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A review of the biology and genetics of sea lice

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Studies of the biology of sea lice have been conducted from various perspectives for two decades. For *Lepeophtheirus* spp., most of the published literature has centred on the economically important *Lepeophtheirus salmonis*, while for *Caligus* spp., research has focused on a wider range of species. The most numerous species of *Caligus* in North Atlantic waters, however, is *Caligus elongatus*, which is also economically important to salmon farming. Since the last review by Pike, A. W., and Wadsworth, S. L. (1999. Sea lice on salmonids: their biology and control. Advances in Parasitology, 44: 234–337.), research on sea lice has developed considerably, including the application of genetic methods. This new research has focused on life history biology, studying developmental stages under different environmental conditions (e.g. temperature and salinity), behaviour, distribution and the dispersal of free-living stages, monitoring practices, population structure, and modelling. The results of this research have informed risk analyses and allowed the refinement of management strategies to reduce sea lice infestations in wild and farmed populations of anadromous salmonids. Molecular techniques have been used to describe population structure and identify differences in genetic characterization of geographically separate populations and population markers. Research has been initiated to understand the parasite–host relationship at a molecular level and to develop a vaccine against sea lice.

Keywords: developmental stages, distribution, life history biology, modelling, molecular biology, monitoring practices, populations, sea lice, susceptibility, vaccine.

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Introduction

Parasitic copepods of the family Caligidae, often referred to as sea lice, are responsible for many outbreaks of disease in marine aquaculture, especially in intensive salmonid aquaculture (Roth *et al.*, 1993; Roth, 2000; Mustafa *et al.*, 2001; Carr and Whoriskey, 2004; Johnson *et al.*, 2004). Sea lice can affect the growth, fecundity, and survival of their hosts because their feeding may cause skin lesions leading to osmotic problems and secondary infections and, if untreated, they can reach a level that is highly detrimental to the fish (Pike and Wadsworth, 1999; Nolan *et al.*, 2000a; Bjørn *et al.*, 2001, 2002; Tully and Nolan, 2002; Heuch *et al.*, 2005). Both wild and farmed salmonids can act as hosts to sea lice, and the possible interaction and cross-infestation of the parasite between farmed and wild fish is causing much concern (Tully, 1992; Birkeland and Jakobsen, 1997; Tully and Nolan, 2002; Marshall, 2003; Morton *et al.*, 2004). Wild anadromous fish in areas with salmon farms may experience severe sea lice infestations, in some cases resulting in their premature return to fresh water or mortality at sea (Birkeland, 1996; Birkeland and Jakobsen, 1997; Tully *et al.*, 1999; Bjørn *et al.*, 2001; Bjørn and Finstad, 2002). The abundance of hosts available in farm cages can result in large sea lice production (Heuch and Mo, 2001; Heuch *et al.*, 2005).

Norway, Chile, Scotland, Ireland, and Canada are the principal producers of farmed salmonids. Johnson *et al.* (2004) reviewed the economic impact of parasitic copepods in marine aquaculture and concluded that the annual cost of sea lice infestations in salmonid aquaculture exceeds US$100 million (approximately GB£60 million).

Pike and Wadsworth (1999) reviewed the literature on sea lice biology and control, but since then four major international conferences and many published studies have focused on sea lice. The present review considers both the biology of sea lice in its widest sense and recent advances in our understanding of sea lice genetics, focusing on the most important species in salmonid aquaculture, *Lepeophtheirus salmonis* and *Caligus* spp. Studies of sea lice biology included in Pike and Wadsworth's (1999) review are not included here except for some key articles, which are discussed where pertinent.

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Biology

The most commonly reported species of sea lice in the brackish and marine environment are members of the family Caligidae. These species are also responsible for most disease outbreaks in aquaculture (Johnson *et al.*, 2004). A literature search of a scientific database (ISI, Web of Science http://portal.isiknowledge.com/) revealed the extent to which studies have focused on different species of sea lice. For the genus *Lepeophtheirus*, 286 of 339 scientific papers involved *Lepeophtheirus salmonis*, while for *Caligus* spp. a wider range of species was studied with 81 of 181 papers involving *C. elongatus*. In the Atlantic Ocean, *L. salmonis* and *C. elongatus* are thought to be responsible for the main problems in salmonid aquaculture. In the Pacific Ocean (i.e. the coasts of Canada and Chile), several species of both genera have been described on salmonids (Boxshall and Bravo, 2000; Bravo, 2003; Johnson *et al.*, 2004; Beamish *et al.*, 2005; Krkošek *et al.*, 2005). *L. salmonis* is often referred to as the salmon louse because it is specific to salmonids, especially Atlantic salmon (*Salmo salar*), while *C. elongatus* is less host-specific and has been reported on 80 different species (Kabata, 1979).

Life history biology

Sea lice have a relatively simple life cycle with attached juveniles and mobile pre-adult and adult stages on the host. Gravid females produce a series of egg strings, which give rise to three free-living planktonic stages before settlement on a host (Heuch *et al.*, 2000). The exact number of stages depends on the species. *L. salmonis* has a total of ten stages, while *C. elongatus*, which does not have pre-adult stages, has eight (Kabata, 1979, 1992; Schram, 1993). In both species, the copepodid is the infectious stage that locates and attaches to the host.

Reproductive output and development

Sea lice have high reproductive capacity, and the number of eggs in egg strings at different times of the year was reviewed by Pike and Wadsworth (1999). Heuch *et al.* (2000) found that egg strings sampled at a low temperature (7.1 °C) had a greater total length and contained more eggs, on average, than egg strings produced at a higher temperature (12.2 °C). However, the individual eggs were significantly smaller in diameter, and the percentage of non-viable eggs was higher at the lower temperature. In an experimental study, female *L. salmonis* survived for up to 191 days, producing as many as 11 pairs of egg strings, with the first egg string being shorter and containing significantly fewer eggs, while the length of subsequent egg strings was relatively constant, and the number of non-viable eggs did not vary from the first to the third egg strings. Mustafa *et al.* (2000a) recorded up to ten pairs of egg strings per female *L. salmonis*, and the females lived for up to 210 days. They also found that fecundity fell over time, with the proportion of active copepodids declining from 75% in the first month to only 5% in the sixth month of the study. The longevity of the females indicates that they can over-winter on the salmonid host in the open ocean and return to coastal areas when the host fish returns to spawn.

Hatching and larval production

It was widely believed in Norwegian salmon farming that *L. salmonis* does not reproduce or grow during the winter, but studies have shown that this is not the case (Hogans and Trudeau, 1989; Hogans, 1995; Boxaspen, 1997). For example, in studies conducted at temperatures between 2 °C and 10 °C, the time to hatching ranged from 45.1 days at 2 °C to 8.7 days at 10 °C, and a large proportion of the nauplii developed into copepodids even at 4 °C (Boxaspen and Naess, 2000). This study clearly demonstrates that *L. salmonis* can develop into the infectious stage during the winter, even though biological processes slow down.

From free-living to attached stages

The time from hatching of the nauplii to the infectious copepodid stage varies with temperature, and the copepodid's lifespan is prolonged at lower temperatures (Boxaspen and Naess, 2000). The copepodid, however, is time-limited by its endogenous energy supply. Tucker *et al.* (2000) found that the energy supply of copepodids of *L. salmonis* was 7800 cal g^{-1} dry weight, comparable with the level found in copepodid stages of other parasitic and free-living copepods during winter. The energy level declined sharply between 1–2-day-old and 7-day-old copepodids, but no statistical difference was found in the development and initial survival after attachment of copepodids of different ages.

The attachment and moulting process from copepodid to the chalimus I stage is of interest. A short and stubby frontal filament is produced in *L. salmonis*, but this filament is longer and more slender in *C. elongatus* (Bron *et al.*, 1991). Studies of the production of the frontal filament in *L. salmonis* have shown that a new filament is produced for each moult (Gonzalez-Alanis *et al.*, 2001). These authors postulated that a potential control measure for parasitic sea lice could be to disrupt the filament production process. Bron *et al.* (2000a) described the major features of the moult sequence, which were generally similar to those of other Crustacea. These authors also described the ultrastructure of the cuticle of chalimus larvae as being very similar to that of free-living copepods, but with some modifications associated with a parasitic existence (Bron *et al.*, 2000b). Knowledge of cuticle processes and its composition might explain the variation in sensitivity of sea lice to pesticides and assist in developing new methods of sea lice control.

Epidemiology of sea lice

Sea lice have co-evolved with their salmonid hosts. Although references to sea lice date back to about 1600 AD (Berland and Margolis, 1983), until recently little was known about the dynamics of transmission (Pike and Wadsworth, 1999). In recent years, this has been addressed to some extent. For example, Todd *et al.* (2004) interestingly found no genetic differences in geographically separated populations of *L. salmonis* within the Atlantic Ocean, suggesting long-distance oceanic transfers of lice on their wild hosts and interchange of larvae from wild to farmed hosts. This is discussed later in the section on the origin of sea lice.

