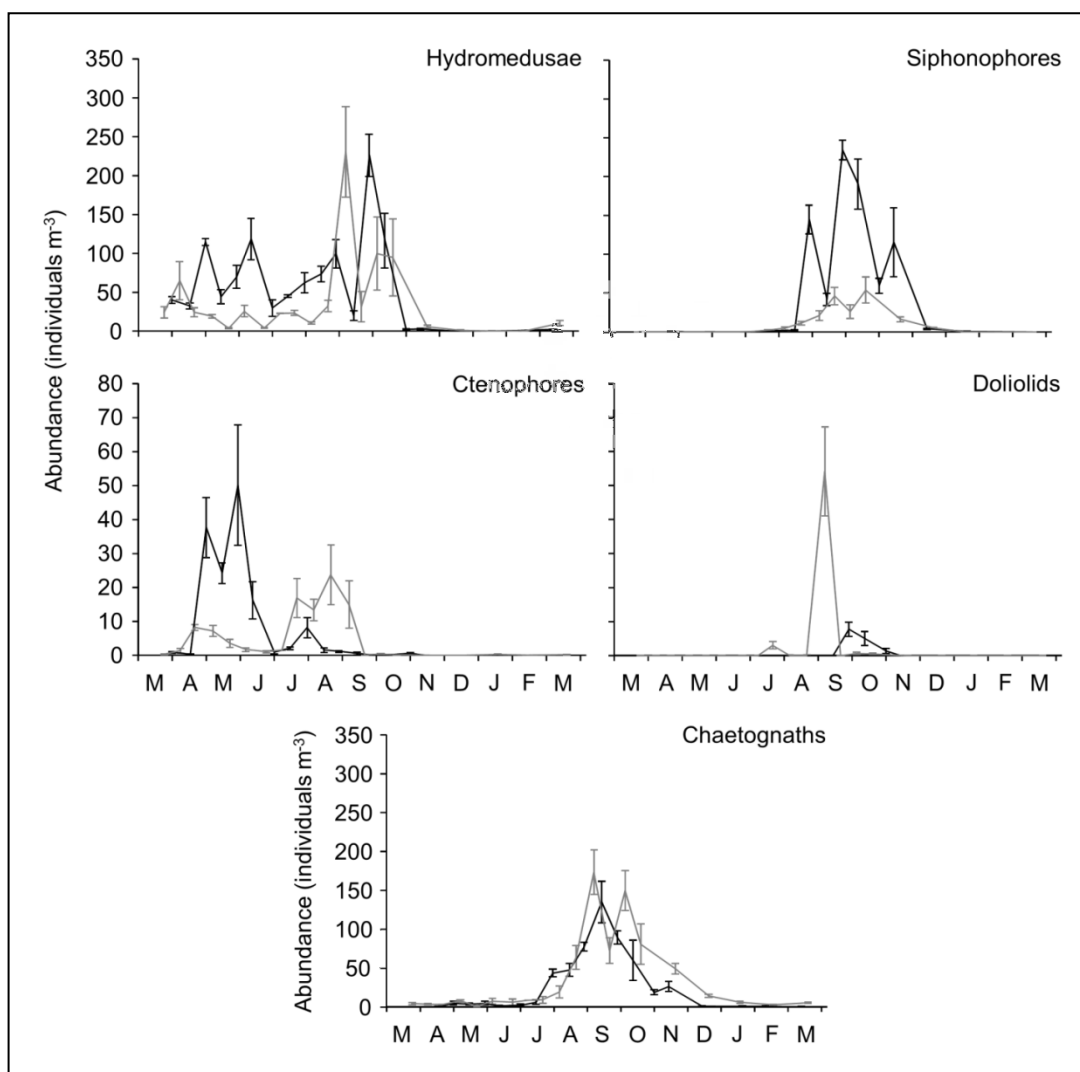


Figure 3.2: Seasonal abundance of the gelatinous taxa recorded around the Bantry Bay farm over two years. Data are means \pm S.E. for Year 1 (black line) and Year 2 (grey line).

The occurrence of *S. corona* was linked to an influx of oceanic water detected from the temperature depth profiles and the simultaneous presence of doliolids, indicators of oceanic water masses. Whilst the oceanic species *S. corona* was entirely absent at the Clifden Bay site, both *M. atlantica* and *P. quadrata* did occur; however, they were scarce when present. There was no significant difference ($p > 0.05$) in total gelatinous zooplankton or jellyfish abundance between samples taken inside and outside the cages at Bantry Bay and Clifden Bay. There was also no significant difference ($p > 0.05$) in zooplankton or jellyfish abundance between the samples taken around the cages at the Bantry and Clifden Bay salmon farms. All taxa (hydromedusae, siphonophores, ctenophores, doliolids, and chaetognaths) occurred over almost the same period though in different abundances in Bantry Bay Year 2 compared to Year 1 (Fig. 3.2). In particular, harmful hydromedusae and siphonophores (*S. corona* and *M. atlantica* respectively) were much less abundant in Year 2 than in Year 1 (Fig. 3.2). *M. atlantica* was still

the most abundant siphonophore in both years and there remained a significant difference with month ($p < 0.001$); with the species being more abundant in September and October compared to other months of the species' occurrence.

From April until the end of August in Year 1, the fish sampled from Bantry Bay had only minor pathological damage. From the end of August, some fish exhibited gill damage from moderate to severe, and the peak severity occurred in October and November. The gill damage included extensive epithelial hyperplasia, multi-focal lamellae fusion, numerous necrotic epithelial cells, focal inflammation, an increase in eosinophilic granular cells in the filaments, focal telangiectasis, haemorrhage, areas of focal sloughing, and loss of the epithelium (Fig. 3.3).

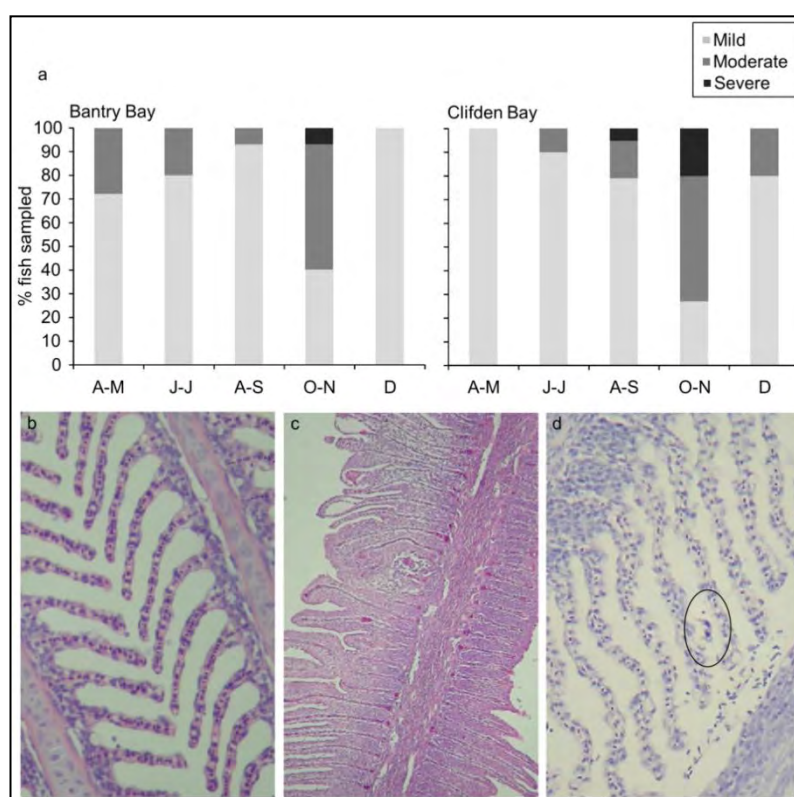


Figure 3.3: Ranked gill damage and histological sections from Bantry Bay and Clifden Bay salmon farms over during the first year of the monitoring programme. a) the seasonal severity of the gill damage at the two farms; b) example of healthy gills - Bantry Bay, May 2009; c) severe gill damage - Bantry Bay September 2009; d) gills with *Trichodina* sp. (circled) - Clifden Bay October 2009. H & E, 200 x.

The salmon in the Clifden Bay farm had only minor histopathological damage to the gills throughout most of the first year of monitoring. On 6 October 2009 the protozoan parasite *Trichodina* sp. was observed on the gills as well as the bacterial pathogen, *Tenacibaculum* sp. The presence of these pathogens was concurrent with an increase in epithelial hyperplasia, fusion, and necrosis. The peak in gill damage at Bantry Bay coincided with the peak in abundance of harmful jellyfish (September to November, abundance: ~ 450 individuals m^{-3} , *M. atlantica* and *S.*

corona). The gill damage observed would have had a significant clinical impact on fish health and its ability to survive. Importantly, a highly significant, positive correlation between the abundance of harmful jellyfish (*M. atlantica*, *P. quadrata* and *S. corona*) and the average daily fish mortality was identified, although pancreas disease also affected fish at this site (Baxter et al. 2011a). These results suggested that *M. atlantica* and *S. corona* were the causative agents of the gill disorders identified in Bantry Bay and may also have had an impact on the observed mortalities.

3.1.3. Conclusions from the monitoring programme

Zooplankton Monitoring:

- A higher density of jellyfish was found in the south west compared to the west coast
- Gill disease in the south west was correlated with abundance of *M. atlantica*, *P. quadrata*, and *S. corona*
- Gill disease was also linked to the occurrence of *Trichodina* sp. and *Tenacibaculum maritimum*
- Seasonal abundance of jellyfish can vary from year to year
- Further investigations into the role of hydroids in the pathogenesis of gill disease are required
- The role of jellyfish as potential vectors of bacteria should be investigated further.

3.2. Challenge Trials

3.2.1. Background

Common jellyfish (*Aurelia* spp.) are among the most cosmopolitan jellyfish species in the oceans, with populations increasing in many coastal areas. The interaction between jellyfish and fish in aquaculture remains a poorly studied area of science. Furthermore, the potential direct health problems posed to marine-farmed salmonids by the biofouling hydroids, and the potentially harmful effects of *in situ* net cleaning to remove fouling organisms, has received little consideration. A recent fish mortality event in Ireland, involving *A. aurita* spurred the investigation into the detrimental effects of the common jellyfish on salmon gill tissues (Mitchell et al. 2011).

For the challenge trials, Atlantic salmon smolts were exposed to macerated *A. aurita* or biofouling hydroids (attached to the nets and loose in the water) for 10-11 hours (before

removal) under experimental conditions. Gill tissues of control and experimental treatment groups were scored with a semi-quantitative gill scoring system according to Mitchell et al, (2012).

3.2.2. Findings from the common jellyfish challenge trial

The results revealed that *A. aurita* rapidly and extensively damaged the gills of salmon, with the pathogenesis of the disorder progressing even after the jellyfish were removed (Fig. 3.4). At 2 hours after exposure, significant multi-focal damage to gill tissues was apparent. The nature and extent of the damage increased up to 48 hours from the start of the challenge. Although the gills remained extensively damaged 3 weeks from the start of the challenge trial, shortening of the gill lamellae and organisation of the cells indicated an attempt to repair the damage suffered. These findings, reported by Baxter *et al.* (2011b), clearly demonstrate that *A. aurita* can cause severe gill problems in marine-farmed fish.

