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The spread of salmon lice (*Lepeophtheirus salmonis* Krøyer) from Norwegian fish farms and their impact on sea trout (*Salmo trutta* L.)

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Summary

The transmission of salmon lice (*Lepeophtheirus salmonis* Krøyer) from farm salmon to wild salmonids and the impact on wild populations are sources of intense debate in all salmon producing countries, and it is currently an issue of high political and economic relevance in Norway. Solid science-based evidence is therefore needed by decision makers to apply sound management strategies. Given the complexity of the systems, coupled with methodological challenges, the causal relation between the in-farm production of lice and increased lice abundances on wild salmonids have been difficult to demonstrate.

In this thesis, mean salmon lice abundances on sea trout (*Salmo trutta* L.) were shown to be directly correlated with the estimated number of lice emanating from nearby fish farms. Although temperature was found to also have an impact on lice epidemiology on both farmed and wild fish, temperature alone could not explain the high numbers of lice parasitizing sea trout in areas were farmed and wild fish coexist in close vicinity. This corroborates the hypothesis that salmon farms act as a main source of lice for sea trout in aquaculture-intensive areas in Norway, even after the effect of temperature has been accounted for. These conclusions were drawn based on the statistical analysis of a large and comprehensive data set combining information on standing stock and mean lice abundances on farmed fish from Norwegian salmon farms, together with observations of lice infestations on wild sea trout collected all along the Norwegian coast between 2010 and 2011. In combination, these two large and comprehensive data sets provided a unique opportunity to investigate the role of salmon farms as a source of lice for local sea trout populations.

Moreover, results from this thesis suggest that the Norwegian National Salmon Fjords, i.e. protected areas subjected to strict limitations on aquaculture activities, can effectively protect wild salmonids against infestations with lice of farm origin, but only if the size and configuration of the protected area is adequate. Lice abundances on sea trout caught inside large National Salmon Fjords were found to be consistently low over time and are assumed to represent natural levels. Lice infestations on sea trout caught inside smaller National Salmon Fjords, on the contrary, showed large variation between years, oscillating in accordance with the variations in stocked biomass in the surrounding farms. Lice prevalence and intensities in these areas occasionally matched and even exceed those previously reported in areas of intensive farming in Norway. These results suggest that the production and transport of planktonic lice larvae from farms situated outside the limits of the protected areas might still be an important source of lice for local populations of sea trout. Although individual studies for each National Salmon Fjord may be necessary to evaluate the degree of protection, one can expect that the capacities of the smallest fjords for preventing the transmission of lice from farm salmon to wild fish are probably limited, and it is reasonable to assume that the protection of large areas contributes best to the protection of wild salmonids. The optimal design will in any case depend on the target species: while sea trout may benefit from the establishment of a local protected area covering their marine home range, the protection

of Atlantic salmon may require the protection of entire fjords or the establishment of “clean corridors” along their migration routes.

Last, this thesis delivers direct evidence of a causal relationship between salmon lice and marine mortality of sea trout in nature. Based on results from exposure field studies, combined with acoustic telemetry, this thesis shows a mortality hazard ratio of 2.7 (95% CI = 1.04−7.13) for lice-infested trout post-smolts compared to non-infested control individuals. In other words, the probability of a trout post-smolt to survive the first summer at sea was reduced by almost two-thirds when infested with a heavy lice load, although there is a big uncertainty around this estimate. These results apply to fish carrying a relative intensity of 2.4 lice g^{-1}, on average. Although such lice intensities are well beyond the estimated mortality-inducing threshold of 0.3 lice g^{-1} (Taranger et al. 2015), they are still representative of lice infestations observed on sea trout in some farm-intensive areas in Norway. Furthermore, this thesis documents an altered migration behavior of lice-infested individuals, including the abrupt interruption of the feeding migration after just a few weeks at sea and a preference for feeding areas closer to the river, in the inner part of the fjord. While control fish spent on average 100 days at sea before return, lice-infested sea trout returned to freshwater after only 18 days at sea, meaning that almost 80% of the feeding time at sea was lost because of salmon lice. This can be expected to significantly reduce marine growth and further have an impact at the population level. Studies that accurately quantify the impact of salmon lice on wild sea trout and their populations are still needed, and this thesis makes an important contribution in this direction.

List of original papers

This thesis is based on the following original papers, which are referred to in the text by their roman numerals:

I. Serra-Llinares RM, Bjørn PA, Finstad B, Nilsen R, Harbitz A, Berg M, Asplin L (2014). Salmon lice infection on wild salmonids in marine protected areas: an evaluation of the Norwegian 'National Salmon Fjords'. *Aquaculture Environment Interactions* 5: 1-16. https://doi.org/10.3354/aei00090

II. Serra-Llinares RM, Bjørn PA, Finstad B, Nilsen R, Asplin L (2016). Nearby farms are a source of lice for wild salmonids: a reply to Jansen et al. (2016). *Aquaculture Environment Interactions* 8: 351-356. https://doi.org/10.3354/aei00181

III. Serra-Llinares RM, Freitas C, Nilsen R, Elvik KMS, Albretsen J, Bøhn T, Karlsen Ø, Bjørn PA (2018). Towards direct evidence of the effects of salmon lice (*Lepeophtheirus salmonis* Krøyer) on sea trout (*Salmo trutta* L.) in their natural habitat: proof of concept for a new combination of methods. *Environmental biology of fishes* 101: 1677-1692. https://doi.org/10.1007/s10641-018-0816-1

IV. Serra-Llinares RM, Bøhn T, Karlsen Ø, Nilsen R, Freitas C, Albretsen J, Haraldstad T, Thorstad EB, Elvik KMS, Bjørn PA (2020). Impacts of salmon lice on mortality, marine migration distance and premature return in sea trout. *Marine Ecology Progress Series* 635: 151-168. https://doi.org/10.3354/meps13199

1 Introduction

General background

Since its start in the early 1970s, Atlantic salmon *Salmo salar* aquaculture has developed into an important industry in several countries, bringing income and employment to local communities and helping meet an increased demand for protein for a growing global population. Norway is currently the largest producer of Atlantic salmon, at approximately 1.3 million metric tons sold in 2018 with a value of >67 billion NOK (Statistics Norway, https://www.ssb.no). Even though the growth of the industry has slowed down in the last few years, the political ambition is to increase the production in Norway to 5 million tons by 2050, provided that the production is determined to be environmentally sustainable (Sandvik et al. 2020).

Salmon farming in open cages at sea presents a series of environmental concerns, both in Norway and in other salmon producing countries like Scotland, Ireland and Canada. Among others, the genetic interaction between farmed escapees and wild conspecifics, together with infestations of salmon lice *Lepeophtheirus salmonis* K. on wild and farmed salmonids, have been identified as major threats for wild salmonids in the North Atlantic (Taranger et al. 2015, Forseth et al. 2017, Fjørtoft et al. 2019). Lice epidemics can negatively impact individuals and populations of wild salmonids (Skilbrei et al. 2013, Thorstad et al. 2015, Vollset et al. 2016). Among these, sea trout (the anadromous form of brown trout *Salmo trutta* L.) may be particularly vulnerable to aquaculture-driven lice infestations because they reside in coastal waters, where most aquaculture sites concentrate, during most part of their marine migration.

The impacts of salmon lice on wild salmonid populations are sources of intense debate in all salmon producing countries, and it is currently of high political and economic relevance in Norway. Solid science-based evidence is therefore needed by decision makers to apply sound management strategies. Given the complexity of the systems (including interactions between hosts, parasites and the environment), coupled with methodological challenges, direct quantitative evidence of the role of salmon farms as a source of lice for wild salmonids and their impact on wild populations has been difficult to obtain. In this context, this PhD thesis pursues two main goals:

1. To investigate the role of salmon farms in regulating lice infestations on wild sea trout in Norway.
2. To investigate the effects of salmon lice on individual sea trout in their natural environment.

Brown trout

Brown trout is an iconic species in the family Salmonidae. It is indigenous to Europe, northern Africa and western Asia, but brown trout's popularity among anglers and its high value as a food resource has resulted in the introduction of this species in many countries outside their natural distribution area (Klemetsen et al. 2003). Nowadays, brown trout is found in all continents except Antarctica. Once it was believed that what we today know as *Salmo trutta* was about 50 different species, because trout vary so much in appearance and life-history expressions (Behnke 1986). Genetic analyses have however shown that brown trout constitutes one single species, displaying a huge genetic variability, which results in an enormous diversity of morphology and life strategies.

Brown trout spawn in freshwater, and seasonal movements between spawning, wintering and feeding areas are typical of their life histories (Klemetsen et al. 2003). These migratory feeding strategies are highly variable, and are best described as a continuum from strict freshwater residency (i.e. populations that complete their entire life cycle within a stream or lake), to potamodromy (i.e. populations that migrate within a river or between river and lakes), to anadromy (i.e. populations with direct contact to the sea where some individuals migrate between freshwater and the sea). In most cases, populations consist of both migrant and non-migrant individuals, a phenomenon typically referred to as "partial migration" (Chapman et al. 2012).

Anadromous populations of brown trout occur in Western Europe, from Iceland to mid-Portugal, with a southern distribution limit at 42° of latitude. The eastern distribution limit extends to the Baltic and White Seas and the Kola Peninsula (Lobón-Cerviá & Sanz 2017). The anadromous morph of brown trout, hereafter referred to as sea trout, usually migrate downstream to the sea as juvenile fish (smolt) in the spring. Once at sea, trout post-smolts (as they are referred to from the moment they enter the marine environment) will typically remain in estuarine and coastal areas during their feeding migration, and they will rarely migrate to the open ocean (Klemetsen et al. 2003). The migration may last from a few months to 4-5 years, and it may occur repeatedly during the rest of the individual's lifetime. Due to enhanced growth at sea, anadromous individuals will normally be considerably larger than their resident counterparts upon return to freshwater. The relationship between body size, egg size and fitness in terms of viable offspring is well documented for female salmonids (Einum & Fleming 1999, Jonsson & Jonsson 2011), and a larger size is also related to a more competitive foraging and reproductive ability in males. Thus, anadromy can be seen as a strategy that takes advantage of the more productive marine environment to increase growth and ultimately increase fitness of the fish. Nevertheless, migrations to sea are also energetically costly, not only because of the journey itself but also because of the physiological and morphological changes needed to adapt from life in freshwater to the marine environment (i.e. smoltification). Mortality is also normally higher at sea compared to freshwater due to predators and diseases/parasites, and it is particularly high shortly after the smolts enter the sea (Thorstad et al. 2012).

The balance between costs and benefits associated with freshwater residency and anadromy may result in different life-history strategies coexisting within the same freshwater system (Jonsson & Jonsson 1993, Ferguson 2006, Jonsson & Jonsson 2006), but only if both residents and migrants have approximately equal fitness. Anthropogenic impacts that reduce the benefits or increase the costs of marine migration may result in selection against anadromy, altered life-history traits and reduced recruitment on a population level (Chapman et al. 2012, Shaw 2016).

Salmon lice

The salmon louse is a marine ectoparasitic copepod from the family *Caligidae*. They occur naturally in marine areas in the northern hemisphere, where they live as specialized ectoparasites of salmonid fishes, on which they depend to complete their lifecycle.

The lifecycle of salmon lice comprises eight stages, including two planktonic nauplii stages, one infective copepodid stage, two attached chalimus stages, two mobile preadult stages and one adult stage (Hamre et al. 2013) (Fig 1). Adult female lice carry eggs in two egg strings at the rear of the body. The lice hatch directly from these eggs as pelagic nauplius I, which molts into pelagic nauplius II, and thereafter to the infective copepodid stage. Once they reach this stage, lice are dependent on finding a suitable host to survive and complete their lifecycle. If failing to find a host within a certain temperature-dependent timeframe, the copepodids will die. Later stages (chalimus I and II, preadult I and II, and adult) live on the host and are divided into sessile (chalimus I and II) and mobile (preadult I, II and adult) lice based on their ability to move around on the fish.

The spread of the lice occurs during the free-living naupli and copepodid stages, as they are drifting with the water currents. Once released in the water, the larvae must establish on a host in the course of approximately 150 degree-days (i.e. 15 d at 10°C) (Stien et al. 2005, Samsing et al. 2016). Within this time, the lice will be transported by the water currents, with the range of the dispersion varying significantly from site to site depending on water temperatures, currents and other hydrodynamic conditions (Asplin et al. 2011, Asplin et al. 2014, Johnsen et al. 2014).

Salmon lice feed on the skin, mucus, blood and muscle of the host fish (Kabata 1974, Wootten et al. 1982) causing tissue damage, especially as a consequence of feeding by the preadult and adult stages. This tissue damage entails further costs for the host, including osmoregulatory problems and physiological stress responses (Birkeland & Jakobsen 1997, Bjorn & Finstad 1997, Dawson 1998), increased susceptibility to secondary infections, reduced swimming performance, reduced body mass and condition factor, reduced reproduction capacity and in worst cases a complete physiological breakdown and death of the host (Wells et al. 2007, Wagner et al. 2008, Tveiten et al. 2010). Changes in migratory behavior have also been documented (Birkeland & Jakobsen 1997, Pert et al. 2009, Gjelland et al. 2014).

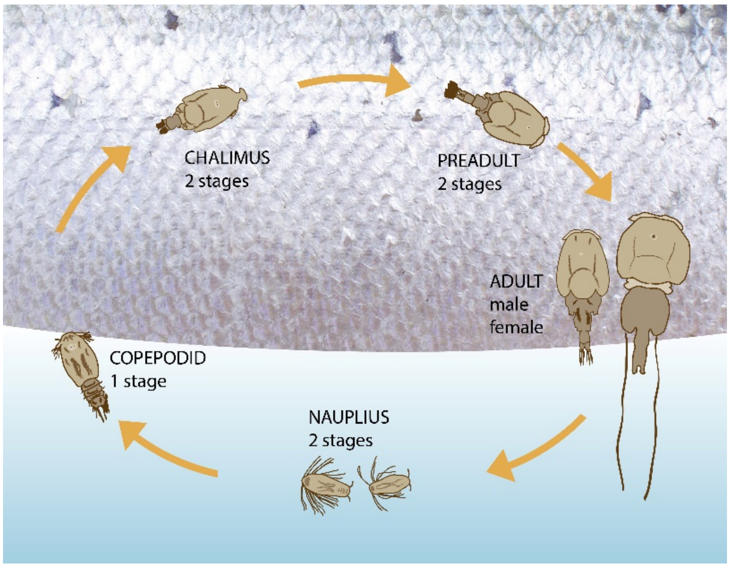

Figure 1. Lifecycle of the salmon louse. Graphic design: Kari Sivertsen, NINA. In Thorstad et al. (2015).

How salmon farming affects the host-parasite dynamics

Salmon lice have historically been observed parasitizing wild salmonids in low numbers, and few adverse effects on the host have been reported. Over the last 30 years, however, unprecedented high lice abundances have been reported in areas with intensive salmon farming in Norway, Ireland and Scotland (see Thorstad et al. (2015) and references therein). This has been mainly attributed to the spread of salmon lice from aquaculture sites.

Salmon farming in open net pens at sea allow the free movement of pathogens between farmed and wild fish and can affect the epidemiology of salmon lice through several mechanisms. First, the increased host density in areas with fish farming promote the transmission and population growth of salmon lice (Heuch & Mo 2001, Jansen et al. 2012, Torrissen et al. 2013). Second, the presence of farmed salmon in the fjords alters the natural temporal pattern of parasite reproduction. Under natural conditions (i.e. in areas without salmon farming) the density of hosts for salmon lice is generally low during the winter months due to the seasonality of salmonid fish migrations, imposing a natural bottleneck for salmon lice populations by restricting the availability of hosts. However, unlike wild salmonids, farmed salmon are present in large numbers in the fjords all-year round, thus de-coupling salmon lice production from wild host densities (Vollset & Barlaup 2014). Third, aquaculture practices may be favoring a rapid human-induced evolution in the parasite's virulence and resistance to treatments, which further amplifies the problem (Mennerat et al. 2012, Besnier et al. 2014). Due to all the factors mentioned above, the density of planktonic salmon louse larvae in areas with intensive farming can reach several orders of magnitude higher than in farm-free areas (Butler 2002, Krkošek et al. 2005, Jensen et al. 2012), resulting in an increased risk of lice infestation for local populations of wild salmonids (Bjørn et al. 2001, Gargan et al. 2003).

Field studies in Ireland, Norway and Scotland have shown a positive correlation between lice infestations on wild fish and intensive salmon farming by showing increased lice prevalence and intensities on sea trout closer to salmon farms (Bjørn et al. 2001, Gargan et al. 2003, Shephard et al. 2016). However, the issue of how closely, and over what spatial scale, lice infestation of trout relates to salmon farming practices has been strongly debated both in Europe and in North America (Revie et al. 2009, Middlemas et al. 2010, Jones & Beamish 2011). One of the reasons behind this controversy is the enormous variation in salmon lice abundances on sea trout observed across farm-intensive areas combined with the lack of precise quantitative data on the in-farm production of lice. This has often forced researchers to rely on indirect indicators such as the distance to the closest fish farms or the presence/absence of aquaculture sites across study sites or periods.

In Norway, all farms keeping salmonid fish in open cages at sea are obligated to report key numbers to the authorities on a regular basis, including standing stock of farmed fish (reported to the Norwegian Directorate of Fisheries, www.fiskeridir.no) and average number of mature female lice per fish (reported to the Norwegian Food Safety Authority, www.mattilsynet.no) among others. Moreover, lice abundances on wild salmonids have been systematically monitored in Norway for the last 20 years, as part of a large monitoring program extending over the whole Norwegian coast. In combination, these two large and comprehensive data sets provide an unique opportunity to investigate the role of salmon farms as a source of lice for local sea trout populations, and hence make the foundation for the first part of this thesis (**papers I and II**).