Dispersal of free-living stages

Following hatching, sea lice disperse immediately into the water column. In the first three life stages (nauplius I, nauplius II, and copepodid), sea lice are planktonic and drift with the current. They are small (0.4−0.7 mm long) and live on their energy reserves. The dispersal of larvae has been of great concern in the debate about the appropriate siting of salmon farms with regard to their distance from wild salmonid rivers (see below). Sea lice larvae are thought to behave like inert particles, drifting with the current and, therefore, the study of hydrographic conditions in relation to sources of sea lice has been a focus of interest in recent years and will probably generate interesting results in the future.

Early efforts to sample sea lice larvae were largely unsuccessful, but recently, more efficient sampling methods have been developed. Costelloe *et al.* (1999) concluded that the distance to a salmon farm is important and that the concentration of copepodids fell by two orders of magnitude within 100 m of farm cages. The use of a cone-shaped plankton net, 830 mm long with a mouth diameter of 300 mm and a mesh size of 140 μm used in horizontal tows at low speed (0.4 m s^{-1}), yielded data on the abundance of free-living *L. salmonis* larvae in the intertidal zone and in open water in a Scottish sea loch. Planktonic sea lice larvae in the intertidal zone were concentrated in the river mouths, but only when gravid females were present on nearby fish farms (McKibben and Hay, 2004). Studies of offshore and sublittoral plankton samples in lochs only found nauplii next to fish farms, although copepodids were also found in open water and at the head of a sea loch (Penston *et al.*, 2004).

Identification of larvae to species can be complicated. Some authors send subsamples for independent species verification (McKibben and Hay, 2004). Schram (2004) concluded that the length and width of *L. salmonis* and *C. elongatus* larvae overlap and cannot be used to distinguish between these two species, which, however, could be distinguished by their pigmentation (i.e. black and brown in *L. salmonis* and red in *C. elongatus*).

Geographical distribution of sea lice and occurrence on their wild hosts

L. salmonis has a circumpolar distribution in the northern hemisphere, whereas *C. elongatus* can be found in both hemispheres. *C. elongatus* is found more commonly in temperate environments, whereas *L. salmonis* thrives in temperate to Subarctic areas. Epizootics of *C. elongatus* are rare in Norway, but both *L. salmonis* and *C. elongatus* epizootics have occurred in Canada, Ireland, and Scotland. A larger number of species occur on farmed Atlantic salmon in Pacific waters off the coasts of Canada and Chile, but *C. clemensi* has been reported in several studies (Carvajal *et al.*, 1998; Boxshall and Bravo, 2000; Bravo, 2003; Johnson *et al.*, 2004).

The occurrence of sea lice on wild anadromous fish may vary with season and geographical location (Table 1). Studies carried out over several years off the southern coast of Norway revealed an increased prevalence of *L. salmonis* on sea trout (*Salmo trutta*), from 20% to 35% in March and April to a peak of 100% in late summer (Schram *et al.*, 1998). The highest prevalence recorded for *C. elongatus* in the same study was 90%. Rikardsen (2004) found similar seasonal variations in prevalence of sea lice in two fjords in northern Norway, although the peak prevalence was recorded 1−2 months later during autumn, probably resulting from lower water temperatures. The optimum temperature range for *L. salmonis* is not fully understood, but this species probably requires temperatures of 4 °C or higher to complete its life cycle successfully (Boxaspen and Naess, 2000). The effects of high temperature on *L. salmonis* are poorly documented, but during summer 1997, the parasite was absent from Norwegian salmon farms when water temperatures exceeded 18 °C (pers. obs.).

The variation in prevalence can be explained by a decline in reproduction and survival during winter followed by growth of sea lice populations under warmer conditions. Research has shown that sea lice prevalence differs between estuaries, fjords, and the open sea. Mo and Heuch (1998) recorded a 50% prevalence of *L. salmonis* on sea trout in a river, but a lower prevalence in the fjord, the difference probably resulting from variation in salinity levels. Todd *et al.* (2000) found higher prevalences of *L. salmonis* on Atlantic salmon from marine coastal waters than from estuarine areas and on two-sea-winter fish compared with one-sea-winter fish. Copley *et al.* (2005) found no differences in prevalence of adult *L. salmonis* on salmon from two locations on the western and northwestern coasts of Ireland, but the prevalence of juvenile lice differed significantly. Heuch *et al.* (2002) found a reduction in the winter population of sea lice on sea trout in the Skagerrak, which they attributed to both low temperature and salinity. The reasons for the observed geographical variation in prevalence are unclear, but temperature and salinity are likely to be important factors.

Murray (2002) developed a model for analysing the epidemiology of sea lice using observed load distribution on sea trout to explain some variation in observed settlement.

When data from western Scotland, western Ireland, Norway, and offshore populations from the English sector of the North Sea were analysed, patchiness of infestation (i.e. the assumption of aggregation of infectious copepodids in certain areas) explained the observed settlement better than any host factor. The results fell into two categories with patchy distribution of lice, where patch intensity was constant, or patchy distribution, where patch intensity was variable. The pattern in coastal areas of all countries is similar, although the offshore samples show slightly less variation. The aggregation of copepodids at hot spots in space and time appeared to explain the observed load patterns in this study. More studies on the dispersal of the free-living stages *L. salmonis* are being undertaken (Asplin *et al.*, 2004), but further studies are needed.

Sea lice and host interactions

Host responses to an infestation of sea lice include changes in appetite and in the levels of haematological parameters. Skin abrasions followed by osmotic problems and secondary infections have also been reported (Nolan *et al.*, 1999; Pike and Wadsworth, 1999; Bowers *et al.*, 2000; Finstad *et al.*, 2000; Tully and Nolan, 2002; Heuch *et al.*, 2005). Tully and Nolan (2002) considered both direct and indirect effects of sea lice on their host and vice versa, and noted the importance of identifying factors, which might be the key to enhancing host rejection of the parasite. Fast *et al.* (2002a) compared several non-specific humoral parameters in rainbow trout (*Oncorhynchus mykiss*), coho salmon (*Oncorhynchus kisutch*), and Atlantic salmon and found that Atlantic salmon had the lowest mucous lysozyme and protease activity, the thinnest epidermal layer, and the least dense distribution of mucous cells. This might explain the higher susceptibility to *L. salmonis* of Atlantic salmon than that found in other species of salmonids (Johnson and Albright, 1992; Dawson *et al.*, 1998; Glover *et al.*, 2001, 2005). Changes in the skin of salmon can also be detected after an infestation. Ross *et al.* (2000) described an increase in protease activity over time following an infestation of *L. salmonis*, indicating that biochemical changes resulting from the infestation occurred in the mucous layer at the site of the host–pathogen interaction.

The specific effects of artificial infections with *L. salmonis* have been studied for different host species. Twenty-one days after an artificially high infection (>100 sea lice per fish), Bowers *et al.* (2000) found that stress indicators, such as cortisol and glucose levels, had increased significantly in Atlantic salmon. Dawson *et al.* (1999) also found a decrease in haematocrit, sodium, and cholesterol in Atlantic salmon 21 days post-infection. Mustafa *et al.* (2000b) found similar increases in cortisol and glucose, but a macrophage respiratory burst and phagocytic activities had decreased by day 21 post-infection. The effects detected on sea trout (*Salmo trutta*) varied with the time of *L. salmonis* exposure after transfer to seawater (Dawson *et al.*, 1998). Trout exposed to sea lice two weeks after seawater transfer were less able to repair skin damage caused by pre-adult *L. salmonis*, leading to increased osmoregulatory problems and mortality compared with those experienced by trout exposed six weeks after transfer to seawater. The intensity of *L. salmonis* infestation did not, however, vary three weeks post-infestation, but both groups had significantly higher chloride levels, which appeared to coincide with the moult from attached stages to pre-adult lice on the fish and may have been the result of higher feeding activity of mobile lice compared with the chalimi stages. Adding a second stressor (confinement) after initial infestation with *L. salmonis* evoked an even greater response, which took the form of raised levels of plasma cortisol and glucose in rainbow trout (Ruane *et al.*, 2000). Nolan *et al.* (1999) concluded that a level of ten *L. salmonis* per fish, which is considered a low level of infestation in nature, is stressful and will render the fish susceptible to secondary infections. Sublethal levels of *L. salmonis* also resulted in lower critical swimming speeds in Atlantic salmon and, after swimming, fish with higher sea lice numbers experienced increased chloride levels, which significantly reduced the overall fitness of the fish (Wagner *et al.*, 2003). These results strongly suggest that even low levels of sea louse infestation will adversely affect salmonid physiology.