3.2.3. Findings from the hydroid challenge trial

On gross examination, fragments of the hydroids were observed in the gills of the salmon from the experimental treatment groups throughout the first hours of the challenge trials (Fig. 3.5). Focal areas of epithelial sloughing, necrosis, and haemorrhage were seen on the gills of the Atlantic salmon smolts exposed to *E. larynx*. A similar degree of damage was also seen in the control groups after several hours of the experiment. However, no gill damage was observed in the smolts sampled prior to the start of the experiment (Fig. 3.6). Therefore, it is likely that the results observed may be due to a contamination of the control groups through the recirculation system used to conduct the challenge trails.

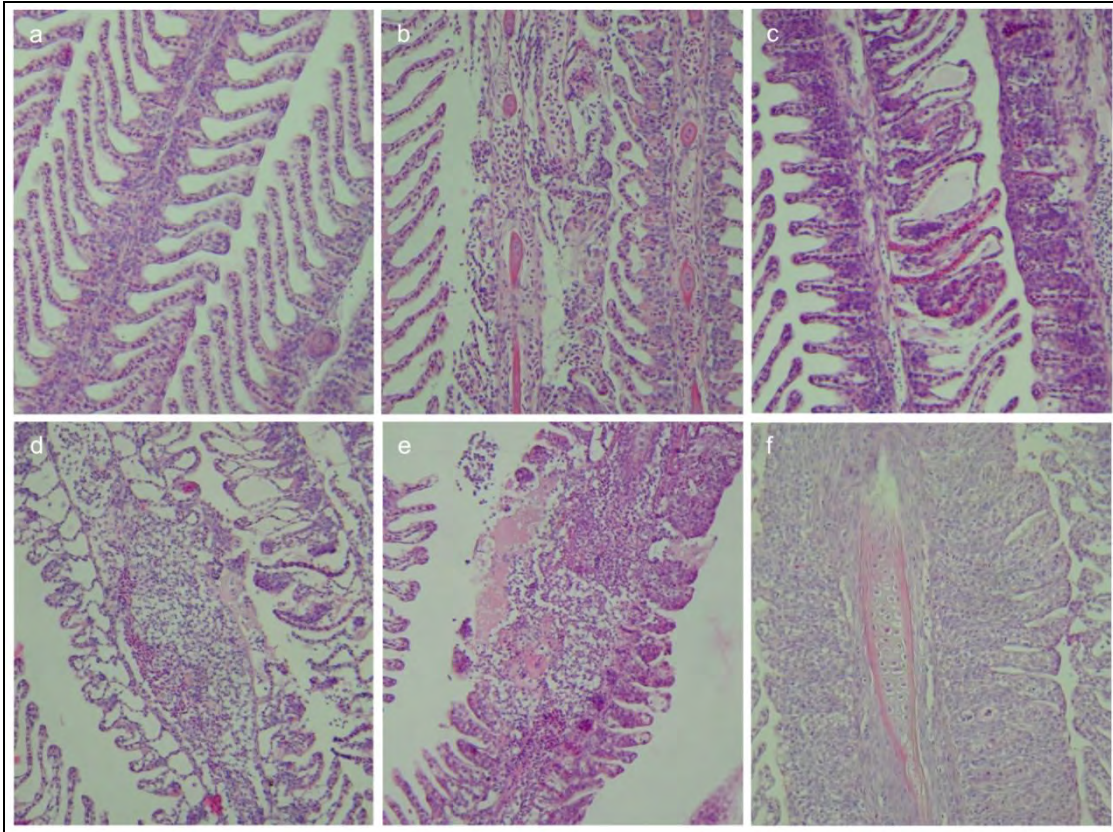


Figure 3.4: Photographic time series of gill damage in Atlantic salmon smolts exposed to *Aurelia aurita* under experimental challenge. Time from start of experiment – a) Healthy gills from control group, b-f) gills from experimental treatment groups. b) 2 hours; c) 6 hours; d) 24 hours; e) 48 hours; and f) 3 weeks. Haematoxylin and eosin at 200 x magnification.

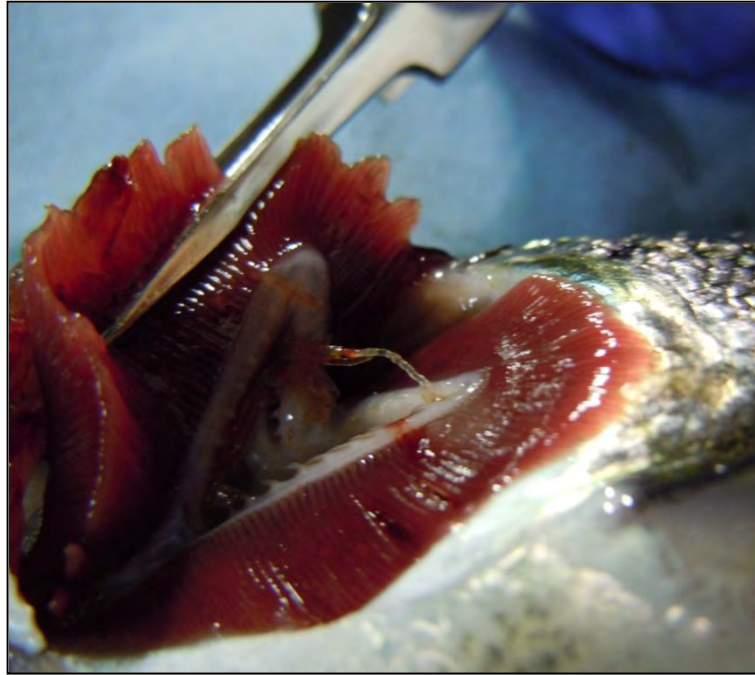


Figure 3.5: Hydroid fragment visible in the gills of an Atlantic salmon smolt during the challenge trials

These results are the first of any investigation into the potential role of biofouling hydroids in the gill disorders of marine-farmed fish, and whilst there were potential limitations to the study that complicated the interpretation of the results, the outcomes suggest that *E. larynx* can damage the gills of *S. salar*. Therefore, further work on this area is vital before the implementation of *in situ* net washing is deemed safe to finfish health.

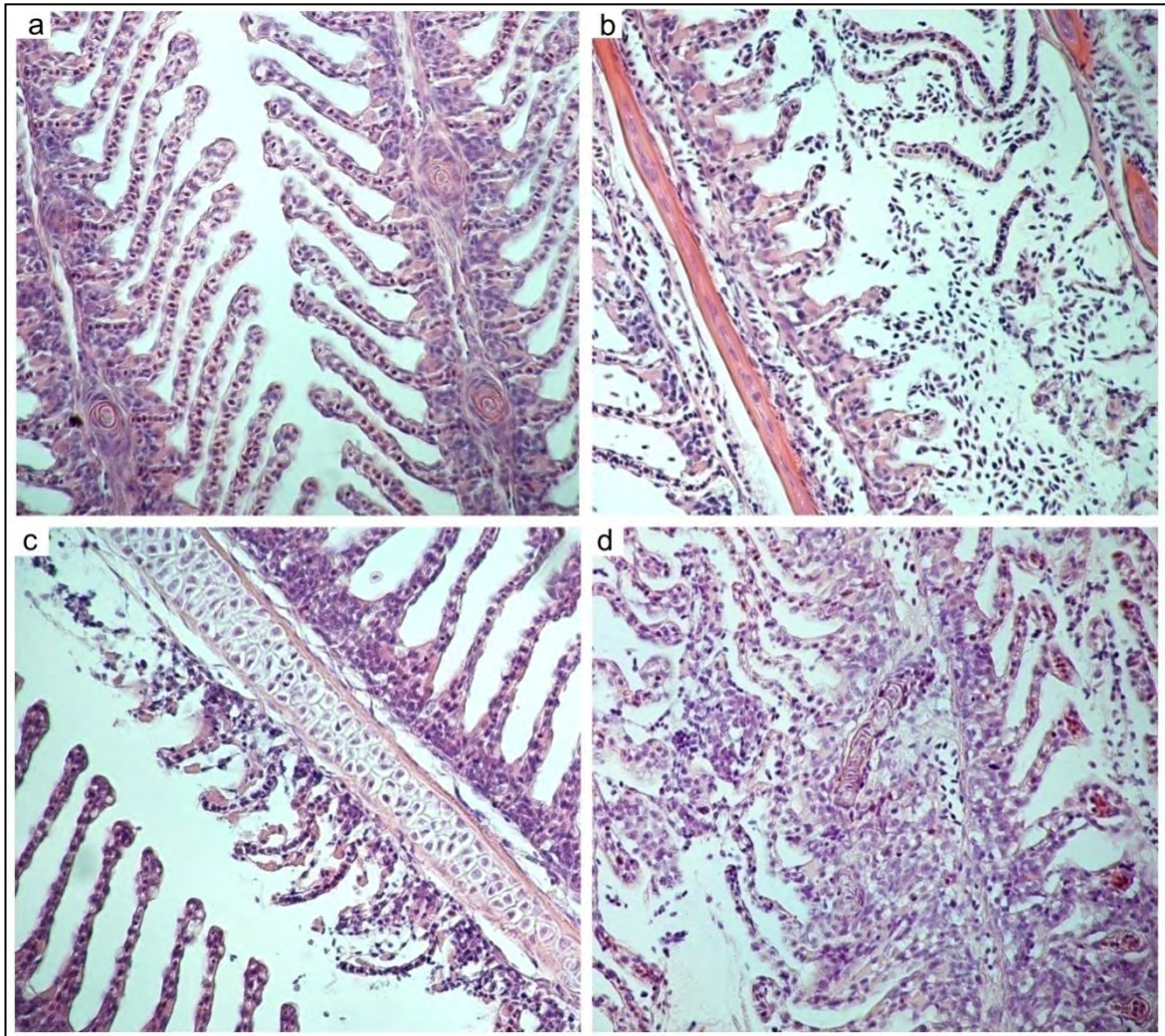


Figure 3.6: Histological sections from Atlantic salmon smolts under experimental challenge with the biofouling hydroid, *Ectopleura larynx*. a-d) Gills from the treatment and control groups with time in hours from the start of the experiment. a) 0 hours; b) control group at 8 hours; c) treatment group with biofouled net baskets at 48 hours; and d) treatment group with cleaned baskets and free of hydroids 48 hours. Haematoxylin and eosin (200x magnification).

3.2.4. Conclusions from the challenge trials

- Both the common jellyfish *Aurelia aurita* and the hydroid *Ectopleura larynx* were shown to cause significant gill damage upon experimental exposure
- The experimental hydroid challenge trial should be repeated to elucidate the effect of *E. larynx* on the gills
- It is likely that biofouling organisms (zooplankton) play a role in gill disease and this should be investigated fully.