Consequences for sea trout individuals and populations

Elevated salmon lice abundances in areas with intensive salmon farming can negatively impact sea trout at the population level. The first indication of such an impact was the decline of Atlantic salmon and sea trout populations in some farm-intensive areas in Ireland, Scotland and Norway, which were attributed to increased salmon lice production from fish farms (Poole et al. 1996, Gargan et al. 2006, Skaala et al. 2014). Several of these studies indicated a severe impact on wild fish populations, mostly by comparisons of catch statistics between farm-intensive and less farm-intensive or farm-free areas or periods. However, the relative role of salmon lice in these declines has been difficult to quantify, not only because field records of lice abundances are often lacking, but also because different factors other than salmon lice may contribute to differences in population size and structure between years, rivers and regions (Thorstad et al. 2015). In a recent and extensive review of the available scientific literature on the impact of salmon lice on sea trout, Thorstad et al. (2015) concluded that the most important knowledge gaps are related to salmon lice impacts at the population level and in quantifying the reduction in wild sea trout populations arising from increased mortality and reduced growth attributable to salmon lice. In order to make sound inferences about the impact of salmon lice on trout populations, quantitative information about these impacts at the individual level is needed.

In the last decades, a series of laboratory experiments have been conducted with the aim of quantifying the impact of salmon lice on individual sea trout and salmon. Based on these results, a classification system has been developed to assess the probability of lice-induced mortality for individual fish based on the number of lice per gram of fish weight (Taranger et al. 2015). For first-time migrating sea trout, this system predicts no additional mortality risk for individuals carrying <0.1 lice per gram of fish weight, 20% additional mortality for individuals carrying 0.1−0.2 lice g^{-1}, 50% for individuals carrying 0.2−0.3 lice g^{-1} and 100% additional mortality for individuals infested with >0.3 lice g^{-1}. This classification system is based on the best available knowledge, and is currently applied for management purposes in Norway. The extrapolation of these threshold levels to wild, free-swimming fish can however be problematic, especially for sea trout. First, the data behind this classification system mainly comes from laboratory studies, which are often short-term and conducted using cultivated fish. Second, free-swimming fish may be exposed to additional stressors that are hard to replicate in a laboratory experiment, such as predators, other diseases and parasites or other environmental factors. These can act in synergy with salmon lice exacerbating the consequences and must therefore be considered when determining critical threshold levels. Last, lice-infested free-swimming sea trout have the possibility of seeking refuge in freshwater to regain osmotic balance, counteracting the impact of lice to increase the chances of survival, although at the expected cost of reduced marine growth and compromised future fitness. In summary, assessing the outcome of a given lice burden on individual sea trout in their natural environment is a complex task, and the extrapolation of threshold levels derived from laboratory experiments requires further validation and verification.

Field studies performing paired releases of control groups and groups treated with an anti-parasitic agent have become a widespread method to separate the impact of salmon lice from other factors regulating mortality and growth of wild fish in nature. This approach has been extensively used in recent years to study the impact of salmon lice on the marine survival of Atlantic salmon (Gargan et al. 2012, Krkošek et al. 2013, Skilbrei et al. 2013), whilst very few similar studies have focused on sea trout (Gjelland et al. 2014, Skaala et al. 2014, Halttunen et al. 2017). Besides, there are some important caveats associated with this approach. These include limitations in the effect of the treatment (in terms of efficacy, variation and duration), unwanted secondary effects (e.g. toxicity or effects on other parasites) and difficulties in predicting final lice abundances in non-protected (control) fish. In a recent article, Vollset et al. (2018a) provide a thorough review of the conceptual and methodological challenges associated with the quantification of the impacts of salmon lice on the marine survival of Atlantic salmon. After enumerating the strengths and caveats of prophylaxis-based field studies, these authors suggest moving from treatment to exposure studies. The second part of this PhD thesis (**papers III and IV**) follows this suggested methodological shift and investigates the causal relationship between salmon lice and marine mortality of sea trout in nature through exposure experiments in the field.

2 Structure and goals of this thesis

According to the two main goals stated earlier (chapter 1), this thesis is divided in two parts.

The first part (**papers I and II**) investigates the correlation between in-farm production of infective lice copepodids and lice infestations on local sea trout populations. This is done based on a unique and comprehensive data set comprising detailed information on lice abundances on both farmed and wild fish in a wide geographical and temporal scale and covering a wide range of environmental scenarios, including areas with both low and high farming intensities. Besides, the data set included a series of sea trout sampling locations situated inside “National Salmon Fjords”, i.e. areas of especial protection where farming activities are restricted in order to protect wild salmonids. Accordingly, the second goal in the first part of the thesis was to evaluate whether these areas were effectively protecting wild salmonids from aquaculture-driven lice outbreaks (**paper I**).

Two years after the publication of **paper I**, Jansen et al. (2016) argued that that the reported correlation between sea louse abundances on wild and farmed fish could potentially occur as a consequence of temperature, a factor that had not been properly accounted for in the original analysis. In response to this question, we reanalyzed the data in **paper I** with the specific aim of separating the roles of fish farms and temperature on the infestation dynamics on sea trout (**paper II**).

In summary, **papers I and II** aimed to answer the following questions:

- Is the spread of infective louse copepodids from fish farms significantly influencing lice infestations on wild sea trout? (**papers I and II**)
- Is the establishment of National Salmon Fjords an effective management tool to prevent the transmission of lice from farm salmon to wild salmonids? (**paper I**)

- Is the covariation in lice infestations on farmed and wild fish mainly due to a temperature effect? Or is the spread of lice from salmon farms the main driver of lice infestations on wild salmonids, even after the effect of temperature is accounted for? (**paper II**)

The second part of the thesis (**papers III** and **IV**) investigates the effect of salmon lice on the mortality and behavior of sea trout in nature. Given the limitations of the prophylaxis-based approach (briefly referred in the previous chapter), a new experimental approach was conceived based on artificial infestation of wild fish in an area with low lice infestation pressure. This was done in combination with acoustic telemetry, which does not depend on the recapture of the tagged individuals to retrieve the data and further allows observation of fish behavior. This innovative approach was first tested in a pilot study; the methodological progress and limitations of the method, together with recommendations for further studies, were published in a "food-for-thought" article (**paper III**). Once the feasibility of the approach was tested and with the lessons learnt, the approach was implemented in a full-scale design where the impact of salmon lice on the mortality and migratory behavior of sea trout post-smolts was investigated (**paper IV**).

In summary, **papers III and IV** aimed at answering the following questions:

- Is the artificial infestation of sea trout with salmon lice a feasible approach for a field study? What are the methodological progress and limitations associated to this change of method? (**paper III**)
- How does salmon lice affect the survival and behavior of first-time migrating sea trout in nature? (**papers III and IV**).

In this thesis, I use conventional parasitological terms to define the frequency and abundance of salmon lice on a sample or population of salmonid fish (Bush et al. 1997). "Prevalence" is defined as the proportion of infested hosts in a sample. "Abundance" and "intensity" are defined as the number of lice parasitizing any host within a sample or only infested hosts, respectively. "Mean abundance" refers to the arithmetic mean of all abundances in a sample, whereas "mean intensity" is the arithmetic mean of lice intensities. "Relative intensity" is used as a mass-specific measure of intensity, i.e. the number of lice on an infested host divided by its weight in grams. Last, the term "infestation" is used to refer to the frequency distribution of parasites in a host sample or population.

Before presenting the results from these studies (chapter 4), this thesis will include some methodological considerations related to *i)* sampling bias and *ii)* acoustic telemetry and associated handling and tagging effects (chapter 3).

3 Methodological consideration

Sampling bias: is our sample representative?

In order to draw inferences about a population based on a sample of individuals, it is paramount that the sample is representative of the whole population. Sampling design and methods must therefore be carefully selected to reduce potential bias. Monitoring parasite infestations is particularly challenging due to the complex interactions between hosts, parasites and the environment (Dobson & Hudson 1986, Hatcher et al. 2006, Tompkins et al. 2011). For example, the premature return of lice-infested individuals to fresh or brackish water is a well-documented phenomenon for sea trout (Birkeland & Jakobsen 1997). Therefore, sampling methods targeting fish at sea alone may fail to secure observations of heavily infested individuals and can lead to a systematic underestimation of the lice infestation within the total population. Inversely, concentrating sampling efforts in inner estuaries or river mouths can lead to the over-estimation of the total infestation if heavily infested fish concentrates here. Samples from estuarine areas are further complicated to interpret, since newly out-migrated fish or fish that have spent a certain amount on time in freshwater to delouse may also concentrate there. Without a deeper insight into individual fish movements, it can be difficult to evaluate whether the sample is or not representative of the local lice infestation pressure.

The data set analyzed in **papers I and II** includes a total of 2959 fish (sea trout and Arctic char *Salvelinus alpinus L.*), sampled at sea using gillnets. With no sampling in rivers or estuaries, this design may have led to a systematic underestimation of lice abundances for the underlying populations, since the more heavily infested fish can be expected to seek refuge in freshwater. Additionally, heavily infested fish may have already died before they could be caught, leaving this group further underrepresented in our samples.

The choice of sampling gear can also lead to sample bias through gear selectivity. With passive capture techniques, for instance, the behavior and movements of the animals themselves determine their capture probability. This can lead to sampling bias if an individual's behavior is affected by parasite infestation. How data collected through the monitoring program may (or may not) be subjected to sampling bias due to the choice of sampling gear is unknown. There is little information on how salmon lice affect the behavior of sea trout beyond the premature return to freshwater, but a possible selection for more active or "less observant" individuals due to the use of passive fishing gear must be kept in mind when interpreting results from these and other studies.

Acoustic telemetry. Tagging and handling effects.

Acoustic telemetry is a tracking method that can be used to acquire long-term, detailed data on movement of aquatic animals. The animal carries a tag transmitting an acoustic signal to a network of nearby receivers. The signal conveys an identity code and may also include information measured and/or stored by the tag, for example depth-use measured by a pressure sensor, or acceleration measured by an accelerometer. Such data can be used to draw inferences on the fate of the tagged individuals, for instance by identifying predation events through interpretation of vertical profiles.

In studies using acoustic telemetry methods, tagged individuals usually experience significant handling, including capture, anesthesia, surgical implantation of the tag and recovery. In some cases, storage of the fish for short periods is also needed. These handling and tagging procedures may cause negative effects on the fish (e.g. reduced swimming performance, increased stress or even increased mortality), which must be mitigated as much as possible by following appropriate routines. Reducing these possible effects is mandatory from the point of view of scientific ethics, but also to ultimately avoid drawing false conclusions of a study due to abnormal individual behavior (Jepsen et al. 2002, Bridger & Booth 2003, Jepsen et al. 2005). All tagging performed in the studies presented here was done with outmost observation of fish welfare guidelines and approved by local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority (FOTS IDs 8601 and 11907). To further prevent a confounding effect due to handling, control fish in our studies received a sham infestation following the exact same procedure as infested fish in a separated tank.

4 Summary of results

Paper I: Salmon lice infection on wild salmonids in marine protected areas: an evaluation of the Norwegian 'National Salmon Fjords'.

In Norway, 29 fjords have been given special protection to prevent negative impacts from aquaculture activities on important populations of wild salmonids. We evaluated the effect of area protection on the lice infestation pressure for wild salmonids based on lice abundances recorded on sea trout and Arctic charr *Salvelinus alpinus* L. caught inside and outside these protected fjords (known as 'National Salmon Fjords') over a 5-year period. Further, we estimated the number of lice emanating from salmon farms in the vicinity of each sampling location between 2010 and 2011 (when data were available) to investigate a possible correlation between the in-farm production of lice and lice infestations on local sea trout populations.

Results from this study highlight the importance of size on the effectiveness of area protection in preventing the transmission of lice from farm salmon to wild salmonids. Infestation levels in terms of prevalence, mean abundance, mean intensity and relative intensity recorded on wild sea trout caught inside large protected areas (i.e. where the distance to the closest fish farm was > 30 km) were consistently low over time, presumably having little impact on the local populations of wild salmonids. These levels were consistent with those reported in other farm-free areas in Norway and represent a natural lice−host interaction, characterized by occasionally high prevalence but low mean intensity. In the presence of one or more salmon farms within a 30 km distance, on the other hand, the mean abundance of lice on sea trout showed a positive correlation with the accumulated daily production of infective lice larvae in neighboring farms, and approximately 41% of the variation in mean lice abundance on wild fish could be explained by the in-farm production of lice, on a log-log scale. The degree of protection provided by small protected areas is therefore questionable especially for salmon smolts, which must swim through different parts of the fjord and therefore through areas with different lice infestation pressure on their way out to the open sea. In order to establish more precise management practices in Norway and elsewhere, the development and validation of accurate planktonic larval distribution and abundance models are needed.

Paper II: Nearby farms are a source of lice for wild salmonids: a reply to Jansen et al. (2016).

Jansen et al. (2016) questioned the regression analysis presented in Serra-Llinares et al. (2014), which correlates lice abundances on sea trout with estimates of infective lice copepodids emanating from neighboring salmon farms, arguing that the observed correlation might not reflect a cause–effect relationship but may instead be a mere artifact of the spatio-temporal covariance in lice abundance on farmed and wild fish driven by temperature. In this Reply Comment we revisited the analysis presented in Serra-Llinares et al. (2014) and further re-analyzed our data using a zero-altered negative binomial (ZANB) GLMM to rule out the potential confounding effect of temperature.

Partial correlation analysis indicated that the spread of lice copepodids from nearby farms by itself explained 21.8 % of the variation in mean lice abundance on wild fish (on the log-log scale) while controlling for the effect of temperature. Results from the ZANB GLMM indicated a significant effect of both temperature and infestation pressure from nearby farms on both the probability of having one or more lice (binary part of the ZANB GLMM) and on the number of lice on infested fish (zero-truncated part of the ZANB GLMM), despite large variation around the fitted values. In agreement with Helland et al. (2015) and Thorstad et al. (2015), results from this re-analysis further suggests the transmission of lice from farm salmon to wild salmonids in systems where both coexist in close vicinity.

Paper III: Towards direct evidence of the effects of salmon lice (*Lepeophtheirus salmonis* Krøyer) on sea trout (*Salmo trutta* L.) in their natural habitat: proof of concept for a new combination of methods.

In this pilot study, a novel methodological design was tested to study the effects of salmon lice on the survival and behavior of wild sea trout in their natural habitat. We artificially infested sea trout post-smolts with salmon lice in a farm-free area in southern Norway, where low abundances of salmon lice have been recorded on sea trout for the last 10 years. The survival and movements of lice-infested individuals were compared with a control (un-infested) group using acoustic telemetry. Additionally, we combined 3D fish positions provided by acoustic telemetry with salinity estimates provided by a hydrodynamic model.

Results from this pilot study showed consistent trends in behavioral differences between artificially infested and control fish. All fish that died or prematurely returned to freshwater were artificially infested fish, although differences between groups were not statistically significant. Power analysis highlighted nevertheless a limited statistical power of this small pilot study. We also found indications of artificially infested fish remaining in shallower waters and within shorter distance to low salinity habitats. These results supported our idea that this shift in method is the way forward for disentangling and finally quantifying the impact of salmon lice on wild sea trout. Some suggested improvements to increase the statistical power of future studies were: *i)* to increase the number of tagged fish to reach a minimum required sample size, *ii)* to capture the fish on their way out of the river, before they enter the sea, and *iii)* to expand the spatial and/or temporal coverage of the study, for instance by combining acoustic telemetry with PIT tagging.

Paper IV: Impacts of salmon lice on mortality, marine migration distance and premature return in sea trout

We investigated the effect of salmon lice infestation on the survival and behavior of wild trout post-smolts (average fork length = 180 mm) during their marine migration. Comparisons of survival and movements were made between an artificially infested group (n = 74) and a control group (n = 71) in an area with low natural lice infestation pressure using acoustic telemetry.

Artificial infestation was estimated to cause 100 % prevalence and a mean intensity of 65 lice fish^{-1} (mean relative intensity of 2.4 lice g^{-1} fish). Survival analysis showed limited statistical power but revealed lice-induced mortality on artificially infested fish, with an estimated hazard ratio of 2.73 (95 % CI = 1.04−7.13) compared to the control group when data from the pilot study were included in the analysis. Surviving individuals in the infested group additionally responded by residing closer to fresh water while at sea, and by prematurely returning to fresh water. On average, infested fish returned to fresh water after only 18 d at sea, while control fish spent on average 100 d at sea. The residency in the inner part of the fjord and the premature return to fresh water represent an adaptive behavioral response to survive the infestation, at the probable cost of reduced growth opportunities and compromised future fitness.

5 Discussion

Transmission of lice from salmon farms to wild sea trout

The causal relation between salmon farming and increased lice infestations on wild fish is a politically controversial subject, and studies that causally link estimates of the in-farm production of infective lice copepodids with lice abundances on wild fish are still scarce. In this thesis, mean abundances of salmon lice on sea trout were proven to be directly correlated with the estimated number of infective lice copepodids emanating from fish farms within a distance of 30 km (**papers I and II**). Using a simple linear model, results from **paper I** showed that approximately 41% of the variance in mean lice abundance on wild fish could be explained by the production of lice in the farms, on a log-log scale. Furthermore, the re-analysis of the data in **paper II** showed that, although temperature played a role in regulating lice epidemiology on both farmed and wild fish, it alone could not explain the high numbers of lice parasitizing sea trout in areas were farmed and wild fish coexist. These results provide solid evidence of the role of salmon farms as a main source of lice for sea trout in aquaculture-intensive areas in Norway. However, further interpretation of these results deserves some clarifications about the strengths and limitations of the data and of the statistical approach used.