L. salmonis produces secretory products, and the morphology, function, and distribution of the glands that are probably associated with this secretion have been described (Bell *et al.*, 2000). Firth *et al.* (2000) studied the effects of *L. salmonis* infection on Atlantic salmon and found several trypsin-like proteases present in the mucus of the fish. Fast *et al.* (2003) described enzymes released by *L. salmonis* in response to the host mucus, and they found that *L. salmonis* secretes larger proportions of low molecular weight proteases on rainbow trout and Atlantic salmon than on coho salmon, winter flounder, *Pleuronectes americanus*, or other marine fish species. Those authors suggest that less-susceptible host species, such as coho salmon, might block the production of these proteases by sea lice, while more susceptible hosts might stimulate their production. The secretory product was identified as Prostaglandin E_2 (Fast *et al.*, 2004), a potent vasodilator, which may protect the parasite from the host's immune response, thereby creating an environment more favourable to the parasite.

Susceptibility to sea lice

Several interacting factors can influence the host's susceptibility to an infestation, including the host's stress and nutritional status, the effectiveness of the host's immune system, and the genetically determined susceptibility of the host (MacKinnon, 1998).

Comparison of the susceptibility of rainbow trout, Atlantic salmon, and coho salmon to *L. salmonis* indicates that

Table 1. Overview of the published literature concerning sea lice prevalence (and abundance and intensity for some studies) on wild salmonids in different geographical areas, for different species of host and sea lice.

Reference	Host species	Parasite species	Geographical area	Time of year	Prevalence (range)	Abundance	Mean intensity
Schram *et al.* (1998)	*Salmo trutta*	*L. salmonis*	Southern Norway	March–April	20–35		<3
	Salmo trutta	*L. salmonis*	Southern Norway	Late summer	100		8
	Salmo trutta	*C. elongatus*	Southern Norway	March–April	0–30	0–2	
	Salmo trutta	*C. elongatus*	Southern Norway	Late summer	90	4–10	
Mo and Heuch (1998)	*Salmo trutta*	*L. salmonis*	Oslo Fjord (Aker River)	Summer/early autumn	<50		19.9
	Salmo trutta	*L. salmonis*	Oslo Fjord (outside Aker River)	Summer/early autumn	85		7.6
MacKenzie *et al.* (1998)	*Salmo trutta*	*L. salmonis*	Scotland	Spring/summer	0–100 (33)*		
	Salmo trutta	*C. elongatus*	Scotland	Spring/summer	0–12		
Tully *et al.* (1999)	*Salmo trutta*	*L. salmonis*	Ireland (no farms)	Not stated	31		
	Salmo trutta	*L. salmonis*	Ireland (areas with farms)	Not stated	65		
Finstad *et al.* (2000)	*Salmo salar*	*L. salmonis*	Trondheim Fjord	Not stated	0–54		
Todd *et al.* (2000)	*Salmo salar*	*L. salmonis*	Firth of Tay (estuarine)	Not stated	84	5.35	
	Salmo salar	*L. salmonis*	Strathy Point (marine coastal)	Not stated	100	25.67	
Bjørn *et al.* (2001)	*Salvelinus alpinus*	*L. salmonis*	Vesterålen (farming)	June	100		
	Salvelinus alpinus	*L. salmonis*	Bogen (non-farming)	July	71		
	Salmo trutta	*L. salmonis*	Vesterålen (farming)	June	88		
	Salmo trutta	*L. salmonis*	Bogen (non-farming)	July	25		
Heuch *et al.* (2002)	*Salmo trutta*	*L. salmonis*	Norwegian Skagerrak coast (south)	Oct/March/April	47/8/25†		
	Salmo trutta	*L. salmonis*	Norwegian Skagerrak coast (south)	Oct/Dec/March	12/43/0†		
Bjørn and Finstad (2002)	*Salvelinus alpinus*	*L. salmonis*	Alta Fjord (farming)	July	92/50/0‡		
	Salvelinus alpinus	*L. salmonis*	Lakse Fjord (non-farming)	July	0/0/0‡		
	Salmo trutta	*L. salmonis*	Alta Fjord (farming)	July	38/38/0‡		
	Salmo trutta	*L. salmonis*	Lakse Fjord (non-farming)	July	0/0/0‡		
Marshall (2003)	*Salmo trutta*	*L. salmonis*§	Laxford Bay, Sutherland	March–October	0–100	0.38–68.4	
Rikardsen (2004)	*Salmo trutta*	*L. salmonis*	Rana and Bals Fjord	Winter/early spring	0–25	<0.5	
	Salmo trutta	*L. salmonis*	Rana and Bals Fjord	September	80–81		
	Salmo trutta	*L. salmonis*	Rana Fjord (non-farming)	September		6.8	8.6
	Salmo trutta	*L. salmonis*	Bals Fjord (non-farming)	September		3.6	4.5

Morton *et al.* (2004)	*Oncorhynchus*	*L. salmonis*	British Columbia (farming)		2.1
	Oncorhynchus	*L. salmonis*	British Columbia (farming)		6.8
	Oncorhynchus	*L. salmonis*	British Columbia (non-farming)		0
Carr and Whoriskey (2004)	*Salmo salar*	*L. salmonis*	New Brunswick	21	
Copley *et al.* (2005)	*Salmo salar*	*L. salmonis*/*C. elongatus*	Northwest Ireland	99/70‖	13.4/2.5‖
	Salmo salar	*L. salmonis*/*C. elongatus*	West Ireland	94/73‖	11.3/3‖

*Total prevalence for the number of fish sampled shown in parenthesis.
†Prevalence for the months stated in the previous column.
‡Prevalence of larval lice/pre-adult lice/adult lice, respectively.
§May include *C. elongatus* chalimus stages.
‖Values for *L. salmonis*/*C. elongatus*, respectively.

infestation was significantly reduced on coho salmon within 7−14 days, although lice persisted on the other two species (Fast *et al.*, 2002b). *L. salmonis* also matured at a slower rate on coho salmon and at a slightly slower rate on rainbow trout than on Atlantic salmon. Significant differences were found in the biochemistry of the mucus of these three species, indicating differences in susceptibility. Comparisons between sea trout and farmed Atlantic salmon indicated lower abundance and slower development of *L. salmonis* on sea trout (Glover *et al.*, 2003). After challenging individually tagged Atlantic salmon with *L. salmonis* on two separate occasions, Glover *et al.* (2004b) found little correlation between infestations on individual fish in the first and second challenges, indicating that the potential for a selection programme for low susceptibility in Atlantic salmon may be limited. However, Glover *et al.* (2005) compared 30 families (full sibling groups) of Atlantic salmon and found significant differences in abundance of *L. salmonis* among the groups, ranging from 3.8 lice per fish to 6.5 lice per fish.

The comparison of three wild and two farmed stocks of Atlantic salmon indicated that the wild Dale River stock had significantly lower levels of infestation of *L. salmonis* than the wild Vosso River stock and the two farmed stocks, the latter having the highest infection levels (Glover *et al.*, 2004a). This may reflect genetic differences in the susceptibility to infestations of *L. salmonis* among the different stocks. Kolstad *et al.* (2005) found that the genetic correlation between the numbers of *L. salmonis* recorded in a challenge test and during a natural infestation was very high ($r(g) = 0.88$) and that the potential for improving resistance to sea lice in Atlantic salmon by selective breeding is high. One approach to identifying the genes that confer resistance would be to develop screens for salmon genes that are activated when the fish is infected with sea lice (Jones *et al.*, 2002).

Integrated pest management and monitoring in farms

Potential interactions between sea lice on farmed salmonids and wild populations has been a matter of considerable controversy (McVicar, 2004), with *L. salmonis* from farmed fish being implicated in the marked decline of wild salmon and sea trout in areas where salmon farms are located (Birkeland and Jakobsen, 1997; Bjørn *et al.*, 2001; Heuch *et al.*, 2005).

In Norway, the largest producer of farmed Atlantic salmon in the world, farmed Atlantic salmon outnumber wild salmon 100-fold (231 million farmed fish compared with 2 million wild fish in 2002; Heuch *et al.*, 2005). The potential number of *L. salmonis* that can be produced on farmed salmonids is large (Heuch and Mo, 2001), and cross-infestation of *L. salmonis* occurs most likely between farmed and wild hosts. It is important to determine if sea lice infesting wild fish originated in fish farms. Butterworth *et al.* (2004) examined levels of stable isotopes of carbon and nitrogen in sea lice and were able to differentiate between *L. salmonis* collected from farmed Atlantic salmon and those from wild coho salmon, and between lice from

commercially reared salmon from the Pacific and Atlantic Oceans. Further studies will examine how long after settlement this difference remains apparent. Sea lice sampled from cultured salmonids could also be distinguished from those on wild salmonids at the same site using analysis of the elements magnesium, vanadium, and uranium (Shinn *et al.*, 2000a). Analysis based on either 28 or 16 elements allowed lice from separate locations to be identified with 100% correct classification, while the use of 12 elements provided 97.3% correct classification. Sea lice are larger on wild fish than on farmed fish, but it is not known whether this difference is genetically determined in the lice or is an expression of phenotypic plasticity. Nordhagen *et al.* (2000) found that *L. salmonis* from a wild source were significantly larger than from farmed fish, but that the offspring of both groups raised under similar conditions had similar growth rates and morphology. The size of *L. salmonis* is, therefore, a poor indicator of origin.