3.3. Molecular Diagnostics

As viral, bacterial, and parasitic species are likely to be involved in gill pathologies, a range of molecular assays were developed:

1. Real time RT-PCR to detect a viral species associated with gill disease in Atlantic salmon, the Atlantic salmon paramyxovirus (ASPV)
2. Real time PCR to detect *Piscichlamydia salmonis*, an organism considered to be involved in some of the epitheliocystis cases in marine sites in Ireland
3. Quantitative duplex real time PCR to detect *Neoparamoeba* spp and *Neoparamoeba perurans*
4. Quantitative duplex real time PCR to detect *Tenacibaculum maritimum*
5. Real time RT-PCR to detect *Desmozoon lepeophtheirii* a microsporidian parasite associated with a range of diseases, including gill disease.

Standard operating procedures (SOP) describing the methods were produced (see Appendix II - VI).

3.3.1. Assay development

A molecular diagnostic method for ASPV was validated using published primers and probes targeting the ASPV phosphoprotein gene of ASPV. The ASPV real-time RT-PCR was optimized as a duplex assay that includes primers and probe specific for the Atlantic salmon elongation factor alpha I (ELF) housekeeping gene in the same reaction as internal positive control. The lack of an amplification signal when the ASPV assay was set up on RNA samples positive for infectious pancreatic necrosis virus (IPNV), infectious haematopoietic necrosis virus (IHNV), infectious salmon anaemia virus (ISA), viral haemorrhagic septicaemia virus (VHSV), SVCV, nodavirus and salmonid alphavirus (SAV), showed that the assay was specific for ASPV. No loss

in sensitivity was detected when the assay was run as a single or a duplex reaction, indicating the absence of competition between ASPV and ELF primers and probe. The assay was found to have a high degree of repeatability and reproducibility, to have a dynamic range extending over 5 log₁₀ dilutions, and to have a high efficiency (97%) and linearity (0.995). Due to the absence of clinical disease in the longitudinal sampling done to date within the project, the anticipated field samples could not be tested. Archived RNA samples extracted from gill tissues gathered during other longitudinal studies in Ireland that AFBI have been involved in were tested but no ASPV was detected.

A real time PCR was developed for the diagnosis of *Piscichlamydia salmonis*. The test was shown to be very sensitive, able to detect as little as 16.7 DNA molecules / µl, to have a high degree of repeatability and reproducibility, to have a dynamic range extending over 8 log dilutions, and to have a high efficiency (98%) and linearity (0.998). The validation of real time PCR methods for the detection and quantification of *Neoparamoeba perurans* and *Tenacibaculum maritimum* involved the design of primers and probes targeting the *N. perurans* 18S rRNA and the *T. maritimum* 16S rRNA genes. In both assays the amplification and the quantification of the elongation factor alpha 1 (ELF) housekeeping gene, in a duplex (for *N. perurans*) or single (*T. maritimum*) reaction was not only used as an internal positive control but also as normalizer in the quantification strategy adopted. Both assays were shown to be very sensitive, being able to detect as little as 13.4 DNA copies / µl (*N. perurans*) and 4.8 DNA copies / µl (*T. maritimum*). In addition, both reactions were found to have a high degree of repeatability and reproducibility, to have a linear dynamic range ($R^2 = 0.999$) extending over 5 (*N. perurans*) and 6 (*T. maritimum*) log₁₀ dilutions, and to have a high efficiency (104% for *N. perurans* and 100% for *T. maritimum*).

3.3.2. Testing field samples

The PCR methods for the detection of *N. perurans* and *T. maritimum* were applied to DNA samples extracted from 48 formalin-fixed, paraffin-embedded (FFPE) salmon gill tissues respectively selected on the basis of the absence or presence of gill pathology and amoebic gill disease-type histopathology. For each block a score from 0 to 3 was assigned with 0 as “none”, 1 as “low”, 2 as “moderate”, and 3 as “high” based on the degree of gill pathology present which took into account epithelial hyperplasia, epithelial fusion, epithelial cell necrosis, haemorrhage, irregular epithelial surface, and epithelial sloughing. In addition a score from 0 to 3 was attributed to quantify the degree of amoebic gill disease (AGD), with 0 being “none” up to 3 “severe”, based on the presence and extent of typical AGD histopathology. AGD was diagnosed based on the combination of clinical signs, gross pathology, and typical

histopathology and/or observation of amoeba on fresh smears. The typical histopathology was characterized by hyperplasia, hypertrophy, and lacunae or vesicle formation in the gill lamellae with associated amoeba. The salmon gill tissues were selected from diagnostic samples taken from marine farmed Atlantic salmon reared in net pens in the west of Ireland from 2004 through to 2010.

The comparison of the results obtained by microscopic examination did not show a direct correlation between the gill pathology score and the AGD score. Only 26% of the blocks with severe gill damage showed severe AGD (Table 3.1a), while all the blocks with severe AGD also showed severe gill pathology (Table 3.1b).

Table 3.1: (a) Distribution (%) of amoebic gill disease (AGD) scores for blocks with gill pathology scores 0-3; (b) distribution (%) of gill pathology scores for blocks with AGD scores 0-3.

(a)		Gill pathology score %			
		0 (N.9)	1 (N. 2)	2 (N. 10)	3 (N. 27)
AGD score %	0	100	100	30	63
	1	0	0	20	11
	2	0	0	50	0
	3	0	0	0	26
	TOTAL	100	100	100	100
(b)		AGD score %			
		0 (N.31)	1 (N. 5)	2 (N. 5)	3 (N. 7)
Gill pathology score %	0	29	0	0	0
	1	6	0	0	0
	2	10	40	100	0
	3	55	60	0	100
	TOTAL	100	100	100	100

ELF DNA was detected in all the blocks with a mean Ct value of 22.26 (SD = 1.38). *N. perurans* DNA was detected in all the blocks where AGD was microscopically diagnosed, and in 17 of the 39 blocks (43.59%) showing signs of gill pathology (Table 3.2). However parasitic DNA was detected only in those blocks with moderate or high levels of gill damage, with the highest prevalence detected in association with gill pathology scores of 2 (Figure 3.7). When the parasite load (ETA) was compared with the AGD score no association was found (Figure 3.8). Also, the level of parasite DNA did not increase significantly with increasing gill pathology score (Figure 3.9). This is likely to be consistent with the amoebic burden decreasing with time

as a result of the host response, and reflects findings on the immune response to AGD. Other factors or pathogens might contribute to the high degree of gill damage and AGD observed.

Table 3.2: Percentage of samples tested positive by duplex real time PCR for the presence of *N. perurans* among the blocks in which AGD or gill pathology was diagnosed histologically

	% blocks PCR positive
AGD not detected	0
AGD detected	100
Gill pathology not detected	0
Gill pathology detected	43.59

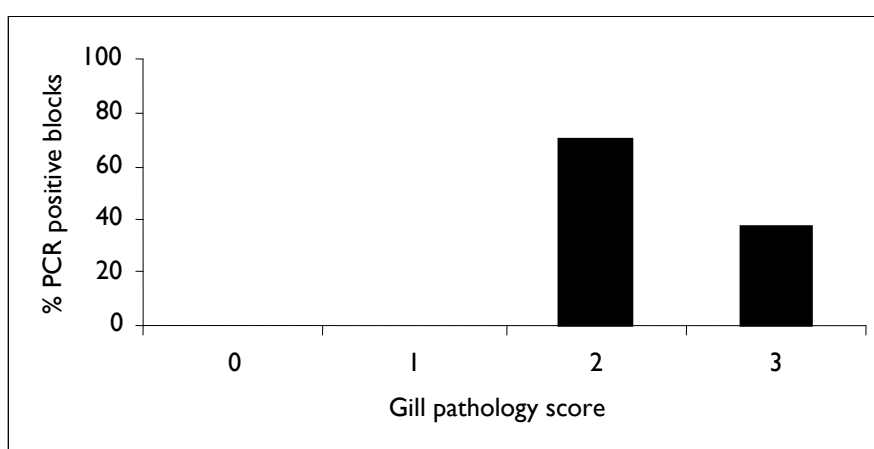


Figure 3.7: Prevalence of *N. perurans* infection among the formalin-fixed, paraffin-embedded (FFPE) blocks showing different degrees of gill pathology.

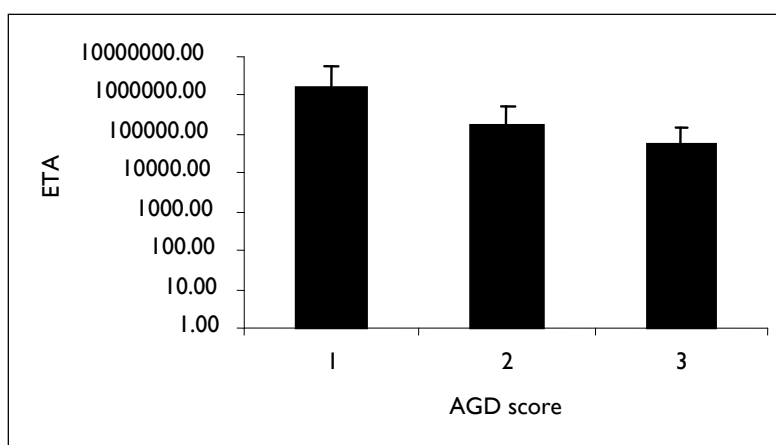


Figure 3.8: Comparison of the *N. perurans* load (ETA equivalent target amount) with the severity of the AGD observed (Error bars = 1 SD)

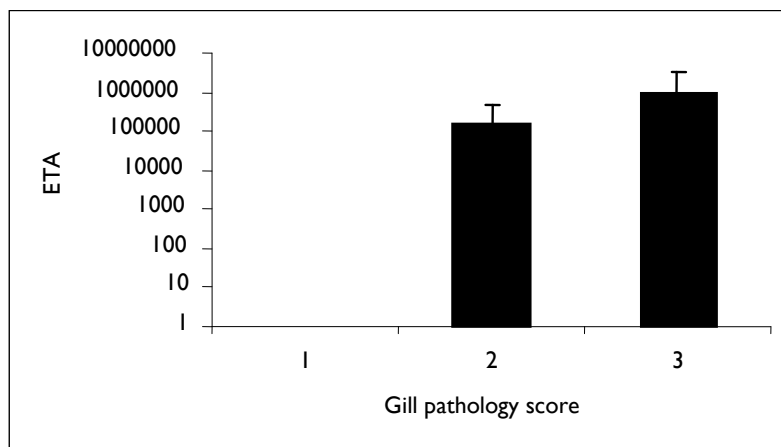


Figure 3.9: Comparison of the *N. perurans* load with the severity of the gill disease observed (Error bars = 1 SD)

Bermingham et al. (2006) described five genera of amoebae, *Platyamoeba*, *Mayorella*, *Vexillifera*, *Flabellula*, and *Nolandella*, detected in conjunction with *Neoparamoeba* sp. in the gill of Atlantic salmon in Ireland. However, the pathogenic role of these protozoa was not investigated.

