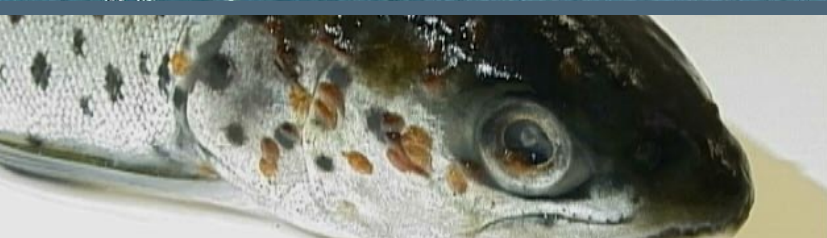


# Sea Lice Working Group Report

Crawford Revie, Larry Dill, Bengt Finstad and  
Christopher Todd



Report from the Technical Working Group on Sea Lice (A sub-group of the Working Group on Salmon Disease) of the Salmon Aquaculture Dialogue.



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## Report

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This report was commissioned by the Salmon Aquaculture Dialogue. The Salmon Dialogue is a multi-stakeholder, multi-national group which was initiated by the World Wildlife Fund in 2004. Participants include salmon producers and other members of the market chain, NGOs, researchers, retailers, and government officials from major salmon producing and consuming countries.

The goal of the Dialogue is to credibly develop and support the implementation of measurable, performance-based standards that minimize or eliminate the key negative environmental and social impacts of salmon farming, while permitting the industry to remain economically viable

The Salmon Aquaculture Dialogue focuses their research and standard development on seven key areas of impact of salmon production including: social; feed; disease; salmon escapes; chemical inputs; benthic impacts and siting; and, nutrient loading and carrying capacity.

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More information on the Salmon Aquaculture Dialogue is available at:  
<http://www.worldwildlife.org/salmondialogue>

## Table of Contents

<b>Extended Summary</b> .....	9
<b>Chapter 1: Sea Lice as Disease Organisms</b> .....	14
1.1 Introduction.....	14
1.2 Defining disease.....	16
1.2.1 Can fish infested by sea lice be considered “diseased”? .....	16
1.2.2 The wider perception of sea lice as benign parasites/disease organism....	17
1.2.3 The salmon louse, <i>Lepeophtheirus salmonis</i> : Pacific & Atlantic.....	20
1.2.4 Establishment of parasitic disease.....	21
Phase I: Initial Colonization.....	22
Phase II: Infection.....	25
Phase III: Disease.....	26
1.2.5 Sea lice infestation and “premature migratory return”.....	26
1.3 Health, Disease and Sustainability .....	27
1.3.1 Stability of the host-parasite association and epizootic outbreaks.....	27
1.3.2 Sustainability of sea lice disease of salmonids.....	33
1.4 Range as a determinant of impact.....	34
<b>Chapter 2: Avoiding Infection</b> .....	38
2.0 Introduction.....	38
2.1 What do we mean by avoid? .....	45
2.2 Different avoidance scenarios .....	46
2.2.1 Endemic in the region but not on farm .....	46
2.2.2 Endemic in the country but not in region.....	46
2.2.3 Exotic diseases that are known elsewhere.....	46
2.2.4 New, emerging infections .....	47
2.3 What must be in place to avoid a disease? .....	47
2.3.1 Herd and individual immunity .....	47
2.3.2 Preventing exposure (of wild fish) .....	47
2.3.3 Knowledge of what might be coming (prediction).....	48
2.4 Prediction: Knowing what to avoid .....	49
2.5 Promoting Resistance .....	50
2.6 Preventing exposure (or transmission to wild fish) .....	50
2.7 Legislation .....	47

<b>Chapter 3: Can we prevent disease/ infestation?</b> .....	51
3.0 Introduction .....	51
3.1 What are the tools for prevention (farmed)? .....	51
3.1.1 Vaccination .....	51
3.1.2 Non-specific immune moderators .....	52
3.1.3 Risk factors for modification .....	52
3.1.4 Genetics .....	52
3.1.5 Nutrition .....	53
3.1.6 Water quality environment .....	53
3.1.7 Treatment (not prophylactic, except from perspective of the wild fish)....	53
3.1.8 Culling/depopulation (compulsory slaughter) of high risk groups.....	54
3.1.9 Screening plus action.....	54
3.2 Herd health and HAACP on farm .....	54
<b>Chapter 4: Can we reduce disease impacts?</b> .....	55
4.0 Introduction.....	55
4.1 Can we reduce impacts on farmed fish? .....	55
4.1.1 Monitoring/surveillance (presence, levels and patterns).....	57
4.1.2 Treatment/control options for sea lice .....	60
4.1.3 Measuring effects of sea lice control .....	69
4.2 Can we reduce impacts on wild fish? .....	70
4.2.1 Monitoring/surveillance .....	73
Effects on populations of sea trout, Arctic charr and Pacific salmon.....	74
Effects running Atlantic salmon post-smolt populations.....	75
4.2.2 Treatment/control options (all interventions).....	76
4.2.3 Measuring effects of disease and disease control.....	77
4.2.4 The issue of farms as a potential bio-magnifier of pathogens in water.....	78
Escaped salmon and rainbow trout .....	78
Density of fish farms .....	79
Cod farming (Norway) .....	79
Marine fish species as potential sources of sea lice to wild/farmed fish.	80
<b>Chapter 5: Disease management systems</b> .....	81
5.0 Introduction.....	81
5.4 Systems management.....	82
5.4.1 Sea lice modeling and empirical data.....	82
Limitations in sampling procedures.....	83

Applying quantitative epidemiological approaches to lice dynamics..... 85  
5.4.2 Minimizing the impacts of disease requires collaboration..... 91

**Chapter 6: Framework for assessing risk – sea lice case studies..... 92**

[**N.B.** This report on sea lice is a ‘partner’ document to a more general Disease report (Hammell et al. 2009) and was developed in tandem as part of the WWF Salmon Aquaculture Dialogue. For this reason it was decided to follow, where sensible, the same section/sub-section layout in both reports.]

**References..... 93**

**Glossary..... 113**

**Acknowledgements..... 117**

## List of Tables and Figures

- Figure 1.1** Abundance of sea lice (*Lepeophtheirus salmonis*, *Caligus elongatus*) on individual wild, one sea-winter, adult Atlantic salmon (*Salmo salar*) (n = 430; length, weight ranges: 49-77.5 cm, 1.1-4.6 kg) captured in fully marine seawater at Strathy Point, N Scotland 1999-2006..... 18
- Figure 1.2** Adult female *Lepeophtheirus salmonis* occupying the skin adjacent to the anal fin of an adult two sea-winter Atlantic salmon (~7 kg) captured in fully marine seawater at Strathy Point, N Scotland. Note the (pink) erosion of the epidermis and associated bleeding of the lesion. [Photo: C.D. Todd] ..... 18
- Figure 1.3** Adult female *Lepeophtheirus salmonis* (most with paired eggstrings) occupying the dorsal midline between the dorsal fin and adipose fin of a wild, one sea-winter adult Atlantic salmon (2.80 kg) captured in fully marine seawater at Strathy Point, N Scotland. The sea louse abundance ('mobile' stages only) on this fish was 52 *L. salmonis* and 2 *Caligus elongatus*. [Photo C.D. Todd]..... 19
- Figure 1.4** Adult two sea-winter Atlantic salmon (~8 kg), captured in estuarine conditions in the Firth of Tay, E Scotland, showing the typical lesions caused by adult female *Lepeophtheirus salmonis* on the scales and skin adjacent to the anal fin. [Photo C.D. Todd]..... 19
- Figure 1.5** The molt stages and life cycle of *Lepeophtheirus salmonis*. The gravid female extrudes a pair of eggstrings containing the developing embryos and these are retained to the hatching stage (Nauplius I). After two planktonic larval molts host infestation occurs at the final free-swimming, copepodid stage and the subsequent four chalimus stages all are attached to the host fish by a thread (frontal filament). The pre-adult and final adult stages all are mobile over the host fish. No further molts occur after the adult status is attained (from Schram 1993)..... 23
- Figure 1.6** (Attached) chalimus stages of *Lepeophtheirus salmonis* infesting a wild post-smolt sea trout (*Salmo trutta*, ~60 g), captured in brackish water in Wester Ross, W Scotland. The chalimi here are attached to the bones of the fin rays of the dorsal fin and their restricted feeding area has led to erosion of the dermal tissue and exposure of the fin ray bones. [Photo: C.D. Todd]..... 26
- Figure 1.7** The 18 Scottish Area Management Agreements (AMAs) signed as at September 2008. (Un-signed areas are shown in hatch)..... 36



<b>Figure 2.1</b>	Map of the Broughton Archipelago, BC, Canada (Courtesy of Google).....	39
<b>Figure 2.2</b>	A Broughton Archipelago, BC pink salmon ( <i>Oncorhynchus gorbuscha</i> ) smolt with several adult <i>Lepeophtheirus salmonis</i> attached. The topmost individual is a gravid female louse with paired eggstrings (courtesy of Alexandra Morton).....	40
<b>Figure 2.3</b>	Total number of sea trout caught by rod angling in the Connemara district between 1974 and 2001 [From report of the Sea Trout Review Group (2002); their Fig. 1]...	42
<b>Figure 2.4</b>	Photograph of a closed containment net-bag being used by Marine Harvest as part of an experimental trial at the Cusheon Cove site in British Columbia.....	46
<b>Table 4.1</b>	Typical sea lice sampling regimes in the major salmon-producing countries.....	58
<b>Figure 4.1</b>	The prevalence and abundance profile, with associated 95% confidence intervals, for <i>Lepeophtheirus salmonis</i> mobile sea lice over two-year production cycles on Scottish farms between 2002 and 2006.....	60
<b>Table 4.2</b>	Strategies for reducing sea lice infestation on salmon farms which do not involve the use of chemical treatments, as adopted by various countries. ....	61
<b>Table 4.3</b>	Treatments that have been, or are being, used to reduce sea lice infestation on salmon farms in various countries .....	66
<b>Table 4.4</b>	Treatment trigger levels which exist in various salmon-producing countries. (In some cases these are legislative requirements while in others they are guidelines adopted by industry.) .....	68
<b>Figure 4.2</b>	Sea lice on sea trout ( <i>Salmo trutta</i> ) [Photo: Bengt Finstad, NINA].....	70
<b>Table 4.5</b>	References for the geographical distribution of <i>Lepeophtheirus salmonis</i> on different species of wild salmonids ( <i>Salmo</i> and <i>Oncorhynchus</i> ) listed by country. [Revised version of Boxaspen et al. (2007)] .....	72
<b>Figure 4.3</b>	A fish farm in Norway [Photo: Eva B. Thorstad, NINA].....	73
<b>Figure 5.1</b>	Graph illustrating the output of three models which attempt to relate salmon skin surface area to fish weight.....	90

### **Comments regarding the combined approach to General Disease and Sea Lice reports**

The term “disease” captures a wide array of physical, physiological and population abnormalities. An exhaustive review of the state of knowledge on fish disease would require an evaluation of multiple disciplines, ranging from molecular biology to pathology to epidemiology as well as the consideration of a range of relevant species. Once the issues of how disease might affect conservation goals or ecosystem functions are added, additional information on ecology and the means to manage disease impacts, including legislation, open up for review. The breadth of such a suite of information is daunting. It is further complicated by the many contradictory findings and important uncertainties that exist around the issue of diseases of farmed salmon and their potential environmental impacts.

The General Disease Technical Working Group (TWG) consisted of 4 scientists from 4 different locations (Hammell from Eastern Canada, Stephen from Western Canada, Evensen from Norway, Bricknell from Scotland/Maine) and the Sea Lice TWG consisted of 4 more scientists (Revie from Scotland/Eastern Canada, Dill from Western Canada, Finstad from Norway, Todd from Scotland). The two groups initially met jointly to outline the breadth of the report and to further define the approach to evaluating sea lice issues in depth. The decision to cover general disease broadly and sea lice as the in-depth case study was the group’s attempt to address the seemingly impossible task of adequately describing the state of knowledge and research gaps for an area of research that spans many different disciplines and diseases across many areas of the world in which salmon is farmed. The Sea Lice report adopted the same basic outline except for specific headings that were irrelevant. The final chapter 6 (Addressing Unknowns in Disease Risk Management) of the General Disease Report contains comments contributed by both groups.

Our approach was based on answering the questions of 1) what is the risk of disease transfer from farmed to wild salmon (i.e. should we be concerned)? 2) can salmon farms avoid disease in their fish? 3) assuming that farms cannot avoid disease, can salmon farms adequately reduce the level of disease in their fish to a level that would reduce the risk of transfer to wild salmon? And lastly, 4) what are the gaps in knowledge regarding the risk of disease in farmed and wild fish when considered separately and when considered in each other’s presence? We then decided that there were 2 important reasons to take sea lice as the one disease to consider in greater detail using the same risk based approach: 1) sea lice issue has had a great deal of attention in peer-reviewed literature paid to ecology and the risk of interactions between farmed salmon and the environment, and 2) sea lice was identified by the Steering Committee (Salmon Aquaculture Dialogue) as a particular issue for focus. Essentially, the reports were generated as stand-alone reports but our combined approach provides breadth (general disease) and depth (sea lice) on which establishment of measurable standards can be discussed in the next stages of the process.

## Extended Summary

The collective term “sea lice” is colloquially used to refer to numerous species of copepod crustaceans of the family Caligidae that are externally parasitic on the skin of marine and anadromous fishes. The most intensively studied species - *Lepeophtheirus salmonis* - is, as its specific name implies, a specialist parasite of salmonid fishes. It is commonly associated with a total of 12 host salmonid fish species of the genera *Salmo*, *Oncorhynchus* and *Salvelinus* in the Pacific and Atlantic Oceans. Along the Pacific coasts of Alaska and British Columbia, *L. salmonis* as well as *Caligus clemensi* and *Lepeophtheirus cuneifer* (both host generalist lice species) have been recorded on wild and farmed salmonids. In British Columbia, Chile and Tasmania Atlantic salmon (*Salmo salar*) is the principal salmonid species in culture. While the Tasmanian industry apparently suffers no especial problems from caligid infestation, the Chilean industry has been heavily impacted by *Caligus* species, initially *C. teres* but more recently and significantly *C. rogercresseyi* (both of which are host generalists). In Japan, *Caligus orientalis* is the most pathogenic sea louse on cultured Pacific salmon, although *L. salmonis* also remains a problem. *L. salmonis* is associated with wild chum and pink salmon in Japan, but also infests cultured coho salmon and rainbow trout. *C. orientalis* – like *C. elongatus* in the North Atlantic – is a host generalist; *C. orientalis* occasionally impacts salmonids, and it is an especial problem to cultured rainbow trout.

The current scientific literature refers to *Lepeophtheirus salmonis* Krøyer as occurring on salmonids in both the North Pacific and North Atlantic Oceans. At first sight it might appear curious that the same species should occur in two separate and geographically distant oceans, but there is convincing geological, molecular and ecological evidence of past trans-Arctic connectivity of the marine fauna of the North Pacific and North Atlantic basins — and specifically of Pacific species having tended to colonize the North Atlantic rather than *vice versa* — following the recent opening of the Bering Strait (~5 million years ago). The presently available molecular (DNA) results cannot provide conclusive evidence regarding the specific status of Pacific and Atlantic *L. salmonis*, but recent DNA sequence analyses do indicate clear genetic distinction between the Atlantic and Pacific lineages. Similarly, there now is a body of mitochondrial DNA sequence and ecological (host association) data indicating that the host generalist parasite, *Caligus elongatus*, actually comprises taxonomically separable entities. It is, however, too early to affirm that there are definitely two (or more) species of either “*C. elongatus*” or “*L. salmonis*”; for clarity and consistency with the contemporary scientific literature we continue in the present report to refer to single species in both cases.

All female caligids undergo internal fertilization of the eggs prior to their extrusion into a pair of external egg sacs or “eggstrings”. The nauplius I is the hatching stage and at this point the eggstring disintegrates and the larvae are released to become planktonic. The nauplius I molts into a nauplius II and then again into the infective copepodid stage. None of the three planktonic stages feed; all the reserves the larvae require to complete development to the infective copepodid are provided by the parent female. Initial attachment for the copepodid

typically occurs on the fins of the host fish (especially the dorsal, ventral and anal fins) or to the scales. Several chalimus stages follow, attached to the host by a sort of tether. Depending upon the species of caligid there then may be two, one, or no pre-adult stages between chalimus IV and the mature adult. The pre-adult and adult stages are all mobile, i.e., able to move about on the host fish's body.

When large numbers of farmed salmon are introduced to the marine environment in open net cage salmon farms, three things are virtually inevitable for these fish:

- they will become hosts to sea lice (*Lepeophtheirus* spp. and/or *Caligus* spp.) since these occur naturally on wild host species in the vicinity of most farms;
- they will become part of a dynamic host-parasite system involving wild hosts, because they can produce large numbers of infective larvae in a restricted spatial area if gravid females are allowed to develop; and
- because they carry sea lice, and because some of these fish may escape from the farms, the dispersal of parasites is likely to be even more widespread on occasion.

Given the above, it may be concluded that it is next to impossible to (1) avoid infection of farmed fish, all of which go into the pens as clean smolts, and (2) also subsequently avoid infection of wild fish that are found in the vicinity ("infective field") of an open cage farm.

A parasitic infection becomes a disease when host behavior and physiology (and ultimately host health, survivorship and fitness) are altered or compromised to an exceptional extent. For example, the increased metabolic demand exerted by the parasite may cause slower host growth, making the wild fish more likely to be captured by predators, or conversely causing them to take greater risks to feed, with the same end result. Reduced host condition also can affect swimming ability, with several negative ecological consequences ranging from reduced competitive ability to slower migration. Slower migration rates through coastal waters might elevate the risk of infestation by sea lice copepodids. Skin damage caused by the feeding behavior of sea lice can increase the physiological cost of osmotic regulation, or provide sites for secondary bacterial or fungal infection. Contrary to typical perceptions that it is not "in the interest" of parasites to kill their host, it is worth noting that sufficiently high sea lice loads will kill individual wild fish, but the definition of "high" will depend on sea louse stage, fish size and developmental stage. While not exhaustive, this list illustrates some of the direct and more subtle indirect ways that sea lice may cause disease, as defined above.

Sea lice abundance on farmed salmon only rarely attains levels where the health or welfare of these fish is negatively affected. When this occurs there are legal and regulatory requirements in many countries that demand treatment, but it is clearly also in the economic interests of the farmer to treat the infection promptly and effectively. For wild fish, disease is likely to be an issue whenever sea lice intensity on individual hosts is sufficiently high as to cause significant stress, or to increase their vulnerability to secondary pathological infection or other mortality agents, as noted above. For example, newly-migrated smolts exposed to the challenge of osmoregulating in saline waters will be physiologically stressed by that

environmental challenge and will be more vulnerable than post-smolts that are older and fully adapted to seawater.

Sea lice disease of wild salmonids is potentially problematic in areas with intensive Atlantic salmon aquaculture in British Columbia, Canada, on the west coasts of Ireland and Scotland, and throughout Norway (Chile lacks endemic species of anadromous salmonids). In British Columbia, the focus of attention has been on the much studied and discussed Broughton Archipelago region, where there is particular concern regarding the impact of *Lepeophtheirus salmonis* on wild stocks, particularly juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon.

Unlike Atlantic salmon, sea trout (the anadromous form of the brown trout, *Salmo trutta*) spend extended periods of time in nearshore or coastal waters, and this feature may render them particularly vulnerable to sea lice infestation. As has been the case in British Columbia, analyses concerning the potential interaction between farmed and wild salmonids in Irish bays, Scottish sea lochs and Norwegian fjords subject to intensive aquaculture have not been without controversy. The circumstantial evidence of farm-produced larval sea lice contributing to parasite loadings on wild sea trout in Ireland is considerable. Correlations have been drawn between abundances of sea lice on wild sea trout and on Irish farms up to 30 km distant. As is the case for sea trout, Arctic charr (*Salvelinus alpinus*) are effectively confined to coastal waters (often in narrow fjord systems) and these areas commonly are home to a high density of captive farmed salmon. Sea trout and Atlantic salmon are also the species of most concern with regard to detrimental effects of sea lice in Norway, although Arctic charr also are impacted by these parasites there.

It has been demonstrated that salmon within a given farm site can be self-reinfesting (because hatched nauplii drift back into the net pens having completed their development to the infective copepodid stage); it is also intuitive that nauplii exported from one farm site will infect salmon being grown in neighbouring farms or free-ranging wild fish in the vicinity. Similarly, wild fish may well infect other wild fish or, if they are resident in coastal waters, adjacent farmed fish. The absolute abundances of farm and wild fish, the absolute abundances of sea lice on those fish and the relative strengths of farm-farm and farm-wild interactions (and any seasonal or annual variation thereof) will determine the overall infestation pressure on individual fish in a given locality.

It is far easier to monitor and assess the outcome of interventory treatment for sea lice infestations for farmed fish than it is for wild fish, and there are potentially many more strategies available to control sea lice on farmed fish. For example, in addition to medicinal treatments, a variety of management (and even informed environmental) decisions can be made which can have impacts on the control of sea lice on farms. The challenges in managing sea lice on farmed and wild fish in an integrated manner should not, however, be underestimated.

As we outline, the evidence is largely indirect or circumstantial that sea lice emanating from salmon farms can and do exert detrimental effects on wild salmonids. It is practically

impossible to track larvae from release to host colonization and therefore to precisely quantify wild-to-farm versus farm-to-wild and wild-wild infestation interactions. Furthermore, in view of the diversity of life-history strategies and differential vulnerability of host species associated with sea lice in both the Pacific and Atlantic Oceans, as well as the geographic differences in the intensity of the industry and its regulation, it is not plausible to draw a single over-riding conclusion regarding the potential negative impacts of sea lice on all wild fish stocks world-wide. Nevertheless, we believe that the weight of evidence is that sea lice of farm origin can present, in some locations and for some host species populations, a significant threat. Hence, a concerted precautionary approach both to sea lice control throughout the aquaculture industry and to the management of farm interactions with wild salmonids is expedient.

It is arguably the case that sea lice are one of the most studied diseases of aquaculture and, as such, thinking in terms of ‘system-wide’ management has been relatively well developed. The principles of Integrated Pest Management (IPM) have been taken from the terrestrial setting and attempts made to apply them to sea lice in an inclusive and comprehensive fashion. In addition, as mentioned earlier, sea lice infestation on salmon farms has been a matter not only of control on farms to maximize cultured fish health and well-being, but of significant public and scientific controversy; these issues arose initially and most notably with respect to wild sea trout populations in Ireland and Scotland, Atlantic salmon and sea trout in Norway and, more recently, for the case of wild Pacific salmon in British Columbia. Management of wild-farm and farm-farm infestation interactions is not a simple challenge, if only because of our present inability to reliably quantify them. Given the impossibility of directly observing and tracking individual sea lice larvae from release by the adult female to ultimate settlement on a host fish, alternative indirect analytical approaches have proven necessary to specifically assess farm-wild interactions. The utility and limitations of these various empirical methods (e.g. molecular genetics and stable isotope markers) has been reviewed. A conceptually different, but complementary, analytical approach has been the development of mathematical models to enable both a better understanding of infection dynamics and to aid decision makers in exploring assumptions regarding underlying management parameters and the effectiveness of potential intervention strategies. Once again, these models are much more diverse and complex than is typical for most pathogens within the aquatic setting. However, in further complicating the debate as to the importance of farm sources of infestation to wild fish, in a number of cases the models themselves have become a source of controversy. This is not necessarily a “bad thing” as it is arguably not the place of mathematical modeling to produce answers/solutions, but rather to encourage policy makers, commercial farmers, sport fishery managers, and scientists to think more carefully about their assumptions and the likely impact of various types of intervention.

Another important issue relates to the optimal location of salmon farms; establishment of “safe sites” should lead to minimizing risks and maximizing benefits to all concerned parties. Indeed, research in this area has led to a number of recent projects – for example, the Hardangerfjord project in Norway or the Finite Volume Coastal Ocean Model in British Columbia – which have attempted to tackle aspects of the problem through the use of fjord/sea loch/archipelago-wide hydrographic modeling to improve our understanding of dispersal and

colonization of sea lice larvae. This has also led to changes in policy, for example, in Scotland the Location/Relocation Working Group (LRWG) of the Scottish Government has the remit to, “prepare criteria to assess whether or not any finfish aquaculture site is poorly located, and make an assessment of the likely benefits and effectiveness of relocation of those farms that are sited close to rivers important for migratory fish” (<http://cci.scot.nhs.uk/Topics/Fisheries/Fish-Shellfish/whatwedo/whatwedo5>).

## Chapter 1: Sea Lice as Disease Organisms

This report on sea lice was written in conjunction with a more general disease report (Hammell et al. 2009) as part of the WWF-coordinated Salmon Aquaculture Dialogue. The authors of both reports decided to follow, where sensible, a common report structure with this document developing in more detail general themes laid out in the main disease report for the case of sea lice. [See the 'Prologue' section of the general disease report for more discussion.]

### 1.1 Introduction

Disease in the human context generally is considered in terms of bacterial or viral infection, which is itself often manifested by the host as recognizable and specific symptoms. Parasites can be considered as pathogens, or organisms capable of causing disease, if the behavior or physiology (and ultimately the health and/or survivorship) of the host organism is altered or compromised to an exceptional extent. The difficulty lies in defining the extent of that impairment. Sea lice are natural parasites of many marine fish species. Can a large, healthy adult fish of several kilograms weight, bearing a single parasitic sea louse (weighing a few milligrams) be considered "diseased"?

In defining whether or not parasitic sea lice present a "disease" problem to wild and farmed fish, it is necessary to assess the infection intensity in relation to the size and species of the host fish. *Lepeophtheirus salmonis* is exceptional among parasite species in infecting adult wild Atlantic salmon (*Salmo salar*) with 100% prevalence. In most host-parasite associations, prevalence typically is much below 100%; random chance effects alone dictate that some individual hosts in a given population will never be encountered or successfully infected by the parasite species. Shaw & Dobson (1995) reviewed quantitative studies for 211 parasite species and of these only 15 species showed prevalence >90% and only two (both endoparasites of grouse and reindeer) displayed 100% prevalence. The infective planktonic larval phase of *L. salmonis* is therefore extraordinarily effective at locating and infecting wild Atlantic salmon, even in the open North Atlantic Ocean. For this reason alone, it is highly likely that *L. salmonis* has the potential to present a disease threat to salmonid fish.

Adult one sea-winter Atlantic salmon returning to the British Isles typically weigh between one and four kilograms at the completion of their marine migration. Not only is the prevalence of *Lepeophtheirus salmonis* at 100%, but their mean abundance typically is high and varies between 17 and 31 *L. salmonis* per fish (Todd et al. 2006). Can these fish be considered "diseased"? Mean values can be misleading in this context because, as is typical of host-parasite associations, *L. salmonis* shows 'over-dispersion' amongst the fish hosts; that is, most hosts carry a low abundance of parasites, but a few individual hosts can carry extremely high



burdens and at the population level the variance in abundance exceeds the mean. As an illustration, seven years of monitoring data for *L. salmonis* on return-migrant wild Atlantic salmon in Scotland showed maximum abundances ranging up to 117 (horizontal axis, Figure 1.1). But it is important to note also that, in some years, mean abundance of *Caligus elongatus* (a host generalist sea louse that also infests salmonids) can equal that of *L. salmonis* (Todd et al. 2006); wild salmon typically are infested by both species because *C. elongatus* prevalence itself ranges from 90 to 100%. Figure 1.1 shows, for example, that certain individual fish carried high burdens of both species (e.g. 63 *L. salmonis* + 37 *C. elongatus*, 55 *L.s.*+83 *C.e.*; 45 *L.s.*+107 *C.e.*; 40 *L.s.*+100 *C.e.* etc). As a generalization, when both species are relatively abundant on the sampled fish (e.g. 2001, 2002, 2003) fish that carried a high abundance of one species tended also to carry a high abundance of the other, perhaps reflecting that individual salmon are similarly vulnerable to either species of sea louse.

