A THESIS SUBMITTED TO THE UNIVERSITY OF STIRLING FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNDERSTANDING KEY FACTORS ASSOCIATED WITH THE INFECTION OF FARMED ATLANTIC SALMON BY THE SALMON LOUSE *LEPEOPHTHEIRUS SALMONIS*

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Declaration

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged, the work described in this thesis has been conducted independently and has not been submitted for any other degree. All images presented in this thesis are original, unless otherwise stated. All experimental procedures and husbandry practices involving animals were conducted in compliance with the Animals Scientific Procedures Act 1986 (Home Office Code of Practice. HMSO: London, January 1997), in accordance with EU regulation (EC Directive 86/609/EEC), and approved by the Animal Ethics and Welfare Committee of the University of Stirling.

Signature of candidate:

Signature of principal supervisor:

Signature of supervisor:

Date:

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Abstract

The objective of the work described in the current thesis was to provide a better understanding of some of the key factors associated with sea louse, *Lepeophtheirus salmonis*, infection of farmed Atlantic salmon.

In Chapter 2, initial work focused on establishing the vertical and horizontal distribution of sea lice copepodids and spatial patterns of on-farm infections. The louse distribution was investigated along the main current gradient across adjacent salmon production pens at three commercial sites. A depth profile for the distribution of larval lice was also established for the top 12 m of the water column at three different locations in close proximity to commercial salmon farms. Within all multi-pen sites there were clear patterns of distribution and infection along the main water current gradient with the abundance of lice in end pens at each site appearing to be different from the central pens. The vertical distribution pattern of free swimming *L. salmonis* larvae (nauplii and copepodids) showed that the surface 6 m harboured 85.5 ± 1.6 % of the lice present in the water body analysed (0 – 12 m depth), irrespective of sampling date and location.

In Chapter 3, further environmental effects / influences on attachment success of the copepodids were analysed using controlled infection challenges. A flume with adjustable flow rates, and controlled light conditions was designed for this study. Flume current velocity was observed to be a significant factor in infection success, with higher infection levels observed at lower current velocities, while higher current velocities were demonstrated to reduce settlement success. At fixed velocity, higher copepodid exposure levels gave rise to higher infection levels, this having a linear relationship suggestive of a lack of competitive

effects for space on the fish. Light was also shown to play an important role in host settlement. A positive correlation between increasing light intensity and higher louse attachment success was found for all tested light spectra / wavelengths (white - Halogen, blue 455 nm, green 530 nm and red 640 nm). Observation of an infecting cohort of copepodids showed maximal infection at four days post-moulting with a tail-off of infection by six days post-moulting. However, even under the optimal conditions represented by a flume challenge, including linear water flow, the constraint of copepodids to pass close to the salmon host and the very high exposure levels of copepodids per fish, louse attachment success was still relatively low.

Chapter 4 examined implementation of a possible management approach based upon some of the environmental influences observed. This chapter described a study in which environmental manipulation of salmon swimming depth was employed on-site in an attempt to reduce farm infection of Atlantic salmon. The effects of submerged artificial lighting in combination with submerged feeding were tested with respect to salmon swimming depth and sea lice infection, following the hypothesis that *L. salmonis* infection in a commercial salmon population could be reduced through exposure to deep lighting and feeding. The results of the study suggest that swimming depth manipulation can indeed be used at a commercial scale to reduce salmon lice burdens on Atlantic salmon by physically minimising spatial interactions between the two animals.

In the final research chapter (Chapter 5), this thesis examines the question of whether ploidy of the host impacts on sea louse infection levels and whether susceptibility of individual fish is consistent between replicate infections. Results

showed that triploid salmon are not subject to higher sea louse infection levels under experimental challenge and farm infection conditions compared to diploid hosts. In addition, triploid fish subject to initial infection, did not become more or less resistant to infection compared to diploids when comparing repeated sea louse infections.

In summary, this thesis describes work conducted to analyse key infection pathways and factors influencing infection of Atlantic salmon by sea lice and suggestions made as to how findings may be exploited to reduce louse burdens in Atlantic salmon farming. The practical solutions presented to exploit the results found in this work are currently under consideration by the Scottish salmon industry.

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CHAPTER 1: General Introduction

The salmon louse *Lepeophtheirus salmonis* is a parasitic temperate marine copepod species. The salmon louse and other sea lice species have affected the salmon aquaculture industry for numerous years and are currently considered the most important parasitic disease problem for maricultured Atlantic salmon, *Salmo salar*. Sea lice are believed to have been in existence for 37–113 million years, with an equally long parasitic association with salmonids (Yasuike *et al*., 2012). The salmon louse was first described as a parasitic copepod on wild Atlantic salmon in 1940 (White, 1940) and on farmed salmon in Norway in the 1960s by Hastein & Bergsjo (1976). It was in the 70s, shortly after the first commercial scale establishment of sea cage farming for salmonids that sea lice emerged as a major problem (Heuch *et al.,* 2005). It has been suggested that over the last 30 years, the increased presence of sea lice in salmon farming has paralleled the growth of industrial scale salmon farming (Krkošek *et al.,* 2005).

In Europe, *Caligus elongatus* (von *Nordmann,* 1832) and *Lepeophtheirus salmonis* (Krøyer, 1837) are the dominant species of sea lice parasitizing salmon. *L. salmonis* is a larger parasite than *C. elongatus* and thus has a larger potential for damage and impact on wild and farmed salmonids in Europe (Bron *et al.,* 1991; Westcott *et al.,* 2004). In wild fish stocks the question remains of whether sea lice cause minimal damage (Bakke & Harris, 1998) or whether they substantially influence wild populations (Krkošek *et al.,* 2011). However, for commercial aquaculture, sea lice are the most damaging parasite of farmed salmonids (Costello, 2006; Penston & Davies, 2009). The Scottish industry alone lost €33 million annually (2009) in terms of lost harvest and the price of

control measures, which is between 0.1 and 0.2€/kg fish produced (Costello, 2009a). This cost has been estimated at 6-10% of the production value (Rae, 2002; Costello, 2009a). The costs related to stress caused by sea lice in Scottish fish farms and a reduced growth rate can be estimated at about 5% per year of the total production output (Costello, 2009a).

A variety of control measures have been developed to reduce sea lice numbers. Treatments against sea lice rely strongly on bath and oral treatments, but are limited by the availability of a few efficacious licensed chemotherapeutants (Shinn & Bron, 2012). Modern bath treatments include the closely related compounds, Excis® and AlphaMax®, cypermethrin and deltamethrin, hydrogen peroxide, and azamethiphos, an organophosphate. The most important in-feed treatment used is emamectin benzoate (Slice®), an avermectin. However, such treatments are extremely costly and may not be 100 % effective. Furthermore, these methods can have impacts on the host fish and the environment at high doses (Salte *et al.,* 1987).

Due to the limited range of medicines available, it is likely that resistance will develop in sea lice to any chemical used over a prolonged period. Development of reduced sensitivity / resistance to previously employed treatments, including organophosphates, pyrethroids, avermectins, and topical treatments such as H_2O_2, has already been documented (Treasurer *et al.,* 2000, Sevatdal & Horsberg, 2003; Fallang *et al.,* 2004; Sevatdal *et al*., 2005). As part of a successful louse management strategy, the avoidance of resistance in the salmon louse depends in part upon the detection of resistance in its start-up phase, avoidance of sub-optimal therapeutant dosing and proper training for the treating site staff. The type of resistance present has to be evaluated by routine

monitoring of resistance, which can be achieved through monitoring of treatment clearance rates and louse survival post-treatment. Furthermore, variations in resistance to therapeutic agents have been found in sea lice from different locations, as a result of phenotypic plasticity and / or genetic variation between sea lice (Sevatdal & Horsberg, 2003, Boxaspen 2006).

As a result, integrated pest management strategies have been researched to reduce sea lice numbers using a range of non-chemotherapeutant methods, such as the use of cleaner fish feeding on adult lice, light lures which exploit the phototactic behaviour of the lice, selective breeding for louse resistance, semiochemical treatments or ultrasound applications. Additional research focuses on the use of fallowing and other general management practices to reduce parasite numbers (Denholm *et al*., 2002; Rae, 2002).

1.1. **The salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837)**

1.1.1 Life history

The salmon louse *Lepeophtheirus salmonis* is a temperate marine copepod species that feeds on the mucus, skin and blood of salmonids, primarily adult salmon *Salmo salar.* As adults, female lice reach up to 18mm and males up to 7mm in length (Pike, 1989; Heuch *et al*., 1995).

Recent re-examination of the life cycle of the salmon louse has shown it consists of 8 stages: two nauplius stages (nauplius I and II), an infective copepodid, two chalimus stages (chalimus I and chalimus II), 2 preadults and the adult stage (*i.e.* six post-nauplius instars) (Ohtsuka *et a*l., 2009; Hamre *et al*., 2013, Venmathi Maran *et al*., 2013).

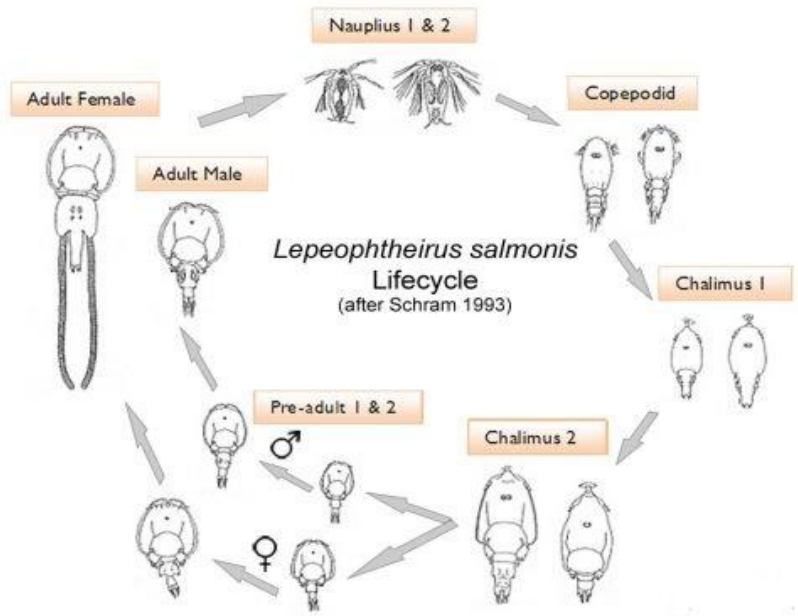


Figure 1.1. The Salmon louse life cycle (from: http://www.marine.ie /Home/site-area/areas-activity/aquaculture/sea-lice/life-cycle-salmon-louse?language=ga, 27th Dec 2014)

The salmon louse nauplius larva hatches from egg-strings extruded by the adult female on the host. The planktonic larvae undergo two stages, nauplius I & II, and disperse throughout the water column during these stages. They are entirely lecithotrophic, relying for energy upon maternally derived lipid reserves stored in the cells of the developing gut and ultimately in large lipid vesicles within the gut epithelium (Bron *et al.*, 1993a). As well as lipids, the larval stages use energy provided from vitellogenins, which are the precursors of salmon-louse egg-yolk glycoprotein (Dalvin *et al*., 2011). The nauplius stages hatch with a lipid containing area, which decreases in size over time, suggesting that the free-swimming larval stages utilise this as an energy reserve (Gravil, 1996).

The free-swimming nauplius stages, nauplius I (180-226µm) and II (182-205µm) (Schram, 2004), undergo 2 moults in the plankton in the course of 2 to 9 days, depending on water temperature, to reach the infective copepodid stage (Bricknell *et al.,* 2006). The hatched planktonic copepodid stages needs to find a host within ~3-5 days, thereafter growing to maturity on the host in four weeks or more depending on temperature (Brandal & Egidius 1979). The infective copepodid stage colonises the host fish, possibly with the help of phototactic cues (Bron & Sommerville, 1998), shadows and flashes from the scales, mechanoreceptors reacting to vibrations of the fish (Bron *et al*. 1991, 1993a, Costello, 2006) or chemotaxis (Gresty *et al*., 1993), and attains the first of the sessile chalimus stages (Bricknell *et al.,* 2006). Copepodids are largely found on fins and other low current-flow protected areas, which allow better attachment success (Pike, 1989; Bron *et al.*, 1991). The settlement of the parasite on the fish occurs in three stages, attachment, exploration and fixation. During the searching phase upon contact with the host, the parasite searches a

small surface area of the fish for a suitable attachment site. In the primary attachment phase the copepodid's antennula (= second antenna) grapples the host and is embedded in the host epithelium through a repeated stabbing action. In the secondary "filament" attachment phase, the chalimus larva attaches to the host through eversion of a proteinaceous frontal filament during the moult from copepodid, this being inserted under the host epidermis and adhered to the basement membrane or other hard substrate using an adhesive basal plate (Bron *et al.*, 1991). Feeding on host tissue starts immediately following attachment to the host and continues following the moult into the permanently attached chalimus stages (Bron *et al.*, 1991; Pike, 1989; Pike & Wadsworth, 1999; Venmathi Maran *et al*., 2013).

The final chalimus stage moults to the first of two mobile pre-adult stages, pre-adult I & II, losing its filament and using water pressure to maintain contact with the host fish (Costello, 2006). Both sexes undergo a final moult to the mature adult stage (Pike & Wadsworth, 1999; Todd *et al.*, 2005; Venmathi Maran *et al*., 2013). Males mature faster than females, reaching adulthood 25 days post hatching, whereas female maturation takes 30 days at 9-10°C (Johnson, 1993). Females can live up to 191 days in controlled situations (Hamre *et al.,* 2009), although age may vary significantly with respect to lice reared on wild fish versus those reared under commercial conditions and according to the season considered (Cook et al., 2010). Females can produce up to 11 egg string pairs during their life (Hamre *et al.,* 2009). After reaching a mobile stage, male sea lice will readily switch hosts to seek female partners (Ritchie, 1997, Murray, 2002). The time to hatching, as well as all subsequent moults, is highly dependent on temperature. *L. salmonis* can grow and reproduce in

temperatures as low as 4°C, although the process is slower than at warmer temperatures (Gravil, 1996; Boxaspen, 2006). Soon after mating, adult female lice start extruding fertilised eggs enclosed in a chitin sac that holds the eggs together as uniseriate egg strings. Egg strings remain attached to the female until hatching but are functionally independent, not requiring any additional energy from the female prior to hatching (Pike & Wadsworth, 1999). The egg strings contain the nauplii encased in two egg membranes. During hatching, the outer membrane bursts within the egg string, the inner one after the membrane has split (Gravil, 1996). Between 100 to 1000 sea lice eggs can be produced by a single gravid female (Costello, 2006).

While the focus of the thesis is aimed at explaining, analysing and interpreting *L. salmonis* and its role in affecting Atlantic salmon, the gaps in literature make it necessary to investigate the role of other ectoparasites and their infection pathways. Additionally, to strengthen findings for *L. salmonis*, the complexity of the species makes it necessary to compare the strategies and life cycles of a range of parasites, both to find parallels and depict discrepancies between findings. To increase understanding of the different parasites presented in the text, an overview is given as follows:

Overview of presented ectoparasites used for comparisons to *L. salmonis* (retrieved from: World Register of Marine Species (WoRMS), 27th June 2015 *http://www.marinespecies.org/index.php*)

Scientific name: *Argulus coregoni* (**Thorell, 1865**)

Crustacea: Branchiura

Environment: Fresh water

Principal hosts: Freshwater species

Geographical distribution: Europe, North America

Scientific name: *Gyrodactylus salaris* (**Malmberg, 1957**)

Platyhelminthes: Monogenea

Environment: Fresh water

Principal hosts: Salmonid species

Geographical distribution: Europe, North America

Scientific name: *Caligus elongatus* (**Nordmann, 1832**)

Crustacea: Copepoda

Environment: Marine

Principal hosts: Marine species

Geographical distribution: North Atlantic, New Zealand

Scientific name: *Lepeophtheirus dissimulatus* (**Wilson, 1905**)

Crustacea: Copepoda

Environment: Marine

Principal hosts: Marine species

Geographical distribution: North Atlantic

Scientific name: *Lepeophtheirus pectoralis* (**Müller, 1776**)

Crustacea: Copepoda

Environment: Marine

Principal hosts: Flatfish

Geographical distribution: North Atlantic, North Sea

1.1.2 Host infection

Initial infection of salmon farms in Scotland with *L. salmonis* occurs via lice originating from wild fish or through transport of louse larvae from neighbouring farms (Penston *et al.,* 2004), with gravid adult female lice on wild fish or farms contributing directly to the number of louse nauplii and copepodids found in the surrounding water body (Penston *et al.,* 2008a). Larval dispersion, with lice originating from a commercial farm, was modelled to be ~ 31 km (13 km - 149 km) and the intensity of infection was seen to fall progressively within this range (Middlemas *et al.,* 2013). Sea lice stages found on wild salmonids in proximity to salmon farms have been suggested to be largely juvenile stages, which has been taken to be indicative of a stationary salmon population with adult lice within the farm (Morton *et al.,* 2004). However, sea louse nauplius and copepodid stages do not act as purely passive particles, and are thought to employ a combination of buoyancy and short swimming bursts to achieve movement (Huse & Holm, 1993). All *L. salmonis* free-swimming larval stages have been demonstrated to display a "hop and sink" swimming pattern (Gravil, 1996), which might be attributed to a sea water density of 1020 – 1050 kg / m^3. Although greater periods of time are spent passively sinking, the speeds obtained during both upward spontaneous and directional swimming means that

a net upward movement of larvae in the water column occurs (Gravil, 1996). A possible method to exploit observed swimming patterns of the lice is described in chapter 4.

1.1.3 Environmental factors affecting host infection

Sea lice use environmental cues to help bring them into areas where they are most likely to encounter potential hosts (Bron *et al*., 1993a, Beamish *et al.*, 2007; Marty *et al.,* 2010). To that end, copepodids remain at the surface between 0 and 4 metres depth (Johannessen, 1978; Hevrøy *et al*., 2003; Murray & Gillibrand, 2006) and respond to light, pressure and salinity (Bron *et al*., 1993a; Heuch *et al*., 1995; Flamarique *et al*, 2000; Bricknell *et al*., 2006) in order to maximise the likelihood that they will encounter a suitable host.

Endogenous tidal rhythms, responses to sunlight, moonlight, salinity or water pressure, but also turbulence associated with high current velocities stimulate upward swimming in the larvae, increasing chance encounters with suitable hosts (Heuch, 1995). However, the dispersion can be affected by salinity or fresh water influx, since low / medium salinities cause a significant decrease in both hatching success and nauplius viability (Gravil, 1996; Costello, 2006). Host-parasite interaction can occur continuously, during sinking and rising migration of the sea lice and / or fish (Lampert, 1989). Farmed salmon are fed at the surface, and therefore may show higher numbers of sea lice than wild fish (Heuch *et al.,* 1995). A study of sea lice larvae dispersion suggested copepodids were in greater abundance in the upper 6m of the water column (Huse & Holm, 1993). This may be supported by the finding that sea trout, *Salmo trutta* morpha *trutta,* referred to as sea trout, which have a pronounced

surface feeding behaviour and specialised feeding patterns in shallower water, can show an accumulation of the parasite (Rikardsen, 2004), although this might also reflect differential susceptibility of the species (Dawson *et al*., 1997).

Low salinity levels in the sea water affect the settlement success of copepodids of *L. salmonis* (Tucker *et al.,* 2000; Bricknell *et al.,* 2006). Copepodids sink faster in the water column at lower salinity levels; in salinity gradients, copepodids demonstrated avoidance of salinities below 27 ppt, both by altering their swimming behaviour and changing the orientation of passive sinking (Gravil, 1996, Bricknell *et al*., 2006). Thus, copepodids are clearly salinity sensitive and orientate themselves towards haloclines, because they use up more energy in low salinity environments (Genna *et al.,* 2005).

As the salmon louse can reproduce over a wide range of temperatures, infection can occur at any time throughout the year (Brandal & Egidius 1979). Larger sea lice are found at lower water temperatures and on wild fish (Tully & Whelan, 1993; Costello, 2006). At lower temperatures egg strings of female adults are longer and carry more eggs, however egg size and survivability are reduced (Gravil, 1996; Heuch *et al.,* 2000). Summer sea lice have less offspring per eggstring, but have shorter generation times and higher fecundity due to the higher temperatures (Costello, 2006). Sea lice on wild fish decline during the winter, however, in general, temperature seems to be of little importance for overall abundance of lice (Heuch *et al.,* 2002). Sea lice are very hardy with respect to temperature and can be expected to produce up to four generations per year in Scottish waters (9-14°C) (Pike, 1989). Seasonality patterns were investigated in chapter 2 by monitoring sea louse copepodid infection dynamics and drawing conclusions to louse dispersion. Additionally, the spatial preference

of sea louse copepodids has been tested in chapter 2 and exploited in chapter 4.

1.1.4 Host factors affecting host infection

Fish do not have uniform numbers of sea lice attached. It is reported that single individuals can have a very high number compared to the bulk of the surrounding fish (Murray, 2002). Even if the overall sea lice infection is low, a few individuals will show very high, potentially lethal, numbers of sea lice. This aggregation of sea lice can arise from host factors, such as attractiveness, susceptibility and selectivity, or patchiness of sea lice occurrence (Salama *et al.,* 2013a, b). High densities of copepodids can cause aggregated lice infection patterns on infected fish. High aggregation of copepodids in the water body in general has been observed in the vicinity of fish farms. As mentioned earlier, lice may become aggregated by vertical swimming following salinity, tidal and light gradients. Active aggregation on hosts would increase the chance of encountering mating partners on infected hosts, however, there is no evidence so far presented to suggest that copepodids detect and respond to conspecifics on hosts. However, high lice burden on a few fish can cause lethal damage to the fish, or a change in behaviour which is lethal to the lice, such as migration up a freshwater stream. In salmon farming, controlling so called infection “hot spots” might help improve sea lice prevention. However, the occurrence of hot spots can be regarded as a by-product of infection dynamics, since planktonic copepodids have very limited swimming capacity (Murray, 2002).

Sea lice reproduce sexually, thus the presence of potential mates on a host fish could strongly influence host selection or residence time on the host. However,

mating opportunities must be balanced by the avoidance of competition for resources. This trade-off is important in habitat selection by free-living animals, but data are lacking from equivalent studies of host selection by facultatively dispersing parasites (Connors *et al*., 2010). Generally, low infection intensities of lice are aggregated among the host population with a few fish carrying the majority of the louse population and most fish carrying few or no lice. As infection intensity increases the distribution of lice in the host population becomes more normal. This pattern conforms to the assumption that motile lice should tend towards an ideal free distribution, but one that is controlled by the availability of mates and resources (Bandilla *et al*., 2007).

There is further evidence to suggest that infection levels observed on individual fish are repeatable. When fish were deloused between infection events, their initial louse burden was a poor predictor of their subsequent burden, when fish were not deloused those with relatively high louse burdens in the first sampling event again had the highest louse burdens in the second sampling event (Glover & Skaala, 2006). This suggests that the infection status of an individual is important in determining its susceptibility to subsequent infections, but this effect could be due to body size effects. Following up on observations described above of individual differences and possible attractions differences, chapter 5 aims at investigating louse infection success in subsequent infection waves. The findings presented in this chapter aim at further strengthening guidelines for stringent louse controls and overall low infection pressure levels.

1.1.5 Mate finding

Behavioural trials have begun to test hypotheses related to mate finding and its role in host selection by adults of both *L. salmonis* and the branchiuran *Argulus coregoni,* recognising the importance of semiochemcals (Mordue (Luntz) 2006). Adult male *L. salmonis* have been shown to respond behaviourally to water conditioned with the odour of female lice (Ingvarsdóttir *et al*., 2002a). Bandilla *et al*. (2007) tested the hierarchy of preferences in *A. coregoni* between light and the chemical cues of the presence of a fish and the availability of mates using a y-maze. While *A. coregoni* can potentially be considered a poor proxy to *L. salmonis*, this study needs still be used to compare the chemiosensory and photosensory capabilities of the parasites due to a lack of literature for *L. salmonis*. Additionally the comparisons can highlight similarities as both species are crustacean ectoparasites on fish, sharing similar life cycles. The study using *A. coregoni* (Bandilla *et al*., 2007) found that female lice were attracted to light and fish odour, but not to male odour, whereas male lice, although attracted to female odour above plain freshwater, preferred fish odour and light when given the choice. The study also found that when lice were placed in a tank with one uninfected fish and one fish infected with potential mates, the parasites showed no preference. Although this suggests that mate availability is not important in host selection by *A. coregoni*, aspects of the experimental design suggest other explanations for this finding. The test louse was a young adult and had been starved for 12 hrs before being introduced to the tank and the experiment did not allow for any subsequent switching behaviour. The authors suggested that finding a mate may become more important to older adult parasites, and it is also highly likely that well-fed compared to hungry parasites will make different

choices. In a similar trial conducted with *L. salmonis* adults, Ritchie (1997) found that a higher, but not significantly higher, proportion of male lice added to a tank attached to fish carrying female pre-adult 2 lice than to uninfected fish. No information on the condition of the male lice is available.

In branchiurans, the sex and stage of the louse appears to be a good predictor of its likelihood to switch to a different host. In male *A. coregoni*, the tendency to move between hosts increases with age, but females of all ages have similar tendencies to move between hosts and all are significantly less likely to move than the adult males (Bandilla *et al*., 2008). If there is no potential mate available on the same host, male *A. coregoni* are more likely to move than if there is, but whether or not a potential mate is available on a nearby fish does not have an effect on this tendency (Bandilla *et al*., 2008). The rate at which females move between hosts does not depend upon the availability of mates, supporting the hypothesis that male *A. coregoni* are the more active mate searchers and do not expend as much energy on pheromonal signalling as females, which sit at the opposite end of the trade-off (Bandilla *et al*., 2007). The authors note that by the end of this experiment many of the lice were mating (Bandilla *et al*., 2008) which, combined with the different effects of the treatments suggests that the search for mates was the principal driver for movement between hosts in this experimental setup. *A. coregoni* can, however, be considered a poor comparison to *L. salmonis* behaviour, but can nonetheless highlight the potential behavioural adaptations employed by other ectoparasites.

Some of these findings have been replicated in *L. salmonis.* Adult female *L. salmonis* move between hosts significantly less frequently than males, although

this sex difference is not evident in the preadult 1 stage (Connors *et al*., 2010). In an experiment using exclusively mated adult lice, Hull *et al*. (1998) found that males had a transfer rate of 62.4%, compared to 17.9% in females, and suggest that males are also more likely to move between hosts multiple times. Ritchie (1997) recorded similar findings from laboratory experiments using adult males and pre-adult 2 females, and also tested the hypothesis that movement between hosts occurs in the more natural setting of a sea cage. It was found that not only did lice move among experimentally infected hosts within the cage, but that there was a high level of migration of lice from the surrounding cages.

1.2. **Host identification by L. salmonis copepodids**

For a copepodid, finding a host can be usefully divided into three components (Heuch *et al*., 2007). Firstly, the copepodid must position itself into an area that maximises its probability of encountering a host. Secondly, it must be able to detect a host fish in the vicinity and locate it accurately enough to make contact with it, and thirdly, it must be able to determine the suitability of that host. In order to successfully complete all three of these components, the parasite depends on a combination of chance and its own sensory capabilities.

Lepeophtheirus salmonis uses a mix of stimuli from the environment to detect suitable hosts. Detection and recognition of the host is achieved by many senses of the parasite simultaneously (Browman *et al.,* 2004); sea lice follow a hierarchy of cues, such as pressure, moving water, light, salinity, temperature and semiochemicals, visual cues, such as a decrease in light intensity resulting from shadows of fish swimming overhead (Flamarique *et al.,* 2000), diffuse chemical cues devoid of a spatial or temporal gradient, such as the odorants

released from a large group of salmon on a migratory run or in sea cages and chemical trails/plumes emitted from single fish. The low molecular weight, water-soluble compounds found in salmon flesh have shown to be an important directional cue for host finding in the parasite (Devine *et al.,* 2000; Fields *et al.,* 2007).

The infective stage of *L. salmonis* uses environmental cues to position themselves in areas where they are most likely to encounter potential hosts (Beamish *et al.*, 2007; Marty *et al.,* 2010). They remain at the surface (Gravil, 1996) mainly between 0 and 4 metres depth (Johannessen, 1978; Hevrøy *et al*., 2003). Non-viable larvae were seen to be deeper in the water column (Gravil, 1996). Light, pressure and salinity (Bron *et al*., 1993a; Heuch *et al*., 1995; Flamarique *et al*, 2000; Bricknell *et al*., 2006) all provide cues by which the copepodids navigate into and remain in waters that apparently maximise the likelihood that they will encounter a suitable host. Infectivity of copepodids is believed to be positively correlated to the size of the lipid reserves, making "younger" copepodids more infective than older ones (Gravil, 1996; Cook *et al.*, 2010).

Copepodids do seem to show diurnal vertical migration, but whether they are shallower at night or during the day is unclear, as contradictory findings have been reported (shallow during the day: Heuch *et al*., 1995; deep during the day: Aarseth and Schram, 1999). Naturally, salmon follow a diel swimming rhythm, following ambient light patterns with migration downwards in the water column at dawn and return to surface waters at dusk and through the night (Juell & Westerberg, 1993; Bjordal *et al*., 1993; Fernö *et al*., 1995; Oppedal *et al*., 2001; Juell & Fosseidengen, 2004). Therefore, it has been suggested that copepodids

may engage in the opposite pattern as the resultant crossing over may facilitate host finding (Heuch *et al*., 1995). Therefore the more likely conclusion will be that sea lice will be found in surface waters throughout daylight and deeper during darkness. In their free-swimming, lecithotrophic state, they are vulnerable to predation and have limited resources for host finding (Karvonen *et al*., 2003). A behavioural pattern in lice was observed, with lice being 3 times more likely to disperse in the dark, when susceptibility to predation was low (Connors *et al.,* 2011).

Whilst maintaining their position in the water body, copepodids must also be able to detect nearby fish. This combined with the temporally and spatially stochastic nature of host-finding events suggests that copepodids are likely to attempt to attach to any fish with which they come into close enough proximity. Since it is easier for the lice to detect and attach to large fish, a positive correlation between lice count and body weight of the hosts has been reported (Gjerde & Saltkjelvik, 2009). Lice are believed to make a detailed assessment once their chemosensory antennules are close enough to the host surface (Bron *et al*., 1993a) and subsequently attach more permanently or leave in favour of a more suitable host (Bron *et al*., 1991).

Evidence in support of this hypothesis of indiscriminate infection comes from sampling infected fish. Bandilla *et al*. (2005) found that the aggregated distribution of *A. coregoni* on rainbow trout, *Oncorhynchus mykiss*, was related to exposure time rather than differences in host suitability occurring between individual fish. Jones *et al*. (2006) reported that three-spine sticklebacks, *Gasterosteus aculeatus*, are infected with *L. salmonis* at higher levels than juvenile pink and chum salmon in the Broughton Archipelago, British Columbia,

Canada, even though subsequent work has demonstrated that louse survival on sticklebacks is significantly lower under laboratory conditions than that on these salmonids (Losos, 2008). Fast *et al*. (2002a) found that *L. salmonis* initially infected cohabiting Coho salmon, *Oncorhynchus kisutch*, Atlantic salmon and rainbow trout at the same density, despite the fact that Coho salmon are resistant to infection and therefore a much less desirable host than the other two species.