Marine bacteria belonging to the *Flavobacteriaceae* have shown a positive correlation with both the prevalence and severity of AGD. The role of these bacteria in the aetiology of AGD, however, remains obscure, although it was suggested that the ability of *Neoparamoeba* sp. to infect filaments and cause lesions might be enhanced in the presence of salmonid gill bacteria. Environmental conditions have also been suggested as risk factors that predispose salmon to colonization by amoebae and ciliates. Salinity is regarded as one of the most important environmental factors in AGD; protracted infections in salmonids have frequently been associated with high salinities.

The application of the *T. maritimum* PCR on FFPE gill samples showed that this bacterium is relatively ubiquitous, being commonly detected in both healthy (89.89% of the blocks with no signs of gill pathology) and diseased FFPE gill tissues samples (97.44% of the blocks with gill damage score ≥ 1). A positive trend was found between detection of *T. maritimum* and gill pathology with an increase of the bacterial load with the increasing of the severity of the gill damage (Fig. 3.10), although the differences were not statistically significant possibly due to the small number of samples analysed.

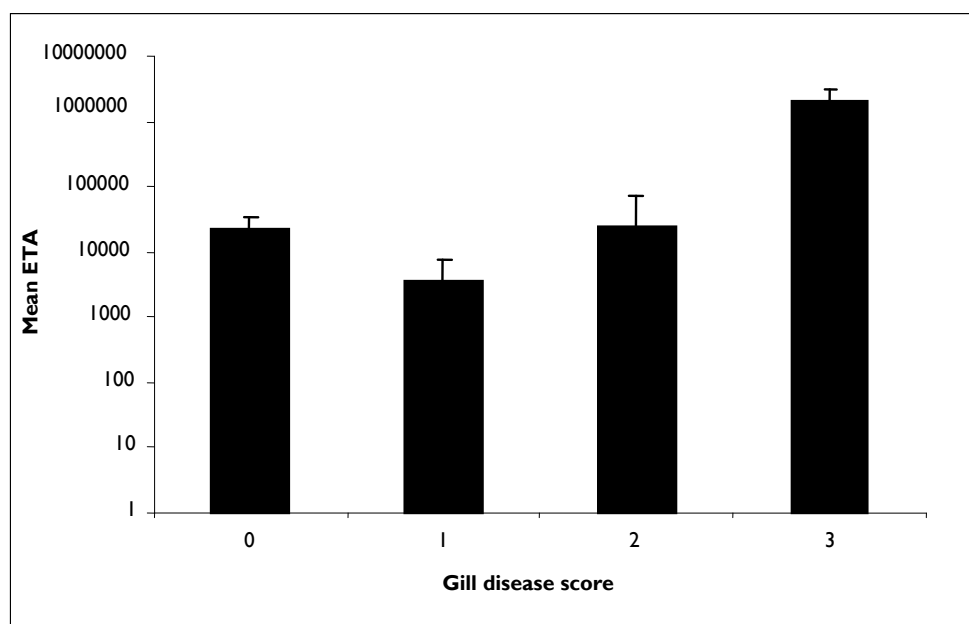


Figure 3.10: Comparison of the *T. maritimum* load with the severity of gill disease observed. Error bars = 1 SD

The detection of *T. maritimum* DNA in samples that did not show any histological damage could indicate an early stage of infection or the presence of an asymptomatic carrier fish. Alternatively, it may suggest that *T. maritimum* is not a primary pathogen for gill disease. Further investigation of this finding is required. This data together with the high bacterial load found in those blocks with no evidence of gill pathology might suggest that *T. maritimum* is not directly responsible but rather may cooperate with other organisms in the development and progression of gill pathology. Filamentous bacteria, with morphology consistent with *Tenacibaculum* spp., were described in only four PCR positive blocks where severe gill disease was histologically observed. This finding clearly indicates the higher sensitivity of this molecular method for detection of *T. maritimum* in comparison to histological examination.

The *T. maritimum* PCR was also applied to DNA samples extracted from 26 jellyfish samples, identified by microscopic examination as *Phialella quadrata* or *Muggiaea atlantica*. The jellyfish were collected between June 2009 and September 2010 from 17 seawater locations around Ireland as part of separate studies (Fig. 3.11, Table 3.3). Each sample contained between 3 and 30 individuals preserved in 4% seawater formalin. Seven of the 17 samples of *P. quadrata* were collected inside a sea cage or within 200 m of that cage on Atlantic salmon farms; the other 10 were collected between 55.4 km and 292.7 km from the nearest farm. All the *M. atlantica* jellyfish were collected inside or within 200 m of the farm cages with the exception of two that were collected 99.1 km and 154.6 km from the nearest farm.

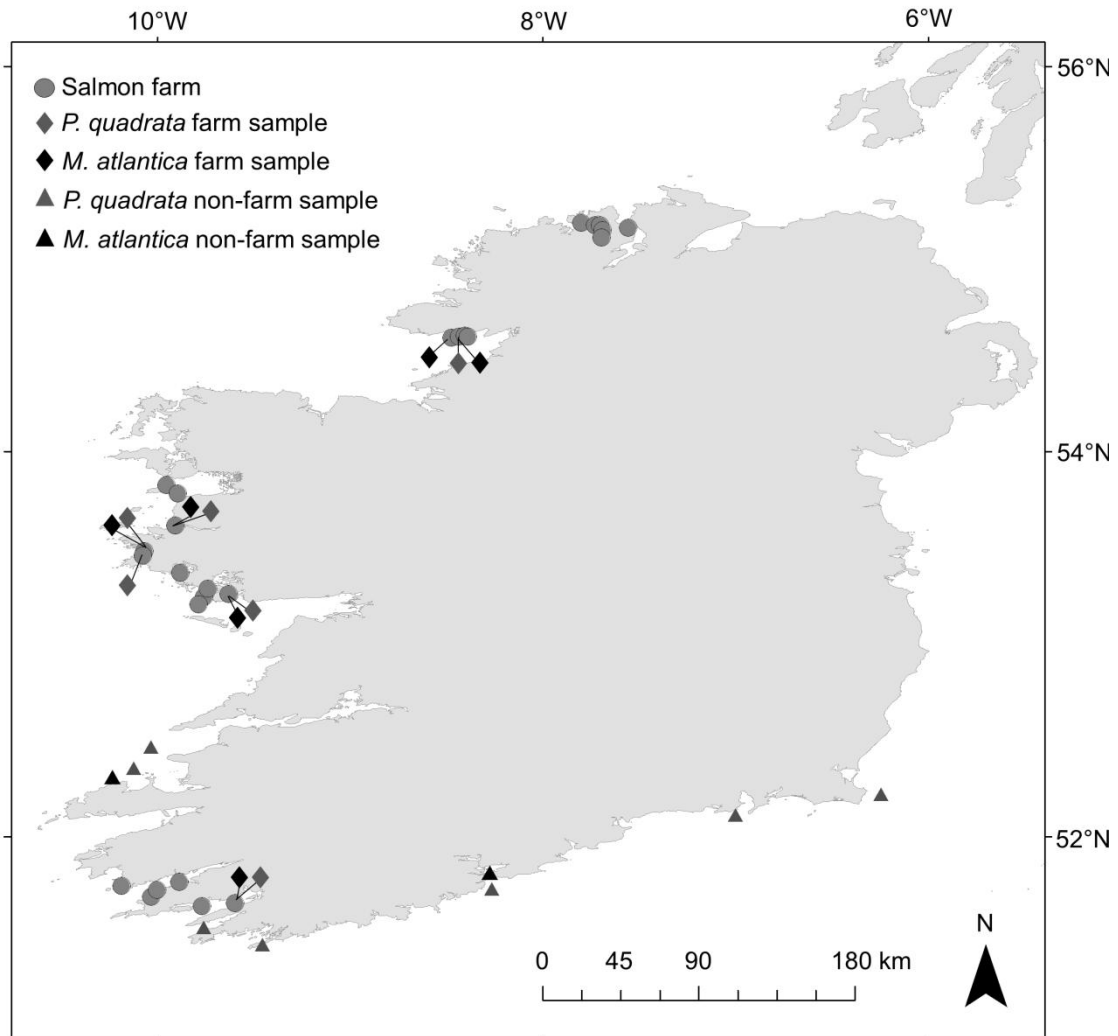


Figure 3.11: Sea water locations where jellyfish were collected. Non-farm samples indicate *P. quadrata* and *M. atlantica* jellyfish collected between 55.4 Km and 292.7 Km from the nearest farm. Farm samples indicate *P. quadrata* and *M. atlantica* jellyfish collected inside a cage or within 200m from that cage.

Table 3.3: Jellyfish tested by real time PCR for the presence of *T. maritimum*. Real time PCR results are included in the last column. In brackets Ct value is reported. PQ: *Phialella quadrata*, MA: *Muggiaea atlantica*

Sample ID	Jellyfish Species	N. of individuals	Date collected	Distance to nearest farm (km)	Real time PCR result
PQ 1.1	<i>P. quadrata</i>	21	Jun-09	90.9	-
PQ 1.2	<i>P. quadrata</i>	21	Jun-09	105	-
PQ 1.3	<i>P. quadrata</i>	7	Jun-09	260	-
PQ 1.4	<i>P. quadrata</i>	8	Jun-09	55.4	-
PQ 1.5	<i>P. quadrata</i>	9	Jun-09	292.7	-
PQ 1.6	<i>P. quadrata</i>	5	Jun-09	147.5	-
PQ 1.7	<i>P. quadrata</i>	3	Jun-09	26.7	-
PQ 1.8	<i>P. quadrata</i>	30	Apr-10	154.6	-
PQ 1.9 (1 of 2)	<i>P. quadrata</i>	12	Jun-10	154.6	+ (40)
PQ 1.9 (2 of 2)	<i>P. quadrata</i>	13	Jun-10	154.6	-
PQ 2.1	<i>P. quadrata</i>	25	Sep-10	On farm	-
PQ 2.2	<i>P. quadrata</i>	10	Jul-09	On farm	-
PQ 2.3	<i>P. quadrata</i>	6	Jun-09	On farm	-
PQ 2.4	<i>P. quadrata</i>	8	Aug-10	On farm	+ (34.32)
PQ 2.5	<i>P. quadrata</i>	12	Aug-10	On farm	-
PQ 2.6	<i>P. quadrata</i>	22	Jun-10	On farm	+ (37.34)
PQ 2.7	<i>P. quadrata</i>	3	Jun-09	On farm	-
MA 1.1	<i>M. atlantica</i>	1	Jun-09	99.1	-
MA 1.2	<i>M. atlantica</i>	25	Sep-10	154.6	-
MA 2.1	<i>M. atlantica</i>	20	Aug-10	On farm	+ (33.93)
MA 2.2	<i>M. atlantica</i>	5	Aug-10	On farm	-
MA 2.3	<i>M. atlantica</i>	22	Aug-10	On farm	-
MA 2.4	<i>M. atlantica</i>	15	Sep-10	On farm	-
MA 2.5	<i>M. atlantica</i>	15	Sep-09	On farm	-
MA 2.6	<i>M. atlantica</i>	4	Sep-09	On farm	-
MA 2.7	<i>M. atlantica</i>	3	Sep-09	On farm	-

Tenacibaculum maritimum DNA was detected at low levels in four of 26 jellyfish tested (Table 3.3). Three of the positive signals were obtained from *P. quadrata* and one from *M. atlantica*.