Salmon lice infestations on sea trout can vary enormously across farm-intensive areas, ranging from infestations resembling those recorded in farm-free areas to those indicating high lice-induced mortalities (see Thorstad et al. (2015) and references therein). This large variability is due to multiple factors. Farming practices such as stocking densities and/or delousing regimes, combined with environmental factors such as water temperature or salinity, can have a huge impact on the number of lice larvae released to the environment from different aquaculture sites or areas and over time (Heuch & Mo 2001, Middlemas et al. 2010). Additionally, differences in hydrodynamic conditions (mainly currents and temperature) between sites and with time can result in different dispersal patterns and transport distances for the planktonic lice stages (Asplin et al. 2011, Salama & Rabe 2013). Furthermore, sea trout may move between areas with high or low infestation risk while at sea, including visits to estuaries and freshwater which can result in delousing, further contributing to variation in lice abundances between individuals. All this complexity, common in ecological studies, is hard to predict or fully monitor, and can explain the occasional report of no relationship between salmon lice levels on sea trout and distance to nearby farms, or between lice levels in wild sea trout and neighboring salmon farms (MacKenzie et al. 1998, Marshall 2003, Thorstad et al. 2015).

In this thesis, some of the above-mentioned factors were to some degree included, whereas others remained unaccounted for. Variation in infestation pressure from farms between areas and periods was calculated using reported data on standing stock, average number of mature female lice per fish and water temperature from each salmon farm of interest, but the temporal resolution of these data was limited (based on monthly reports). The dispersal of lice from fish farms was modeled using a simple down-weighting function, where the contribution of each farm in terms of infective lice stages for a particular sampling location decreased linearly as a function of distance. This is a simplification of the mechanisms driving the dispersal of lice. This simplistic approach assumes that salmon lice larvae spread evenly in all directions away from the fish farm, independently of local hydrodynamic conditions, and it also sets a fixed maximum dispersal distance (in this case of 30 km), beyond which the influence of fish farms is considered negligible. Despite its limitations, and in the absence of detailed lice dispersion models applicable to each study location and time, this approach was expected to sufficiently capture the variation in lice infestation risk between sampling events. Fish movements prior to capture were unknown and thus it was impossible to know exactly what lice infestation risk the fish had been exposed to. Added up, all these unaccounted sources of variability can add considerable noise to the data set, potentially weakening posterior analyses.

The choice of parameters used to describe lice infestations and the choice of statistical approach are important for reaching valid and biologically interpretable results. Lice infestations on hosts are often presented as aggregated group values, such as prevalence, mean abundance and/or mean intensity, in order to contain the variation among individuals and facilitate comparisons between groups or populations (Rózsa et al. 2000). As a draw-back, such parameters are highly sensitive to the number of individuals included in each group, especially when the variation between individuals is large. In addition, important biological information such as fish size, sex, etc. may be lost when aggregated into group values, and the biological interpretation of results based on grouped statistics can be difficult. Lice abundance on individual fish, on the other hand, is a more intuitive measure that retains most of the biological information. However, salmon lice infestations are typically over-dispersed and exhibit an excess of zeros, and there are statistical limitations to what kind of methods that can cope with such distributions (Helland et al. 2015).

In **paper I**, salmon lice infestations on sea trout were summarized as mean abundances prior to analysis. Despite the large variability between individuals and the loss of information due to the choice of group values, results still showed a positive correlation between lice on sea trout and lice emanating from nearby farms. However, the biological interpretation of these results is not straightforward. Given the typical over-dispersed distribution of salmon lice among hosts, mean abundance values are little informative unless they are combined with some measure of variance and/or frequency distribution within the host sample. Moreover, as temperature was not explicitly accounted for in this analysis, it was not possible to rule out the possibility of this correlation occurring as a result of the temporal covariation of lice intensities on farmed and wild fish mediated by temperature.

Results from **paper II**, where the same data set were reanalyzed using zero-altered negative binomial (ZANB) models, showed a significant effect of both temperature and infestation pressure from nearby farms on the probability of having one or more lice and on the intensity of the infestation. This further corroborates the role of fish farms as a main factor influencing lice infestations on wild fish, even after the effect of temperature had been accounted for. Despite large variation around the fitted values, the model could explain approximately 25 % of the variance, which is a rather good fit given all the noise in the data set. The model further identified a significant interaction between infestation pressure from farms and water temperature on the probability of a fish having one or more lice, indicating that the potential impact of the infestation pressure imposed by salmon farms was exacerbated in warmer years. No such interaction was detected when modeling the intensity of the infestation (i.e. number of lice parasitizing infested fish). However, this can as well be due to sampling bias, since the more heavily infested fish can be expected to die or seek refuge in freshwater and thus be systematically underrepresented in the samples.

A few papers investigating the causal relation between salmon lice emanating from fish farms and lice infestations on wild fish have been published during the writing of this thesis. Both Helland et al. (2015) and Vollset et al. (2018b) used various statistical methods to analyze data comparable to those used in this thesis and reached similar conclusions. Helland et al. (2015) concluded that, even after correcting for temperature and salinity, infestation pressure from salmon farms significantly increased the probability of wild sea trout having salmon lice. The probability of infestation was also shown to increase with fish body length and temperature and to decrease with increasing freshwater influence. Furthermore, Helland et al. (2015) also found a significant interaction between temperature and infestation pressure from salmon farms, indicating that when the infestation pressure from farms was low, temperature had a strong positive effect on the probability of infestation, but as the infestation pressure from farms increased, temperature gradually became less important. Vollset et al. (2018b) found that the numbers of attached lice on sea trout increased with temperature when the infestation pressure from fish farms was high but not when the infestation pressure was low. Furthermore, nearly all of the effect of rising temperature was indirect and mediated through the infestation pressure from near-by farms.

Direct comparison of results between the above-mentioned studies and those from the current thesis is difficult due to differences in the choice of parameters and statistical approaches. However, the main message is clear: temperature may increase the natural infestation levels on wild sea trout, but these levels will increase much more rapidly when the infestation pressure from surrounding fish farms is high. A significant effect of water temperature on salmon lice infestation dynamics is not surprising, since the rate of development, the *per capita* reproductive rate of female lice and the infestation success, among other parameters, are known to be temperature-dependent (Stien et al. 2005, Samsing et al. 2016). However, since this applies to lice parasitizing both farmed and wild salmonids, and given that the number of farmed salmon can exceed 10 000 times the number of wild Atlantic salmon present in some

Norwegian fjords (Skaala et al. 2014), salmon farms still ought to represent a main source of lice for wild salmonids, even when the numbers of female lice per farmed salmon remain low. Importantly, it has been recently documented that lice collected from wild salmonids in farm-dense areas often carry resistance to organophosphates in a frequency that mimics those in nearby farms, providing further proof that salmon farms are a primary driver of the salmon louse infection dynamics (Fjørtoft et al. 2017, Fjørtoft et al. 2019).

Evaluation of the National Salmon Fjords

Area protection is one of the main strategies adopted by Norwegian authorities to protect local populations of Atlantic salmon, sea trout, and Arctic charr from negative impacts of aquaculture activities (Aasetre & Vik 2013). Within a National Salmon Fjord, the strict limitations imposed on fish farming activities are presumed to minimize the risks for lice being transmitted from farm salmon to wild salmonids.

Lice abundances reported on sea trout inside large National Salmon Fjords (i.e. where the distance to the closest fish farm was > 30 km) in this thesis (**papers I and II**) were found to be consistently low over time, presumably having little effect on the local sea trout populations. Lice infestations in this areas were comparable with those reported in other farm-free areas in Norway (Schram et al. 1998, Rikardsen 2004) and represent a natural lice–host interaction, characterized by sometimes high prevalence but low mean intensities (Tingley et al. 1997).

On the other hand, high lice abundances were occasionally observed on wild sea trout caught inside the smallest National Salmon Fjords. Large variations between years were also observed in these areas, with lice abundances oscillating in accordance with variations in the stocked biomass in the surrounding farms. Lice infestations in these areas sometimes matched and even exceeded those previously reported in areas of intensive farming in Norway (Birkeland 1996, Bjørn et al. 2001, Bjørn et al. 2011). These results suggest that the production and transport of planktonic lice larvae from farms situated outside the limits of the protected areas might still be an important source of lice for local populations of sea trout. The efficacy of small protected areas seems therefore limited.

On the other hand, the main reason for the establishment of the National Salmon Fjords in Norway was the protection of important Atlantic salmon populations. Unfortunately, sampling Atlantic salmon post-smolts at sea is difficult and costly (Finstad et al. 2000) and thus lice burdens on sea trout have often been used as proxy for lice burdens on Atlantic salmon. Nevertheless, the effects of area protection might not be the same for both species owing to significant behavioral differences. Unlike sea trout, which spends most of their marine migration in littoral areas often within a few kilometers of their native rivers (Jensen 1968, Berg & Berg 1987, Thorstad et al. 2016), Atlantic salmon post-smolts must swim through different parts of the fjords in their journey from their natal river to the open sea. During this migration, which may last several weeks (depending on the location of the river, length of the fjord and water currents), the fish will be exposed to different infestation pressures, with the total risk pressure depending on the time spent in different fjord stretches (Thorstad et al. 2004, Finstad et al. 2005, Harvey et al. 2019). Since the size of the protected areas is limited and rarely cover the whole length of a fjord system, this cannot be the only tool used to protect Atlantic salmon against the transmission of salmon lice from fish farms.

In summary, results from this thesis indicate that the Norwegian National Salmon Fjords can effectively protect wild salmonids against infestations with lice of farm origin, but only if the size and configuration of the protected area is adequate. The area of influence of a given fish farm (i.e. the expected spatio-temporal distribution of infective salmon lice emanating from it) is strongly dependent on hydrodynamic conditions, and it can only be predicted through the development and validation of accurate planktonic larval distribution models. At present there are 29 National Salmon Fjords in Norway, encompassing a large disparity in size and latitude, position within the fjord (deep inside the fjord or in the outer coast) and proximity to aquaculture sites. Although individual studies of each National Salmon Fjord may be necessary to evaluate the degree of protection, one can expect that the capacities of the smallest fjords of withstanding lice infestation from fish farms are probably limited, and it is reasonable to assume that the protection of large areas contributes best to ensure the protection of wild salmon. The optimal design will in any case depend on the target species. While sea trout may benefit from the establishment of a local protected area extending over their marine home range, the protection of Atlantic salmon may require the protection of entire fjords or the establishment of “clean corridors” through their migration routes.

Impacts of salmon lice on individual sea trout in their natural environment

Scientific evidence that salmon lice can impact the marine survival of sea trout is robust. Laboratory studies have shown that salmon lice can cause direct mortality of the host, and mortality thresholds have been drawn based on such studies. However, free-swimming fish face several additional challenges, such as predation, competition or exposure to other pathogens and parasites (Krkošek et al. 2011, Godwin et al. 2015), and thus mortality at sea results from a combination of factors. Additionally, salmon lice may further impact mortality through indirect mechanisms, by modulating the impact of other factors affecting the marine survival of sea trout. For instance, salmon lice may impair the swimming ability of the host, which will expose the fish to a higher risk of being eaten by a predator. Thus, laboratory studies where the impact of lice is examined in isolation from all other factors affecting the marine mortality of sea trout are likely to underestimate the real impact on free-swimming fish. A few field studies using released groups of treated and untreated fish have been used to try to quantify effects of salmon lice on sea trout (Gjelland et al. 2014, Skaala et al. 2014, Halttunen et al. 2017), but these have often been limited by low numbers of returning individuals, large natural variation in mortality and methodological caveats. As a consequence, some of these studies have failed to find a significant association between salmon lice and the marine survival of sea trout (Gjelland et al. 2014, Halttunen et al. 2017), and thus proof of a causal relationship has remained elusive. Vollset et al. (2018a) have recently reviewed different conceptual and methodological aspects related to the quantification of the impacts of salmon lice on the marine survival of Atlantic salmon, much of which can also be applied to sea trout. In this exhaustive article, the authors provide a thorough discussion on the strengths and caveats of prophylaxis-based field studies, and further suggest to move from treatment to exposure studies.

The studies presented in this thesis (**papers III and IV**) pick up the baton and embrace the suggested methodological shift. Since the baseline marine mortality of sea trout post-smolts is known to be high, especially right upon entrance to the sea, acoustic telemetry was added to the design to alleviate the dependence on returns or recaptures, which could be anticipated to be limited. Another way to increase statistical power of the study would be to significantly increase the number of fish released in the experiment. However, the release of a large number of artificially infested sea trout in an otherwise relatively lice-free area may have ecological and ethical implications that, in any case, would require thorough consideration. Using a similar approach, Sivertsgård et al. (2007) compared the marine mortality of hatchery-reared sea trout equipped with acoustic transmitters in one of three groups: i) artificially infested, ii) control and iii) lice-protected. No differences in mortality where observed between the three groups during fjord migration in this study. However, the study was poorly designed to monitor sea trout movements and extended only over a short time period, during which the salmon lice could develop only to the chalimus stage.

Based on exposure field studies, combined with acoustic telemetry, this thesis (**papers III and IV**) reveals a causal relationship between salmon lice and marine mortality of sea trout in nature. Results from these studies show a mortality hazard ratio of 2.7 (95% CI = 1.04−7.13) for lice-infested trout post-smolts compared to non-infested control individuals. In other words, the probability of a trout post-smolt to survive the first summer at sea was reduced by almost two-thirds when infested with a heavy lice load, although there was a large uncertainty around this estimate. In this case, a heavy lice load implied an average relative intensity of 2.4 lice g^{-1}, well beyond the estimated mortality-inducing threshold of 0.3 lice g^{-1} (Taranger et al. 2015). Although very high, these lice levels are nowadays not implausible in Norway, where the percentage of sea trout carrying relative intensities >2 lice g^{-1} has occasionally been registered to be as high as 70 % in some areas with intensive fish farming. Birkeland and Jakobsen (1997) also reported extremely high lice levels on sea trout in Samnangerfjorden, western Norway. In their study, control post-smolts that returned after only one day in the sea carried a median of 175 lice (or >6 lice gr^{-1}), and after four days in the sea the median infestation was 450 lice (16 lice/gr^{-1}). Additionally, it must be noted that, in this study, lice abundances on infested fish refer exclusively to newly attached copepodids. Given that some lice mortality can be anticipated between developmental stages, and given the high growth potential of sea trout at sea, lice burdens (estimated as lice/gr^{-1}) can be assumed to decrease gradually during the weeks following infestation unless the fish is exposed to new infestation.

In summary, results from this thesis indicate that, in areas with high lice infestation pressure, the number of trout post-smolts surviving the first season at sea may be significantly reduced. These results are in agreement with Skaala et al. (2014), who showed that the survival of sea trout protected against lice was nearly doubled compared to non-treated fish in a large-scale field experiment in a fjord with intensive salmon farming. To our knowledge, this thesis is the only other study providing proof of a causal relationship between salmon lice and the marine survival of wild sea trout in their natural environment. In any case, the shape of the relationship between lice abundance and marine mortality of sea trout can be expected to vary across years and locations, and thus further studies over a number of years and under different environmental conditions are still needed.

Direct mortality effects are not the only way salmon lice can impact sea trout populations. In this thesis, we document an altered migration behavior of lice-infested individuals, including the abrupt interruption of the feeding migration after just a few weeks at sea and a preference for feeding areas closer to the river, in the inner part of the fjord. Premature return to fresh water of lice-infested trout has been previously documented in Ireland, Scotland and Norway (Tully et al. 1993, Birkeland & Jakobsen 1997, Pert et al. 2009), and is seen as a behavioral adaptation of the fish to delouse and regain osmotic balance in order to survive the infestation (Birkeland & Jakobsen 1997, Bjørn et al. 2001). In this thesis (**paper IV**), lice-infested sea trout returned to freshwater after only 18 days at sea, whereas control fish spent on average 100 days at sea before return. This implies a loss of almost 80 % of the feeding time at sea as a consequence of salmon lice. Since growth in the sea is generally better than in fresh water (L'Abee-Lund et al. 1989, Frier 1994), and growth is positively correlated with the duration of the sea sojourn (Berg & Jonsson 1990), an abrupt interruption of the feeding migration after just a few weeks at sea, as reported in this study, may result in a significant loss of feeding and growth opportunities and further impact other life history traits such as fecundity or competitive ability (Einum & Fleming 1999, Jonsson & Jonsson 2011). Also, reduced summer growth may additionally reduce the probability of surviving through the following winter (Jensen et al. 2018). How these impacts may affect the whole population could in theory be quantified using e.g. matrix population models or spawner-recruit analyses. However, quantitative data to feed into such models are still largely missing for sea trout, making their practical application still difficult.

All in all, results from this thesis (**papers III and IV**) highlight the benefits of moving from treatment to exposure field studies to quantify the impacts of salmon lice on sea trout in nature. Besides, prophylaxis-based studies are by definition not suitable for describing the infestation intensity—

mortality relationship, as they only test the effect of treatment (with an unknown efficacy) against unknown infestation intensities of salmon lice on untreated individuals (Vollset et al. 2018a). Further exposure studies in the field, with larger data sets over a number of years and under different environmental conditions are therefore warranted. Such studies should ideally include lice counting on individually tagged fish, so that the impact of salmon lice on marine mortality could be studied across a range of lice abundances.

6 Future prospects

Reduced marine survival and growth as a result of increased lice abundances can shift the balance between costs and profits of anadromy and ultimately lead to a decrease in the frequency of sea-run brown trout in areas of intensive farming (Gargan et al. 2006). The likelihood of moderate heritability of anadromy as a trait, combined with the higher reproductive potential of larger sea trout, can ultimately lead to a lower abundance of brown trout and reduced recruitment (Thorstad et al. 2015). Anadromy also gives populations the possibility to escape poor environmental conditions, such as extreme cold, dry-out periods, warm water or low oxygen levels to name a few. Smaller catchments that regularly experience such conditions during parts of the year will therefore be especially vulnerable to changes in the marine environment and are at risk of losing their brown trout populations if the marine mortality is persistently high.