Studies of the sensory capabilities of the copepodid stage of parasitic copepods similarly argued against this stage differentiating between hosts before settlement. Copepodids carry both chemosensory aesthetascs, organs used to determine the concentration and direction of a smell, and mechanosensory setae on their antennules to detect changes in water movement around them (Gresty *et al*., 1993), and, also, have a complex eye structure with three ocelli (Bron & Sommerville, 1998). It is likely that information from all three senses is used at different stages during the attachment to the host, but investigations of the sensitivity of each sense supports the theory that copepodids initially attach to fish indiscriminately.

Photoreception is clearly important in maintaining the copepodid in the surface waters, and during diurnal vertical migration (Heuch *et al*., 1995), but is unlikely to inform settlement behaviour. It is well documented that copepodid *L. salmonis* are positively phototactic (Bron *et al*., 1993a; Heuch, 1995; Flamarique *et al*, 2000), and apparently insensitive to shadows (Bron *et al*., 1993a; Flamarique *et al,* 2000). The positive phototaxis decreases with age. Adult stages are significantly less sensitive to light than copepodids, in *Lepeophtheirus pectoralis* (Boxshall, 1976) and *Lepeophtheirus dissimulatus*

(Lewis, 1963). This suggests that maintaining copepodid position in the water column could be the principal, and perhaps only, role of vision in these species, and possibly also *L. salmonis.* Copepodids show a positive phototactic response to a wide spectrum of light wavelengths (300-700 nm) with a peak response at 550 nm (Bron *et al.,* 1993a). Light response was positively correlated to light intensity between 2.4 – 240 lux with immobilisation occurring at 20,000 lux (Bron 1993a). Intermediate (300 lux) light levels produced higher settlement of copepodids on Atlantic salmon smolts than high (800 lux) or low (10lx) levels (Genna *et al*., 2005), which does suggest a possible role of light. However, Heuch *et al*. (2007) found that there was no difference between dark (infrared) and light (infrared and white light) trials in the rate at which copepodids attacked a model fish head. Similarly, there was no difference in infection level between tanks held under polarised or UV-A light or darkness (Browman *et al*., 2004), and infection under laboratory conditions in complete darkness can also be successful (Johnson & Albright, 1991; Bron *et al*., 1993a). Following the suggestions, that light may be employed by copepodids to assist or drive host finding, chapter 3 investigates in depth the attachment success under changing light intensities and frequencies.

Research investigating the role of chemical information in host finding behaviour gives similarly mixed results.

Copepodids are able to discriminate between seawater conditioned by Atlantic salmon and plain seawater (Bailey *et al*., 2006). They do not, however, demonstrate a preference between water conditioned by host and non-host fish (Bailey *et al*., 2006), lending further support for the hypothesis of indiscriminate initial attachment. Chemosensory detection is apparently not important in

settlement behaviour: copepodids can settle on hosts without chemical stimulation (Heuch *et al*., 2007), but do not remain settled on immobile hosts, despite the presence of abundant chemical information (Bron *et al*., 1993a).

Information about movements in the water is therefore likely to be the most important cue guiding copepodids to attack fish. Their antennules support setae that are sensitive to the infrasonic movements produced by fish (Heuch & Karlsen, 1997), and these movements cause copepodids to attack even model fish (Heuch *et al*., 2007). Although highly sensitive, this mechanosensory ability is unlikely to allow the copepodid to determine the suitability of the host (although the model was based on *Atlantic salmon*).

It is highly likely, however, that during the period of temporary attachment, chemical testing of the host surface is the method by which copepodids assess host suitability (Bron *et al*., 1993a). As discussed below, the major histocompatibility complex (MHC) genes possibly contribute to an individual's susceptibility to infection with sea lice (Gharbi *et al.,* 2009). MHC molecules are periodically lost from the cell surface and are excreted in saliva, sweat and urine (Singer *et al.*, 1997), thus genotype can be assessed chemically, and may be important during the copepodid's host selection process.

1.3. **The role of Atlantic salmon as a host for L. salmonis**

L. salmonis is, after initial indiscrimate attachment to any fish, highly host specific, specialised in infecting salmonid hosts. However, *L. salmonis* does not appear to discriminate between cues from different families of teleosts, let alone between salmonid species at initial attachment. Chemotaxis towards the source of water conditioned with the odour of Atlantic salmon in both the copepodid

(Bailey *et al*., 2006) and adult (Devine *et al*., 2000; Ingvarsdóttir *et al*., 2002b; Losos, 2008) life stages is not detected when the choice is between salmon odour and water conditioned by a non-host fish (copepodid: Bailey *et al*., 2006; adult: Losos, 2008). This may lead to attachment to atypical hosts. Three spined stickleback, pacific sand lance, *Ammodytes hexapterus*, white sturgeon, *Acipenser transmontanus*, ling, *Molva molva*, cod, *Gadus morhua*, sea bass, *Dicentrarchus labrax* and saithe, *Pollachius virens*, have all been described as potential atypical hosts (Bruno & Stone, 1990; Jones *et al*., 2006, Pert *et al.,* 2009). However, louse infection on most of these species (possibly with the exception of three-spine stickleback in some regions) can be expected to be erroneous and not viable for the parasite (Campbell *et al.*, 2009).

Comparing various salmonid species, this host specificity can also be seen comparing salmonid species to each other. Bjørn *et al.* (2007) found 0% prevalence of sea lice infection in wild Atlantic salmon in Norwegian fjords, whereas prevalence in sympatric Arctic charr, *Salvelinus alpinus*, and brown trout, reached as high as 88%, with mean infection intensity reaching 19-27 lice per fish. Fast *et al.* (2002a) found that Coho salmon were essentially resistant to infection with *L. salmonis*, whereas lice were able to complete their life cycle on both rainbow trout and Atlantic salmon. This is in accordance with the finding of Jackson *et al.* (1997) that numbers of *L. salmonis* were higher on Atlantic salmon than rainbow trout kept in adjacent sea cages. Lice also develop fastest on Atlantic salmon, followed by rainbow trout and Coho salmon (Fast *et al*., 2002a). Similarly, Johnson (1993) found faster maturation rates in lice grown on Atlantic than on Chinook salmon, *Oncorhynchus tshawytscha*, and that *L. salmonis* produces approximately twice as many eggs on Atlantic salmon than

on Chinook salmon. Also, Glover *et al*. (2003) demonstrated that lice abundance and density were higher on Atlantic salmon than on sea trout during controlled challenge experiments.

1.3.1 Host physiology affecting susceptibility to infection

Susceptibility to infection with the parasite is dependent on the life history of the host species. Due to the respective life-cycles, some species will expose themselves physically to the parasite, both spatially and behaviourally *e.g.* Atlantic salmon spend relatively short periods of time in inshore coastal waters and fjords before migrating to the sea (Rikardsen *et al.,* 2004), and therefore spend less time in areas with potentially large numbers of infective copepodids, than littoral feeding charr and trout (Bjørn *et al.,* 2007).

Host specificity also reflects the nutritional requirements of the parasite and / or its ability to adapt and overcome the innate immunological defence mechanisms of a potential host species (Kabata, 1979). There are no significant differences in the physiological parameters of the blood between the different salmonid species (Fast *et al.*, 2002a). Therefore it seems likely that the difference in susceptibility is mediated by differences in the epidermis and mucus. In this respect, a practical application (Marine Harvest, Scotland, 2007/2008) of the glycoprotein feed additive, Bio-Mos®, which is extracted from the yeast cell wall of *Saccharomyces cerevisiae***,** showed an increase in mucus production in Atlantic salmon and a detectable decrease in lice numbers (Cockerill, D. (2011), Pers. Comm.). However, Holm *et al.* (2015), suggest, that a lower infection number depends on the genetic ability to avoid immunosuppression and that the mucus layer does not, per se act as a barrier to infection which might

attribute to lower louse infection numbers. That said, it is recognised, that the mucus layer is a dynamic boundary and generally considered the first line of defence between a fish and its environment (Deplancke & Gaskins, 2001). Mucus viscosity is dictated by the opposing forces of secretion and sloughing, or shedding (Akiba *et al*., 2000). Fast *et al*. (2002b) showed that there are differences between rainbow trout, Atlantic and Coho salmon in terms of the enzyme activity in their mucus, the thickness of the mucus layer and epidermis and found that Coho salmon was the only species with sacciform cells in the epidermis. Atlantic salmon, the most susceptible of the three species tested, had the thinnest mucus and epidermis and the lowest enzyme activity of the three (Fast *et al*., 2002b). Atlantic salmon also demonstrate a lower tissue response to *L. salmonis* compared to other species (Johnson & Albright 1992a). Fast *et al*. (2003) found that lice secrete low molecular weight proteases when incubated with mucus from Atlantic salmon and rainbow trout, but not with mucus from Coho salmon, flounder, *Platichthys flesus*, or with sea water alone. Despite these differences, there is no evidence to suggest any significant genetic differentiation between *L. salmonis* on different host species, *Atlantic salmon, brown trout or rainbow trout* (Todd *et al*., 2004), unlike the closely related *C. elongatus* (Øines *et al*., 2006), which shows different genotype proportions on different hosts.

Body size seems to be important. Studies using both cage and tank trials contribute to the understanding that large fish tend to be more heavily infected (Jaworski & Holm, 1992; Glover *et al*., 2001; Tucker *et al.*, 2002; Glover *et al*., 2003, 2004a, 2004b, Genna *et al*., 2005; Glover *et al*., 2007). There are, however, several studies that found no effect of body size on louse abundance

(Todd *et al*., 2000; Glover *et al*., 2005). Glover & Skaala (2006) found that the relationship between body size and infection level is highly variable even when the same fish are exposed to the lice under the same environmental conditions. As high growth rate and low susceptibility to infection are two highly desirable factors in selective breeding programs, it is important to determine the extent to which they are linked.

As well as body size, fish behaviour changes and within-species behavioural variations may cause differences in susceptibility to infection. For example, larger, older fish in the wild seem to, actively or passively, develop behavioural patterns that reduce their exposure to the infective copepodid life stages (Bjørn *et al*., 2007).

A number of studies, often with contradictory conclusions, have been undertaken to determine whether physiological stress makes fish more attractive hosts to sea lice. The response of Atlantic salmon to stressors varies between individuals and is heritable, with fish in “high stress” families having higher rates of mortality during bacterial infection events (Fevolden *et al*., 1993). Similarly, Coho salmon implanted with cortisol developed a reduced inflammatory response and epithelial hyperplasia, and thus increased susceptibility to *L. salmonis* (Johnson & Albright, 1992b). Stress response associated with smoltification may explain the higher lice abundance on brown trout from a land locked population than from a naturally anadromous population (Glover *et al*., 2001). Other ectoparasites respond differently to the levels of cortisol in their host’s blood. Mustafa (1997) implanted cortisol into Atlantic salmon and found that they became more heavily infected with

C. elongatus than control fish. Conversely, Krasnov *et al*. (2012) did not find any correlation between cortisol level and sea louse infection numbers.

Another important factor contributing to an individual's susceptibility to sea lice is its infection history. Atlantic salmon have been suggested by some authors to develop specific antibodies to sea lice over time (Grayson *et al*., 1991; 1995), but the resultant acquired immune response is apparently weak. This may reflect response to immunosuppressive agents released by the parasite, as suggested for *A. coregoni*, or alternatively, the parasite may exploit hosts whose immune systems have been compromised by other pathogens (Bandilla *et al*., 2005). Further, pathogens may make a host more vulnerable to secondary infection by altering other aspects of its physiology, morphology, or behaviour, or a combination of these. Both observations, that stress caused by infection and infection history might influence infection severity with sea lice, chapter 5 aims to investigate repetitive sea louse infections to compare naïve against pre-infected hosts.

Genetic differences of salmon have also been investigated and analysed with respect to sea louse susceptibility. Comparisons between wild and farmed strains of Atlantic salmon have shown as much as 70 % variation between the highest and lowest infected family strains, highlighting the potential of selective breeding and family selection and different susceptibilities based on the genetic make-up of the fish (Glover *et al.,* 2004a, Glover *et al.,* 2005; Gharbi *et al.,* 2009).

One area of interest, following from the potential for use of triploid fish under commercial conditions, concerns potential host ploidy effects on louse attraction and susceptibility (O'Flynn *et al.,* 1997). Differences in performance, physiology,

behaviour and morphology between triploid and diploid fish are widely described (Piferrer *et al.,* 2009; Taylor *et al.,* 2011), and these differences could conceivably contribute to differential susceptibility to sea lice infection.

Some previous studies have suggested that triploids could be more susceptible to pathogen or parasite infection due to reduced immune activity as compared to diploids (Ojolick *et al.,* 1995; Hakoyama *et al*., 2001; Langston *et al*., 2001; Johnson *et al.,* 2004; Halačka *et al*., 2010). For example, triploid Atlantic salmon have been found to be more susceptible than their diploid counterparts to infection by *Gyrodactylus salaris*, a monogenean ectoparasite (Ozerov *et al*., 2010), although this study failed to take account of host size effects. Since no studies have investigated the effect of triploidy on sea louse infection, chapter 5 investigates the performance of triploid Atlantic salmon under repeated infection instances.

1.4. **Existing control methods and management**

Parasite control in aquaculture can involve management of a broad range of environmental / water quality parameters and host-related parameters. In Scotland and Norway, treatments are highly controlled and subject to the constraints of economics, requirements for aquaculture sustainability and environmental protection (Shinn & Bron, 2012).

Numbers of mobile stages of the lice usually increase in the last quarter of the first year in a production cycle and then drop and increase again gradually towards the end of the production cycle, in spite of treatment efforts. Sea louse counts provided by the industry can give a good indication of overall louse pressure, and according to one recent study, there is no evidence of systematic

bias arising from the use of farm staff counting sea lice compared with dedicated independent counting teams (Heuch *et al.,* 2011). Treatments in the second year of production typically have to follow a 6 week intervention cycle (Revie *et al.,* 2002). An effective treatment against sea lice in Scotland is defined as a treatment where the abundance of motile *L. salmonis* falls to <40% of their pre-treatment level at some point in the 13 weeks post-treatment (Saksida *et al.,* 2010).

With respect to sea louse treatments, the Scottish Code of Good Practice for Scottish Finfish Aquaculture (CoGP) (2013), the officially recognised guideline for salmon production within the UK states: “In general, treatments should be guided by the build-up of pre-adults as indicated by weekly counts, the objective being to prevent the development of gravid females. Suggested criteria for the treatment of sea lice on individual farm sites are: An average of 0.5 adult female L. salmonis per fish during the period 1st February to 30th June inclusive. An average of 1.0 adult female L. salmonis per fish during the period 1st July to 31st January inclusive. “

The Norwegian authorities enforce a limit of 0.5 adult female lice per fish, above which it is mandatory that a fish farming site should be treated with a delousing medicine or cleaner fish within 14 days (Heuch *et al*., 2009). This threshold does not account for spatial or temporal heterogeneity in host densities, which was described to be a main determinant of sea lice abundance (Jansen *et al*., 2012).

Treatments against sea lice rely strongly on oral or bath treatments, but are limited by the availability of few efficacious licensed products (Shinn & Bron, 2012). Modern bath treatments include the closely related compounds, Excis®

and AlphaMax®, cypermethrin and deltamethrin, hydrogen peroxide, and azamethiphos, an organophosphate. The most important in feed treatment used is emamectin benzoate, Slice®, a derivative of avermectin, a potent anthelmintic lactone derivative.

Additional research focuses on the success of fallowing, integrated pest management and general management issues to reduce parasite numbers (Denholm *et al*., 2002; Rae, 2002). Computer modelling of sea lice infection, such as seasonal trends, over a 2 year grow out cycle in commercial salmon farms can be used to predict increases in sea lice dynamics (Revie *et al.,* 2005; Robbins *et al.,* 2010). *C. elongatus* abundance is seasonally dependent, and unlike *L. salmonis,* is lower in second year salmon production cycles (McKenzie *et al.,* 2004). This seasonality was attributed to stocking dates of commercial salmon farms. Spring stocked sites experienced increasing infestation toward the end of the first year and on average, counts remained elevated thereafter, whereas autumn stocked sites averaged lower sea lice counts throughout most of the production cycle until the latter part of the second year when these escalate rapidly (Gettinby *et al*, 2011). By dealing with the predicted, early stages of sea lice, the stress response and damage to the fish can be minimised. Also, secondary infections through lesions and wounds, or weakness due to high stress levels, can be reduced (Bowers *et al.,* 2000).

It has been found that water at high currents can reduce infection levels (Jaworski & Holm, 1992). Faster water currents, or lower stocking densities leading to faster swimming by fish, can lead to a decreased success in pre-settlement of the parasite. Also, placing fish outside the halocline or surface waters, where settlement largely occurs, can decrease the number of parasites

on the host (Genna *et al.,* 2005). This can be achieved by a feeding regime that influences salmon swimming depth. If feeding can be achieved at deep water layers (< 5 m depth), infection with sea lice may be reduced (Lyndon & Toovey, 2000).

One of the biggest problems in modern salmon farming is the proximity of salmon farms and associated cross contamination. Gravid sea lice levels on fish farms can influence the abundance of sea lice in a given system. At a trial conducted in Scotland, the relocation of a salmon farm was shown to significantly reduce the production of *L. salmonis* larvae (Penston *et al.*, 2011). However, the density of the infectious copepodid stage at the vacated farm site was not reduced, which was suggested to be due to influx from neighbouring farms (Penston *et al.*, 2011). Synchronised sea lice treatment in a system can prevent the spreading of lice infestations between farms, which was suggested to occur to a distance of 5 - 8 km (McKibben & Hay, 2004; Penston *et al.,* 2011). Also, synchronised fallowing of a complete system/fjord/loch can vastly decrease sea lice numbers in a system (Werkman *et al.,* 2011). Coordinated fallowing down the main wind gradient has been suggested to increase the benefits of fallowing sites (Murray & Gillibrand, 2006). Fallowing and use of single-year class farming systems are very easy and effective ways to decrease sea lice numbers. Longer periods of fallowing are more effective than short periods. A minimum time span for fallowing which is longer than the maximum possible survival time of the parasite has to be used (Bron *et al.,* 1993b). The widely accepted official regulations in Scotland state that a minimum fallowing period of 4 weeks at the end of each cycle needs to be implemented (Code of Good Practice, Scotland, 2013).

Other approaches, which aim at achieving reduced settlement effects are currently being developed, which exploit the preference of sea louse larvae for surface waters. These include the use of “snorkel” cages, which employs surface access for the salmon only by use of a plankton net cylinder (Oppedal *et al*., 2014), and plankton net barrier nets, which block plankton access in the surface top 6 metres of the water body (Stien *et al.,* 2012) and electrified skirt nets employed in the surface 6 metres to kill incoming sea louse larvae (Bredahl, 2014).

In summary, the observed resistance built-up of the L. salmonis to all currently available therapeutical treatments and a better understanding of sea louse behavioural patterns and life cycle implications has led to a shift in louse control strategies. The approaches described in this thesis, exploiting the behaviour and biological strategies of the louse itself, will lead to an even better understanding of infection dynamics and ultimately lead to alternative treatment methods and treatment regimes on a pro-active rather than, as currently employed, reactive response to sea louse infection.

1.5. **Aims of the thesis**

The objective of the current thesis project was to provide a better understanding of the key factors associated with sea louse infection of farmed Atlantic salmon. So far, Chapter 1 (Introduction) of the thesis has outlined the 'state of the art' regarding knowledge about the natural habitat and behaviour of sea lice, and existing management practices on farms. The following experimental chapters focus on further identifying where the lice are and how they get there, the mechanisms by which they come into contact with their host fish. Furthermore, the work looks into the methods of exploiting natural habitats and behaviours to reduce louse fish interactions under farming conditions.

The specific objectives of the work described in this thesis can be summarised as follows:

1. To investigate the vertical and horizontal distribution of sea lice copepodids and establish spatial patterns of on-farm infection (Chapter 2)
2. To examine the effect of infective dose, light and current flow parameters on infection success (Chapter 3)
3. To determine whether modified lighting and feeding regimes on farms might be employed as a tool for reducing louse infection levels (Chapter 4)
4. To determine whether ploidy impacts sea louse infection levels and whether susceptibility of individual fish is consistent between replicate infections (Chapter 5)

CHAPTER 2: Investigation of longitudinal and vertical salmon louse distribution in commercial Atlantic salmon farms in Scotland

2.1. Introduction

The salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837), has two free-swimming nauplius stages, nauplius I and II (Kabata, 1979). From hatching, these undergo two moults in the plankton over a period of two to nine days depending on the water temperature in order to reach the infective copepodid stage (Bricknell *et al.,* 2006). At this larval stage, sea lice show behavioural responses to light intensity and wavelength, salinity, water pressure, turbulence associated with high current velocities or host movement, sex pheromones released by potential louse mates resident on the host and possibly host kairomones, these factors all having been described to play a role with respect to host location (Pike, 1989; Bron *et al.*, 1991; Ingvarsdóttir *et al.*, 2002a; Bandilla *et al.*, 2007). Generally, the larval stages keep within the surface layer of the water body by short swimming bursts to maximise their chances of physically encountering suitable salmonid hosts (Johannessen, 1978; Hevrøy *et al*., 2003; Costello, 2006; Murray & Gillibrand, 2006). Larval *L. salmonis* also show diel migration, gathering close to the surface during daylight hours and moving deeper in the water column in darkness (Lampert, 1989). As such, by altering the swimming behaviour of Atlantic salmon to reduce their use of surface waters where sea louse settlement is largely considered to take place, sea lice numbers on host fish can be reduced (Genna *et al.,* 2005, chapter 4).

Similarly, technologies that involve closed-pen farming possibly using pumped water from greater depths (Byrne *et al.,* 2010) or placing plankton nets around cages to exclude infecting larvae (Stien *et al.,* 2012), can serve to reduce infection pressure.

The dispersion of planktonic sea louse stages from wild or farmed sources has been reported to range from 4.6 km (McKibben & Hay, 2004) to up to 100 km from the original hatching source facilitated by hydrodynamic currents and wind forcing (Penston *et al.,* 2004; Penston *et al.,* 2008b, Middlemas *et al.,* 2013; Salama *et al.,* 2013b). Consequently, free swimming planktonic *L. salmonis* can be transported into or out of salmon cages, affecting wild and/or farmed salmon (Costello 2009b). The exposure of a given site to copepodids from outside sources will be affected by a range of factors including proximity to neighbouring farms (Revie *et al.,* 2003), exposure to migrating wild salmonids (Johnson & Albright, 1991; Penston & Davies, 2009), prevalent environmental factors such as current direction and speed, water body turnover, site location and hydrodynamics, and general weather conditions, *e.g*. wind, freshwater runoff (Penston *et al.,* 2004, 2008b; Middlemas *et al.,* 2013; Salama *et al.,* 2013b). At low current velocities, parasite-host exposure time will be higher, the depth of the boundary layer overlying the host skin greater and drag/turbulence for settled copepodids lower, giving rise to higher infection levels (chapter 3). Jaworski & Holm (1992) have also noted that water at high currents can cause sea lice (post-chalimus stages) to become detached from their hosts.

Since it will take copepodids two to nine days in order to reach the infective copepodid stage from hatch (Bricknell *et al.,* 2006), the origin of the infective copepodids affecting a given fish stock can be difficult to determine. Butler

(2002), for example, suggested that the Atlantic salmon farms he investigated within his study site were the primary source (i.e. 78-94%) of sea lice establishing on the resident farm fish. In another study area, Aldrin *et al*. (2013) found that 66% of the sea lice at a commercial farm stemmed from self-infection within the farm, 28% from neighbourhood farms and 6% from non-specified sources of infection. The relative importance of different sources of infection is, however, likely to be highly site specific.

Copepodid prevalence around commercial Atlantic salmon farms has been described to follow seasonal patterns and to change according to the stocking date of the fish. Spring stocked sites experience increasing infection towards the end of the first year and remain elevated thereafter (Gettinby *et al*, 2011), with the majority of female lice believed not to mature before November. Autumn stocked sites, however, were found to have on average lower sea lice burdens throughout most of the production cycle until the latter part of the second year when these escalate rapidly (Gettinby *et al*, 2011). For both ambient S1 and out of season S0 smolt cohorts, infection pressure will be highest during the second year of the grow-out cycle, which can be considered to be due to louse build-up and self-infection at the farming location.

Anecdotally, in Scottish salmon farms, it has been reported that end pens, *i.e*. salmon pens located at either extremity of a pen group positioned along the current gradient, tend to have higher louse numbers than pens located in the middle of the group (Cockerill, D. (2011), Pers. Comm.). Thus, the aims of the present study were to investigate sea louse density and dispersion patterns across a number of commercial salmon farms in order to examine infection patterns within a farm. The results could then be used to exploit existing water

current patterns / speeds to reduce the impact louse dispersion might have on salmon farms. Additionally, depth dispersion of lice through the water column was examined at various salmon farms, following the suggestions that louse larvae can primarily be found in surface waters.

2.2. **Materials and Methods**

2.2.1 Longitudinal/transect louse profile

Longitudinal sea louse infection counts were performed across sea cages at three different Atlantic salmon farms in Scotland. Farm 1 is located in the Farm Management Area (FMA) M-33, as described by the "Code of good practice for Scottish finfish aquaculture" and was sampled in November 2011. Farm 2 is located in FMA M-36 and was sampled in February 2013. Farm 3 is located in FMA M-21 and was sampled in September 2013.

To accurately define predominant current dynamics around each sample sites, three current impeller meters (Model BFM 105, Valeport Limited, UK) were placed along the main current gradient at both ends of the farms and centrally in between cages 15 days prior to the water sampling date (25th September 2011, 12th February 2013 and 3rd September 2013 for farms 1, 2 and 3, respectively). The current meters were checked daily for biofouling and / or seaweed blockage to ensure accurate measurement. Current speed and direction were logged at 20 minute intervals. The average current speed at 5 m depth was measured over 15 days prior to sampling, due to practical reasons, and was found to be 0.14 m s^{-1} (farm 1), 0.07m s^{-1} (farm 2) and 0.11m s^{-1} (farm 3).

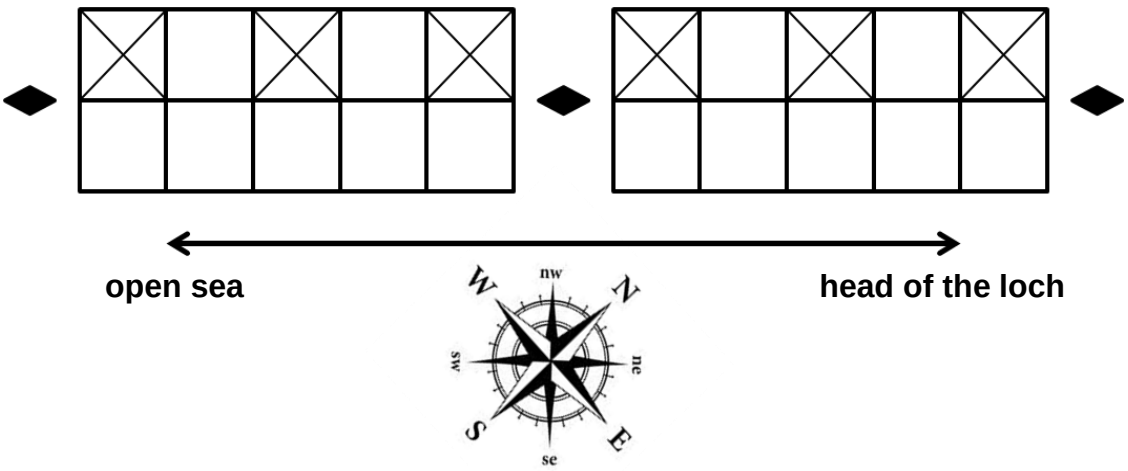
The farm configurations are given in Figure 2.1a-c. Farm 1 had 20 stocked square cages (24 × 24 m) in three separate cage groups. Two of the cage

groups comprised 10 cages each (2*5 configuration). Transect samples were taken in 6 pens following the current gradient through the farm. The two pens located at the very end of each cage groups and the two centrally positioned cages were sampled for sea lice. At the time of the trial, the site was fully stocked with a mean stocking density of 15 kg m^{-3} with second year sea-water salmon (mean weight 7405.9 ± 21.9 g). The cage depth was 15 metres with a mesh size of 15mm.

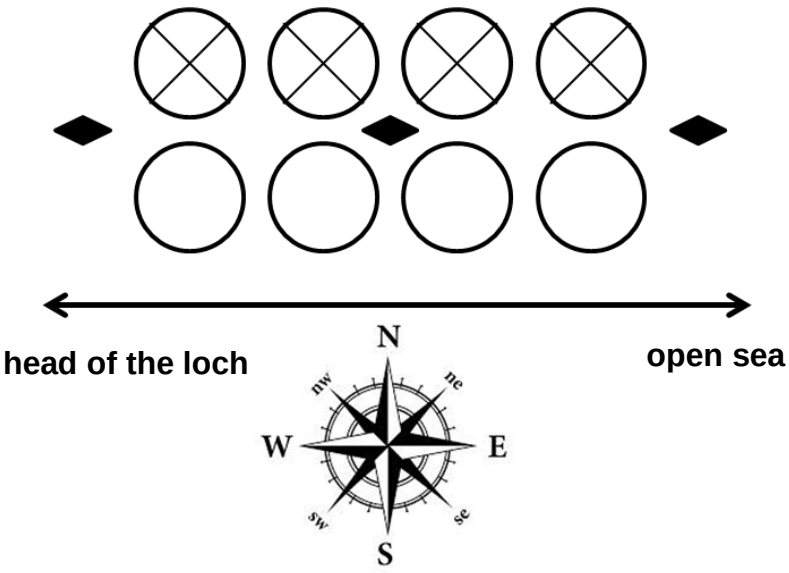
At farm 2, every pen along the main current gradient was sampled to give a complete picture of lice dynamics between pens. The farm had 8 circular cages (100 m circumference Polarcirkel^{TM} cages, Akva) in a single cage group of 8 pens (2*4 configuration). At the time of the trial, the site was in full use and fully stocked with a mean stocking density of 11 kg m^{-3} with second year sea water salmon (average size 3379.3 ± 24.0 g). The cage depth was 15 m (+ bottom cone) with a mesh size of 15mm.

Farm 3 had 12 circular cages (100 m circumference "Polarcirkel^{TM}" cages, Akva) in two separate cage groups of 6 pens (2*3 configuration). At the time of the trial, the site was fully stocked with a mean stocking density of 3.31 kg m^{-3} with first year sea water salmon (mean size 924.7 ± 6.4 g). Samples were taken in every pen along the main current gradient. The cage depth was 15 m (+ bottom cone) with a mesh size of 15mm.