In 2008, *P. quadrata* was implicated in heavy mortality of sea-caged Atlantic salmon in Scotland. *T. maritimum* DNA was detected by conventional PCR in a single jellyfish sample collected around affected sea-cages. Phylogenetic analysis of bacterial DNA sequences extracted from the jellyfish and the salmon gills showed that they were almost identical, suggesting that jellyfish may act as a carrier for this pathogen. *Tenacibaculum maritimum* may therefore be responsible for a secondary bacterial infection characterised by severe necrotic lesions and infarction of the lamellae and filaments. Recently another species of jellyfish, *P. noctiluca*, was associated with mass mortalities of sea-caged fish. The four positive jellyfish in the current study were collected during summer (June and August) 2010 and only one, *P. quadrata*, was not collected inside a sea cage but rather 154.6 km from the nearest farm. This is the first study describing the detection of *T. maritimum* from jellyfish collected at 154.6 km from the nearest farm. However, *T. maritimum* was only found in one of two samples from this site. It is also important to note that these samples were taken inside Cork Harbour where there may be other potential hosts for *T. maritimum* and not from open areas of coastline. For two of the four positive samples the level of bacterial DNA could not be estimated as the Ct values (37.34 and 40.00) were beyond the reproducible limit of detection and therefore outside the linear dynamic range of the assay. The other two, collected on the same farm, were weakly positive with Ct value of 33.93 (*M. atlantica*) and 34.32 (*P. quadrata*) corresponding to a concentration of 9.10 and 6.94 copies/ μ l respectively.

This is the first study that describes the detection of *T. maritimum* in the jellyfish *M. atlantica* suggesting that this siphonophore might be added to the list of organisms that are able to host *T. maritimum*. However, further investigation is required to understand the relationship between the bacterium and the jellyfish, and the potential role of the latter as a vector for *T. maritimum* as this relationship cannot be confirmed purely on the basis of *T. maritimum* being present on both Atlantic salmon and jellyfish.

3.3.3. Longitudinal studies

Two longitudinal studies were undertaken on the presence, prevalence, and quantity of three of the pathogens (*T. maritimum*, *P. salmonis*, and *N. perurans*) in salmon gills using the quantitative RT-PCR and assessed against the gill histopathology scores (Mitchell et al. 2012). These were undertaken on the same two farms that were monitored for zooplankton (see Section 3.1) and were sampled every two weeks from March to November/December 2009. A total of 173 fish were screened between the two sites. The results indicated that in the Bantry Bay site in 2009 there was no evidence for *N. perurans* throughout the sample period, but both

T. maritimum and *P. salmonis* were present throughout, although both pathogens decreased in loading on the gills with higher gill histopathology scores. In the Clifden Bay site gill scores increased significantly through the sample period and all three pathogens were present from August, although both *T. maritimum* and *P. salmonis* were present from March. There was a significant increase in the load of both *P. salmonis* and *N. perurans* during the sample periods and a significant increase in the load of *P. salmonis* with the increasing gill histopathology score. *N. perurans* load also increased with increasing gill score, although this was not statistically significant.

The findings indicate that *T. maritimum* appears to be associated with gill tissue as soon as salmon are in the marine farm environment, however, the load of bacteria did not appear to increase significantly with increasing gill pathology in this study. *N. perurans*, in contrast, was not present in both sites but loads did increase with increasing gill pathology, although not to a statistically significant degree in this study. *P. salmonis* appeared associated with gill tissue in the marine environment and loads of this organism increased significantly in one of the two sites with increasing gill pathology. The question remains as to whether this apparent increase is primary or secondary to the increase in gill pathology.

3.3.4. Conclusions

- The Atlantic salmon paramyxovirus was not detected in any samples tested
- *Neoparamoeba perurans* was detected in fish with amoebic gill disease and/or severe gill pathology
- The load of *N. perurans* in the gill tissue increased in one of the longitudinal studies along with increasing gill pathology
- *Tenacibaculum maritimum* was found in the majority of fish tested regardless of the presence of gill disease
- The bacterium was also found in a number of jellyfish suggesting that they may play a role transmitting the bacteria as potential vectors
- *Piscichlamydia salmonis* was associated with gill tissue and the load of this organism increased significantly in one site with increasing gill pathology.

4. MANAGEMENT AND MITIGATION

4.1. Management Options

4.1.1. Challenges

There are many challenges facing the aquaculture industry in relation to the management of gill diseases. These include the lack of information on the causative agents, treatments, and the very nature of farms being located in the marine environment where naturally occurring blooms take place. The GILPAT project proposes that management of gill disease should consist of a combination of early warning systems through zooplankton and pathogen monitoring, combined with a range of mitigation measures in the event of, for example, a zooplankton bloom. As the investigation of all these factors was outside the scope of the GILPAT project, it has focussed primarily on the development of early warning systems outlined below. To address mitigation measures a review was carried out and a number of potential measures are described below. These options could include a greater understanding of biofouling, the use of flocculating agents, air bubble curtains, or the use of closed containment systems. However it must be noted that while all these options have potential, their use in reducing the impacts of blooms on farmed fish should be investigated in a proper scientific manner.

4.2. Early Warning Systems

4.2.1. Zooplankton monitoring

Extensive phytoplankton monitoring is conducted around many shellfish and finfish aquaculture sites to rapidly identify harmful species which may pose a threat to the health of humans and that of the stock; however, other than the zooplankton monitoring programme initiated by the GILPAT project, no such system exists for the monitoring of jellyfish populations. The routine monitoring of gelatinous zooplankton and harmful jellyfish around marine finfish farms, conducted throughout the project, has greatly helped to elucidate the understanding of the links between jellyfish blooms and detrimental effects on the fish. Furthermore, it has been shown that information at a site-specific level is essential to identify the seasonal and inter-annual abundance and occurrence of detrimental species, highlighting risk periods for each location.

As there is no national monitoring programme in place for zooplankton it is imperative that training of industry workers in the area of zooplankton sampling and identification occurs. The

GILPAT project ran training workshops and provided industry participants with the necessary equipment and tools to perform basic monitoring on their own sites. To complement the workshops, a guide to the identification of the most common jellyfish species in Irish waters was also produced. This has resulted in a number of sites regularly sampling and monitoring the zooplankton species occurring around the cages on an ongoing basis. It is recommended that training of marine farm operatives to monitor and identify harmful jellyfish blooms is continued. It is also recommended that co-operation between salmon farmers is developed whereby information on the occurrence of blooms is shared by all producers.

4.2.2. Screening for pathogens

This study has shown that the Atlantic salmon paramyxovirus does not appear to be involved in the development of gill pathology in Ireland. However the role of epitheliocystis and the bacteria *Tenacibaculum maritimum* in the development of gill pathologies needs further investigation. Both have been shown to be associated with severe cases of gill disease, yet at this stage it is not known whether they are the primary pathogens involved or simply act as secondary opportunistic pathogens. This project developed robust molecular assays which are available for use by the industry to screen their fish for the presence or absence of these pathogens. The value of this work was apparent in 2011 when there was a significant increase in amoebic gill disease cases on marine sites. The *N. perurans* assay will prove valuable in any future investigations into this disease.

4.3. Mitigation Measures

4.3.1. Reducing biofouling

The potential for aquaculture structures to unintentionally aid jellyfish populations is one of concern in times where both aquaculture operations and jellyfish populations appear to be increasing their footprint in coastal regions worldwide (Richardson et al. 2009). Removal of biofouling is a labour and capital intensive task for the aquaculture industry. Current methods of dealing with biofouling in Ireland are based on replacing nets for cleaning or in-situ cleaning with power washers such as the Idema Net Cleaning System (AkvaGroup). Replacing nets has the effect of causing stress on the salmon while power washing results in bits of fouling drifting in to the cages where they may cause gill problems in the salmon (see section 2.2.5).

Coatings can be applied to nets to deter or kill biofouling organisms. However most coatings used today are based on copper oxide. Alternative non-toxic coatings suitable for use on organic farms should be developed. For example, Hodson et al. (2000) found that silicone-

coated netting significantly reduced the amount of fouling at a salmon farm in Tasmania. However current silicone coatings have the disadvantage of lack of abrasion resistance and shear strength meaning that handling and cleaning of the nets may result in damage to the coating.

Other possibilities include improving hydroid removal with net washing by using single filament nets which may hinder the strong attachment of hydroids under loose filaments (Carl et al. 2010). Carl et al. (2010) suggested that the development of net washers to a design that collects any removed material, thus reducing settlement (and removing harmful polyps from the water), could also be a potential mitigation against harmful biofouling hydroids. The Marine Inspector and Cleaner (MIC) system, which is a vacuum based net cleaning system, has been developed by an Australian salmon farm, Tassal, for nets that have anti-fouling applied (MIC, 2009). Like the water jet system for cleaning nets, this system is operated onsite and does not require removal of the nets. It has an advantage over the water jet system in that the biofouling can be removed and disposed of away from the nets.

4.3.2. Flocculating agents

In a number of areas around the world flocculating agents have been used as a management tool against, primarily, phytoplankton blooms. A number of different types of compounds have been used such as clays, aluminium sulphate, polyaluminium chloride, slaked lime etc. The main objective of these applications is to immobilise the cells through physico-chemical interactions with the flocculant. The newly developed cationic polyacrylamides (CPAM's) are widely used in the water industry and have a number of advantages over the traditional flocculants used such as lower coagulant dose requirements, more effective, lower cost, and they are also biodegradable. A recent study by Jančula et al. (2011) has shown that they are highly effective against phytoplankton species under laboratory conditions. The use of these agents should be investigated against zooplankton/jellyfish species to determine their suitability.

4.3.3. Bubble curtains

Air bubble curtains have long been suggested to prevent jellyfish blooms entering salmon cages but there is little evidence of their effectiveness. Small scale trials using water filled ping pong balls to stimulate jellyfish have indicated that bubble curtains may be effective in pushing jellyfish to the surface where they would accumulate at a float and collected using a suction pump (Lo 1991). A pilot field trial was carried out in Ireland in 2004 and while there was not a significant difference in the numbers of *M. atlantica* inside and outside the cage, the numbers of non-harmful zooplankton species outside the curtain was significantly higher (Marine Harvest

Ireland, pers. comm.). Further field trials are required in order to assess the effectiveness of the system and the best layout for the system. The energy required to run a bubble curtain may also rule out its use, especially where there is a strong current as costs may be high. However, if a comprehensive large scale bubble curtain trial is conducted and the results are significant, the bubble curtain system may offer the best solution to protect salmon cages from large jellyfish events over short periods (i.e. days to weeks). Integrating bubble curtains with a source of renewable energy (such as wave, wind or tidal energy devices) is an area which should be looked into.