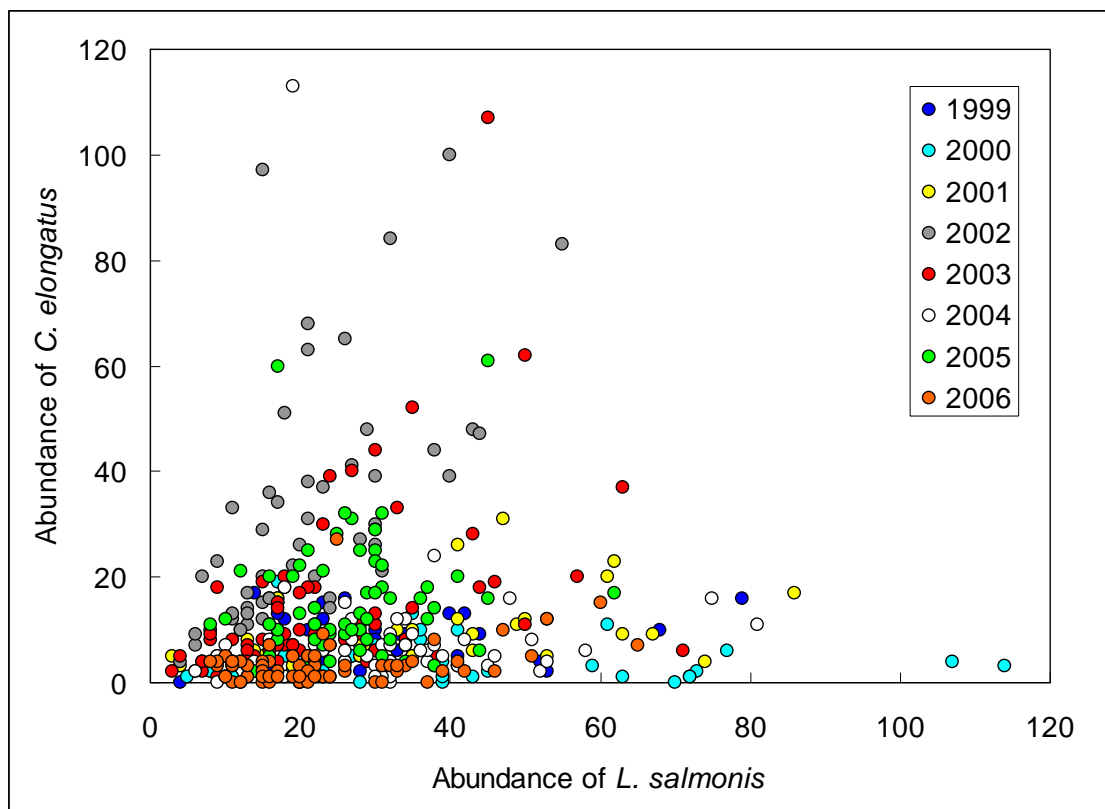


Figure 1.1 Abundance of sea lice (*Lepeophtheirus salmonis*, *Caligus elongatus*) on individual wild, one sea-winter, adult Atlantic salmon (*Salmo salar*) (n = 430; length, weight ranges: 49-77.5 cm, 1.1-4.6 kg) captured in fully marine seawater at Strathy Point, N Scotland 1999-2006. [Data are for 'mobile' stages only and do not include chalimus larval stages.]

## 1.2 Defining disease

### 1.2.1 Can fish infested by sea lice be considered “diseased”?

The most straightforward operational definition of a “diseased” individual fish is if the host is behaviorally or physiologically compromised by sea louse infestation. Physiological compromise can be measured objectively as suppression of the immune system, osmoregulatory dysfunction, or stress. Several recent studies have focused on either the characterization or quantification of the immune response to sea lice infestation (e.g. Fast et al. 2004) or physiological biomarkers of stress (e.g. Grimnes & Jakobsen 1996, Bjørn & Finstad 1997, 1998; Wells et al. 2006, 2007). Harmful infestation levels of *Lepeophtheirus salmonis* to host smolts and post-smolts have been estimated both in the laboratory (Bjørn & Finstad 1997) and in the laboratory and field (Finstad et al. 2000). Finstad et al. (2000) showed that once *L. salmonis* attained the pre-adult and adult stages this led to an osmoregulatory imbalance for sea trout (*Salmo trutta*) and salmon (*Salmo salar*) smolts and to the mortality of sea trout smolts. Bjørn & Finstad (1997) estimated that infestations exceeding 90 attached chalimi or 50 pre-adult or adult (colloquially ‘mobile’) *L. salmonis* could kill 60 g sea trout post-smolts. Finstad et al. (2000) reported that >30 chalimus stage larvae could kill 40 g salmon smolts once they developed into pre-adults and became mobile over the host body surface; the relative intensity of ~0.75 (lice.g<sup>-1</sup> fresh weight) therefore indicated that only 11.3 chalimus larvae may have a detrimental effect on a wild salmon smolt of 15 g. This level of infection also caused mortality of wild salmon smolts in aquarium experiments (Finstad et al. 2000). Note, however, that mass of salmonids increases approximately as the cube of body length so for larger fish beyond the smolt stage this detrimental weight-specific loading will probably differ markedly with increasing size. Because in the natural environment only survivors generally remain available for capture and sampling by observers, Holst et al. (2003) suggested that a lack of wild smolts with >11 lice in Norway may be explained by mortality of more heavily-infested hosts.

In subsequent physiological studies, and utilizing a suite of stress markers and empirical measurements, Wells et al. (2006) concluded that 13 mobile sea lice per fish was the critical abundance which elicited sub-lethal stress responses in wild post-smolt sea trout (*Salmo trutta*) in the weight range 19-70 g (mean 37 g). Thus, while juvenile sea trout with a burden of 14 sea lice could then be objectively categorized as significantly stressed, and hence “diseased”, that argument would not extend to a 4 kg adult Atlantic salmon also infected with 14 sea lice, but which would not be significantly stressed. However, even though at the individual fish level that 4 kg host salmon would not be considered “diseased” it is clear that, in an epidemiological context, at the population level that fish should be considered diseased because the sea lice it carries have the capacity to infect other farmed and wild salmonids. Thus, the categorization of sea lice as a “disease” can vary according to the context in which the term is being applied.

None the less, it must be acknowledged that even a single sea louse infecting an otherwise healthy adult salmon still can cause damage to the host skin, as a result of the parasite's feeding activity. The skin and its mucus covering is a critically important barrier to ion exchange with the surrounding water and while that damage might not in itself induce osmoregulatory compromise or dysfunction, such lesions of the fish epidermis can expose the host fish to pathological secondary bacterial infection. Furthermore, there are indications and reports that sea lice themselves may also be vectors of microbial and bacterial diseases (e.g. Infectious Salmon Anaemia (ISA), Nylund et al. 1993; Pancreatic Disease (PD), Marian McLoughlin, pers. comm. 2008) though this has yet to be demonstrated conclusively.

An additional concern when assessing whether or not a sea louse-infected salmonid is diseased is that there is clear evidence that different species of salmonids vary in their susceptibility to infestation by *Lepeophtheirus salmonis* (e.g. Fast et al. 2002). Nagasawa (1987) and Nagasawa et al. (1993) reported on differences in prevalences and abundances of *L. salmonis* infecting six Pacific species of salmonids captured in the open ocean: pink salmon (*Oncorhynchus gorbuscha*) showed the highest prevalence and abundance of *L. salmonis*, followed by chinook salmon (*O. tshawytscha*) and steelhead trout (*O. mykiss*). Whereas they recorded relatively high levels of infestation on coho (*O. kisutch*) and chum (*O. keta*) salmon, they did find sockeye (*O. nerka*) to be relatively rarely infected (cf. Atlantic salmon; Section 1.0 above) but whether these can be considered compromised or "diseased" remains unclear. Beamish et al. (2005) recorded "sea lice" (i.e. *L. salmonis* plus *Caligus clemensi*) at high intensities and 98-100% prevalence on pink (*Oncorhynchus gorbuscha*), chum (*O. keta*), sockeye (*O. nerka*), chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon in coastal waters of British Columbia. However, because Beamish et al. (2005) did not numerically distinguish the two caligids within hosts in their tabulations it is not possible to derive the prevalences or abundances of either parasite on particular host species. On the basis of the relative abundances of the various host species, and their typical parasite loadings, Nagasawa et al. (1993) considered pink and chum salmon to be the most important host species in the North Pacific. Multiple host species laboratory infestation trials also have indicated clear differences in susceptibility of salmonid species, and coho are seemingly relatively resistant to initial infection (Fast et al. 2003); Atlantic salmon appear to be more susceptible than Pacific species (e.g. chinook and coho salmon; Johnson & Albright 1992) and *Salmo trutta* may be more susceptible than is *S. salar* (Dawson et al. 1997).

### **1.2.2 The wider perception of sea lice as benign parasites or as a disease organism**

In terms of describing sea lice as a "disease" the very nature of the term disease has negative connotations, in the sense that we conceptualize disease as having a detrimental or

debilitating effect on the host organism. It is pertinent, therefore, to note that sport anglers still consider the occurrence of sea lice on a fish captured in freshwater as being a positive indicator of the “freshness” and (assuming only a moderate number of parasites) the quality of the fish. This is because female caligids rapidly lose their eggstrings when the host fish re-enters freshwater, and the occurrence of so-called “long-tailed sea lice” on a captured salmon still is widely considered to be the ultimate indicator of freshness.

Infestations of *Lepeophtheirus salmonis* can cause visibly obvious skin erosion and lesions if the intensity of the infestation is moderate to high. For moderately to heavily infested fish these lesions are especially obvious along the dorsal midline between the dorsal fin and the tail, and on the skin adjacent to the anal fin, where the adult females tend to aggregate (Todd et al. 2000; Figures 1.2, 1.3 and 1.4).



Figure 1.2 Adult female *Lepeophtheirus salmonis* occupying the skin adjacent to the anal fin of an adult two sea-winter Atlantic salmon (~7 kg) captured in fully marine seawater at Strathy Point, N Scotland. Note the (pink) erosion of the epidermis and associated bleeding of the lesion. [Photo: C.D. Todd]

Many sport anglers today still erroneously interpret the erosion adjacent to the anal fin (e.g. Figure 1.4) as being “running marks” where the fish has been scraping its body over stones and rocks as it ascends the river. Again, as with the presence of adult females still bearing their eggstrings, capture of a fish with “running marks” commonly is viewed positively as an indicator of freshness and quality.



Figure 1.3 Adult female *Lepeophtheirus salmonis* (most with paired eggstrings) occupying the dorsal midline between the dorsal fin and adipose fin of a wild, one sea-winter adult Atlantic salmon (2.80 kg) captured in fully marine seawater at Strathy Point, N Scotland. The sea louse abundance ('mobile' stages only) on this fish was 52 *L. salmonis* and 2 *Caligus elongatus*. [Photo C.D. Todd]

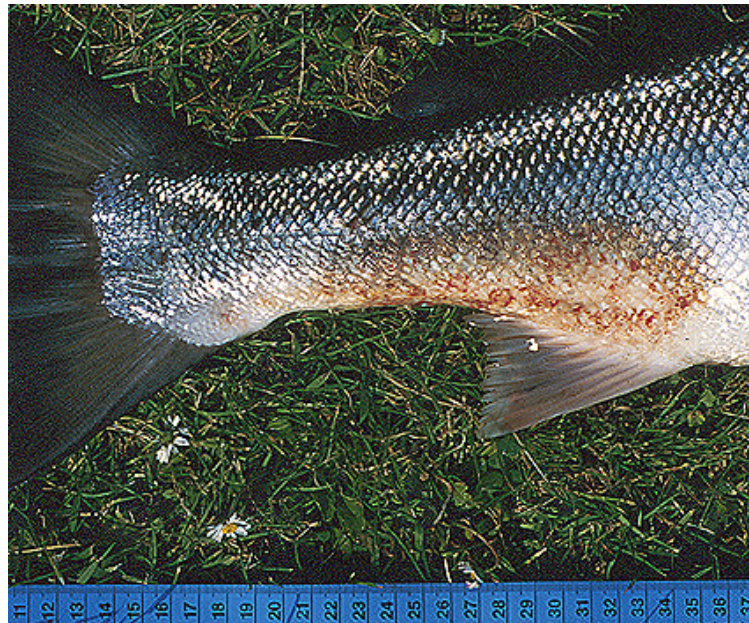


Figure 1.4 Adult two sea-winter Atlantic salmon (~8 kg), captured in estuarine conditions in the Firth of Tay, E Scotland, showing the typical lesions caused by adult female *Lepeophtheirus salmonis* on the scales and skin adjacent to the anal fin. [Photo C.D. Todd]

### 1.2.3 The salmon louse, *Lepeophtheirus salmonis*: Pacific and Atlantic populations

The current scientific literature refers to *Lepeophtheirus salmonis* Krøyer as occurring both in the North Pacific and North Atlantic Oceans and infesting salmonids (*Salmo* spp., *Oncorhynchus* spp., *Salvelinus* spp.). There is convincing geological, molecular and ecological evidence of past trans-Arctic connectivity of the marine fauna of the North Pacific and North Atlantic basins (e.g. Dodson et al. 2007) — and specifically of Pacific species having tended to colonize the North Atlantic rather than *vice versa* — following the recent opening of the Bering Strait (~5 million years ago; Marinovich & Gladenkov 2001). There have been repeated re-openings of the Bering Strait but many trans-Arctic invasions apparently occurred ~3.5 million years ago. The predominance of Pacific species invading the Atlantic seems to be explained by the bulk of water flowing through the Strait to the north, perhaps with concomitant effects on planktonic larval transport. Morphologically, Pacific and Atlantic *L. salmonis* are apparently indistinguishable, but certainly there are genetic differences at the ocean basin level. Microsatellite DNA loci include tandem repeat sequences of the four nucleotide bases (T,A,G,C) of the DNA molecule. These loci typically are highly variable and are generally considered to be selectively neutral (i.e. not adaptive); they offer a powerful and commonly used means of assessing and comparing the genetic structure of populations. Analyses of microsatellite DNA variation (Todd et al. 2004) showed no significant differentiation of *L. salmonis* populations sampled from wild and farmed salmonids throughout the North Atlantic (E. Canada to N. Norway). There were, however, significant differences in microsatellite allele frequencies at all the loci studied, and hence overall population genetic structure, for *L. salmonis* from a Pacific (British Columbia) farm population compared to the pooled data from all sites and all three host species sampled for the North Atlantic population. Very closely related, but distinct, species can share microsatellite loci and it is relevant that *C. elongatus* shares none of these six *L. salmonis* microsatellite DNA sequences.

Those DNA results cannot provide conclusive evidence regarding the specific status of Pacific and Atlantic *Lepeophtheirus salmonis*, but recent DNA sequence analyses (Yazawa et al. 2008) do indicate clear genetic distinction of the Atlantic and Pacific lineages. Together with other evidence, Yazawa et al. (2008) conclude that the reduced genetic diversity specifically of the 16S rRNA and *COI* gene sequences for Pacific *L. salmonis* are indicative of an Atlantic origin for this species. Whether or not North Pacific and North Atlantic *L. salmonis* lineages have diverged sufficiently to now comprise closely-related and morphologically similar, but taxonomically distinct species remains unclear. But the likelihood is that genetic differences between Pacific and Atlantic “*L. salmonis*” might well be sufficient to warrant taxonomic separation into two distinct species. Similarly, there now is a body of genetic (mitochondrial DNA sequence) and ecological (host association) data to indicate that the host generalist parasite, *Caligus elongatus*, actually comprises taxonomically separable entities (Øines & Heuch

2005, 2007). It is, however, perhaps too early to affirm that there definitely are two (or more) species of “*Caligus elongatus*” and we have, for the purposes of the present report, continued to refer to the one species of *C. elongatus*.

### **Host associations of *Lepeophtheirus salmonis* and other caligids**

While recognizing that there are probably two species of “*Lepeophtheirus salmonis*” in the North Pacific and North Atlantic Oceans here we assume only the one species in the interests of clarity and in order to remain consistent with the published scientific literature to date. Whether Pacific and Atlantic *L. salmonis* are one and the same species is not simply of semantic importance. For example, much of the apparent among-host species variability in susceptibility of salmonids to *L. salmonis* infestation may be explained by Atlantic and Pacific *L. salmonis* being distinct species. However, given the assumption of the one species, *L. salmonis* is associated with a total of 12 host salmonid fish species of the genera *Salmo*, *Oncorhynchus* and *Salvelinus* (Costello 2006). Along the Pacific coasts of Alaska and British Columbia, *L. salmonis*, *Caligus clemensi* (a host generalist), and *Lepeophtheirus cuneifer* (also a host generalist) all have been recorded on wild and farmed salmonids. In British Columbia, Chile and Tasmania (Australia) *Salmo salar* is the principal salmonid species in culture. While the Tasmanian industry apparently suffers no especial problems from caligid infestation of farmed salmon (but is impacted significantly by Amoebic Gill Disease, AGD), the Chilean industry is heavily impacted by *Caligus teres* and especially *Caligus rogercresseyi* (both host generalists). In Japan, *Caligus orientalis* is the most pathogenic sea louse on cultured Pacific Salmon, although *L. salmonis* also remains a problem (Nagasawa 2004; Costello 2006). *L. salmonis* is associated with wild chum and pink salmon in Japan, but also infests cultured coho salmon and rainbow trout. *C. orientalis* – like *C. elongatus* in the North Atlantic – is a host generalist which occasionally impacts upon salmonids but it is an especial problem to cultured rainbow trout (Nagasawa 2004).

#### **1.2.4 Establishment of parasitic disease**

In the General Disease Report, the distinction is made between infectious (bacterial, viral, fungal) disease and parasitic disease (their Section 1.2). Three phases of infectious disease (1, initial colonization; 2, infection; 3, pathological disease manifestation) are distinguished. The same three criteria, or phases, can be extended to sea lice as pathogens and their establishment (and categorization) as disease organisms on host fish.

### Phase I: Initial colonization

All female caligids undergo internal fertilization of the eggs prior to their extrusion into a pair of external egg sacs or “eggstrings” for further embryonic development towards hatching (Figure 1.5). The female retains the eggstrings as the embryo develops to the hatching stage. The nauplius I is the hatching stage and at this point the eggstring disintegrates and the larvae become planktonic. Planktonic organisms, by definition, cannot swim directionally against the current; they drift passively but do have the ability to adjust their vertical depth in the water column. Caligid larvae do not feed in the plankton and all energetic requirements for planktonic development to the infective stage are provided by the female during vitellogenesis and pre-fertilization maturation of the egg. The free-swimming nauplius I molts into a nauplius II and then again into the infective planktonic copepodid stage. Planktonic development from hatching to the copepodid takes 2-14 days according to species and ambient temperature. During this planktonic phase the larvae drift on currents and may migrate vertically in the water column, perhaps with a diurnal rhythm (e.g. Heuch et al. 1995; Aarseth & Schram 1999; Flamarique et al. 2000; Hevrøy et al. 2003). There is evidence that suggests *Lepeophtheirus salmonis* also may modulate its vertical migratory behavior in response to changing salinity (e.g. Heuch 1995).

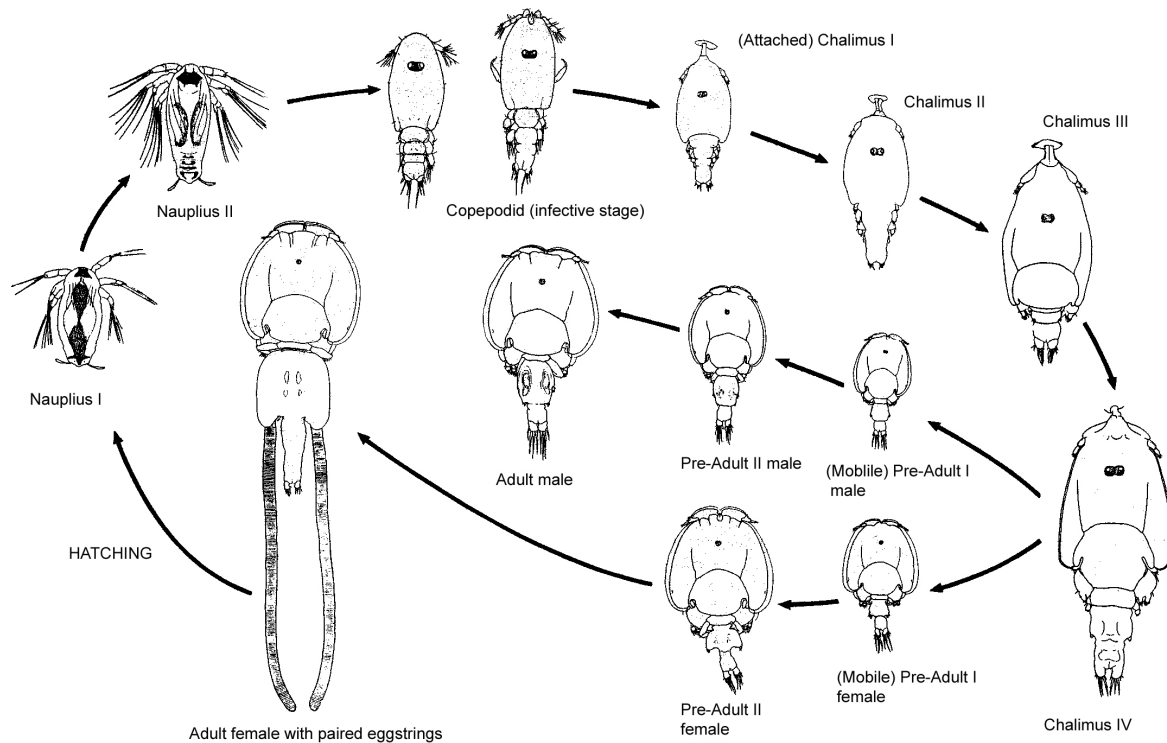




Figure 1.5 The molt stages and life cycle of *Lepeophtheirus salmonis*. The gravid female extrudes a pair of eggstrings containing the developing embryos and these are retained to the hatching stage (Nauplius I). After two planktonic larval molts host infestation occurs at the final free-swimming, copepodid stage and the subsequent four chalimus stages all are attached to the host fish by a thread ('frontal filament'). The pre-adult and final adult stages all are mobile over the host fish. No further molts occur after the adult status is attained (from Schram 1993).

Typically, planktonic organisms reside at greater depths during daylight hours and migrate upwards at night. However, in general the vertical migratory behavior of the planktonic stages of sea lice (and their responses to, for example, salinity changes) is poorly understood and also probably critical to an understanding of the infestation dynamics between host and parasite (e.g. Heuch et al. 1995; Hevrøy et al. 2003). Given the "through-flow" nature of salmon farm pens and net cages, it clearly is not possible for the larval stages of sea lice to be retained throughout their planktonic development period within their cage of origin (but see Costelloe et al. 1996 for heavily-fouled net meshes) — the planktonic larvae will be uncontrollably exported from the cage and they therefore have the potential to infect (1) host fish in other cages within the same farm, (2) other farms within drift-distance of the farm of origin, and (3) wild fish passing sufficiently close to the farm. By the same token, larvae emanating from free-ranging wild fish clearly have the potential to infect caged farm populations if those larvae are released in coastal waters. Because of their finite longevity and being limited by maternally-provided reserves of energy, larvae hatching from salmonids occupying their offshore oceanic feeding areas will be able to infect only other wild hosts and will not impact farmed salmonids held in sheltered or semi-enclosed coastal waters. In this sense it is inescapable that the initial infestations of cultured salmonids in the earliest days of the industry must have been attributable to wild fish resident in, or migrating through, coastal waters. Taking the specific example of the Scottish industry, this was initially developed during the 1960s by Unilever at a site near Lochailort. The first commercial smolts were put to sea in 1972, and by a date as early as 1976 it became apparent that *Lepeophtheirus salmonis* was problematic to the industry (Rae 2002).

Initial colonization by sea lice in the natural environment and in farm pens is very poorly understood. In fjordic systems, in NW Europe the infective copepodids appear to concentrate at salinity discontinuities ("fronts", or sharp discontinuities in temperature or salinity) near to the outfall of rivers (e.g. McKibben & Hay 2004; Penston et al. 2004), and it is here that coastal sea trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) — which remain in inshore waters throughout their marine residence — are perhaps especially vulnerable to initial colonization.

However, Bricknell et al. (2006) have proposed that colonization of newly-migrating smolts occurs in the coastal zone away from river outfalls, and at salinities that are closer to oceanic levels, and >27 practical salinity units (psu). Certainly, this seems to extend to fish in sentinel cages in the Norwegian Hardangerfjord system (Finstad et al. 2007). Whether it be for wild smolts first entering salt water, or farmed fish held within net pens, or even free-ranging wild adult fish in offshore waters and the open ocean, the likelihood is that it is the host fish that “finds” the parasite (infective copepodid) and not the parasite that “finds” the fish (see, e.g., Hevrøy et al. 2003). That is, as a result of diurnal vertical migration, and perhaps also responses to local changes in salinity, the infective copepodid probably positions itself at the appropriate depth in the water column where the host salmonid tends to forage or migrate and thereby enhances the potential for encounter.

Less is known about how the copepodid attaches initially in the natural environment but it is likely from laboratory investigations that the infective larva can respond to physical stimuli (light, shadow, vibration changes), and perhaps biological cues (e.g. Ingvarsdóttir et al. 2002; Genna et al. 2005), indicating the proximity of a potential fish host and to attach itself temporarily. In the case of *Lepeophtheirus salmonis*, the copepodid clearly has the capacity to chemically distinguish between a salmonid and a non-salmonid fish once it has initially attached (e.g. Ingvarsdóttir et al. 2002). Although no published laboratory observations are available of settlement and attachment responses of *L. salmonis* copepodids to non-salmonid hosts, semiochemical studies with adult male *L. salmonis* (e.g. Ingvarsdóttir et al. 2002; Genna et al. 2005) do indicate a clear ability for *L. salmonis* to distinguish salmonid from non-salmonid fish. The expectation is, therefore, that the specificity of the *L. salmonis*-salmonid host association is maintained by positive responses of the larval and post-larval stages to salmonids only. Initial attachment for the copepodid typically occurs on the fins of the host fish (especially the dorsal, ventral and anal fins) or the scales. If the host fish is of an appropriate species the copepodid clasps the host tissue and then undergoes a molt to the first sessile stage in the life cycle. At this stage the parasite attaches itself typically to a bony fin-ray or a calcified scale by means of a ‘frontal filament’ (penetrative thread) which punctures the epidermis. This stage is termed the chalimus and successful molting of the copepodid and permanent larval attachment of the chalimus stage can be considered the completion of initial colonization.