Sea trout have historically contributed significantly to the culture and economy of coastal communities, supporting important fisheries and incentivizing river conservation (Elliott 1989). Unfortunately, a marked decline in sea trout stocks has been documented in Ireland, Scotland and Norway, and have been linked to infestation of lice from salmon farms (Tully et al. 1993, Tully et al. 1999, Bjørn et al. 2001, Gargan et al. 2003, Butler & Walker 2006). Despite trout being a culturally, socially and economically important species, there are still important knowledge gaps on its population biology and potential responses to parasites. This is partly explained by a complex and flexible life history with a wide range of strategies within and between populations. Notwithstanding the challenges, this information is urgently needed.

Understanding salmon lice infestations on wild salmonids require advanced statistical methods and large datasets in order to handle the natural variation in such complex systems (Helland et al. 2015). Although complex statistical models combining detailed information on the production and dispersal of infective lice larvae from aquaculture sites, wild fish movements, infestation rates and populations dynamics may become central in the management of salmon lice and aquaculture in the near future, extensive monitoring programs generating comprehensive datasets for risk assessment will continue to be central for understanding and managing the impact of salmon lice on wild salmonids until the uncertainties mentioned in this thesis can be significantly reduced.

Improvements in sampling design to reduce bias, the acquisition of more accurate estimates of relevant environmental data and a deeper understanding of fish movements can all contribute to reduce the noise and improve the models. Especially relevant is the development and validation of accurate planktonic larval distribution. In the last 20 years, the development of coupled biological−hydrodynamic lice dispersion models has become widespread (Salama et al. 2013). These kinds of models combine detailed information about water currents and other forcing and boundary values with lice behavior and development parameters to predict the distribution of infective salmon lice copepodids from a given source in time and space. Such models constitute today the basis for more precise management practices of lice in fish farms along the Norwegian coast, but their full potential is still to be reached.

Last, studies that accurately quantify the impact of salmon lice on wild sea trout and their populations are still urgently needed. Exposure-based field studies including lice counting on individually tagged fish are arguably the best option for this enterprise. Such studies, replicated over a number of locations and time periods, are likely to deliver much sought-after quantitative data on the impact of salmon lice, allowing the formulation of more accurate threshold levels for wild sea trout in nature. The impacts of salmon lice on wild salmonids will continue to be an important topic for marine aquaculture and conservation of wild salmon in the years ahead, and robust scientific knowledge on the impact of lice on individuals and populations is key to the implementation of sound conservation strategies.

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Impacts of salmon lice on mortality, marine migration distance and premature return in sea trout

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ABSTRACT: Brown trout *Salmo trutta* (L.) is a facultative anadromous species, where a portion of individuals in populations with access to the sea perform migrations to use the richer feeding resources. We investigated the effect of salmon lice *Lepeophtheirus salmonis* (Krøyer 1837) infestation on the survival and behaviour of wild trout post-smolts (average fork length = 180 mm) during their marine migration. Comparisons of the marine migratory behaviour were made between an artificially infested group (n = 74) and a control group (n = 71) in an area with low natural lice infestation pressure. Artificial infestation was estimated to cause 100% prevalence and a mean intensity of 65 lice fish^{-1} (mean relative intensity of 2.4 lice g^{-1} fish). Survival analysis showed limited statistical power but revealed lice-induced mortality, with an estimated hazard ratio of 2.73 (95% CI = 1.04–7.13) compared to the control group, when data from a previous pilot study were included. Surviving individuals in the infested group additionally responded by residing closer to fresh water while at sea, and by prematurely returning to fresh water. On average, infested fish returned to fresh water after only 18 d at sea, while control fish spent on average 100 d at sea. The residency in the inner part of the fjord and the premature return to fresh water represent an adaptive behavioural response to survive the infestation, at the probable cost of reduced growth opportunities and compromised future fitness.

KEY WORDS: Parasite · Salmon louse · *Lepeophtheirus salmonis* · Sea trout · *Salmo trutta* · Epizootic · Acoustic telemetry · Mortality · Behaviour · Field experiment

1. INTRODUCTION

Migration is a behavioural adaptation that occurs in many animal taxa, enabling individuals to use the best-suited habitat during different life stages and seasons and thereby leading to an increase in individual fitness (Lucas & Baras 2001, Dingle & Drake 2007). Partial migration, where only a fraction of the individuals in a population perform migrations, is common, and the balance between the costs and benefits of migration versus residency is thought to affect the tendency to migrate (Chapman et al. 2012, Sahashi & Morita 2018). Salmonid fish spawn in fresh water, but in many of the species, some or all individuals perform migrations to use the richer feeding resources at sea (Gross et al. 1988). Among salmonids, brown trout *Salmo trutta* is an especially adaptable and flexible species, showing considerable life-history variation within and among populations (Klemetsen et al. 2003), including partial migration. For sea trout (i.e. the anadromous form of brown trout), the advantages of marine migration include

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the opportunity to access more food, which in turn enhances growth and fecundity. On the other hand, costs may be related to increased predation risk and physiological adjustment to different salinities. The balance of costs and benefits associated with freshwater residency and anadromy may result in different life-history strategies coexisting within the same water system (Jonsson & Jonsson 1993, 2006, Ferguson 2006). Females tend to adopt the anadromous life-history strategy more than males (Jensen 1968, Pratten & Shearer 1983, Euzenat et al. 1999, Knutsen et al. 2004), arguably because females have more to gain by increasing body size in terms of higher fecundity (Thorstad et al. 2016). Anthropogenic impacts that reduce the benefits or increase the costs of migration may result in selection against migration, altered life-history traits, reduced body size of individuals and reduced recruitment on a population level (Chapman et al. 2012, Shaw 2016). Studies of how human activities influence animals during their migration are therefore necessary to evaluate consequences for wildlife and biodiversity, both at the individual and population levels, and for the consideration of management measures.

Atlantic salmon *Salmo salar* (L.) farming has become a large industry, negatively impacting wild salmonids through the occurrence of farmed salmon escapees and the spread of salmon lice *Lepeophtheirus salmonis* (Krøyer,1837) and infectious diseases (Taranger et al. 2015, Forseth et al. 2017). Salmon lice are marine parasites that occur naturally on wild salmonids, but salmon farming has increased the number of potential hosts for lice in coastal areas, resulting in an increased infestation pressure on wild salmonids (Heuch & Mo 2001, Krkošek et al. 2005, Jansen et al. 2012). Sea trout are particularly at risk of experiencing unnaturally high infestation rates as a result of salmon farming, because they typically remain inside the fjords or in coastal waters during their entire marine migration, where most salmon farms are situated.

Salmon lice are ectoparasites that feed on the mucus, skin, muscle and blood of the host fish, causing tissue erosion, osmoregulatory dysfunction, physiological stress, reduced feeding and growth, and increased mortality (Birkeland & Jakobsen 1997, Bjørn & Finstad 1997, Dawson 1998, Bjørn et al. 2001, Wells et al. 2006). Salmon lice-induced stress responses and mortality have been documented for both hatchery-reared and wild trout post-smolts under laboratory conditions (Bjørn & Finstad 1997, 1998, Wells et al. 2006, 2007). Equivalent physiological disturbances, including an integrated stress response and osmoregulatory disfunctions, have been shown on free-swimming wild trout post-smolts both feeding at sea and prematurely returned to fresh water (Poole et al. 2000, Bjørn et al. 2001). Based on threshold levels shown to induce mortality in laboratory experiments, wild trout carrying potentially deadly lice loads are at times reported in Scotland, Ireland and Norway (Thorstad et al. 2015). However, conclusive results from field experiments are still scarce, and quantitative knowledge on the effects of lice on sea trout in their natural habitat is still limited. Moreover, free-ranging sea trout have the capacity to modify their behaviour to mitigate physiological stress and osmoregulatory dysfunction by prematurely returning to fresh water (Birkeland 1996, Bjørn et al. 2001, Gjelland et al. 2014). This would enable infested fish to regain osmotic balance and increase chance of survival at the probable cost of reduced growth.

Knowledge of the impacts of lice on trout is extensive, but field studies on wild fish that examine population-level effects are still needed (Thorstad et al. 2015). Disentangling the relative role of lice from other factors impacting mortality and fitness of wild fish in nature is challenging. Population-level effects have been quantified in Atlantic salmon in large-scale field studies by comparing the survival of fish chemically protected against lice to that of untreated control fish (Gargan et al. 2012, Vollset et al. 2014). Few such studies have been performed on trout (Skaala et al. 2014). These studies have primarily relied on the natural lice infestation level in the study area, which can be highly variable in intensively farmed areas (Serra-Llinares et al. 2014, 2016, Helland et al. 2015). This, in combination with the typically skewed distribution of lice among individuals and the limitations of the prophylactic treatment, make it difficult to deduce the actual infestation level of the experimental fish. An alternative approach is to compare artificially infested fish with non-infested control fish in a farm-free area with low natural lice levels. This approach does not rely on the limited duration of the effect from the prophylactic drug and allows for better control of the levels of lice on the studied fish. Moreover, other secondary effects associated with the treatment are not expected. Serra-Llinares et al. (2018) performed a pilot study to test this new method using wild trout post-smolts (n = 29) caught in bag nets shortly after they entered the sea in Sandnesfjord, southern Norway. The authors concluded that, despite limitations in the statistical power rendered by the study, results indicated consistent trends in behavioural differences between control and artificially infested fish, suggesting that this shift in method (i.e. using artificial infestation instead of prophylaxis) is a promising novel approach to study impacts from lice on wild free-swimming sea trout. Furthermore, the authors made recommendations on how to increase the power of an eventual follow-up study by (1) increasing the number of individuals tagged, (2) capturing the fish before they enter the sea, to ensure lice-free individuals and (3) expanding the spatial/temporal coverage of the study by use, for instance, of a combination of acoustic telemetry and PIT-tagging. This approach with suggested improvements is followed up in the present study.

Here, we used acoustic telemetry methods to investigate the survival and habitat use of wild trout post-smolts during their marine migration in an area without fish farms and with a low lice infestation pressure. The main aim was to study the impacts of lice on (1) survival, (2) rate and timing of freshwater returns and (3) migration behaviour (in terms of horizontal and depth use of the fjord) by comparing the movements of trout artificially infested with lice and non-infested control fish. Fish in both groups were tagged with acoustic transmitters with depth sensors, enabling recording of both horizontal and vertical fish movements. We hypothesized that (1) lice-infested fish would have a lower survival probability during the first summer at sea compared to control fish; (2) infested fish would return to fresh water earlier than control fish, to regain osmotic balance and de-louse, and (3) infested fish would remain closer to the river and/or swim higher up in the water column, seeking areas/layers with lower salinity.

2. MATERIALS AND METHODS

2.1. Study area

The study was performed in Sandnesfjord, southern Norway (58.6943° N, 9.1488° E, Fig. 1) from June to November 2017. There is no salmon farming activity in this fjord, with the nearest active salmon farm located more than 85 km (sea-way distance) away. Consistently low lice levels were recorded on wild sea trout in Sandnesfjord in the period 2010–2017, with a mean prevalence of 30% (range 0–98%) and a mean intensity of 2.5 lice per infested fish (range 1–13) (Karlsen et al. 2018).

The river Storelva flows into Sandnesfjord through a transition area (Songevatn estuary and Nævestadfjord, Fig. 1). The salinity in Sandnesfjord is commonly above 20 psu, but the inner part can have a shallow layer of fresh or more brackish water. The transition area is characterized by a strong salinity gradient, increasing from close to 0 psu at the surface to over 30 psu at 50 m depth. In the upper 3 m, salinity is commonly between 0 and 15 psu, depending on depth, freshwater supply and the hydrodynamic properties of the coastal current (Tjomsland & Kroglund 2010, Haraldstad et al. 2016).

Atlantic salmon and brown trout use the lowermost 20 km of the river Storelva as spawning and nursery habitats. The catchment area is 409 km^2, with an average annual water discharge of 12 $m^3 s^{-1}$ measured at the outlet of Lake Lundevatn (Norwegian Water Resources and Energy Directorate ID: 18.4.0, HYDRA database NVE, www.nve.no/hydrologi/hydrologiske-data/historiske-data/data-i-hydra-ii-databasen/). The local trout population has been monitored for several years. The smolts descend during April and May, with an average annual total length ranging from 150 – 190 mm (Haraldstad et al. 2017). Sea trout in this system usually spend 2 growth seasons at sea before first returning to the river (Haraldstad & Güttrup 2015). Annual sea survival from smolt to first-time spawning ranges from 14–18%, while survival from one spawning to another ranges from 30 – 60% (Haraldstad et al. 2018). During a study by Olsen et al. (2006), the age of the sea trout caught in the Skagerrak coastal area, to which the Storelva catchment belongs, between 1998 and 2003 ranged from 2–8 yr, with more females (64%) than males. The average growth during the first season at sea is about 150 mm (K. Bleeker pers. comm.).

2.2. Hydrographic conditions

Estimates of temperature and salinity to describe the hydrographic properties in Sandnesfjord were retrieved from a hydro-dynamical model simulation using the Regional Ocean Modeling System (ROMS, http://myroms.org; Shchepetkin & McWilliams 2005, Haidvogel et al. 2008) applying 32 m × 32 m horizontal resolution. The highest-resolution model was based on a four-fold nested model system where the horizontal grid was refined from 4 km (the operational forecast model for the Nordic Seas provided by the Norwegian Meteorological Institute and accessible at http://thredds.met.no) to 800 m (see e.g. Albretsen et al. 2011) and 160 m, all model systems using ROMS. Tides were added to the boundaries of the 800 m model and interpolated from the global TPXO7.2 (Egbert & Erofeeva 2002). Applications evaluating and using 160 m model systems are

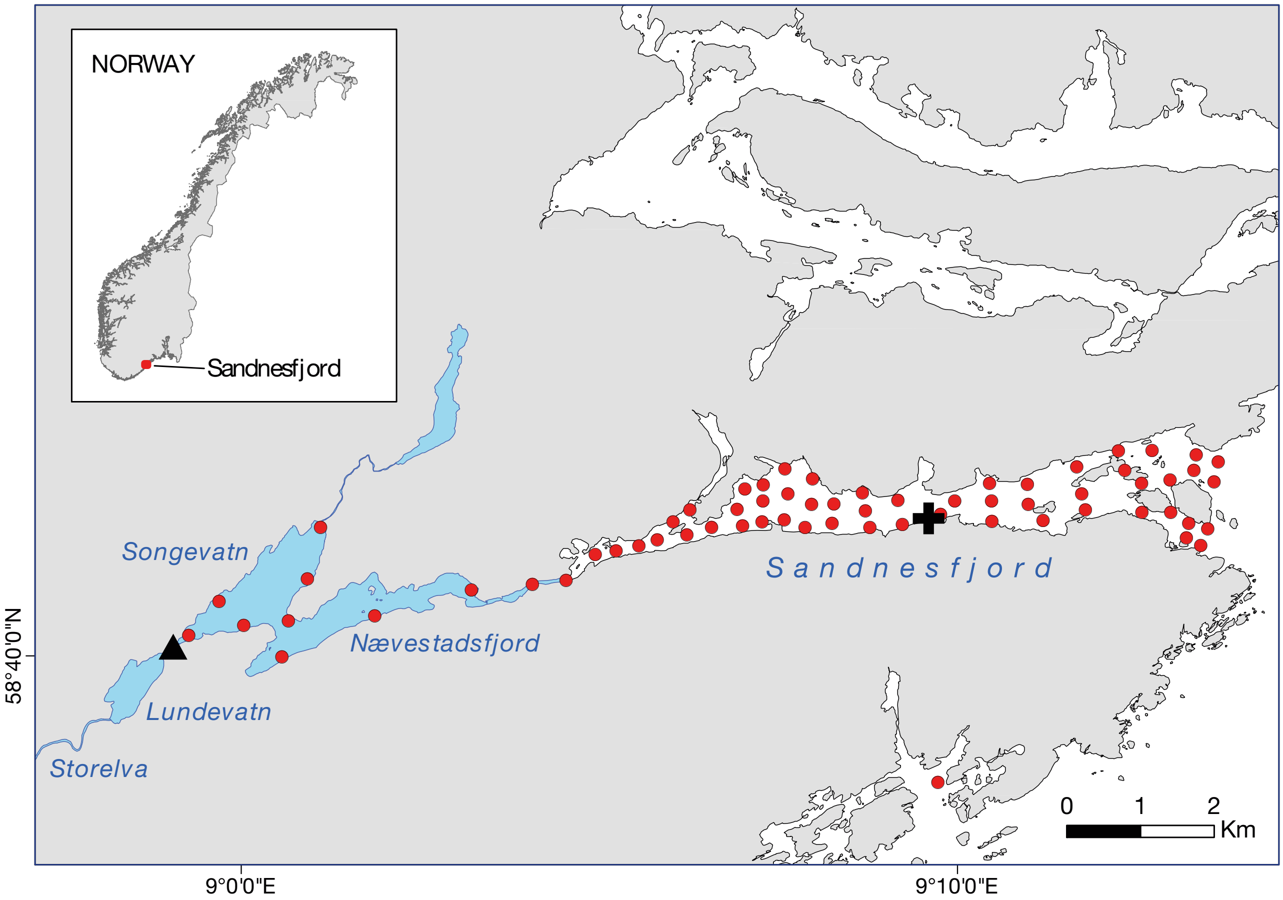


Fig. 1. Study area. Freshwater and transition areas are indicated in blue. The positions for the acoustic receivers (red dots), the fish trap and PIT antenna (black triangle) and the tagging and release location (black cross) are indicated. Note: the farthest receiver, situated approximately 10 km further south from the outermost receiver in the map, is not shown

described by Espeland et al. (2015) and Huserbråten et al. (2018). The 800, 160 and 32 m models applied high-resolution atmospheric forcing from the non-hydrostatic 2.5 km AROME MetCoOp regional atmospheric model (Müller et al. 2017) provided by the Norwegian Meteorological Institute and accessible at http://thredds.met.no. The freshwater runoff from Storelva was inputted in the model downstream of the narrow strait of Lagstrømmen (the transition zone denoted with a change from blue to white in Fig. 1), and volume fluxes for all rivers applied in the ROMS models were based on daily measurements from the Norwegian Water Resources and Energy Directorate.