Farm 1


open sea
head of the loch
nw
ne
sw
se

Farm 2


head of the loch
open sea
N
nw
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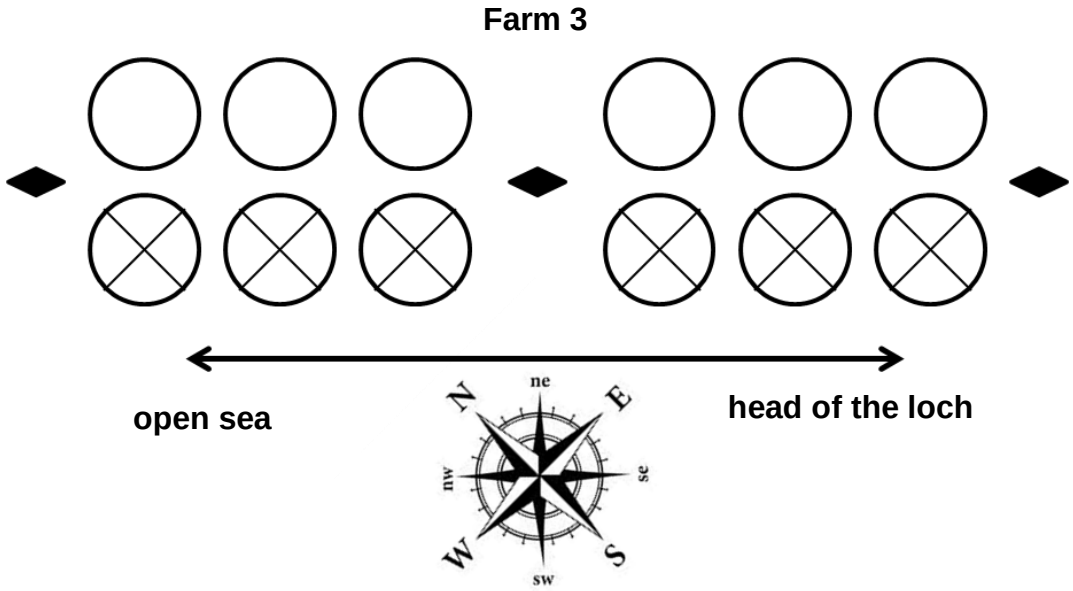


Figure 2.1a-c. Farm 1-3 layout of the three sampled Atlantic salmon farms. Square and round structures are pens, crosses indicate sampled pens. Black diamonds show positioning of current meters and arrows indicate the principal current flow axis.

At all sites, a random sample of salmon were caught in a box net (Marine Harvest, box dimensions 4×6×10 m, catch volume/sample 240 m^{-3}). Thirty anaesthetised (MS222, 50 ppm) fish per cage were lengthed, weighed and their sea louse numbers and stages determined. Pen position in this chapter is described in terms of: "end pens sea", these being pens located on the edge of the fish farm which is closest to the open sea; "end pens land", these being pens located in the edge of the fish farm located closest to the land-end of a sea loch; "central pens" refers to those pens located away from either edge of the fish farm.

2.2.2 Historical louse infection data

Historical louse infection data comprised twice weekly counts of sea lice, made by farm staff, from five randomly caught fish in three randomly chosen pens/sites. These data were analysed to provide context for the results obtained by manual sampling at the same farming locations during this study (see above). Five sites (Farm A-E) with comparable hydrodynamics, farm configurations and farming practices were also investigated to validate louse infection findings with respect to current and hydrodynamics. Only fish of the second year cohort, with higher louse numbers were considered, due to typically very low louse numbers during the first year of production cycles (Gettinby *et al*, 2011). Data analysis considered treatment dates and effects, but also considered pen position and seasonality to describe louse dynamics in the analysed farms. Winter is defined as December-February, spring as March-May, summer as June-August, and autumn as September-November. Farm A is located within the FMA M-36, Farm B, C and D within FMA M-21 and Farm E within M-22 as described by the "Code of good practice for Scottish finfish aquaculture" (*http://www.thecodeofgoodpractice.co.uk/farm/farms-introduction*, acessed 29th Dec 2014).

2.2.3 Vertical larval louse profiles

Water samples for vertical larval lice profile analysis were obtained from the same three Atlantic salmon farms sampled in Scotland, as described above: Farm 1 was sampled in September 2013, farm 2 in February 2013 and farm 3 in September 2012. Farms 2 and 3 were fully stocked at the time of sampling. At

farm 1, 50 % of the cages had already been harvested out at the time of sampling due to the site being at the end of the commercial grow out cycle.

To accurately define predominant current dynamics around the sampled sites, three current impeller meters, Model BFM 105, Valeport Limited, UK, were placed between cages along the main current gradient at both ends of the farms and centrally 15 days prior to the water sampling dates, due to practical reasons, on 20th August 2013 (farm 1), 12th February 2013 (farm 2) and 28th August 2012 (farm 3). The impeller meters were checked daily for biofouling and / or seaweed to ensure accurate measurement. Current speed and direction were logged at 20 min intervals. The average current speed at 5 metres depth were found to be 0.12m s^{-1} in farm 1, 0.09m s^{-1} in farm 2 and 0.12m s^{-1} in farm 3.

Plankton samples were pumped (Honda Water Pump WB20) from various depths of the water body, using a weighted hose (2 inches in diameter). Samples were taken directly adjacent to salmon pens, as centrally as possible. All samples were taken during daytime between 9.00 and 16.00 hours. A sample was taken at the surface and then at 2 m intervals to a maximum depth of 12 m. The pump was flushed at the respective depth prior to the sample to minimise contamination, and subsequently used to collect 500 L of seawater in a rinsed collection bin. Subsequently the sample was filtered through a 53 micron plankton net (Educational Field Equipment UK Ltd, EFE and GB Nets, UK). The collected plankton was stored in 10 mL of absolute ethanol. Subsequently, 1 mL of the mixed sample was placed in a Sedgewick-Rafter counting cell (D50) (Graticules, Pyser SGI Limited, UK) under a dissecting microscope (VMT BHS 313, Olympus, UK) and the number of *L. salmonis*

nauplii and copepodids present in the sample was counted; a total of five replicates was taken from each sample.

2.2.4 Calculations and statistical analysis

All current data logging and analysis was performed using Data Log Version 1.65-0300742H, Valeport Limited, UK. Sea lice comparisons for the depth profile were carried out by performing GLM tests after confirming homogeneity of variance, using Levene's test, and normality of the data using the Ryan-Joiner tests (Minitab, Version 16.1.0.). All fish and sea lice comparisons for the transect were similarly checked for normality, using Ryan-Joiner tests, and homogeneity of variance, using Levene's test (Minitab, Version 16.1.0). Fish performance comparisons were carried out using one-way ANOVAs (Minitab, Version 16.1.0.). Sea lice prevalence comparisons were carried out using Fisher's exact test (Minitab, Version 16.1.0.).

2.3. **Results**

2.3.1 Longitudinal/transect louse profile

A similar and statistically significant spatial infection pattern was observed at farms 1 and 2 (Table 1, Figure 2a & b). Significantly higher numbers of sea lice ($p < 0.05$) were found on fish located in "end pens sea" for both farms. The transect sampling at farm 3 did not show a significant difference between pen locations (Figure 2c).

Table 2.1. Longitudinal louse profile for three Scottish salmon farms: Summary of mean fish size and sea louse infection statistics for salmon at three point observation sampling points, comparing pen position (land: pens closest to the land-end of the sea-loch, central: pens located in the middle of the farm, sea: pens closest to the open sea). Data expressed as mean ± SEM (30 fish sampled/pen). Statistical comparisons (fish performance and lice abundance: ANOVAs, louse prevalence: Fisher's exact tests) are indicated using superscript letters with significant effects indicated by differing letters.

Date/Treatment	Fish parameters		Sea louse parameters		
	Length (cm)	Weight (g)	Prevalence (%)	Louse abundance	Range
Farm 1 November 2011					
End pens land	81.7 ± 0.1 [a]	7445.9 ± 21.2 [a]	99.2 ± 0.6 [a]	9.6 ± 0.1 [a]	0-33
Central pens	82.4 ± 0.1 [a]	7403.1 ± 19.9 [a]	98.4 ± 0.0 [a]	6.8 ± 0.1 [b]	0-17
End pens sea	82.7 ± 0.1 [a]	7368.8 ± 24.6 [a]	96.7 ± 3.5 [a]	4.4 ± 0.0 [c]	0-11
Farm 2 February 2013					
End pens land	58.7 ± 0.3 [a]	3431.0 ± 36.2 [a]	76.7 ± 2.4 [a]	1.7 ± 0.1 [a]	0-6
Central pens	60.0 ± 0.1 [a]	3392.5 ± 12.6 [a]	58.3 ± 13.0 [ab]	1.2 ± 0.0 [a]	0-4
End pens sea	60.1 ± 0.1 [a]	3314.3 ± 23.2 [a]	43.3 ± 7.1 [b]	0.6 ± 0.0 [b]	0-3
Farm 3 September 2013					
End pens land	38.1 ± 0.1 [a]	1021.7 ±9.6 [a]	43.3 ± 1.7 [a]	0.5 ± 0.0 [a]	0-3
Central pens	35.5 ± 0.0 [b]	810.7 ± 2.6 [b]	26.7 ± 0.7 [a]	0.4 ± 0.0 [a]	0-3
End pens sea	37.0 ± 0.1 [a]	941.8 ± 6.9 [a]	33.3 ± 1.6 [a]	0.5 ± 0.0 [a]	0-2

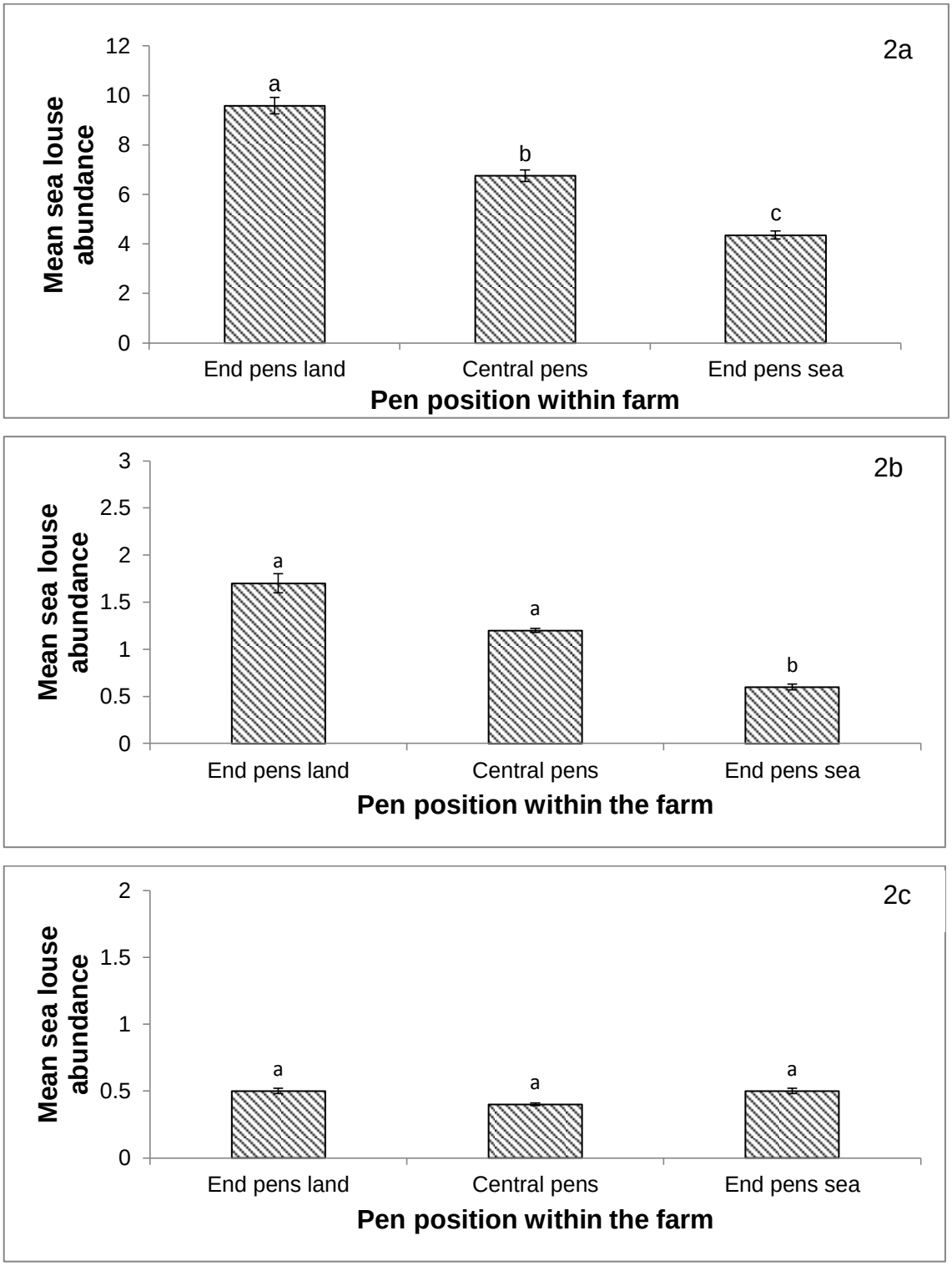


Figure 2.2a-c. Mean sea louse abundance with respect to pen position within the three sampled salmon farms (2a: Farm 1, 2b: Farm 2, 2c: Farm 3). Data expressed as mean ± SEM (30 fish sampled/pen in duplicates). Statistical comparisons (ANOVAs) are indicated using letters with differing letters indicative of significant differences at $p<0.05$

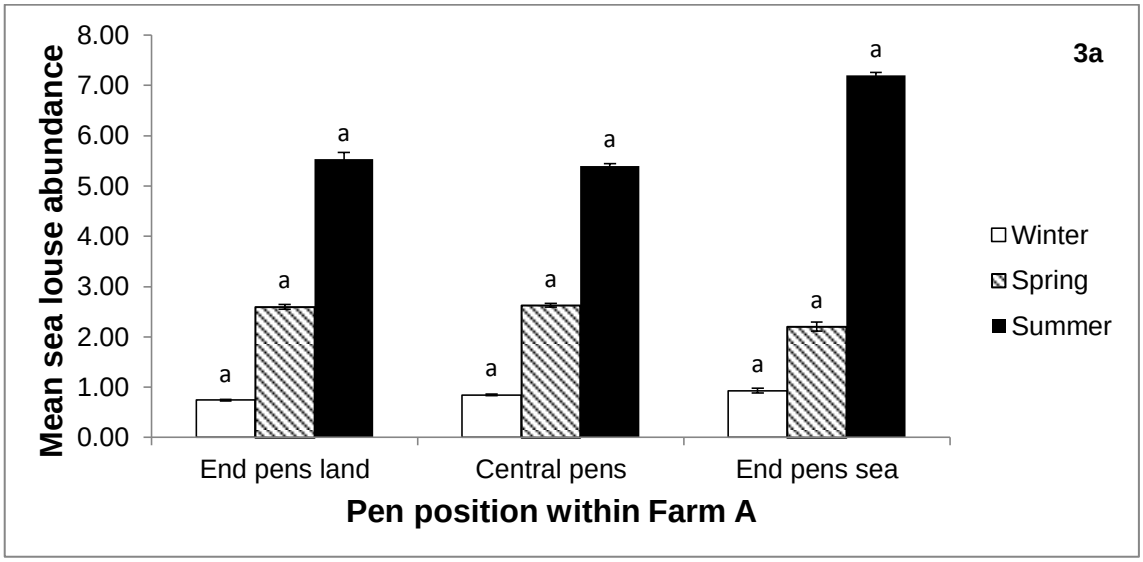
2.3.2 Historical louse infection data

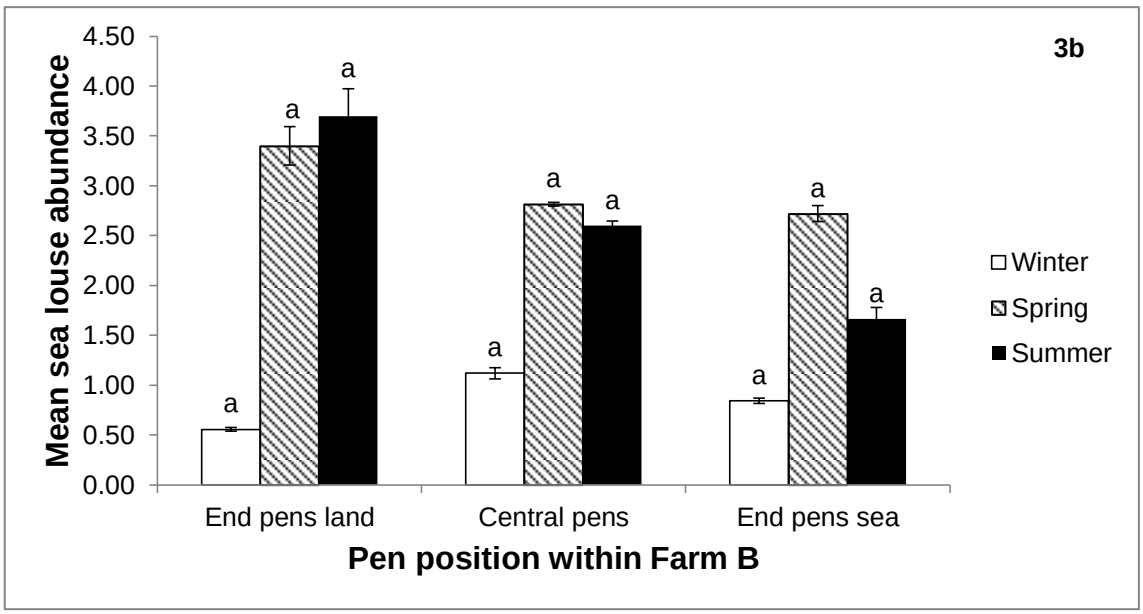
Table 2.2 presents historical louse patterns at five salmon farming sites in Scotland. A strong effect of season was observed for all analysed farms, with lowest louse numbers in winter, rising to peak numbers in the summer-time (Figure 3a-e).

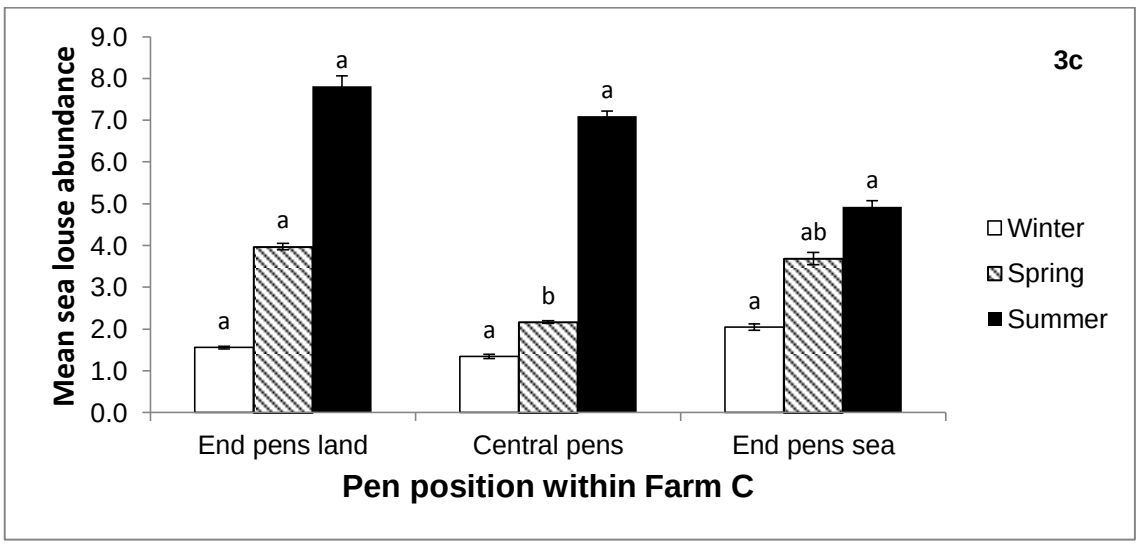
Table 2.2. Longitudinal louse profiles: Summary of sea louse infection statistics at five commercial Scottish salmon farms from historic data, comparing pen position and seasonal louse trends. Data expressed as mean ± SEM. Statistical comparisons (ANOVAs) of louse abundance are indicated using superscript letters with significant effects indicated by differing letters.

Site/ Pen position	**Sea louse abundance**			
Farm A	**Winter 2013**	**Spring 2013**	**Summer 2013**	**Overall mean abundance**
End pens land	0.7 ± 0.0 [a]	2.6 ± 0.1 [a]	5.5 ± 0.1 [a]	2.4 ± 0.0 [b]
Central pens	0.9 ± 0.0 [a]	2.6 ± 0.0 [a]	5.4 ± 0.1 [a]	3.3 ± 0.0 [b]
End pens sea	0.9 ± 0.1 [a]	2.2 ± 0.1 [a]	7.2 ± 0.1 [a]	*4.9 ± 0.0* [a]
Farm B	**Winter 2012**	**Spring 2012**	**Summer 2012**	**Total**
End pens land	0.6 ± 0.0 [a]	3.4 ± 0.2 [a]	3.7 ± 0.3 [a]	2.6 ± 0.2 [a]
Central pens	1.1 ± 0.1 [a]	2.8 ± 0.1 [a]	2.6 ± 0.1 [a]	2.2 ± 0.0 [a]
End pens sea	0.8 ± 0.0 [a]	2.7 ± 0.1 [a]	1.7 ± 0.1 [a]	1.7 ± 0.1 [a]
Farm C	**Winter 2012**	**Spring 2012**	**Summer 2012**	**Total**
End pens land	1.6 ± 0.0 [a]	4.0 ± 0.1 [a]	7.8 ± 0.3 [a]	4.4 ± 0.1 [a]
Central pens	1.4 ± 0.1 [a]	*2.2 ± 0.0* [b]	7.1 ± 0.1 [a]	3.5 ± 0.1 [a]
End pens sea	2.0 ± 0.1 [a]	3.7 ± 0.1 [ab]	4.9 ± 0.1 [a]	3.6 ± 0.1 [a]

Farm D	**Winter 2012**	**Spring 2012**	**Summer 2012**	**Total**
End pens land	1.4 ± 0.0 [b]	4.7 ± 0.1 [a]	14.2 ± 1.6 [ab]	6.8 ± 0.6 [b]
Central pens	*3.6 ± 0.1* [a]	5.3 ± 0.1 [a]	14.9 ± 0.2 [a]	*7.9 ± 0.1* [a]
End pens sea	1.1 ± 0.0 [b]	5.5 ± 0.1 [a]	*10.1 ± 0.2* [b]	5.6 ± 0.1 [b]
Farm E	**Winter 2010**	**Spring 2010**	**Summer 2010**	**Total**
End pens land	0.8 ± 0.0 [a]	1.0 ± 0.0 [a]	3.3 ± 0.1 [a]	1.7 ± 0.0 [a]
Central pens	*0.6 ± 0.0* [a]	0.9 ± 0.0 [a]	*1.3 ± 0.0* [b]	0.9 ± 0.0 [b]
End pens sea	0.5 ± 0.0 [a]	0.8 ± 0.0 [a]	2.3 ± 0.0 [ab]	1.2 ± 0.0 [ab]


3a
Mean sea louse abundance
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
a
a
a
a
a
a
a
a
a
Winter
Spring
Summer
End pens land
Central pens
End pens sea
Pen position within Farm A


3b
Mean sea louse abundance
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
a
a
a
a
a
a
a
a
a
Winter
Spring
Summer
End pens land
Central pens
End pens sea
Pen position within Farm B


3c
Mean sea louse abundance
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
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b
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ab
a
Winter
Spring
Summer
End pens land
Central pens
End pens sea
Pen position within Farm C

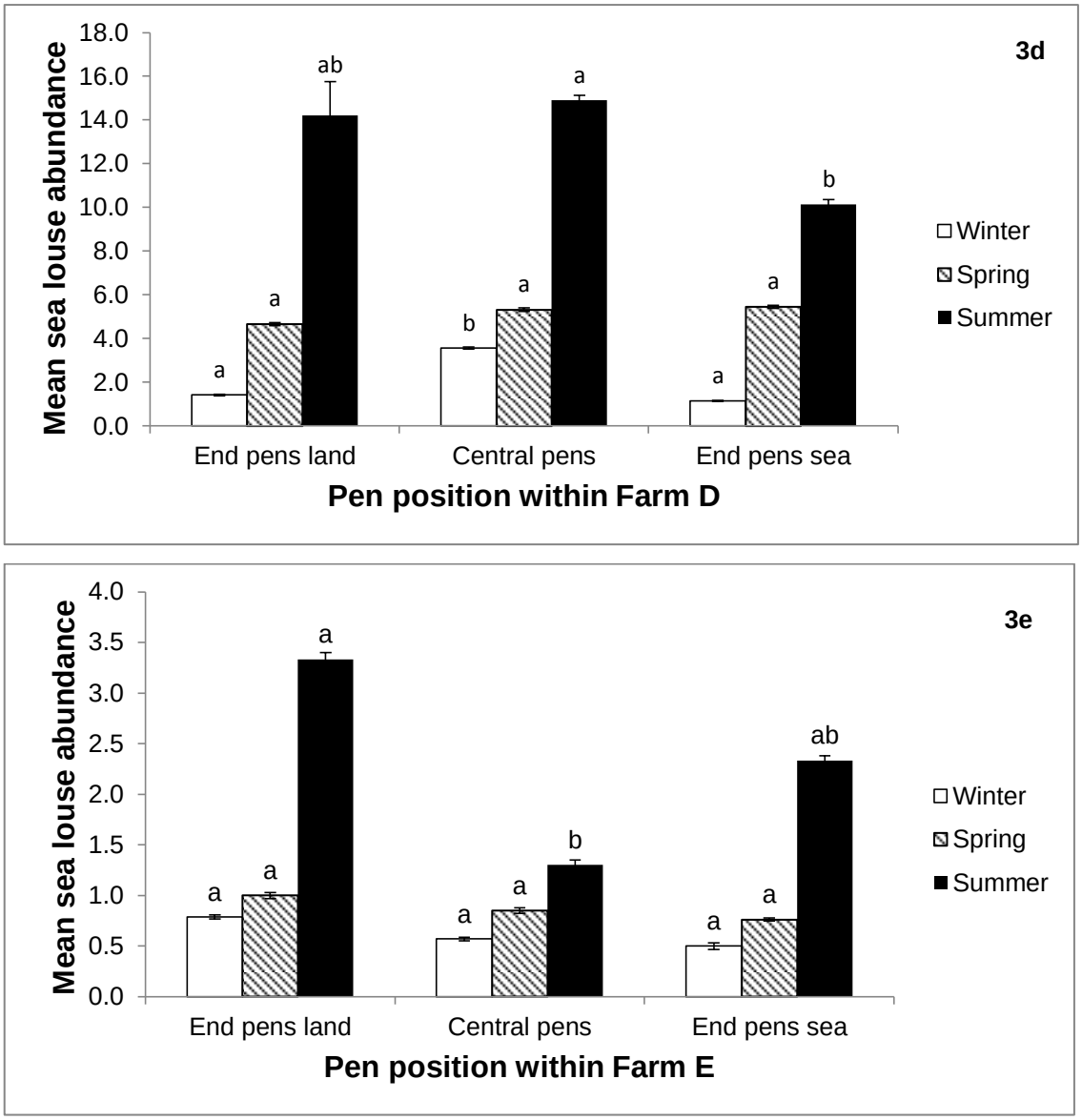
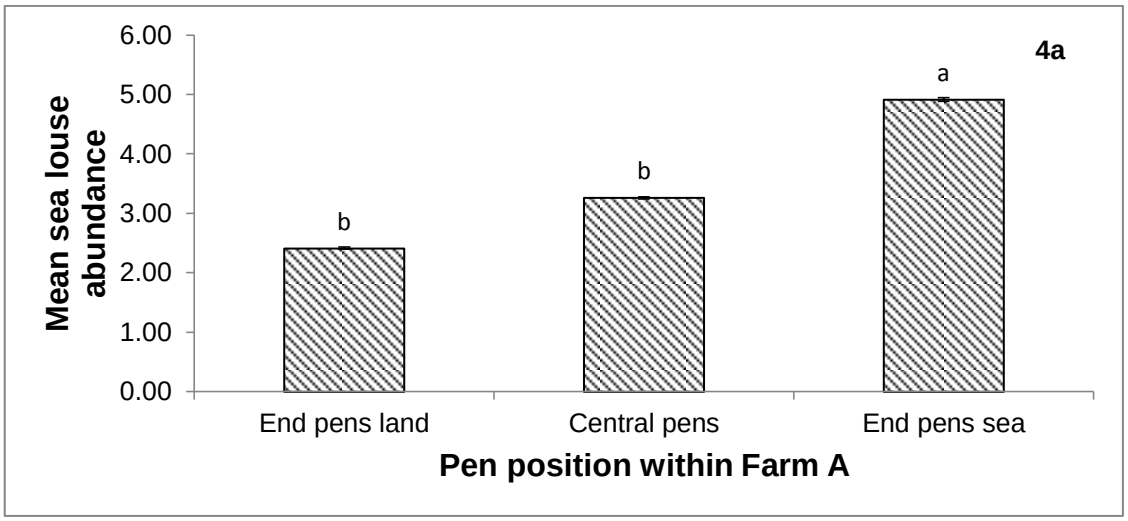
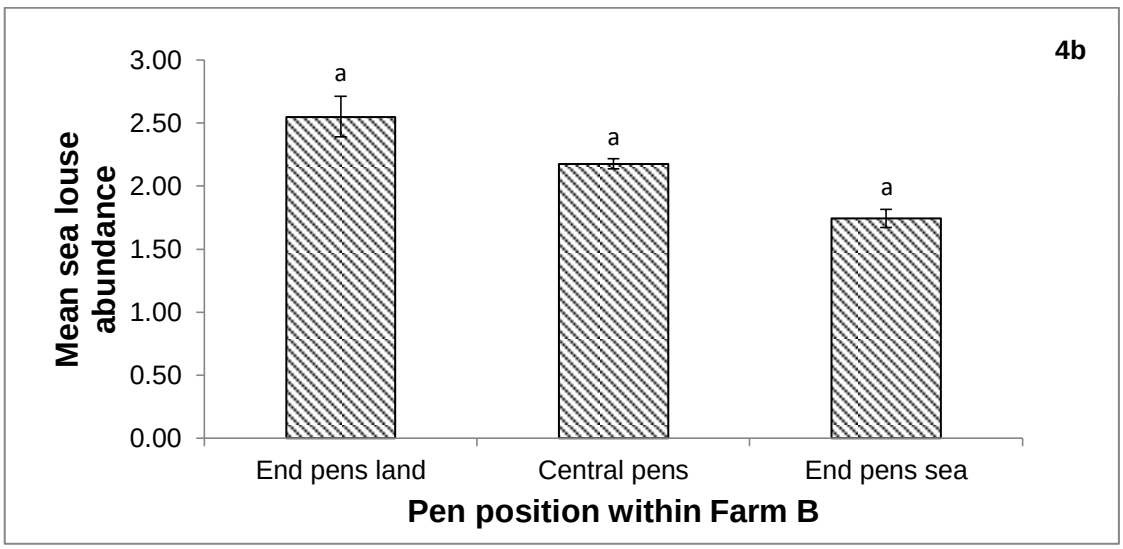
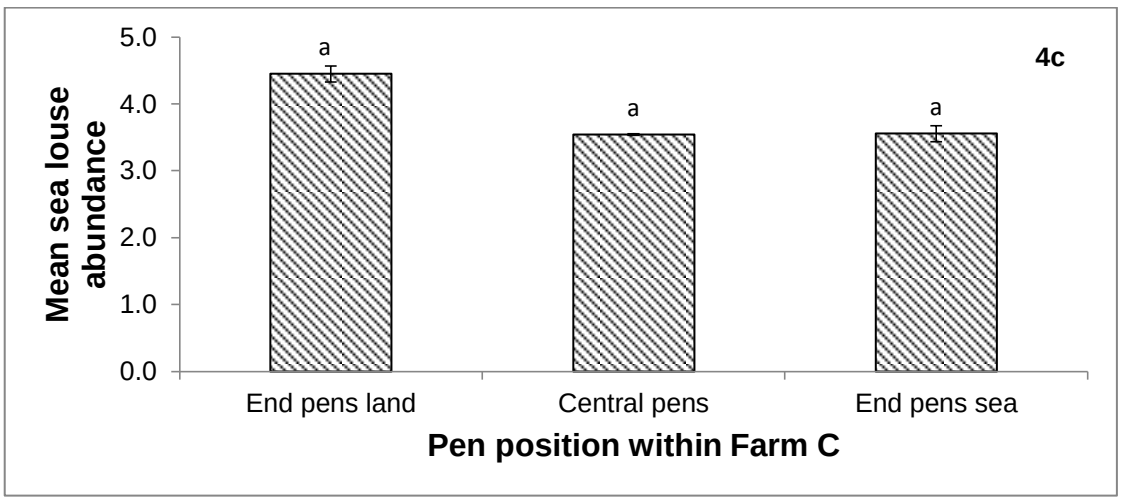


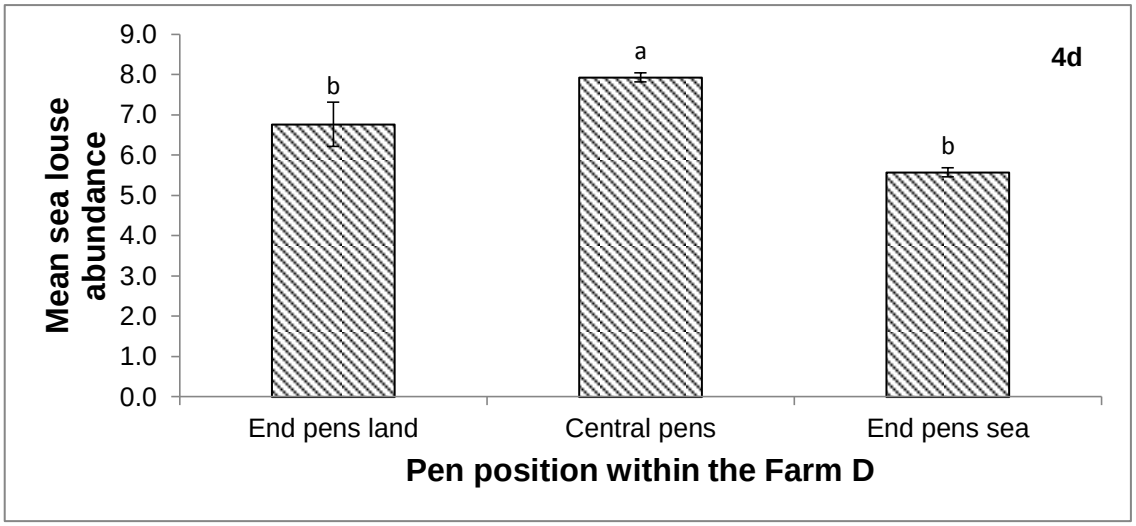
Figure 2.3a-e. Mean sea louse abundance at selected salmon farms with respect to pen position comparing seasonal differences in abundance. Current runs along the longitudinal axes of the farms, with farm positioning along the main current axis parallel to shorelines. Data expressed as mean ± SEM. Statistical comparisons (ANOVAs) are indicated using letters with significant effects indicated by differing letters.