## **Phase II: Infection**

Penetration of epidermal tissues by the frontal filament can elicit a localized inflammatory response by the host fish, and this may be the reason some *Oncorhynchus* spp. can successfully reject attached chalimi (Wagner et al. 2008). For juvenile *Salmo trutta* the site of attachment can be visibly recognizable as a small black spot. Host tissue responses to

attachment and feeding of sea lice appear to be limited, although the details of the host immune response to sea lice infestation do vary among *Salmo* spp. and *Oncorhynchus* spp. and only now are beginning to emerge (Wagner et al. 2008). Pink and chum salmon appear to differ in their susceptibility to initial infestation by copepodids (e.g. Jones et al. 2007) and non-specific, localized immune responses of both of these species to initial colonization can be effective in ridding experimental single-pulse infestation before the larvae molt on to the fourth and final chalimus stage (Wagner et al. 2008). However, this resistance does not appear to be fully developed until the fish exceed about 0.7 g in weight (Jones et al. 2008). Single-pulse challenge is the simplest and most typically used experimental protocol, whereby copepodids are introduced to the experimental tank just once. This will differ from the natural environment in that fish there generally will be exposed to much lower densities of larvae but perhaps repeatedly so and over an extended period of time. Depending upon the species of caligid there may be two, one, or no pre-adult stages between chalimus IV and the mature adult. *Lepeophtheirus salmonis*, for example, undergoes two pre-adult stages, *Caligus clemensi* has one pre-adult stage, while *C. elongatus* has none and molts directly from the chalimus IV to the mature adult. Irrespective of the presence/absence or number of pre-adult stages, the key aspect of this point in the sea louse life-cycle is that the chalimus attachment now is permanently lost and the parasite becomes free-ranging (colloquially, “mobile”) over the body of the host fish. The potential for parasite-induced damage of the host epidermis as a result of feeding activity is markedly increased once the parasite becomes ‘mobile’ over the host body.

### **Phase III: Disease**

Although attached chalimi can cause severe erosion of host fins and scales if infection intensity is especially high (e.g. Figure 1.6), it is generally at the so-called “mobile” stages that pathological effects of sea lice become especially profound for the host fish. This is attributable to both the degree and extent of epidermal and dermal damage caused by the feeding activity of the mobile stages of the parasite (e.g. Figure 1.4). As skin damage and lesions develop and extend, the fish is liable to suffer osmoregulatory dysfunction and physiological stress, and also to become increasingly vulnerable to secondary microbial infection. Depending upon the species and size of the host fish, and the number of established sea lice, it is at this point that the fish can be considered “diseased”.



Figure 1.6 (Attached) chalmus stages of *Lepeophtheirus salmonis* infesting a wild post-smolt sea trout (*Salmo trutta*, ~60 g), captured in brackish water in Wester Ross, W Scotland. The chalimi here are attached to the bones of the fin rays of the dorsal fin and their restricted feeding area has led to erosion of the dermal tissue and exposure of the fin ray bones. [Photo courtesy of Sally Northcott]

### 1.2.5 Sea lice infestations and “premature migratory return”

For wild fish, return to freshwater results in the loss of all attached and mobile stages because of the inability of sea lice to survive long-term in freshwater (e.g. Connors et al. 2008). Adult female *Lepeophtheirus salmonis* initially drop the eggstrings in response to fresh water but attached chalmus larval stages can survive a few further days depending on host species and temperature (Finstad et al. 1995). For *Salmo trutta* in NW Europe the phenomenon of “premature migratory return” of infested fish to freshwater has been recorded extensively in recent years (e.g. Tully et al. 1993a; Birkeland 1996; Birkeland & Jakobsen 1997; MacKenzie et al. 1998; Bjørn et al. 2001), and has been attributed to be a behavioral response of the host fish to excessive sea lice burdens. For juvenile *S. trutta* this behavior may result in a short-term improvement in survival, but growth potential inevitably is compromised by the reduced period at sea and the fish also is exposed to the increased risk of secondary infection of lesion-exposed tissue by micro-organisms in freshwater (Wells et al. 2007). So, while there may well be a short-term benefit (in terms of osmoregulatory function and reduced stress) of premature return (e.g. Birkeland 1996; Bjørn et al. 2001; Wells et al. 2007), the longer-term benefits in relation to survivorship and fitness are uncertain. Irrespective of that uncertainty, from a management perspective such disruption of behavior is sufficiently extreme to warrant the deduction that

sea lice can and do present a clear disease problem to juvenile salmonids in these circumstances.

### 1.3 Health, Disease and Sustainability

#### 1.3.1 Stability of the host-parasite association and epizootic outbreaks

Salmonids infested with sea lice can show clear pathological damage and bleeding of the epidermis in areas where the sea lice tend to aggregate on the host fish (Figures 1.2 and 1.4). As far as can be judged, however, the natural salmonid host-sea lice association is stable: for example, adult one sea-winter Atlantic salmon (~1-4 kg) returning to Scotland from the open ocean have been recorded with sea lice burdens (*Lepeophtheirus salmonis* plus *Caligus elongatus*) as high as 150 (Figure 1.1) and yet appear to be in good physiological condition. Wild salmonids were associated with sea lice prior to the development of the salmon aquaculture industry and intensive aquaculture has not introduced 'new' sea lice species to wild salmonids within the bounds of their natural geographic distribution. In the North Atlantic, for example, it is very likely that *L. salmonis* and *C. elongatus* have been associated with Atlantic salmon, sea trout and Arctic charr since at least the last glacial maximum. In the Pacific, the introduction of farmed Atlantic salmon has undoubtedly extended the potential host range for indigenous Pacific sea lice (e.g. *L. salmonis*, *Lepeophtheirus cuneifer*, *Caligus clemensi*, *Caligus rogercresseyi*) but, with the exception of *C. rogercresseyi*, those same sea lice species probably already were variously associated with the native Pacific species of *Oncorhynchus*. For example, before the establishment of salmon aquaculture in British Columbia, the host generalist, *C. clemensi*, had been recorded from about 14 species of pelagic fish in British Columbia, including *Oncorhynchus* spp. (Parker & Margolis (1964). The debate is therefore not about aquaculture extending the host range of sea lice impacting wild salmonids, but of possible amplification of the quantitative elements of these natural host-parasite associations, perhaps to the extent of them comprising exceptional epizootic outbreaks.

The mobile adult male and pre-adult male and female stages of *Lepeophtheirus salmonis* tend to prefer the head and anterior dorsal areas of the skin of wild Atlantic salmon; by contrast, the adult females tend to occupy the skin areas adjacent to the anal fin (Figures 1.2 and 1.4), and the dorsal midline between the dorsal and caudal fins (Figure 1.3) (Todd et al. 2000). For salmonids infested with high numbers of sea lice, the feeding activity of the parasites can be locally intensified to the point that muscle tissue, fin rays or skull bone is exposed. Infestation of wild juvenile sea trout with large numbers of attached chalimi can result in the erosion of dorsal and paired fins and exposure of the fin-ray bones (e.g. Tully et al. 1993b; Fig. 1.6).

For individuals such as the fish illustrated in Figure 1.6, the attribution of “diseased” status is visibly obvious. But such instances appear to be rare in Irish, Scottish, and Norwegian areas remote from commercial salmon farming, and from the late 1980’s this was the starting point of the public controversy and scientific debate in Europe concerning the detrimental impacts of sea lice on wild salmonid stocks, and the implication of intensive salmon aquaculture as a causal or contributory factor (e.g., for Scotland and Ireland see McVicar et al. 1993; Tully et al. 1993a, 1999; Whelan 1993; Northcott & Walker 1996; Dawson et al. 1997, 1998; McKenzie et al. 1997; McVicar 1997, 2004; Costelloe et al. 1998; Butler 2002). In British Columbia the situation is rather different, due largely to the extremely small sizes of juvenile pink and chum salmon. For these fish (which are diminutive and weigh <1 g in early spring [Morton et al. 2005]), short-term mortality rates of juveniles may be elevated by infestations as low as 1-3 mobile sea lice per fish (Morton & Routledge 2005), although the authors included no fish mass data to qualify this lethal loading. Infestation with as few as 10 mobiles certainly appears to be potentially lethal, with death commonly occurring before extensive lesions and fin erosion develop. Morton et al. (2004) recorded >90% of sampled juvenile pink and chum salmon adjacent to farms in the Broughton Archipelago (British Columbia, Canada) as carrying sea lice burdens exceeding a lethal limit of  $1.6 \text{ lice.g}^{-1}$ , but abundances were near zero away from farms.

The debate concerning sea lice infestation interactions between farmed and wild fish in British Columbia currently remains unresolved, and to a large extent polarized. We discuss in detail below those studies from British Columbia which focus on detrimental effects of sea lice on juvenile Pacific salmonids, and the implication of farmed salmon as the infestation source. But it is important at this juncture to emphasize also that there are reports which conclude that wild Pacific salmonid populations can co-exist with farmed Atlantic salmon in W Canada (Beamish et al. 2006). However, it should also be noted that this conclusion is based on survival rates of the 2003 smolts, which went to sea during a period when one their major migration corridors was fallowed as part of the Provincial Action Plan. With specific reference to the wild-farm debate in W Canada, the recent scientific literature has been reviewed for the Pacific Salmon Forum in a constructive and balanced manner (Harvey 2008). The present report draws no overall conclusion regarding these important issues, but acknowledges that problems with sea lice for both wild and farmed salmonids persist and outlines recent progress (for example in objectively determining management tools, based upon threshold levels of sea lice infestation that can cause significant stress to host fish) which might assist in the application of the precautionary principle in conserving and maintaining wild salmonid stocks. As we outline in the report, the evidence is largely indirect or circumstantial that sea lice emanating from salmon farms can and do exert detrimental effects on wild salmonids. That is not to denigrate or detract from the quality of the various observational, experimental and theoretical approaches adopted by scientists in addressing this important environmental issue. Rather, it is

an objective acknowledgment that it is practically impossible to precisely quantify wild-to-farm versus farm-to-wild and wild-wild infestation interactions (see also Section 5.0). Given the diversity of life-history strategies and differential vulnerability of host species associated with sea lice in the Pacific and Atlantic Oceans, as well as the geographic differences in the intensity of the industry and its regulation, it is not plausible to draw a single over-riding conclusion regarding the potential negative impacts of sea lice on all wild fish stocks world-wide. However, on the basis of the range of available information (from the Pacific and Atlantic Oceans, and from both Hemispheres), we believe it is clear that the weight of evidence is that sea lice of farm origin can present, in some locations and for some host species populations, a significant threat. Hence, a concerted precautionary approach both to sea lice control throughout the aquaculture industry and to the management of farm interactions with wild salmonids is expedient.

Recent physiological studies (Wells et al. 2006, 2007; Finstad et al. 2007b), and advances in our understanding of the host immune response of salmonid fish to *Lepeophtheirus salmonis* infestation (reviewed by Wagner et al. 2008), have led to our ability to ascribe sub-lethal threshold abundances of sea lice causing significant stress to the host fish. The objective derivation of these target levels is seen to be crucial to wild fishery managers in ascribing particular populations as being subject to excessive sea lice infestation pressure. Building upon the early studies of Grimnes & Jakobsen (1996) and Finstad et al. (2000) on hatchery-reared Atlantic salmon smolts, it has proved possible to identify target levels of infestation of *L. salmonis* that define threshold levels for sub-lethal stress of host fish. Two caveats are, however, necessary: (1) most experimental studies involving sea lice challenge of naïve hosts concern single-pulse infestations (see Section 1.2.4 above) of laboratory fish; and, (2) the stress threshold levels will vary with both species and size of host fish as well as with the conditions under which the experiment is conducted. The importance of these caveats is that in the natural environment host fish will be exposed to repeated infestation pressure, perhaps at a lower level but over an extended period of time. Stress responses to intense, single-pulse infestation are likely to differ from responses to protracted and repeated infestation pressure at lower levels.

Wild fish are vulnerable to repeated infestation of sea lice once they have entered seawater, but to experimentally mimic this in a controlled fashion in the laboratory will be extremely challenging. Migration from freshwater presents salmonid smolts with the demanding and physiologically stressful requirement to osmoregulate in a hypersaline environment. Exposure to potentially rapid and intensive infestation by sea lice presents a contemporaneous source of additional physiological stress for smolts and juvenile salmonids (perhaps especially the diminutive pink and chum salmon) as soon as they enter the marine environment. For wild juvenile sea trout post-smolts (weight range 19-70 g; mean 37 g),

simultaneously challenged by transfer to seawater and a single-pulse infestation of *Lepeophtheirus salmonis*, an abundance of 13 mobile lice.fish<sup>-1</sup> was found to be a threshold for multiple physiological markers of stress (Wells et al. 2006). But how that target might be applied perhaps is a matter for discussion and consideration on a local basis. For example, as an ecological rule, parasites tend to be “over-dispersed” among host individuals – typically many hosts have zero or low intensity infestations but a few individuals carry extremely high burdens (Section 1.1). This inevitably raises the subjective question of what proportion (10%? 50%?) of a wild population should exceed the target figure in triggering further interventory measures on adjacent salmon farms. In the specific case of Norway, an objective of < 10 lice per wild salmonid smolt has been recommended to ensure no negative effect of salmon lice on wild salmonid populations (Bjørn et al. 2008; 2009). It is relevant to note that the “<10 lice per salmonid smolt” recommendation is not a Norwegian legislative requirement but a suggestion based on experimental results (Wells et al. 2006; Bjørn et al. 2008,2009). Given the scale of the Norwegian industry and the size of modern farms (see Section 1.3.2), in order to achieve that it would seem to be necessary both to reduce the sea lice level on each farmed fish and to further optimise delousing strategies.

For somewhat larger farmed Atlantic salmon smolts (weight range 50-160 g) — the size typically transferred to sea for on-growing by the industry — the comparable threshold sub-lethal infestation level of *Lepeophtheirus salmonis* was found to be 20 mobile sea lice (Wells et al. submitted). For larger adult sea trout and Atlantic salmon (weighing upwards of 1 kg) the threshold levels will undoubtedly be considerably higher, whereas for the very small chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) juveniles the comparable thresholds will be markedly lower. For example, long-term monitoring of return-migrant, one sea-winter adult Atlantic salmon (1-4 kg) captured in coastal marine waters in Scotland has shown that the average abundance of *Lepeophtheirus salmonis* is ~25 mobile sea lice per fish (Todd et al. 2006): although some individual fish showed abundances of *L. salmonis* and *Caligus elongatus* in excess of 100 (Figure 1.1) it is most unlikely that any of these fish could be considered “diseased” with respect to the criteria discussed in 1.2.1 above. However, by definition, observers generally can sample only surviving fish in the natural environment. The field and laboratory study by Morton & Routledge (2005) in British Columbia is especially valuable in this context in that they were able to sample free-swimming juvenile salmonids prior to their possible death in the natural environment and to retain them and monitor their survivorship in experimental facilities. Notwithstanding that particular study, it generally is the case that wild fish subject to excessive infestation may simply not survive long enough to be captured and assessed. It therefore follows that it is impossible to state with any certainty that sea lice never kill host fish at “natural” levels of infestation and, as White (1940) reported on the southeast coasts of Nova Scotia (Canada), epizootic outbreaks have been recorded 30 years prior to the development of intensive salmon aquaculture.



Scottish catches of wild sea trout (*Salmo trutta*) have shown a general pattern of long-term decline since detailed catch records were initiated in the early 1950s. But the late 1980s and early 1990s was a period of particularly marked declines, and even crashes in many populations. For Scotland as a whole there are important geographic differences in population trends for sea trout: whereas eastern Scotland catches have shown short term fluctuations but no overall trend over the period 1950 to present, western Scotland has shown a general downward trend over this same period and including the marked decline of ~20 years ago (Anon. 2008a). Data of comparable duration are not available for Ireland, but in Connemara the catches of sea trout were remarkably steady through the 1970s and 1980s before the catastrophic collapse in the late 1980s-early 1990s (Sea Trout Review Group 2002). None the less, for the Burrishoole system in particular there has been a pattern of progressive decline of sea trout smolt production from the early 1970s to the turn of the millennium (Byrne et al. 2004). The temporal patterns of sea trout stock sizes in Ireland and Scotland are therefore somewhat complex, but there can be no doubt that both western Ireland and western Scotland showed contemporaneous (and locally catastrophic) decline of certain stocks in the late 1980s and early 1990s.

The observations of heavily lice-infested juvenile sea trout coinciding with the continued swift decline of stocks in Ireland, Scotland, and Norway in the late 1980's and early 1990's were the starting point of the "wild-farm" controversy in Europe. The conclusions from several key studies in Ireland, Scotland and Norway on sea trout and Atlantic salmon (e.g. Tully et al. 1993a; Whelan 1993; Bjørn et al 2001; Butler 2002; Butler & Watt 2003; Gargan et al. 2003) were consistent: (1) sea lice were infesting juvenile salmonids to an unusually high extent, (2) physiological and behavioral responses all indicated these fish to be "diseased", and (3) adjacent salmon farms were deduced to be the likely source of these excessive infestations. Because of our inability to directly track individual sea louse larvae from initial hatching through planktonic development to ultimate attachment on the host fish, the initial scientific approach to assessing wild-farm interactions was correlative: sea lice epizootic outbreaks on wild fish apparently occurred only in areas subject to intense salmon farming and therefore the two inevitably were connected. It is regrettable that routine monitoring and detailed information on sea lice abundances on wild salmonids prior to the development of the salmon farming industry are lacking, for both the North Atlantic and North Pacific. The value of such data to informing the present debates about wild-farmed interactions for Atlantic salmon and sea trout in Europe, and pink and chum salmon in British Columbia would have been considerable.

Notwithstanding the historical observations of sea lice epizootics of White (1940), and given the lack of comprehensive "pre-salmon-farming" data on sea lice abundances, scientists and fishery managers are presented with considerable difficulties in ascribing instances of sea lice disease specifically to the aquaculture industry. In the absence of such "before/after" data,

experimental and analytical difficulties remain even in comparing “exposed” (= farmed) and control (= “no farm”) areas or fjords because of the problem of replication of areas/fjords. A comparison of a single farmed fjord with a single unfarmed fjord is flawed by a lack of replication and within farmed or non-farmed areas different sea lochs or fjords cannot be considered replicates because they will differ in size, depth, hydrography, geology of catchment, wind exposure, etc.

Two particular problems thus emerge in terms of informing the current wild-farmed debate: the lack of pre-salmon farming data on sea lice prevalences and abundances (or even reliable data on present prevalences and abundances on wild adult fish (e.g. Beamish et al. 2007)), and sample bias due to the fact that observers can generally only sample fish that survive infestation to be captured. In the latter regard, however, progress has been made both in British Columbia and Norway. For example, Atlantic salmon smolts have been sampled and tracked in Norwegian Fjords (e.g. Sivertsgård et al. 2007; Thorstad et al. 2007), and in British Columbia juvenile pink salmon have been sampled extensively in coastal waters along their migratory routes (Krkošek et al. 2005a, 2006a; Morton & Routledge 2005; Morton et al. 2004, 2005). The common outcome of these studies is that coastal migratory transit times for juveniles are such that early and rapid infestation in inshore waters can lead to fish that will become unequivocally “diseased”, within a matter of days or weeks of first migration to sea. The challenge remains to confirm the interaction strengths between wild fish and salmon farms as sources of infestation and sea lice as a population-regulating factor in local declines of wild salmonids. This requires progression beyond correlative studies and computer model predictions to affirm direct links (or lack thereof) between larval sea lice production from farms, infestation of wild fish, and wild population declines.

### **1.3.2 Sustainability of sea lice disease of salmonids**

As discussed in Section 4.2 the question of sustainability of salmon farming must embrace sea lice abundances on farmed fish in terms not only of the welfare and husbandry of cultured fish, but also in relation to larval sea lice production and export from those farms. Larval sea lice production from farm sites can, in many senses, be viewed as an “outfall” or “effluent” that perhaps should be subject to regulatory control, just as are chemical and organic pollutants. The mechanisms by which control is applied differ between countries. In Scotland, for example, the regulatory regime specifies a range of actions to manage the levels of sea lice. These include the Aquaculture and Fisheries (Scotland) Act of 2007 which requires farmers to take satisfactory measures in relation to the control, prevention and reduction of sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*), and is a legal requirement with the provision of the Scottish Government to take enforcement action where considered necessary. In

addition, farmers must demonstrate satisfactory measures are in place to control sea lice levels. This includes maintaining records in accordance with the Fish Farming Businesses (Record Keeping) (Scotland) Order 2008; this concerns records in relation to training, weekly counts, treatments, other methods of control (other than treatment) and records in relation to management area operation. Sea lice levels and their management must also accord with the standards of assessment for satisfactory measures to manage these risks, based upon the Code of Good Practice for Scottish Finfish Aquaculture (SSPO 2006; see also Section 4.1.1). This is in contrast to the situation in Norway where it is clear that the regulatory levels of sea lice abundances on the Norwegian salmon farms (most notably, a mean of 0.5 adult female or <3 mobile stages of *Lepeophtheirus salmonis*.fish<sup>-1</sup>; discussed in detail in Section 4.1) are sufficiently low to preclude individual farm fish from ever being categorized as “diseased”. Nonetheless, the sheer scale of commercial salmon aquaculture is such that even extremely low abundances of gravid females per fish can still result in massive levels of larval sea lice export from those cages, given that several hundreds of thousands fish may be stocked in each cage (Heuch & Mo 2001).

The fundamental requirements of fish welfare and husbandry dictate that sea lice abundances on salmon farms should be maintained at levels sufficiently low as to preclude those fish ever being categorized “diseased”. Furthermore, from a precautionary standpoint, sea lice abundances (and total larval sea lice production per net pen or per farm) should be maintained at even lower levels to minimize possible detrimental effects on adjacent wild salmonid populations. While acknowledging the lack of certainty about wild-farm interactions (e.g. Harvey 2008), there is a considerable body of evidence from the North Atlantic and North Pacific to indicate that sea lice can be severely detrimental to wild salmonids. This should therefore, be very much a case of applying the precautionary principle: in Norway the ‘encouragement’ of farmers to further de-louse their fish when infestations of local sea trout exceed 10 mobile sea lice per fish (Bjørn et al. 2008) has to be seen as a positive precautionary gesture by the industry, and not an overt admission of responsibility or guilt. Total eradication of sea lice on salmon farms is impossible, but farms could be enabled and required to minimize abundances through interventory treatment especially during periods of seaward migration of wild juvenile salmonids (as is an objective of Area Management Agreements [Section 1.4; Figure 1.7] in Scotland). Migrating smolts of some Atlantic salmon populations in northern Norway may be protected from coastal infestation by their natural mis-match of migration timing and the peak of larval sea lice production (Bjørn et al. 2007), but these quite probably are the exception to the rule.

Chemotherapeutant treatment is costly to the industry, has other environmental and human health implications, and is itself closely regulated in the Northern Hemisphere. In Scotland, for example, a given farm is authorized with a prescribed annual discharge consent

for specific chemotherapeutants and once that amount has been used it cannot be increased within that year. That constraint, in addition to concerns of the development of resistance through treatment over-use, and the increased reliance in both hemispheres on a single treatment, SLICE<sup>®</sup> (emamectin benzoate), presents real risks and also places practical limits on how farms can manage sea lice infestations in a sustainable manner. To date, there is no new classes of chemotherapeutant treatments on the worldwide market that are in a position to succeed SLICE<sup>®</sup>. Should resistance to SLICE<sup>®</sup> develop and become widespread, the industry would apparently have no option but to revert to older control agents (e.g. organophosphates, pyrethroids, chitin inhibitors, peroxide) though these could perhaps be beneficially utilized within a management framework of careful and rational product rotation.

#### 1.4 Range as a determinant of impact

Unlike many contagious bacterial or viral diseases, infestation of one host fish with sea lice by another host fish does not require physical contact. The inclusion of three free-swimming, planktonic larval stages in the life cycle – requiring several to many days to attain the infective copepodid stage – dictates that initial colonization and infestation occurs remotely. As a consequence, larvae released by adult sea lice infesting a wild salmonid may ultimately infect another wild host or a penned salmon in a culture cage, perhaps up to tens of kilometres distant from the point of release of the first nauplius stage. For sea lice larvae hatching from cultured hosts, the export of larvae from the source cage, and the possibility of farm-wild interaction, is unavoidable and inevitable: the through-flow of seawater in cages and duration of larval development time dictates that it is highly unlikely that those larvae will be retained within the cage (but see also Costelloe et al. 1996 for heavily fouled net meshes). In Scotland, Penston et al. (2008a,b) recorded the greatest planktonic densities of nauplius stages adjacent to farms, but found that copepodids were more widely dispersed in the fjord. Direct self-reinfestation of a given cage population is therefore highly unlikely because of the low probability of the copepodid re-entering that same cage some days later. However, because salmon farms have traditionally been located in semi-enclosed fjords and bays (where they are sheltered from wind and wave action) the relative lack of hydrographic flushing can result in the potential for self-reinfestation at the ‘whole farm’ level as a result of the indirect re-importation of released larvae (Revie et al. 2003). Similarly, geographically adjacent farms within the one fjord are liable to infest each other ([www.mattilsynet.no](http://www.mattilsynet.no) - The Norwegian Food Safety Authority - keyword "lakselus"; [www.fhf.no](http://www.fhf.no) - The Fishery and Aquaculture Industry Research Fund - keyword "hardangerfjorden", including *Salmon Lice Project in the Hardangerfjord 2004-2007*; Bjørn et al. 2008) and it is this which has led to the range of co-ordinated actions among adjacent farms in Norway, Ireland and Scotland in particular.

In Scotland, Integrated Pest Management (IPM) for sea lice led in 1999 to the formation of the Tripartite Working Group (STWG; <http://www.tripartite-workinggroup.com>). The STWG includes representatives of the Scottish Government, the aquaculture industry and wild fishery interests. One of the major achievements of the STWG was the development of formal Area Management Agreements (AMAs) involving the industry and wild fishery interests on a local scale (Figure 1.7). AMAs include co-ordinated fallowing of cage sites, the stocking of single year-classes, and synchrony of sea lice treatments within fjords, often involving co-operation between different aquaculture companies. These AMAs are undertaken with a view to specifically minimizing farm-farm interactions and also reducing the potential impacts of farmed sea lice larval production on local wild stocks. In the latter regard, it is important to note that the objective of zero ovigerous (egg-bearing) salmon lice on farms during the critical wild smolt migration period (February-June) is precisely that – an objective, but not a statutory requirement. In Ireland, the comparable co-operative policy adopted within the industry is referred to as 'single bay management'. [Growers in eastern Canada have adopted a similar approach creating of a number of Bay Management Areas; these relate to a broader range of species. The writers of this report could find little in the 'grey' or peer-reviewed literature on the details or impact of this initiative which began around 2001. Personal conversations with veterinarian and farm health managers in the area confirmed that these are bays of substantive geographical size and involve innovative strategies such as fallowing a given bay for a full year so it will be interesting to see what effects this has of sea lice management when data are forthcoming. A brief outline can be found on the New Brunswick Salmon Growers' Association web site at - <http://www.nbsga.com/farmedsalmon.php?view=29>.]