The sample in the text box overleaf is a calculation for the energy requirements of a bubble curtain protecting one cage, 60 m in diameter and 10 m in depth, from one current direction. The energy requirements will be dependent on the current speed and operating pressures, depth of operation, air volume flows, and length of manifold. More energy would be required if a suction pump were used in combination with the bubble curtain to remove the jellyfish. Field trials of the bubble curtain are required in order to assess the actual energy costs for running the system and a cost benefit analysis carried out.

Bubble Curtain Energy Consumption Sample

Design Assumptions:

- The length of the bubble curtain is the same as the diameter of the salmon cage (60 m)
- The depth of the curtain is the same as that of the cage (10 m)
- The curtain only needs to protect the cage from current in one direction at any time
- Surface Velocity = 0.5 m/s
- Design Pressure = 2.5 bars
- From Lo (1991): U_{\max} (Max horizontal current) = V_{\max} (max vertical velocity) = $K(gq)^{1/3}$

Constants:

- $K = 1.4$
- $K_{\text{air}} = 1.4 \text{ J/kg}^\cdot K$
- $R_{\text{air}} = 286.9 \text{ J/kg}^\cdot K$
- $g = 9.81 \text{ ms}^{-2}$

Energy Calculations:

Required Air flow rate = $U_{\max}^3/gK^3 = 0.5^3/9.81 \cdot 1.4^3 = 0.005 \text{ m}^3/\text{s}$ per unit width

- For 60 m manifold, $V = 60 \cdot 0.005 = 0.279 \text{ m}^3/\text{s}$
- Mass flow rate = Density * flow = $1.225 \cdot 0.279 = 0.341 \text{ kg/s}$
- Specific Work, $w = K_{\text{air}} / (K_{\text{air}} - 1) R_{\text{air}} T_1 [(p_2/p_1)^{(K_{\text{air}} - 1)/K_{\text{air}}} - 1]$
- $w = 1.4 / (1.4 - 1) \cdot 286.9 \cdot 293 \cdot [(2.5/1.01)^{(1.4-1)/1.4} - 1]$
- $w = 87.5 \text{ kNm/kg}$
- Power = $w \cdot \text{Mass flow rate} = 87.5 \times 10^3 \cdot 0.341 = 29.8 \text{ kW}$

⇒ **1 hour energy requirement for 1 cage = 29.8 kWh**

⇒ **24 hour energy requirement for 1 cage = 717.1 kWh**

Diesel Requirement:

Assume diesel engine efficiency = 50%

Specific Energy of diesel = 38.6 MJ/l = 10.7 kWh/l

Diesel requirement = $(29.8/0.5) / 10.7 = 5.6$ litres per cage per hour

4.3.4. Closed containment systems

Closed containment systems may not be cost competitive with conventional salmon cages, but the technology is successfully used for a range of other species such as cichlids, catfish, and

flatfish. Closed containment systems, either offshore or onshore units, offer a potential solution for the removal/reduction of the threat from harmful zooplankton.

The main reason for the costs of closed containment aquaculture being so high are the energy costs associated with pumping water. This needs to be reduced in order for it to become competitive. Concepts where renewable energies are used to supply aquaculture energy requirements should be developed, and the economics of combining the two industries studied.

Given the requirement for seawater in the salmon aquaculture industry, there is an opportunity to use wave energy devices to supply seawater. Many wave energy devices pump water to produce electricity and could be modified to supply water to an aquaculture development. Combined aquaculture and wave energy concepts should be developed, and assessed economically and for their practicality. It may be a number of years before commercial wave energy devices are available to supply water to salmon farms. Further development of ocean energy technologies in terms of reliability, survivability, and cost is required before it becomes attractive to the aquaculture industry.

Combining Aquaculture with Ocean Energy

Some wave energy devices are being developed to pump water to an onshore reservoir where the water is passed through a turbine to generate electricity. In future it could be possible to use this reservoir of water to supply an onshore aquaculture facility and any excess energy produced by the system could be sold to the national grid (see Figure 4.1).

Examples of these include the Aquamarine Power's 'Oyster' (www.aquamarinepower.com/technology) and Carnegie Wave Energy's CETO (www.carnegiecorp.com.au).

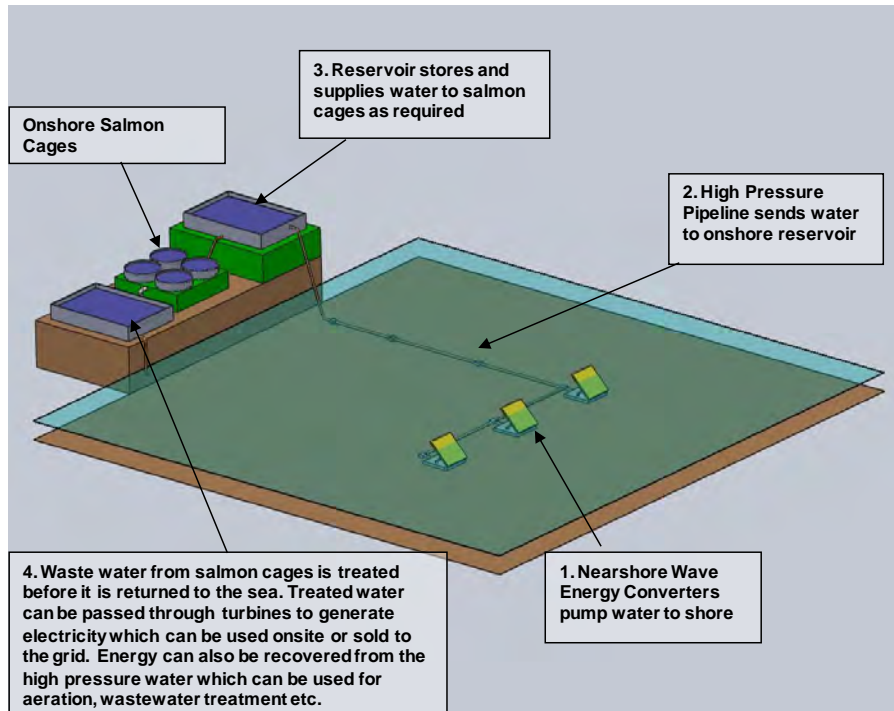


Figure 4.1: An onshore aquaculture concept using wave energy to supply seawater to a flow-through facility.

5. CONCLUSIONS AND FUTURE RESEARCH PRIORITIES

5.1. Conclusions

The GILPAT project was a multidisciplinary project investigating the causes of gill disease primarily affecting marine farmed Atlantic salmon in Ireland. The project had many elements including epidemiology, zooplankton monitoring, experimental challenge trials, and molecular diagnostics. Some of the main conclusions of each section are summarised below.

GILPAT CONCLUSIONS

Epidemiology:

- In the period 2004-2008 gill disease was a significant cause of mortality
- Diseases such as epitheliocystis may exacerbate the effects of gill disease
- The role of hydroids (biofouling) and alternative net cleaning methods should be investigated further.

Zooplankton Monitoring:

- Gill disease in the SW was correlated with abundance of *M. atlantica*, *P. quadrata* and *S. corona*
- In some cases the disease was linked to the occurrence of *Trichodina* sp. and *T. maritimum*
- The role of jellyfish as potential vectors of bacteria should be investigated.

Challenge Trials:

- The common jellyfish *A. aurita* and the hydroid *E. larynx* were shown to cause significant gill damage
- It is likely that biofouling organisms play an important role in the development of gill disease.

Molecular Diagnostics:

- Sensitive and specific molecular diagnostic assays for five potential pathogens were developed
- The bacteria *T. maritimum* appears to be ubiquitous in the marine environment
- Higher levels of *N. perurans* and *P. salmonis* were often associated with more severe gill disease.

5.2. Future Research Priorities

As mentioned in section 4.1, the work from the GILPAT project outlines the importance of developing early warning systems in conjunction with suitable mitigation measures which can be put in place when a harmful jellyfish bloom is imminent. Early warning systems should be based on the detection of harmful zooplankton species or pathogens, which are outlined below along with research requirements and expected outcomes (Table 5.1).

Table 5.1: Proposed early warning systems and the recommended research requirements for each as proposed by the findings of the GILPAT project.

Objective	RTDI Requirements	Outcomes
Monitoring and predicting jellyfish movements and abundance	Continuous training in zooplankton identification, develop models to predict movement	Knowledge of jellyfish blooms, abundance, and movements
Investigate the role of jellyfish as vectors of pathogens	Screening jellyfish for pathogens, experiments to determine transmission and pathogen viability	Knowledge of which jellyfish species can transmit specific pathogens
Trials to determine the pathology associated with jellyfish species e.g. hydroids	Experimental challenge trials	Knowledge of which species are harmful or benign
Investigate the role of specific pathogens in the development of gill pathology	Investigations determining the risk factors, aetiology, mode of disease transmission of epitheliocystis, amoebic gill disease, and tenacibaculosis	Knowledge of how other diseases interact with the onset of gill pathology
Development of treatments	Investigate the potential of treatments such as vaccines or specialised diets	Treatments to reduce the effects of gill disease

Table 5.2 outlines some of the key research requirements which need to be investigated in order to determine the feasibility and suitability of the proposed mitigation measures. It should be noted that there are any number of potential mitigation measures used by the industry, most commonly cessation of feeding and oxygenation of cages. The measures, while useful, just serve to reduce the severity of gill disease on the fish and are not preventative in nature. True mitigation measures will prevent the harmful agents from entering the cages, however at present there are no validated methods being used. Therefore the mitigation measures proposed will need to undergo extensive testing, initially at the experimental level, before final testing on marine sites to determine their feasibility.

Table 5.2: Proposed mitigation measures and the recommended research requirements for each as proposed by the GILPAT project.

Mitigation Measure	Research Requirements
Biofouling	<ul style="list-style-type: none"> • Environmentally friendly coatings • Vacuum based net cleaning systems • Investigate the pathology associated with hydroids
Flocculating Agents	<ul style="list-style-type: none"> • Cationic polyacrylamide agents • Field trials to determine suitability
Bubble Curtains	<ul style="list-style-type: none"> • Field trials to determine suitability • Methods of increasing energy efficiency
Closed Containment Systems	<ul style="list-style-type: none"> • Onshore site development • Offshore cage design

APPENDIX I: PROJECT OUTPUTS

Peer-reviewed Publications

- Bastian, T., Stokes, D., Kelleher, J.E., Hays, G.C., Davenport, J. and Doyle, T.K. (2011). Fisheries bycatch data provide insights into the distribution of the mauve stinger (*Pelagia noctiluca*) around Ireland. *ICES Journal of Marine Science*, **68**, 436-443.
- Baxter, E.J., Walne, A.W., Purcell, J.E., McAllen, R. and Doyle, T.K. (2010). Identification of jellyfish from Continuous Plankton Recorder samples. *Hydrobiologia*, **645**, 193-201.
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- Baxter, E.J., McAllen, R., Allcock, A.L. and Doyle, T.K. (2013). Abundance, distribution and community composition of small gelatinous zooplankton in southern Irish coastal waters. *Biology & Environment: Proceedings of the Royal Irish Academy B* DOI: 10.3318/BIOE.2012.01.
- Baxter, E.J., Sturt, M.M., Ruane, N.M., Doyle, T.K., McAllen, R. and Rodger, H.D. (2012). Biofouling of the hydroid *Ectopleura larynx* on aquaculture nets in Ireland: implications for finfish health. *Fish Veterinary Journal*, **13**, 17-29.
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- Mitchell, S.O., Baxter, E.J. and Rodger, H.D. (2011). Gill pathology in farmed salmon associated with the jellyfish *Aurelia aurita*. *Veterinary Record*, **169**, 609.
- Mitchell, S.O., Baxter, E.J., Holland, C. and Rodger, H.D. (2012). Development of a novel histopathological gill scoring protocol for assessment of gill health during a longitudinal study in marine-farmed Atlantic salmon (*Salmo salar*). *Aquaculture International*, **12**, 813-825.