2.3. Fish tagging and artificial infestation

Wild trout post-smolts (n = 145; average ± SD fork length [FL] = 180 ± 14 mm; range 155–226 mm) were captured in a rotary screw fish trap located in the river mouth (Haraldstad et al. 2017) (Fig. 1) between 18 and 24 May 2017. A rotary screw trap is a sampling gear that takes advantage of flowing water to gently capture and retain downstream migrating fish (Chaput & Jones 2004). The trap was monitored once or twice a day, depending on capture rates. Captured fish were identified to species, and trout post-smolts were held for a maximum of 48 h before further handling.

Early-stage lice are highly sensitive to fresh water (Wright et al. 2016). Consequently, the fish were transported by boat in a tank with oxygenated water from the capture site in fresh water to the more saline central fjord prior to tagging and artificial infestation (Fig. 1). First, the fish were moved into a large tank with circulating fjord water, where they could recover and acclimatize for several hours prior to tagging. The fish were then tagged with individually coded acoustic tags with depth sensors produced by

Thelma Biotel (model ADT-LP-7.3, size: 22 × 7.3 mm; 1.1 g in water; depth sensor with 0.2 m resolution and maximum depth of 51 m, random pulse repetition intervals of 30–90 s; typical battery life of 5 mo). We anaesthetized the fish by a 3 min immersion in an aqueous solution of benzocaine (0.1–0.2 ml Benzoak® l^{-1}). We then made a small incision on the ventral surface posterior to the pelvic girdle, through which we inserted the acoustic transmitter. Additionally, a PIT tag (23 mm, half duplex, Oregon RFID) was also inserted into the body cavity via the same incision, which was closed using a single silk suture (4/0 Ethicon). After tagging, the fish were randomly assigned to 1 of 2 groups: a control group (n = 71, average ± SD FL: 181 ± 14 mm; range 156–220 mm) or an infestation group (n = 74, FL: 180 ± 14 mm; range 155–226 mm) and placed into a small net-pen at sea with 2 separated compartments for a recovery period prior to infestation (1–5 d).

Salmon lice copepodids ('LsGulen' family, 30/31 generation in the laboratory) used for artificial infestation were provided by the Institute of Marine Research in Bergen. Approximately 35 000 copepodids were collected in the laboratory and sent to the field site in Sandnesfjord. Incubation and quantitative assessment were performed as described by Hamre et al. (2009).

For artificial infestation, all fish in the infested group were placed in an infestation tank (1 × 1 × 0.8 m fibreglass tank) with circulating fjord water (13°C, 25 psu), where they were exposed to approximately 200 copepodids each (i.e. approximately 14 800 copepodids were added to the infestation tank). During infestation, water circulation in the tank was stopped and the level was kept at approximately 10 cm for 30 min. The oxygen content was continuously monitored and regulated during the process. After infestation, the water circulation was re-established in the tanks, where the fish could recover for 12 h prior to release. To reduce the risk of a potential handling effect impacting our results, the control group received a parallel sham infestation following the same procedure as infested fish in a separate tank. After recovery, all control and infested fish were released in the middle part of the fjord (Fig. 1).

To evaluate the success of the artificial lice infestation, additional 30 post-smolts (FL: 151 ± 18 mm; range 119–196 mm) were used as laboratory reference groups. These were not tagged with acoustic transmitters. Half of them were infested with lice together with the infested fish which were released, as described above. After infestation, both infested and non-infested reference groups were transported by boat in a tank with 2 compartments to the nearby research facilities in Flødevigen, where they were placed in 2 separate tanks (1 per group). After 1 wk, their lice levels were recorded.

2.4. Fish tracking

The movements of tagged fish were monitored from release (25 May 2017) to the end of the study (29 October 2017) through an array of receivers (Vemco VR2W, which recorded depth, ID and the time when tags were within receiver range) covering the study area both in the fjord and in the transition areas. Range tests indicated an optimal detection range of up to at least 150–200 m (85 % of the signals were still recorded at this distance). Based on this, we designed a hydrophone array consisting of 66 receivers, of which 55 were placed in sea water and 11 in the transition area (Fig. 1). Migration out of the fjord was monitored by a double array of receivers at the fjord mouth, enabling recording of the movement direction of the fish in this area.

Movements of fish in the river were detected using 2 swim-through PIT-antennas located 150 m upstream of the river mouth (Haraldstad et al. 2017). At this location, the river is 9 m wide and 0.9 m deep. The 2 antennas were installed 2.5 m apart and wired to 2 remote tuner boards, one for each antenna. The tuner boards were connected to an antenna reader box (TIRIS RI-CTL MB2A; Oregon RFID) and supplied with a 12V battery. When a tagged fish passed through the antenna loop, the PIT-tag number, antenna number, date and time were recorded and logged by the reader box.

2.5. Fish fate assessment

Individual fish fates were assessed by examination of individual detection plots. Based on their vertical profile and horizontal movements, the fish were classified as:

(1) Dead, when the vertical profile indicated that a tag became stationary. When a diving pattern inconsistent with trout vertical swimming behaviour was observed prior to the tag becoming stationary, the fish was considered to have been eaten by a predator (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m635p151_supp.pdf). The final individual trout record was defined as the last transmitter detection before the tag became stationary or before the predation event. Transmitter detections after this time were removed before further analyses.

(2) Returned, for fish swimming into the transition area and/or the river (hereafter referred to as fresh water) and remaining there for the rest of the study (i.e. never observed in the main fjord again). Returns before 1 August 2017 were further categorized as early returns, as less than 25% of first-time migrants return to the river Storelva before that date (Haraldstad & Güttrup 2015).

(3) Migrated, when last detections occurred in the outer double receiver array in an outwards direction.

(4) Alive at sea, for fish detected alive in the main fjord by 1 October 2017 unless another fate could be assigned after that date. This date was chosen based on the expected life span of the acoustic tags.

(5) Unknown, for fish disappearing inside the study area before the end of the study and before the end of the expected lifetime of the tag.

2.6. Statistical analyses

All analyses were performed using the R software version 3.6.0. The receivers recorded 2166380 detections with IDs matching those from tagged trout, and 1601 detections with IDs not corresponding to any tagged fish (which were consequently discarded). A single receiver accumulated 27% of these unidentified detections. To filter out additional false detections with IDs matching those from fish tags, single detections within a 24 h window were considered as spurious and discarded. A more restrictive 2 h filter was applied to the receiver with the most false detections. In total, 1242 spurious detections were removed following this method. Finally, we examined individual vertical and horizontal plots to detect and remove remaining false detections, based on spatial inconsistency.

Fish positions (1 h intervals) were estimated using center-of-activity locations (Simpfendorfer et al. 2002). The center-of-activity location for a given time interval is the mean position of the hydrophones that detected the animal at that time interval, weighted by the number of times the animal was detected at each hydrophone (Simpfendorfer et al. 2002). A mean depth value was also associated with each center-of-activity position, so that they consisted of a 3D position with latitude, longitude and depth. In addition, we calculated sea-way distance to fresh water (i.e. to the Nævestadfjord outlet) for each center-of-activity position using the 'Spatial Analyst' package from ArcGIS.

Differences in mortality and return to fresh water between control and infested fish were analysed using Cox proportional hazard (Coxph) models, fitted with the 'survival' package (Therneau 2014) in R. For analysis of mortality, the fate/status of each individual fish was set as 1 (died) for fish having a vertical profile indicating mortality, and as 0 (alive) for fish for which mortality could not be identified. Fate time (t) was specified as the number of days between tagging and the observed fate and specified as right-censored data. By using right-censored data, we could use information on all fish, including fish that migrated out of the study area, which were alive at least until they left. For fish that died by fishing, the fate was set as 0 and the fishing date was used as the fate date. The following Coxph model was used to model the hazard of death at time t, as a function of group (control/ infested) and fish length (FL):

$$h(t) = h_0(t) \times e^{(\beta_1 \mathit{Group} + \beta_2 \mathit{Fish\ Length} + \beta_3 \mathit{Group} \times \mathit{Fish\ Length})} \quad (1)$$

where $h(t)$ is the hazard of the event (death) at time t, and $h_0(t)$ is the baseline hazard (i.e. the value of the hazard when all exposure variables are equal to 0).

Power analysis using the function 'ssizeCT' from the R library 'powerSurvEpi' (Qiu et al. 2012) was used to assess the ability of the fitted Coxph model to detect differences between infested and control fish in this study. We performed scenario testing to estimate the minimum sample size required to detect different hazard ratios (HR) under the premise of 3 fixed parameters: power (i.e. power to detect the magnitude of the hazard ratio as small as that specified by HR) = 0.8, alpha (i.e. type I error rate) = 0.05, and k (ratio of participants in each group) = 1. In other words, we calculated the minimum sample size required to have an 80% probability of detecting a HR with a confidence of 95%, given the same number of fish in both groups, and using our own data to estimate the remaining parameters such as the expected total number of events in each group.

In anticipation of power analysis indicating insufficient statistical power for the survival analysis, a second Coxph model was specified including data from both the current study (n = 143) and data from the pilot study (n = 29; Serra-Llinares et al. 2018). Both studies were performed in the same fjord in consecutive years and are to a high degree comparable when it comes to methods and study design. However, there are some small differences that must be accounted for when results from both studies are to be combined. First, fish used in the pilot study were caught at sea in mid-June and therefore (1) they were slightly bigger (mean FL = 185 ± 18 mm) than fish in the present study, (2) they could have spent different amounts of time at sea prior to capture, and

(3) initial mortality immediately after migration to sea, when the fish are the smallest and thus most vulnerable to predation, was not properly captured in the pilot study. Second, fish in the pilot study were not completely lice free prior to tagging: 96 % of the fish were infested with 1 or more lice, with intensities ranging from 2–42 lice fish^{-1}. Thus, control fish in the pilot study could arguably be affected to some degree by the lice they carried, possibly obscuring the differences between treatment groups. Last, artificial infestation in the pilot study resulted in more moderate lice loads (estimated relative intensities after artificial infestation between 0.24 and 0.74 lice g^{-1} fish) compared to the present study, and thus differences in survival between control and infested fish can also be expected to be smaller. Based on all of the above, and to account for potential differences between the 2 studies, Year was added as a covariate in the model, which was finally expressed as:

$$h(t) = h_0(t) \times \mathrm{e}^{(\beta_1\, Group\, +\, \beta_2\, Fish\ Length\, +\, \beta_3\, Group\, \times\, Fish\ Length\, +\, \beta_4\, Year)} \tag{2}$$

For analysis of return to fresh water, fate/status was set to 1 (returned) for fish that returned to fresh water and were never observed at sea again afterwards, and to 0 (not returned) for fish having their last detection at sea. Fate time t of returned fish was set as the number of days between tagging and fate date. Fate time t for fish that did not return to fresh water was defined by the last observation and specified as right-censored data. Analysis of the Schoefeld residuals showed that the effect of Group (control/infested) shifted after approximately 60 d, showing a time dependency. We therefore applied a stratified analysis before and after this 60 d threshold (Period) using the formula:

$$h(t) = h_0\,(t) \times \mathrm{e}^{(\beta_1\, Group\, +\, \beta_2\, Group\, \times\, Strata\ (Period)\, +\, \beta_3\, Fish\ Length)} \tag{3}$$

Here, the term $h(t)$ denotes the hazard of returning to fresh water at time t.

Differences in distance to fresh water and depth use during the marine migration (i.e. before final return to fresh water) were investigated using mixed models. Daily values (calculated as averages of hourly positions) were used to avoid severe autocorrelation in model residuals. Group (control/infested), fish length (FL in mm) and their interaction term were used as covariates in all models, while time of day (day/night) was additionally used for analysis of swimming depths. For this, daytime was defined as the time between sunrise and sunset using data from the Astronomical Applications Department of the US Naval Observatory (www.usno.navy.mil) for the coordinates 58° 41′ N, 9° 07′ E. Night was defined as the time between sunset and sunrise. Individual daily mean swimming depths were calculated for day and night separately. Fish ID was used as a random effect in all models, as well as a correlation structure (auto-regressive model of order 1) to account for the temporal correlation between consecutive daily data from the same fish.

Distance to fresh water was first modelled using the above described linear mixed model. Exploration of the residuals showed a strong non-linear temporal pattern for infested fish, and consequently week was included as a smoother, which was allowed to differ between experimental groups. The final generalised additive mixed-effect model (GAMM) used was fitted according to the formula (full model):

$$\begin{aligned} Dist_{ij} = \alpha + \beta_1\ Group_i + \beta_2\ Fish\ Length_i + \beta_3\ Group_i \\ \times\ Fish\ Length_i + f\,(Week\_\ Group_i) + ID_i \end{aligned} \tag{4}$$

where $Dist_{ij}$ denotes the mean distance to fresh water for individual i on day j, $Group_i$ is the experimental group (control or infested) of individual i, $Fish\ Length_i$ is FL and $f\,(Week\_Group_i)$ is the week-effect smoother for the corresponding experimental group. ID_i is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2.

For analysis of daily mean swimming depth, the variable was first log-transformed in order to normalize the residuals (prior to log-transformation, 0.2 m was added to have all observations above 0; this was subtracted when back-calculating model coefficients). Daily mean swimming depth showed a nonlinear temporal pattern, and therefore week was included as a smoother, which was allowed to differ between day and night. The final GAMM used was fitted according to the formula (full model):

$$\begin{aligned} Log\,(Depth_{ij} + 0.2) = \alpha + \beta_1\ Group_i + \beta_2\ Fish\ Length_i + \beta_3 \\ Time\ Of\ Day_{ij} + \beta_4\ Group_i \times Fish\ Length_i + \beta_5\ Group_i \\ \times\ Time\ Of\ Day_{ij} + f\,(Week\_\ Time\ Of\ Day_{ij}) + ID_i \end{aligned} \tag{5}$$

The term $Depth_{ij}$ denotes the mean depth of individual i on day j, $Group_i$ is the experimental group (control or infested) of individual i, $Fish\ Length_i$ is FL of individual i, $Time\ Of\ Day_{ij}$ denotes the time of the day (day/night) of individual i on day j, and $f\,(Week\_\ Time\ Of\ Day_{ij})$ is the week-effect smoother for the corresponding time of day. ID_i is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2.

Each global model (as specified above) was used to generate a model set of all possible sub models, using the function 'dredge' in the R package MuMIn (Bartón 2007). Models were ranked by Akaike's information criterion corrected for small sample size (AICc). The model with the lowest AICc value was considered as 'best fit' and was used to estimate model coefficients. Models with ΔAICc < 2 were considered competing models. Complete model selection tables are presented in the Supplement (Tables S2–S5).

3. RESULTS

3.1. Artificial infestation

Examination of the laboratory reference groups (Table 1) revealed significant differences in lice loads between control and artificially infested fish. Control fish (n = 14) showed a 78% prevalence (i.e. number of fish carrying 1 or more lice divided by the number of fish examined), indicating either that the fish had time to get infested with lice during the short acclimation period at sea, or that there was a cross-infestation between the 2 tank compartments during the transport by boat to our lab facilities. However, mean intensity (i.e. average number of lice on infested fish) and mean relative intensity (i.e. average number of lice per g of fish weight) remained low in this group (mean ± SD intensity = 1.9 ± 1.3 lice fish^{-1}; mean relative intensity = 0.05 ± 0.05 lice g^{-1}). Prevalence in the infested group (n = 16) was 100%, with a mean intensity of 65.4 ± 30.6 lice fish^{-1} and a mean relative intensity of 2.4 ± 0.9 lice g^{-1}.

3.2. Hydrography

According to model simulations, surface salinity in Sandnesfjord was lowest in the inner part and increased progressively towards the ocean (Fig. 2). There was a surface layer of brackish water in the inner fjord, with salinities remaining under 15 psu during most of May and June whilst increasing to above 20 psu in July. In the middle fjord, surface salinity remained between 15 and 25 psu during May and June and increased to 30 psu in July. Surface salinity in the outer part of the fjord was between 25 and 30 psu from May to September. Surface temperature was relatively homogeneous throughout the whole length of the fjord and increased progressively from 12°C on the release day to a maximum of 19°C in late July (Fig. 2).