Monitoring the level and development of sea lice in farms is an important factor in managing the sea louse problem (Jackson *et al.*, 2000; Westcott *et al.*, 2004; Heuch *et al.*, 2005). Pike and Wadsworth (1999) suggested monitoring at least 20 farmed fish per cage and two cages per farm. Treasurer and Pope (2000) outlined a design for a monitoring programme and guidelines for the selection of host sample numbers in farmed Atlantic salmon. They found it impractical to record sea lice on more than 30 fish and suggested sampling in multiple cages. Revie *et al.* (2005) evaluated the effect of clustering in sea lice numbers and proposed a monitoring procedure for randomly sampling a large number of cages using a small number of fish from each cage.

Heuch and Mo (2001) noted that for Norway, with a high production of farmed salmon, the production of egg strings from farmed fish could easily outnumber the production from the estimated wild sources if not controlled to an acceptable level. Similarly in Scotland, farmed Atlantic salmon in their second year in the sea accounted for 98% of the sea lice population (Butler, 2002). Because the infestation pressure will always be the product of the number of hosts in the system and the number of lice on each host, it is important to appreciate that an increase in the number of hosts will invariably need to be matched by a reduction in the number of gravid females per host.

Norway has developed a National Action Plan against *L. salmonis* on salmonids, ratified by law and enforced by the Norwegian health authorities. This plan gives authorities jurisdiction to gather monthly reports, make unannounced checks on farms, and demand delousing if levels exceed the target levels in the plan. Thresholds for late winter and early spring are currently 0.5 gravid females or two mobile lice per fish. The plan was implemented in 1997, and Heuch *et al.* (2005) found a significant reduction in *L. salmonis* on wild salmon smolts in Sognefjord, a fjord with substantial farming activity from 2001 to 2002; this trend continued in 2003 and 2004 (Anon., 2005). Heuch *et al.* (2005) also noted that escaped farmed Atlantic salmon may account for a large percentage of the sea lice in a system. Skilbrei (2005) recommended that fishing for escapees should be allowed in coastal areas during the periods when wild salmonids are either at sea or in the rivers.

There has been considerable speculation about the factors which might affect the abundance of sea lice. Recent improvements in monitoring have made it possible to analyse large data sets with more variables. Revie *et al.* (2002) analysed the results from extensive monitoring in Scotland, data covering 33 fish farms for the period 1996–2000. This revealed extremely wide variations in sea louse abundance from year to year that could not readily be explained. Seasonal and annual variations in sea louse abundance on farmed salmon followed a pattern of slow build-up on the fish during their first year in the sea, with an occasional reduction in abundance in winter followed by a more variable and abundant level of lice during their second year at sea. Chemical treatment of sea louse infestations, especially for fish in their second year at sea, led to pronounced cycles of infestations. Fish origin, geographical region, and coastal exposure did not affect mean levels of abundance in this study. Revie *et al.* (2003) used general linear models to test more than 20 management and environmental variables in a study of 40 Scottish farm sites during the same period. The level of treatment, type of treatment, cage volume, current speed, loch flushing time, and levels of sea lice preceding the period analysed were identified as key explanatory variables. Heuch *et al.* (2003) compared data from Scotland and Norway and found that the levels of *L. salmonis* were significantly higher in Scotland during the period of study, even when the data set was corrected for differences such as pen sizes or stocking density. The higher water temperatures in Scotland may have increased the reproductive capacity of *L. salmonis*.

Stien *et al.* (2005) considered the possibility of integrating available experimental information on *L. salmonis* biology relevant to the demographic rates of the functional stages of *L. salmonis* into a set of models. These included developmental rate and mortality related to temperature for the different stages of the parasite and female fecundity. They identified several areas in which experimental data are lacking, such as stage-specific development under varying conditions, and mortality rates at low temperatures ($<7°C$).

Sea louse genetics

Methods based on developments in molecular biology have opened new opportunities in sea lice research. These include the opportunity to characterize different populations of sea lice in the search for genetic variation to assist in understanding sea lice–salmonid host relationships. The controversy concerning wild and farmed sources of *L. salmonis* also stimulated research into sea lice genetics. There is also considerable concern about the availability of a limited range of chemical treatments for sea lice in fish farming,

and it is likely that resistance will develop in sea lice to any chemical used over a prolonged period. Research into methods to control sea lice without chemical treatment is a priority, particularly the possibility of developing a vaccine against sea lice.

Origin of sea lice – identification on a geographical scale

Variations in size, fecundity, and resistance to therapeutic agents can be found in sea lice. Whether this is a result of phenotypic plasticity or genetic variation has been investigated using various research methods. Isdal *et al.* (1997) assessed the genetic variation in groups of *L. salmonis* along the Norwegian coast at the allozyme level, using starch gel electrophoresis (SGE) and isoelectric focusing. Using four polymorphic loci and using sea lice from six geographical locations, two distinct populations (southern and northern) could be detected among samples of sea lice from six geographical locations. Todd *et al.* (1997) found evidence of genetic differentiation among *L. salmonis* populations from the eastern, northern, and western coast of Scotland. The DNA polymorphism was quantified by Polymerase Chain Reaction (PCR) and Random Amplification of Polymorphic DNA (RAPD) analysis. Samples were taken from wild and farmed Atlantic salmon, wild sea trout, and farmed rainbow trout. *L. salmonis* from wild Atlantic salmon and sea trout showed genetic homogeneity, although the samples from farmed Atlantic salmon and rainbow trout showed highly significant levels of genetic differentiation. In a subsequent study, sea lice from nine localities in Scotland, with both farmed and wild salmonid hosts, showed a greater similarity between ITS-1 sequences after sequencing of specific nucleotide regions within farms than for wild population sources (Shinn *et al.*, 2000b).

Nolan *et al.* (2000b) used DNA preparation and PCR techniques to develop four microsatellite-PCR assays, two of which proved to be useful. The initial conclusion was that the method produced allele frequencies that differed between populations and, thus, could be used for studies of sea louse ecology and population structure. Dixon *et al.* (2004) used RAPD-PCR analysis for genetic characterization of 15 sea lice populations in Scotland. The analysis yielded two distinct clusters of samples with one group subdividing further into two sections. However, these samples did not exhibit a structured geographical pattern. The larger grouping contained most of the west coast farmed salmon sites, but no clear differentiation between lice from farmed and wild salmon was possible. The authors speculated that Todd *et al.* (1997), who found differences in this geographical area using the same methods, might not have starved their lice and that the additional bands observed were the result of non-sea lice DNA in the gut. However, the technique is probably not sensitive enough at this level. Todd *et al.* (2004) carried out a wider-ranging geographical comparison, with samples taken from wild and farmed salmonids in Scotland, wild sea trout in Norway, and farmed Atlantic salmon in eastern Canada. *L. salmonis* from farmed Atlantic salmon from the west coast of Canada were also included. No evidence of isolation of populations was found for the sea lice from wild Atlantic fish, suggesting that these wild hosts must have exchanged *L. salmonis* in the ocean for thousands of years. The non-migratory fish in aquaculture and the decline in wild Atlantic salmon populations could thus promote heterogeneity of sea lice in the Atlantic owing to the possible lower levels of exchange. Population genetic differentiation, however, was found between North Atlantic and North Pacific Ocean populations of *L. salmonis*. The authors speculated that, although these basins have been largely isolated since the Cenozoic era, the opening of the Bering Strait during the Pliocene (five million years ago) might have facilitated the migration of *L. salmonis* around North America.

In conclusion, it is not currently possible to identify the origin of *L. salmonis* as being farmed or wild using the genetic methods currently available. This might be attributed to the documented rather open gene flow in *L. salmonis* populations in the Atlantic Ocean. The conclusion of Tully and Nolan (2002) that the structure of possible metapopulations in the North Atlantic remains vague, still stands.

Oines and Heuch (2005) have developed a molecular assay for investigations of the population genetic structure of *C. elongatus*. Preliminary results indicate two distinct clades and possibly two closely related species. The two genotypes did not appear to be associated with sample site or host species.

Development of vaccines against sea lice

Experience with terrestrial parasites has shown that a successful vaccine must consist of one or more antigens. Such antigens may be rare and show little or no homology to other organisms. Detailed knowledge of the life cycle of sea lice at the molecular level is thus vital to vaccine development. Ectoparasitic sea lice feeding on host mucus, skin, and blood have only limited contact with the host immune system. Pike and Wadsworth (1999) summarized studies of immune modulation and noted that the younger stages, which have a more intimate association with host tissues, might be a target for vaccines. They also report on immunohistochemical screening of monoclonal antibodies. Raynard *et al.* (2002) noted that research to develop vaccines against sea lice is still in its infancy. A vaccine has been developed against the blood-feeding cattle tick, *Boophilus microplus*, but the assumption that arachnid and insect physiology are directly comparable with that of sea lice is not proven, and success in developing a sea louse vaccine will depend on a better understanding of sea louse digestive biology (Raynard *et al.*, 2002).