No consistent sea louse infection pattern, comparing end and central pens, could be found comparing the five investigated farms (Figure 4a-e).









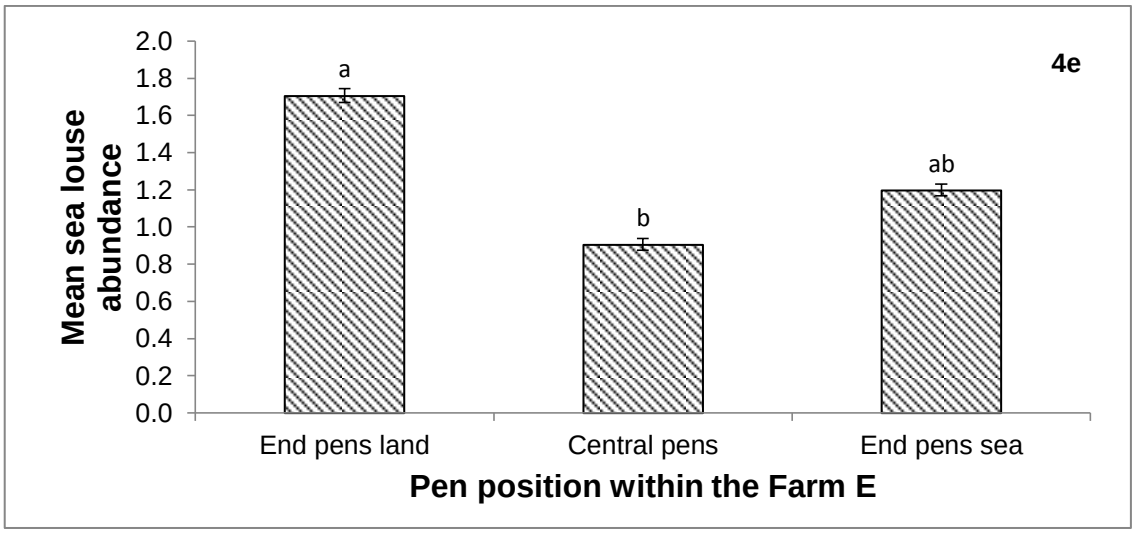


Figure 2.4a-e. Mean sea louse abundance at various salmon farms with respect to pen position comparing overall sea louse abundance over the 2nd year sea water grow out cycle. Current runs across the longitudinal axes of the farms, with farm positioning along the main current axis parallel to shorelines. Data expressed as mean ± SEM. Statistical comparisons (ANOVAs) are indicated using letters with significant effects indicated by differing letters.

2.3.3 Vertical larval louse profile

An overview of absolute louse abundance in terms of percentage of lice at a given water depth and cumulative louse abundance present at increasing water depth is given in Table 3.

Table 2.3.

Abundance of sea louse larvae with increasing water depth, comparing sea louse larval counts (500L filtered sea water) and calculated abundance percentages at the analysed water depths (0-12 metres depth).

Water depth (m)	Number of sea louse larvae (mean ± SEM)			Percentage of total sea louse larvae (%)			Cumulative percentage of total sea louse larvae with depth (%)		
	Farm 1	*Farm 2*	*Farm 3*	*Farm 1*	*Farm 2*	*Farm 3*	*Farm 1*	*Farm 2*	*Farm 3*
0	1.0 ± 0.3	0.7 ± 0.1	3.4 ± 0.1	5.6	4.0	9.9	5.6	4.0	9.9
2	6.0 ± 0.3	7.0 ± 0.7	11.1 ± 0.5	33.3	41.6	31.9	38.9	45.5	41.8
4	4.3 ± 0.2	2.5 ± 0.2	7.0 ± 0.2	24.1	14.9	20.1	63.0	60.4	61.9
6	5.0 ± 0.6	4.0 ± 0.4	6.8 ± 0.2	27.8	23.8	19.6	90.7	84.2	81.5
8	1.0 ± 0.0	1.2 ± 0.2	2.7 ± 0.1	5.6	6.9	7.8	96.3	91.1	89.3
10	0.7 ± 0.2	1.5 ± 0.2	2.3 ± 0.1	3.7	8.9	6.7	100.0	100	96.0
12	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.1	0.0	0.0	4.0	100.0	100	100.0

Significantly higher sea louse larvae (nauplii and copepodids) numbers were found in the surface 6 m at all sampling sites (Table 4).

Table 2.4. Effects of water depth on presence of sea louse larval stages (nauplii and copepodids) T-test comparisons (n=12)

Trial/Source	Mean louse abundance	% of total lice	t-value	p-value
Farm 1				
0 - 6 m water depth	4.08 ± 0.4	86.4 %	8.37	*< 0.005*
6 – 12 m water depth	0.56 ± 0.1	13.6 %		
Farm 2				
0 - 6 m water depth	3.54 ± 0.6	74.9 %	3.79	*0.002*
6 – 12 m water depth	0.89 ± 0.2	25.1 %		
Farm 3				
0 - 6 m water depth	7.10 ± 0.6	69.7 %	5.97	*< 0.005*
6 – 12 m water depth	2.15 ± 0.4	30.3 %		

Significant effects ($p < 0.05$) are indicated by *italics*.

The highest numbers of lice were found at 2 m depth, however, lower louse numbers were found above 2 m, directly at the surface (Figure 5). Highest louse larval abundance was found at 2 m water depth (35.6 ± 1.7 % of all lice present in the analysed water body). Cumulatively, 61.8 ± 0.4 % of the sea lice larvae could be found in the surface top 4 m of the analysed water body. Sea louse larvae abundance increases to 85.5 ± 1.6 % of all lice present within the top 6 m of the water body.

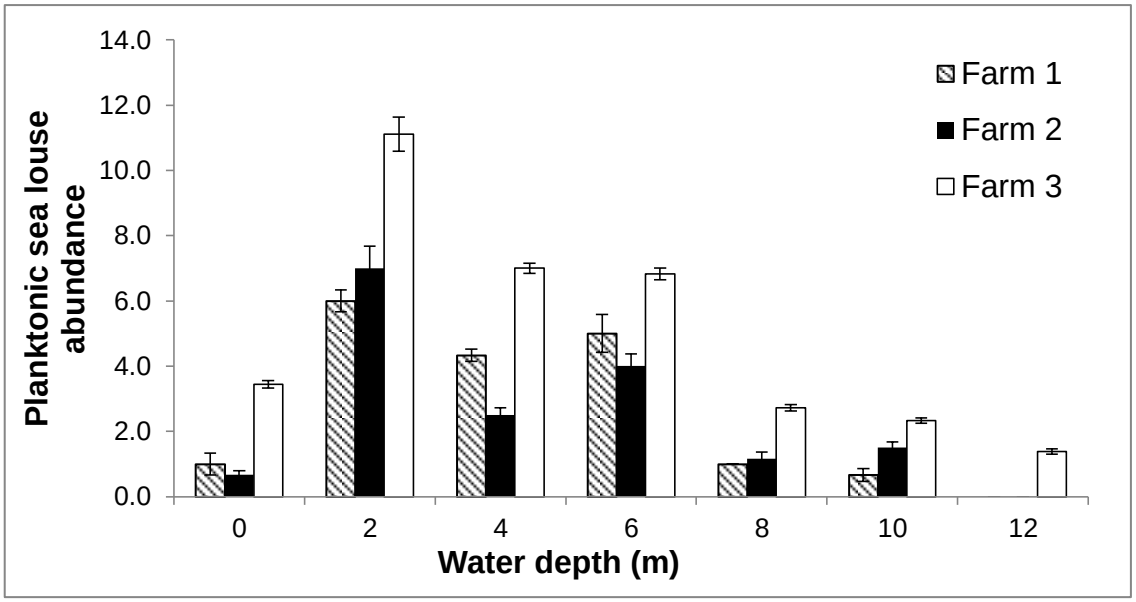


Figure 2.5. Mean planktonic sea louse abundance with respect to water depth at the three sampled salmon farms. Data expressed as mean ± SEM (500 litres of water filtered, n=5)

2.4. **Discussion**

In the current study, the louse abundance and larval density / distribution patterns across various commercial salmon farms were tested to examine infection patterns within a farm. Longitudinal infection patterns across a farm were seen to be highly variable and inconsistent comparing salmon farms with similar hydrodynamics. Additionally, the distribution of larval lice with depth was examined following the suggestions that louse larvae can primarily be found in surface waters and that therefore initial infection occurs in surface layers (Huse & Holm, 1993; Genna *et al.,* 2005). This study confirms that sea louse larvae were mainly found in surface layers, with peak abundance at 2 m water depth, which can be concluded to be due to distinct migration patterns deployed by the parasite.

The current study investigated the question of whether sea louse infection levels were structured with respect to the principal axis of flow in sea loch salmon pens. The results suggest that infection within the analysed farm sites cannot be derived from analysing louse numbers transactional through a salmon farm. However, a combination of hydrodynamic and seasonal infection patterns might give an indication to possible external sea louse sources. Although not verified by this study, since self-infection of a farm has been described as the highest louse contributor (Aldrin *et al.,* 2013), end pens at the loch end position can potentially hold higher louse numbers. This could be due to planktonic lice stages getting picked up by the incoming current along the farm and delivered to the end pens with the outgoing current, with the result being that the farm is most likely to re-infect itself. Other authors have shown that wind driven sea louse accumulation is capable of having an even higher effect than tidal or oceanic current patterns, dependent on context (Penston *et al.,* 2008b; Costello, 2009b; Salama *et al.,* 2013b). The effect of wind driven surface currents were not analysed as part of this study, so the additional effects of wind could not be ascertained, however, this may be an additional factor influencing re-infection. Neighbouring farms, sharing the same overall loch system and current gradient would, in all likelihood, contribute to, and intensify the observed effects, although the area management activities undertaken by all major salmon producers in Scotland, which include the use of synchronised sea lice treatments and fallowing of all sites in a system, can decrease the extent of inter-farm transmission of lice (McKibben & Hay, 2004; Murray & Gillibrand, 2006; Penston *et al.,* 2011). The sites analysed in this study were chosen for their longitudinal current gradient and in that they lacked

major perturbations in the principal directed current flow. Thus, any deviations in the direction of the transport of lice outside the principal current direction are likely to be minimal and lice infection occurring in the end pens will be highly affected by the main direction of flow. It is important, however, to stress that the effect of wind forcing, as described earlier, might be considerable and therefore affect the described results.

The investigation of historical louse counts at five Scottish salmon farms showed a range of infection patterns, which were highly dependent on seasonality and possibly other environmental effects such as fresh water influx, or wind direction influencing surface current patterns. The lack of significant results and trends might be explained by infection patterns being clearly site dependent. A close investigation of louse numbers could give an indication as to which end pens will show highest infection numbers and possibly act as louse reservoirs from incoming / outgoing currents.

The second part of this study examined depth distribution of larval sea lice from 0 – 12 m, as sea lice infection is believed to occur primarily in the surface waters. Light, pressure and salinity (Bron *et al*., 1993a; Heuch *et al*., 1995; Flamarique *et al*, 2000; Bricknell *et al*., 2006) all provide cues by which the copepodids navigate into and remain in waters that apparently maximise the likelihood that they will encounter a suitable host. Also, salmon pen depths in Scotland extend down to a maximum depth of 15 m (+ bottom cone). This study shows that the highest concentrations of planktonic sea lice stages were found at 2 m below the water surface. The numbers of sea lice found below 6 m, however, was seen to fall dramatically. This study thus provides confirmation that the main region of occupation of sea louse larval stages is the surface

water layers in Scotland. This is in line with the observations made within the study by Huse & Holm (1993) suggesting that copepodids were in greater abundance in the upper 6 meters of the water column compared to deeper water layers down to 20 meters. These findings are further supported by findings from wild stock investigations, which suggested that the highest infection risks occur in shallow waters and for low-depth swimming fish species or life-cycle stages (Johnson & Albright 1992a; Dawson *et al*., 1997; Bjørn *et al*., 2007). In the current study, low numbers of lice were found directly below the surface (within the top 10 cm, as required for accurate pumping). As sea louse copepodids show avoidance behaviour with respect to areas of low salinity (Genna *et al.,* 2005), low surface numbers might be attributable to sub-optimal salinity levels at the surface due to fresh water run-off. However, the current study does not take into account salinity gradients. Additionally, the time of sampling could not be synchronised between the different sites, due to practical limitations. This, combined with weather effects will have affected the light intensity and brightness and may thus affect larval distribution within the water body.

The concentration of infective larval stages in surface waters provides an opportunity for industry to minimise infection through modification of salmon depth distribution or physical exclusion of larvae. Studies based on the surface preference of sea louse larvae have investigated the use of “snorkel” cages, which employs surface access for the salmon only by use of a plankton net cylinder (Oppedal *et al.,* 2014), plankton net barrier nets blocking plankton access in the surface top 6 m of the water body (Stien *et al.,* 2012) and

electrified skirt nets also employed in the surface 6 m to kill incoming sea louse larvae (Bredahl, 2014).

Conclusively, this study shows that sea louse abundance is indeed increased in the surface layer of the water body. By altering salmon behaviour or physically separating fish from parasite environments, the infection with sea louse can be minimised. A successful approach to this theory will be further investigated in chapter 4. Longitudinal sampling across a salmon farm might be used to identify infection sources and potential high risk areas within a farm. The distribution of sea lice within a farm, however, is highly influenced by environmental factors and the described method was seen to be indicative only, due to the lack of significant trends observed.

2.5. **Acknowledgements**

The authors would like to thank Marine Harvest Scotland for kindly funding the described work. Thanks also go out to all the staff at Marine Harvest Scotland and especially those at the sampled sites for their invaluable support, feedback and help with the performed sampling.

CHAPTER 3: Copepodid attachment success of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon (*Salmo salar* L., 1758) under experimental flume challenge conditions

3.1. Introduction

Success of copepodid attachment to host Atlantic salmon by the salmon lousedepends upon a complex interaction between host parameters, ambient environmental factors and the physiological, sensory and behavioural properties of the sea louse. The research presented in this chapter seeks to improve understanding of the influence of environmental factors upon copepodid attachment. New data provide a basis for improving management of sea lice on salmon farms through use of passive, e.g. farm siting / pen design, or direct measures, e.g. manipulation of environmental conditions such as light.

The salmon louse, L. salmonis, has two free-swimming nauplius stages, nauplius I and II, and from hatching these undergo two moults in the plankton over a period of 2 to 9 days in order to reach the infective copepodid stage (Bricknell et al., 2006). Following infection, a series of moults produces the adult stages, which mate on the host to continue the life-cycle. In their free-swimming state, sea lice larvae are non-feeding and lecithotrophic and thus entirely dependent upon finite maternal lipid reserves, having limited resources for host finding (Karvonen et al., 2003). This, combined with the temporally and spatially stochastic nature of host-finding events, dictates that copepodids must attempt to attach indiscriminately to any fish that comes into sufficiently close proximity

(Heuch et al., 2007). It is likely that copepodids use a combination of mechanosensory, chemosensory and visual cues at different stages during the process of host location and attachment (Gresty et al., 1993; Bron & Sommerville, 1998) and it is clear that the age of copepodids affects its ability to infect a host. Freshly moulted copepodids are not maximally infective, requiring 1-2 days of maturation to achieve peak infectivity (Tucker, 1998). The length of the infective window and the probability of copepodid infection success is also considered to be positively correlated to available lipid reserves, making younger copepodids more infective than older ones (Gravil, 1996; Tucker, 1998).

Wind and current have been described as the main factors affecting dispersion of the planktonic stages of the sea louse (Penston et al., 2004). Sea louse naupliar and copepodid stages, however, do not act as purely passive particles, and are thought to employ a combination of buoyancy and short swimming bursts to keep within the surface layer of the water column (Huse & Holm, 1993). They are also considered to use environmental cues to help bring them into areas where they are most likely to encounter potential hosts (Bron et al., 1993a; Beamish et al., 2007; Marty et al., 2010). To that end, copepodids are reported to remain at the surface between 0 and 4 metres depth (Johannessen, 1978; Heuch *et al*., 1995; Hevrøy *et al*., 2003; Murray & Gillibrand, 2006, chapter 2) and to respond to light, pressure and salinity (Bron et al., 1993a; Heuch et al., 1995; Flamarique et al., 2000; Bricknell et al., 2006) in order to maximise the likelihood of encountering a suitable host.

Current speed is recognised to be a key factor mediating host attachment. The faster the water flow across the host surface, the shallower the drag-induced

boundary layer of reduced flow overlying the fish skin becomes and therefore the greater the ambient flow and drag experienced by the attaching copepodid (Bron, 1993). For this reason, faster water current speeds and faster host swimming speeds make it more difficult for copepodids to attach to the host in the first place and to remain anchored before attaching more permanently using a frontal filament (Bron, 1993). This phenomenon also explains the higher incidence of copepodid settlement on dorsal and paired fins, whose fin rays, lying perpendicular to the current, provide sheltered areas in their lee and hence deeper boundary layers, which serve to protect the copepodid from the ambient flow (Bron, 1993). As a result of such effects, faster water currents in salmon cages have been shown to decrease louse attachment success (Genna et al., 2005).

Light and photic conditions have also been shown to influence swimming behaviour of copepodids. Sea lice have well-developed photoreceptive capabilities (Bron et al., 1998), however, the role of light in host location and infection success remains unclear to date. It is well documented that copepodids are positively phototactic (Bron et al., 1993a; Heuch, 1995; Flamarique et al., 2000), and apparently insensitive to shadows (Bron et al., 1993a; Flamarique et al., 2000). For *Lepeophtheirus dissimulatus* (Wilson, 1905) and *Lepeophtheirus pectoralis* (Müller, 1777) and also possibly for *L. salmonis*, it was suggested that maintaining copepodid position in the water column could be the principal, and perhaps only role of vision (Lewis, 1963; Boxshall, 1976). Intermediate (300 lux) light levels were observed to produce higher settlement of copepodids on Atlantic salmon smolts than high (800 lux) or low (10 lux) levels (Genna et al., 2005), although the tested levels were very

low in comparison to full daylight (> 30,000 lux). Heuch et al. (2007), however, found that there was no significant difference in the rate at which copepodids attacked a model fish head when exposed to 'dark' (infrared) or light (infrared and white light) conditions. This model approach though does not consider tactile or chemosensory factors. Under laboratory conditions, it was seen that infection in complete darkness can be successful (Johnson & Albright, 1991; Bron et al., 1993a), indicating that light is not essential for infection.

Taken together, previous research therefore indicates that environmental factors have a strong influence on the attachment success of salmon louse copepodids and this underlines the importance of gaining a better understanding of their influence on settlement.

The aim of this study was therefore to test the effects of selected environmental factors upon the attachment success of sea louse copepodids infecting Atlantic salmon hosts under controlled flume conditions.

3.2. **Materials and Methods**

3.2.1 Flume design

To scientifically investigate copepodid attachment under controlled conditions, a flume with controllable water current speed, louse introduction and light regime measurements was designed. The flume was designed to hold a single fish in position at any given time and testing attachment success of by-flowing copepodids. Additionally the system was designed to be modular to meet the requirements of easy transport and quick on/site set-up. Therefore a new design of a closed circular flume was built to conduct this study (Figures 1, 2). Water was supplied from a reservoir tank (500 litres) holding fresh filtered sea

water, which passed water to the flume via a water pump (Argonaut AV250-3DN-S; 400V/50Hz).

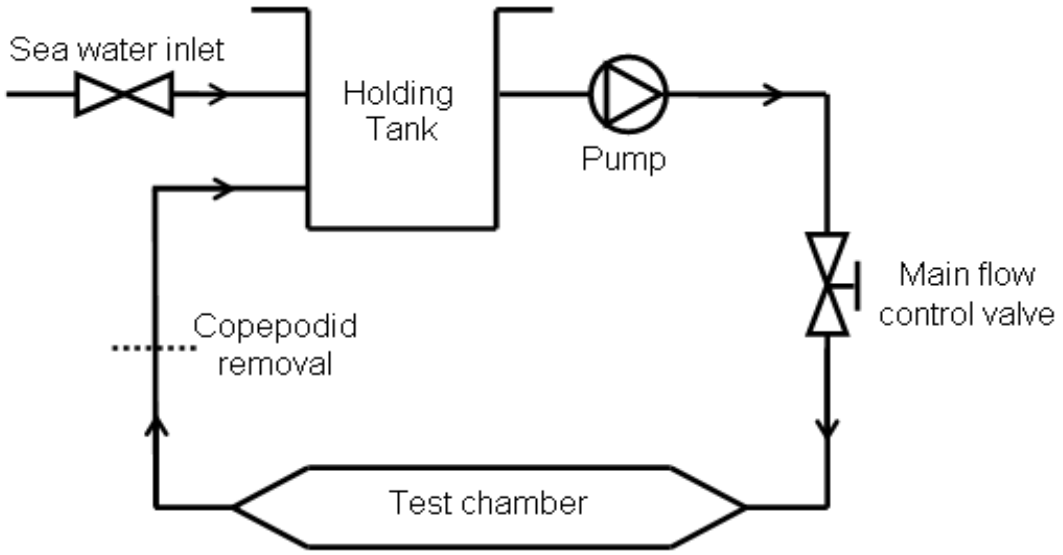


Figure 3.1. Schematic diagram of flow control for the designed flume

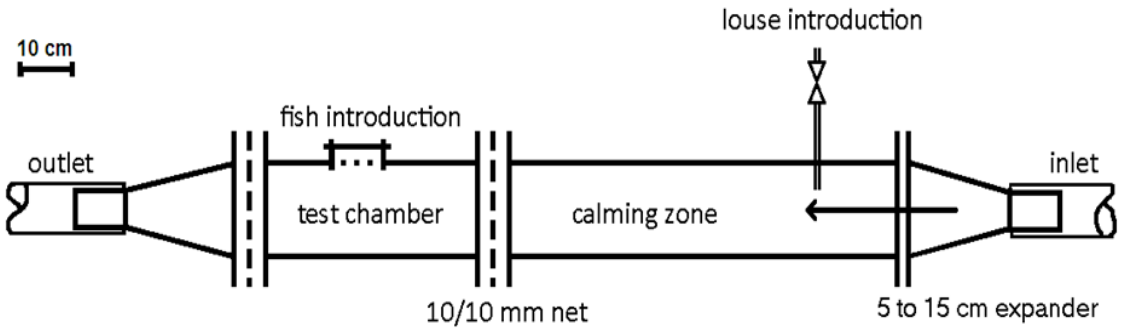


Figure 3.2. Schematic diagram of the designed flume, including transparent fish infection test chamber and louse and fish introduction apertures

The flume (Figure 2) was connected to the pump via a 2 inch (5 cm) hose and a flow-control valve, used to control current speeds in the test chamber (Figure 1). The flume opened up into the testing chamber, a 15 cm diameter pipe, through

a 5 ► 15 cm pipe diameter expansion. Directly adjacent to the pipe expansion, a louse injection port (evacuation / dispensing pump syringe, 1 litre max. volume) was connected. The turbulence created by the pipe expansion ensured mixing of the water (containing copepodids) injected into the flume during the trials. The louse-water mixture then passed through a section of the pipe long enough to create a laminar flow (calculated by pipe length : diameter ratio), allowing natural orientation of the copepodids in the flume. An interchangeable transparent fish test chamber (15 cm pipe diameter) was fitted after this calming zone, with a 10 x 10 mm nylon net on each end to keep the fish within the chamber. A fish introduction / removal window (opening diameter of 10 cm) was installed on top of the fish chamber. After passing through the flume, the outlet water was filtered using a 53 micron mesh plankton net (Educational Field Equipment UK Ltd, EFE and GB Nets, UK) to collect unattached sea louse copepodids and prevent cross contamination between samples.

Water current speed through the chamber was tested by measuring flow rate at the outlet of the flume. A maximum current speed of 32.6 cm s^{-1} was obtained at maximum pump output. The pump performance was chosen to allow for comparison to natural conditions around commercial salmon farms located on the west coast of Scotland - high current speed salmon farms in Scotland show a mean current speed of ~14.5 cm s^{-1} and peak current speeds of ~ 50 cm s^{-1} (Cockerill, D. (2013) Pers. Comm.). A “residual current” speed through the flume of 2.2 ± 0.2 cm s^{-1}, associated with low level water movements owing to fish motion, was established by estimating the best fit of infection data against current speed and used as a baseline for calculating all stated current speeds. Accordingly, a minimum current speed through the testing chamber of 2.4 cms^{-1}

was found to be achievable through the use of manually-controlled flow rate valves. The total water volume in the fish test chamber was calculated to be 3.22 litres.

3.2.2 Experimental Atlantic salmon and salmon lice

Fish used in the trials were produced at the Institute of Aquaculture's Howietoun Fishery in 2012 and smolts were transferred to the Institute of Aquaculture marine facilities (Machrihanish Marine Environmental Research Laboratory (MMERL) on the 13th May 2013. Fish were on-grown in sea water until the start of the experiment in June 2013. Each trial comprised 5 replicate runs, with new lice added for each run. The number of replicates was constrained by the quantity of copepodids required per run to achieve a suitable resolution for assessing infection success. Sea lice copepodids for the challenge trials were produced at MERL's sea louse farm according to standardised protocols: Gravid *L. salmonis* females were collected from infected stock fish and the caligid eggstrings subsequently hatched under ambient temperature, salinity (i.e. 33 ppt; 14°C) and artificial light conditions (50:50 mixture of light produced from LM TS183/36W coolwhite and Osram L36W/840 Lumilux coolwhite fluorescent tubes). After hatching, the larvae were separated into hatching cohorts to ensure a uniform age distribution of louse batches. The hatched larvae were subsequently incubated under ambient water temperatures to reach the nauplius II and finally copepodid stage. For each copepodid batch, stage checking and counting were carried out for 5 replicate sub-samples using a standard Bogorov zooplankton counting chamber (10 mL aliquots) observed under a dissecting microscope (VMT BHS 313, Olympus, UK).

3.2.3 Standard trial methodology

For each trial run, the flume was set up to the required trial design parameters (flow, light, louse challenge). All trials were performed under ambient temperature (14.0 ± 0.2 °C). Single fish were introduced into the test chamber at minimal handling time to reduce scale loss. Following fish introduction, the chamber was locked tight using a stainless steel clamp. Fish were given an experimental acclimation period of 3 min under the given current and light set-up as required by the trial run. Thereafter, copepodids were introduced to the system via the louse injection port. The fish remained in the testing chamber for a period of three minutes following louse introduction, ensuring that all lice had passed the fish and reached the flume outlet. Fish were then captured using a small plastic bag to minimise dislocation of freshly attached copepodids and sacrificed. The total number of copepodids on the fish and in the water from the plastic bag was counted using a dissecting microscope (VMT BHS 313, Olympus, UK); this value was used for all given copepodid counts in the described trials. All experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986 UK under the approval of the local ethical committee.