3.3. Acoustic tracking and fate assessment

A total of 143 fish (70 control and 73 infested) provided depth and movement data (2 fish did not provide any data) (Table S1). A substantial proportion of the individuals (approximately 60% in both groups) moved towards the ocean within the first 2 wk after release and were never observed again in the study area (Fig. 3). We found that 35 fish visited fresh water at least once during the study; of these, 18 individuals (6 control and 12 infested) had the last detection in fresh water and were therefore classified as returns. Twelve of the returns (1 control and 11 infested) returned before 1 August and were therefore classified as early returns. Among early returning fish from the infested group, 6 (of 11) individuals remained in the transition area at least until August 1st, 2 of which were later detected migrating back to the river (the remaining 4 individuals had the last detection as living fish in the transition area); the remaining 5 indi-

Table 1. Size and lice counts from sea trout in the reference groups after artificial infestation. FL: fork length; W: weight; N lice: number of lice; Rel. int: relative intensity of infestation (number of lice per g fish weight)

	FL (mm)	W (g)	N lice	Rel. int.
Control	167	45	1	0.02
	153	37	0	0.00
	145	32	1	0.03
	141	25	5	0.20
	139	25	1	0.04
	180	45	1	0.02
	137	23	1	0.04
	158	34	0	0.00
	187	68	1	0.01
	132	23	0	0.00
	184	60	2	0.03
	128	21	2	0.10
	140	29	4	0.14
	148	35	2	0.06
Infested	132	22	46	2.09
	153	31	74	2.39
	146	28	67	2.39
	150	29	38	1.31
	143	27	66	2.44
	125	23	34	1.48
	114	14	28	2.00
	137	24	118	4.92
	140	25	24	0.96
	128	18	47	2.61
	118	15	31	2.07
	136	25	97	3.88
	160	41	92	2.24
	163	37	102	2.76
	170	46	89	1.93
	154	35	94	2.69

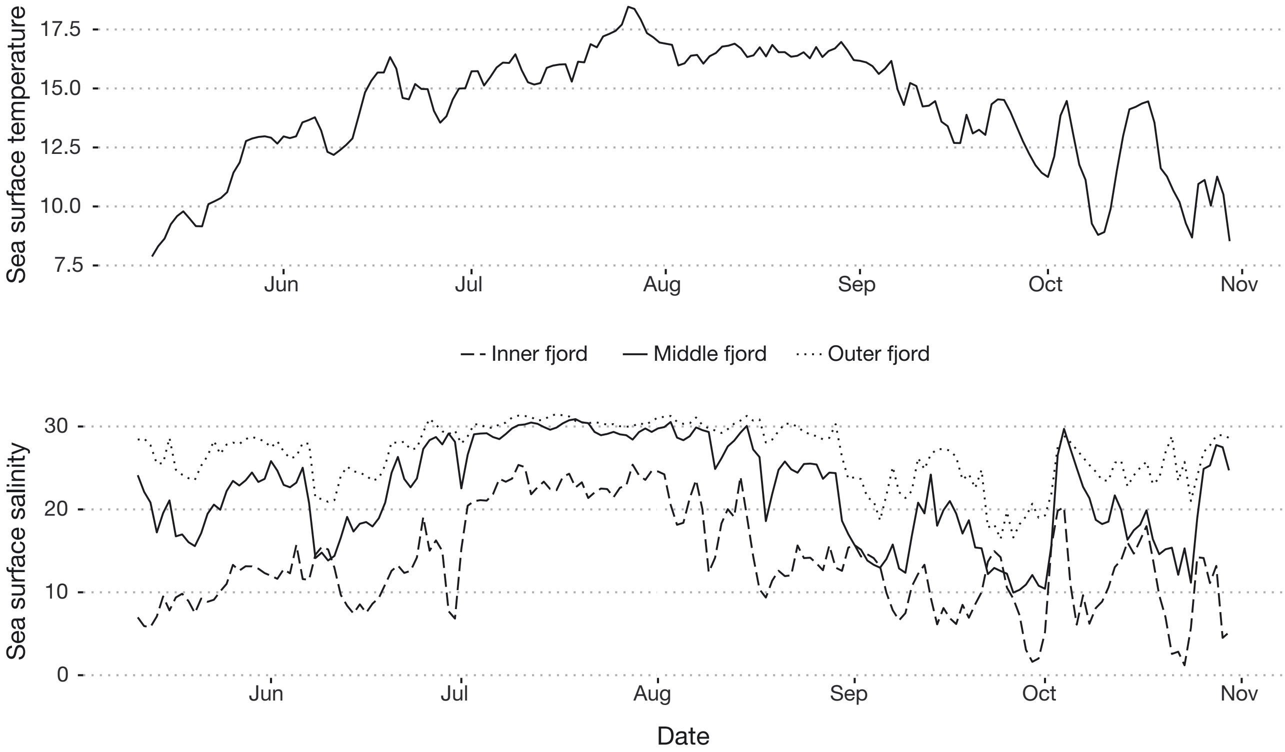


Fig. 2. Modelled sea surface temperatures and salinities in the inner, middle and outer part of Sandnesfjord. Only middle-fjord data are shown for temperature due to little variation along the fjord length

viduals either died (4) or disappeared (1) shortly after premature return to freshwater. A total of 19 individuals, 7 control and 12 infested, were classified as dead. Eleven fish (9 control and 2 infested) were observed alive at sea by 1 October. Nine fish (6 infested and 3 control) were detected at the first and closest receiver outside Sandnesfjord, situated approximately 16 km (sea-way distance) from the innermost part of the fjord. Another 6 fish (4 infested and 2 control) were further detected at the most distant outer receiver, situated another 9 km farther south along the coast.

All fish detected in the PIT antenna returning during the first season at sea could also be positioned at the river mouth through detections by the corresponding acoustic receiver. The latest of these detections were used for analysis. None of the fish that disappeared during the study period was later detected in the PIT antenna.

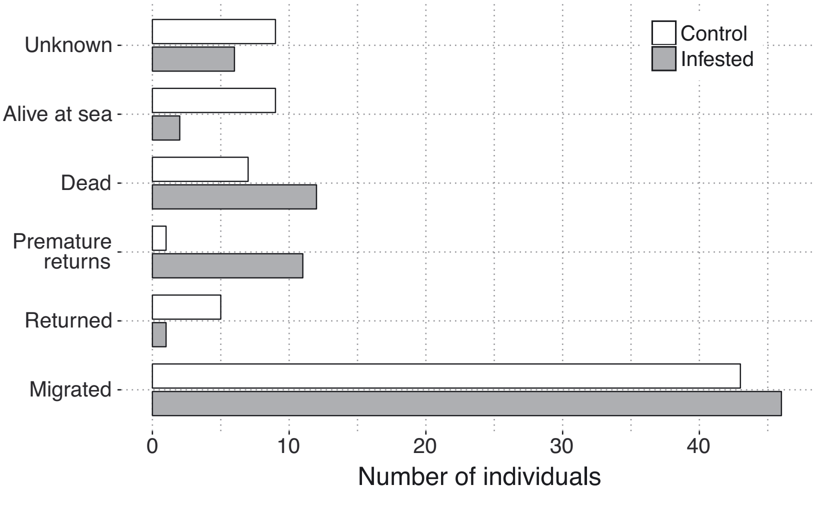


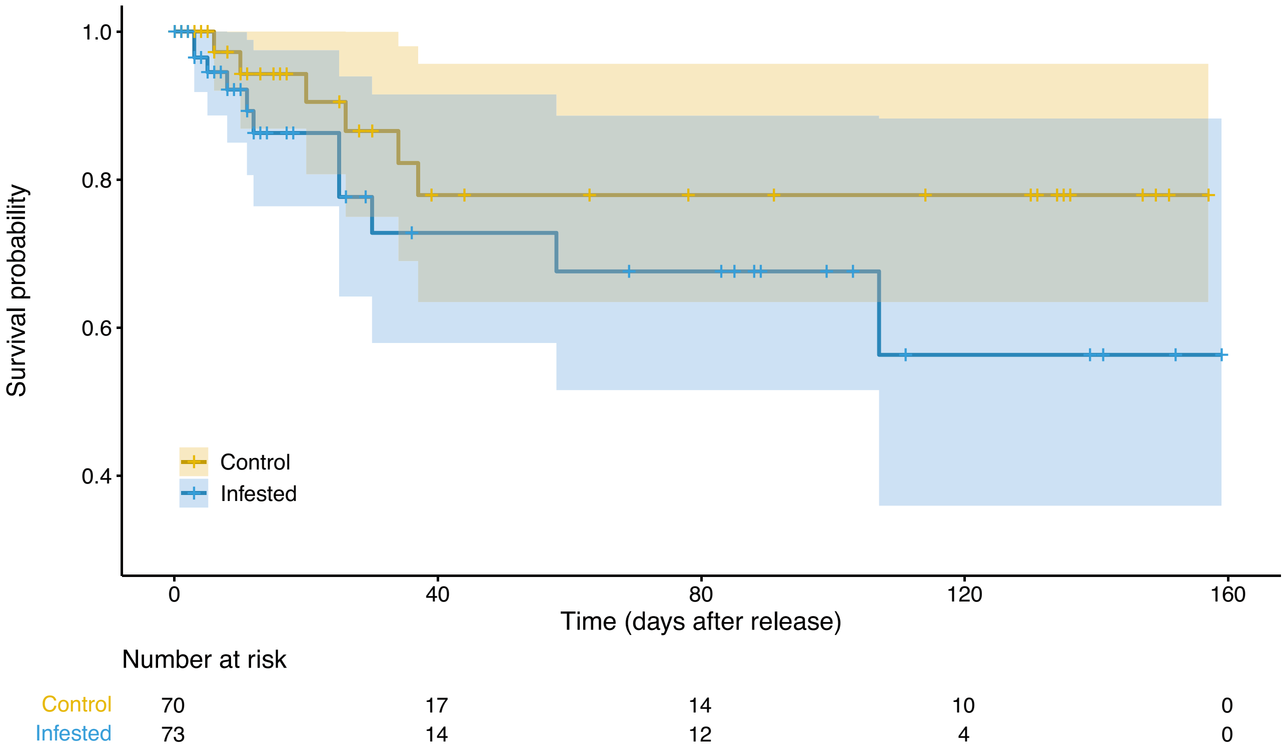
Fig. 3. Summary of fish fates. See Section 2 for further details on fate assessment and Table S1 in the Supplement for details on individual fates

3.4. Survival

None of the covariates tested had a statistical effect on the mortality of the fish in 2017 according to the 'best fit' model, which included only the intercept (Table S2a). The top competing model included the variable 'group,' and was practically indistinguishable from the 'best fit' model (ΔAICc = 0.22). This competing model showed higher, albeit statistically non-significant, mortality for lice-infested fish compared to controls (Coxph, 0.70 ± 0.51 SE; hazard ratio = 2.01, 95% CI = 0.74–5.46, z =

1.37, p = 0.17; Figs. 4 & 5a). Power analysis revealed that, given the structure of the data in this study, a sample size of 362 fish (181 in each group) would be needed to detect an HR between infested and control fish of 2 (as suggested by our results) with an 80% probability and a confidence of 95%. Furthermore, our actual sample size (~70 fish per group) would only be able to provide statistical significance given an HR between infested and control fish of 2.9 or higher.

Survival analysis on the merged dataset (2016 and 2017 data) revealed a higher mortality risk for lice-infested trout compared to control individuals (Coxph, 1.00 ± 0.49 SE; hazard ratio = 2.73, 95%



	0	40	80	120	160
Control	70	17	14	10	0
Infested	73	14	12	4	0

Fig. 4. Survival curves with 95% confidence bands for control (yellow) and artificially infested (blue) sea trout post-smolts in 2017. Crosses represent censoring points. The table under the graphic shows the number of individuals at risk (not censored) at each time step

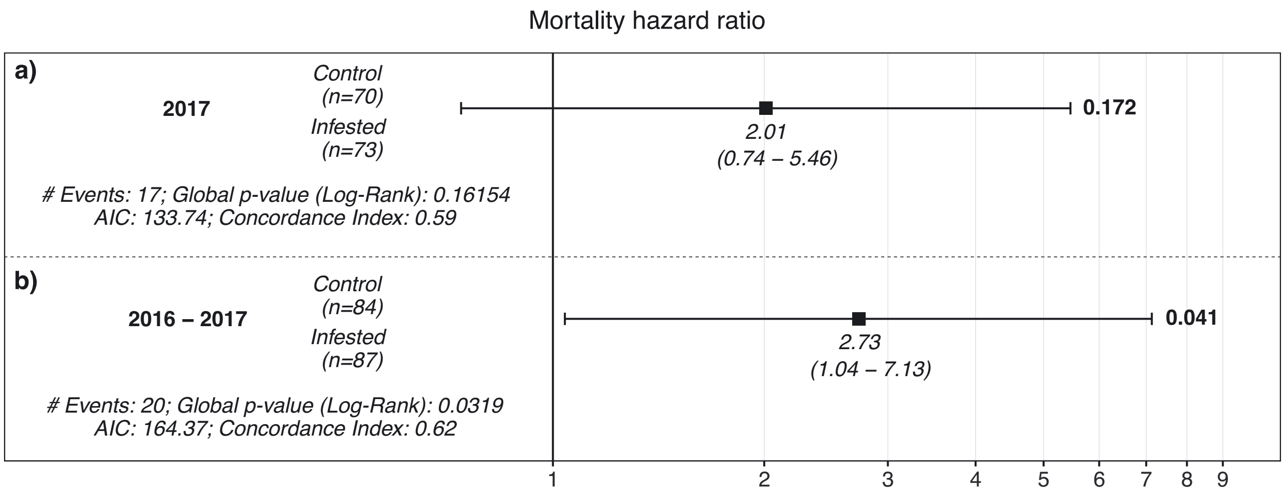


Fig. 5. Mortality hazard ratio between control (reference level) and artificially infested sea trout in (a) the present study (2017 data) and (b) the merged dataset including data from the pilot study. Solid squares and error bars show estimates and 95% CI, respectively (also noted under the corresponding symbols). Associated p-values are given as annotations on the right side of each graphic. AIC: Akaike's information criterion

CI = 1.04–7.13, $z = 2.04$, p = 0.041; Fig. 5b). Competing models included the effect of year on the mortality hazard ratio between control and infested fish (Table S2b) in the direction of a bigger (although not statistically significant; $p > 0.05$) difference between groups in 2017. Neither the 'best fit' model nor competing models included fish length as a covariate.

3.5. Return to fresh water

Eighteen fish (6 control, 12 infested) returned to fresh water. The timing of the returns was significantly earlier for infested (18 ± 26 d after release) than for control fish (100 ± 49 days; *t*-test, p = 0.003), with 12 individuals (1 control and 11 infested) returning before 1 August. The 'best fit' model included both fish length and the interaction group:strata (period) (Table S3), both having a significant effect on the probability of return. During the first 60 d of the migration (period 1), infested fish had a higher probability of returning to fresh water compared to control fish of the same size (Coxph, 2.43 ± 1.05 SE; hazard ratio = 11.33, 95% CI = 1.45–88.4, $z = 2.32$, p = 0.021; Figs. 6 & 7). After 60 d (period 2), the return probability for infested fish decreased until becoming lower, although not statistically significant, than that of their control counterparts (Coxph, −1.16 ± 1.12 SE; hazard ratio = 0.31, 95% CI = 0.03–2.84, $z = 1.03$, p = 0.30; Figs. 6 & 7). In general (both periods), bigger fish had a higher probability of returning to fresh water (Coxph, 0.03 ± 0.02 SE; hazard ratio = 1.04, 95% CI = 1.01–1.07, $z = 2.31$, p = 0.021).

3.6. Distance to fresh water

Lice-infested trout remained closer to the estuary compared to control fish while at sea (i.e. before they eventually terminated their sea migration and moved back to fresh water or into the transition area) (Table 2). This difference was especially noticeable after the first 3 wk post release and remained a consistent pattern for the rest of the study period (Fig. 8). There was a significant interaction between group and fish length, with fish length having a small positive effect for control fish and a stronger and negative effect for infested fish. The average distance to fresh water for a control fish of average size (FL = 180 mm) was approximately 6 km, with smaller fish staying slightly closer (approximately 80 m for each 1 cm increase in body length). For an infested fish of the

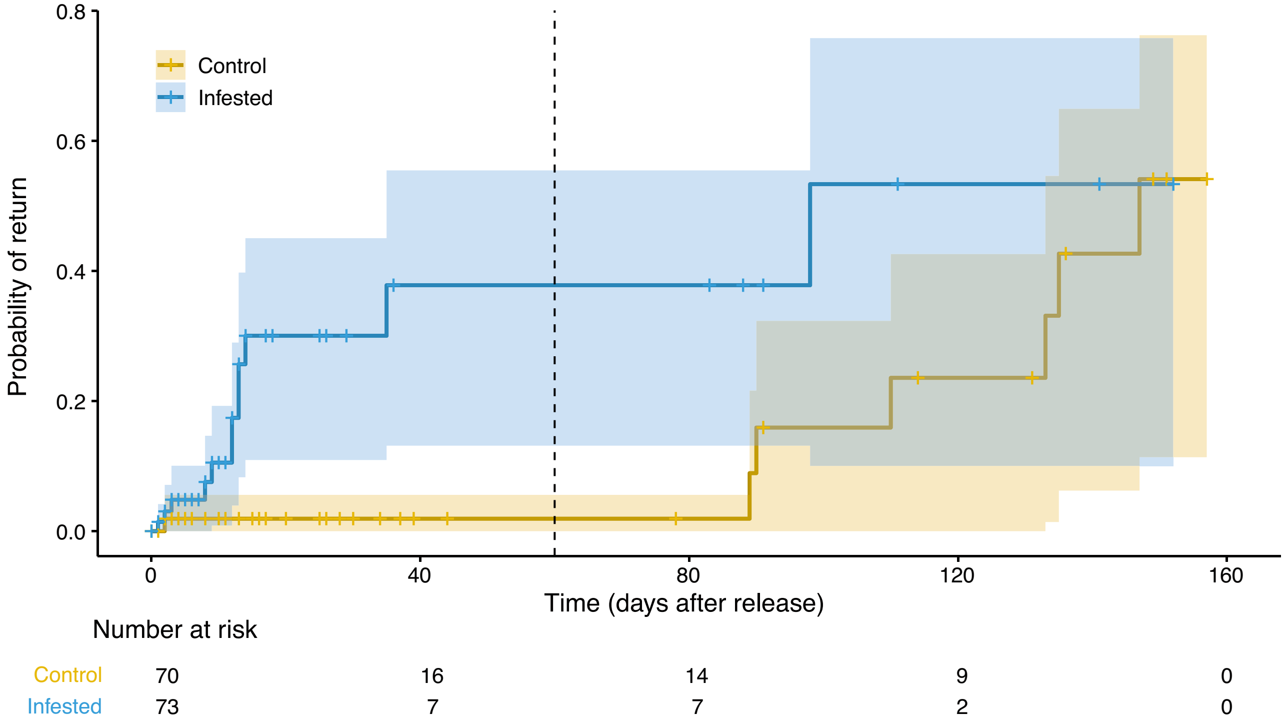


Fig. 6. Probability of return to fresh water with 95% confidence bands for control (yellow) and artificially infested (blue) sea trout post-smolts. Crosses represent censoring points. Dotted line indicates Day 60 post release, separating period 1 from period 2 in the stratified analysis. The table under the graphic shows the number of individuals at risk (not censored) at each time step