Trypsins in the gut

L. salmonis consistently consume significant blood meals, as suggested by the red gut seen in adult females. The gut is

a major interface between sea lice and the host, and a possible strategy to arrest sea lice propagation is to repress its protein digestion. Roper *et al.* (1995) fractionated homogenates from adult *L. salmonis* females and made antisera from those that were enzymatically active. The antisera induced immunostaining of the louse gut, and the stained substances were thought to be digestive enzymes. The new molecular techniques described below appear to substantiate these findings. Johnson *et al.* (2002) cloned and sequenced seven trypsin-like components from a cDNA library prepared from whole body pre-adult female and male *L. salmonis* and found that these forms differ in their regulation and function but are very similar to other crustacean trypsins and insect hypodermins. Kvamme *et al.* (2004a) cloned and characterized three variants of an LsTryp1 open reading frame in *L. salmonis*, and their results strongly suggested that these were serine proteases with trypsin-like specificity similar to the sequences published by Johnson *et al.* (2002). Measured by RT-PCR, the serine proteases were detected in all attached stages of *L. salmonis* but not in free-living stages. This indicates that it is up-regulated at attachment to the host. Another four novel trypsin-like S1A peptidase transcripts (LsTryp2−5) and one LsTryp1 trypsin were further characterized based on analyses of 1918 sequence tags from two adult female libraries (Kvamme *et al.*, 2004b). Phylogenetic analyses showed that the five sea lice peptidases form a monophyletic group with other crustacean trypsins. Higher transcript levels were found from the planktonic through to adult stages (Kvamme *et al.*, 2004b). Sequencing the genomic DNA surrounding the previously described trypsins and using PCR analysis, Kvamme *et al.* (2005) conservatively estimated the presence of 22 trypsin genes, of which 18 were most similar to the trypsins. These biological studies of differentially expressed genes in *L. salmonis* on a functional molecular level will probably assist in the search for prophylactic or therapeutic strategies against sea lice (Kvamme, 2005).

Gene expression

Biological studies of differentially expressed genes in sea lice are becoming increasingly important. Quantitative PCR (Q-PCR) can be used to measure how a regulated gene is expressed compared with an unregulated reference gene. Truly unregulated genes (housekeeping genes) are generally always expressed and thought to be involved in routine cellular metabolism. However, it is important that the chosen reference gene is truly unregulated within the biological samples employed. Frost and Nilsen (2003) have validated several candidate reference genes for transcription profiling in *L. salmonis*. Harvesting the different developmental stages of *L. salmonis* throughout the life cycle after an infestation yielded lice that were all from the F1 generation. Three standard genes, structural ribosomal protein S20 (RPS20), the translation elongation factor 1α (eEF1α), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were evaluated against 18S rRNA. The results indicated that GAPDH exhibited up to sixfold variation during the *L. salmonis* life cycle, while the other two genes exhibited less than twofold variation. 18S rRNA was detected ten PCR cycles earlier than other genes. Therefore, eEF1α and RPS20 are recommended as reference genes for *L. salmonis* studies. Skern *et al.* (2005), however, showed that different analytical approaches may lead to conflicting biological conclusions. Using the $2-\Delta\Delta CT$ method (Livak and Schmittgen, 2001), transcript levels of LsTryp1 decreased following starvation and return to normal adult levels in *L. salmonis* upon access to food. When the DART method (Peirson *et al.*, 2003) was employed, the LsTryp1 transcript levels decreased by a factor of two or three when the lice were starved and remained low even on access to food. Caution, however, is needed in interpreting these findings. Several aspects of the novel research on *L. salmonis* genetics from this Norwegian group are summarized by Nilsen (2004).

Mitochondrial DNA (mtDNA) from *L. salmonis* has been found to be 15 445 bp in length and the gene order is very different from that observed in other crustaceans (Tjensvoll *et al.*, 2005). In *L. salmonis,* both DNA strands contain coding regions, in contrast to *Tigriopus japonicus*, the other copepod characterized, in which only a few genes overlap. In a phylogenetic analysis using an alignment of mitochondrial protein sequences, *L. salmonis* groups together with *T. japonicus*, but genetic distance trees show that they are farther apart than other crustaceans included in the study. The very different structure of *L. salmonis* DNA compared with other similar arthropod organisms might suggest that studies on physiology and susceptibility to treatment in these organisms have low transfer value and are not particularly applicable to sea lice.

Genetic target sites for resistance

Problems with sea lice in salmonid farming are kept under control by reliance on a few chemotherapeutants. This is not considered a sustainable approach to pest management because several hundred pest species are documented as being resistant to one or more chemical classes of pesticides (Denholm *et al.*, 2002). Reduced sensitivity of sea lice to chemical treatment has been reported for various pesticides (Treasurer *et al.*, 2000; Denholm *et al.*, 2002; Sevatdal and Horsberg, 2003; Fallang *et al.*, 2004; Sevatdal *et al.*, 2005). Fallang *et al.* (2004) developed a biomolecular rate assay and demonstrated the presence of two acetylcholinesterase (AChE) enzymes in *L. salmonis*. AChE is the target of a major pesticide family (organophosphates, extensively used in Atlantic salmon farming between 1975 and 1985) and a major mechanism for the development of resistance in arthropods. The two AChE enzymes identified showed different sensitivity towards azamethiphos, the first report of target site resistance to organophosphates found in Crustacea. Another family of pesticides used in the treatment of sea lice is

pyrethroids; knockdown resistance (kdr) is caused by point mutations in the pyrethroid target site, the para-type sodium channel of nerve membranes. Fallang *et al.* (2005) PCR amplified and sequenced the sodium channel gene of *L. salmonis* but failed to identify any of the mutations within this region. However, a novel glutamine to arginine mutation (Q945R) in transmembrane segment IIS5 was consistently found in *L. salmonis* from populations exhibiting reduced sensitivity to pyrethroids.

Concluding remarks

Research on sea lice continues to develop, but individual research groups appear increasingly focused in their scope. This necessitates an open environment in which collaboration between various groups results in a holistic and multidisciplinary approach. I endorse the statement that progress requires interdisciplinary research (Tully and Nolan, 2002). Cooperation should involve fish and marine biologists to study both the host and parasite in detail, physical oceanographers to develop a complete model of the dispersal of sea lice larvae, and mathematicians to analyse large data sets, describe epidemiological models, and offer advice regarding the best ways to advance in integrated pest management. Progress in developing methods and the various approaches made in genetic studies is also very important if host–parasite interactions are to be understood. Then, new and preferably prophylactic or therapeutic strategies can be developed to arrest the propagation of these parasites.

References

Anon. 2005. Sea lice as a population regulating factor in Norwegian salmon: status, effects of measures and future management. Institute of Marine Research, Bergen. 46 pp.

Asplin, L., Boxaspen, K., and Sandvik, A. D. 2004. Modelled distribution of sea lice in a Norwegian fjord. ICES CM 2004/P:11.

Beamish, R. J., Neville, C. M., Sweeting, R. M., and Ambers, N. 2005. Sea lice on adult Pacific salmon in the coastal waters of central British Columbia, Canada. Fisheries Research, 76: 198–208.

Bell, S., Bron, J., and Sommerville, C. 2000. The distribution of exocrine glands in *Lepeophtheirus salmonis* and *Caligus elongatus* (Copepoda: Caligidae). Contributions to Zoology, 69: 9–20.

Berland, B., and Margolis, L. 1983. The early history of "lakselus" and some nomenclatural questions relating to copepod parasites of salmon. Sarsia, 68: 281–288.

Birkeland, K. 1996. Consequences of premature return by sea trout (*Salmo trutta*) infested with the salmon louse (*Lepeophtheirus salmonis* Krøyer): migration, growth, and mortality. Canadian Journal of Fisheries and Aquatic Sciences, 53: 2808–2813.

Birkeland, K., and Jakobsen, P. J. 1997. Salmon lice, *Lepeophtheirus salmonis*, infestation as a causal agent of premature return to rivers and estuaries by sea trout, *Salmo trutta*, juveniles. Environmental Biology of Fishes, 49: 129–137.

Bjørn, P. A., and Finstad, B. 2002. Salmon lice, *Lepeophtheirus salmonis* (Krøyer), infestation in sympatric populations of Arctic charr, *Salvelinus alpinus* (L.), and sea trout, *Salmo trutta* (L.), in areas near and distant from salmon farms. ICES Journal of Marine Science, 59: 131–139.

Bjørn, P. A., Finstad, B., and Kristoffersen, R. 2001. Salmon lice infection of wild sea trout and Arctic charr in marine and freshwaters: the effects of salmon farms. Aquaculture Research, 32: 947–962.