3.2.4 Experimental challenge trials

3.2.4.1 Flow rate challenge trial

The flow rate challenge aimed to investigate the relationship between current speed through the flume and attachment success of copepodids. The current

speeds tested ranged from 0.0 to 32.6 cm s^{-1}. This compares to average current speeds observed in chapter 2 ranging from 7 – 14 cm s^{-1} and chapter 3 with observed current speeds from 0.0 - 12.1 cm s^{-1}. Static conditions were achieved by closing both the inflow and outflow valves. For static conditions the sea lice were loaded through the fish port and left to infect the fish for a time period of 3 minutes. This time period was chosen randomly. Minimum flow was defined as the minimum achievable flow through mechanical valve control. The maximum flow was obtained by opening all valves fully at maximum pump performance. Based on the best attachment results obtained, the minimum flow, a current speed of 2.4 cm s^{-1}, was chosen for all following trials. Trials were carried out under ambient light conditions (8.7 W m^{-2}). The mean fish size for this challenge was 88.0 ± 4.8 g (weight ± SD) and 18.5 ± 0.3 cm (length). The current speed was set to the tested flow rates by controlling the inflow valve. Current speed was measured using volume / time measurements at the outflow side (triplicates).

3.2.4.2 Louse dose challenge trial

For the louse dose calibration trial, sea louse challenges comprising different doses of 0, 1000, 2500, 5000 to 7500 copepodids per replicate were tested. One dose consisted of a 1 litre syringe filled with 1 litre of water loaded with the relevant copepodid amount and injected into the current flow over the course of 3 seconds. Based on results obtained from the flow rate challenge trials, the valves were adjusted to allow minimum flow (2.4 cm s^{-1}) through the flume. Each dose was tested on 5 replicate fish. Trials were carried out under ambient

light conditions (8.7 W m^{-2}). The mean fish size during the challenge was 75.2 ± 5.5 g (weight) and 17.9 ± 0.3 cm (length).

3.2.4.3 Copepodid cohort challenge trial

For the copepodid cohort trial, a single 24 hour batch of copepodids was tested over a period of six days to examine the profile of attachment success as a product of copepodid infectivity and mortality over time. A fixed water volume, containing 2500 newly moulted copepodids from this single batch, was employed for the first day challenge. Thereafter, with pre-sample mixing to ensure homogeneity of copepodids, an identical water volume was tested each day for a period of 6 days post copepodid moult (8 days post hatch, DPH), to establish an infection curve reflecting louse mortality and infectivity. Trials were carried out under ambient light conditions (8.7 W m^{-2}). The fish size during the challenge was 103.6 ± 5.3 g (weight) and 19.3 ± 0.3 cm (length).

3.2.4.4 Light challenge trials

Experimental LED lamps (Intravision Aqua AS, Oslo, Norway) equipped with dimmers were used to test blue (peak at 455 nm), green (peak at 530 nm) and red (peak at 640 nm) narrow bandwidth light (Figure 3).

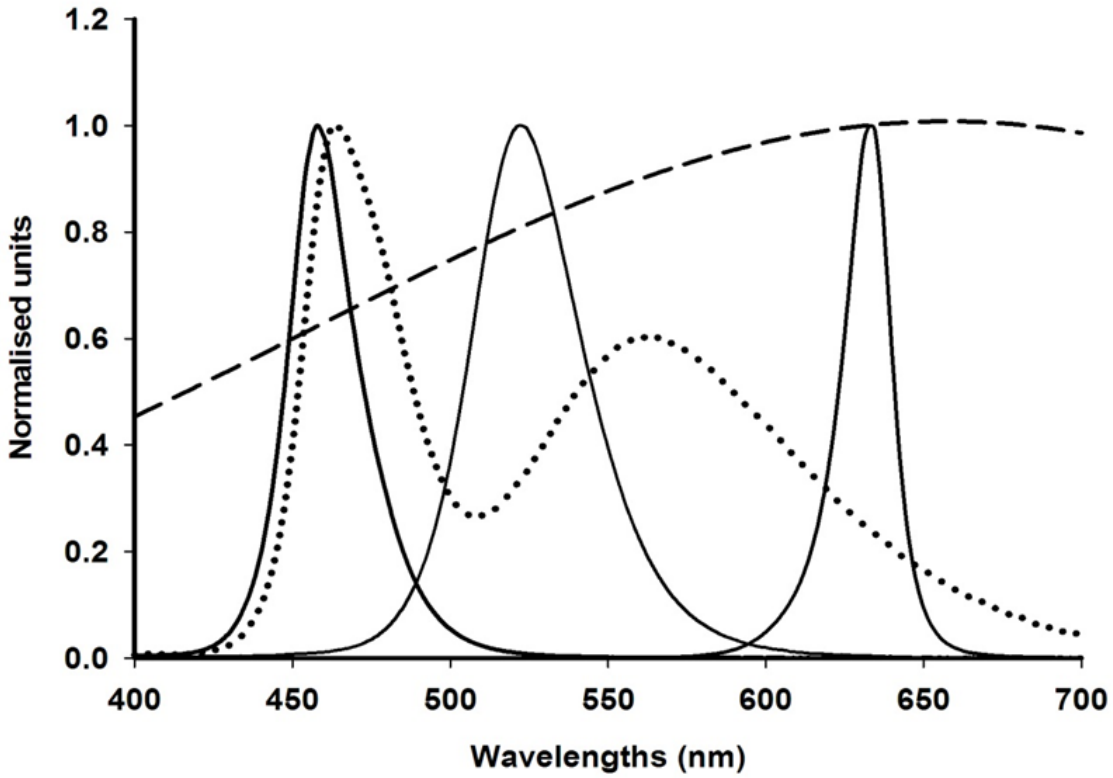


Figure 3.3. Spectral scans of the light units used during the light trials. Coloured light – solid curves: blue light spectrum (peak at 455 nm), green light spectrum (peak at 530 nm), red light spectrum (peak at 640 nm), dashed curve – bright halogen light spectrum (peak at 660 nm), dotted curve – ambient fluorescent tube lighting.

LED lamps were placed centrally outside the testing chamber, to illuminate a maximum volume of the testing chamber. Artificial (white) light was produced by standard fluorescent tube lighting (50:50 mixture of light produced from LM TS183/36W coolwhite and Osram L36W/840 Lumilux coolwhite fluorescent tubes) mounted 1.1 m above the tanks. Bright white light was produced by shining a halogen flood light (500W R7s halogen lamp) directly at the testing chamber. Light irradiance (Watts m^{-2}) for all treatments was measured with a

calibrated single channel light sensor (Skye Instruments Ltd., Powys, UK). Spectral composition (Figure 3) using a portable spectroradiometer (Stellarnet Inc, USA). The light intensities tested for the coloured LEDs were: a maximum intensity of 5.4 W m^{-2}, a medium intensity of 0.54 W m^{-2} and a minimum intensity 0.054 W m-2. The white light intensity for the bright light produced by the halogen flood light was 70.5 W m^{-2}, ambient light produced by the fluorescent tubes was 8.7 W m^{-2} and darkness readings were < 0.005 W m^{-2}. Darkness conditions were achieved by covering the test chamber with black plastic acrylic sheet. Trials were carried out under ambient water temperature (14.0 ± 0.2 °C) and fish size during the trial was 172.8 ± 10.3 g (weight) and 22.3 ± 0.4 cm (length).

3.2.5 Calculations and statistical analysis

Challenge comparisons were carried out using ANOVA tests after confirming homogeneity of variance using Levene's test, and normality of data using the Ryan-Joiner tests (Minitab, Version 16.1.0.). Regression analysis confirming linearity between current speed / louse dose and copepodid attachment success in the challenge trials was carried out using linear regression (Minitab, Version 16.1.0.).

The experiment was carried out in accordance with the Animal (Scientific Procedures) Act 1986 UK under the approval of the local ethical committee.

3.3. **Results**

3.3.1 Experimental challenge trials

3.3.1.1 Flow rate challenge trial

Attachment success of copepodids was seen to increase significantly with a decrease in current speed through the flume. The highest attachment of sea lice to the salmon host was seen in a static environment, followed by the minimum stable flow (current speed 2.4 cm s^{-1}) through the flume. Lowest louse attachment was observed at the highest current speed tested (32.6 cm s^{-1}) (Figure 4). Regression analysis comparing flow rates and attached copepodids gave a significant linear relationship between flow rate and attachment success ($p = 0.042$, $R^2 = 0.729$).

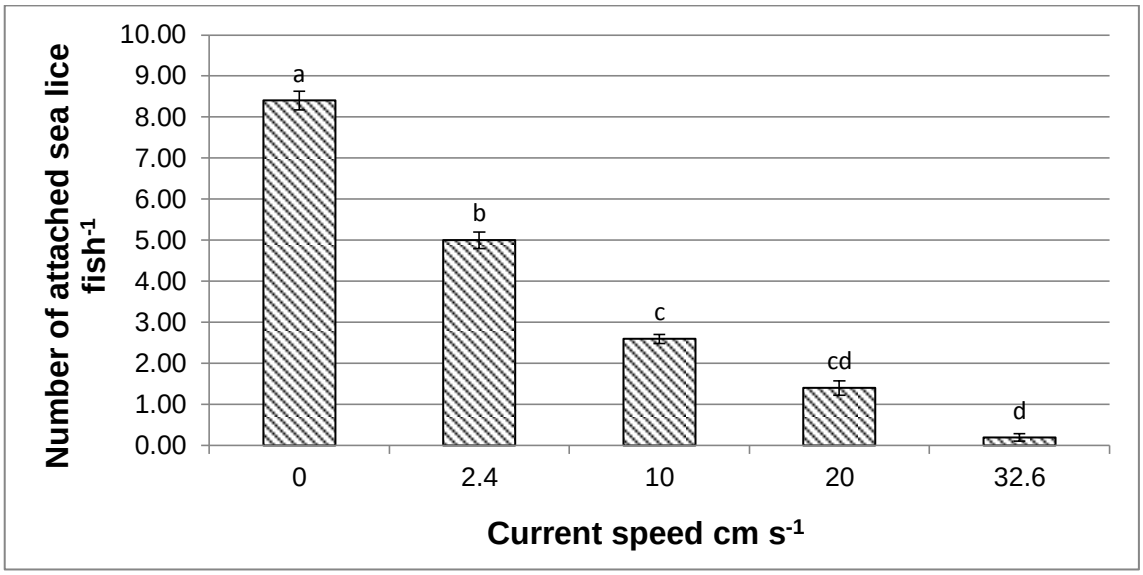


Figure 3.4. Louse attachment success at varying pre-set current speeds. Louse dose 2500 copepodids $fish^{-1}$. Data expressed as mean ± SEM (n = 5); significant differences ($p < 0.05$) are denoted by differing letters over the bars.

3.3.1.2 Louse dose challenge trial

Based on the results obtained from the flow rate challenge trial, the flow rate through the flume was adjusted to the minimum flow speed of 2.4 cm s^{-1}. It was observed, not unexpectedly, that higher sea louse doses led to higher infection of the fish (Figure 5). The percentage attachment success for all doses, however, was similar, and was found, for louse doses of 0, 1000, 2500, 5000 and 7500 lice $fish^{-1}$, to be 0.0%, 0.0%, 0.2%, 0.1% and 0.1% respectively. An optimal challenge dose of 2500 copepodids (0.2 % attachment success) per fish was used for all subsequent trials, this providing sufficient louse attachment for statistically valid comparisons (mean louse infection of 5.0 ± 0.2 lice $fish^{-1}$). Regression analysis comparing louse dose and attachment success demonstrated a significant linear relationship ($p = 0.010$, R^2 value = 0.895).

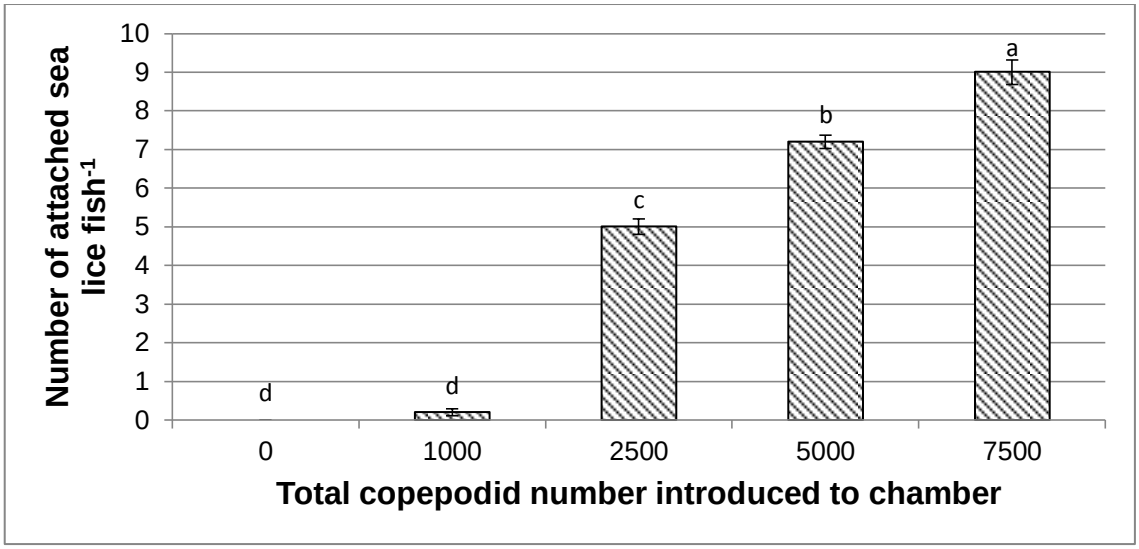


Figure 3.5. Louse attachment success for different total copepodid challenge numbers. Fixed current speed of 2.4 cm s^{-1}. Data expressed as mean ± SEM (n = 5); significant differences ($p < 0.05$) are denoted by differing letters over the bars.

3.3.1.3 Copepodid cohort challenge

Post-moult age of a single cohort significantly affected attachment success. Newly moulted copepodids (0 days post-moult - DPM) showed significantly lower attachment success than older copepodids at 2 and 4 DPM ($p < 0.05$). A peak attachment rate was reached with 4 DPH copepodids followed by a significant ($p < 0.05$) drop in attachment success when using 6 DPH copepodids (Figure 6).

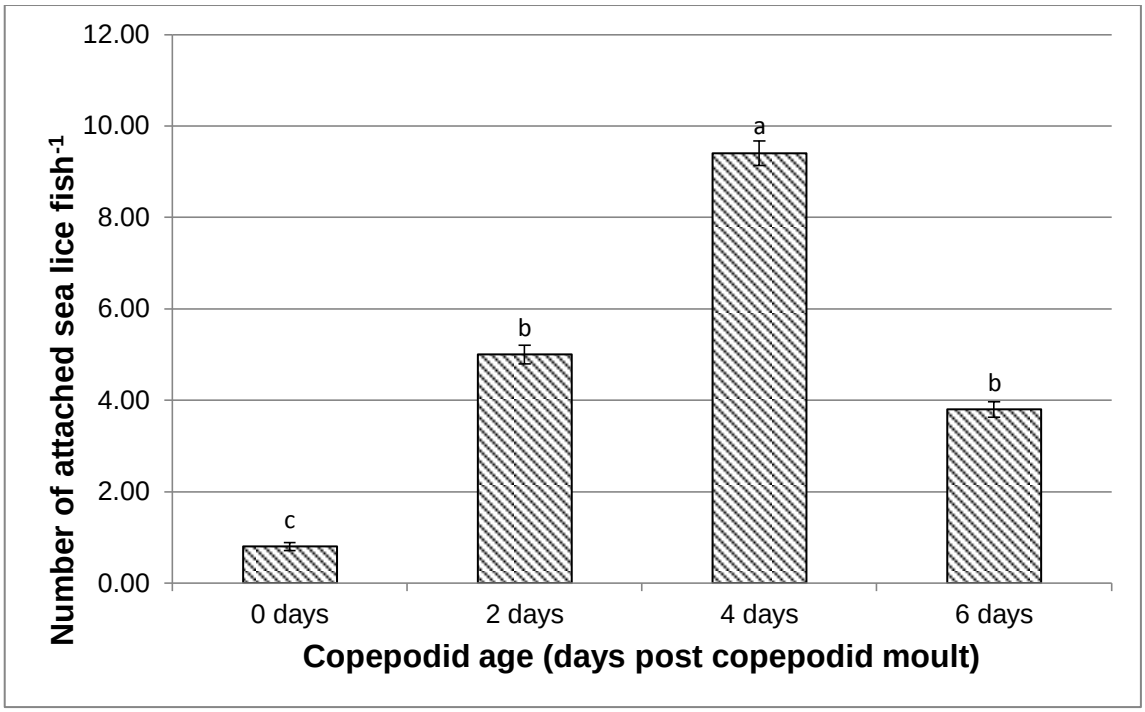


Figure 3.6. Infection by a single cohort of copepodids followed over 6 days. Copepodid age is given as days post moulting into the copepodid stage (water temperature 14.0 ± 0.2 °C). Data expressed as mean ± SEM (n = 5); significant differences ($p < 0.05$) are denoted by differing letters over the bars.

3.3.1.4 Light trials

Overall, irrespective of the white light source, sea louse attachment success was significantly positively correlated to increasing white light intensities. A 20× higher attachment success was seen under bright white light conditions (70.5 W m^{-2}) compared to darkness (< 0.005 W m^{-2}) ($p < 0.05$). An 8× higher attachment success was seen under ambient white light conditions (8.7 W m-2) compared to darkness (< 0.005 W m^{-2}) ($p < 0.05$). The attachment success was 0.5 % for bright white light, 0.2 % for ambient white light and 0.0 % in darkness (Figure 8). The enhanced attachment success under increasing light intensities was confirmed for all narrow bandwidth light tested (Figure 8).

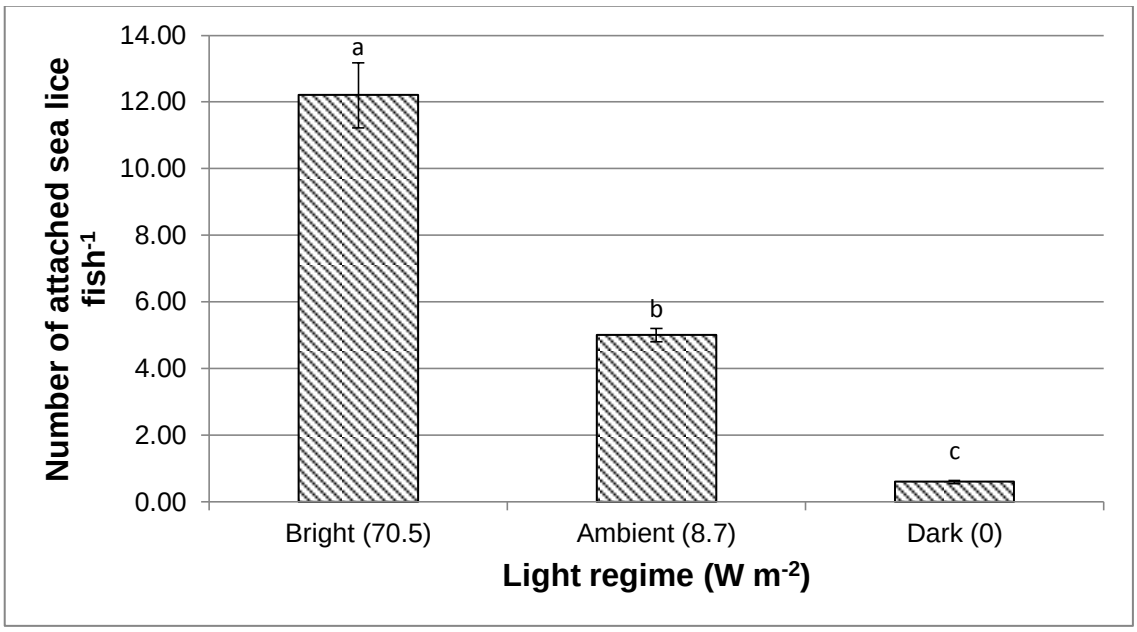


Figure 3.7. Copepodid attachment success under varying white light intensities. Fixed current speed 2.4 cm s^{-1}, Louse dose 2500 copepodids fish-^{1}. Data expressed as mean ± SEM (n = 5); significant differences ($p < 0.05$) are denoted by differing letters over the bars.

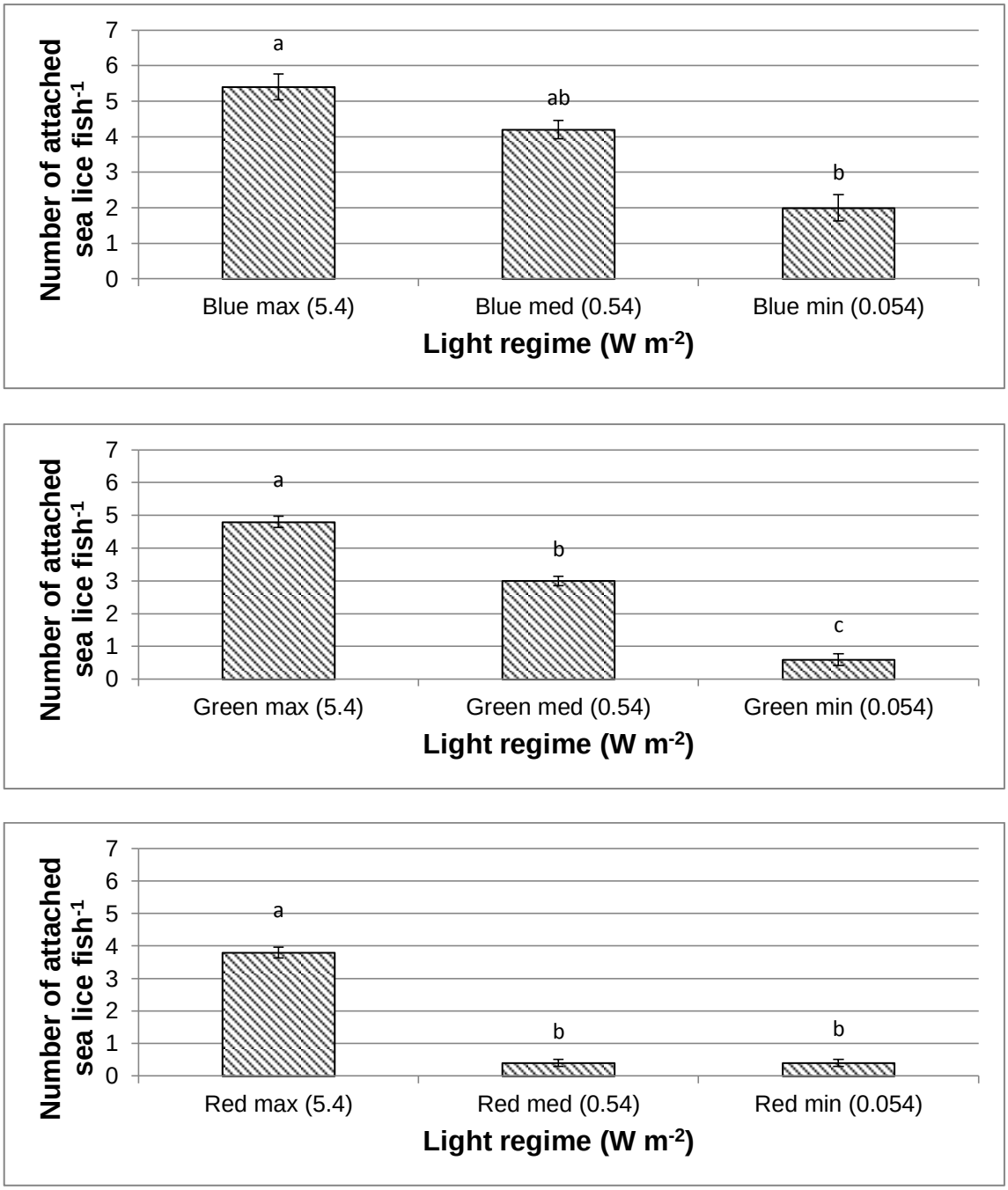
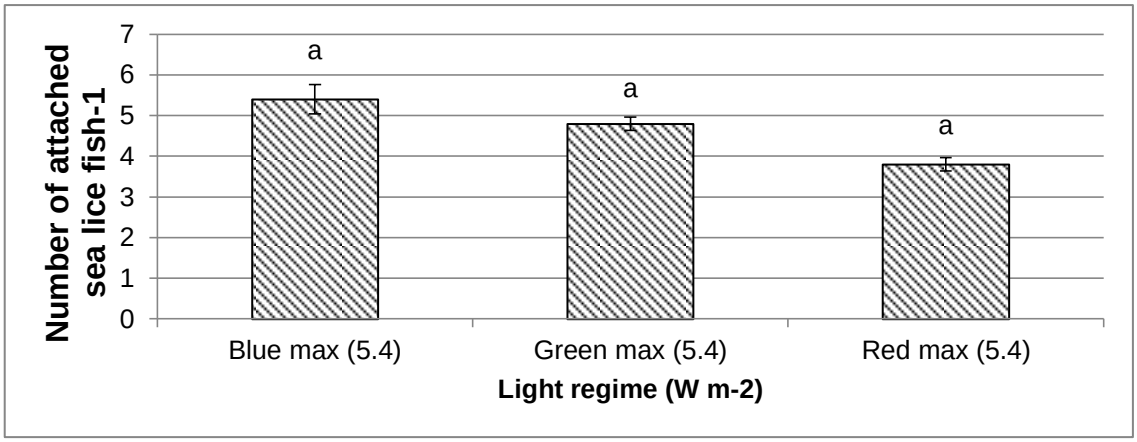
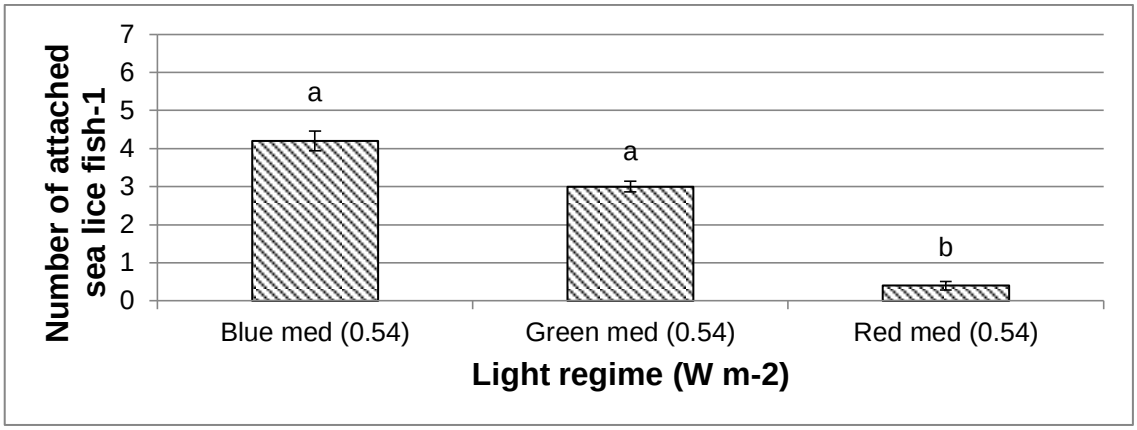


Figure 3.8a-c. Copepodid attachment success under varying light conditions comparing blue, green and red LED lights at 3 different intensities (maximum intensity 5.4 W m^{-2}, medium intensity 0.54 W m^{-2} and minimum intensity 0.054 W m^{-2}). Fixed current speed of 2.4 cm s^{-1}; dose 2500 copepodids $fish^{-1}$. Data expressed as mean ± SEM (n = 5); significant differences ($p < 0.05$) are denoted by differing letters over the bars.

No significant differences were found in attachment rate between wavelengths at a high light intensity (5.4 W m^{-2}) (Figure 9a). Under lower light intensities (medium intensity, 0.54 W m^{-2}), however, attachment rate was significantly lower in the red treatment (0.4 ± 0.1 lice / fish; all $p < 0.05$, Figure 9b) compared to green and blue treatments (3.0 ± 0.1 lice / fish and 4.2 ± 0.3 lice / fish. respectively, Fig. 9b). At the lowest tested intensity (0.054 W m^{-2}), no significant differences were found, although attachment rate under blue light appeared to be higher than in the green and red treatments.





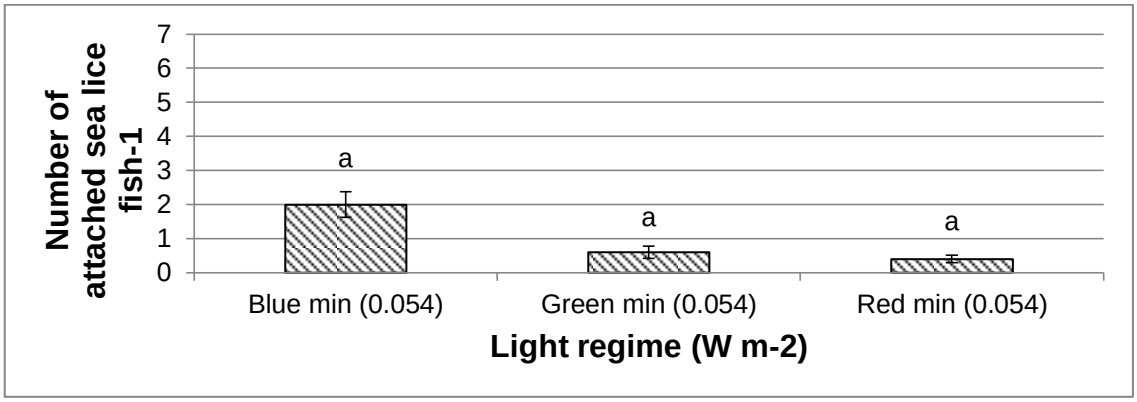


Figure 3.9a-c. Louse attachment success under varying light conditions comparing blue, green and red LED lights to each other. The three graphs show the tested intensities (maximum intensity: 5.4 W m^{-2}, medium intensity: 0.54 W m^{-2} and minimum intensity: 0.054 W m^{-2}). Fixed current speed 2.4 cm s^{-1}; dose 2500 copepodids $fish^{-1}$. Data expressed as mean ± SEM (n = 5); significant differences ($p < 0.05$) are denoted by differing letters over the bars.

3.4. **Discussion**

The use of a dedicated flume system proved a productive approach to studying the effects of a range of parameters upon copepodid infection success on Atlantic salmon hosts. Across all trials, the proportion of copepodids successfully attaching to salmon hosts was shown to be very low, being 0.2 % at best. The conditions promoting highest louse infection levels in this study were: high parasite dose, low current speed, high light intensity with a blue spectrum. The age of the copepodid cohort also impacted on the numbers successfully attaching to the host.