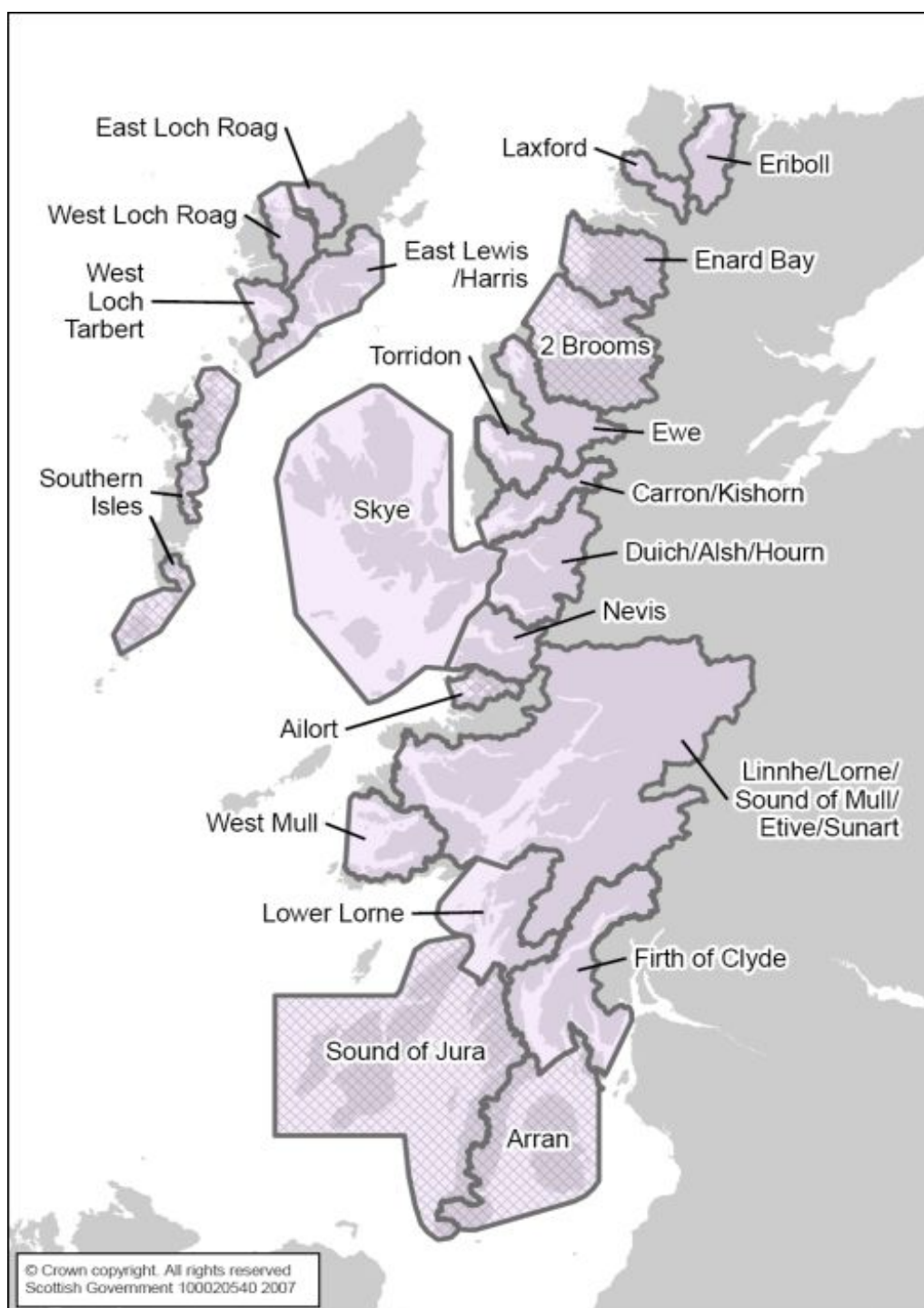


Figure 1.7 The 18 Scottish Area Management Agreements (AMAs) signed as at September 2008. (Un-signed areas are shown in hatch)

<http://www.tripartiteworkinggroup.com/content.asp?ArticleCode=25>

While IPM within the context of AMAs in Scotland, or more informal agreements in Ireland and Norway, has been developed and enacted within definable fjord systems – some of them very large and extensive (e.g. Hardangerfjord in Norway) – the maximum scale of impact of sea lice remains uncertain. The key here is the maximum distance over which larvae can be passively transported on coastal and tidal currents, and yet still succeed in encountering a host and successfully complete metamorphosis to the attached chalimus stage (see Figure 1.5). Whether co-ordination of actions against sea lice could or should be extended to multiple adjacent fjord systems remains open to argument, though hydrographic modeling (see Asplin 2004, 2008, Finstad et al. 2007a,b for more modeling information) can be readily applied knowing the tidal circulation patterns, variation in wind-stress effects and the temperature/development time relationship (and hence larval dispersal potential) for sea lice. Nevertheless, it is abundantly clear that local wind effects can markedly alter or over-ride the effects of basic hydrography in influencing the dispersal and aggregation of the planktonic larval stages of caligids in fjordic environments (e.g. Murray & Gillibrand 2006) and our understanding of variation in larval behavior still is very incomplete. Moreover, daily and seasonal variations in freshwater runoff also can significantly alter circulation and tidal flushing patterns in fjords and sea lochs, perhaps to the extent that simple hydrographic models based on tidal parameters alone may provide inadequate descriptors or predictors at a local level.

The ecological scale of impact of sea lice larvae emanating from culture pens also varies according to sea louse species. In the North Atlantic, *Caligus elongatus* is a host generalist, as are *Caligus clemensi* (Alaska, British Columbia), *Caligus orientalis* (Japan) and *Caligus rogercresseyi* (Chile) in the Pacific. The additional complexities of the scale of farm-farm and farm-wild interactions are evident from their multiple host fish species, which can act either as infestation sources or sinks for these generalist caligids. Of further concern in NW Europe is the recent development and expansion of Atlantic cod (*Gadus morhua*) farming. Cod naturally are host to a wide range of copepod parasites, including sea lice (notably *Caligus curtus* and perhaps *C. elongatus*), and as intensive cod aquaculture expands it is highly likely that there will be an increasing problem with *C. elongatus* both on cod and salmon farms, and interaction between these because of the scale of larval transport potential. *Lepeophtheirus salmonis* will not, however, itself present a problem to the cod farming industry because *L. salmonis* is a salmonid specialist. Although *C. elongatus* is of more immediate concern to farmers in the warmer waters of Ireland and Scotland than to those in Norway, there are indications that *C. elongatus* farm-farm, farm-wild and salmon-cod interactions are likely to become important in the near future (e.g. Øines et al. 2006). One specific concern is that whilst there are restrictions on the location of salmon farms in Norway (e.g. “safe sites”, as in the Hardangerfjord project) in relation to designated rivers and wild salmon stocks, those restrictions do not extend to the establishment of cod farms in those same fjords.

## Chapter 2: Avoiding Infection

### 2.0 Introduction

When large numbers of salmon are introduced to the natural (marine) environment in open net cage salmon farms, three things are virtually inevitable for these fish:

- 1) they will become hosts to sea lice (*Caligus* spp. and/or *Lepeophtheirus* spp.), since these occur naturally on wild host species in the vicinity;
- 2) they will become part of a dynamic host-parasite system involving wild hosts, because they can produce large numbers of infective larvae in a restricted spatial area if gravid females are allowed to develop; and
- 3) because they carry sea lice, and because they potentially will escape from farms, the spread of parasites could occur even more widely.

From these points it may be concluded that it will be next to impossible to avoid infection of farmed fish, all of which go into the pens as “clean” smolts and also to avoid infection of wild fish that are found in the vicinity (“infective field”) of an open cage farm.

As established in Chapter 1, a parasitic infection becomes a disease when host behavior and physiology (and ultimately health/survivorship and fitness) are altered or compromised to an exceptional extent (see also Section 1.2.1.) For example, the increased metabolic demand exerted by the parasite may cause slower host growth, making the fish more likely to be captured by predators, or causing it to take greater risks to feed (Milinski 1985; Mages 2008), with the same end result. Reduced host condition can also reduce swimming ability (Wagner et al. 2003; Mages 2008), with several negative ecological consequences, ranging from reduced competitive ability to slower migration. As discussed in Chapter 1, skin damage caused by the feeding behavior of the sea louse can increase the physiological cost of osmotic regulation (Webster et al. 2007), or provide sites for secondary bacterial or fungal infection; sea lice may also vector other diseases that can kill the host fish directly (e.g., Nylund et al. 1991, Rolland & Nylund 1998). While not exhaustive, this list does illustrate some of the direct and the more subtle indirect ways that sea lice may cause disease, as defined above.

Sea lice abundance on farmed salmon only rarely attains levels where the health of these fish is negatively affected. Recent exceptions to this appear to have involved concurrent disease challenge, as has been the case in Chile since 2006 with both salmonid rickettsial septicaemia (SRS) and infectious salmon anaemia (ISA). More recently (January 2009), in the Shetland Islands of Scotland ISA was detected partly as a result of inspectors responding to a call to investigate high sea lice infestations. When this occurs there are legal and regulatory



requirements in many countries that demand treatment, but it is clearly also in the economic interests of the farmer to treat the infection promptly and effectively, for example by administering SLICE<sup>®</sup> or other chemicals.

For wild fish, disease is likely to be an issue whenever sea lice intensity on individual hosts is sufficiently high as to cause significant stress, or to increase their vulnerability to secondary pathological infection. Vulnerability is itself difficult to define, because it will vary with host fish species, time of year (e.g. in response to seasonal temperature), size of host fish and their physiological or immunological state. For example, smolts newly exposed to the challenge of osmoregulating in saline waters will be physiologically stressed by that environmental challenge and will be more vulnerable than post-smolts that are just a few weeks older but fully adapted to seawater. Sea lice disease of wild salmonids is particularly problematic in areas with intensive Atlantic salmon aquaculture in British Columbia, Canada, on the west coasts of Ireland and Scotland, and throughout Norway (Chile lacks endemic species of anadromous salmonids).

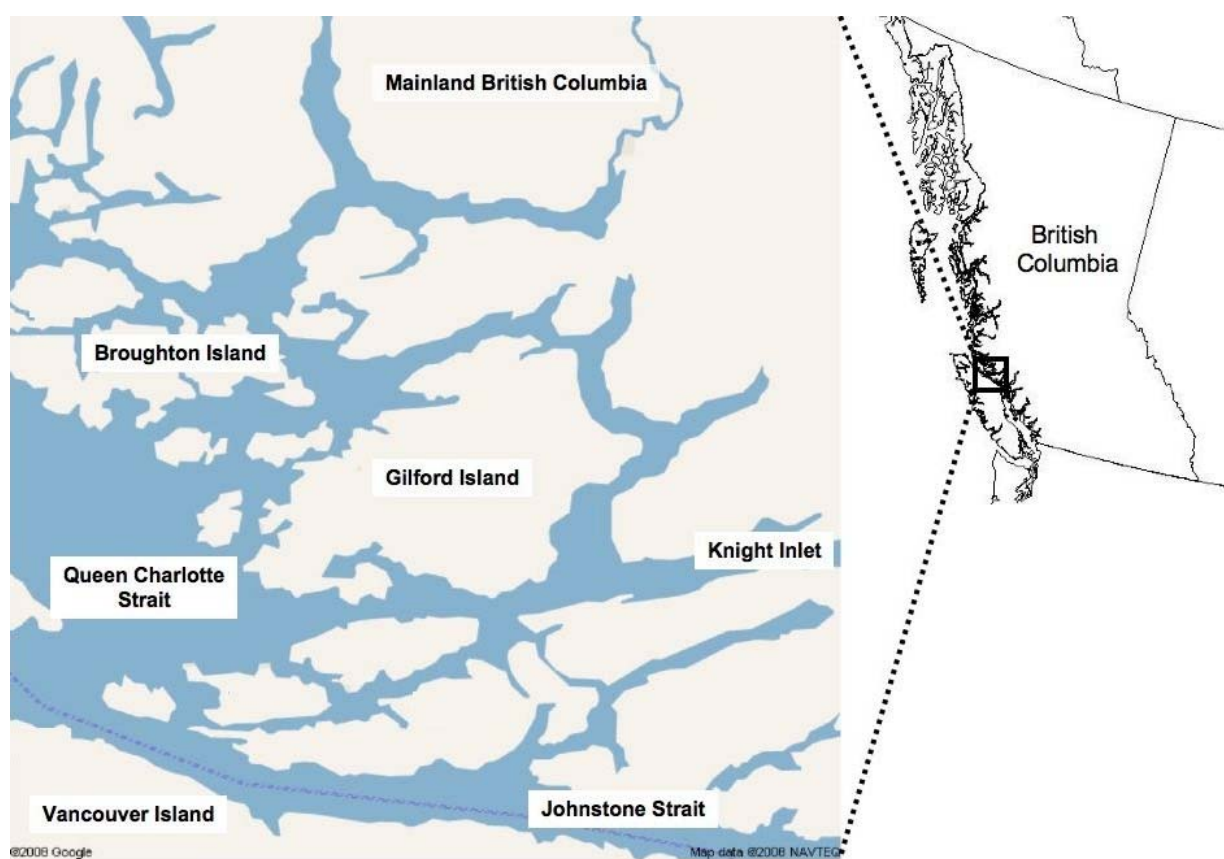


Figure 2.1 Map of the Broughton Archipelago, British Columbia, Canada (Courtesy of Google)

In British Columbia, the focus of attention has been on the much studied and discussed Broughton Archipelago region (Figure 2.1), where there is especial concern regarding the impact of *Lepeophtheirus salmonis* on wild stocks, particularly juvenile pink (*Oncorhynchus gorbuscha*, Figure 2.2) and chum (*O. keta*) salmon.



Figure 2.2 A juvenile pink salmon (*Oncorhynchus gorbuscha*) smolt with several adult *Lepeophtheirus salmonis* attached (Broughton Archipelago, British Columbia, Canada). The topmost individual is a gravid female louse with paired eggstrings. (Photo courtesy of Alexandra Morton).

As outlined in Chapter 1, *Lepeophtheirus salmonis* is a salmonid-specific parasite (see Section 1.2.3). The natural history of the species requires inter-generational transmission of an infective stage, either the copepodid larva, or the mobile pre-adult and adult stages. In the North Pacific, it is likely that this occurs when adult salmon, returning from the open ocean, and typically laden with sea lice, pass in close proximity to out-migrating juvenile salmon. Many, but not all adult salmon bear sea lice (Nagasawa 1993, 2004) and, with rare exceptions (e.g., Alberni Canal in a very warm summer; Johnson et al. 1996), natural levels of infestation apparently do not present a problem for them (for a more complete discussion of this point see Section 1.2.1). Transmission of sea lice to the succeeding host generation occurs in coastal waters in late summer, when these juvenile fish are relatively large and healthy, and able to

withstand low intensities of sea lice infestation. Those sea lice remaining on the returning adult salmon die when the fish re-enter freshwater to spawn and die themselves; there is no evidence for a sea louse resting stage overwintering in near shore habitats. Some of the eggs produced by *L. salmonis* in near shore environments may develop into larvae that infect three spined sticklebacks (*Gasterosteus aculeatus*); large numbers of chalimus larvae and a very few pre-adults have recently been found on this species (Jones et al. 2006), but there is no evidence that *L. salmonis* can complete its lifecycle on sticklebacks (Losos 2008) or on any species other than a salmonid host.

Since the advent of open net pen aquaculture in the Broughton Archipelago region of British Columbia, wild adults approaching spawning streams must travel through narrow coastal inlets that contain millions of farmed salmon hosts (primarily Atlantic salmon, *Salmo salar*) held in open net cages. It is likely that infection of these farm fish occurs at this time (late summer-fall). Those farmed fish which remain in cages over the winter will support sea lice populations, growing through at least one generation *in situ* and producing significant numbers of eggs (Saksida et al. 2007; Orr 2007), resulting in large numbers of infective copepodid larvae. In the spring (starting usually in late-March) the out-migrating pink and chum salmon juveniles pass by these cages on their way to the open sea through these same narrow migration corridors. It is at this point that they are likely to first become infected. These small (frequently under <0.5 g), scale-less fish which are adapting to the physiological challenge of osmoregulating in a new hyper-osmotic environment are particularly vulnerable and frequently become host to large numbers of sea lice (DFO Canada has reported >100 on a single fry). This is precisely the set of conditions in which an infection can become a disease which compromises fish survival and creates potentially negative consequences for local populations. There is considerable evidence that the farms are a foci of infection for juveniles (Krkošek et al. 2005b, 2006a), and there are those who argue strongly that local pink and chum salmon populations in this region have declined more than those elsewhere since the advent of commercial scale net pen aquaculture (Krkošek et al. 2007b; but see Section 5.4.1). As discussed in Chapter 1, wild population declines of wild salmonids do appear to be a recent worldwide phenomenon, but these may be due to a multitude of possibly interacting causes other than, or in addition to, sea lice.

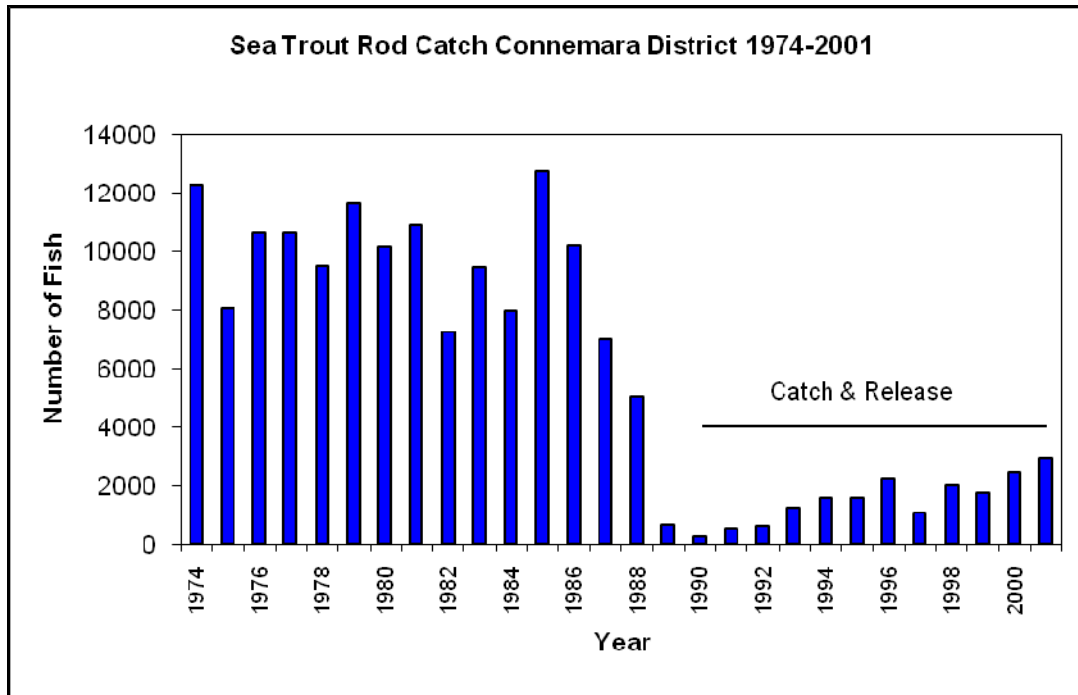


Figure 2.3 Total number of sea trout caught by rod angling in the Connemara district between 1974 and 2001 [From report of the Sea Trout Review Group (2002); their Fig. 1]

Note that there also are areas of the British Columbia coast (such as Johnstone Strait) where high infection intensities on juvenile sockeye salmon (*Oncorhynchus nerka*) and even Pacific herring (*Clupea pallasii*), have been reported and may be of conservation concern (Morton et al. 2008). The authors note that *Lepeophtheirus salmonis* were “essentially absent” on Pacific herring; most sea lice observed were *Caligus clemensi*.

In other parts of the world the vulnerable wild salmonid species are different. In Ireland most concern has been for anadromous brown trout, or “sea trout” (*Salmo trutta*). Irish sea trout populations have shown declines and, in some cases, catastrophic collapse in certain streams and catchments, particularly those entering into small bays containing fish farms. For example, the Irish Sea Trout Review Group showed in their 2002 report (Figure 2.3), the striking decline in catches of sea trout in Connemara over the period 1989-2001.

Unlike Atlantic salmon, sea trout spend extended periods of time in nearshore or coastal waters, and this feature may render them particularly vulnerable to sea lice infestation. Analyses concerning the potential interaction between farmed and wild salmonids in Irish bays subject to intensive aquaculture have not been without controversy (Tully 1993; Tully & Whelan

1993; Tully et al. 1993a,b; Jackson et al. 1997; Costello 2006; plus several un-refereed grey literature reports). The circumstantial evidence of a causal link between farms and some impact on wild sea trout is considerable. Tully et al. (1999) suggested that infestation pressure was spatially variable around Irish coasts and they deduced little movement either of sea trout or sea lice larvae between bays, leading them to conclude that there was an overall pattern of increased infestation of sea trout in bays occupied by salmon farms. Correlations also have been drawn between abundances of sea lice on wild sea trout and on farms up to 30 km distant (Tully & Nolan 2002; Gargan et al. 2003). Sea trout is also the species of most concern with regard to detrimental effects of sea lice in Norway, however Arctic charr (*Salvelinus alpinus*) also are impacted by sea lice (Bjørn & Finstad 2002). As is the case for sea trout, Arctic charr are effectively confined to coastal waters (often in narrow fjord systems) and, as described in Section 4.2.1, these areas commonly are home to a high density of captive farmed salmon.

In Scotland both sea trout and salmon are ecologically and economically important species which support significant game angling industries. As in Ireland and Norway, the late 1980s and early 1990s were characterized by marked declines in some Scottish sea trout stocks, especially those on the west coast where the salmon aquaculture industry is concentrated. From a comparatively early date in the development of the salmon aquaculture industry it became apparent that short-term fallowing of production sites (to break the host-parasite life cycle on farmed fish) and the culture of single year classes of fish assisted in reducing the infestation levels of *Lepeophtheirus salmonis* on farms. For example, Bron et al. (1993) compared three fallowed farms with an un-replicated, non-fallowed farm and reported reduced farm infestations with fallowing for *L. salmonis*, but not for *Caligus elongatus*. In the light of results such as these, and as discussed in Section 1.4, the contemporary approach by the industry is for single bay management (Ireland) and the co-ordination of farm cycles and lice treatments on an area-wide basis (Area Management Agreements; Scotland). Culture of single year-classes of farmed salmon through the two-year production cycle should, therefore, lead to years of low larval lice abundances within a fjord (sea loch) alternating with years of higher larval abundances (in year two when farmed adult salmon are harvested).

Abundances of wild Atlantic salmon in the North Atlantic have declined steadily since the mid 1970s, and the over-riding problem appears to be mortality at sea. Sea trout populations also have declined in abundances but unlike Atlantic salmon (which peaked in abundance in the 1960s) sea trout numbers have declined markedly since records began in Scotland in 1951 (Anon. 2008a [Fisheries Research Services; Scottish salmon and sea trout catches, 2007. ISSN 0264-116X]). Analysis of abundance trends and patterns is complicated, according to the geographical scale applied: data for west *versus* east coasts, regions within coasts, and river catchments within regions show no simple or clear pattern. But if the east and west Scotland data are separated at that broadest level, distinct contrasts in long-term pattern

can be detected. East coast populations show a complex pattern, or perhaps no consistent long-term trend in abundances, whereas west coast sea trout have shown steady declines. The challenge for scientists and fishery managers is to explain and understand these broad- and fine-scale contrasts in sea trout population trends. Heavy infestations of juvenile sea trout with *Lepeophtheirus salmonis* were repeatedly recorded from western Scotland during the years of severe population declines. Perhaps it was inevitable that an early conclusion drawn in many quarters was that sea lice and salmon aquaculture were the primary causal agents (see, for example, Butler 2002), if only because of the clearest and most marked recent declines in sea trout (and intense sea lice epizootics) having occurred in areas of salmon aquaculture. Monitoring of sea lice infestations on wild sea trout was identified as a particular priority for the West Coast Fishery Trusts, which were set up from the early 1990s in collaboration with riparian owners specifically to support the management, conservation and development of freshwater fisheries in Scotland. Early reports of lice monitoring results (e.g. Butler 2002) indicated a connection between sea lice abundances and the two year production cycle on local farms.

The earliest substantive reports assessing sea lice infestations and sea trout population declines were provided by McVicar et al. (1993) and Sharp et al. (1994); they highlighted the levels of popular concern regarding this issue in Scotland and that concern was further emphasized by Northcott & Walker (1996). Sharp et al. (1994) reported the heaviest sea lice infestations of wild sea trout to be from the northwest of Scotland, but the conclusions of other analyses of extensive surveys of juvenile sea trout and sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) infestations (e.g. MacKenzie et al. 1998) did not include support for an unequivocal connection between sea lice loadings on wild salmonids and nearby salmon farms. That latter conclusion was based on their finding a lack of clear evidence, on a location by location basis, that sea lice loadings of wild sea trout bore any relationship to the proximity of salmon farms. But this begs the question: on what spatial scale (kilometres, 10's of kilometres, 100's of kilometres?) should one seek correlations between farm lice larval production and infestation levels on free-ranging wild salmonids? Indeed, is the simple correlative approach adequate in this context?

As a consequence of the ongoing controversy concerning declines in sea trout on the west coast of Scotland, and the possible interactions with sea lice originating from farmed salmon, the government-funded Fisheries Research Services (now Marine Scotland, a Directorate of Scottish Government) in Scotland initiated an intensive programme of monitoring for the planktonic larval stages of sea lice in Loch Torridon, Wester Ross. The early results (from 1999) showed peaks in larval abundances that coincided with the second year of salmon production in local farms, and a virtual absence of larvae during the first year of production. These surveys were continued by Penston and colleagues (e.g. Penston et al. 2004,

2008) up to 2006 and affirmed the established “on-off” biennial pattern. Despite focusing on just the one un-replicated fjord and in the absence of contemporary “control” data for a comparable fjord lacking salmon farm activity, these surveys do provide persuasive evidence of a connection between local salmon farming activity and larval sea lice production to which wild fish are susceptible. It is unarguable that wild salmonids are an infection source for farmed salmonids and *vice versa*: the controversy lies in the extent of the imbalance of that interaction and the potential exposure of wild salmonids to levels of infestation that are “unnaturally high” (however that is defined) and potentially lethal (see, for example, Wells et al. 2006, 2007). But the above mentioned plankton survey data, of themselves, cannot provide unequivocal evidence that sea lice are the sole, or even the primary, causal agent for sea trout population declines, or that salmon farms are primarily responsible. Nevertheless, given all the foregoing evidence, and in light of sea trout declines having been coincident with observations of sea lice epizootics and the proximity of intensive salmon aquaculture, the logical conclusion should lead to the invocation of the precautionary principle.

A different louse species, *Caligus rogercresseyi*, infects caged Atlantic salmon in Chile, sometimes at levels which appear to leave the fish more susceptible to concurrent disease such as SRS or ISA. (Indeed some have suggested that lice may vector *Rickettsia* or the ISA virus but this has not been rigorously demonstrated.) Though these probably originate on a variety of local wild fish species (*Caligus* are host generalists), the impact on wild species of the high density of lice on the farms has so far not been examined.

## 2.1 What do we mean by avoid?

Avoidance of sea lice infection of farmed fish would only be possible only if the rearing facility were not open to the surrounding environment, and all water entering the site were filtered to remove sea lice. Moreover, avoidance of infection of wild fish would be possible only if all water passing through the facility were adequately filtered or disinfected. Clearly, the only way to achieve this is through closed containment technology (see Figure 2.4). Of course, as noted above, infection is not synonymous with disease, and avoiding disease in wild fish is possible if the number of infective stages produced from the farm is kept to a sufficiently low level, and care is taken to not expose those most vulnerable components of the wild populations. In what follows, we take “avoid” to mean “*avoid disease of local wild hosts*”.



Figure 2.4 Photograph of a closed containment net-bag being used by Marine Harvest as part of an experimental trial at the Cusheon Cove site in British Columbia. ([http://www.al.gov.bc.ca/fisheries/technology/marine\\_harvest.htm#gallery](http://www.al.gov.bc.ca/fisheries/technology/marine_harvest.htm#gallery))

## **2.2 Different avoidance scenarios**

### **2.2.1 Endemic in the region but not on farm**

Proper siting of farms, or coordinated treatment of farms in a local area, can prevent spread of sea lice from farm to farm, and re-infection from local reservoirs. This may reduce the need for chemical treatment, and lessen the spread of sea lice to wild hosts. This is facilitated in Ireland by Bay Management Agreements and in Scotland by Area Management Agreements, as discussed in Section 1.4 (see also Figure 1.7).