Bjørn, P. A., Tveiten, H., Johnsen, H. K., Finstad, B., and McKinley, R. S. 2002. Salmon lice and stress: effects on reproductive performance in Arctic charr (poster paper). *In* AquaNet II, Second Research Conference and Annual Meeting of AquaNet, Moncton, New Brunswick, Canada. Ed. by M. Riehl and M. Struthers. Delta Beauséjour, Moncton, New Brunswick.

Bowers, J., Mustafa, A., Speare, D., Conboy, G., Brimacombe, M., Sims, D., and Burka, J. 2000. The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. Journal of Fish Diseases, 23: 165–172.

Boxaspen, K. 1997. Geographical and temporal variation in abundance of salmon lice (*Lepeophtheirus salmonis*) on salmon (*Salmo salar* L.). ICES Journal of Marine Science, 54: 1144–1147.

Boxaspen, K., and Naess, T. 2000. Development of eggs and the planktonic stages of salmon lice (*Lepeophtheirus salmonis*) at low temperatures. Contributions to Zoology, 69: 51–55.

Boxshall, G., and Bravo, S. 2000. On the identity of the common *Caligus* (Copepoda: Siphonostomatoida: Caligidae) from salmonid netpen systems in southern Chile. Contributions to Zoology, 69: 137–146.

Bravo, S. 2003. Sea lice in Chilean salmon farms. Bulletin of the European Association of Fish Pathologists, 23: 197–200.

Bron, J., Shinn, A., and Sommerville, C. 2000a. Moulting in the chalimus larva of the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Contributions to Zoology, 69: 31–38.

Bron, J., Shinn, A., and Sommerville, C. 2000b. Ultrastructure of the cuticle of the chalimus larva of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). Contributions to Zoology, 69: 39–49.

Bron, J. E., Sommerville, C., Jones, M., and Rae, G. H. 1991. The settlement and attachment of early stages of the salmon louse, *Lepeophtheirus salmonis*, (Copepoda: Caligidae) on the salmon host, *Salmo salar*. Journal of Zoology, 224: 201–212.

Butler, J. R. A. 2002. Wild salmonids and sea louse infestations on the west coast of Scotland: sources of infection and implications for the management of marine salmon farms. Pest Management Science, 58: 595–608.

Butterworth, K. G., Li, W. M., and McKinley, R. S. 2004. Carbon and nitrogen stable isotopes: a tool to differentiate between *Lepeophtheirus salmonis* and different salmonid host species? Aquaculture, 241: 529–538.

Carr, J., and Whoriskey, F. 2004. Sea lice infestation rates on wild and escaped farmed Atlantic salmon (*Salmo salar* L.) entering the Magaguadavic River, New Brunswick. Aquaculture Research, 35: 723–729.

Carvajal, J., Gonzales, L., and George-Nascimento, M. 1998. Native sea lice (Copepoda: Caligidae) infestation of salmonids reared in netpen systems in southern Chile. Aquaculture, 166: 241–246.

Copley, L., Tierney, T. D., Kane, F., Naughton, O., Kennedy, S., O'Donohoe, P., Jackson, D., and Mcgrath, D. 2005. Sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus*, levels on salmon returning to the west coast of Ireland, 2003. Journal of the Marine Biological Association of the United Kingdom, 85: 87–92.

Costelloe, M., Costelloe, J., O'Donohoe, G., Coghlan, N., and O'Connor, B. 1999. A review of field studies on the sea louse, *Lepeophtheirus salmonis* Krøyer, on the west coast of Ireland. Bulletin of the European Association of Fish Pathologists, 19: 260–264.

Dawson, L. H. J., Pike, A. W., Houlihan, D. F., and McVicar, A. H. 1998. Effects of salmon lice, *Lepeophtheirus salmonis*, on sea trout, *Salmo trutta*, at different times after seawater transfer. Diseases of Aquatic Organisms, 33: 179–186.

Dawson, L. H. J., Pike, A. W., Houlihan, D. F., and McVicar, A. H. 1999. Changes in physiological parameters and feeding behaviour of Atlantic salmon, *Salmo salar*, infected with sea lice, *Lepeophtheirus salmonis*. Diseases of Aquatic Organisms, 35: 89–99.

Denholm, I., Devine, G. J., Horsberg, T. E., Sevatdal, S., Fallang, A., Nolan, D. V., and Powell, R. 2002. Analysis and management of resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Pest Management Science, 58: 528–536.

Dixon, B. A., Shinn, A. P., and Sommerville, C. 2004. Genetic characterization of populations of the ectoparasitic caligid, *Lepeophtheirus salmonis* (Krøyer 1837) using randomly amplified polymorphic DNA. Aquaculture Research, 35: 730–741.

Fallang, A., Denholm, I., Horsberg, T. E., and Williamson, M. S. 2005. Novel point mutation in the sodium channel gene of Pyrethroid-resistant sea lice *Lepeophtheirus salmonis* (Crustacea: Copepoda). Diseases of Aquatic Organisms, 65: 129–136.

Fallang, A., Ramsay, J. M., Sevatdal, S., Burka, J. F., Jewess, P., Hammell, K. L., and Horsberg, T. E. 2004. Evidence for occurrence of an organophosphate-resistant type of acetylcholinesterase in strains of sea lice (*Lepeophtheirus salmonis* Krøyer). Pest Management Science, 60: 1163–1170.

Fast, M. D., Burka, J. F., Johnson, S. C., and Ross, N. W. 2003. Enzymes released from *Lepeophtheirus salmonis* in response to mucus from different salmonids. Journal of Parasitology, 89: 7–13.

Fast, M. D., Ross, N. W., Craft, C. A., Locke, S. J., MacKinnon, S. L., and Johnson, S. C. 2004. *Lepeophtheirus salmonis*: characterization of prostaglandin E-2 in secretory products of the salmon louse by Rp-Hplc and mass spectrometry. Experimental Parasitology, 107: 5–13.

Fast, M. D., Ross, N. W., Mustafa, A., Sims, D. E., Johnson, S. C., Conboy, G. A., Speare, D. J., Johnson, G., and Burka, J. F. 2002a. Susceptibility of rainbow trout, *Oncorhynchus mykiss*, Atlantic salmon, *Salmo salar*, and coho salmon, *Oncorhynchus kisutch*, to experimental infection with sea lice, *Lepeophtheirus salmonis*. Diseases of Aquatic Organisms, 52: 57–68.

Fast, M. D., Sims, D. E., Burka, J. F., Mustafa, A., and Ross, N. W. 2002b. Skin morphology and humoral non-specific defence parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. Comparative Biochemistry and Physiology A – Molecular and Integrative Physiology, 132: 645–657.

Finstad, B., Bjørn, P. A., Grimnes, A., and Hvidsten, N. A. 2000. Laboratory and field investigations of salmon lice (*Lepeophtheirus salmonis*, Krøyer) infestation on Atlantic salmon (*Salmo salar* L.) post-smolts. Aquaculture Research, 31: 795–803.

Firth, K., Johnson, S., and Ross, N. 2000. Characterization of proteases in the skin mucus of Atlantic salmon (*Salmo salar*) infected with the salmon louse (*Lepeophtheirus salmonis*) and in whole-body louse homogenate. Journal of Parasitology, 86: 1199–1205.

Frost, P., and Nilsen, F. 2003. Validation of reference genes for transcription profiling in the salmon louse, *Lepeophtheirus salmonis*, by Quantitative Real-Time PCR. Veterinary Parasitology, 118: 169–174.

Glover, K. A., Aasmundstad, T., Nilsen, F., Storset, A., and Skaala, O. 2005. Variation of Atlantic salmon families (*Salmo salar* L.) in susceptibility to the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus*. Aquaculture, 245: 19–30.

Glover, K. A., Hamre, L. A., Skaala, O., and Nilsen, F. 2004a. A comparison of sea louse (*Lepeophtheirus salmonis*) infection levels in farmed and wild Atlantic salmon (*Salmo salar* L.) stocks. Aquaculture, 232: 41–52.

Glover, K. A., Nilsen, F., and Skaala, O. 2004b. Individual variation in sea lice (*Lepeophtheirus salmonis*) infection on Atlantic salmon (*Salmo salar*). Aquaculture, 241: 701–709.

Glover, K. A., Nilsen, F., Skaala, O., Taggart, J. B., and Teale, A. J. 2001. Differences in susceptibility to sea lice infection between a sea run and a freshwater resident population of brown trout. Journal of Fish Biology, 59: 1512–1519.

Glover, K. A., Skaala, Ø., Nilsen, F., Olsen, R., Teale, A. J., and Taggart, J. B. 2003. Differing susceptibility of anadromous brown trout (*Salmo trutta* L.) populations to salmon louse (*Lepeophtheirus salmonis* (Krøyer, 1837)) infection. ICES Journal of Marine Science, 60: 1139–1148.