Copepodid dose (~ density in the water column) clearly affects the level of infection. More importantly, however, attachment success as a proportion of infective dose was observed to be strongly influenced by current speed. Sea louse attachment was negatively correlated with current speed, agreeing with the study of Genna et al. (2005), who found maximal settlement at the lowest tested host velocity of 0.2 cm s^{-1}. Initial copepodid attachment to the host is achieved through grappling using the paired hook-like antennae (Bron et al., 1993a). The negative correlation of attachment success with current speed is likely to reflect the capacity of the settling copepodid to remain attached to the fish. This is likely to be due to less protection offered by the reduced-flow boundary layer around the fish at higher water velocities. Lower current conditions will increasingly protect the newly attached copepodids from the current, reducing drag and thus increasing attachment success (Bron et al., 1991). Current speed can also affect the time of exposure of the fish host to the infective copepodid, particularly in the highly linear flow conditions created by the flume. Under field conditions, however, host infection will largely follow from

interception of a passing host in response to detection of vibrations by the copepodid (Bron et al., 1993a; Heuch et al., 1995). Under these conditions, infection will tend to occur at a point rather than along a transect, hence minimising the effects of exposure time.

Another factor that has been suggested to impact on attachment success is the age of copepodids (Tucker, 1998) since the parasites take time to mature to maximal infectivity and have finite energy reserves that give them a short window for infection to occur before they die. In this trial, a single louse cohort was observed to initially increase attachment success up to 4 days post-moulting, before infection tailed off, which might have been due to or a combination of mortality, energy depletion or inactivity, as observed in the incubators in the louse farm (personal observation). Unfortunately the exact cause was not determined. The highest infectivity was found at 4 days post hatch. These results confirm previously published data indicating that freshly moulted copepodids are not maximally infective and require 1-2 days to achieve peak infectivity (Tucker, 1998), with copepodid infectivity lasting only for a short time window (maximum infectivity after 4 days at 12 °C (Wootten el al., 1982; Gravil, 1996). Based on findings in this trial, it is suggested, that future infection trials need to take copepodid age post moult into consideration. This time span is rarely reported in past research but seems to play an underestimated and important role for copepodid infection success. For other parts of this study copepodids at 3 days post moult had to be used, due to spatial and practical shortcomings in the trial set-up.

Copepodids use a range of mechanosensory, chemosensory and visual cues to attach to the salmon host. While photoreception is known to have a significant

impact on louse behaviour (Bron et al., 1993a; Heuch et al., 1995; Flamarique et al., 2000), the effects of light upon copepodid infection success has been largely overlooked. The lensed dorsal ocelli of copepodids are relatively sensitive to light, displaying a pigmented cup and reflective tapetum, which allows accurate location of a light source or detection of shadow (Bron et al., 1991). This may also allow detection and response to polarised light (Bron et al., 1998). An unlensed ventral ocellus and extraoptic photoreceptors provide additional photoreceptive capacity (Bron et al., 1998), making lice highly likely to employ light cues at various stages in their life-cycle. In the present study, the light regime to which both the salmon host and lice were exposed, including intensity and wavelength, significantly impacted on attachment success. In all trials, louse attachment was clearly increased when light intensity was elevated. Louse attachment under conditions of complete darkness did occur, but at a much lower level than under brighter light conditions (20-fold decrease compared to bright white light). These findings do not support previously published results where intermediate (300 lux ~ 2.37 W m^{-2}) light levels were observed to produce higher settlement of copepodids on Atlantic salmon smolts than high (800 lux ~ 6.32 W m^{-2}) or low (10 lux ~ 0.8 W m^{-2}) levels (Genna et al., 2005). However, the differences in light intensity (high 70.5 W m^{-2} and low 8.7 W m^{-2}) in this study have been much higher than in the comparable study by Genna et al. (2005).

The present study clearly showed the impact of light wavelength on louse attachment success. Greater levels of infection were apparent when fish and lice were exposed to blue light, this being followed by green and then red light. Similar to the case for white light, a positive correlation was found between

attachment success of copepodids and increasing light intensities for all wavelengths tested. The differences in attachment success between light spectra could potentially be caused by behavioural differences of the host and / or the parasites due to the light conditions. Only empirical observations of host behaviour were carried out in the present study and therefore this factor cannot be ruled out as in the results obtained until tested further. The high attachment rate seen at shorter wavelength light (blue-green) may reflect the maximum absorbance wavelengths observed for sea louse photo pigments and in turn their greater phototactic response at these wavelengths (Bron, 1993). These responses have been suggested (Bron & Sommerville, 1998) to show adaptation to the transmission properties of the principal marine environments inhabited by sea lice and their hosts. In the open ocean, blue wavelengths (470nm) penetrate deeper through the water layer, while in coastal areas particles in suspension and substances originated from the decay of organic matter affect water transparency and spectral absorbance shifting the ambient spectral profile to longer wavelengths (500 – 550 nm) (Dartnall, 1975; Jerlov, 1976). Also, the findings from the current study might suggest, that sea lice are possibly equipped for host detection at deeper water depths, but this effect will be overruled within the comparably shallow water bodies used for Atlantic salmon farming in Scotland. In can thus be considered, that within shallower water bodies, sea louse larvae might depend more frequently on salinity changes and visual contrasts, rather than detection within the blue/green spectrum.

In all the trials described in this chapter, a high density of infectious parasites (2500 copepodids) was used to infect a single fish, under a highly linear flow

regime and with the fish confined to a small space (3.2 litres of water). All the copepodids in the present study were constrained by the flume design to pass close to the fish host. Attachment success observed in the present study is therefore likely to represent a maximum value not reflective of field infection success. Swimming speed of fish under farmed and wild conditions, including burst-swimming, coupled with ambient current flows, mean that the water velocities tested here are likely to be exceeded in the field and will thus further reduce attachment success. In addition, this study observed numbers of copepodids still attached, however insecurely, immediately after each trial was completed, whereas under normal circumstances a proportion of these would be lost before moulting to the filament-attached chalimus stage. The findings of this study corroborate anecdotal / unpublished observations concerning effects of environmental variables upon salmon farm louse settlement levels and can help to suggest or support new approaches for on-farm louse management. It is generally, though not universally, observed, that farm sites with high current flows have lower louse infection levels and that sites with particularly low flows have higher louse numbers. Also, light has been recognised to play a role in louse infections, at least insofar as the infective stages are most often found in the top 4 m of the water column (Johannessen, 1978; Hevrøy et al., 2003). This thesis further aims to exploit the use of artificial lighting and / or feeding to affect distribution of salmon in cages in next chapter 4. During daytime lighting conditions (full daylight > 30,000 lux, ~ 237 W m^{-2}) both copepodids and salmon may be found near the surface, particularly when salmon are fed continuously throughout the day with surface feed-spreaders.

In summary, the influence of environmental parameters, such as current speed, light conditions, and louse specific conditions, such as copepodid density and age, are shown to affect attachment success to Atlantic salmon hosts. Through the manipulation and control of the light regime within pens, current dynamics around salmon production sites and louse influx minimisation, unfavourable conditions for louse attachment, as outlined in this chapter, might be employed as a non-invasive method to reduce sea louse infection.

3.5. **Acknowledgements**

The authors would like to thank all the staff at the Marine Environmental Research Laboratory at Machrihanish. Special thanks go to Sally Boyd for invaluable support, feedback and help with the performed sampling and organisation of the trials. Many thanks are also due to Professor Anton Edwards for his comments and calculations concerning louse exposure time.

CHAPTER 4: Manipulation of farmed Atlantic salmon swimming behaviour through the adjustment of lighting and feeding regimes as a tool for salmon lice control

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4.1. **Introduction**

Sea lice are among the most economically costly parasites of marine farmed salmonids (Costello, 2009a). The annual cost of two sea lice species, *L. salmonis* and *C. elongatus* , including harvest losses and therapeutant costs, has been estimated at €300 million globally, which is equivalent to 0.1 to 0.2 € kg^{-1} fish produced or 6-10 % of the total production value (Rae, 2002; Costello, 2009a). Sea louse control is therefore critical if productivity is to be maximised. To date, use of veterinary drugs remains a key component of integrated control strategies (Rae, 2002). This is problematic as, for instance, the UK is restricted in the number of licensed anti-sea lice medicines available and the few therapeutants available are largely becoming less effective due to development of drug resistance by the parasite (Shinn & Bron, 2012). The current study tested an alternative control strategy which relies upon manipulation of fish swimming depth.

The life-cycle of salmon lice consists of eight host-associated stages, and two free swimming nauplius stages (Pike, 1989; Heuch *et al*., 1995). It has been suggested that sea lice larvae remain within the first four metres of the water surface by performing short swimming bursts (Johannessen, 1978; Heuch *et al*., 1995; Hevrøy *et al*., 2003; Murray & Gillibrand, 2006, chapter 2). The upward swimming behaviour of lice larvae counters their negative buoyancy; however, copepodids do seem to show diurnal vertical migration (Heuch *et al*., 1995; Aarseth & Schram, 1999). The principal cue employed to make contact with swimming fish is the vibration of passing hosts, detected using an array of mechanoreceptors (Bron *et al.,* 1993a; Heuch *et al.,* 2007). Additionally, *L. salmonis* may also uses phototactic cues, such as shadow and potentially light

reflection from the scales of host fish, to colonise the host (Bron *et al*., 1993a; Bron & Sommerville, 1998; Genna *et al.,* 2005, chapter 3).

In an earlier small scale trial, infection rate was observed to increase in fish swimming at shallow depths compared to deeper water (Hevrøy *et al*., 2003). Similarly, another study showed that salmon kept in cages with deep net pens (20 m depth) had lower louse infection than salmon kept in shallow pens (6 m; Huse & Holm, 1993). This depth preference of the sea louse larvae may therefore provide an opportunity for sea lice control on salmon farms through the manipulation of salmon swimming behaviour and depth (Oppedal *et al.,* 2011).

Salmon swimming behaviour is mainly dictated by environmental factors such as seasonal and daily changes in lighting conditions, temperature, salinity and oxygen, as well as by the mode of feeding employed in a commercial setting (Oppedal *et al.,* 2011). Salmon are positively phototactic and therefore they seek out light sources in order to display their preferred schooling swimming behaviour (Juell *et al*., 2003; Juell & Fosseidengen, 2004; Oppedal *et al*., 2007; Dempster *et al*., 2009). Naturally, salmon follow a diel swimming rhythm, following ambient light patterns with migration downwards in the water column at dawn and return to surface waters at dusk and through the night (Juell & Westerberg, 1993; Bjordal *et al*., 1993; Fernö *et al*., 1995; Oppedal *et al*., 2001; Juell & Fosseidengen, 2004). Photoperiod regimes acting through the use of high intensity submerged lights, which are routinely used to suppress early sexual maturation in Atlantic salmon during the on-growing phase, impact directly on fish swimming behaviour, with schooling at night around the submerged light units (Oppedal *et al*., 2007). Strategic deployment of

submerged lights can therefore be employed to attract fish to specific water layers (Juell *et al*., 2003; Juell & Fosseidengen, 2004; Oppedal *et al*., 2007).

Commercially reared salmon are normally fed a pellet diet through surface spreading and the fish respond by changing swimming speed and direction, showing horizontal and vertical scattering towards the pellets (Ang & Petrell, 1998). The fish will remain up in the water column in the feeding corridor until satiated (Juell *et al*., 1994; Fernö *et al*., 1995; Ang & Petrell, 1998). Appetite and feeding are the strongest behavioural cues in fish with regards to swimming behaviour and they usually override any sub-optimal conditions, whether environmentally or artificially induced (*e.g*. phototaxis or water temperature) (Oppedal *et al*., 2007).

In the current study, the effects of submerged artificial lighting (placed at 10 m depth) in combination with submerged feeding (delivering feed at 5 m depth) were tested to examine elective salmon swimming depth and associated sea louse infection. The submerged lighting was installed to attract fish to deeper water levels during night time and the submerged feeding was installed to attract salmon away from a surface feeding corridor below the nominal principal infective louse layer. The hypothesis being tested was that sea lice infection in a commercial salmon population could be reduced by exposure to deep lighting, and further decreased by deep lighting and deep feeding. This is based on two assumptions suggested by previous studies, firstly that infective sea louse copepodid larvae remain in the water surface layer and secondly that deep lighting and feeding can be employed to attract salmon to deeper water depths.

4.2. **Materials and Methods**

4.2.1 Fish stock and farm set up

Eggs were produced and incubated by Landcatch Natural Selection (Hendrix Genetics) until transferred at the eyed stage to the Inchmore Marine Harvest Hatchery. On 5th April 2011, smolts (75.9 ± 7.6 g) were transferred into seawater at Marine Harvest Ardentoul salmon farm. Fish were on-grown according to current industry standards. On the 9th December 2011, fish were transferred to Marine Harvest Duich salmon farm (57° 14' 55.93" N, 5° 29' 57.24" W) and stocked into cages with a mean stocking density of 5.35 ± 1.23 kg / m^3. The health status of the fish stock was monitored as per Marine Harvest standard protocol. No evidence of disease was reported in the stock prior to the experiment.

4.2.2 Farm set up

The mean water depth at the Loch Duich salmon farm is 30 m and the farm has 12 circular cages (100 m circumference circular Polarcirkel™ cages, Akva) in two separate cage groups of 6 pens each with nets (Nylon, 29 mm, MøreNot) of a working depth of 16 m. All pens are normally equipped with surface spreading feeders (Rotor spreaders CF90, Akva) connected with feed pipes to an automated feed control unit and storage system at the "SEA-CAP" feed barge. Daily feeding to satiation was carried out according to standard commercial practice and guidelines.

All cages were exposed to constant light from January – April 2012 using four 400 W Metal halide submersible lights (BGB Engineering Ltd.) per pen. The

lights were deployed evenly across the pen and held at 4 and 8 m depths, in order to prevent maturation according to standard industry practice for Atlantic salmon. Water temperature during seawater grow-out ranged between 4 to 12 °C, due to seasonality. Fish were fed to satiation using a standard commercial diet (Biomar ELR 12 mm 16PF).

4.2.3 Experimental set up

On the 23rd March 2012, the lights for the experimental pens (6 pens with a mean of 45,078 ± 1,165 fish pen^{-1}) were adjusted from the standard light depths (at 4 and 8 m) to the experimental light depths.

Two pens were equipped with 4 lights each at 1.5 m depth. Four pens were equipped with 4 lights placed at 10 metres depth (Figure 1). The light intensity was measured on the 7th of June during night-time using a single channel light sensor (Skye instruments, Powys, UK) in the fully stocked experimental pens. Light intensity was measured at 1 m intervals horizontally and vertically across the pens along the maximum (directly across light sources) and minimum (centrally between light sources) intensity planes with fish present. This gave indication of average illumination per depth as an average over the whole plane of the pen at a given depth. This was performed following the assumption, that fish will typically deploy a doughnut shaped swimming pattern in a single plane in the absence of feeding (Stien, 2013, Pers. Comm.).

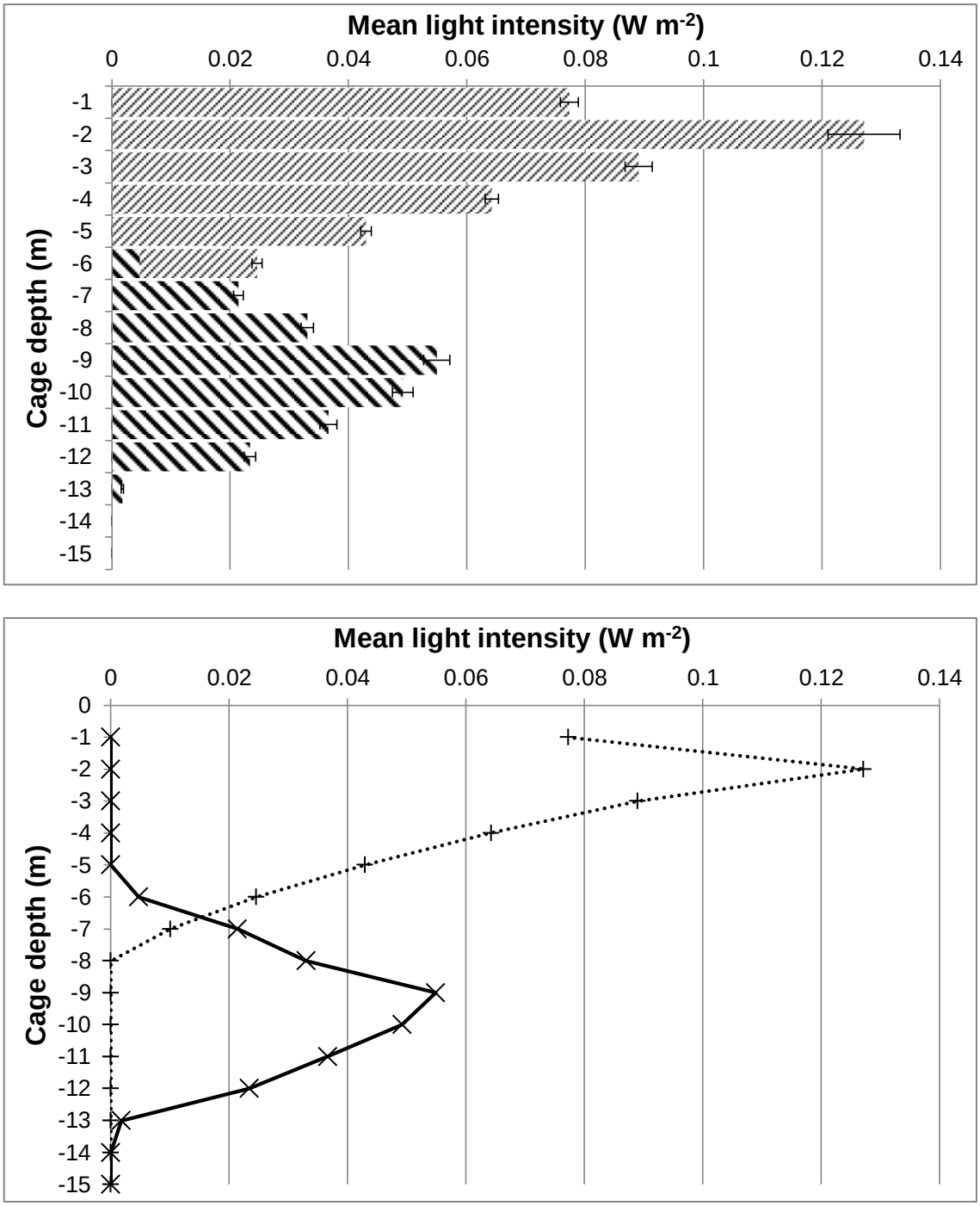

Figure 4.1 a & b. Mean light intensity (W m^{-2}) at different depths during night time (7th June 2012) for the experimental lighting regimes with fish present. Narrow hatching & dotted line: pens with shallow lights (1.5 metres depth); wide hatching & solid line: pens with deep lights (10 metres depth). Graphs show mean ± SE

Prior to the start of the trial, fish were treated against sea lice with a certified standard delousing agent, Alphamax™, to ensure that sea louse numbers at the farm were at a low and uniform baseline level (< 1.0 louse / fish). The fish were treated using a tarpaulin bath treatment at a dose aimed to achieve 2 mg deltamethrin per litre of sea water. The effectiveness of the treatment was measured by comparing pre and post treatment louse numbers (treatment successful at a reduction of > 90% of all lice). Fish were acclimated to the rearing conditions and on 17th May, two out of the 4 pens equipped with lights at 10 m depth were further equipped with submerged feeders (LiftUp®, Akva AS). The submerged feeders were designed to deliver the same quantities of feed as the surface spreaders to a water depth of 5 m. Overall, the experiment included 3 replicate treatments consisting of surface feeding / surface light (SS), surface feeding / deep light (SD) and deep feeding / deep light (DD). Fish were exposed to the experimental treatments until 13th July. On the 15th May and 11th June, fish were treated against sea lice using a commercial dose of Alphamax™ (achieve uniform baseline level < 1.0 louse / fish). Due to an early harvest following the standard commercial harvest plan, one of the surface light/surface feeding (SS) treatment pens had to be subsequently excluded from the trial. Fish in the deep light / deep feeding pens were passively graded (top crop) on the 3-4th June due to commercial demand.

Sea lice stages and numbers were recorded at least two times per week in random pens and at three sampling points (28th May, 20th June and 9th July, with trial “period 1”, “period 2” and “period 3” covering the period prior to each sampling point respectively). On each of these dates a random sample of salmon was caught in a box net (Marine Harvest, box dimensions 4×6×10 m,

catch volume/sample 240 m^3) enabling vertical sampling of the whole water column in a cage. Thirty anaesthetised (MS222, 50 ppm) fish per cage were checked for length, weight and sea louse numbers and stages. The lice were staged according to the life-cycle stages described by Kabata (1979). For the comparison of louse numbers on the fish, only ”juvenile” *L. salmonis* stages, *i.e.* copepodids, chalimus I-IV, pre-adults I & II, were considered so as to include only infections having occurred during the intervals between samples. Due to commercial operations on the sampling day, lice numbers for one replicate DD pen were not established correctly for sampling period 1.

The feeding rates in the pens were kept stable and followed the standard, projected, commercial grow-out schedule used by Marine Harvest Scotland. Submerged cameras (Akva Group) were used to monitor feeding response in the experimental cages. During daylight hours, fish in the surface feeding treatment were fed continuously throughout the day. In the deep feeding treatments, feed was delivered in batches, twice a day, for practical reasons.

Water temperature was measured daily (OCEA Weather sensor station) and rose steadily throughout the experiment (Figure 2). Oxygen was monitored daily (Portable Meter, ProfiLine Oxi 3205) and remained stable throughout the experiment and throughout the water column (87.6 - 100.3 % , 7.6 – 8.6 mg l^{-1} O_2). Visibility was measured by daily Secchi disk readings, dropping to 3 m after prolonged rainwater influx due to freshwater and / or run-off build-up in the loch and rising to 11 m during periods of good weather (Figure 2).

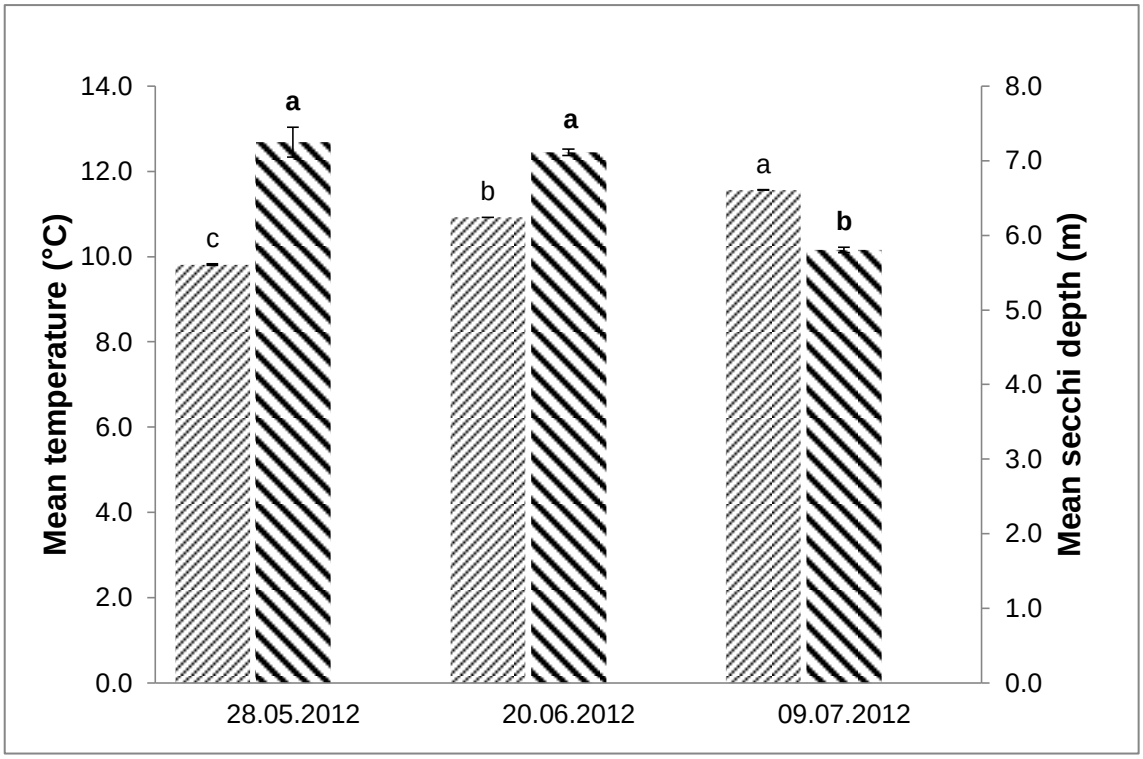

Figure 4.2. Water quality parameters during the three experimental periods prior to the louse sampling points shown; Graph showing mean water temperature (°C) (at 5 metres water depth) (narrow hatching) and mean Secchi visibility depth as a measure of water turbidity (wide hatching). Graphs show mean ± SE

The current follows a longitudinal pattern (North-West ↔ South-East) along the loch with a measured mean current speed of 5.6 ± 6.5 cm s^{-1} (Figure 3).

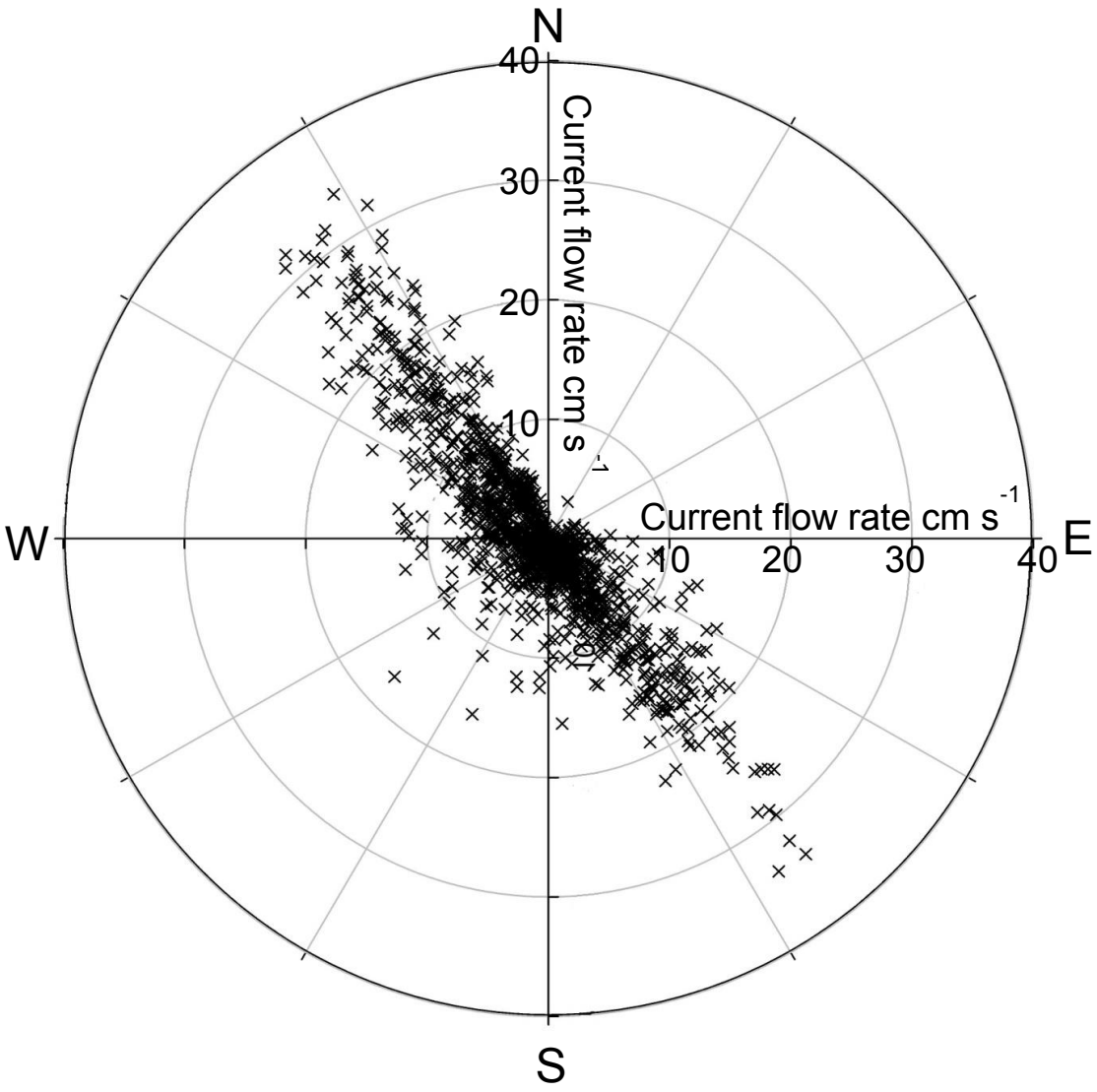

Figure 4.3. Polar plot of current speed and current direction across the experimental pens and the sampling site at Loch Duich during the trial period.

4.2.4 Echo sounder and swimming activity analyses

The vertical fish distribution was observed continuously using a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway) in the SD and DD

cages. Due to restrictions in equipment availability, the echo sounder system could not be installed in the SS pens. A full description of this system is given in Bjordal *et al.* (1993). Upward-facing transducers with a 42° acoustic beam were mounted in gimbals and positioned inside the pens at 15 m to enable recording of the top 12 m of the water column. Four transducers were deployed in total to investigate fish movements throughout the pens. The transducers were measuring upwards in a cone shape, which allowed accurate measurements of fish densities in the vertical water column above the transducer. Data were analysed following the assumption that fish not being picked up in the surface metres of the pen, will, therefore, be at deeper water depths. The data are presented for the surface metres, as the trial was designed to determine whether fish in surface waters are more likely to be exposed to sea lice infection. This follows from the assumption that lice are predominantly found in the upper layer of the water body.

4.2.5 Calculations and statistical analysis

Comparisons of temperature and Secchi depth data during the experimental period were carried out using ANOVA tests (Minitab, version 16.1.0.). Lice prevalence comparisons were carried out using Fisher's exact test (Minitab, version 16.1.0.). Fish weight comparisons and stocking densities were carried out using ANOVAs (Minitab, version 16.1.0.). The lice data were analysed using a quasi-Poisson generalised linear model for each experimental period, with data being corrected for fish size (R version 2.15.0 of the R Foundation for Statistical Computing, function "glm"). This was performed due to the assumption, that larger fish with bigger surface areas harbour higher louse

numbers. The effect of fish size on louse density was examined using permutational multivariate analysis of variance (Permanova software, Department of Statistics, University of Auckland, 2005; Anderson, 2001; McArdle & Anderson, 2001). The Permanova software was used in order to correct for high 0 values with respect to louse numbers on a majority of fish, being more robust than comparable non-parametric tests.

From analysis of the echo sounder data, a total echo intensity response of ~100,000 (exact value depending upon specific cage) indicated that 100 % of all the fish in the respective cage were positioned above the transducer. This was used to estimate percentage fish in the surface 4 meters during different periods for the SD and DD cages, and the non-normal distributed percentage-values compared using Wilcoxon rank sum test, following relevant Bonferroni corrections.

The experiment was carried out in accordance with the Animal (Scientific Procedures) Act 1986 UK under the approval of the local ethical committee.