- Rodger, H.D., Henry, L. and Mitchell, S.O. (2011). Non-infectious gill disorders of marine salmonid fish. *Reviews in Fish Biology & Fisheries*, 21, 423-440.
- Rodger, H.D., Murphy, K., Mitchell, S.O. and Henry, L. (2011). Gill disease in marine farmed Atlantic salmon at four farms in Ireland. *Veterinary Record*, 168, 668.
- Rodger, H.D., Fringuelli, E., Baxter, E.J., Mitchell, S.O., Ruane, N.M., Gordon, A. and Graham, D.A. (in prep.). Gill pathogens of Atlantic salmon (*Salmo salar* L.): longitudinal studies in marine farms in Ireland.

Theses

- Baxter, E.J. (2011). *Gelatinous zooplankton in Irish waters: Ecology and role in the gill disorders of marine-farmed salmon*. Ph. D. University College Cork, Ireland.

Presentations

- Baxter (de Clive-Lowe), E.J. and Doyle, T. (2009). Investigating the role of gelatinous zooplankton in gill disorders of marine reared finfish. In: *9th TriNation PD & Gill Disease Seminar*. Stirling, Scotland February 2009.
- Baxter, E.J., Rodger, H.D., McAllen, R. and Doyle, T.K. (2010). Jellyfish and potential impacts on aquaculture. In: *3rd International Jellyfish Blooms Symposium*. Argentina July 2010.
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- Doyle, T.K. and Hays, G.C. (2010). The rise of slime. Jellyfish invasions: a consequence of failed management of our seas? In: *Address to the European Parliament's Intergroup 'Seas and Coastal Affairs'*. Brussels, Belgium September 2010.
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Anon. (2010). How jellyfish can sting coastal economies. *Marine Times*, 1 Feb. p20.

Viney, M. (2011). Why the Forty Foot jellyfish could be a bad sign for the sea, *Irish Times*, 28 May.

Miscellaneous

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Training Courses

- Phytoplankton and zooplankton sampling and identification workshop, Marine Institute, March 2009
- Zooplankton and sea lice sampling and identification workshop, Marine Institute, April 2010
- Sampling methods and identification of harmful jellyfish, National Maritime College of Ireland, May 2011

APPENDIX II: ATLANTIC SALMON PARAMYXOVIRUS

ASSAY

DETECTION OF ATLANTIC SALMON PARAMIXOVIRUS (ASPV) IN FISH TISSUES AND CELL CULTURES BY ONE STEP REAL TIME RT-PCR

Atlantic salmon paramyxovirus (ASPV) is an RNA virus that may be associated with proliferative gill inflammation (PGI) in salmonids, a significant disease of Atlantic salmon during the marine phase in Norwegian aquaculture. This SOP describes the performance of a duplex Taqman real time RT-PCR assay in a one step procedure, for the detection of ASPV infection in tissues or cell culture supernatant.

PRIMERS AND PROBE

The sequence of the primers and probe targeting ASPV (Watanabe et al. 2006) are:

- PAR6 forward primer = 5' CCC ATA TTA GCA AAT GAG CTC TAT CTT3'
- PAR6 reverse primer = 5' CGT TAA GGA ACT CAT CAT TGA GCT T 3'
- PAR6M2 probe = FAM 5' AGC CCT TTT GTT CTG C 3' MGB

The sequence of the primers and probe amplifying 54 bp within the ELA housekeeping gene (Christie et al. 2007) are:

- EL.1A-ELA for = 5' CCC CTC CAG GAC GTT TAC AAA 3'
- EL.1A-ELA rev = 5' CAC ACG GCC CAC AGG TAC A 3'
- EL.1A-ELAMI = VIC 5' ATC GGT GGT ATT GGA AC 3' MGB

MASTERMIX TABLE

Mix components	Volume / 20 µl reaction	Final concentration
2x QuantiTect Multiplex RT-PCR NoROX Master Mix	10 µl	X1
Template RNA	2 µl	10 pg – 1 µg
PAR6 Primer forward	1.2 µl	0.6 µM
PAR6 Primer reverse	1.2 µl	0.6 µM
PAR6 probe	0.8 µl	0.4 µM
ELA Primer forward	0.8 µl	0.4 µM
ELA Primer reverse	0.8 µl	0.4 µM
VIC ELA probe	0.4 µl	0.2 µM
QuantiTect RT mix	0.2 µl	-
RNase-free water	2.6 µl	-
<i>Total Volume</i>	20 µl	-

PREPARATION AND ADDITION OF TEMPLATE RNA

Add 2 µl of RNA from each sample or control to an 18 µl aliquot of master mix. Include appropriate controls in each run. Typically these should comprise:

- Extraction positive control (usually a cell culture-grown virus pool) and tested in duplicate
- Negative extraction water controls (at least one per 10 samples)
- No template control (NTC i.e. simply add RNase free water instead of template RNA).

Samples may be tested singly or in duplicate as designated by the technical manager or as agreed with the customer.

CYCLE DESCRIPTION

Cycle Description	Temperature	Time	Number of cycles
cDNA synthesis (reverse transcription)	50°C	20 minutes	1
<ul style="list-style-type: none"> • activation of the hotStart Taq DNA polymerase • inactivation of the reverse transcriptase • denaturation of the cDNA template 	95°C	15 minutes	1
Denaturation	94°C	15 seconds	45
Annealing / extension (acquisition of fluorescence in FAM and VIC channels)	56°C	15 seconds	
Final extension	72°C	4 minutes	1

APPENDIX III: NEOPARAMOEBA PERURANS ASSAY

DETECTION OF *Neoparamoeba perurans* IN FRESH AND FORMALIN FIXED FISH TISSUES AND CELL CULTURES BY DUPLEX TAQMAN REAL-TIME PCR

Neoparamoeba perurans is the aetiological agent of amoebic gill disease (AGD), an ectoparasitic condition of farm-reared marine fish. AGD, responsible for significant direct losses in Atlantic salmon, is characterized by multifocal lesions that appear as pale gill tissue. This SOP describes the performance of a duplex Taqman real time PCR assay for the detection of *N. perurans* in fresh and formalin fixed paraffin embedded (FFPE) tissues.

PRIMERS AND PROBE

The sequence of the primers and probe amplifying a 139bp product of *N. perurans* are:

- Peru forward primer = 5' GTTCTTTCGGGAGCTGGGAG 3'
- Peru reverse primer = 5' CTTTTGTGCCGCGATAGTTC 3'
- Peru probe = FAM 5' CAATGCCATTCTTTTCGGA 3' MGB

The sequence of the primers and probe amplifying 66 bp product within the Atlantic Salmon Elongation Factor alpha I gene (Bruno et al. 2007) are:

- SAL ELF for = 5' GGC CAG ATC TCC CAG GGC TAT 3'
- SAL ELF rev = 5' TGA ACT TGC AGG CGA TGT GA 3'
- SAL ELF probe = VIC 5' CCT GTG CTG GAT TGC CAT ACT G 3' MGB

MASTERMIX TABLE

Mix components	Volume / 20 µl reaction	Final concentration
2x QuantiTect Multiplex RT-PCR NoROX Master Mix	10 µl	X1
Template DNA	1.5 µl	10 pg – 1 µg
Peru Primer forward	0.6 µl	0.3 µM
Peru Primer reverse	0.6 µl	0.3 µM
Peru probe	0.3 µl	0.15 µM
ELF Primer forward	0.3 µl	0.15 µM
ELF Primer reverse	0.3 µl	0.15 µM
ELF probe	0.15 µl	0.75 µM
Nuclease-free water	6.25 µl	-
<i>Total Volume</i>	20 µl	-

PREPARATION AND ADDITION OF TEMPLATE DNA

Add 1.5 µl of DNA from each sample or control to an 18.5 µl aliquot of master mix.

Include appropriate controls in each run. Typically these should comprise of:

- Extraction positive control (usually a positive tissue) and tested in duplicate
- Negative extraction water controls (at least one per 10 samples)
- No template control (NTC i.e. simply add Nuclease free water instead of template DNA)

CYCLE DESCRIPTION

Cycle Description	Temperature	Time	Number of cycles
Activation of the hotStart Taq DNA polymerase Denaturation of the DNA template	95°C	15 minutes	1
Denaturation	95°C	15 seconds	45
Annealing / extension (acquisition of fluorescence in FAM and VIC channels)	56°C	20 seconds	
Final extension	72°C	4 minutes	1

APPENDIX IV: *TENACIBACULUM MARITIMUM* ASSAY

DETECTION OF *Tenacibaculum maritimum* IN FISH TISSUES BY TAQMAN REAL-TIME PCR

Tenacibaculum maritimum, a Gram-negative filamentous bacterium, has been described as the aetiological agent of an ulcerative disease known as tenacibaculosis in a large number of marine fish species. This SOP describes the method of a TaqMan real-time PCR assay for the detection of *T. maritimum* in fish (the ELF assay is run separately but has the cycle conditions).

PRIMERS AND PROBE

The sequence of the primers and probe amplifying a 155bp sequence of 16S rRNA gene are:

- MAR4 forward primer = 5' TCG CTT CTA CAG AGG GAT AGC C 3'
- MAR reverse primer = 5' CTA TCG TTG CCA TGG TAA GCC G 3'
- MAR probe = FAM 5' CAC TTT GGA ATG GCA TCG 3' MGB

The sequence of the primers and probe amplifying 66 bp product within the Atlantic Salmon Elongation Factor alpha 1 gene (Bruno et al. 2007) are:

- SAL ELF for = 5' GGC CAG ATC TCC CAG GGC TAT 3'
- SAL ELF rev = 5' TGA ACT TGC AGG CGA TGT GA 3'
- SAL ELF probe = VIC 5' CCT GTG CTG GAT TGC CAT ACT G 3' MGB

MASTERMIX TABLE

Mix components	Volume / 20 µl reaction	Final concentration
2x QuantiTect Multiplex RT-PCR NoROX Master Mix	10 µl	X1
Template DNA	2 µl	10 pg – 1 µg
MAR4 Primer forward	1 µl	0.5 µM
MAR Primer reverse	1 µl	0.5 µM
MAR probe	0.1 µl	0.05 µM
ELF Primer forward	0.2 µl	0.1 µM
ELF Primer reverse	0.2 µl	0.1 µM
ELF probe	0.1 µl	0.05 µM
Nuclease-free water	5.9 µl (MAR) / 7.5 µl (ELF)	-
<i>Total Volume</i>	20 µl	-

PREPARATION AND ADDITION OF TEMPLATE DNA

Add 2 µl of DNA from each sample or control to an 18 µl aliquot of master mix.