### **2.2.2 Endemic in the country but not in the region**

Norway has created “National Salmon Fjords” from which all salmon farms are excluded, in the hope that at least some populations of wild fish can avoid infection by lice. This is treated in more detail in Section 4.2.1.

### **2.2.3 Exotic diseases that are known elsewhere**

NA



#### **2.2.4 New, emerging infections**

There is the possibility of sea lice species not previously of concern, becoming a problem for both farmed and wild salmon. For example, *Caligus elongatus* appears to be increasing in abundance on Norwegian farms (Bengt Finstad, pers. comm., February 2009) [perhaps due to warming of coastal waters] and there is the potential for cod (*Gadus morhua*) farms to act as “bio-magnifiers” for non-host specific sea lice species (see Sections 1.4, 4.2.4).

### **2.3 What must be in place to avoid a disease?**

As mentioned above, the only way to completely avoid infection (and thus potential disease) would be to switch to closed containment. As desirable as this may be from the perspective of wild fish population management, it is likely to be a number of years before the technological and economic issues of closed containment can be solved, and there is no guarantee that this approach to salmon farming will become a practical large-scale option. It is therefore important to explore other ways to reduce the likelihood of lice infection of farm fish and prevent the production of large numbers of infective stages.

#### **2.3.1 Herd and individual immunity**

It is known that sea lice infestation causes immune responses both in Atlantic and Pacific salmon (Fast et al. 2002, 2007). Because sea lice go through several generations on a single farm cohort of salmon, both individual and herd immunity could, in theory, be increased through vaccination (but see General Disease report, Section 2.5.1), by the administering of diet additives, or by reducing stress levels (see the General Disease report and also Section 4.1.2).

Evidence of the benefits of dietary nucleotides in the laboratory (Burrells et al. 2001) has not been replicated in controlled field studies. There have been unsubstantiated suggestions that dietary additives can induce a thicker mucus coating on salmon, thereby providing partial immunity by making it difficult for larval sea lice to attach and feed, but the evidence for this is weak (Wallace et al. 2008).

#### **2.3.2 Preventing exposure (of wild fish)**

Closed containment is the only option that could reduce risks of exposure to zero. Failing that, and given the current technological and economic issues with closed containment, there are several other options to significantly decrease exposure and subsequent risk of

disease that are worthy of discussion.

One option is to place farms in areas where vulnerable stages of wild hosts are not present. For instance, in British Columbia farms might not be sited along migration routes, or they could be located sufficiently seaward that the wild fish will have grown to a size where they are no longer so vulnerable to sea lice infestation. Periodic fallowing of farms along a migration corridor could ensure that infected fish are not present on the farms during the migration window of wild fry, and is another management option currently under discussion in British Columbia.

Treating farmed fish when sea lice counts rise above a threshold, or trigger level, is normal practice and this level is legislated in a number of countries (see Table 4.4). In this instance, the concern in many jurisdictions is not so much for the farm fish but for the local wild populations. Some attempts have been made to base these farm treatment triggers on infection levels on wild fish (Section 4.2.3). While it is much easier to count sea lice than assess fish for viral or bacterial infections, this often will be impractical due to the difficulty of seeing and counting the earlier stage chalimus larvae and the time lag between recognizing a problematic infestation level on a farm and reducing it by interventory treatment. Wells et al. (2006) sought to empirically derive a critical infestation intensity for *Lepeophtheirus salmonis* impacting wild sea trout smolts. They assessed changes in an array of stress physiological markers for wild smolts (weight range 19-70 g) that were simultaneously challenged by experimental transfer from freshwater to seawater and a pulse infestation of *L. salmonis* copepodids. A consistent outcome, indicative of sub-lethal stress, for a range of markers was a threshold abundance of 13 mobile (pre-adult or adult) *L. salmonis* per fish. While this threshold intensity of sea lice was readily determinable, the actual application of this as a tool in managing wild salmonids is less clear. Should an extra farm treatment be invoked once the mean abundance of sea lice on wild sea trout exceeds ~10 sea lice per wild fish? Given that parasites are over-dispersed among fish hosts (variance greater than the mean) should an arithmetic or geometric mean be calculated? Should treatment be applied if a certain percentage of wild fish exceed burdens of 10 sea lice? As a direct result of the analyses reported by Wells et al. (2006), Bjørn et al. (2008, 2009) (see Section 1.3.1), recommend achieving the aim of <10 sea lice per wild sea trout. In order to attain that objective it would seem to be necessary both to reduce the sea lice level on each farmed fish below currently applied threshold levels as well as further optimizing de-lousing strategies on farms.

### 2.3.3 Knowledge on what might be coming (prediction)

The presence of farms is a recent perturbation of a co-evolved host-parasite system and can be expected to have several evolutionary consequences. For example, the presence of large numbers of farm hosts in close proximity to one another, as well as to free-ranging wild hosts (implying minimal dispersal costs) creates precisely the conditions under which increased parasite virulence might be expected to evolve (Ewald 1994). This could have negative consequences both for farmed and wild fish.

In the longer term, continued mortality of wild fish will likely select for resistance to lice, and the use of chemotherapeutants may select for parasite resistance to these (see Section 3.1.7). Given the difference in generation times and reproductive potential of host and parasite, sea louse resistance to chemotherapeutants is likely to evolve first. However, whether this will occur, and how quickly, will depend on the level of gene flow between treated and untreated segments of the sea louse population (and there is evidence that this is considerable; Todd et al. 2004) as well as the fitness cost of resistance genes in unexposed salmonid hosts.

From a relatively early stage in the development of the salmon farming industry there were anecdotal reports of “reduced sensitivity” of sea lice to various chemical treatments (e.g. organophosphates [target site, acetyl cholinesterase (AChE) enzymes] and pyrethroids [target site, *para*-type sodium channel proteins in neuron membranes]). For example, for organophosphates, Jones et al. (1992) showed some experimental evidence of reduced sensitivity of *Lepeophtheirus salmonis* to dichlorvos, and subsequently Fallang et al. (2004) reported differential sensitivities of two AChE enzymes which may have been implicated in resistance of *L. salmonis* to both dichlorvos and azamethiphos on Norwegian farms in the 1990s: one of the two AChE enzymes was rapidly inactivated by organophosphate, but the other only slowly inactivated. Resistance to pyrethroid insecticides (“knockdown resistance”) arises from mutations in the protein, and typically in domain II of the molecular sequence. Fallang et al. (2005) found no mutations in the so-called domain II (S4-S6) region – which is the location of other typical resistance mutations in insects – but they did report a novel mutation in segment II S5, which they tentatively attributed as being a site of possible knockdown resistance in *L. salmonis*.

### 2.4 Prediction – knowing what to avoid

Sea louse population levels can be expected to be greatest on both farm and wild salmon hosts when temperature and salinity levels are optimal for development (Johnson & Albright 1991). Some level of prediction is thus possible from knowledge of regional snow packs and predicted local weather events. However, a number of farm-based studies (Revie et al.

2005; Saksida et al. 2007) have shown that, particularly for sea water temperature, it is difficult to detect any significant effect at the population level. Moreover, seasonally intense variation in migration of adult wild salmonids into coastal waters may well elevate local infestation pressures in a predictable manner, albeit perhaps only for relatively restricted periods in the year.

(See also previous section.)

## **2.5 Promoting resistance**

Covered above

## **2.6 Preventing exposure (or transmission to wild fish)**

Covered above

## **2.7 Legislation**

See Sections 4.1.2 and 4.2.3.

## Chapter 3: Can we prevent disease/infestation?

### 3.0 Introduction

This section assumes that farm fish are inevitably exposed to sea lice and deals with preventing disease/infestation in the net cages, thereby interrupting the farm-wild fish infection interaction. It also is assumed that salmon within a given farm site can be effectively self-reinfesting (if hatched nauplii drift back into the net pens after developing to the infective copepodid stage), or can infect other farms or free-ranging wild fish in the vicinity. Similarly, wild fish may well infect other wild fish or adjacent farmed fish. The absolute abundances of farm and wild fish, the absolute abundances of sea lice on those fish and the relative strengths of farm-farm and farm-wild interactions (and any seasonal or annual variation thereof) will determine the overall infestation pressure in a given locality. As pointed out in Section 1.2.1, a farmed fish with even a single ovigerous louse can be considered “diseased” when viewed in this wider epidemiological context.

### 3.1 What are the tools for prevention (farmed)?

#### 3.1.1 Vaccination

According to a recent review (Raynard et al. 2002), there has been no successful development of a vaccine against sea lice. However, a number of drug companies and academic researchers in both Canada and Norway are continuing research to develop an effective vaccine as we write. Development of vaccines against parasites like the salmon louse is a challenging task due to the complexity of the target organism. This makes it very difficult to identify vaccine targets that provide sufficient protection upon vaccination. A key activity in the research so far has been to develop tools that facilitate the search for potential vaccine targets and so far a range of molecular methods (e.g. microarrays, RNAi) have been established for salmon lice. In addition, a clinical model system to evaluate vaccine effects is necessary to assess vaccine antigens. Once a model is established it can be continuously improved to increase the power of the experiments. Frank Nilsen (University of Bergen; pers. comm., January 2009) has conducted vaccine experiments that significantly reduce the number of sea lice on vaccinated fish. However, it is uncertain if the antigens used can be produced at an industrial level. To identify new vaccine targets, key biological processes in female *Lepeophtheirus salmonis* need to be studied to identify regulatory points that could be targeted by a vaccine.

### 3.1.2 Non-specific immune modulators

Discussed in Section 2.5.1 of the General Disease report, and in Section 2.3.1 above.

### 3.1.3 Risk factor modification

Discussed in Section 4.1.2 below.

### 3.1.4 Genetics

If genetic variability for resistance exists, then it may be possible to select for this in Atlantic salmon broodstock. Some attempts to do this are underway in Norway and Scotland (Glover & Skaala 2006; Gharbi 2008). Selective breeding of Atlantic salmon for traits conducive to intensive culture has been a feature throughout the development of the industry. The extension of this principle to include research specifically into innate resistance of farmed salmon to sea lice infestation has a more recent history. The likelihood of genetic resistance to disease agents, and especially sea lice, amongst salmon species was perhaps indicated by early observations of differing infestation intensities of *Lepeophtheirus salmonis* on Pacific salmon species (e.g. Nagasawa 1987). More recently, Jones et al. (2007) have shown relatively enhanced innate resistance to *L. salmonis* infection for juvenile pink salmon compared to juvenile chum salmon. Given the strong environmental concerns over sea lice infestations of juvenile wild salmonids in British Columbia, it is notable that a recent publication (Jones et al. 2008) indicates that this innate resistance develops rapidly in juvenile pink salmon; there is, however, a particular “vulnerability window” until the post-emergent juvenile pink salmon attain a body mass of ~0.7g (Jones et al. 2008).

For Atlantic salmon, natural resistance to sea lice infestation amongst “stocks” has been established (e.g. Jones et al. 2002) and Kolstad et al. (2005) provided encouraging evidence that resistance to sea lice could be enhanced by selective breeding. For experimentally challenged Atlantic salmon post smolts, Glover et al. (2005) showed as much as 70% variation in *Lepeophtheirus salmonis* infestations among 30 families and significant heritability; more recently, Glover et al. (2007) have indicated a link between susceptibility to *L. salmonis* infestation and genetic variation in the Major Histocompatibility Complex (MHC) Class II. All these results lend some encouragement to the possibilities of selectively breeding Atlantic salmon for resistance to sea lice, but whether such outcomes result in stocks that generally perform well in commercial culture remains unclear.

MacKinnon (1998) reviewed the evidence that Pacific salmon may be more resistant to

sea lice infection than Atlantic salmon. This might, for example, suggest a benefit to the Canadian industry in switching the species being cultured in British Columbia to chinook (*Oncorhynchus tshawytscha*) or coho salmon (*O. kisutch*), though this would lead to other problems of disease and escape and the commercial product itself might not be favoured by international consumers. It may also be possible to genetically modify Atlantic salmon by inserting genes from Pacific salmon, though any pleiotropic effects would need to be carefully assessed before placing such fish in the wild, even in cages, due to the inevitable problem of escapes.

### **3.1.5 Nutrition**

Nutrition-based approaches to the prevention of infestation appear to be unlikely to be productive, since the healthier the individual fish the more suitable a host it presents to parasites. That is, well-nourished farm salmon may well support larger numbers of sea lice, reproducing at a higher rate, than less well-nourished individuals, and thus produce more nauplii to infect wild fish. Of course, if immune function is improved in well-nourished fish, this could have the opposite effect.

### **3.1.6 Water quality improvement**

Siting of net pens and cages in less saline areas could reduce sea lice levels, but the growth and stress consequences for the farm salmon themselves may render this impractical. It would also have the potentially deleterious effect of placing the farms close to shore, where small juveniles might be more seriously impacted than if the farms were moved more seaward (as suggested in Sec 2.3.2 above). Ensuring that the aquatic environment of the wild fish remains of high quality (i.e., unpolluted) might reduce their susceptibility to infection by sea lice emanating from aquaculture facilities, but this is generally the case because successful farms themselves require high quality waters.

### **3.1.7 Treatment (not prophylactic, except from the perspective of the wild fish)**

A number of chemotherapeutants have been used to treat sea lice on farm salmon (see Table 4.3). In many countries, the chemical of choice remains SLICE<sup>®</sup> (emamectin benzoate), a neurotoxin supposedly specific to invertebrates. SLICE<sup>®</sup> is provided to fish as an “in feed” additive to pellets and the recommended dose is 50µg.kg<sup>-1</sup> fish biomass per day for 7 consecutive days. While effective in killing sea lice, this approach has several issues with its use:

- 1) To minimise any human health risks due to chemical residues fish must be held for a withdrawal period prior to slaughter. Withdrawal periods are relative short in most countries (less than two weeks) though the fact that SLICE<sup>®</sup> is only available through Emergency Drug Release (EDR) in Canada means that the withdrawal period is set to 68 days prior to the fish product being put on the market. Amongst other factors, this can result in farmers being reluctant to treat when their fish are close to optimal market size.
- 2) There are concerns (so far unsubstantiated) that other crustaceans in the vicinity of farms, such as shrimp and prawns, may be killed by the treatment. There also is the possibility that zooplankton populations, on which small wild fish feed, may also be impacted negatively, though this has not been studied in detail. The only large-scale study to date (Willis et al. 2005) found only limited effects from both bath and in-feed treatments. Indeed in the main PAMP (Post Authorisation Monitoring Project) project report the authors specifically state that, “there was no evidence that Slice adversely affected the macrobenthic community” (PAMP 2005).
- 3) Perhaps most seriously, the high mortality caused by the treatment puts strong selection on the sea lice to develop resistance. Based on experience with earlier treatments for sea lice, as well as with antibiotic and anthelmintic resistance in other species, this seems almost a certainty (see Sections 2.3.3 and 4.1.2) in the long run. If resistance evolves, there are currently no more tools in the chemical toolbox.

### **3.1.8 Culling/depopulation (compulsory slaughter) of other high-risk groups**

NA (not applicable)

### **3.1.9 Screening plus action**

NA

## **3.2 Herd health & HAACP on farm**

Discussed in Chapter 2



## Chapter 4: Can we reduce disease impacts?

### 4.0 Introduction

In this section we focus on a range of issues relating to the reduction of impacts from sea lice. We look at how disease challenge from sea lice on salmon farms can be monitored, at the range of mitigation strategies that exist and at measuring the effectiveness of such interventions. We then discuss the more complex question, both in terms of taking action and of measuring any impact reduction, of the interactions between wild and farmed fish as they relate to the issue of disease impact.

### 4.1 Can we reduce impacts on farmed fish?

As noted in earlier chapters, sea lice infestation is endemic on salmon farms in most countries which practice commercial aquaculture. The extent to which these infestations become a direct problem for farmed fish has varied over time and across species. For example, in the early days of the aquaculture industry in eastern Canada, *Caligus elongatus* was found to be a serious problem (Hogans & Trudeau 1989b). However, by the mid 1990s most papers discussing sea lice reduction on salmon farms in eastern Canada, and in Europe, focused almost exclusively on the other major species that occurs in Atlantic waters, *Lepeophtheirus salmonis*. This also is the species of predominant concern in western Canada, though the host generalist *Caligus clemensi* occasionally is noted as a potential problem, as is the case for *C. elongatus* in Scotland and Ireland. In Chile early reports following the emergence of the salmon industry noted the presence of *Caligus teres* (Reyes & Bravo 1983) on some farms (particularly those raising coho salmon), although more recently the species of predominant concern has been *Caligus rogercresseyi*. Thus a variety of sea lice species parasitize a range of salmonid hosts, with variation in patterns of infestation apparent across geographical locations and through time.

The issue as to when infestation should be considered “disease” is not a straightforward matter, as discussed in Chapter 1. Disease status will vary with the size of fish being considered because larger, more mature fish are able to tolerate higher levels of infection. It will vary also with the species implicated in the infestation. For example, *Caligus elongatus* is seen to be a mostly transient phenomenon on Scottish farms, with the adults often disappearing from fish as suddenly as they appear, and as such are seldom treated against specifically. This is to an extent explained by the fact that it is a host generalist parasite, although the exact mechanisms of on-farm development of populations of this species in Scotland are much less well understood than is the case for *Lepeophtheirus salmonis*. But it also has to be recognised that *C.*

*elongatus* differs strikingly from *L. salmonis* in molting directly from chalimus IV to the mature, mobile adult – there are no mobile pre-adult stages in the life cycle of *C. elongatus*. Given that distinguishing of *C. elongatus* and *L. salmonis* chalimi requires microscopic observation and specialist taxonomic expertise it is inevitable that routine farm monitoring of the sessile chalimus stages of sea lice infestations will be imprecise. The “sudden” appearance of *C. elongatus* adults on farmed fish is therefore absolutely to be expected and may not, in fact, be attributable to vagile adults transferring from passing, non-salmonid wild hosts as is suggested by some.

In addition, even when levels of sea lice infestation on fish farms might be well below those which would be considered to confer “disease” on the farmed fish, control may still be put in place to minimize the spread of parasites to the surrounding environment and the potential for creating disease on wild fish. This is precisely the reason why the “treatment trigger” level set for farms in many countries is well below a level that would cause disease issues for salmon being raised commercially (see Section 4.1.2).

Because of this potential controversy in agreeing on what might be meant by “disease” with respect to sea lice, the sections which follow will focus upon the much simpler definition of the presence and level of infestation by parasites. Thus it might be more appropriate for the chapter heading to have read, “can we reduce the level of sea lice infestation” (particularly in the case of farmed fish). The sections follow those of the main Salmon Aquaculture Dialogue working group Disease Report in so far as monitoring and surveillance must provide the starting point; from that, any decisions regarding appropriate treatment (or other strategies which might be adopted) to control disease can be evaluated and the effects of any disease and its control can be assessed. It is far easier to monitor and assess the outcome of interventions for farmed fish than it is for wild fish, and there are potentially many more strategies available to control sea lice on farmed fish. For example, in addition to medicinal treatments, a variety of management and even informed environmental decisions can be made which can have impacts on the control of sea lice on farms. The challenges in managing sea lice on farmed and wild fish in an integrated manner should not, however, be under-estimated. Even the designation of farm infestation trigger levels that are viewed as potentially being detrimental to wild fish are totally subjective judgements: there is no scientific means of determining what level of farm infestation (and larval sea lice output) in a given bay or fjord presents a disease risk to adjacent wild salmonid populations.

#### 4.1.1 Monitoring/surveillance (presence, levels and patterns)

The monitoring of sea lice on salmon farms is an important activity which is regularly and routinely conducted on most farms to detect the emergence and development of levels of infestation through time. Fortunately the observation of the mobile adult stages of these ectoparasites is a relatively straightforward matter which requires only minimal training of site personnel. No complex diagnostic or off-site laboratory tests are required. However, as alluded to above, if the various developmental life cycle stages and species of sea lice are to be properly and consistently reported then rather more experience and expertise is required. In response to this many companies arrange training sessions for farm staff to ensure consistency of identification and to emphasize the importance of regular and accurate counting in order to maximize the effectiveness of interventory management of sea lice populations.

What is actually enumerated during sea lice counting varies between countries. For example, some counts will differentiate between adult females and “gravids” (i.e. those with egg strings), while in other counts only the total numbers of adult females will be recorded. The latter is based on the rationale that a mature female may have egg strings one day but none the next (following hatching), and that the “ gravid” subdivision is thus artificial. Most counts also will enumerate the numbers in the “chalimus” stages but are unlikely to differentiate those to stages I – IV. This level of detail normally is associated only with laboratory-based experiments. Furthermore, if multiple species are being counted it is very likely that these are not differentiated for chalimi. In Scotland, for example, only adults are recorded for *Caligus elongatus* and by implication the chalimus load which is noted for *Lepeophtheirus salmonis* will be over-estimated, perhaps especially during the spring and summer months. Despite the limitations of field-based counting on live salmon (compared to microscopic observation in the laboratory of dead samples) a range of interesting observations on the spatial and temporal infection patterns of lice on farmed salmon can be gained from such monitoring (e.g. Revie et al. 2002a, 2002b; Saksida et al. 2007; Lees et al. 2008c).

The frequency and extent of counting is an issue that has been under discussion for some time within both the farming and scientific communities. Some countries (e.g. Norway and Ireland) promote the counting of a larger number of fish from fewer cages while others suggest that fewer fish should be sampled from a larger number of pens (see Table 4.1). A recent study (Revie et al. 2007) has demonstrated that in the presence of significant clustering (over-dispersion) of parasites among individual fish, as was shown to be the case on a range of farms in Scotland and Norway, the choice of a few fish from many cages is a much more efficient way to gain a meaningful farm-level average estimate. However, this assumes that the effort involved in sampling across many cages is similar to selecting just a few pens; i.e. that a sampling effort of “45 fish” is the same whether it is 15 fish from three cages or 5 fish from nine

cages. This clearly is not the case, especially where isolated, large polar circle pens – as opposed to rows of contiguous ‘rectangular’ cages – are in use, or where a seine net is used to obtain a subsample of fish from which the final monitoring sample is randomly selected.

Notwithstanding the statistical implications of sampling protocols, the “best” approach will sometimes have to be modified in the light of practical sampling conditions on site.

	Canada (E)	Canada (W)	Chile	Ireland	Norway	Scotland
<b>No. of cages</b>	2 – 6	2 – 4	2	2	2 – 3	4 – 8
<b>No. of fish/cage</b>	5 to 20	20	10	30	20	5 to 10
<b>Frequency</b>	Bi-weekly	Bi-weekly	Bi-weekly	Bi-weekly or monthly	Bi-weekly	Weekly
<b>Source</b>	Westcott et al (2004)	BCMAL (2005)	<a href="http://www.sernapesca.cl">http://www.sernapesca.cl</a>	O'Donohoe et al (2005)	<a href="http://www.mattilsynet.no">http://www.mattilsynet.no</a>	SSPO (2006)

Table 4.1 Typical sea lice sampling regimes in the major salmon-producing countries.

[N.B. “Canada (E)” includes the eastern USA. There will inevitably be variation from producer to producer within each country; the table attempts to capture the most ‘normal’ practice or protocol mandated by the regulatory authority.]

It also is the case that some countries state that the objective of surveillance is not to provide average sea lice estimates for a given farm, but to determine whether an infection threshold had been breached to the extent that interventory treatment would be required. In this context it is argued that selecting the “worst cage” within a farm is a useful mechanism and this is the approach adopted by both Ireland and Norway. It is an assumption of this approach that the farmer will gain some understanding over time as to which are the “worst” cages on the farm, but in the absence of farm-wide sampling this cannot be tested. A recent study has suggested that, in Norway at least, it is not easy to identify these “worst” cages and so this strategy has come into question (Peter Heuch, pers. comm., September 2008). This is not to say that selecting a small number of cages cannot be justified on other, practical, grounds – it

merely questions the assumption that including a “worst” cage makes this a more appropriate approach.

In Norway, routine monitoring of sea lice on fish farms is carried out by farm staff and in recent years has been based on counts of parasites taken every second week (once sea water temperatures exceed 4°C); the results are reported to the Norwegian Food Safety Authority (<http://www.mattilsynet.no> – and relevant documents therein; keyword “lakselus”). In western Canada there is a monitoring program in British Columbia for farmed fish, but not for wild fish (except by independent scientists). The farm monitoring is done by the farms themselves, and periodically verified by veterinarians from British Columbia’s Ministry of Agriculture, Fisheries, and Food (Saksida et al. 2007). In Norway the situation is similar where farms carry out their own counts with occasional cross-verifications. In Scotland counting is the sole responsibility of the farms though the Code of Good Practice for Scottish Finfish Aquaculture (CoGP) requires that “auditing compliance against the provisions of the strategy will be carried out by independent UKAS [United Kingdom Accreditation Service] accredited inspection bodies as part of the audit process for the CoGP for Scottish Finfish Aquaculture” (SSPO 2006; <http://www.scottishsalmon.co.uk/aboutus/codes.asp>). Ireland provides an alternative approach, whereby all farms have counts carried out by government fish health professionals (O’Donohue et al. 2005) to supplement those routinely carried out by farm staff. However, it must be emphasized that the Irish industry is only a tenth the size of that in Scotland and a fraction of that of Norway. To put this into context, production in Hardangerfjord alone parallels that of the entire Irish industry.

Another issue that must be considered when deciding on the number of fish to be sampled from a cage is the ‘metric’ to be used to measure infestation. In the past, *abundance* – the average number of lice per fish (calculated as total lice counted divided by number of fish sampled) – was the standard metric used (Bush et al. 1997). This is subtly different from *intensity* – the average number of lice on infected fish – though these values are identical when all fish are infected (i.e. *prevalence* = 100%). The *prevalence* metric itself is not very informative at high levels of prevalence (>80%) but recent work has shown that at the lower levels of infestation now typically seen on salmon farms there is an interesting relationship between abundance and prevalence (Baillie et al. 2009). Indeed it appears that prevalence, which can be very readily obtainable, acts as an excellent ‘proxy’ for abundance (see Figure 4.1). In circumstances where there is a need to minimize the handling of fish, or if automated lice counting is being applied, prevalence may be the preferred measure; fish simply are scorable as “infested” or “not infested”. In these cases, however, at least ten fish would have to be taken from each pen to achieve a ‘granularity’ of 10% intervals on the prevalence estimates.

Perhaps the most important issue to note in all of this discussion is that regular and consistent monitoring is the key to successfully tracking, intervening, and managing sea lice infestation patterns on salmon farms through time. The data are inherently 'noisy' and the costs – both in terms of economic loss and stress to fish – of taking large samples, together with difficulties in avoiding various types of sampling bias, will ensure that this is always likely to be the case. If regular (say, weekly) sampling is being carried out it is much more likely that a coherent picture of the underlying infestation patterns will emerge and that 'outliers' can be identified and dealt with appropriately. Regular monitoring also is important to ensure that the outcome of interventions can be assessed and to alert the farmer to the potential of reduced efficacy or failing of intervention strategies (see Section 4.1.3).