Gonzalez-Alanis, P., Wright, G. M., Johnson, S. C., and Burka, J. F. 2001. Frontal filament morphogenesis in the salmon louse, *Lepeophtheirus salmonis*. Journal of Parasitology, 87: 561–574.

Heuch, P. A., Bjørn, P. A., Finstad, B., Holst, J. C., Asplin, L., and Nilsen, F. 2005. A review of the Norwegian "National action plan against salmon lice on salmonids": the effect on wild salmonids. Aquaculture, 246: 79–92.

Heuch, P. A., Knutsen, J. A., Knutsen, H., and Schram, T. 2002. Salinity and temperature effects on sea lice over-wintering on sea trout (*Salmo trutta*) in coastal areas of the Skagerrak. Journal of the Marine Biological Association of the United Kingdom, 82: 887–892.

Heuch, P. A., and Mo, T. A. 2001. A model of salmon louse production in Norway: effects of increasing salmon production and public management measures. Diseases of Aquatic Organisms, 45: 145–152.

Heuch, P. A., Nordhagen, J. R., and Schram, T. A. 2000. Egg production in the salmon louse [*Lepeophtheirus salmonis* (Krøyer)] in relation to origin and water temperature. Aquaculture Research, 31: 805–814.

Heuch, P. A., Revie, C. W., and Gettinby, G. 2003. A comparison of epidemiological patterns of salmon lice, *Lepeophtheirus salmonis*, infections on farmed Atlantic salmon, *Salmo salar* L., in Norway and Scotland. Journal of Fish Diseases, 26: 539–551.

Hogans, W. 1995. Infection dynamics of sea lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae) parasitic on Atlantic salmon (*Salmo salar*) cultured in marine waters of the lower Bay of Fundy. Canadian Technical Report of Fisheries and Aquatic Sciences, 2067. iv+10 pp.

Hogans, W., and Trudeau, D. J. 1989. Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtus* and *Lepeophtheirus salmonis* (Copepoda: Caligoida) parasitic on the cage-cultured salmonids in the lower Bay of Fundy. Canadian Technical Report of Fisheries and Aquatic Sciences, 1715. iv+14 pp.

Isdal, E., Nylund, A., and Nævdal, G. 1997. Genetic differences among salmon lice (*Lepeophtheirus salmonis*) from six Norwegian coastal sites: evidence from allozymes. Bulletin of the European Association of Fish Pathologists, 17: 17–22.

Jackson, D., Hassett, D., Deady, S., and Leahy, Y. 2000. *Lepeophtheirus salmonis* (Copepoda: Caligidae) on farmed salmon in Ireland. Contributions to Zoology, 69: 71–77.

Johnson, S. C., and Albright, L. J. 1992. Comparative susceptibility and histopathology of the response of naive Atlantic, chinook and coho salmon to experimental infections of *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms, 14: 179–193.

Johnson, S. C., Ewart, K. V., Osborne, J. A., Delage, D., Ross, N. W., and Murray, H. M. 2002. Molecular cloning of trypsin Cdnas and trypsin gene expression in the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitology Research, 88: 789–796.

Johnson, S. C., Treasurer, J. W., Bravo, S., Nagasawa, K., and Kabata, Z. 2004. A review of the impact of parasitic copepods on marine aquaculture. Zoological Studies, 43: 229–243.

Jones, C. S., Lockyer, A. E., Verspoor, E., Secombes, C. J., and Noble, L. R. 2002. Towards selective breeding of Atlantic salmon for sea louse resistance: approaches to identify trait markers. Pest Management Science, 58: 559–568.

Kabata, Z. 1979. Parasitic Copepoda of British Fishes. The Ray Society, British Museum, London. 468 pp.

Kabata, Z. 1992. Copepods Parasitic on Fishes. Synopses of the British Fauna. New Series. Universal Book Services, Oegstgeest, The Netherlands. 264 pp.

Kolstad, K., Heuch, P. A., Gjerde, B., Gjedrem, T., and Salte, R. 2005. Genetic variation in resistance of Atlantic salmon (*Salmo salar*) to the salmon louse, *Lepeophtheirus salmonis*. Aquaculture, 247: 145–151.

Krkošek, M., Lewis, M. A., and Volpe, J. P. 2005. Transmission dynamics of parasitic sea lice from farm to wild salmon. Proceedings of the Royal Society of London, Series B, 272: 689–696.

Kvamme, B. O. 2005. Trypsins from the salmon louse (*Lepeophtheirus salmonis* Crustacea: Copepoda). DSc thesis, University of Bergen. 58 pp.

Kvamme, B. O., Frost, P., and Nilsen, F. 2004a. The cloning and characterization of full-length trypsins from the salmon louse, *Lepeophtheirus salmonis*. Molecular and Biochemical Parasitology, 136: 303–307.

Kvamme, B. O., Kongshaug, H., and Nilsen, F. 2005. Organization of trypsin genes in the salmon louse (*Lepeophtheirus salmonis*, Crustacea: Copepoda) genome. Gene, 352: 63–74.

Kvamme, B. O., Skern, R., Frost, P., and Nilsen, F. 2004b. Molecular characterization of five trypsin-like peptidase transcripts from the salmon louse (*Lepeophtheirus salmonis*) intestine. International Journal for Parasitology, 34: 823–832.

Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using Real-Time Quantitative PCR and the $2(T)(-\text{Delta Delta } C)$ method. Methods, 25: 402–408.

MacKenzie, K., Longshaw, M., Begg, G. S., and McVicar, A. H. 1998. Sea lice (Copepoda: Caligidae) on wild sea trout (*Salmo trutta* L.) in Scotland. ICES Journal of Marine Science, 55: 151–162.

MacKinnon, B. M. 1998. Host factors important in sea lice infections. ICES Journal of Marine Science, 55: 188–192.

Marshall, S. 2003. The incidence of sea lice infestations on wild sea trout compared to farmed salmon. Bulletin of the European Association of Fish Pathologists, 23: 72–79.

McKibben, M. A., and Hay, D. W. 2004. Distributions of planktonic sea lice larvae, *Lepeophtheirus salmonis*, in the inter-tidal zone in Loch Torridon, Western Scotland in relation to salmon farm production cycles. Aquaculture Research, 35: 742–750.

McVicar, A. H. 2004. Management actions in relation to the controversy about salmon lice infections in fish farms as a hazard to wild salmon populations. Aquaculture Research, 35: 751–758.

Mo, T. A., and Heuch, P. A. 1998. Occurrence of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on sea trout (*Salmo trutta*) in the inner Oslo Fjord, southeastern Norway. ICES Journal of Marine Science, 55: 176–180.

Morton, A., Routledge, R., Peet, C., and Ladwig, A. 2004. Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences, 61: 147–157.

Murray, A. G. 2002. Using observed load distributions with a simple model to analyse the epidemiology of sea lice (*Lepeophtheirus salmonis*) on sea trout (*Salmo trutta*). Pest Management Science, 58: 585–594.

Mustafa, A., Conboy, G. A., and Burka, J. F. 2000a. Lifespan and reproductive capacity of sea lice, *Lepeophtheirus salmonis*, under laboratory conditions. Aquaculture Association of Canada, Special Publication, 4: 113–114.

Mustafa, A., MacWilliams, C., Fernandez, N., Matchett, K., Conboy, G., and Burka, J. 2000b. Effects of sea lice (*Lepeophtheirus salmonis* Krøyer, 1837) infestation on macrophage functions in Atlantic salmon (*Salmo salar* L.). Fish and Shellfish Immunology, 10: 47–59.

Mustafa, A., Rankaduwa, W., and Campbell, P. 2001. Estimating the cost of sea lice to salmon aquaculture in eastern Canada. Canadian Veterinary Journal, 42: 54–56.

Nilsen, F. 2004. http://www.st-andrews.ac.uk/~sumbaws/Frank%20Nilsen.pdf.

Nolan, D. V., Martin, S. A. M., Kelly, Y., Glennon, K., Palmer, R., Smith, T., McCormack, G. P., and Powell, R. 2000b. Development of microsatellite PCR typing methodology for the sea louse, *Lepeophtheirus salmonis* (Krøyer). Aquaculture Research, 31: 815–822.

Nolan, D. T., Reilly, P., and Bonga, S. E. W. 1999. Infection with low numbers of the sea louse, *Lepeophtheirus salmonis*, induces stress-related effects in post-smolt Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences, 56: 947–959.

Nolan, D. T., Ruane, N. M., van der Heijden, Y., Quabius, E. S., Costelloe, J., and Bonga, S. E. W. 2000a. Juvenile *Lepeophtheirus salmonis* (Krøyer) affect the skin and gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum), and the host response to a handling procedure. Aquaculture Research, 31: 823–833.

Nordhagen, J., Heuch, P., and Schram, T. 2000. Size as indicator of origin of salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Contributions to Zoology, 69: 99–108.

Oines, O., and Heuch, P. A. 2005. Identification of sea louse species of the genus *Caligus* using mtDNA. Journal of the Marine Biological Association of the United Kingdom, 85: 73–79.