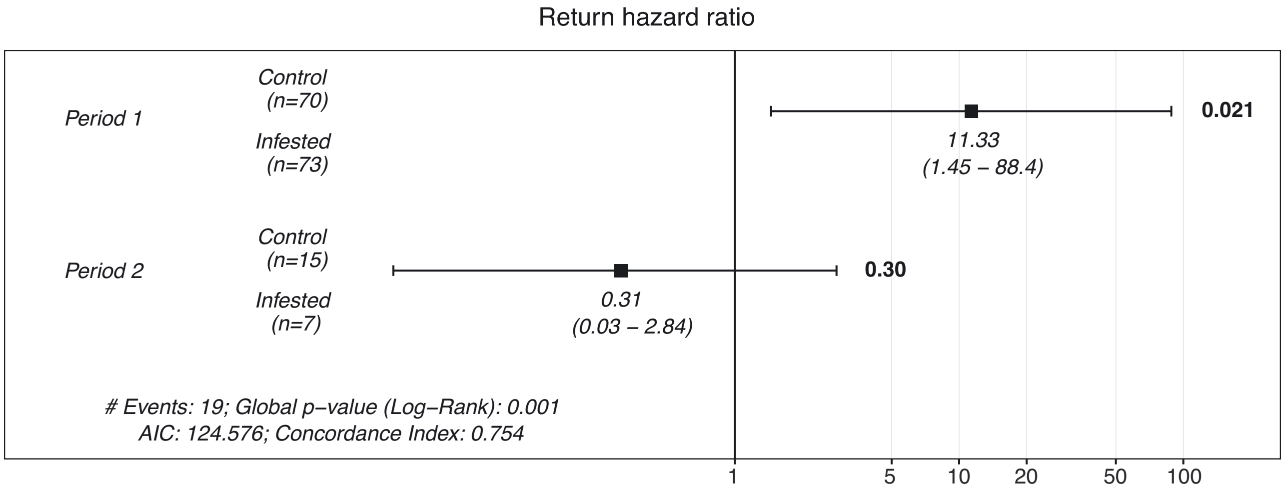


Fig. 7. Hazard ratio for freshwater return between control (reference level) and artificially infested sea trout before (period 1) and after (period 2) 60 d post release. Solid squares and error bars show estimates and 95% CI, respectively (also noted under the corresponding symbols). Associated p-values are given as annotations on the right side of each graphic. AIC: Akaike's information criterion

same size, the corresponding distance was estimated at approximately 2.5 km, with larger fish remaining closer than smaller fish (approximately 565 m closer for each 1 cm increase in body length). The model explained 46% of the variance. All candidate models evaluated are shown in Table S4.

3.7. Depth use

Trout resided close to the surface during the whole marine migration, with more than 98% of all detections recorded at depths shallower than 3 m. Only 8 fish (6%) were detected performing 1 or more dives >15 m during the whole study. Mean swimming depth during the whole marine migration was 0.78 m (range of means for individuals: 0.04–2.54 m), with individuals staying shallower at night than during the day (mean swimming depths were 1.01 and 0.55 m for day and night time, respectively). Infested fish tended to swim slightly shallower (approximately 15 cm) than control fish during the day, whilst both groups occupied approximately the same depth at night (Table 2, Fig. 9). Fish size also had an effect on swimming depth, with bigger fish swimming slightly deeper than smaller fish (approximately 7 cm deeper for each 1 cm increase in fish length) independently of lice infestation. All candidate models evaluated are shown in Table S5.

Table 2. Results from generalised additive mixed-effects models (GAMMs) for distance to fresh water and swimming depth, modelled as a function of fish length (fork length in mm), group (control/infested) and time of day (day/night; included only in the analysis of swimming depth). *Significant values ($p < 0.05$)

	Estimate	SE	t	p(>|t|)
Distance to fresh water				
(Intercept)	4781.07	2414.21	1.980	0.048*
Fish length	8.08	13.46	0.600	0.548
Group (infested)	7481.47	3306.21	2.263	0.024*
Fish length: Group (infested)	−56.51	18.30	−3.088	0.002*
Log (Swimming depth + 0.2)				
(Intercept)	−0.746	0.331	−2.256	0.024*
Fish length	0.005	0.002	3.010	0.003*
Group (infested)	−0.134	0.052	−2.576	0.011*
Time of day (night)	−0.656	0.011	−59.809	0.000*
Group (infested): Time of day (night)	0.122	0.019	6.510	0.000*

4. DISCUSSION

In this study, we documented parasite-induced mortality and significant behavioural changes in trout post-smolts as a consequence of a heavy lice infestation. The lice levels artificially imposed on experimental fish in our study were estimated at 65 lice fish^{-1} on average, or a relative intensity of 2.4 lice g^{-1}. This is well above the expected mortality-inducing threshold for trout, estimated at 0.3 lice g^{-1} (see Taranger et al. 2015), and individuals carrying such high lice loads represent only a small fraction (between 4 and 5%) of all trout sampled in the Norwegian Salmon Lice Surveillance Program (R. Nilsen pers. obs.). However, in some areas with intensive fish farming, the percentage of fish carrying

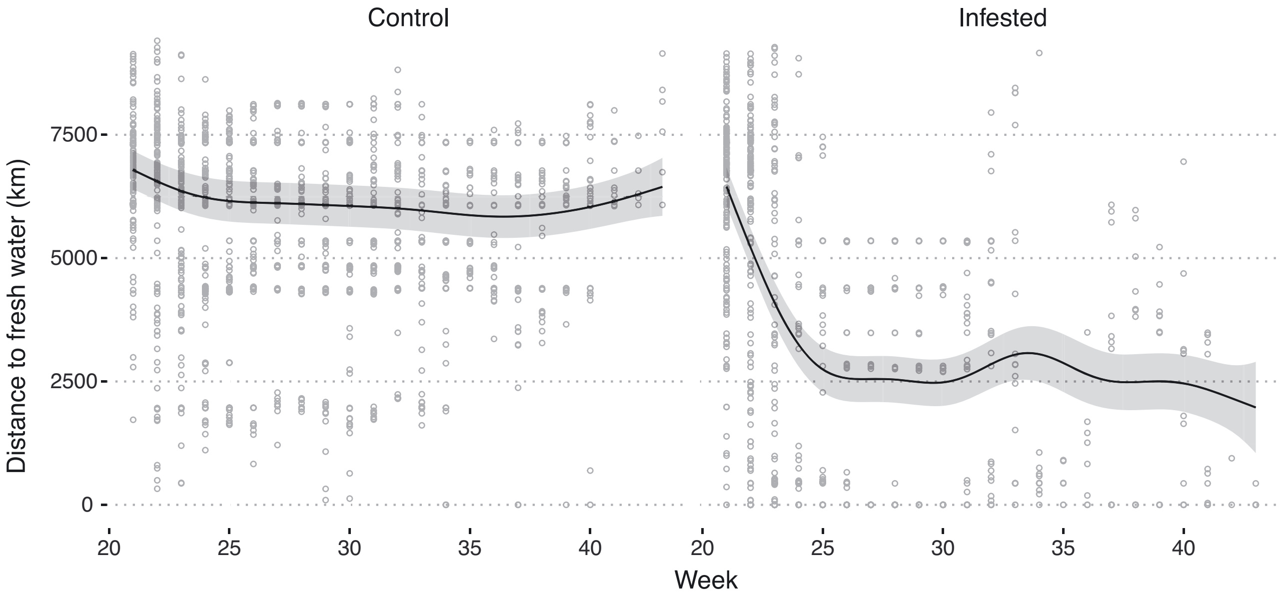


Fig. 8. Observed (points) and predicted (lines and shaded areas, representing estimates with 95 % CI) distance to fresh water for individual fish by experimental group (control or infested). Predictions are made for a fish of average size (180 mm fork length)

>65 lice (or relative intensities >2 lice g^{-1}) has occasionally been registered to be as high as 70 % of all fish sampled (R. Nilsen pers. obs.). Thus, lice loads such as that in this study may be extreme and represent perhaps only a 'worst-case' situation, but they are not implausible under the current scenario in Norway.

4.1. Role of lice in post-smolt mortality

Dead and moribund trout observed in estuaries have been linked to lice infestations (Tully et al. 1993a,b, Birkeland 1996), but proof of a causal relationship remains elusive (Thorstad et al. 2015). Direct observation of mortality in free-ranging fish at sea is

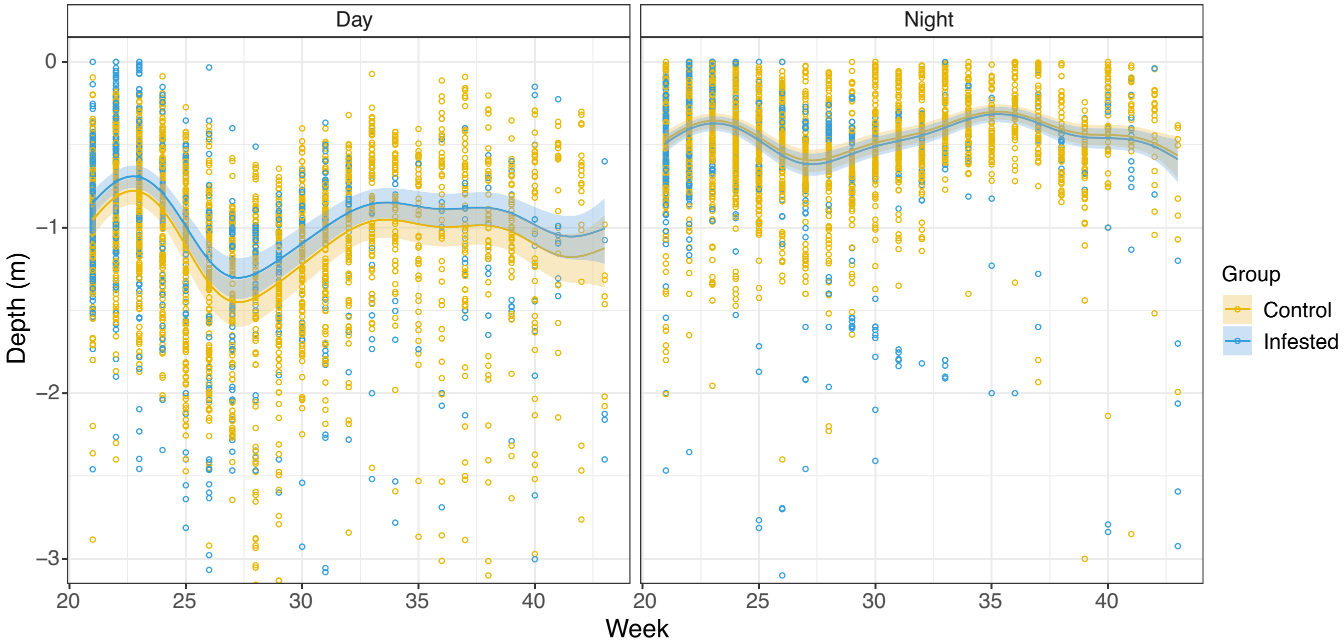


Fig. 9. Observed swimming depths for control (yellow dots) and infested (blue dots) sea trout post-smolts during day and night. Lines and shaded areas represent predicted swimming depths and 95 % CI, respectively, for a fish of average size (180 mm fork length)

difficult, and studies are often limited by low numbers of returning individuals and large natural variation in mortality. In this study, we document a higher mortality of lice-infested trout post-smolts compared to non-infested control individuals, with an estimated HR of 2.7 (95% CI = 1.04–7.13). In other words, the probability of a trout post-smolt to survive the first summer at sea was reduced by almost two-thirds when infested with a heavy lice load. These results are in agreement with Skaala et al. (2014), who showed that the survival of sea trout protected against lice was nearly doubled compared to non-treated fish, in a large-scale field experiment involving PIT-tagging of 2000 fish over 2 yr in a fjord with intensive salmon farming in Norway. To our knowledge, this is the only other study showing the impact of lice on the survival of wild sea trout in their natural environment. Other studies have not found a correlation between prophylactic treatment and marine survival of trout post-smolts, but these are (as stated by the authors of those studies) likely constrained by methodological caveats and low statistical power (Gjelland et al. 2014, Halttunen et al. 2018).

4.2. Salmon lice as a causal agent for premature return

In addition to an increase in mortality for lice-infested individuals, lice infestation triggered the almost immediate return of the fish to fresh water in our study. Most of the infested fish that did not rapidly disperse from the study area actively sought fresh or brackish waters within the first few days post release. Of these, almost half were never detected again at sea, having spent on average only 18 d feeding in the marine environment. Given a water temperature of around 12–13°C during that period, the attached copepodites had presumably reached the preadult stage around that time (Stien et al. 2005). Premature return to fresh water of lice-infested trout has been previously documented in Ireland, Scotland and Norway (Tully et al. 1993a, Birkeland & Jakobsen 1997, Pert et al. 2009). This return to fresh water is suggested to allow the fish to delouse and regain osmotic balance in order to survive the infestation (Birkeland & Jakobsen 1997, Bjørn et al. 2001). Even though preadult and adult lice are the most detrimental stages for the host fish (Bjørn & Finstad 1998), heavy infestations with copepodites and chalimus stages can also cause skin damage and hydromineral imbalance and can trigger a stress response in trout (Bjørn & Finstad 1997, Poole et al. 2000, Bjørn et al. 2001). Thus, heavily infested individuals may return to fresh water even before the offset of severe lice-induced physiological effects. Additionally, larger fish had a higher probability than smaller fish of returning to fresh water in both groups throughout our study. Higher return rates for larger trout post-smolts can be expected due to size-dependent mortality (Thorstad et al. 2015, 2016, Flaten et al. 2016). According to results from our survival analysis, the effect of fish size on the probability of return to fresh water cannot be attributed to size-dependent mortality, since no effect of fish size was detected on the probability of surviving the sea journey. However, this could be an artifact of low statistical power combined with large inter-individual variability.

4.3. Other behavioural adaptations to lice infestation

Of the 11 lice-infested fish that returned prematurely to fresh water in our study, 7 remained in the transition area for the rest of the migration. This is likely because the costs of ionic regulation are lower in brackish water than in both fresh and sea water (Rao 1968, Otto 1971, Brett & Groves 1979). Lice-infested individuals that did not prematurely return to fresh water spent most of the feeding migration in the innermost part of the fjord, characterized by lower surface salinities and shorter distance to the native river. Unlike lice-infested individuals, most control fish exploited the feeding grounds in the outer fjord and spent on average 100 d feeding at sea before returning to fresh water.

The vertical profiles of the trout movements showed a strong preference for the upper 3 m of the water column. Average swimming depths were slightly shallower at night than in the daytime, indicating small-scale diel vertical movements. These results agree with other studies investigating the behaviour of wild trout in the marine environment (Lyse et al. 1998, Eldøy et al. 2017, Kristensen et al. 2018). However, most of these studies have focused on veteran migrants, mainly due to technological constraints such as tag size for telemetry studies. Thus, our findings provide a new insight into the behaviour and habitat use of trout post-smolts during their sea journey. Lice-infested fish resided slightly closer to the surface compared to control fish during daytime in our study. Lice-infested trout have been previously reported to swim closer to the surface (Gjelland et al. 2014, A. Mohn unpublished data). Because the upper water layers are typically more influenced by rivers and other freshwater sources, this can be seen as a behavioural adaptation to counteract the osmoregulatory problems derived from the parasitic infestation. All in all, it seems likely that lice-infested individuals in our study were seeking fresh and brackish water by adjusting both their horizontal and vertical use of the fjord.

4.4. Indirect and delayed impacts

Growth in the sea is generally better than in fresh water (L'Abee-Lund et al. 1989, Jonsson & L'Abée Lund 1993, Frier 1994), and growth is positively correlated with the duration of the sea sojourn (Berg & Jonsson 1990). Thus, an abrupt interruption of the feeding migration after just a few weeks at sea, as reported in this study, may result in a significant loss of feeding and growth opportunities. Birkeland (1996) observed prematurely returned, lice-infested trout re-entering the sea after a median stay of 38 d in fresh water. By that time, they had lost 23.5 % of their body mass. Similarly, Fjørtoft et al. (2014) calculated a 20–40 % reduction in summer growth in the Etne River, western Norway, during a period of intensive farming, and suggested lice as a possible cause. In Ireland, proximity to salmon aquaculture and associated louse infestation pressure have been demonstrated to reduce weight in wild trout post-smolts by up to 9 g (at an average length of 18 cm), thus reducing Fulton's condition factor by ca. 0.15 (Shephard et al. 2016). Reduced summer growth may additionally reduce the probability of surviving through the following winter, since individuals that have not attained a critical size or sufficient energy stores may be unable to meet minimum metabolic requirements during winter and die (Jensen et al. 2018). Also, as fecundity increases with body size (Jonsson 1985, L'Abée Lund & Hindar 1990) and age at first maturity is influenced by growth rate at sea (L'Abée-Lund 1994, Vollset et al. 2014), reduced growth and energy reserves as a result of louse infestation may reduce fecundity and reproductive success.