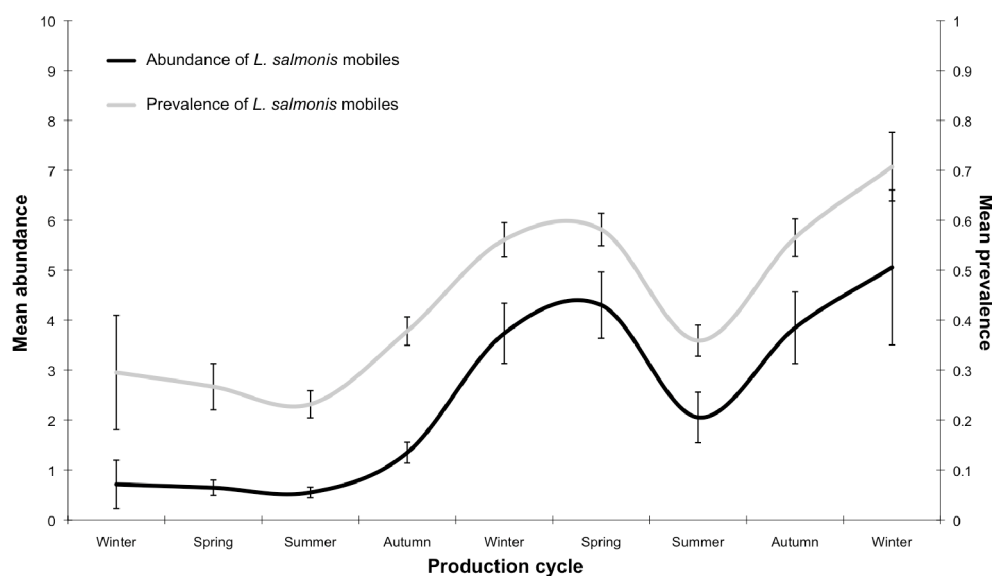


Figure 4.1 The prevalence and abundance profile, with associated 95% confidence intervals, for *Lepeophtheirus salmonis* mobile sea lice over two-year production cycles on Scottish farms between 2002 and 2006.

[From Figure 6 in Baillie et al. (2009), used with permission.]

#### 4.1.2 Treatment/control options for sea lice

A number of control methods have been adopted to reduce the level of sea lice infestation on salmon farms. The most obvious of these are chemotherapeutants, a range of

which have been available for use on farms over the past 30 years. However, before considering this form of intervention it is worth noting some alternative methods for reducing sea lice levels on farms. These relate mostly to management strategies which can be adopted on salmon farms or in the initial positioning of such farms – a summary of these alternative control strategies is given in Table 4.2.

Control Strategy	Description	Approximate adoption dates
Single year class stocking	The practice of having only one year class of stock present in a site at any one time (in contrast to multiple year classes – ages of fish – being present in different cage groups at the same time on a given site). This is much more of an imposition on small operations which may have only a few farms but still wish to have a range of fish sizes available to send to market. For larger operations it is much more straightforward and in most countries these producers have adopted this approach.	Norway (1991); Scotland (1994); Ireland (1995); E. Canada (2001); W. Canada (2002); Chile (2006)  [N.B. These dates are by no means exact and within each country there were 'early adopters' as well as some – mostly smaller – producers who have yet to use this practice.]
Fallowing periods	The decision to leave sites free of fish for a period leads to the practice of "fallowing". In many cases this period will be around 4-8 weeks, though in a few cases it is more analogous to "fallowing" in the agricultural sense of leaving a field unplanted for a year (or at least growing season). For example, in British Columbia (BC) some sites were left empty for a full wild salmon running season while in New Brunswick (NB) a 'rotation' plan is in plans which should result in areas being fallowed every third year.	Because of the interlinked nature of this practice with the use of single year class stocks the dates will be very similar to those noted above. (i.e. it is unlikely that all fish will be removed from a site when multiple age classes are present and so fallowing is not really an option.) The BC experiment happened in 2003, while the NB rotation plan was introduced in 2006.
Synchronised production	In many ways this is a natural extension to the two practices noted above – the major difference being that a number of sites are involved. Once sites were stocking with only one year class and practicing fallowing (essentially to break the cycle of 'self infestation') it made sense to coordinate this with neighbouring sites (as these were the next most likely sources of cross infestation in the absence of significant challenge from wild, as is largely the case in E Canada, Ireland and Scotland). It has also increasingly involved synchronised treatment interventions (see below).	This was introduced from around the mid-1990s in Scotland and Ireland as part of their respective Area Management Agreement and Co-ordinated Local Aquaculture Management System processes. In Canada, and elsewhere, it has also greatly increased with the consolidation of production (i.e. it is much easier to coordinate when one owns all of the sites involved). The trend is more recent and more limited in Norway (e.g. Hardangerfjord from 2003), while the first recorded attempt at such an approach in Chile was not documented until 2007.
Biological control	The only successful 'natural' control measure so be used to date on a commercial scale is the use of cleaner fish. These are discussed below but have been largely restricted to the north east Atlantic	All of Norway, Scotland and Ireland made reasonably extensive use of cleaner fish in the mid 1990s. However since 1998 the only place

	<p>countries – partly because of the more ready availability of the relevant wild species.</p> <p>A number of alternative biological control measures have been suggested but so far with no evidence of effectiveness. These include the use of pumping fresh water into cages (Stone et al. 2002) and even the use of garlic in feed (Boxaspen &amp; Holm 1992).</p>	<p>where significant and regular use has been made is Norway. (Even here they are typically only used when salmon are in their first year of production. Nor have they been used extensively in northern Norway due to low water temperatures and the short summer season.)</p>
Site location	<p>As noted in the discussion below there are a number of aspects of site location that are likely to affect lice levels on farms. For example, locating a farm in a position with fast flowing water or with swift water exchange in the surrounding area is likely to lead to lower lice infestation. Sites located in areas of lower salinity are also likely to be associated with reduced sea lice infestation pressure.</p>	<p>While each country has planning requirements that must be satisfied before salmon farms become active, the authors know of no formal requirement to consider likely effects of location on sea lice levels as part of this planning process.</p>
Production/Design	<p>There have been a number of cage design or production initiatives that have been suggested as being valuable in reducing sea lice infestations. These include the use of light traps (Pahl et al. 1999), automated feeding systems (Lyndon &amp; Toovey 2000), and even a device which emits electromagnetic waves (<a href="http://aquafind.com/info/bioemitter2.php">http://aquafind.com/info/bioemitter2.php</a>).</p>	<p>While a number of these production designs have been taken up by the industry for other reasons (e.g. automated feeders) the authors know of no evidence to support claims that they reduce sea lice infestation.</p>

Table 4.2 Strategies for reducing sea lice infestation on salmon farms which do not involve the use of chemical treatments, as adopted by various countries.

Two of the earliest management strategies to gain acceptance in Scotland, and which were subsequently accepted in most other production countries, were the practices of having single year-class stocks and ensuring a fallow period between production cycles. (Of course many of these practices developed in multiple places as the industry matured so it is difficult to say exactly when something was initiated and/or became “widely adopted” – see also note in Table 4.2. The dates noted in the text below are guided by when various reports of control strategies were published in the peer-reviewed literature.) It was noted by the late 1980s that on Scottish farms the practice of holding more mature fish at the same production site into which smolts were being introduced (a so-called “mixed year class” situation) often led to early and heavy sea lice infestations on the newly introduced fish. The evidence for a beneficial effect of “single year-class” production (also referred to as “all-in, all-out”) in terms of sea lice numbers was provided by Bron (1993a). His study found that smolts introduced into a single year-class setting tended to take longer to develop lice infestations and that when they occurred the initial levels of abundance were lower. This was explained in terms of the absence of a large background ‘reservoir’ of infection provided by the older fish. However, this led also to the additional suggestion (Bron et al. 1993b) that single year-class management could be



enhanced further if a period of fallow was observed between the production cycles. Their proposal was that at least 4 weeks be left between final harvest and the introduction of new smolts. This time was based on the natural life cycle of the lice, adults of which can survive without a host only for a limited period of time (a few days), and the larval development time. Both of these are dependent on water temperatures and other environmental variables and 4-6 weeks appears to be a suitable compromise to ensure breakage of the life cycle without placing undue constraints on farm production and profitability.

The principle of the single year-class production cycle with an intervening fallow period approach has now been adopted in most salmon producing countries. It has been argued that increasing the fallow period beyond 4-6 weeks may reduce the levels of sea lice infection even further. Extension of the period of fallowing may indeed have beneficial environmental effects but there is no evidence that this will aid sea lice control. Indeed in the only large-scale study that has addressed this issue, Revie et al. (2003) found no significant difference between the levels of sea lice found on Scottish farms which had used a “long” (~11 week) period compared to sites which had used a “short” (~6 week) fallow period. An extension of the single-year-class-with-fallowing approach has been commonly applied in Scotland and Ireland over the past decade where the timing of production cycles and fallowing periods are coordinated over a wider area – typically a zone thought to share common sea water exchange. In Scotland this has been increasingly implemented as part of the Area Management Agreements (see Section 1.4), while in Ireland it was initially referred to as “Single Bay Management” and latterly by the acronym CLAMS (Co-ordinated Local Aquaculture Management Systems). In Norway there is no country-wide approach, partly due to the extensive nature of the industry. However, in certain major fjords, e.g. Hardangerfjord, similar moves to co-ordinate production and fallowing (as well as treatment – see later in this section) have become increasingly common.

With almost as long a history of experimentation, though with less universal success or on-going use, has been the adoption of biological control measures using so-called “cleaner fish”. In Europe five species of wrasse (family Labridae) have been variously used as cleaner fish (e.g. Treasurer 1993; Costello 1993, 2006), with the goldsinny wrasse (*Ctenolabrus rupestris*) the most widely used in Ireland, Scotland and Norway. In an un-replicated study, Treasurer (1994) affirmed that goldsinny were effective in controlling sea lice (1-8 sea lice per fish in a wrasse-populated cage, *versus* up to 40 per fish in a control cage). In Eastern Canada, an early assessment of the efficacy of cunner (*Tautoglabrus adspersus*) in controlling *Caligus elongatus* found a significant effect in laboratory trials, but no significant impact after 12 weeks of these wrasse being placed in marine pens (MacKinnon 1995). The conclusion drawn by MacKinnon (1995) was that the natural biofouling of the net pen mesh provided the more attractive foraging resource for the wrasse. Similarly, Deady et al. (1995) observed enhanced cleaning activity of corkwing wrasse (*Crenilabrus melops*) and goldsinny in Ireland when biofouling of

cage meshes was controlled. Since the early trials, millions of goldsinny have been routinely introduced annually to cages in the Norwegian industry but it has to be acknowledged that this is, in all probability, unsustainable because there is total reliance on wild-caught wrasse for this purpose. The use of cleaner wrasse in Scotland had a long history, dating from 1989 (Treasurer 1994), but their use there was curtailed in 1998 following an outbreak of ISA and an unwillingness of farmers to introduce wild wrasse to the net pens (Treasurer 2002). There are no native labrids in British Columbia; however, three-spine sticklebacks will clean lice from young salmon in aquaria (Losos 2008).

Revie et al. (2003) were the first to undertake a comprehensive, large-scale evaluation of a range of management approaches within the context of environmental risk factors for sea lice infestation. Their study included information from over 40 farms in Scotland, over the period 1996 – 2000, and comprised ~120 ‘site-years’ of data (not all farmed sites had fish at sea in all years studied). They found only weak evidence for any effect of a number of management strategies (notably density of fish, overall biomass, or volume of cages) which had been anecdotally suggested as being likely to be relevant and important to the control of sea lice. There were indications of a limited effect for some of these factors based on univariate analysis alone, but these were lost when a fuller multi-variable model was applied. By contrast, evidence was found that a number of environmental factors linked to the local hydrographic dynamics of sites played a role in determining sea lice infestation levels. Specifically, higher sea lice numbers were seen at sites or fjords that were subject to lower current speeds (and which took a longer time to ‘recirculate’ their water), and where the tidal flushing patterns were “mixed” or complex as opposed to flowing continuously in one direction.

The foregoing results raise important questions as to whether sea lice control should be considered at the level of a single farm only, or as part of some broader strategy. Thus, for example, while it was seen that sites with higher current speeds and faster flushing exhibited lower sea lice infestations it is plausible that this might create a greater potential for infection on other farms in close proximity, and/or to wild fish in the surrounding environment. Therefore a factor which is positive in terms of allowing improved control of sea lice within a given farm may be seen to be less so when viewed from the perspective of the overall patterns of infection within the broader coastal habitat. The issue of the most appropriate location of salmon farms and how this can best be achieved to minimize risks and maximize benefits to all concerned parties is complex. This has led to a number of recent attempts to tackle aspects of the problem through the use of fjord/sea loch/archipelago-wide hydrographic modeling (Asplin 2008; Amundrud & Murray 2009; Foreman & Stucchi, In Prep. – for an outline of approach see - <http://www.pacificsalmonforum.ca/pdfs-all-docs/2008ResearchProgram.pdf>).

More limited risk factor studies have subsequently been carried out in Chile (Zagmutt-Vergara et al. 2005) and British Columbia (Saksida et al. 2007). Other than the well-known relationship between the length of time in sea water and level of sea lice infestation (i.e. larger/older salmon tend to carry more sea lice), the Chilean study indicated no clear risk factors, though the quality of the data set was very variable with >85% of the planned data having to be excluded due to incomplete records. The study from British Columbia also found fish size to be a risk factor as well as there being some marked spatial variation. However, neither salinity nor water temperature was found to be a relevant risk factor in this Canadian study. The finding for water temperature was consistent with the results of Revie et al. (2005, 2007) and is something of a paradox because laboratory-based studies have shown a clear link between larval development times and water temperature. In the case of salinity, there have been a number of laboratory and field-based studies which appear to indicate that lower levels of salinity act to reduce sea lice levels (laboratory-based: Heuch 1995, Tucker et al. 2000, Bricknell et al. 2006; field-based: Bravo et al. 2008a, Heuch et al. 2009); why this did not extend to the British Columbia study remains unclear, although the authors did note that the lack of accuracy in salinity measurement may at least partly explain this anomaly.

While this range of 'alternative' control methods has been explored, and to varying extent applied in different countries, by far the most common method to reduce the levels of lice on salmon farms are topical or in-feed chemotherapeutants. Over the past 30 years a range of treatments has been available to salmon farmers (Table 4.3) including a variety of classes of chemical compounds and active ingredients. For a reasonably comprehensive discussion of the historical context including the use of some of the 'older' treatment options the reader is pointed to Roth et al. (1993), Branson (1996), Roth (2000) or Grant (2002). The present discussion is focused on the synthetic pyrethroid (topical, or "bath") and emamectin benzoate (in-feed) treatments because these are the main options in use at the time of writing (2009). However, it should be noted that hydrogen peroxide has recently been used in Norway and Chile, and that azamethiphos has recently become more widely available.

It is possible to categorize the recent treatment approaches into those countries which have been using only a single compound, emamectin benzoate – as has been the case in Canada and the USA (due to regulatory limitation) or in Chile (due to management choice) – and those such as Norway, Ireland and Scotland which have continued to use more than one compound. In the latter countries the use of the in-feed treatment (emamectin benzoate; marketed as SLICE<sup>®</sup>) is predominant in the early part of the production cycle, whereas older fish are more likely to receive a topical treatment (cypermethrin or deltamethrin) because the use of in-feed treatment becomes relatively expensive for larger fish. Anecdotal reports from industry of reduced efficacy (or "treatment failure") of particular compounds and the generic issue of sea lice developing resistance to chemical treatments has been a source of ongoing

concern in a number of countries. The likelihood of such resistance occurring could be predicted from terrestrial studies but typically is difficult to demonstrate. The most convincing case of such resistance having occurred relates to emamectin benzoate in Chile. Resistance has been demonstrated through bioassays (Bravo et al. 2008b) taken from fish subsequent to 'failed' treatments, and would also appear to be clear from field-based data where more than 10 treatments with emamectin benzoate have been administered on some sites within a single

Active compound	Trade-name	Chemical class/Mode of action	Notes on availability/use
Dichlorvos	<i>Aquagard</i>	Organophosphate / Blocks acetylcholinesterase at synapse and interferes with nerve transmission.	Effective against "mobile" pre-adult and adult lice only. Evidence of resistance (Jones et al. 1992). Discontinued in most countries.
Azamethiphos	<i>Salmosan</i>	as above	Similar comments as above, though an application to bring this drug back to the market was recently approved in the UK (2008).
Hydrogen peroxide	<i>Paramove;</i> <i>Salartect</i>	Reactive oxidizer / Oxygen bubbles form within sea lice and disrupt tissues. Toxic also to salmon – low therapeutic index, particularly as sea water temperature rises.	Used in a significant way in parts of Scotland around 2000 when access to appropriate therapeutants was limited. Recent use in Chile, Scotland and Norway as a potential rotation option.
Cypermethrin	<i>Excis</i>	Synthetic pyrethroid / Blocking of sodium channels interferes with nerve transmission.	Used since the late 1990s these bath options still are a part of the core rotation strategy in areas which are not restricted to in-feed options. <i>ALPHA MAX</i> was recently approved for use in Chile (2007) and is available under the 'cascade'/EDR use in Ireland.
Cis-Cypermethrin	<i>Betamax</i>	as above	
Deltamethrin	<i>ALPHA MAX</i>	as above	
Emamectin benzoate	<i>SLICE</i>	Avermectin / Disruption of chloride ion movement within nerve cells.	This has been the most widely used treatment intervention since around 2000 – see the main text for details/discussion.
Ivermectin	<i>Ivomec</i>	as above	This was a swine pre-mix, never designed for use on fish. (Some use prior to availability of <i>SLICE</i> but with poor results.)
Teflubenzuron	<i>Calicide;</i> <i>Ektobann</i>	Insect growth regulator (IGR) / Disrupts ecdysis	By inhibiting the production of chitin these can halt the development of crustaceans - thus effective only against chalimus and pre-adults. Not available in most national markets since around 2003.
Diflubenzuron	<i>Lepsidon</i>	as above	This preparation was used to a limited extent in Norway from 1996 to 2000.

Table 4.3 Treatments that have been, or are being, used to reduce sea lice infestation on salmon farms in various countries.

year (Sandra Bravo pers. comm. April, 2008) – far more than would be needed if the 8-10 weeks of protection noted for SLICE<sup>®</sup> were in fact being achieved. However, in Chile the picture is somewhat confused by the fact that the treatments used have typically involved generic versions of emamectin benzoate (rather than the proprietary SLICE<sup>®</sup> product), and it may be that these products are not genuine equivalents. However, recent reports from Scotland (Lees et al. 2008a, b) indicate that the number of ‘failed’ SLICE<sup>®</sup> treatments has been rising over the past few years and that the clearance rates achieved by fish subsequent to in-feed treatments are falling.

These concerns have led to an increased focus on the need for product rotation where more than one treatment is available. They have highlighted also the need to strategically time treatment interventions to maximize impact (Revie et al. 2005c). In addition there have been moves – particularly in Scotland and Ireland, but more recently in Norway – to synchronize treatments within geographically linked areas. Indeed this is a core element of most AMAs in Scotland and is encouraged in the CLAMS initiative in Ireland. A project aim of the recent Salmon Lice Project in the Hardangerfjord (Finstad et al. 2007a) was to gather empirical evidence to demonstrate the value of synchronising treatments across a relatively large-scale area within a Norwegian fjord. While there are differing costs associated with varying treatments, the main determinants in terms of treatment selection are becoming the necessity to ensure rotation of product and the avoidance of resistance. In all cases treatments must be authorized by veterinarians.

There are circumstances in which attempts to plan and rationalize treatments so as to maximize their impact come into conflict with the “treatment trigger” approach to intervention. This involves the setting of some threshold level(s) above which it is mandatory (in the case of instigation by the regulatory authority) or strongly suggested (in the case of industry guidelines) that a treatment be initiated. For example, in Norway over the period December 1st to July 1st, the average number of sea lice on farmed fish must not exceed 0.5 adult females or a total of 5 mobile lice. On exceeding this limit the farmer has to delouse. (In the Troms and Finnmark districts of northern Norway this period extends from November 1st to July 1st). From July 1st to December 1st the numbers of sea lice must not exceed 2 adult females or 10 mobiles. Again, on exceeding this limit the farmer must apply a delousing medication. If these regulations are not followed the Norwegian Food Safety Authority can impose financial penalties on the fish farmer, within certain guidelines. This fine increases daily and starts from the day the fine was applied until the farmer starts delousing. [As of late 2008 a new regulation is being

implemented that will require that the numbers of sea lice not exceed 0.5 adult females or 3 mobile stages per fish per net pen during the whole year.] In Scotland it is the aquaculture industry rather than the regulatory authorities which sets the thresholds, but in practice the intervention strategies are very similar. The Scottish Salmon Producers' Organisation recommends in its *Code of Good Practice for Scottish Finfish Aquaculture* (SSPO 2006) that farmers adhere to the National Treatment Strategy for sea lice. This includes clear recommendations on target sea lice loadings and requires weekly monitoring of 5 fish from each of 5 net pens. During February-June inclusive (embracing the wild smolt migration period) the target level is a mean of 0.5 adult female *Lepeophtheirus salmonis* per fish. During July-January inclusive this increases to 1 adult female *L. salmonis* per fish. Treatment for "... episodic *Caligus elongatus* infestations should be applied, as appropriate, to protect the welfare of farmed salmon". Note that there is no explicit connection made here between wild salmonids and *C. elongatus* infestation. (Table 4.4 provides a more extensive summary of some of the current treatment triggers/thresholds that exist in a number of countries.)

Country	During period of wild smolt migration	Other times of the year	Notes
Canada (E)	not known	not known	
Canada (W)	> 3 total mobiles	> 3 total mobiles	Reduced from 6 mobiles at "other" times in 2006.
Chile	not applicable (no wild salmonids)	> 6 mobiles, (including gravid females)	Sernapesca noted <i>Caligidosis</i> as a List-2 disease in 2007, with increased surveillance and intervention. The application of this trigger is therefore novel.
Ireland	> 0.3-0.5 gravid females	> 2 gravid females	No reason stated as to why a range is given during smolt migration.
Norway	> 0.5 adult females, or, > 5 total mobiles	> 2 adult females, or, > 10 total mobiles	Reducing to 0.5 females or 3 mobiles at all times of the year (2008/9).
Scotland	> 0.5 adult females	> 1 adult female	

Table 4.4 Treatment trigger levels which exist in various salmon-producing countries. (In some cases these are legislative requirements while in others they are guidelines adopted by industry. The exact timing of the "smolt migration" period will vary from country to country, and even within countries – e.g. Finnmark in Norway.)

### 4.1.3 Measuring effects of sea lice control

As noted above, measuring the effect of control interventions in the case of ectoparasites is more straightforward than with many other pathogens. However, to ensure the efficacy of treatments it is critical that regular monitoring of sea lice be maintained so that trends in infestation can be clearly observed and interpreted accordingly. In a recent paper assessing the effectiveness of SLICE<sup>®</sup> treatments in Scotland (Lees et al. 2008a) it was noted that ~40% of the interventions could not be included in the analysis because of inadequate data collection either prior to or subsequent to treatment. Evaluating the impact of less direct interventions, such as the moving of cages to a higher energy site or increasing the length of fallow period, is even more difficult to achieve. Such evaluations typically require large sets of spatially and temporally dispersed data to take account of the natural variation in infestations that occurs across sites and through time. While many interesting research questions remain in terms of risk factors and effective strategies which require on-going data collection by far the most pressing reason to continue such monitoring activity is to ascertain whether there is any reduced sensitivity of lice to current treatments. In addition to the epidemiological evidence that can be gained from changing sea lice numbers on farms it is important that regular bioassay monitoring is carried out to back up field-based observations (which are subject to high levels of ecological 'noise') with laboratory findings on the underlying mechanisms (Westcott et al. 2008).

There is a range of costs associated with sea lice infestation – a review of which has recently been published (Costello 2009). At low levels of infestation these are unlikely to include direct mortality of cultured stock, but will tend to lead to inappetance, lower growth rates and potential downgrading of fish at harvest. These costs are seldom quantified by farmers in any formal manner. As for the potential 'costs' associated with impact on the environment, or increased levels of sea lice on wild fish, these are again very difficult to quantify. To our knowledge no formal cost-benefit analysis is practiced in terms of determining effectiveness of any interventions.

#### 4.2 Can we reduce impacts on wild fish?

Epizootic outbreaks of *Lepeophtheirus salmonis* have occurred in wild sea trout in Ireland (Tully & Whelan 1993), Norway (Bjørn et al. 2001) and Scotland (Butler 2002) and have been associated with significant host pathology (Heuch et al. 2005; Boxaspen et al. 2007). In Norway, heavily infected wild sea trout postsmolts have been captured in areas where salmon are farmed (Birkeland & Jacobsen 1997; Schram et al. 1998, Heuch et al. 2005, Bjørn et al. 2008). Higher infestation rates of sea lice were also found on juvenile pink and chum salmon in British Columbia near fish farms than on those sampled in areas not exposed to fish farming (Morton et al. 2004). In the late 1970s, the development of Atlantic salmon farming industry began along Canada's east Coast in the Bay of Fundy region but there are no specific reports of epizootics on wild fish from this region. In Ireland (Tully et al. 1999; Gargan et al. 2003), and in Scotland (Butler & Watt 2003), significantly higher infestations of sea lice have been recorded on wild fish in bays that also contained sea lice-infested farmed salmon compared to non-farmed areas. In Chile, marine salmonid aquaculture began in the 1980s with the introduction of commercial-scale coho salmon production (e.g. see Johnson et al. 2004) but here there are no endemic salmonids and no specific reports of epizootics on wild fish which are subject to infestation by the host generalist *C. rogercresseyi*.



Figure 4.2 Sea lice on sea trout (*Salmo trutta*) [Photo: Bengt Finstad, NINA]



For wild Atlantic salmon in Scotland, Todd et al. (2000) recorded greater abundances of *Lepeophtheirus salmonis* on older fish and consistently 100% prevalence on wild one sea-winter salmon (Todd et al. 2006). Wild salmon returning to the coast of Ireland had a prevalence of >94%, including some juvenile sea lice (Copley et al. 2005). In Norway, Schram et al. (1998) found *L. salmonis* prevalence rose from 20-35% in March and April to 100% in autumn in a coastal area distant from salmon farms. Prevalence and intensity of sea lice on adult returning salmon in Norway (Bjørn et al. 2003) showed an average of 15 adult *L. salmonis* at high prevalences in the period from May to July. Elsewhere in Norway, Bjørn & Finstad (2002) reported a lower prevalence of *L. salmonis* on sea trout and Arctic charr but noted, in one year, lower levels of sea lice in an area distant from fish farms compared to an area with fish farms. Similar correlations have been reported subsequently (Bjørn et al. 2008, 2009).

Wild salmonids maintain a population of sea lice throughout the year (Tingley et al. 1997; Todd et al. 2000; Copley et al. 2005) and all life stages of sea lice are found on salmon on their oceanic feeding grounds. In the Norwegian Sea, north of the Faroes, Jacobsen & Gaard (1997) reported *Lepeophtheirus salmonis* prevalence at >99%. The abundance and density of sea lice was higher on one sea-winter farm escapees but was similar on two sea-winter fish, suggesting cross infection on the high seas. The finding of all developmental stages of sea lice on high seas fish affirms that infestation occurs commonly in the open ocean as well as in coastal waters.