4.3. **Results**

4.3.1 Fish performance

As this was a commercial site, stocking densities ranged from 12 to 15 kg m^{-3} with no significant differences between treatments ($p = 0.541$). Salmon growth and biological FCR (bFCR) over the trial period were comparable across all treatments (bFCR = 1.28 ± 0.02; $p = 0.988$). Mean fish weights ranged from 3.7 to 4.3 kg at the start of the trial with the SS group having a significantly higher mean weight than the other two groups SD and DD ($p < 0.001$). At trial end mean fish weights ranged from 3.9 to 4.5 kg, with SD group fish being larger

than the SS and DD groups ($p < 0.001$). Mortality was low (< 1%) throughout the experimental period and did not differ across pens ($p = 0.158$).

4.3.2 Fish swimming depth

Comparisons between day-time and night-time densities are presented in Figure 4: For both SD and DD groups, fish density in the surface waters was significantly higher during day-time than night-time (all $p < 0.01$), indicating a shift in fish density to deeper water layers during the night. No shift in fish density to deeper water levels was found comparing the SD and DD treatments during day-time,

Day-time fish density in period 1 was found to be significantly higher (SD $p < 0.01$; DD $p < 0.01$). than in period 2 and 3 (Figure 4).

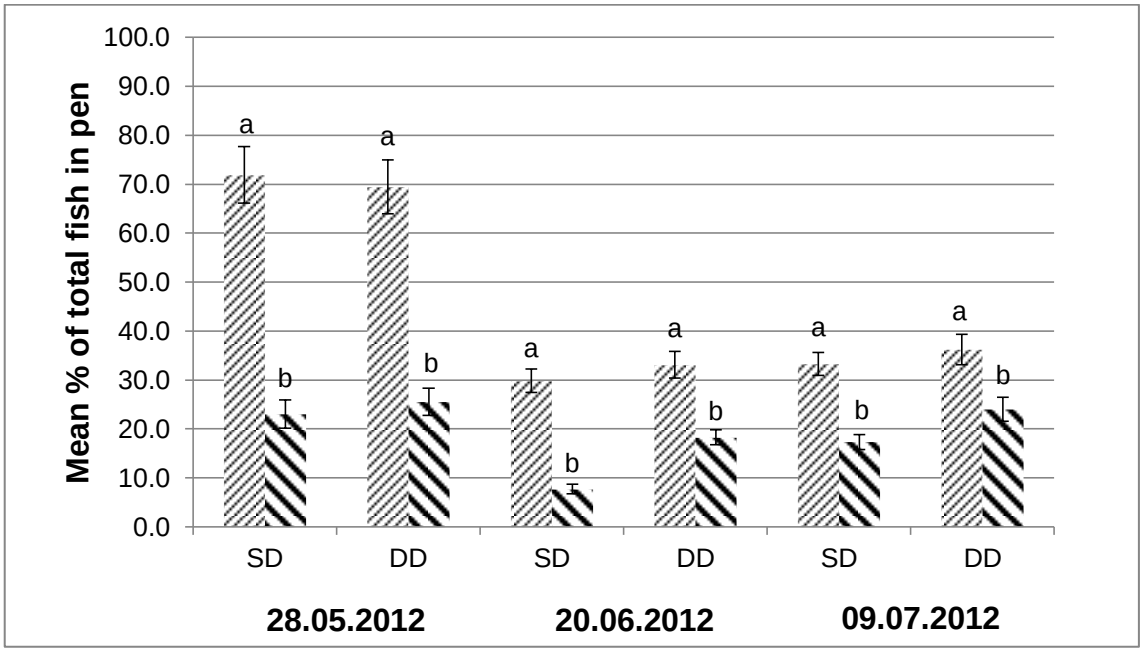

Figure 4.4. Mean % of fish density ± SEM in the surface 4 metres of the water layer derived from echo intensity. Data are presented as day-time fish density averages (narrow hatching) and night-time density averages (wide hatching) for the SD and DD treatment groups for the three experimental periods prior to the lice sampling time points shown.

4.3.3 Sea lice infection

Fish were tested for possible size effects on louse infection (weight and length), but no significant effects were found for any group at any of the three samplings (all $p > 0.05$). Prevalence of lice on the fish was generally high (67.0 ± 2.5 %) with numbers of lice on single fish ranging from 0 - 63 (Table 1).

Table 4.1. Mean fish size and sea louse infection data (copepodid, chalimus 1-4, preadult 1 & 2) for salmon at the three sampling points of the three experimental treatments (SS, SD, DD). Data are expressed as mean ± SEM (n=2 pens, 30 fish sampled/pen/date). DD pens were passively graded (top crop) on the 3-4th June.

Date/Treatment	Fish parameters		Sea louse parameters		
	Mean length (cm)	*Mean weight (g)*	*Prevalence (%)*	*Louse abundance*	*Range*
28th May 2012					
Surface Feeding, Shallow Light (SS)	63.4 ± 0.7 *a*	3656.0 ± 127.3 *b*	89.6 ± 2.8 *a*	1.9 ± 0.2 a	0-6
Surface Feeding, Deep Light (SD)	64.5 ± 0.5 *a*	4053.9 ± 92.4 *a*	77.8 ± 6.3 *a*	2.2 ± 0.2 a	0-10
Deep Feeding, Deep Light (DD)	65.0 ± 0.8 *a*	4254.0 ± 183.8 *a*	79.9 ± 0.9 *a*	2.2 ± 0.2 a	0-14
20th June 2012					
Surface Feeding, Shallow Light (SS)	65.6 ± 0.7 *a*	4198.5 ± 138.6 *a*	58.3 ± 7.7 *a*	2.9 ± 0.1 a	0-63
Surface Feeding, Deep Light (SD)	65.0 ± 0.6 *a*	4103.5 ± 124.5 *ab*	30.0 ± 2.4 *b*	0.4 ± 0.1 b	0-4
Deep Feeding, Deep Light (DD)	64.4 ± 0.8 *a*	3789.3 ± 148.4 *b*	33.3 ± 4.7 *b*	0.5 ± 0.1 b	0-3
9th July 2012					
Surface Feeding, Shallow Light (SS)	66.7 ± 0.7 *ab*	4460.5 ± 157.1 *b*	98.3 ± 0.6 *a*	7.1 ± 0.5 a	0-18
Surface Feeding, Deep Light (SD)	68.6 ± 0.8 *a*	4922.7 ± 168.8 *a*	65.0 ± 2.9 *b*	1.1 ± 0.1 b	0-5
Deep Feeding, Deep Light (DD)	65.7 ± 0.8 *b*	3916.5 ± 163.1 *c*	81.7 ± 2.9 *b*	2.7 ± 0.6 b	0-34

For the first period (sampling date 28th May) sea lice abundance did not vary between treatments. In period 2 (20th June) as well as period 3 (9th July) a significantly higher number of lice were observed on the SS treatment group compared to all other groups (all $p < 0.05$; Figure 5).

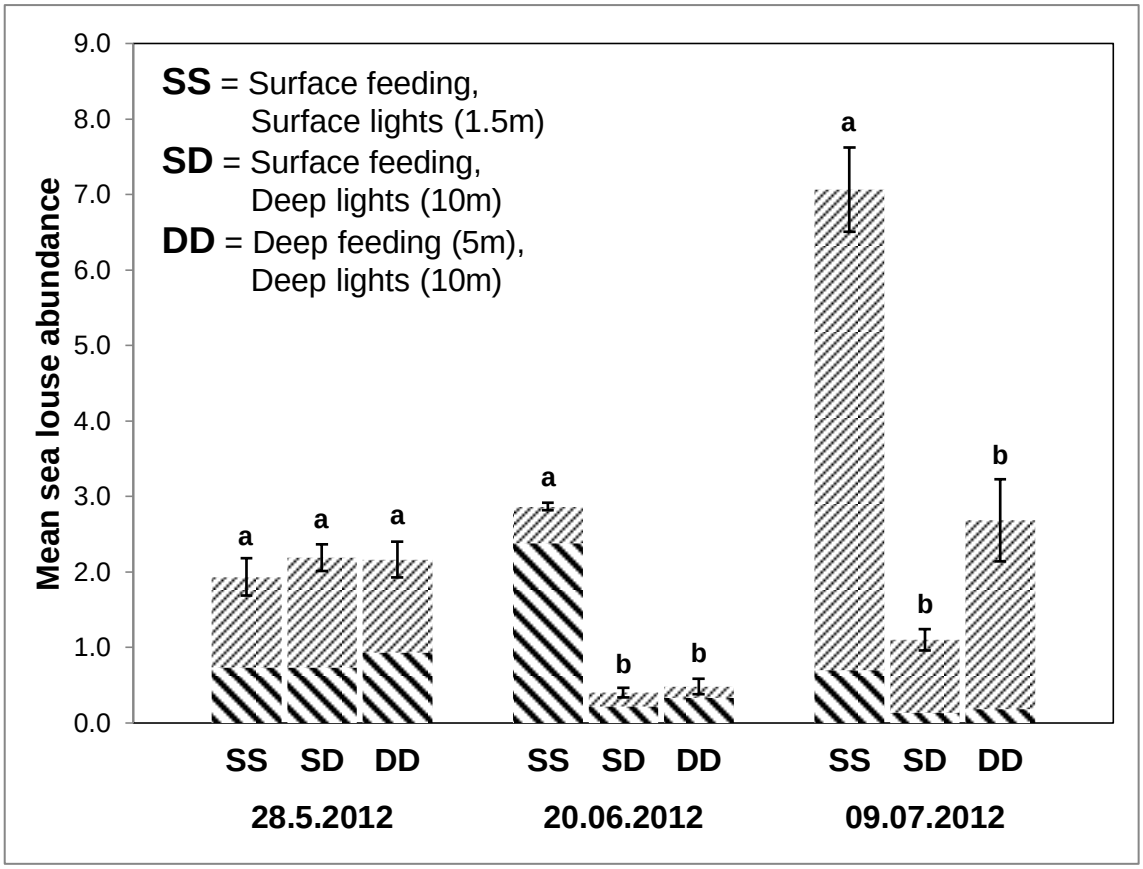

Figure 4.5. Mean sea louse abundance expressed as mean juvenile lice per fish in the three experimental treatments. Wide hatching: copepodids & chalimus 1-4; Narrow hatching: preadult 1 & 2, male and female. Data expressed as mean ± SEM (30 fish sampled/pen/date) (n=2).

4.4. **Discussion**

This trial showed that infection with sea lice was significantly reduced in pens equipped with deep (10 m) compared to shallow (1.5 m) lights. The use of combined deep lights and deep feeding provided no significant additional lice reduction in comparison to surface feeding in conjunction with deep submerged lights.

Artificial lights are routinely used in the salmon industry to suppress early maturation (Hansen *et al*., 1992, Oppedal *et al*., 1997; Porter *et al*., 1999;

Migaud *et al.,* 2010) and to boost salmon growth due to the alteration of the seasonal growth cycle (Oppedal *et al*., 2003; Nordgarden *et al*., 2003). The standard practice in Scotland is to place light units within the first 5 m of the water column. Atlantic salmon are positively phototactic and show a strong attraction to light sources (Juell *et al*., 2003; Juell & Fosseidengen, 2004; Oppedal *et al*., 2007; Dempster *et al*., 2009). Salmon seek out light sources, artificial or natural, in order to display their preferred swimming behaviour, schooling, which requires the fish to see each other and correct their swimming paths actively, and also feeding behaviour as they are visual predators (Juell *et al*., 2003; Juell & Fosseidengen, 2004; Oppedal *et al*., 2007). Previous studies suggested that initial sea lice infection occurs in the surface layers of the water body and that therefore keeping fish lower in the water column should reduce the levels of infection, although prior to this study there had been no scientific confirmation of this at a commercial scale (Bjordal *et al.,* 1993; Juell *et al*., 2003). Light is an important cue by which the copepodids navigate into and remain in waters that maximise the likelihood of encounter with a suitable host (Bron *et al*., 1993b; Heuch *et al*., 1995). Attachment success might be impaired in low light intensities, or increased by lice being more active at higher light intensities. Responses to ambient light sources, such as sunlight and moonlight are believed to increase chance encounters with suitable hosts (Heuch, 1995). Artificial lights might therefore also increase chances for attachment of the louse. Indeed, sea lice abundance differed between treatments, with fish exposed to deep submerged lights having less lice attached than fish exposed to shallow submerged lights. It can therefore be hypothesised that fish exposed to shallow submerged lights might display higher densities in the surface layer

of the water column at night-time and therefore were more likely to be exposed to infective copepodids.

The echo sounder technology employed allowed approximate fish density measurements to be obtained for the surface 4 m of the water column. The fish swam deeper in period 2 and 3 compared to period 1. Migration to the surface waters due to ambient light was observed in treatment period 1, which was potentially increased by temperature stratification in the water body, with surface waters being warmer than deeper levels. The thermotactic behaviour of salmon has been reported to, at least partially, override the phototactic swimming behaviour of salmon (Oppedal *et al*., 2011). A temperature change as little as 1°C could be detected by the fish and trigger a behavioural response (Oppedal *et al*., 2007). A slight temperature change could have contributed to the day-time surface attraction of a majority of the fish. Interestingly, no differences in fish densities were found comparing different feed delivery methods in the presence of deep lights (10 m). During submerged feeding, fish were spread out throughout the water column below the feed delivery point at 5 m depth, as expected by the spiralling feeding behaviour described for batch-feeding fish species (Huse & Holm, 1993; Fernö *et al*., 1995). The results indicate that after satiation the fish returned from the feeding corridor below 5 m depth to the surface water layers. The feeding regime (twice daily) and feed delivery at only 5 m depth in the cage might not have been enough to reduce parasite-host encounters. Critically, the differences in lice numbers caused by deep lights were apparently much higher than the reduction caused by the deep feeding. It can be speculated that the underwater feeding did lead to a certain under feeding in these two cages, resulting in more swimming towards the

surface searching for food. But, at this stage, it is still difficult to disentangle the relative effects of light and feeding on lice reduction and further trials are needed. In a further trial, continuous, reliable submerged feeding needs to be achieved in order to give the fish incentive to stay at deeper water layers throughout the feeding period (typically daytime, 8.00 – 17.00 hours). To date no reliable underwater feeder unit exists to test the hypothesis further.

The results of this trial support previous suggestions that copepodid infection of salmon would occur in the surface water layers, although further study is required to confirm and study sea lice infection zones. Results indicate that a change in swimming depth of the fish can result in a significant reduction in sea louse numbers attached to salmon. The swimming behaviour of the fish is mainly dependent on the feed delivery method, however, upon satiation, other abiotic factors such as water temperature, oxygen saturation and phototaxis influence fish swimming behaviour. Additional trials to keep fish at deeper water layers for longer periods of the day should be carried out in order to confirm the findings of this experiment and to determine if lice burden can be further reduced. From a commercial point of view, the use of submerged lights placed deeper in the water column could provide an additional tool to support the industry's integrated pest management strategy.

4.5. **Acknowledgements**

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CHAPTER 5: Triploid and diploid Atlantic salmon show similar susceptibility to infection with salmon lice Lepeophtheirus salmonis.

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5.1. **Introduction**

Increasing drug resistance in sea lice (Treasurer *et al*., 2000; Denholm *et al.,* 2002; Shinn & Bron, 2012), affect susceptibility to many available treatments such as organophosphates, pyrethroids, avermectins and topical disinfectants (Fallang *et al.,* 2004), much research has been dedicated to the development of new control methods and the understanding of the sea louse life-cycle (Revie *et al.,* 2005; Robbins *et al.,* 2010). These include management practices such as integrated pest management, which encourages synchronised fallowing and lice treatment at different farms in a particular system (Brooks, 2009). Other control strategies being investigated for marine copepod parasites include the development of new therapeutants (Skattebøl *et al.,* 2004) and vaccines (Carpio *et al.,* 2011) and the use of controls involving aspects of chemical ecology (Ingvarsdóttir *et al.,* 2002a; Ingvarsdóttir *et al.,* 2002b; Brooker *et al.,* 2013). These strategies, however, are not likely to be commercially available within the short to medium term. Encouraging results have also been recently obtained with respect to breeding programmes for genetic resistance to sea lice in commercial Atlantic salmon populations, with heritability of up to 0.3 reported (Kolstad *et al.,* 2005; Glover *et al.,* 2005). Differences in susceptibility to sea lice infection and abundance of sea lice on wild and farmed strains of Atlantic salmon have been investigated, with as much as 70 % variation between the highest and lowest infected family strains, showing the potential of selective breeding and family selection (Glover *et al.,* 2004a; Glover *et al.,* 2005; Gharbi *et al.,* 2009). It was reported, that susceptibility to louse infection could be dependent on a Major Histocompatibility Complex (MHC) genotype, although a major effect is unlikely (Glover *et al.,* 2007). The effect may be due to variation

in quantitative trait loci associated with MHC class II regions through linkage disequilibrium (Gharbi *et al.,* 2009). It is, however, untested that QTLs (quality trait loci) moderate the effect of MHC genes, but the discovery of a genetic element that conclusively affects susceptibility to lice is an exciting development. However, results from studies of salmon susceptibility to both *L. salmonis* and *A. coregoni* suggest that there are many interacting factors that contribute to the extent of a louse infection on an individual, thus the role of genetic factors may be small. Selection for salmon resistance, however, is a long term goal and only reduced sensitivity to sea lice is likely to be achieved.

Finally, another relevant strategy is the use of cleaner fish, such as wrasse (*Ctenolabrus* and *Labrus* spp.) in polyculture (Treasurer, 1994; Bricknell *et al.,* 1996; Sayer *et al.,* 1996; Tully *et al.,* 1996; Rae, 2002; Treasurer, 2002) In line with the principles of integrated pest management, the best solutions to the sea lice problem are likely to involve the co-ordinated use of a broad range of these and other strategies.

In recent years there has been a dramatic increase in the production of farmed Atlantic salmon, raising concerns about the environmental impact of these activities. One particular area of concern is escapees, which have been documented to cause genetic changes in native populations as a result of interbreeding (Glover *et al.,* 2012; Skaala *et al.,* 2006). Although considerable technological advances have been made in the design of cages, escapes through natural disaster, human error or mechanical failure are small, but inevitable risks and salmon being on grown in the marine environment are reproductively competent. For these reasons, the production of sterile fish to mitigate the environmental impact of escapees and potential inter-breeding with

wild stocks, is receiving ever-increasing attention. The use of triploid salmon in commercial Atlantic salmon aquaculture is the only commercially acceptable means of sterility to address the environmental impacts of escapees (O'Flynn *et al.,* 1997; Peruzzi *et al.,* 2004; Piferrer *et al.,* 2009). Differences in performance, physiology, behaviour and morphology between triploid and diploid fish are well described (Piferrer *et al.,* 2009; Taylor *et al.,* 2011) and these differences could conceivably contribute to differential susceptibility to sea lice infection. A range of factors contribute to the susceptibility of Atlantic salmon to infection and may signal host suitability to the parasite, such as nutritional condition and size / morphology (Jaworski & Holm, 1992; MacKinnon, 1998).

Triploid fish have three sets of chromosomes instead of two, which leads to larger, but fewer cells in all tissues and organs (Benfey & Tillmann, 1999). Behavioural differences between fish of different ploidies have been described, with reduced aggressiveness, inferior overall performance in sub-optimal conditions, feeding at deeper water depths and lower responsiveness to environmental stimuli reported for triploid Atlantic salmon (Benfey & Tillmann, 1999; Tiwary *et al.,* 2004). Studies have also suggested that triploids could be more susceptible to pathogen or parasite infection due to reduced immune activity as compared to diploids (Ojolick *et al.,* 1995; Hakoyama *et al.,* 2001; Langston *et al.,* 2001; Johnson *et al.,* 2004; Halačka *et al.,* 2010). For example, triploid Atlantic salmon have been found to be more susceptible than their diploid counterparts to infection by *G. salaris*, (Ozerov *et al.,* 2010). The authors suggested this might be due to compromised complement-dependent immune pathways in triploid salmon. It is uncertain, however, as to what extent the observations in these various studies relate to ploidy *per se* rather than to the

interaction of ploidy with particular genotypes. In many of the previously published trials, fish size was unaccounted for. Results from tank and cage trials have shown that large fish tend to be more heavily infected (Jaworski & Holm, 1992; Tucker *et al.,* 2002; Genna *et al.,* 2005). Given that triploid smolts show higher growth potential than diploid salmon smolts (Taylor *et al.,* 2011), the size of the fish can be a potential confounding factor with respect to comparison of sea lice infection levels. However, it needs to be noted, that triploid salmon in mixed ploidy populations show decreased growth potential and reduced performance compared to triploid only populations. The results from a study by Taylor *et al.* (2014) show that triploids perform very differently when reared in the presence or absence of diploid conspecifics. This finding is absent in previous studies and might influence findings on compromised immune responses such as found by Ojolick *et al.* (1995) and Halačka *et al.* (2010).

Salmonid fish are capable of generating an immune response to salmon lice (Grayson *et al.,* 1991), however, no acquired protection against re-infection has been observed. Two studies (Fast *et al.,* 2006; Glover *et al.,* 2004b) tested individually tagged diploid salmon in separate challenges with sea lice and demonstrated that the infection level for a single salmon in one challenge is a poor predictor of its infection level in a subsequent challenge. Persistent infection may lead to compromised host immunity and tissue damage followed by a period of hypo-responsiveness and delayed healing (Glover *et al.,* 2004b). It has been suggested that weakening of the animal could be expected to be more pronounced in triploid fish compared to diploid fish due to reduced immune activity (Johnson *et al.,* 2004).

The aim of the present study was to compare the susceptibility of triploid and diploid Atlantic salmon to infection by the salmon louse *L. salmonis* in several experimental and commercial settings in Scotland and Norway. In addition, a re-infection trial was undertaken to determine if a correlation existed between the outcomes of infection events for individual fish.

5.2. **Material and Methods**

5.2.1 Experiment 1: Tank sea lice challenge and re-challenge in Scotland

5.2.1.1 Fish stock

On November 25th 2009, 78 female two-sea-winter broodstock from the five generation Landcatch Natural Selection (LNS) Atlantic salmon breeding program were stripped and milt collected from 26 unrelated males at Landcatch Ltd., Ormsary UK. A sub sample of 70g of eggs (~300 eggs) per female was removed, fertilized by a different unrelated male, each male being crossed with three different females (*i.e.* male 1 crossed with females 1-3, male 2 with females 4-6 *etc.*), giving three half-sibling families per male. Following fertilization egg batches were sub-divided into two (150 eggs/cross), pooled into batches of six females and two males (1800 eggs/pool) and water hardened at 10°C. Triploidy was induced in one batch by applying a hydrostatic pressure shock (in-house custom built vessel) 30 min post fertilization at 655 bar (9500 psi) for 5 min (Taylor *et al.,* 2011) leaving the other half of the batch untreated as diploids controls. Fertilisation rates were approx. 85%. The process was repeated a further five times. Eggs were incubated at ambient water temperature (6°C) in 6 separate silos per ploidy (~11,000 ova / ploidy) until

eyeing, before transfer to on-growing hatchery (Gairloch Hatchery, Rosshire, Scotland). Survival to hatch (March 2010) was ~65% (~5600 fry/ploidy), at which point silos were pooled per ploidy and split between two tanks per ploidy, and fry on-grown to 2g before transfer to Institute of Aquaculture freshwater facilities (Niall Bromage Freshwater Research Facility) in July 2010. Fish were reared in two circular 1.8m^3 tanks per ploidy, under simulated natural photoperiod and fed a commercial diet (Skretting) during daylight hours to manufacturer's recommendations using 6 L Arvotec T Drum feeders controlled by a computer aided PC system. Ambient water temperature ranged from 1.5°C in winter to 15.5°C in summer). Mortality during freshwater from first feeding to smolt was <1.5% in both ploidy, and presence of externally visible deformity were <1% in both ploidy at time of sea transfer. At end of freshwater rearing diploid and triploid smolts weighed 66.5 ± 3.0g and 91.8 ± 2.8g respectively (P>0.05). Fish were transferred to Institute of Aquaculture marine facilities (Machrihanish Marine Environmental Research Laboratories, MERL) in mid-April 2011 and stocked in two 3m^3 stock tanks (one / ploidy).

On 17th June 2011, after 3 months in seawater, 200 fish from each ploidy (mean weight ± SD of 108.6 ± 20.6 g and 144.2 ± 22.9 g, respectively for diploids and triploids) were intramuscularly PIT-tagged (8 mm passive inductive transponder-tags (PIT-tags), Trovan Ltd., Identify UK Ltd., Hessle, UK) and transferred to 600 L tanks (2 replicate tanks per ploidy, 100 fish / tank, stocking density of 20 kg m^{-3}). Fish were acclimated in the new tank system for 3 weeks prior to the start of the sea lice challenge.

5.2.1.2 Sea lice challenge and sampling

On the 4th July 2011, fish (mean weight: diploids 107.4 ± 21.2; triploids 143.9 ± 22.8) were crowded and challenged with sea lice, *L. salmonis*, copepodids (30 lice per fish, or 5 lice L^{-1} water). Water temperature during the trial was 14 ± 1°C with a 12 h light: 12 h dark light regime. Fish were fed to satiation. Following successful settlement (infection abundance ~ 10 lice $fish^{-1}$), sea lice numbers were recorded on the 12th July 2011 (chalimus I & II) following light anaesthesia with MS222 (50 ppm). The fish (mean weight: diploids 105.5 ± 22.4; triploids 142.8 ± 25.7) were subjected to a second infection on the 19th July 2011 with the same dose of sea lice copepodids, simulating a second wave of infection. A control group of "naïve" sibling diploid fish (mean weight of 188.3 ± 27.0 g), previously uninfected, was also infected at the same time in 2 replicate tanks. After settlement, sea lice numbers were again recorded on 23rd July 2011 (chalimus I & II for second sea lice challenge; chalimus IV and pre-adults for initial challenge). Lice recordings were conducted by a single scientist at all times to avoid variance between counting due to human error. Ploidy was confirmed microscopically by red blood cell smears, within 24 hours after sampling. All experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986 UK under the approval of the local ethical committee.

5.2.2 Experiment 2: Tank sea lice challenge in Norway

5.2.2.1 Fish stock

Fish used in this trial were produced at Matre Research Station, Norway. On 3rd November 2009, ~200,000 eggs were produced from twelve Atlantic salmon females (12,000 - 22,500 eggs female^{-1}). Eggs were fertilized by three different males (Aquagen stock, Trondheim, Norway), each male being crossed with four different females (male 1 crossed with females 1-4, male 2 with females 5-8 *etc.*), giving four half-sibling families per male and three groups of full- sibling families. Hydrostatic pressure (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada) was used to create triploids (37.5 min post-fertilization, 9500 psi (655 bar) for 6 min 15 s at 8 °C) (Fast *et al.,* 2006), giving a total of twelve groups per ploidy. Thereafter, each group was incubated in isolation in an UV-treated, flow-through system in darkness. On the 22nd July 2010, all fish within each ploidy were mixed and randomly allocated to 6 fibreglass tanks (total of 12 tanks; 2 × 2 m, n = 6 tanks ploidy^{-1}). On the 27th October 2010, all fish were injected intraperitoneally with 0.1 mL of a multivalent oil-adjuvant vaccine (Minova 6 Vet., Norvax (r), Intervet International B.V., Boxmeer, Netherlands) using a vaccination pistol (Dosys^{TM} 173 classic, Socorex Isba S.A., Renes, Switzerland). Fish were transferred to seawater on the 4th May 2011, with 90 random fish from each ploidy (mean weight of 79 ± 20 and 91 ± 24 g in diploid and triploid, respectively) being allocated to three 1.5 × 1.5 m tanks, with 30 fish from each ploidy in each tank (total of 60 per tank). Fish were reared under continuous light (LL) from May 2010 until October 2010, at which point the photoperiod was switched to simulated natural. Seawater temperature was 8.8 ± 1.0 ^{o}C.

5.2.2.2 Sea lice challenge and sampling

Lice used for the infection were produced from an outbred laboratory strain that had been maintained at approximately 9 ± 1 °C at the Institute of Marine Research Bergen hatchery (Hamre *et al.,* 2009). Fish (diploids 313.3 ± 35.9 g, triploids 345.6 ± 41.6 g) were crowded (all three tanks to approximately half of their volume) and challenged on 18th August 2011. Copepodids (10 days post-hatch, estimated total of 13,250 copepodids, 74 lice fish^{-1}) were added to the tanks and tank water level returned to normal after 1 hour exposure time. The challenged fish were examined on 16th September 2011 (29 days post-infection). Fish were sacrificed by lethal anaesthesia, their lengths and weights measured and lice numbers counted (stages: pre-adult 1 and 2). The experimental protocol was approved by the Norwegian Animal Research Authority.

5.2.3 Experiment 3: Natural sea cage infection in Scotland

5.2.3.1 Fish stock

On November 28th 2008, 45 females and 15 males from 2 sea-winter (unrelated) Atlantic salmon broodstock were stripped of gametes by Landcatch Natural Selection Ltd., Ormsary, UK. A sub sample of ~180 eggs per female was removed, fertilized by a different unrelated male, each male being crossed with three different females (*i.e.* male 1 crossed with females 1-3, male 2 with females 4-6 *etc.*), giving three half-sibling families per male. Fertilisation rates varied between 79.1- 91.1%. Triploidy was induced as in experiment 1 (Piferrer *et al.,* 2009) with a total of 6 females and 2 males shocked at any one time.

Post-water hardening eggs were stocked into 20 L silos (3 silos / ploidy). Eggs incubated at Landcatch hatchery (Ormsary, UK) until transfer at the eyed stage to the Inchmore Hatchery, Invermorriston, Marine Harvest Scotland on March 12th 2009. On hatching (25th April 2009) family batches of alevins were pooled and split between two first feeding tanks / ploidy. First feeding fry (diploid (2N) = 2453 fish; triploid (3N) = 2166 fish; 31st May) were exposed to continuous light (LL) and fed for 24 h using Arvotec automatic feeders until the summer solstice (21st June 2009), and were subsequently reared under an ambient photoperiod regime to produce S1+ smolts and were fed during daylight hours. At ~ 5 g, fry were transferred to two freshwater pens (1 pen / ploidy, Glenfinnan, Marine Harvest Scotland) and reared until smoltification. Mortality from first feeding to smolt was 9.4% and 8.5% for diploids and triploids respectively, with externally visible deformity <1% in both ploidy. On June 11th 2010, diploid (n = 1213) and triploid (n = 986) smolts were transferred into seawater at Marine Harvest Ardnish fish farm with an average weight of 66.1 ± 3.6 g and 86.1 ± 7.4 g for diploids and triploids respectively (P>0.05). Both groups were transferred into single net pens (10 × 10 × 10 m). On January 18th 2011, fish were graded into two sizes per ploidy (mean weight of 2109 ± 0.05 g and 1541 ± 0.04 g for diploid large and small grades; 2305 ± 0.1 g and 1695 ± 0.7 g for triploid large and small grades, respectively). Fish were stocked into a total of eight 5 × 5 × 5 m pens corresponding to two replicate pens per ploidy and grade. All groups were exposed to constant light (LL) using a single 400W metal halide (BGB Engineering Ltd., Grantham, UK) submersible light per pen in order to prevent maturation according to standard industry practice for Atlantic salmon. During seawater grow-out, the water temperature ranged between 6 to 15 °C. Salmon

were fed a range of different commercial diets (Skretting Optiline) according to manufacturer's recommendations. Sea lice infections occurred naturally.