Although the *T. maritimum* and ELF assays are run separately, they can be put on the same plate as the cycle conditions are identical.

Include appropriate controls in each run. Typically these should comprise of:

- Extraction positive control (usually a positive tissue) and tested in duplicate
- Negative extraction water controls (at least one per 10 samples)
- No template control (NTC i.e. simply add Nuclease free water instead of template DNA)

CYCLE DESCRIPTION

Cycle Description	Temperature	Time	Number of cycles
Activation of the hotStart Taq DNA polymerase Denaturation of the DNA template	95°C	15 minutes	1
Denaturation	95°C	20 seconds	45
Annealing / extension (acquisition of fluorescence in FAM and VIC channels)	52°C	20 seconds	
Final extension	72°C	4 minutes	1

APPENDIX V: PISCICHLAMYDIA SALMONIS ASSAY

DETECTION OF *Piscichlamydia salmonis* IN FISH TISSUES BY DUPLEX TAQMAN REAL-TIME PCR

Piscichlamydia salmonis (order Chlamydiales), is a chlamydia-like bacterium associated with epitheliocystis in farmed Atlantic salmon (*Salmo salar*), a condition that causes heavy mortality and reduced growth of survivors. This SOP describes a method for detection of the bacteria in fish tissues by duplex TaqMan real-time PCR.

PRIMERS AND PROBE

The sequence of the primers and probe amplifying a 150bp sequence of 16S rRNA gene (Steinum et al. 2010) are:

- 16S forward primer = 5' CCG CAA GGA CAA CTA CAC 3'
- 16S reverse primer = 5' ATC GAC TTA GGC AGT CTC G 3'
- 16S probe = FAM 5' CTT CCT CTG CTC GGC GAG CAC G 3' MGB

The sequence of the primers and probe amplifying 66 bp product within the Atlantic Salmon Elongation Factor alpha 1 gene (Bruno et al. 2007) are:

- SAL ELF for = 5' GGC CAG ATC TCC CAG GGC TAT 3'
- SAL ELF rev = 5' TGA ACT TGC AGG CGA TGT GA 3'
- SAL ELF probe = VIC 5' CCT GTG CTG GAT TGC CAT ACT G 3' MGB

MASTERMIX TABLE

Mix components	Volume / 20 µl reaction	Final concentration
2x QuantiTect Multiplex RT-PCR NoROX Master Mix	10 µl	X1
Template DNA	1.5 µl	10 pg – 1 µg
16S Primer forward	0.6 µl	0.3 µM
16S Primer reverse	0.6 µl	0.3 µM
16S probe	0.3 µl	0.15 µM
ELF Primer forward	0.3 µl	0.15 µM
ELF Primer reverse	0.3 µl	0.15 µM
ELF probe	0.15 µl	0.75 µM
Nuclease-free water	6.25 µl	-
Total Volume	20 µl	-

PREPARATION AND ADDITION OF TEMPLATE DNA

Add 1.5 µl of DNA from each sample or control to an 18.5 µl aliquot of master mix.

Include appropriate controls in each run. Typically these should comprise of:

- Extraction positive control (usually a positive tissue) and tested in duplicate
- Negative extraction water controls (at least one per 10 samples)
- No template control (NTC i.e. simply add Nuclease free water instead of template DNA)

CYCLE DESCRIPTION

Cycle Description	Temperature	Time	Number of cycles
Activation of the hotStart Taq DNA polymerase Denaturation of the DNA template	95°C	15 minutes	1
Denaturation	95°C	15 seconds	45
Annealing / extension (acquisition of fluorescence in FAM and VIC channels)	61°C	15 seconds	
Final extension	72°C	4 minutes	1

APPENDIX VI: DESMOZON LEPEOPHTHEIRII ASSAY

DETECTION OF *Desmozon lepeophtheirii* (*Paranucleospora theridion*) IN FRESH AND FORMALIN FIXED FISH TISSUES AND CELL CULTURES BY DUPLEX ONE STEP REAL-TIME RT-PCR

Desmozon lepeophtheirii is a microsporidian parasite associated with a range of important diseases (PGI, HSMI, CMS, PD). This SOP describes the performance of a duplex Taqman one-step real time RT-PCR assay for the detection of *D. lepeophtheirii* in fresh and formalin fixed paraffin embedded (FFPE) tissues.

PRIMERS AND PROBE

The sequence of the primers and probe amplifying a 105bp product of *D. lepeophtheirii* are:

- MICROSP forward primer = 5' GGC AGT GCA TCC TGA TAG C 3'
- MICROSP reverse primer = 5' GTG AGT GTG TGT ATT CAA CCC 3'
- MICROSP probe = FAM 5' TTG CAC TGT GCA CTG T 3' MGB

The sequence of the primers and probe amplifying 54 bp product within the Atlantic Salmon Elongation Factor alpha I gene (Christie et al. 2007) are:

- SAL ELA for = 5' CCC CTC CAG GAC GTT TAC AAA 3'
- SAL ELA rev = 5' CAC ACG GCC CAC AGG TAC A 3'
- SAL ELA probe = VIC 5' ATC GGT GGT ATT GGA AC G 3' MGB

MASTERMIX TABLE

Mix components	Volume / 20 µl reaction	Final concentration
2x QuantiTect Multiplex RT-PCR NoROX Master Mix	10 µl	X1
Template DNA	2 µl	10 pg – 1 µg
MICROSP Primer forward	1 µl	0.5 µM
MICROSP Primer reverse	1 µl	0.5 µM
MICROSP probe	0.4 µl	0.2 µM
ELA Primer forward	0.8 µl	0.4 µM
ELA Primer reverse	0.8 µl	0.4 µM
ELA probe	0.4 µl	0.2 µM
QuantiTect RT mix	0.2 µl	
RNase-free water	3.4 µl	-
<i>Total Volume</i>	20 µl	-

PREPARATION AND ADDITION OF TEMPLATE RNA

Add 2 µl of RNA from each sample or control to an 18 µl aliquot of master mix.

Include appropriate controls in each run. Typically these should comprise of:

- Extraction positive control (usually a positive tissue) and tested in duplicate
- Negative extraction water controls (at least one per 10 samples)
- No template control (NTC i.e. simply add Nuclease free water instead of template DNA)

CYCLE DESCRIPTION

Cycle Description	Temperature	Time	Number of cycles
cDNA synthesis (reverse transcription)	50°C	20 minutes	1
<ul style="list-style-type: none"> • activation of the hotStart Taq DNA polymerase • inactivation of the reverse transcriptase • denaturation of the cDNA template 	95°C	15 minutes	1
Denaturation	94°C	15 seconds	45
Annealing / extension (acquisition of fluorescence in FAM and VIC channels)	49°C	15 seconds	
Final extension	72°C	4 minutes	1

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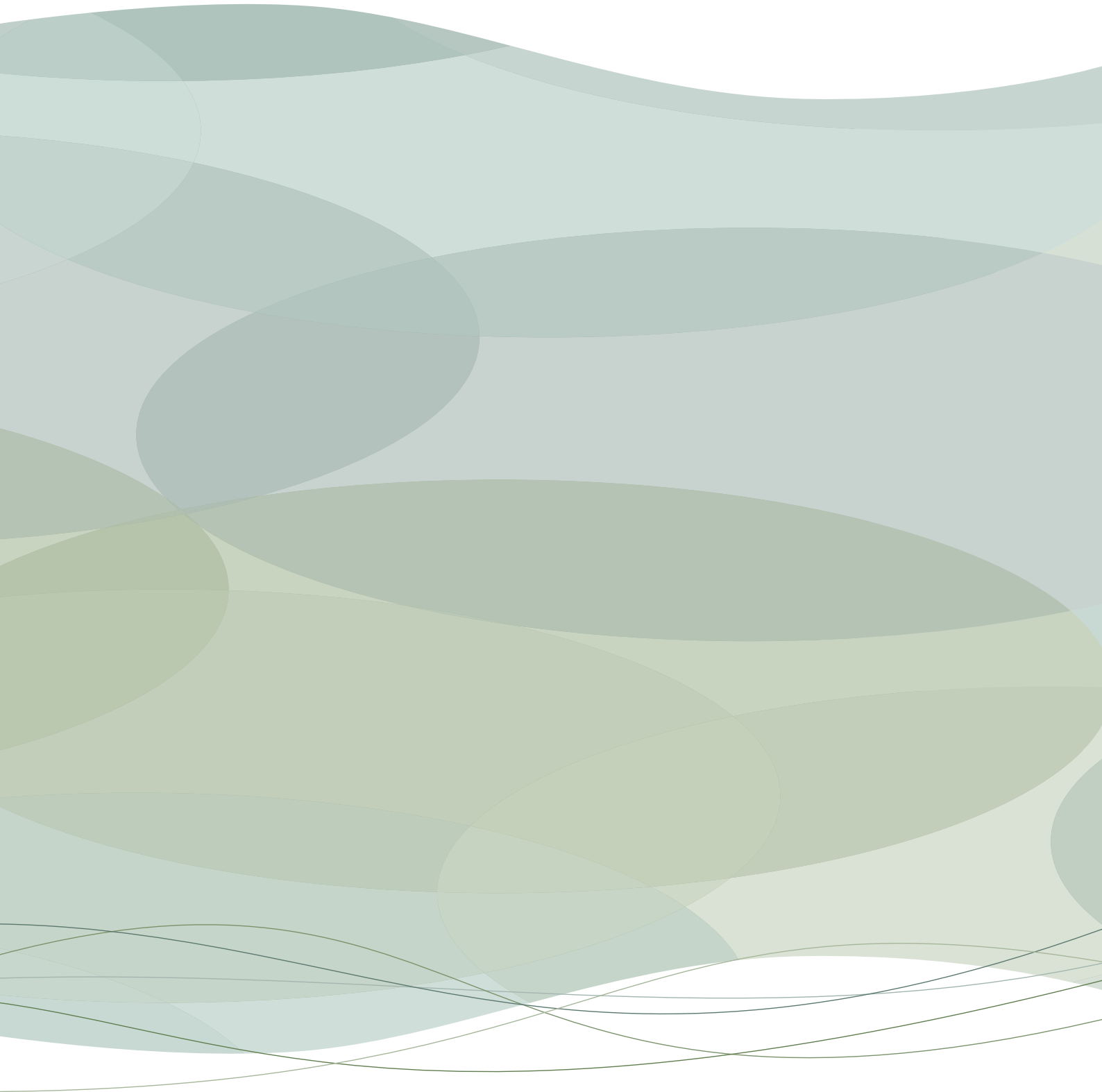
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