Peirson, S. N., Butler, J. N., and Foster, R. G. 2003. Experimental validation of novel and conventional approaches to Quantitative Real-Time PCR data analysis. Nucleic Acids Research, 31(14): e73.

Penston, M. J., McKibben, M. A., Hay, D. W., and Gillibrand, P. A. 2004. Observations on open-water densities of sea lice larvae in Loch Shieldaig, Western Scotland. Aquaculture Research, 35: 793–805.

Pike, A. W., and Wadsworth, S. L. 1999. Sea lice on salmonids: their biology and control. Advances in Parasitology, 44: 234–337.

Raynard, R. S., Bricknell, I. R., Billingsley, P. F., Nisbet, A. J., Vigneau, A., and Sommerville, C. 2002. Development of vaccines against sea lice. Pest Management Science, 58: 569–575.

Revie, C. W., Gettinby, G., Treasurer, J. W., Rae, G. H., and Clark, N. 2002. Temporal, environmental and management factors influencing the epidemiological patterns of sea lice (*Lepeophtheirus salmonis*) infestations on farmed Atlantic salmon (*Salmo salar*) in Scotland. Pest Management Science, 58: 576–584.

Revie, C. W., Gettinby, G., Treasurer, J. W., and Wallace, C. 2003. Identifying epidemiological factors affecting sea lice, *Lepeophtheirus salmonis*, abundance on Scottish salmon farms using general linear models. Diseases of Aquatic Organisms, 57: 85–95.

Revie, C. W., Gettinby, G., Treasurer, J. W., and Wallace, C. 2005. Evaluating the effect of clustering when monitoring the abundance of sea lice populations on farmed Atlantic salmon. Journal of Fish Biology, 66: 773–783.

Rikardsen, A. H. 2004. Seasonal occurrence of sea lice, *Lepeophtheirus salmonis*, on sea trout in two north Norwegian fjords. Journal of Fish Biology, 65: 711–722.

Roper, J., Grayson, T. H., Jenkins, P. G., Hone, J. V., Wrathmell, A. B., Russell, P. M., and Harris, J. E. 1995. The immunocytochemical localization of potential candidate vaccine antigens from the salmon louse, *Lepeophtheirus salmonis* (Krøyer 1837). Aquaculture, 132: 221–232.

Ross, N., Firth, K., Wang, A., Burka, J., and Johnson, S. 2000. Changes in hydrolytic enzyme activities of naive Atlantic salmon, *Salmo salar*, skin mucus due to infection with the salmon louse, *Lepeophtheirus salmonis*, and cortisol implantation. Diseases of Aquatic Organisms, 41: 43–51.

Roth, M. 2000. The availability and use of chemotherapeutic sea lice control products. Contributions to Zoology, 69: 109–118.

Roth, M., Richards, R. H., and Sommerville, C. 1993. Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. Journal of Fish Diseases, 16: 1–26.

Ruane, N., Nolan, D., Rotllant, J., Costelloe, J., and Bonga, S. 2000. Experimental exposure of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to the infective stages of the sea louse, *Lepeophtheirus salmonis* (Krøyer), influences the physiological response to an acute stressor. Fish and Shellfish Immunology, 10: 451–463.

Schram, T. A. 1993. Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *In* Pathogens of Wild and Farmed Fish, pp. 30–47. Ed. by G. A. Boxshall, and D. Defaye. Ellis Horwood, New York.

Schram, T. A. 2004. Practical identification of pelagic sea lice larvae. Journal of the Marine Biological Association of the United Kingdom, 84: 103–110.

Schram, T. A., Knutsen, J. A., Heuch, P. A., and Mo, T. A. 1998. Seasonal occurrence of *Lepeophtheirus salmonis* and *Caligus elongatus* (Copepoda: Caligidae) on sea trout (*Salmo trutta*), off southern Norway. ICES Journal of Marine Science, 55: 163–175.

Sevatdal, S., Copley, L., Wallace, C., Jackson, D., and Horsberg, T. E. 2005. Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modelling. Aquaculture, 244: 19–27.

Sevatdal, S., and Horsberg, T. E. 2003. Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Krøyer) against the pyrethroid deltamethrin using bioassays and probit modelling. Aquaculture, 218: 21–31.

Shinn, A. P., Banks, B. A., Tange, N., Bron, J. E., Sommerville, C., Aoki, T., and Wootten, R. 2000b. Utility of 18s rDNA and ITS sequences as population markers for *Lepeophtheirus salmonis* (Copepoda: Caligidae) parasitizing Atlantic salmon (*Salmo salar*) in Scotland. Contributions to Zoology, 69: 89–98.

Shinn, A., Bron, J., Gray, D., and Sommerville, C. 2000a. Elemental analysis of Scottish populations of the ectoparasitic copepod *Lepeophtheirus salmonis*. Contributions to Zoology, 69: 79–87.

Skern, R., Frost, P., and Nilsen, F. 2005. Relative transcript quantification by quantitative PCR: roughly right or precisely wrong? BMC Molecular Biology, 6: 3 pp.

Skilbrei, O. T. 2005. Romt laks og regnbueorret bor fiskes opp. (Escapees of salmon and trout should be subjected to fishing). *In* Kyst og havbruk, pp. 110–111. Ed. by K. Boxaspen. Institute of Marine Research, Bergen (in Norwegian).

Stien, A., Bjørn, P. A., Heuch, P. A., and Elston, D. A. 2005. Population dynamics of salmon lice, *Lepeophtheirus salmonis*, on Atlantic salmon and sea trout. Marine Ecology Progress Series, 290: 263–275.

Tjensvoll, K., Hodneland, K., Nilsen, F., and Nylund, A. 2005. Genetic characterization of the mitochondrial DNA from *Lepeophtheirus salmonis* (Crustacea: Copepoda): a new gene organization revealed. Gene, 353: 218–230.

Todd, C. D., Walker, A., Hoyle, J., Northcott, S., Walker, A., and Ritchie, M. 2000. Infestations of wild adult Atlantic salmon (*Salmo salar* L.) by the ectoparasitic copepod sea louse, *Lepeophtheirus salmonis* Krøyer: prevalence, intensity and the spatial distribution of males and females on the host fish. Hydrobiologia, 429: 181–196.

Todd, C. D., Walker, A. M., Ritchie, M. G., Graves, J. A., and Walker, A. F. 2004. Population genetic differentiation of sea lice (*Lepeophtheirus salmonis*) parasitic on Atlantic and Pacific salmonids: analyses of microsatellite DNA variation among wild and farmed hosts. Canadian Journal of Fisheries and Aquatic Sciences, 61: 1176–1190.

Todd, C. D., Walker, A. M., Wolff, K., Northcott, S. J., Walker, A. F., Ritchie, M. G., Hoskins, R., Abbott, R. J., and Hazon, N. 1997. Genetic differentiation of populations of the copepod sea louse, *Lepeophtheirus salmonis* (Krøyer), ectoparasitic on wild and farmed salmonids around the coasts of Scotland: evidence from RAPD markers. Journal of Experimental Marine Biology and Ecology, 210: 251–274.

Treasurer, J. W., and Pope, J. A. 2000. Selection of host sample number and design of a monitoring programme for ectoparasitic sea lice (Copepoda: Caligidae) on farmed Atlantic salmon, *Salmo salar*. Aquaculture, 187: 247–260.

Treasurer, J. W., Wadsworth, S., and Grant, A. 2000. Resistance of sea lice, *Lepeophtheirus salmonis* (Krøyer), to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar* L. Aquaculture Research, 31: 855–860.

Tucker, C., Sommerville, C., and Wootten, R. 2000. An investigation into the larval energetics and settlement of sea louse, *Lepeophtheirus salmonis*, an ectoparasitic copepod of Atlantic salmon, *Salmo salar*. Fish Pathology, 35: 137–143.

Tully, O. 1992. Predicting infestation parameters and impacts of caligid copepods in wild and cultured fish populations. Invertebrate Preproduction and Development, 22: 91–102.

Tully, O., Gargan, P., Poole, W., and Whelan, K. 1999. Spatial and temporal variation in the infestation of sea trout (*Salmo trutta* L.) by the caligid copepod *Lepeophtheirus salmonis* (Krøyer) in relation to sources of infection in Ireland. Parasitology, 119: 41–51.

Tully, O., and Nolan, D. T. 2002. A review of the population biology and host–parasite interactions of the sea louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitology, 124 (Suppl.): 165–182.

Wagner, G. N., McKinley, R. S., Bjørn, P. A., and Finstad, B. 2003. Physiological impact of sea lice on swimming performance of Atlantic salmon. Journal of Fish Biology, 62: 1000–1009.

Westcott, J. D., Hammell, K. L., and Burka, J. F. 2004. Sea lice treatments, management practices and sea lice sampling methods on Atlantic salmon farms in the Bay of Fundy, New Brunswick, Canada. Aquaculture Research, 35: 784–792.