5.2.3.2 Sampling

On 7th-8th March 2011, fish from all pens (1170 and 959 diploid and triploid salmon respectively) were anaesthetised using MS222 (50 ppm), however, only 814 triploids were assessed due to recorder error. Unbalanced numbers between ploidies originated from an original miscount of fish transferred from the hatchery to the freshwater pens. Fish length, weight and experimental group were recorded before the lice were counted from each fish (lice attached to the fish as well as lice in the anaesthetic bath) by a dedicated louse counting team over 2 days. Sea lice developmental stages were also recorded.

5.2.4 Calculations and statistical analysis

Due to the observed differences in body size, adjustment of lice numbers with respect to calculated fish surface area, as defined by O'Shea *et al.,* 2006, was performed for all the observed / directly counted sea lice numbers using the following formulae, and averaging the result of both length and weight:

Fish-Length (cm): $\boldsymbol{S = 0.72\ L^{1.88}}$

Fish-Weight (g): $\boldsymbol{S = 14.93\ W^{0.59}}$

S, surface area; L, length (cm); W, Weight (g)

All datasets were checked for normality / goodness of fit and homogeneity of variance using the Ryan-Joiner test and Levene's test respectively (Minitab, Version 16.1.0). Due to non-normality and unequal variances, all mortality,

length and weight comparisons were undertaken using Mann–Whitney U tests. The lice data were analysed with non-parametric ANOVA tests, permutational multivariate analysis of variance (Permanova software, Department of Statistics, University of Auckland, 2005) (Anderson, 2001; McArdle & Anderson, 2001), due to lack of normality. *T*-statistics, based on distances, were used to carry out pair-wise *a posteriori* tests to identify possible tank/pen effects in all three trials using the Permanova software and as described by Department of Statistics, University of Auckland (2005) (Anderson, 2001; McArdle & Anderson, 2001). Spearman's rank correlation coefficient was used to compare the infection levels of the challenged fish (Minitab, Version 16.1.0.). Mortality comparisons were carried out using 2-tailed unpaired t-tests (GraphPad InStat, Version 3.10).

5.3. **Results**

5.3.1 Experiment 1: Tank sea lice challenge and re-challenge in Scotland

Triploid fish were significantly larger (weight diploids 108.7 ± 20.6 g, triploids 144.2 ± 22.9 g, $p<0.001$, Table 1) than diploid fish at initial infection, which made it important to correct lice numbers for fish size. During the course of the trial there were mortalities over both infections for both diploids (3.5%) and triploids (6.5%) (Table 1), which have been excluded from the results, as accurate louse numbers could not be established for these fish (mortality was not significantly different between diploids and triploids, $p = 0.169$).

Table 5.1. Summary of size and sea louse infection statistics (non-parametric ANOVA) for the diploid and triploid salmon in the three trials. Superscripts indicate significant differences.

Trial/ploidy		Fish parameters							Louse parameters				
	n	*Number of individuals assessed / replicate*	*Mortality (%)*	*Louse Prevalence (%)*	*Length (cm)*	*Weight (g)*	*Calculated surface area (cm^2)*		*Abundance*	*p-value*	*Range*	*Density (abundance / surface area)*	*p-value*
Tank trial Scotland													
Diploid	2	193	3.5	97.4	23.3 ± 1.3	108.7 ± 20.6	200.2 ± 18.9		7.6 ± 7.2 ***a***	0.906	0-105	0.038 ± 0.035 ***a***	0.990
Triploid	2	187	6.5	98.4	26.1 ± 1.2	144.2 ± 22.9	254.4 ± 16.9		10.4 ± 8.7 ***a***		0-58	0.040 ± 0.033 ***a***	
Tank trial Norway													
Diploid	3	93	0.0	100	29.4 ± 1.4	313.3 ± 35.9	442.6 ± 29.9		7.0 ± 3.4 ***a***	0.594	1-17	0.016 ± 0.008 ***a***	0.828
Triploid	3	86	0.0	100	30.7 ± 1.1	345.6 ± 41.6	468.8 ± 33.5		7.7 ± 3.1 ***a***		3-17	0.016 ± 0.007 ***a***	
Cage trial Scotland													
Diploid	4	293	n/a	43.4	54.0 ± 3.6	1952.5 ± 445.0	1300.7 ± 166.3		0.6 ± 0.9 ***a***	0.388	0-6	0.0005 ± 0.0007 ***a***	0.543
Triploids	4	210	n/a	56.3	54.8 ± 4.0	1967.3 ± 495.9	1321.0 ± 187.5		0.8 ± 1.0 ***a***		0-7	0.0007 ± 0.0008 ***a***	

No significant difference in infection severity was observed between triploid and diploid fish before or after correcting for fish body size (lice abundance: p = 0.906; lice density: p = 0.990) (Table 1). Within ploidies, tank effects were observed for sea lice abundance and density, however, no significant ploidy × replicate interactions were found.

Triploid fish were significantly larger than diploid fish at re-infection and lice numbers were therefore corrected for fish size (Table 1). No significant differences in sea lice abundance or density were found for either the chalimus count from the second infection wave, or the count of remaining lice from the first infection wave, or when comparing overall lice abundance on the fish (Table 2).

Table 5.2. Effects of ploidy on successive sea louse infection challenges in the reinfection tank trial (assessed by non-parametric ANOVA)

Trial/source	F	p-value
1st Challenge louse abundance	0.670	0.581
1st Challenge louse density	0.274	0.834
2nd Challenge louse abundance	0.839	0.498
2nd Challenge louse density	0.612	0.560
Overall Statistics		
Overall louse abundance	0.587	0.585
Overall louse density	0.129	0.880

No significant difference in infection intensity, measured by the abundance of chalimus between control naïve diploid (uninfected prior to second challenge)

and pre-infected diploid fish was found (F = 4.27, p = 0.105). The correlation between initial and repeat infection levels (measured as lice density) was marginally significant for individual diploid fish, (Spearman's ρ = 0.158; p-value = 0.0429; R^2 = 0.0249) (Figure 1a), with just 2.5% of the observed variation in the second infection caused by the first infection. No significant correlation was found for individual triploid fish (Spearman's ρ = 0.012; p-value = 0.867; R^2 = 0.0002) (Figure 1b).

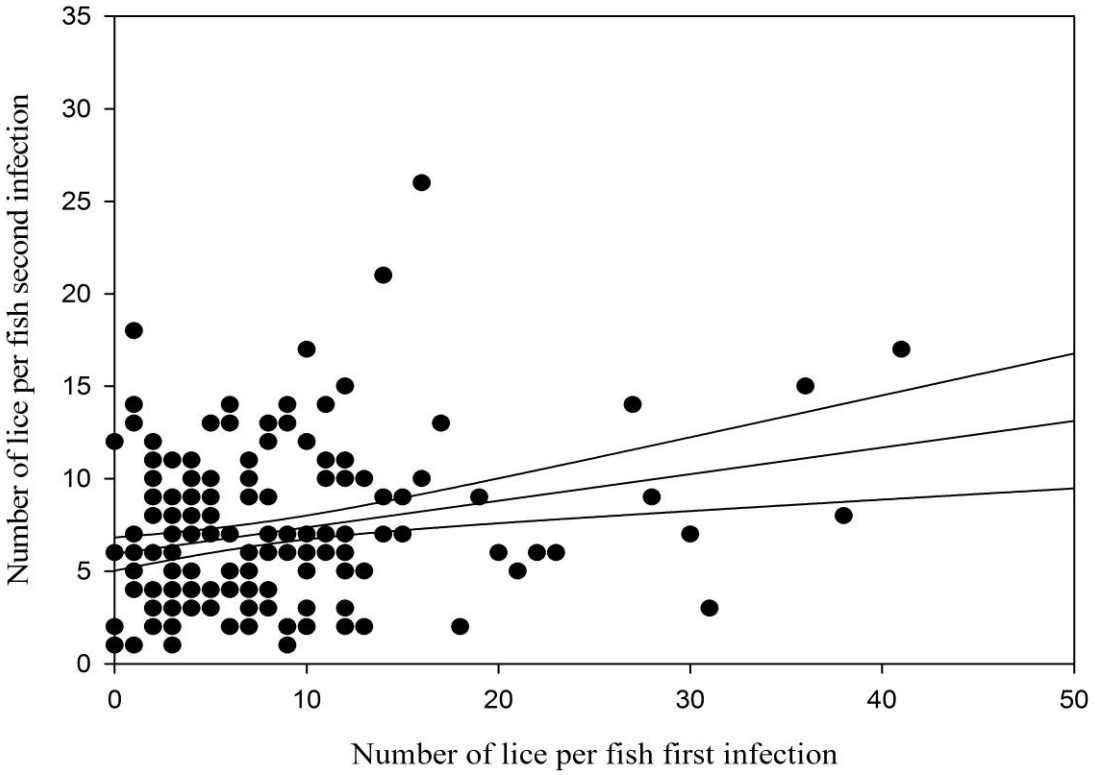

Figure 5.1a. Linear regression of sea louse abundance on individually tagged diploid salmon comparing a first and second infection with sea lice in a tank trial.

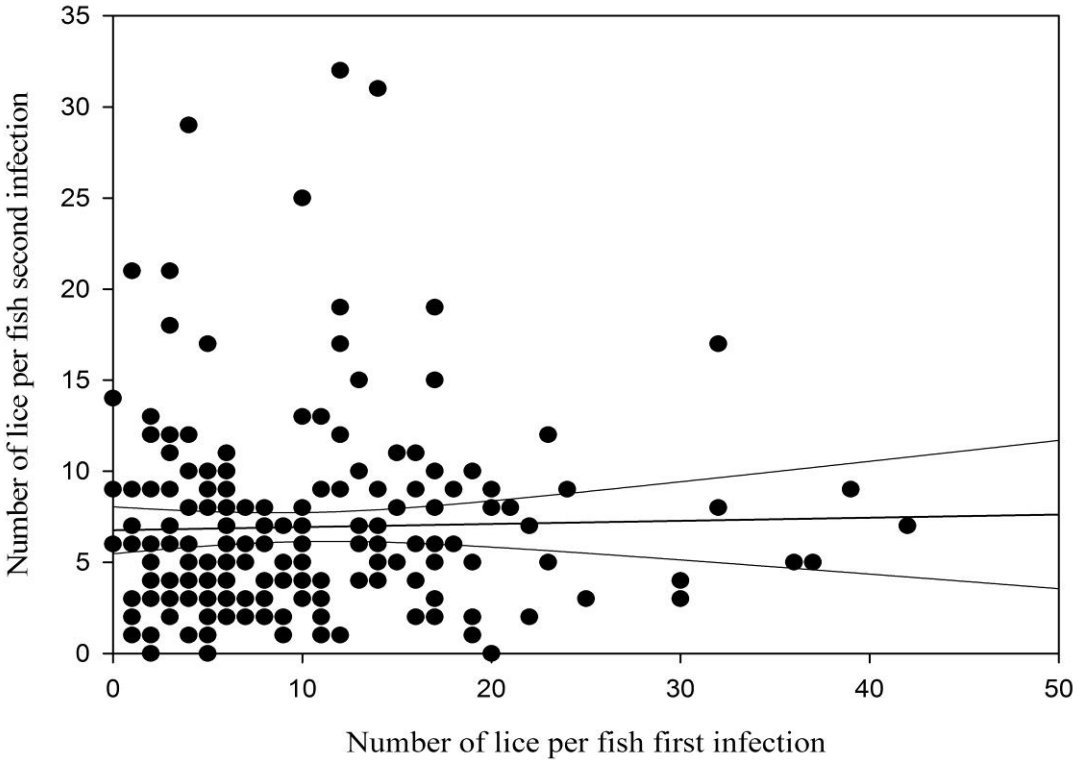

Figure 5.1b. Linear regression of sea louse abundance on individually tagged triploid salmon comparing a first and second infection with sea lice in a tank trial. *n.b*. fitted regression line is not sensitive to removal / inclusion of highest infected fish from first infection.

Mortality was significantly higher (p = 0.0009) in pre-infected diploid fish subjected to a second infection (25 fish, 13.0 %) than naïve diploid fish similarly infected (7 fish, 3.5 %), most likely caused by combined handling stress and louse attachment.

5.3.2 Experiment 2: Tank sea lice challenge in Norway

Triploid fish were significantly larger than diploid fish at initial infection (Table 1). No difference in infection intensity was observed between triploid and diploid

fish before (p = 0.594) or after (p = 0.828) correcting for fish body size (Table 1). Within ploidies, tank effects were observed for sea lice abundance and density, however, no significant ploidy × replicate interactions were found.

5.3.3 Experiment 3: Natural sea cage infection in Scotland

The lice abundance in the cage trial was much lower than in the tank trials (Table 1) (mean lice $fish^{-1}$ ± SE; diploid 0.64 ± 0.88; triploid 0.84 ± 0.98). Prevalence of sea lice (49.9 %) was lower than that seen in the artificial sea lice challenge conditions. No difference in infection intensity was observed between triploid and diploid fish before or after correcting for fish body size (Table 1). Within ploidies, cage effects were observed for sea lice abundance and density, however, no significant ploidy × replicate interactions were found.

5.4. **Discussion**

Farmed escaped Atlantic salmon have successfully introgressed and caused genetic changes in native Atlantic salmon populations (Glover et al., 2012; Skaala et al., 2006). The feasibility of using triploid salmon in commercial production is currently being investigated in terms of a number of key farm traits including survival to hatch, size at hatch, deformity prevalence, early stage growth performance (Taylor *et al.,* 2011), smoltification, survival and growth in salt water, heart morphology and severity of cataract (Leclercq *et al.,* 2011). Evidence for cellular and physiological differences between diploid and triploid salmon, but also evidence for different behavioural patterns (Benfey & Tillmann, 1999; Tiwary *et al.,* 2004) is changing views about the farming of triploids

leading to improved guidelines for farming triploid salmon. One aspect of particular interest is the potential for differential susceptibility to disease between diploids and triploids. In order for triploid salmon to become more widely used by the industry, it is essential that their susceptibility to infection by sea lice with respect to diploid stocks is established.

This study has examined the potential for differential susceptibility to sea lice between diploid and triploid salmon. No difference in susceptibility between ploidies was found in the tank trials performed in Scotland and Norway and in the cage trial in Scotland. This finding contrasts with an earlier study looking at the susceptibility of Atlantic salmon to another ectoparasite, *G. salaris*, which suggested that triploid salmon had higher infection levels (Ozerov *et al.,* 2010). This latter study, however, did not account for different fish sizes resulting from different ploidies.

The fish used in the present tank experiments were obtained from two different selected stocks and the trials were carried out in separate locations (Norway and Scotland) with different fish sizes and infection intensities. Since the findings from the tank trials were similar, this study provides evidence that diploid and triploid salmon do not differ in susceptibility to sea lice infection pressure.

Although no differences in sea lice infection between ploidies were observed, there is the possibility that different families may show different susceptibility, similarly to growth and condition performance effects comparing diploid and triploid families of Atlantic salmon (Taylor *et al.,* 2011). In all experiments presented in the current study, a high number of families were used, representative of a commercial cage population. Genotype × Environment

interactions could not be tested in the present study given the use of three different stocks in different locations and different sizes at the time of challenge. Importantly, there is the possibility of potential different inheritance patterns between ploidy due to the increased chromosome copies in triploids, and determining maternal or paternal inheritance patterns would be essential to determine heritability for selective breeding.

Previous results from tank and cage trials have shown that large fish tend to be more heavily infected (Jaworski & Holm, 1992; Tucker *et al.,* 2002; Genna *et al.,* 2005). Although triploid fish were significantly larger than diploids in the tank trials in Norway and Scotland, no indication was found that larger fish had a higher lice burden than smaller animals. The different light regimes used (tank trial Scotland: 12h:12h light regime; tank trial Norway: simulated natural light August – September; cage trial Scotland: constant artificial submerged light) could play a role in the attachment success of the sea lice on the fish. It was shown that *L. salmonis* may use phototactic cues, such as shadow and potentially light reflection from the scales of host fish, to colonise the host (Bron *et al.,* 1993a; Bron & Sommerville, 1998). Thus, a constant light regime may aid lice attachment compared to day/night rhythms.

In order to reflect a more natural situation, where fish already infected with sea lice are re-infected with fresh lice over the production cycle, a re-infection trial was performed. For the second wave of infection, no differences were found between triploid and diploid salmon. The conclusions of Halačka *et al.* (2010) and Johnson *et al*. (2004) demonstrated that triploid fish could show lower immune activity than diploids and might therefore be more susceptible, which was not supported by the results of the current tank study, in terms of sea lice

infection. When comparing sea lice infection success on naïve (no prior infection) and pre-infected diploid salmon, no significant differences were observed following a second challenge, with equivalent numbers of chalimus attached to fish. The infection levels of naïve fish were more overdispersed (variance > mean) compared to pre-infected fish, this being indicative of higher aggregation of lice in the naïve stock. Even if the overall sea lice infection level is low, a few individuals will show very high, potentially lethal numbers of sea lice. This aggregation of sea lice may arise from host factors, such as attractiveness, susceptibility and selectivity, or patchiness of sea lice occurrence (Murray, 2002). Comparing infection levels for initial infection and re-infection, a significant correlation was found for diploid fish. For triploid fish, no correlation was found. Neither triploid nor diploid fish in this trial, subject to initial infection, became refractory to subsequent sea lice infection.

A significantly higher mortality was observed for pre-infected diploid fish subject to a second infection. This may indicate that the worst affected fish may have received a second severe infection, with possibly lethal consequences to the already weakened animals. The question remains, of whether the few highly infected fish, which had to be taken out of the trial due to lethal lice loads, would have shown a similar pattern of infection in subsequent infections.

The current tank trials were performed with high mean lice numbers, which rarely occur in commercial fish farms under a strict pest management strategy. This study was extended by examining corresponding infection levels on a commercial salmon production site. Following current salmon production as well as environmental guidelines and good management practice, sea lice numbers at the site were kept at a low level (range 0-7 lice / fish). Under farm conditions,

which include a variety of additional factors not seen in controlled tank trials, no significant difference in susceptibility was observed between triploid and diploid salmon in sea cages. Observed cage effects within the ploidies may have been due to positioning of pens with respect to environmental factors. For example, anecdotal evidence suggests that the upstream pens of a cage group might have higher lice loads than pens located in the middle of a cage group. Tidal rhythms, salinity, water flow and turbulence associated with high current velocities have also been described to affect sea louse behaviour in the wild (Heuch, 1995). Further research under commercial conditions is required to identify the main environmental parameters that explain louse burden differences.

Overall, this study clearly demonstrates that there are no intrinsic differences in susceptibility to sea lice infection between diploid and triploid Atlantic salmon.

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CHAPTER 6: General Discussion

6.1. **Overview**

The work discussed in the present thesis aimed to provide a better understanding of the key factors associated with sea louse, *L. salmonis*, infection of farmed Atlantic salmon. Specifically, this project focused on investigating two relevant aspects: 1) the environmental conditions driving the distribution and infection success of sea lice, and 2) the effects of manipulating host salmon behaviour and physiology on the infection success of sea lice.

The first aspect of the thesis work aimed to establish both the distribution of sea louse larval stages in the water body surrounding Atlantic salmon farms and their capacity to infect salmon in different areas of the farm. To determine this, a study was carried out to investigate the depth distribution of sea louse larvae around Scottish Atlantic salmon farms and their infection profiles according to cage position (Chapter 2). A second study was done to quantify the infection success of lice larvae under various environmental conditions, testing a series of light and current conditions using an experimental flume (Chapter 3).

The second aim of the thesis work aimed to investigate the impact of modifying host fish behaviour and physiology on the severity of infection. This work was based on the findings of earlier components of the thesis work, which established that sea lice are transported by current and water movements and keep to the surface layer of the water body. A trial was therefore carried out with the objective of reducing the exposure time of salmon to high risk zones of louse infection. This was achieved by manipulating the swimming depth of salmon through the combined use of artificial lighting and submerged feeding systems outside the established sea louse infection zones (Chapter 4). Finally,

repeat infection trials using diploid and triploid salmon were performed to replicate infection exposure conditions of commercially reared salmon, these being exposed to repeat waves of infection, according to the interaction of factors such as production-cycle timing, season and tide, current, wind-forcing and planned sea louse treatments. This experiment was conducted to determine whether levels of infecting lice are determined by characteristics of individual fish and to show whether high individual infection levels observed after a first infection wave would consequently result in high infection levels after a second infection. Furthermore, in light of promising results obtained in triploid salmon farming, this study aimed to determine if sea lice infection rate differed between ploidies under experimental and farm conditions (Chapter 5).

6.2. **Summary of Main Findings**

6.2.1 Environmental drivers

The findings suggested that higher infection numbers were seen on fish held in sea pens placed at either end of a pen group. In other words, higher infection rates were seen in pens lying at extremities of the axis of the main current gradient, these being closest to any incoming or returning currents upon tide change. Previously, an accumulation of sea lice on fish held at the head of a loch was also observed in a study using sentinel cages (Pert *et al.*, 2014). The authors suggested that this result may have been due to wind forcing, wild fish gathering at the river at the head of the loch and also louse accumulation caused by prevailing tidal currents. Either way, it may be concluded that wind and water current play an important role in the transportation of sea lice into salmon farms.

The highest concentration of larvae, both nauplii and copepodids, were found at a depth of 2 metres. Overall, 86% of louse larvae were found within the top 6 metres. This was in line with previous observations, where salmon staying deeper within a farm environment have lower lice numbers (Hevrøy *et al*., 2003). This has also been supported by previous studies: It has been found that *L. salmonis* copepodids are attracted to higher light intensities and move into areas of lower water pressure (Bron *et al*., 1993a). This suggests that copepodids may accumulate in the surface layers of the water column during daylight hours. Heuch *et al*. (1995) observed this daily diel vertical migration, with copepodids sinking in the water column at night and rising at sunrise, thus increasing chances of host encounter and potentially minimising predation risks. Interestingly, in the present work, high light intensity was also shown to increase the chances for louse infection to a salmon host. Specifically, better illumination, possibly coupled with higher contrast may thus increase the infection success of the lice, highlighting the importance of visual stimuli in host infection. This is in contrast to previous findings by Heuch *et al*. (2007), who found that there was no difference, between darkness and light in the rate at which copepodids attacked a model fish head. Also, no decrease in infection success was seen at higher light intensities, contradicting findings presented by Genna *et al*. (2005).

The highest attachment success was seen in static conditions, where sea lice could use their full host detecting and swimming potential to infect fish, and lice were less likely to be detached by ambient currents. Short exposure times of copepodids to fish, *i.e*. fast currents transporting the lice and thus faster swimming speed of the fish, or, as described by previous studies, smaller

surface areas of smaller fish (Gjerde & Saltkjelvik, 2009), will all decrease the chances of sea lice being able to infect the host initially.

Overall, although louse infection success was generally found to be very low, high light intensity (in the blue / green spectrum), low current speed conditions and high copepodid density were shown to increase the chances for the louse to infect a salmon host.

6.2.2 Behavioural and physiological effects

Salmon have been described to be highly positively phototactic, occupying a swimming region proximal to light sources (Oppedal *et al.*, 2007, Oppedal *et al.,* 2011). This behaviour was also described in the present work, where swimming behaviour in proximity to light sources was observed and quantified through the use of sonar technology. It was therefore hypothesised that in the absence of other over-ruling behavioural cues, such as feeding, bright sunlight or temperature gradients, placing strategic lights at 10 metres depth would attract fish to deeper water layers. As a consequence of the previous findings that sea lice remain in the top 6 metres of the water column, there would be a reduction in infecting sea louse numbers. Indeed, the results showed that deep mounted lights and surface feeding were seen to attract salmon to deeper water depths during darkness and to the surface during daylight. In the trial pens, submerged feeding, below the predominant louse occupation zone, together with deep mounted lights was employed to contain salmon in a deeper water depths. The trial was carried out following a successful sea louse treatment (>90% clearance of all louse stages), and was able to reduce novel sea louse infection and new settlement significantly.

In addition, the comparison of louse infection differences between diploid and triploid Atlantic salmon has been investigated. This trial was carried out based on commercial interest for making triploid salmon a feasible alternative to diploids, due to capacity for increased growth, reduced maturation and minimisation of possibilities for cross-hybridising with wild stocks. Results indicated that no major differences in infection success of lice were observed between the two ploidies. This finding is in contrast to predicted differences between the two ploidies based on findings with respect to other parasites (Ozerov *et al*., 2010). Additionally, it can be assumed, that triploid fish, if to be tested in the flume, as described for diploids in chapter 3, would perform similarly. A further trial testing this hypothesis with the described set-up might further strengthen findings of similar performance regarding to louse infection between the ploidies.

A weak correlation was found for individual diploid fish with respect to infection severity following two separate infection waves: it was found that if individual fish had a higher infection rate in the first infection wave, they had a higher infection rate after the second infection wave, which indicates a possible further genetic effect. The possibility of these more susceptible fish repeatedly succumbing to higher sea louse infections, and increasing local infection pressure for populations of farmed salmon, can therefore not be excluded and should be investigated further.

6.3. **Practical applications**

Overall, the work done in this thesis has served to increase knowledge of louse infection pathways and patterns for farmed Atlantic salmon. Understanding of major infection zones in a given water body, coupled with infection success mediated by environmental and behavioural cues can provide a basis for establishing healthier salmon populations with lower louse abundances. In the face of increasing resistance to currently employed medicines for louse treatment, a delayed, or ideally prevented initial infection with copepodids must be of the highest priority.

Observations in this thesis, that sea lice are predominantly found in the surface 6 metres of the water body and apparently use light as a major cue for host finding and struggle to attach to hosts under higher current conditions need to be addressed and exploited practically.

As a first practical implementation of the work presented in this thesis, a deeper lighting regime is currently being employed by the industry, with the expectation of preventing salmon from spending darkness hours close to surface waters. As no major differences in infection severity between diploid and triploid salmon were observed, a shift to triploid salmon production would not cause higher sea louse infection pressure. A weak correlation, possibly suggestive of the impact of individual fish susceptibility, was found for individual diploid fish with respect to infection severity following two separate infection waves. The method of sick grading of small and / or wounded fish is already employed to possibly remove highly susceptible and infected fish from commercial populations (Cockerill, D. (2012), Pers. Comm.). However, such methods are invasive and may be time consuming.

Genetic differences of salmon have previously been investigated and analysed with respect to sea louse susceptibility. Comparisons between wild and farmed strains of Atlantic salmon have shown as much as 70 % variation between the highest and lowest infected family strains, highlighting the potential of selective breeding and family selection and different susceptibilities based on the genetic make-up of the fish (Glover *et al*., 2004a; Glover *et al.,* 2005; Kolstad *et al.*, 2005; Gharbi *et al.,* 2009). Specific antibodies against sea lice have been found in Atlantic salmon, however, factors contributing to the severity of the infection are multiple, random and apparently unidentified (Glover *et al*., 2007). However, commercially, selective breeding programs to increase the resistance of Atlantic salmon to infection with sea lice have been initiated and are currently on-going (Cockerill, D. (2014), Pers. Comm.).

6.4. **Limitations and future perspectives**

In conclusion, the work described in this thesis supports the argument that sea louse management in Atlantic salmon aquaculture needs to take greater account of a broad range of farm, host and environmental factors, incorporating improved understanding of the complex interactions of louse and fish biology under the environmental conditions experienced by individual farms. As such, integrated pest management strategies employing a range of different control mechanisms, rather than single medication solutions, need to be employed to control louse numbers on farms.

To achieve this goal, further trials are urgently required to gain a better understanding of the role that light and visual cues play in allowing sea lice to successfully infect the host. While the results presented in this thesis provide

evidence that infection success is improved under high light intensities, this finding should furthermore be trialled on a commercial scale. For example, employing low intensity lighting systems, preferably outside the blue light spectrum should be trialled to achieve maturation and growth benefits, as currently achieved by employing high intensity white light sources. Additionally, as sea lice can be expected to attach under bright sunlight conditions, salmon should ideally be encouraged to spend sunlight hours at deeper water levels by submerged feeding. However, due to the set-up tested in chapter 4, using the tested submerged feeding regime and equipment did not show a reduced sea louse burden.

In turn, the use of submerged feeding as a means of attracting salmon to deeper water layers should also be more widely tested, and if efficacious in reducing louse burdens, practically employed. Due to the prototype feeder used in the described trial, no continuous feeding throughout the day was practically possible, and fish were seen to readily return to the surface after feeding, thus spending a period of daylight time in the surface waters. Currently no established commercial product / system exists, which can reliably and continuously feed fish at deeper water depths.

Other approaches, which aim at achieving similarly reduced settlement effects are currently being developed, which exploit the preference of sea louse larvae for surface waters. These include the use of “snorkel” cages, which employs surface access for the salmon only by use of a plankton net cylinder (Oppedal *et al*., 2014), and plankton net barrier nets, which block plankton access in the surface top 6 metres of the water body (Stien *et al.,* 2012) and electrified skirt nets employed in the surface 6 metres to kill incoming sea louse larvae

(Bredahl, 2014). It is still unknown, if sea lice can develop resistance, adaptation or avoidance strategies to mechanical and technical solutions described above and the solutions presented in this thesis. Due to fast lifecycle and high fecundities, it can be assumed, that sea louse adaptation to any method needs to be expected, but an integrated approach using several methods combined will reduce louse numbers on salmon effectively for many years.

Following observations that that individual fish may have consistently higher susceptibility to infection with sea lice, possibly due to behavioural patterns or physiological peculiarities, additional trials should be carried out to examine re-infection patterns. While the results observed reflect over dispersed infection patterns observed in salmon farms, they cannot, however, be used to establish whether individual fish can have drastically different susceptibility to repeated infections in the first place. Highly infected individuals might have been immuno-compromised due to their high infection levels in the first wave and thus had all lice been removed, they might not therefore have shown high susceptibility in the second wave of infection.

6.5. **Overall conclusion**

Taken together this thesis has provided a better understanding of sea louse infection and the methods employed by the lice to find and infect the host. This study also opened up practical solutions to exploit the biological requirements and complex interactions between Atlantic salmon and *Lepeophtheirus salmonis*. The approaches described in this thesis are highly relevant and

useful for practical on-farm applications. They have, to some extent, already been tested on a commercial scale by the Scottish salmon industry.

The main applied findings from this thesis are:

- Copepodid density is highest in the top 6 metres of the water column and highly dependent on wind and current movements,
- Various environmental factors, especially light intensity, play a major role for copepodids in host finding,
- Keeping salmon at lower water depths through modified lighting and feeding regimes can reduce infection levels with copepodids,
- Infection levels do not differ between diploid or triploid salmon hosts.

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