The severity of the infection of *Lepeophtheirus salmonis* for an individual fish depends upon infection intensity, the developmental stage of the parasites (Grimnes & Jakobsen 1996; Bjørn & Finstad 1998) and the size and physiological status of the host (Finstad et al. 2000). A series of laboratory studies has revealed the effect of *L. salmonis* on host physiology, and the number of sea lice that can cause mortality has been estimated (reviewed by Tully & Nolan 2002; Heuch et al. 2005). Major primary and secondary physiological effects (Wendelaar Bonga 1997), including elevated levels of plasma cortisol and glucose, reduced osmoregulatory ability and reduced non-specific immunity in the host, occur when the sea lice develop from the final sessile chalimus IV stage to the mobile first pre-adult stage. Sublethal tertiary effects, such as reduced growth, reduced swimming performance and impaired immune defense, have also been reported (Bjørn & Finstad 1997; Nolan et al. 1999, 2000; Finstad et al. 2000; Wagner et al. 2003; Heuch et al. 2005) causing the fish to be more susceptible to predation and diseases. In the case of Arctic charr, impacts on host reproduction have been shown: *L. salmonis* infections may decrease the number of reproducing fish, delay the maturation of survivors and reduce fecundity (Bjørn et al. 2002b). *L. salmonis* has been reported from multiple wild fish species both in the Atlantic and Pacific Ocean (Table 4.5), as reviewed by Pike & Wadsworth (1999), Tully & Nolan (2002), Johnson et al. (2004), Costello (2006), Boxaspen et al. (2007) and Heuch et al. (2005).

Species	Canada	Ireland	Japan	Norway	Scotland	Other
<i>Salmo salar</i>	Carr & Whoriskey 2004	Copley et al. 2005		Holst et al. 1993,2003; Berland 1993; Finstad et al. 1994, 2000; Bjørn et al. 2001, 2008; Glover et al. 2004	Todd et al. 2000; Butler & Watt 2003	Jacobsen & Gaard 1997 (Open Ocean Atlantic)
<i>Salmo trutta</i>		Tully et al. 1999; Tully & Whelan 1993; Gargan et al. 2003		Schram et al. 1998; Mo & Heuch 1998; Bjørn et al. 2001, 2008; Bjørn & Finstad 2002; Heuch et al. 2002; Rikardsen 2004	MacKenzie et al. 1998; Butler 2002; Butler & Watt 2003; Marshall 2003	
<i>Salvelinus alpinus</i>				Bjørn et al. 2001; Bjørn & Finstad 2002		
<i>Oncorhynchus gorbuscha</i>	Morton et al. 2004		Nagasawa & Takami 1993			
<i>Oncorhynchus masou</i>			Nagasawa & Takami 1993			
<i>Oncorhynchus</i> sp	Morton et al. 2004 ( <i>O.keta</i> ); Krkošek et al. 2005b, 2006a, 2008. DFO has also found <i>L. salmonis</i> on wild coho ( <i>O. kisutch</i> ) and chinook ( <i>O. tshawytscha</i> ) salmon in BC (Larry Dill, pers. comm. 2008)		Nagasawa 1985, 1987			

Table 4.5 References for the geographical distribution of *Lepeophtheirus salmonis* on different species of wild salmonids (*Salmo* and *Oncorhynchus*) listed by country. The information for *Oncorhynchus* spp. refers only to juveniles (fry, smolts) as they are well known to be present on adults. [Revised version of Boxaspen et al. (2007)]

#### 4.2.1 Monitoring/surveillance

In Norway a national monitoring programme on wild fish started at the beginning of 1990 and is ongoing (Bjørn et al. 2008, 2009). Monitoring has been based on registrations of sea lice on wild sea trout and Atlantic salmon captured at sea (postsmolt trawling, gill-netting and bag net fishing) and electrofishing in estuaries/rivers, in addition to experimental cage studies with smolts deployed in fjord systems and releases of individually tagged fish protected and not-protected (population effects) against sea lice by prophylactic treatment. The intensity of the monitoring programme has increased over recent years due to the evaluation and establishment of national salmon fjords; these areas are specifically designated with a view to protecting wild fish from sea lice (Anon. 2006).



Figure 4.3 A fish farm in Norway [Photo: Eva B. Thorstad, NINA]

Other countries, such as Ireland, also started their monitoring of wild fish by gill netting (Gargan et al. 2003). In Scotland, sweep netting is used for routine monitoring of sea lice on wild salmonids. This work is undertaken by the Regional Development Officers of the Scottish Tripartite Working Group (STWG) in conjunction with the biologists of the Fishery Trusts. This work is funded by the Scottish Government as a part of its financial support to STWG. In

Canada's Broughton Archipelago, Fisheries and Oceans Canada (DFO) has implemented sea lice monitoring on wild fish by use of beach and purse seine surveys (e.g. Jones & Hargreaves 2007).

### Effects on populations of sea trout, Arctic charr and Pacific salmon

As discussed in Section 4.2, sea lice can exert impacts on individual wild salmonids and ultimately on population structure. Field monitoring of wild fish should take place both in rivers, estuaries and in adjacent salt water systems. Prematurely returned sea trout will, during their stay in freshwater, either reduce or totally lose their sea lice infection. Freshwater is lethal to sea lice, and most mobile stages of the parasite are lost within a matter of hours or days of river entry (McLean et al. 1990; Finstad et al. 1995). Following premature migratory return the host may subsequently die, or it may re-establish its physiological stasis and return to sea (Birkeland 1996). Thus, even for specific rivers in Norway, Scotland and Ireland which have provided comprehensive fish trap records of return-migrant fish, catches of prematurely returned fish alone will not provide information on the consequences of sea lice infestation for comparable fish which had remained throughout in salt water. Similarly, counts of sea lice on post-smolt sea trout captured in brackish water may not accurately reflect, or be representative of, local sea lice infestation levels because of the propensity for sea lice to be lost from the host in brackish water. Todd et al. (2006) have indicated that *Caligus elongatus* may be especially sensitive to brackish water and detach from the host fish more readily than does *Lepeophtheirus salmonis*. *C. elongatus* has, therefore, probably been historically under-recorded on wild sea trout in Scotland because the host fish tend to congregate in brackish waters and it is there that they are more easily captured and sampled for sea lice. In a similar vein, most commercial netting of wild salmon in Scotland is now focused on estuarine ("in river") locations and sea lice registrations from these brackish areas are liable to be severe under-estimates of true abundances (Todd et al. 2000; see also Urquhart et al. 2008).

Despite the limitations noted above in Scotland, targeted gill-netting for sea trout throughout the coast of northern Norway has been shown to be successful for monitoring sea lice loadings on these fish (Bjørn et al. 2008, 2009). The use of sentinel cages containing naïve smolts has been used successfully in several fjord systems in Norway for estimating the local infestation pressures on wild salmonids (Finstad et al. 2007; Bjørn et al. 2008, 2009; Boxaspen et al. 2008). It has also been shown that sea lice abundance on fish in sentinel cages provides a good correlation with abundances recorded for fish taken by gill-nets at the same locations (Bjørn et al. in prep).

Work by Krkošek and co-workers in the Broughton Archipelago has combined mathematical models with field data to address three fundamental issues in sea lice and

salmon ecology: transmission dynamics, juvenile salmon mortality, and salmon population viability. To study transmission dynamics, Krkošek et al. (2005b, 2006a) modeled sea lice abundances on juvenile pink and chum salmon migrating past salmon farms. Krkošek et al. (2005b) found that a single salmon farm increased sea lice exposure of juvenile pink and chum salmon over a distance as great as 30km along a migration corridor. Farm-origin sea lice larvae raised the infection risk by up to 4 orders of magnitude above ambient. To further assess the corresponding mortality of infected juvenile salmon, Krkošek et al. (2006a) modeled the population dynamics of sea lice and juvenile salmon collected from the field and reared in ocean enclosures. They estimated that 9-95% of juvenile pink and chum salmon were killed by farm-origin lice, with variation depending on year, season, length of migration corridor, and the number of salmon farms per migration corridor. Krkošek et al. (2007b) then assessed the effects of annually recurrent sea lice infestations on the productivity and viability of wild pink salmon populations. By using a standard fisheries model to compare pink salmon escapement data between the Broughton Archipelago and a reference area to the north, they controlled for confounding factors (e.g. climate) and found that infestations were associated with depressed and declining pink salmon populations. Taken together, the findings of Krkošek and colleagues indicate that salmon farms increase sea lice exposure of juvenile salmon, that sea lice increase mortality of juvenile salmon, and that recurrent infestations contribute to declines in wild salmon populations. The details of the work have been contested in several critiques (see Section 5.4.1, and Riddell et al. 2008), but the original authors have attempted to address the concerns in a series of responses that substantiate the basic conclusions of increased transmission, mortality, and decline (see, most recently Krkošek et al. 2008). All of these effects are likely mediated by biotic (e.g. predator abundance), abiotic (e.g. temperature), and management (e.g. chemical treatment) factors that are not yet well understood.

A significant body of data therefore shows that high salmon louse infection levels on wild sea trout, Arctic charr and Pacific salmon can have important negative effects on populations or local stocks. Detailed monitoring in Norway and Ireland has revealed premature return of sea trout in salmon-farming areas (Birkeland & Jakobsen 1997). However, further controlled laboratory investigations and long-term field experiments, in addition to routine monitoring, is required to assess any population-level effects of salmon lice on these salmonids, and to estimate a “sustainable” infection pressure.

### **Effects on running Atlantic salmon post-smolt populations**

Out-migrating wild salmon smolts in Norwegian fjords have been monitored using a specially designed trawl, the “Ocean Fish Lift”, which catches and retains live smolts within a sealed box and with minimal scale loss, thus retaining their natural ectoparasitic infections

(Holst & McDonald 2000). This technology offers reliable estimates of sea lice infection levels, and also allows estimates of mortality due to sea lice using the methods detailed above. In addition to Norway, this technology has been deployed in Canada, Ireland and USA, and it remains an important component of the ongoing Norwegian monitoring programme (e.g. Holst et al. 2000b, 2003a; Bjørn et al. 2002a, 2003, 2008). Results to date have shown large variations in *Lepeophtheirus salmonis* prevalence and mean intensity between years and between fjords. The full range from zero to >100 sea lice per fish has been observed, giving mortality estimates varying from zero to >90% of fish in the migratory run (Bjørn et al. 2001b, 2002a, 2003; Holst et al. 2003b; Bjørn et al. 2008, 2009).

Mortality as a consequence of sea lice infestation may be either additive or compensatory (Tompkins & Begon 1999). To investigate this aspect, individually Carlin-tagged salmon and sea trout smolts in Norway have been protected against sea lice either by an in-feed medication or by topical (bath) treatment (Substance EX, Pharmaq), and released in the vicinity of their native river. Substance EX protects the fish for several weeks, and provided the treatment does not interfere with the fish in other ways, these experiments can give estimates of population effects of sea lice on wild salmonids. Data from Agdenes (Sør-Trøndelag County, central Norway) and Daleelva (Hordaland County, SW Norway) show that in years of high salmon lice infection pressure, the returns of protected fish have been higher than returns of unprotected control groups (Finstad & Jonsson 2001; Hvidsten et al. 2007). These studies also are supported by Skilbrei & Vennevik (2006). Complimentary studies with SLICE<sup>®</sup>-treated salmon smolts also have been performed in Ireland (Hazon et al. 2006) and support these findings. However, the long-term effects of sea lice on wild populations are still uncertain, and it is not known at what magnitude the observed salmon louse epizootics may impact host population size.

#### **4.2.2 Treatment/control options (all interventions)**

Prophylactic treatment of smolts by means of Substance EX, SLICE<sup>®</sup> or potential vaccines can be applied to trapped fish in specific rivers where wild populations are endangered. Feeding treatment of pre-smolts in rivers and prior to their migration to sea might protect fish from emigrating to sea in a sub-optimal physiological condition but probably is impractical in relation to the necessary scale of intervention. For specific rivers with effective fish traps, migrating smolts may be trapped by a fish lift system in the river and/or the estuary and thereafter treated topically or by an in-feed substance (e.g. SLICE<sup>®</sup>). However, this approach is extremely labour-intensive and expensive; rivers with effective fish traps are few in number, and this strategy could be applied only to small river systems for which a suitably large proportion of the wild smolt run can be intercepted and treated. Moreover, in the case of in-

feed treatments, an extended period of time (possibly weeks) in captivity is necessary in order to wean fish onto pellets and to allow their uptake of a sufficient dose of the active ingredient. Delaying the natural migration of wild smolts may lead to mortality, loss of physiological condition and, in extreme cases, physiological “de-smoltification”; the latter two themselves have negative effects on subsequent survival prospects for the fish at sea irrespective of subsequent sea lice infestation.

Pollution from agricultural farms and acid rain can both lead to reduced condition, or “quality” of salmon smolts and it has been shown (Finstad et al. 2007b) that smolts of sub-optimal condition on reaching the sea are more at risk of sea lice than are smolts reared under optimal conditions. Fish that are sublethally stressed may be more sensitive to microbial or viral disease and parasite attacks and can show reduced tolerance to additional stressors (Iversen et al. 2005). Salmon populations on the western coast of Norway may experience both moderately acidified conditions in rivers (Kroglund et al. 2002) and subsequent salmon lice challenge on entering seawater (Birkeland & Jakobsen 1997; Holst et al. 2001; Heuch et al. 2005). Therefore, year to year variations both in acidification and salmon lice infection pressure can singly, and in combination, explain at least some of the temporal variation in postsmolt survival, and hence the variation in Atlantic salmon year-class strength in Norwegian rivers.

#### **4.2.3 Measuring effects of disease and disease control**

Annual salmon lice epizootics on wild salmonids in areas with intensive fish farming activity, together with the detrimental effects of escaped farmed salmon, were the most important arguments for the establishment of the Norwegian National Salmon Fjords: these are protected fjord areas in which salmon farming is prohibited. Some of the Norwegian National Salmon Fjords, especially the larger ones, appear to have been effective at protecting local wild salmonid populations, but a longer time series is needed before firm conclusions can be drawn (Bjørn et al. 2008, 2009). Migrating salmonids do, however, commonly encounter a relatively high infection pressure from sea lice once they have migrated to the outer fjords and coastal areas. In areas subject to intensive salmon aquaculture, the total biomass of farmed salmon in a given region (or complex of inter-connected fjords), may be so high that even ensuring very low levels of sea lice on each farmed fish may not be sufficient to reduce the overall infection pressure to a sustainable level. Therefore, in addition to the enactment of National Salmon Fjords, it would appear to be necessary to both reduce even further the lice level on each farmed fish and to optimise delousing strategies if the management aim of <10 sea lice per wild fish (and thence no negative effects on wild salmonid populations) is to be achieved as described in Sections 1.3.1 and 2.3.2 (see also Bjørn et al. 2008, 2009).

Parasitic copepods may serve as vectors of viral and bacterial diseases of fish (Nylund et al. 1991, 1993) and in laboratory studies *Lepeophtheirus salmonis* has been able to function as a vector for the viral agent responsible for infectious salmon anaemia (ISA) (Nylund et al. 1993, 1994). However, there is no evidence from field studies that *L. salmonis* can act as vector for diseases and specific attention must be given to this important possibility.

#### **4.2.4 The issue of farms as a potential bio-magnifier of pathogens in the water**

Bio-magnification can be defined as the capacity of aquaculture populations of host fish to locally amplify or magnify the potential of a parasite or pathogen to infect adjacent wild populations of host fish. For any assessment of the potential of farmed fish to present a potential source of bio-magnification of disease threat to wild fish stocks it is imperative to appraise the relative abundances of wild and farmed salmonids within a given area. In British Columbia, the populations of wild fish far exceed those on salmon farms (total production in 2007 ~50,000 tonnes) on a province-wide basis, although the ratio can be reversed in local areas, such as the Broughton Archipelago, where farms are especially abundant and wild salmon populations are depressed. On the east coast of Canada farm production is slightly lower (~40,000 tonnes) but there the stocks of wild Atlantic salmon are extremely low (ICES 2008; <http://www.ices.dk/reports/ACOM/2008/WGNAS/WGNAS2008.pdf>). In Norway the situation is very different from British Columbia in that farmed fish greatly outnumber wild salmon. In 2007 there were 280 million farmed fish (~810,000 tonnes of Atlantic salmon and rainbow trout – Anon 2008b) compared to an estimated stock of ~2.5 million wild salmon, while the wild catch of Atlantic salmon in Norway in 2007 was a mere 608 tonnes. In Scotland, farmed fish production was ~140 000 tonnes (2007) compared to a wild fish catch of 346 tonnes (Anon. 2008a).

#### **Escaped salmon and rainbow trout**

Escaped farmed salmon and rainbow trout may carry significant numbers of adult female sea lice (Grimnes et al. 2000). According to official statistics, in 2006 a total of at least 920,000 fish escaped from farms in Norway (Hansen et al. 2008). Given that the reporting of fish escapes is compulsory, it is clear from the repeated incidental catches of escapees in areas where no escapes have been reported that these official statistics are an under-estimate. If each of these escaped fish carries a mean of 10 adult female sea lice (Grimnes et al. 2000; Bjørn et al. 2003), the salmon lice infestation pressure from escapees to the wild fish may be substantial in several fjord systems. While this assumption regarding mean infestation levels seems somewhat high in the context of current treatment trigger levels in Norway (see Section



4.1.2), it is clearly impossible to treat these escaped fish and they may therefore provide a constant source of infection for both wild and farmed fish throughout the year. As an illustration of the severity of this potential infestation source, Heuch & Mo (2001) have assumed different mean abundances of lice on farmed salmonids and calculated estimates of the number of eggs produced by adult female salmon lice on different hosts between April 1 and June 1 1999. For Norwegian coastal areas including the counties of Vest-Agder and Nordland, they showed that escaped farmed hosts could be a significant source of salmon lice.

### **Density of fish farms**

The density of fish farms in different Norwegian fjord systems varies considerably and should be taken into account when assessing the sea lice infestation pressure on wild fish. In extensive and heavily-farmed fjord systems, such as Hardangerfjord in Norway, production amounts to ~60,000 tonnes (nearly 10% of the total Norwegian industry) and the sea lice infestation pressure for wild fish from such a high density of farmed fish is substantial (Finstad et al. 2007a). Examples such as this illustrate the crucial importance of considering not the average sea lice loadings per fish, but total loadings (and their consequential absolute levels of larval production) when attempting to manage farm-wild interactions of sea lice infestation. But as discussed in Section 4.1, there is no scientific means of determining what level of total farm larval sea lice output in a given fjord comprises a disease risk to adjacent wild salmonid populations. That remains a subjective judgement and the level will vary among fjords (or bays) according to local hydrography, wind conditions, and the precise coastal migratory routes of wild salmonids in relation to farm siting.

### **Cod farming (Norway)**

In 2007, a total of 8,900 tonnes of cultured cod (*Gadus morhua*) was produced in Norway (Anon. 2008b). Contrary to the fact that there shall be siting of no new fish farming activity of salmonids in National Salmon Fjords, cod farming has been allowed to be established in these protected fjords. Both *Caligus elongatus* and *Caligus curtus* are natural ectoparasites of cod and the former are reported to infect salmonids, so there are inevitably growing concerns regarding the expanding and developing cod farming industry and its potential impacts on wild salmonid populations.

### **Marine fish species as potential sources of sea lice to wild/farmed fish**

The potential spread of *Caligus elongatus* from other marine fish species to wild fish and/or fish in farms is of increasing concern, especially as the new cod farming industry expands and develops. *C. elongatus* is associated with more than 80 host fish species and *Caligus curtus* also is a host generalist, having been recorded from 32 species as hosts (including both teleosts and elasmobranchs). Jones et al. (2006a) first reported high levels of *Lepeophtheirus salmonis* on three-spine sticklebacks (*Gasterosteus aculeatus*), but few mobiles and no adult females have been found on thousands of fish examined. Furthermore, laboratory studies (Jones et al. 2006b; Losos 2008) confirm that *L. salmonis* cannot complete its life cycle on sticklebacks. In addition, at least in the laboratory, sticklebacks clean lice from pink salmon that are placed in the same tank (Losos 2008).

## Chapter 5 Disease management systems

### 5.0 Introduction

In writing this 'matching' section to the general disease report the Sea Lice Working Group have decided to pick up on the issues of systems management and disease modeling as they relate to sea lice. This is partly because many of the other issues addressed in Chapter 5 of the main report are fairly generic in nature. However, it is also arguably the case that sea lice are one of the most studied diseases of aquaculture and thinking in terms of 'system-wide' management has been relatively well developed. As such it was felt that reviewing in detail some of the important research in these areas would be of more value than giving cursory mention to a wider range of general management issues.

The principles of Integrated Pest Management (IPM) have been taken from the terrestrial setting and attempts made to apply them to sea lice, more so than for any other parasitic disease of fish. In addition, sea lice infestation on salmon farms has been a matter of significant controversy, initially most notably with respect to wild sea trout populations in Ireland and Scotland, Atlantic salmon in Norway and, more recently, for the case of wild Pacific salmon in British Columbia. Given the impossibility of directly observing and tracking individual sea lice larvae from release by the adult female to ultimate settlement on a host fish, alternative indirect analytical approaches have proven necessary to assess farm-wild interactions. The utility and limitations of these various empirical methods (e.g. molecular genetic and stable isotope markers) has been reviewed (Todd 2006) and molecular DNA markers appear to offer the most promising potential of informing estimates of dispersal distances and population connectedness. A conceptually different, but complementary, approach has been the development of mathematical models to enable both a better understanding of infection dynamics and to aid decision makers in exploring assumptions regarding underlying management parameters and potential intervention strategies. Once again, these models are much more diverse and complex than is typical for most pathogens within the aquatic setting. However, in a number of cases the models themselves have become a source of controversy. This is not necessarily "a bad thing" because it is arguably not the place of mathematical modeling to produce answers/solutions, but rather to encourage policy makers, commercial farmers, sport fishery managers, and scientists to think more carefully about their assumptions and the likely impact of various types of intervention. In this section a range of these "systems" issues are explored.

## 5.4 Systems management

### 5.4.1 Sea lice modeling and empirical data

A growth in the number and complexity of mathematical models has been a recent feature in the development of an increased understanding of sea lice dynamics on farmed and wild salmonids. However, modeling the dynamics of sea lice populations on wild fish has made limited progress, due largely to the relative lack of detailed empirical data when compared to the information available for farmed fish. A fundamental requirement of any modeling exercise is confidence in the observational data and sampling sea lice on wild fish is not easy. In cases where the fish are to be returned alive to the natural environment there are time and handling limitations. For observations made in the field, the lack of opportunity for microscopic inspection makes it impossible to count the infective copepodids and to confidently separate all four chalimus stages. In NW Europe and E Canada, where both *Caligus elongatus* and *Lepeophtheirus salmonis* infest fish, or in British Columbia where both *Caligus clemensi* and *L. salmonis* are present, it is impossible to distinguish these species at the chalimus stages without detailed microscopic examination and without anaesthetizing the fish. Anaesthesia and potential mortality raises ethical questions for endangered wild populations and in some countries anaesthesia of fish for scientific purposes requires legal authorization. In the United Kingdom, for example, both personal and project Home Office licences are necessary for anaesthesia because this falls under the Animals (Scientific Procedures) Act 1986.

While developments in the modeling of lice infestation dynamics are welcome and have moved knowledge beyond that based on simple observational studies to a more comprehensive understanding of lice populations and their likely interactions, they have not been without controversy. In particular, the models which have been developed to explain or predict the linkages between salmon farming and infections on wild Pacific salmon smolts have been a source of scientific debate. It is beyond the scope of this report to fully discuss the issues involved and the interested reader is pointed to a recent report by an independent scientist commissioned by the BC Pacific Salmon Forum which addresses this more fully (Harvey 2008). In his conclusions Harvey makes the following statement about the place of models in both our current and likely future understanding of the domain:

“Much of the current controversy over sea lice in the Broughton comes from using different mathematical models on different portions of the available data. Each model has its proponents, who feed their models with different data. Hence all the conclusions that flow from the models – farms contribute most of the sea lice, farms are a minor contributor, and everything in between – are equally ‘right’.” (Harvey 2008: p.8)

### Limitations in sampling procedures

A major factor concerning the reliability and validity of monitoring counts of sea lice relates to the method of fish capture and the environment from which fish are sampled. For gill-netted fish it is almost inevitable that the fish will be killed by the netting process or following removal from the net. Anecdotally, there is a great deal of suggestive evidence that sea lice will readily detach from even recently dead host fish. [This also raises questions over the validity of sea lice data obtained from commercial or even scientific long-line fisheries in both the North Atlantic and Pacific Oceans (e.g. Nagasawa et al. 1993, Jacobsen & Gaard 1997, Nagasawa 2001), in which hooks are deployed for extended periods and fish may die prior to retrieval.] Gill nets are a commonly used and convenient method of capturing salmonids and have the advantage that they can be set in very shallow water and close to the shoreline (e.g. Bjørn & Finstad 2002). Because of the tendency for gill-netted fish to die quickly it is therefore expedient that nets be deployed for only brief “soak times” (hours) to preclude loss of sea lice from intercepted fish. This can be very labour-intensive and will restrict the number of nets or locations to be fished in any one area. Gill-netted fish may lose scales when the fish is actually captured. Fish should therefore be sampled within a few hours, taken very carefully out of the net when sampled and scissors used to clip the fish out of the net. Thus, whilst acknowledging the difficulty of preventing the loss of mobile adults during fish handling, counts of attached chalimi for dead fish taken to the laboratory may be markedly error-prone if scale loss is considerable. However, this is a well-accepted sampling method if the protocols are followed carefully and sampling performed by trained field personnel.

The Norwegians designed and developed the FISH-lift trawl technology for post smolts (Holst & McDonald 2000); these juvenile fish that have a very high propensity to drop or lose scales. This modified trawl retains the captured fish in a rigid box which remains water-filled even when retrieved to the ship deck.

Seine, or sweep-netting, of fish (e.g. MacKenzie et al. 1998; Marshall 2003; Butterworth et al. 2008) also has marked drawbacks, due again to the abrasion and loss of both mobile stages and attached chalimi as the net is gathered and fish tend to thrash in the shallow water. In some circumstances, welfare considerations necessitate the rapid killing of the fish – typically by a blow to the head. This can have important consequences for sampling of, for example, *Lepeophtheirus salmonis* because the sides and back of the head are the preferred locations of adult males and pre-adults of both sexes infesting wild Atlantic salmon (Todd et al. 2005).

The tendency of both *Lepeophtheirus salmonis*, and perhaps especially *Caligus elongatus*, to detach from the host fish in brackish or estuarine water has obvious implications for the reliability of count observations of sea lice infestations on wild fish. In many areas of Ireland, Scotland and Norway juvenile post smolt sea trout often tend to congregate in brackish

waters and at the outfalls of small rivers and streams. It is in these locations that the fish are most easily and reliably caught (usually by sweep-netting from the shoreline) but the validity of sea lice data (e.g. Marshall 2003) in these reduced salinity circumstances may vary. For larger adult sea trout and salmon captured in large river estuaries or hyposaline fjords the same question applies (Todd et al. 2000).

Time-series data have been collated for *Lepeophtheirus salmonis* and *Caligus elongatus* abundances on wild 1SW salmon captured in coastal bag nets in fully marine waters off the north coast of Scotland (Todd et al. 2006). Over a seven-year period abundances of mobile *L. salmonis* ranged from 17 to 34 sea lice per fish, whereas *C. elongatus* was generally rarer, at 3 to 24 adults per fish. These probably are the most reliable and representative sea lice data available for return-migrant adult Atlantic salmon because of the lack of an estuarine influence and because the fish typically remain alive and free-swimming in these traps. Urquhart et al. (2008) quantified *L. salmonis* and *C. elongatus* on wild sea trout captured in commercial “jumper” net traps deployed on a beach near the estuary of a major salmon river in the east of Scotland. Fish are commonly caught “hung” (= gilled) in these nets but generally are retained in the open bag section of the trap. None the less, given that it is well-known that salmonids captured in or near estuaries may have recently lost significant proportions of their sea lice prior to capture because of brackish salinities (e.g. Todd et al. 2000), the abundances of sea lice in such circumstances are probably unreliable and will almost certainly be considerable underestimates.

Unlike Pacific salmon species, Atlantic salmon cease feeding on their migratory return and cannot be captured in coastal waters with baited lines or by trolling. In terms of capture methodology, the most reliable and detailed quantitative data for *Lepeophtheirus salmonis* infesting wild adult Pacific salmonids are those presented by Nagasawa and colleagues (e.g. Nagasawa 2004; Nagasawa et al. 1993, 2001) and by Beamish et al. (2005). Nagasawa et al. (1993, 2001) sampled *L. salmonis* from chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), steelhead (*O. mykiss*), pink (*O. gorbuscha*), chum (*O. keta*) and sockeye (*O. nerka*) salmon from long-lines in offshore waters, and Beamish et al. (2005) captured all these host species except steelhead by trolling in the coastal waters of British Columbia and quantified both *L. salmonis* and *Caligus clemensi*. In all these studies, captured fish were individually bagged and processed and the parasite prevalences and abundances recorded are therefore probably the best attainable. Several contrasts can be seen among these studies which raise important questions regarding the infestation dynamics of not only *L. salmonis*, but also *C. clemensi*. For example, Nagasawa et al. (1993) found that sockeye were rarely infested with *L. salmonis*, whereas Beamish et al. (2005) noted markedly higher intensities of adult female *L. salmonis* on sockeye than on the other four species. Also, it is notable that >70% of the *C. clemensi* recorded by Beamish et al. (2005) for pink, sockeye, chum and coho salmon were of chalimus stages,

indicating recent or very recent infestation. However, an important detraction from the data reported by Beamish et al. (2005) is that the prevalences and intensities of the two parasite species are not distinguishable – they refer only to “sea lice” (i.e. *L. salmonis* plus *C. clemensi*), and within host species they provide only the percentages of the various developmental stages noted.

Use of sentinel cages in various fjord systems has been applied (Finstad et al. 2007a; Bjørn et al. 2008, 2009). In these cages groups of naïve smolts are placed for up to three weeks at 1 m depth in different sections of the fjord system and used to assess local settlement rates for sea lice larvae. Standardized knotless mesh nets are used and the data obtained from caged fish can be compared with sea lice prevalences and intensities for gill-netted/trawled salmonids in the same area (Bjørn et al. 2009).

### **Applying quantitative epidemiological approaches to sea lice dynamics**

A number of research efforts have been associated with modeling work on sea lice and, in a few cases, more generally for aquatic parasites. These bring a range of formal quantitative and mathematical approaches to bear on critically important issues including population dynamics, parasite dispersal and risk factor identification. Most of the explicit modeling articles in the published literature date from the last five years, indicating a maturation of quantitative research within this domain. However, credit should go to scientists working in Ireland under the guidance of Oliver Tully for stimulating thinking in this area, and his was arguably the first paper to consider specific modeling issues in the context of sea lice dynamics (Tully 1992). While Tully dealt specifically with *Lepeophtheirus salmonis*, his work presented general principles of infection dynamics and highlighted important parameters which must be examined before any prediction of infection levels on either wild or cultured salmonids can be achieved. Tully (1992) built on his earlier exploration of development times and successive generations (Tully 1989) and in a sense provided the earliest formulation, albeit in a non-mathematical format, of a ‘model’ of host-parasite interactions and population dynamics for sea lice.

The other researcher, not often mentioned in the context of sea lice modeling as he was working in the more general area of ecological modeling, but whose early work should be noted, is Simon Wood. In particular he was interested in techniques for predicting structured population trajectories (Wood 1994) and discussed the application of these techniques to aquatic organisms (Ohman & Wood 1995), before looking specifically at parasitic copepods (Ohman & Wood 1996, Nisbet & Wood 1996). Wood remains involved with researchers applying population estimation techniques in copepod research (e.g. Asknes et al. 1997; Eiane

et al. 2002), while continuing research on approaches to modeling complex population dynamics (Kendall et al. 1999; Wood 2001).

Despite Tully's early interest in the underlying knowledge required to create population models, and the work by Wood and others on theoretical mechanisms to simulate such population dynamics, when Tully & Nolan (2002) reviewed current knowledge on sea lice population biology a decade after his initial paper there still were no specific mathematical models created for either *Lepeophtheirus salmonis* or *Caligus elongatus*. This would change dramatically over the succeeding five years as discussed below, as the work of Tucker, Krkošek, Stucko, Stien, Murray, Revie, Todd and colleagues made its way into the scientific literature. This situation was not unique to sea lice research; detailed modeling existed for only a few aquatic species. Granovitch et al. (2000), investigating host-parasite interactions of trematodes in the White Sea, noted that, "detailed data about the distribution of [aquatic] parasites in host populations and the population aspects of host-parasite interactions are still scarce". Indeed, whereas the principles of terrestrial modeling should be readily applicable (see, for example, Smith & Grenfell 1985 on parasitic nematodes), it often is the unique challenge of obtaining sufficiently detailed data sets which has been the stumbling block. In giving an overview of the area Peeler et al. (2005) state that, "mathematical modeling has been used in human and terrestrial animal studies to improve understanding of factors influencing disease transmission; however, in aquatic animals it has been little used". Despite this, progress on the understanding required to model parasitic copepods continued to be made at the levels both of detailed host-parasite distribution estimation (Nie & Yao 2000) as well as theoretical epidemiology (Morand et al. 2002; Murray 2002).

Over the past seven years a number of researchers have begun work on mathematical modeling of sea lice population dynamics, and in the past four years an increasing number of studies have been published. The first delay differential equation model to be produced for *Lepeophtheirus salmonis* was a single cohort model which was parameterised using data from tank infections (Tucker et al. 2002b). This was reasonably successful in modeling the staged development of sea lice in a laboratory setting, but as the authors state, "clearly the model presented here is very simple and is designed specifically to describe the carefully controlled experimental system for which data was available".

At the 5th Sea Lice Conference in 2003 a poster presentation was made of some new and interesting modeling work by a group based at the University of Alberta (Krkošek et al. 2003). This was the first in a series of outputs resulting from their modeling research and which has been widely discussed over the past five years, partly due to the highly controversial nature of the debate around the issues of sea lice and wild salmonids in British Columbia. The approach adopted was a system of advection-diffusion equations and made many simplifying



assumptions regarding sea lice biology (Krkošek et al. 2005b). Despite these simplifications the paper made strong claims on the predictive validity of the model and stated that infection pressures around farms was up to “73 times greater than ambient levels” and that “the composite infection pressure exceeds ambient levels for 75 km on the two (juvenile salmon) migration routes”. It was, arguably, this combination of the strength of claims made by the modelers and the relative simplicity of the model which generated much of the subsequent debate (see references below). In a subsequent analysis, Krkošek et al. (2007b) concluded a 99% likelihood of sea lice-induced collapse of pink salmon runs in part of the Broughton Archipelago within 4 salmon generations, and this too has generated some considerable debate (e.g. Brooks & Jones 2008; Riddell et al. 2008).

There is insufficient space here to cover the full extent of the debate which has occurred in discussion forums and in the trade press as well as scientific journals and this is on-going. A useful set of exchanges which captured many of the core issues being debated can be found in a series of articles in the journal *Reviews in Fisheries Science*. The interested reader is directed to these articles because it is impossible in this report to do justice to over 50 pages of detailed debate and, at times, intensive exchange. The first article (Brooks 2005b) provided an overview of the effects of water temperature, salinity and currents within the Broughton Archipelago on infective copepodids, focusing specifically on *Lepeophtheirus salmonis*. The conclusion was that the hydrographic and surface salinity profiles typically found in this water body, together with sea lice stage development patterns at typical water temperatures, provide a “natural control [against sea lice] in the archipelago”. This clearly was not only at odds with the work of Krkošek and colleagues, but led also to a diametrically opposed viewpoint which made only limited attempts to incorporate existing empirical data sets.

Given these conclusions/criticisms, Krkošek and colleagues (Krkošek et al. 2006b) published *A Rebuttal to Brooks*. They issued a number of challenges, but the main focus of their defence and criticism is on a model of sea lice larval diffusion due to Dario Stucchi (and used by Brooks) which, they claim, “overestimates the transport of copepodids primarily because Stucchi’s model overestimates advective flow”. They also asserted that the empirical evidence over the years 2003-2005 was not consistent with Brooks’ implication of “natural control” against significant levels of on-farm sea lice infestation. In turn, Brooks & Stucchi (2006) published *A response of the rebuttal of Krkošek et al.* Once again, a number of detailed arguments were made but the key criticism of Krkošek’s model remained focused on insufficient consideration of hydrodynamic data from areas of the Broughton Archipelago, together with their failure to include “important biological factors describing the life history of sea lice” – in particular the crucial event of molting from nauplius II to the infective copepodid stage. This series of exchanges has been valuable in its own right and has served to open up the debate as to the true nature of sea lice problems within British Columbia, and in particular the

likely detrimental effects (or otherwise) of fish farming on wild juvenile pink and chum salmon. It has also served to illustrate the potential, as well as the limitations, of the modeling approach to describing parasite populations in general. Because the debate has focused on the dynamics and parameters within the various models it has uncovered the key assumptions being made by both 'sides' in this argument and this will ultimately lead to a better understanding of the science. However, it also has illustrated the difficulties of validating any model created, and the problems of gaining access to sufficiently detailed and complete empirical data sets.

Another important feature of the work from British Columbia is that it attempts to bring together sea lice population and hydrographic models into an integrated framework. While such fully integrated models must remain the ultimate goal, work on specific pieces of the 'puzzle' are still required and are being advanced by a variety of research groups. For example, the work on hydrographic modeling taking place in Scotland, specifically in the Loch Torridon area, has become well established (see, Gillibrand et al. 2002; Murray & Gillibrand 2006). Recent work on copepodid dispersal at different water temperatures (Boxaspen 2005) has been incorporated into a similar approach being used as part of the *Hardangerfjord Project* in Norway (Asplin et al. 2004, 2008; Finstad et al. 2007a,b).

Recent work based on formal mathematical modeling, specifically involving delay-differential equation models, has been carried out in Norway (Stien et al. 2005) as well as by the group based in the University of Strathclyde (Revie et al. 2005c). The work by Stien et al. (2005) is useful in that it reviews the current state of knowledge regarding a range of population parameters required in any modeling activity. As in the case of Tucker et al. (2002b) it is limited to experimental, laboratory-based data. However, it does embrace a reasonably wide range of such studies to investigate whether a proposed model can fit a range of situations. The research of Revie et al. (2005c) differs from previous modeling work on sea lice in attempting to fit a relatively simple delay-differential, stage development model to data collected from a wide range of farmed production settings in Scotland.

Among the refinements needed in the Strathclyde model (Revie et al. 2005c), and those using similar techniques, is the identification of risk factors so that important site-specific parameters can be modeled. In the first paper to assess risk factors on farms in Chile (Zagmutt-Vergara et al. 2005) the point is made that in a number of previous studies in the northern hemisphere, incorporating various environmental and management factors, relatively simple univariate analyses were used which "ignore the nested structure of the data sets coming from farms". They argued that "a method that takes into account the nested nature of the sea lice infestation on farmed salmonids is multilevel analysis such as linear mixed effects models (LME)" and applied such an approach to their data set. Unfortunately the quality of the data in this study was rather poor, with < 20% of the available samples actually of use. Their

conclusions appear to be confused in places, with little clarity as to which stages (and indeed species) are being referred to. Nevertheless, their general point on the need for more sophisticated multiple variable analyses is valid and only a few studies had previously applied these principles. One of these (Revie et al. 2003) pre-dated Zagsmutt-Vergara and colleagues' article by two years. Another paper to report a multiple variable approach (Jones & Nemec 2004) investigated *Caligus clemensi* in British Columbia and involved the creation of a logistic regression model which identified host size, salinity and water temperature as risk factors for elevated levels of chalimi.

Another approach which has potential value in increasing our understanding of sea lice dynamics is time-series modeling. McKenzie et al. (2004) is to date the only application of this technique to sea lice research. This approach proved useful in describing the typical pattern of infection seen for *Caligus elongatus* on Scottish salmon farms, though a similar approach was found not to be effective in describing *Lepeophtheirus salmonis* dynamics. The analysis focused on patterns within years (or production cycles) but it is possible also to assess infection across years. Some informal 'time-series' models have been suggested by those concerned with the patterns of lice infection on wild salmonids and farmed production activity within sea lochs (see, for example, Butler 2002; Marshall 2003). As more complete data sets over longer time periods become available it may be possible to formalise these analyses.

Recently, Frazer (in press) has developed a simple probability model to predict the levels at which farm lice levels must be maintained (i.e., the chemotherapeutic treatment threshold) in order that wild fish populations remain at pre-farm equilibrium levels. Using a purely theoretical approach he concludes that wild fish decline in proportion to the ratio of lice abundance on farm fish to lice abundance on wild fish.

In a position paper for the BC Pacific Salmon Forum entitled, *Protocols for Modelling*, Brooks (2005a) categorised models as variously being "conceptual", "statistical" and "mechanistic" among others. While these are helpful distinctions, he noted also the interaction between these model types. As a brief illustration of how even a very simple model can be viewed at these different levels, consider the issue of a model to estimate the body surface area of salmon available for occupation by ectoparasitic sea lice. While abundance normally is noted simply in terms of numbers of sea lice per fish there are occasions, for example with fish of very different size or when comparing between different production systems across countries (see, for example, Heuch et al. 2003), where it may be useful to consider parasite density, i.e. sea lice per cm<sup>2</sup> of salmon skin. It would seem likely that there exists a *mechanistic* model which relates surface area, which is not easy to measure, to fish weight, which is. A number of authors have proposed *statistical* models with parameters fitted to data sets, the outputs of which are shown in Figure 5.1.

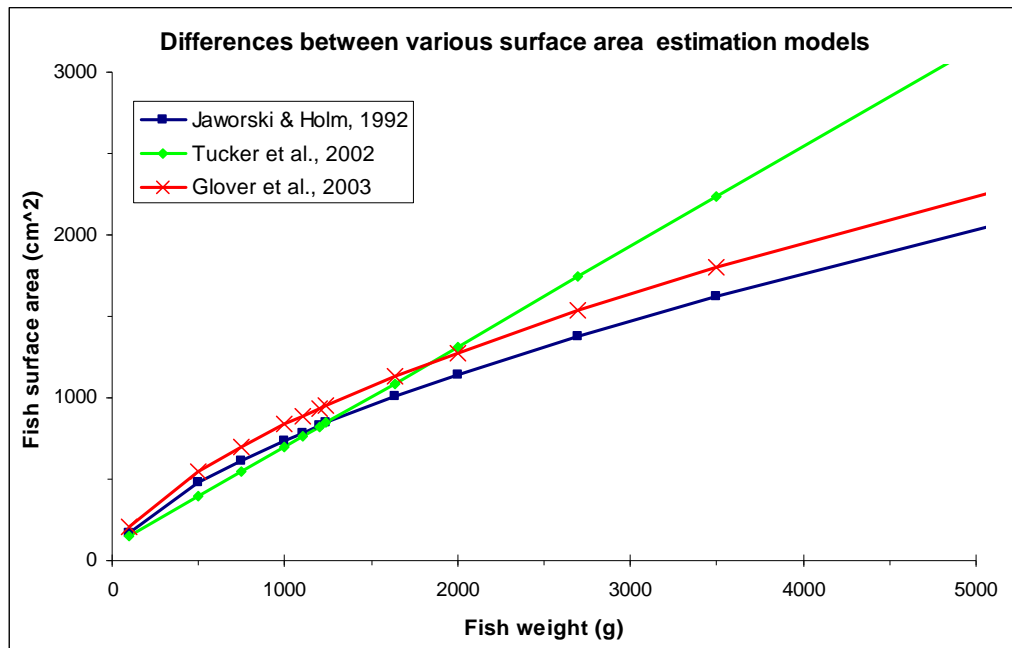


Figure 5.1 Graph illustrating the output of three models which attempt to relate salmon skin surface area to fish weight

In assessing the most appropriate model from those proposed, the underlying *conceptual* model (i.e. that a fish is in some sense a 'sphere') would tend to support one of the alternatives that uses a power relationship (i.e. that of Glover and colleagues, or Jaworski & Holm) rather than that (Tucker et al. 2002) which assumes the increase in surface area is linear with weight. Of course, for small fish all three models appear to provide a reasonable fit to the data and the fact that one is less 'conceptually' credible is of little consequence; once again, this illustrates the point that the usefulness of a model rather than its correctness may be the ultimate criterion against which it is judged.

Brooks (2005a) pointed out that the development of satisfactory mechanistic models will enable the development of more quantitatively oriented risk analysis and risk assessment models. He stated that "there are no quantitative risk models for diseases of marine fish and few qualitative models". This is largely borne out by the few publications which exist (Georgiadis et al. 2001; Munro et al. 2003; Peeler et al. 2004), with the possible exception of the salmonid gill parasite, *Gyrodactylus salaris*, for which several quantitative risk models have been created (Paisley et al. 1999; Høgåsen & Brun 2003). The systemic approach used within risk assessment models also can be seen in a number of attempts to use simple 'spreadsheet' style modeling to estimate the overall impact of lice at sea loch (Butler 2002), regional (Beamish et al. 2005) and national (Heuch & Mo 2001a) levels.

#### 5.4.2 Minimizing the impacts of disease requires collaboration

The need for collaboration is obvious within the context of sea lice, if only because initially (i.e. historically) the farm infestations were attributable to wild fish and once they became established on farms the potential was there for infestations to be spread back to wild fish and/or to other farms. It also is unarguable that in areas where wild and farmed stocks co-occur wild fish have continued to infect farm fish, and *vice versa*, ever since the inception of the industry, and probably in every geographical location that open-net salmon cages are placed in the marine environment. The only major question, or source of specific controversy, lies in the frequency and relative and absolute strengths of this bi-directional and dynamic interaction. In Ireland the “single bay management” approach led to a sharing of data and common management interventions – in terms of stocking, harvesting and chemotherapeutant treatment synchronization – between a number of farms. In Scotland, “Area Management Agreements” (AMAs; Section 1.4) took this a stage further by including wild salmon interests in the planning and collaboration process. But in Scotland farm sea lice data commonly are retained as being commercial-in-confidence, are used only for local management within the AMA structure, and are not publicly available for application or consideration in a wider context. In Norway such collaborations have a longer and less confrontational history and continue to be specifically addressed by fish farmers and biologists with interests in wild fish in a number of the larger salmon-producing fjords. The current Hardangerfjord project is arguably the most comprehensive example (Finstad et al. 2007a,b). A similar collaborative effort was recently seen for the first time in Chile in the vicinity of Colaco Bay (Asencio et al. 2008). Any assessment of the success of such collaborative efforts is necessarily more complex than when considering interventions at a single site; however robust multi-variable and multi-level epidemiological models exist to make such evaluations possible.

## **Chapter 6: Framework for assessing risk – sea lice case studies**

Rather than develop a matching section with case study material on sea lice this has been integrated into the final chapter of the main diseases report (Hammell et al. 2009).

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## Glossary

**Abundance** - the average number of sea lice per fish sampled (i.e. total sea lice counted divided by the number of fish sampled) (cf. “Intensity” and “Prevalence”)

**AChE enzymes** - acetylcholinesterase enzymes: enzymes involved in nerve transmission and specifically the biochemistry of the neurotransmitter acetylcholine.

**AMA’s (Area Management Areas)** - pertaining to integrated management of sea lice in Scotland involving primarily the industry and wild fishery interests on a geographically localized basis.

**Azamethiphos** - an organophosphorous insecticide

**Biomagnification** - (*in the specific context of this report*) the capacity of aquaculture populations of host fish to locally amplify or magnify the potential of a parasite or pathogen to infect adjacent wild populations of host fish.

**Biomarker** - a distinctive biological or biologically-derived indicator (such as a metabolite) of a process, event, or condition (such as aging, disease, or oil formation)

**Burden** - colloquial term used in this report to indicate the level of parasite challenge or infestation on a host fish. (More precise measures of burden include: “abundance”, “intensity” and “prevalence”.)

**Burrishoole system** - the Burrishoole Fishery lies in the west of Ireland some 5km north west of the town of Newport, Co. Mayo. Owned and run by the Marine Institute ([www.marine.ie](http://www.marine.ie)), it is one of Ireland’s premier lough (loch) fisheries for salmon.

**Caligids** - parasitic copepod crustaceans of the Family Caligidae. Typically external parasites (= ectoparasites) on the skin and body fins of marine fish, these often are referred to colloquially as “sea lice”. With specific respect to farmed and wild salmonids, caligid species of the genera *Caligus* and *Lepeophtheirus* are the most important ectoparasites.

**Chalimus (plural, chalimi)** - post-settlement larval stage of caligid copepods. Caligid copepods hatch at a free-swimming nauplius stage (nauplius I). This molts to nauplius II and then the infective copepodid. All three initial larval stages are planktonic. Once the copepodid has attached to a host fish it molts to the first of four chalimus larval stages and attaches to the host fish by means of a small thread (the frontal filament); chalimi cannot therefore move over the host body. The final (fourth) chalimus stage breaks the thread connection and molts (according to species) either to the mobile pre-adult or adult stage; thus, for example, *Caligus elongatus* molts directly from chalimus IV to the mobile adult, whereas *Lepeophtheirus salmonis* molts from chalimus IV to the first of two pre-adult stages prior to undergoing its final definitive adult molt.

**Chemotherapeutants** - chemicals such as dichlorvos, cypermethrin, emamectin benzoate etc used to treat sea lice infestations on farmed salmon.

**COI gene sequence** – DNA base sequence of the mitochondrial cytochrome *c* oxidase subunit 1

**Dichlorvos** - an organophosphorous insecticide widely used in the early development of the aquaculture industry as a chemotherapeutant treatment against sea lice infestation. It is no longer used in Scotland.

**Domain II (of molecular sequence)** - the voltage-gated sodium channel protein of nerve cell membranes is the primary site of action of pyrethroid insecticides. This protein is important to conduction of nerve signals. The so-called domain-II region in the DNA gene sequence for this protein is the site of most mutations that have been found to confer resistance to pyrethroid chemotherapeutants.

**Ectoparasite** - a parasite that lives on the exterior of its host. Caligid ectoparasites feed on the skin, mucus, tissue and blood of host fish, but are not specialist “blood suckers” as such. Rather, they may feed on blood as it leaks from skin lesions caused by their feeding on the skin.

**EDR (Emergency Drug Release)** - although the details of this process will vary by jurisdiction, most countries provide a mechanism through which veterinarians can apply to use a medication that has not achieved full marketing authorization to be available for routine use in animals.

**Eggstrings** - female caligids copulate with males and store sperm in internal sacs. The eggs are fertilized within the female’s body and she then extrudes the eggs into a pair of uniseriate strings which remain attached to the female until they hatch. The developing embryos have the appearance of a single stack of coins, or discs, within the transparent wall of the eggstring.

**Endoparasite** - a parasite that lives within the body cavity, or in the internal organs or tissues of its host.

**Epizootic** - an outbreak of disease affecting many animals of one kind at the same time to the extent that the disease may be considered an epidemic.

**FISH-lift trawl technology** - a newly-developed modified trawl net which is towed at the surface and retains captured fish in a rigid box. The advantage is that the fish remain free-swimming when retrieved to the deck of the ship and are minimally damaged by the capture process.

**Gravids** - a colloquial term used by the aquaculture industry to refer to adult female sea lice bearing eggstrings.

**Heritability** - the fraction of the total phenotypic variance that remains after the exclusion of the variability due to environmental effects; that is, the amount of variation in a trait which can be ascribed specifically to genetic variation.

**Infective Field** - a spatially-defined area within which hosts are potentially at risk from infection by parasites. The size of the infective field is determined by the dispersal potential of the parasite itself and the mobility of hosts bearing reproductive parasites. An individual migrating salmon may swim over large distances and thus have a large infective field. By contrast, if one ignores farm escapees, the infective field of salmon confined in farm pens is determined by the dispersal range of the parasite itself.

**Intensity** - the average number of lice on infected fish (cf. “Abundance” and Prevalence”)

**IPM (Integrated Pest Management)** - management of agricultural and horticultural pests that minimizes the use of chemicals and emphasizes natural and low-toxicity methods (such as the use of crop rotation and beneficial predatory insects).

**Locus (plural loci)** - a position on a chromosome occupied by a particular gene

**MHC (Major Histocompatibility Complex)** - a group of genes in mammals that code for cell-surface polymorphic glycoprotein molecules which display antigenic peptide fragments for T cell recognition and aid in the ability of the immune system to determine self from non-self.

**Microarrays** - technology used in molecular biology, consisting of an arrayed series of thousands of microscopic spots each containing tiny amounts of a specific DNA sequence used as a probe to screen large numbers of samples

**Microsatellite loci** - the genetic locus on a chromosome including a particular microsatellite sequence (see below).

**Microsatellite allele** - diploid organisms carry two copies (alleles) of each gene. Microsatellite loci typically are characterized by many different alleles (characterized by the number of tandem repeats of nucleotides within the gene sequence; see Microsatellite DNA), but the individual carries only two of those possible copies. When assessing population structure using microsatellite loci, one analytical approach is to compare the frequencies of all alleles at multiple loci for different populations. An alternative approach is to measure the incidence among populations of very rare (so-called “private”) alleles which might be diagnostic of particular populations.

**Microsatellite DNA** - microsatellite DNA loci include tandem repeat sequences of the four nucleotide bases (T,A,G,C) of the DNA molecule. These loci typically are highly variable and are generally considered to be selectively neutral (i.e. not adaptive); they offer a powerful and commonly used means of assessing and comparing the genetic structure of populations.

**Natal** - relating to site or location of birth, or origin.

**Nematodes** - any of a phylum (Nematoda) of elongated cylindrical worms parasitic in animals or plants or free-living in soil or water — called also *roundworm*.

**One sea-winter** - a maturity-grouping term used for Atlantic salmon. Broadly, Atlantic salmon mature and return to spawn after either one or more winters at sea. One sea-winter (= 1SW) salmon are therefore differentiated from multi sea-winter (MSW) fish.

**Ovigerous** - bearing eggs; oviferous (see also “gravid” above)

**Plasma cortisol** - cortisol is the fish corticosteroid hormone that is transported in blood plasma, and the concentration of which increases in response to stress.

**Pleiotropic** - producing more than one effect; *especially*: having multiple phenotypic expressions. That is, a single gene can influence more than one phenotype.

**Practical Salinity Units (psu)** - a measurement of salinity similar to part per thousand (ppt) of total salts dissolved in a given volume of water

**Prevalence** - the proportion (or percentage) of fish in a sample that is infected by a parasite or pathogen

**Pyrethroids** - any of various synthetic compounds that are related to the pyrethrins and resemble them in insecticidal properties.

**Single pulse infestation** - in experimental laboratory trials, scientists typically infest fish with sea lice larvae with a single dose, or single exposure to copepodids. The seawater in the experimental tank is usually flushed with clean sea water to remove any unattached sea lice larvae and hence the infestation can be tracked over time in the knowledge that the sea lice are all of the same age.

**Stable isotope markers** – many elements have several stable (i.e. non-radioactive) isotopes. The proportions of certain isotopes (e.g. carbon and nitrogen) can be used as markers to quantitatively measure the movement of materials in biological processes.

**Trematodes** - any of a class (Trematoda) (Phylum Platyhelminthes) of parasitic, usually hermaphroditic, flatworms including the flukes.

**Tri-partite Working Group (TWG)** - in Scotland the TWG was set up as a collaborative body including representatives from government, industry and wild fish interests in managing the perceived or potential environmental impacts of the salmon aquaculture industry.

**Vagile** - free to move about

**Virulence** - a measure of the severity of a disease or parasite's impact on its host's fitness

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