4.5. Consequences for populations

It has been suggested that an increase in marine mortality and a reduction in sea growth due to lice or other factors affecting trout in the marine environment can result in a population shift in life-history strategy (Thorstad et al. 2015). Gargan et al. (2016) showed significant changes in quantitative life-history traits in the trout population in the Erriff River, western Ireland, after the establishment of salmon farming in the local estuary. These changes included a reduction in the number and size of trout kelts, the estimated number of eggs deposited, the sea trout rod catch, the proportion of older (1+ and 2+ sea age) fish and the frequency of repeat spawners. Similarly, Butler & Walker (2006) recorded a collapse in sea trout rod catch in the River Ewe/Loch Maree system, Scotland, in 1988. This collapse was related to an apparently unprecedented reduction in marine growth and survival, changes that were at least partly attributable to lice epizootics from nearby salmon farms. Reduced marine survival and growth as a result of lice epizootics may also shift the balance between costs and profits of the marine migration and ultimately lead to a decrease in the frequency of sea-run brown trout (Gargan et al. 2006).

4.6. Concluding remarks

In summary, our study reports a reduced survival of trout post-smolts as a direct consequence of a heavy lice infestation, suggesting that in areas with high lice infestation pressure, the number of trout post-smolts surviving the first season at sea may be significantly reduced. Furthermore, we document an altered migration behaviour of lice-infested individuals, including the abrupt interruption of the feeding migration after just a few weeks at sea. This interruption could result in a significant loss of feeding and growth opportunities for migrating fish, further affecting later survival and fecundity and ultimately having a potential impact at the population level. Despite trout being a culturally, socially and economically important species in Norway and elsewhere, there are still important knowledge gaps on its population biology and potential responses to parasites. This is partly explained by a complex and flexible life history with a wide range of strategies within and between populations. Notwithstanding the challenges, this information is urgently needed. Salmon lice epizootics are currently regarded as a major threat for wild salmonids in Norway (Costello 2009, Taranger et al. 2015, Forseth et al. 2017) and other salmon-producing countries, and robust scientific knowledge on the impact of lice on individuals and populations is key to the implementation of sound conservation strategies.

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REPLY COMMENT

Nearby farms are a source of lice for wild salmonids: a reply to Jansen et al. (2016)

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ABSTRACT: Jansen et al. (2016; Aquacult Environ Interact 8:349–350) question the regression analysis presented in Serra-Llinares et al. (2014; Aquacult Environ Interact 5:1–16), which correlates lice abundances on farmed and wild fish. Jansen et al. (2016) argue that the correlation might not reflect a cause–effect relationship but be instead a mere artifact of the spatio-temporal covariance in lice abundance on farmed and wild fish driven by temperature. In this Reply Comment we revisit the analysis presented in Serra-Llinares et al. (2014) and further re-analyze our data following the statistical approach used by Helland et al. (2015; Aquacult Environ Interact 7:267–280), to rule out the potential confounding effect of temperature. We conclude that Jansen et al. (2016) were correct in conveying part of the observed correlation to the effect of temperature; however, there is solid evidence of a significant influence of lice originating from nearby farms on the observed lice abundances on wild fish, even after the effect of temperature is accounted for.

KEY WORDS: Temperature · Salmon lice · Wild salmonids · Salmon farms · *Lepeophtheirus salmonis*

Introduction

Serra-Llinares et al. (2014) presented a correlation between the abundance of salmon lice *Lepeoptheirus salmonis* Kröyer on wild salmonids (sea trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* L.) and the infestation pressure imposed by nearby farms, expressed as the daily release of copepodites (the infective stage of *L. salmonis*) within a 30 km distance from farmed sites. In their Comment Jansen et al. (2016) question these analyses, arguing that the correlation at issue might not reflect a cause–effect relationship, but merely be an artifact of the spatio-temporal covariance in lice abundance on farmed and wild fish driven by temperature (under consideration of pairwise timing and location of the data sets). In addition, Jansen et al. (2016) compare our results with those obtained by Helland et al. (2015), who were not able to predict lice numbers on wild salmonids using lice densities in nearby salmon farms when temperature was included in the analysis. Based on this premise, Jansen et al. (2016) question the validity of our conclusions regarding fish farms acting as a main source of lice for wild salmonids.

Salmon lice development rates are strongly dependent on water temperature; and lice levels on both farmed and wild fish fluctuate seasonally with temperature. Higher lice loads are typically recorded in summer compared to spring, regardless of distance to the nearest fish farms. This seasonal effect can indeed be seen in Fig. 2 and Tables 2 & 3 in Serra-Llinares et al. (2014). In addition, a spatial component might affect lice abundances on wild (Serra-Llinares et al. 2014) and farmed fish (Jansen et al. 2012). Hence we agree with the argument presented by Jansen et al. (2016): the need to account for the effect of temperature when analyzing factors that affect lice numbers on wild salmonids. We welcome

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the chance to correct our analysis, and further use this opportunity to re-analyze our data following the statistical approach used by Helland et al. (2015) for direct comparison.

Revisiting the analyses in Serra-Llinares et al. (2014)

The role of temperature in the observed correlation between the infestation pressure from nearby farms and the observed lice abundances on wild salmonids in Serra-Llinares et al. (2014) was re-evaluated. The linear regression model presented in Serra-Llinares et al. (2014) was re-fitted using both the production of copepodites in nearby farms (calculated as specified in Serra-Llinares et al. 2014) and water temperature as predictors for the mean abundance of lice (all stages) on wild salmonids. In addition, partial correlation analysis on the same data was used to elucidate how much of the variance explained can be accounted for by infestation pressure itself. Prior to analysis mean lice abundances on wild fish (log+1) and estimated production of copepodites in nearby farms (log) were log-transformed. Data exploration (pairplots, Pearson's correlation and Variance Inflation Factors [VIF]) indicated no major collinearity between copepodite production in nearby farms and temperature ($r = 0.4$, VIF = 1.17) (Zuur et al. 2010).

Results from the regression analysis revealed that both temperature and infestation pressure imposed by nearby farms (i.e. daily production of copepodites) have a significant effect on the mean abundance of lice observed on wild salmonids ($df = 72$, temperature: $p = 0.004$, log-transformed daily production of copepodites: $p < 0.001$, $n = 75$), and together explained 37 % of the variation on the log-log scale. Moreover, partial correlation analysis indicated that the production of copepodites in nearby farms by itself explained 21.8 % of the variation in mean lice abundance on wild fish (on the log-log scale) while controlling for the effect of temperature ($p < 0.001$, $n = 75$). Model predictions for 3 different temperatures (6, 8 and 10°C), representative of the range of temperatures present in the analyzed data set, show that the mean lice abundance on wild salmonids increased significantly with increasing infestation pressure from nearby farms, with temperature having an additive effect on this correlation (Fig. 1).

A potential pseudo-replication effect due to repeated sampling in the same locations was ruled out by comparing the results from a linear mixed effects model (using location as random intercept) and a generalized least squares model (i.e. a linear regression model without a dependency structure). Results indicate that adding a dependency structure did not improve the model (Akaike information criteria: 225 for the linear mixed effects model vs. 224 for the generalized least squares model), implying that the observed correlation between infestation pressure from farms, temperature and mean lice abundance on wild salmonids is not merely an artifact of pseudo-replication.

Note the difference in sample size between this re-analysis ($n = 75$) and the analyzed sample size ($n = 71$) published in Serra-Llinares et al. (2014). Both analyses are based on the same data set (i.e. 75 data points), but 4 data points were removed from our original analysis (Serra-Llinares et al. 2014): 2 data points were mistakenly identified as outliers, and another 2 were lost after the direct log-transformation of the observed lice abundances on wild fish (without the addition of any constant prior to transformation). These errors have been corrected in this re-analysis, and the results continue to support our original conclusion that lice of farm origin are a main source of lice for wild salmonids.

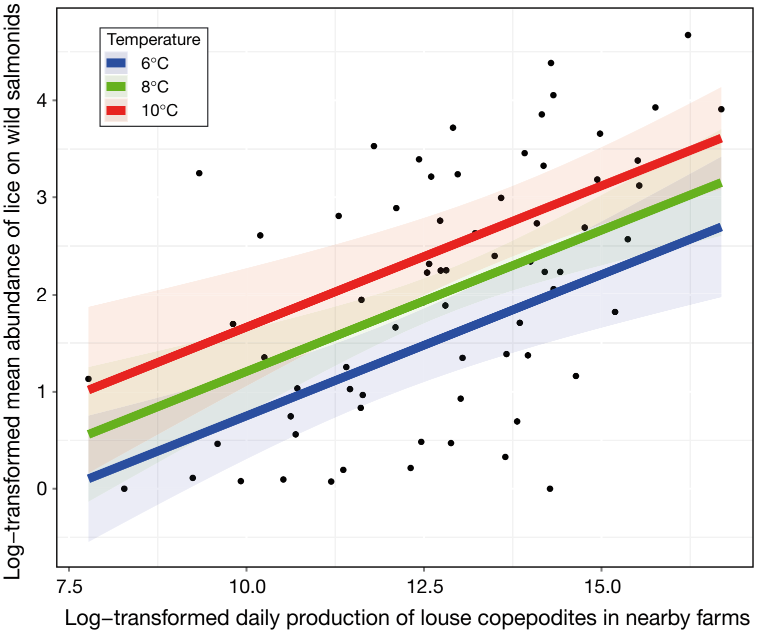

Fig. 1. Predicted mean abundances (fitted values with 95 % CI) of lice (all stages) on wild salmonids as a function of the infestation pressure imposed by nearby farms (expressed as daily production of copepodites, calculated as explained in Serra-Llinares et al. 2014) at 3 different temperatures

Re-analysis using generalized linear mixed models

Jansen et al. (2016) cite Helland et al. (2015) to argue that, once temperature has been included in the analysis, the relationship between the infestation pressure imposed by nearby farms and lice abundances on wild fish appears to be weaker than claimed by Serra-Llinares et al. (2014). Helland et al. (2015) and Serra-Llinares et al. (2014) analyzed comparable data sets. However, Helland et al. (2015) followed a very different statistical approach. Instead of using a summary statistic to describe the lice infestation levels observed on wild fish (such as mean lice abundance, as used by Serra-Llinares et al. 2014), Helland et al. (2015) used the actual number of lice (all stages) observed on each individual fish as the response variable in a generalized linear mixed model (GLMM), in which fish length, temperature, salinity and infestation pressure (expressed as the total number of mature female lice) from nearby farms were used as explanatory variables. We use this opportunity to re-analyze our data using GLMMs to directly compare our results to Helland et al. (2015) and to assess whether the concerns raised by Jansen et al. (2016) are justified.

We re-analyzed the data published in Serra-Llinares et al. (2014) using a zero-altered negative binomial (ZANB) GLMM. In line with the zero-inflated negative binomial (ZINB) GLMM used by Helland et al. (2015), the ZANB GLMM deals with the high occurrence of zeros and the high over-dispersion in the observed data. The main distinction between the 2 approaches is the way the models interpret and analyze zero counts (more details are given in the Supplement at www.int-res.com/articles/suppl/q008p351_supp.pdf).

The observed number of attached stages of lice (copepodites and chalimi, hereafter referred to as ‘attached lice’) on wild salmonids was used as response variable in the ZANB GLMM, instead of the total number of lice (all stages) used by Helland et al. (2015). Attached lice were preferred over the total number of lice since they are indicative of a recent and therefore more local infestation, whilst the total number of lice can be the result of an infestation pressure spanning much longer in time and space (Stien et al. 2005). Fish length, temperature, infestation pressure from nearby fish farms and year (categorical variable with the levels 2010 and 2011) were used as covariates in the ZANB GLMM. The interactions between each covariate and fish farm infestation pressure were also included. Location was used as a random effect to account for repeated measurements at the same location. No temporal auto-correlation was included in the analyses, because attached lice counted in Period 1 would have developed into pre-adult or adult stages by the time the second round of lice counting (Period 2) took place and thus both lice counts could be regarded as independent.

To make our results more comparable to those from Helland et al. (2015), we changed our infestation pressure variable from daily production of copepodites to total numbers of female lice in nearby farms. As in Serra-Llinares et al. (2014), only farms located less than 30 km from each wild fish sampling location were included in the calculation, and a linear correction was applied to account for the effect of distance. All wild fish sampling locations were included in this analysis, including those assumed to be free from lice of farm origin (i.e. those situated more than 30 km from the nearest fish farm). All details about the fitting and validation of the ZANB GLMM are given in the Supplement.

The ZANB GLMM was used to investigate the following 2 questions: What is driving the absence and presence of lice? And when lice are present, what is driving their numbers? Results from this analysis identified a significant effect of both temperature and infestation pressure from nearby farms on both the probability of having one or more lice (binary part of the ZANB GLMM) (Table 1 and Fig. 2) and on the intensity of the infestation (zero-truncated part of the ZANB GLMM) (Table 2 and Fig. 3), despite large variation around the fitted values.

Table 1. Summary from the Bernoulli generalized linear mixed model explaining the probability of presence of attached lice (measured as a binary response of either lice or no lice) on wild salmonids. Estimates for fish length (mm), temperature (°C) and infestation pressure refer to standardized values. Infestation pressure is expressed as the total number of female lice on nearby farms, linearly corrected by distance as explained in Serra-Llinares et al. (2014), and was square-root transformed prior to standardization

	Estimate	SE	*z*	p
Intercept	−0.5937	0.31	−1.89	0.058
Fish length	0.0906	0.05	1.74	0.082
Temperature	0.8134	0.09	9.06	<0.001
Infestation pressure	0.2695	0.15	1.81	0.071
Year 2011	0.6176	0.12	5.28	<0.001
Fish length × Infestation pressure	−0.1848	0.05	−3.51	<0.001
Temperature × Infestation pressure	0.3060	0.09	3.33	<0.001
Infestation pressure × Year 2011	0.5900	0.15	4.06	<0.001

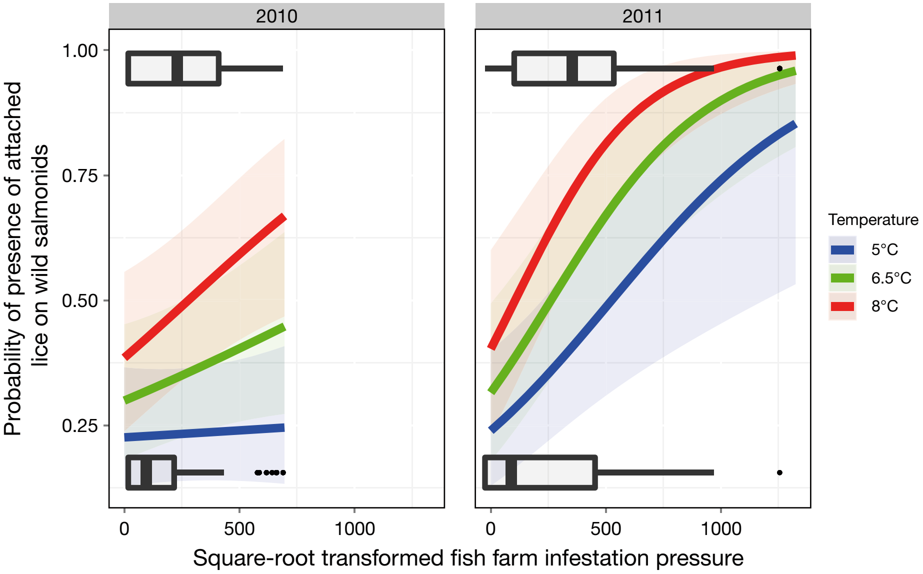

Fig. 2. Probability of presence of attached salmon lice on wild salmonids plotted versus infestation pressure imposed by nearby farms (<30 km) at 3 different temperature values, as predicted by the binary part of the zero-altered negative binominal generalized linear mixed model. Fish farm pressure is expressed as the total number of female lice on nearby farms, linearly corrected by distance as explained in Serra-Llinares et al. (2014). Boxplots show observed data: upper boxes represent fish farm infestation pressure values (square-root transformed) for fish with one or more attached lice; lower boxes infestation pressure values (square-root transformed) for fish with no attached lice. Dark horizontal lines represent median values, boxes the 25th and 75th percentiles, whiskers extreme values and black dots outliers

Although Helland et al. (2015) were not able to predict lice counts on wild fish, they state that 'even after correcting for the temperature effect, our results show that infestation pressure from salmon farms significantly increases the probability of wild sea trout having salmon lice' (p. 267). Thus, our results are in overall agreement with the findings of Helland et al. (2015). Here we provide further evidence of a significant influence of lice originating from nearby farms on the lice loads observed on wild salmonids, even after the effect of temperature is accounted for. There are many unaccounted sources of noise in the analyzed data set, which have the potential to reduce the statistical power of the analysis: variations in salinity among sites, which have not been accounted for; variations in fish behaviour and host–parasite interactions among sampling sites; inaccuracy of temperature and lice infestation estimators (based on monthly reports); density-dependent effects on lice survival and/or host mortality; or potential for other wild fish species to act as reservoirs or vectors for sea lice, among others. A major limitation of the data is the over-simplification made when estimating the infestation pressure imposed by fish farms. On the one hand, a simple linear correction for distance does not reflect the real distribution of planktonic lice in the fjords, which is highly uneven and patchy as a result of their transportation with water currents (Asplin et al. 2014). On the other hand, neglecting the import and export of copepodites over distances >30 to 40 km might represent a critical error, particularly for locations closer to the coast (Asplin et al. 2014, Johnsen et al. 2014). There is currently no tool available to quantify the R^2 of a ZANB GLMM (A. F. Zuur pers. comm.). However, fitted values from our models tend to be comparable to those obtained by a Poisson GLMM, which in this case explained 25% of the variance (see Supplement). This can be interpreted as a rather good fit given all the noise in the data set.

Table 2. Summary from the zero-truncated negative binomial generalized linear mixed model predicting numbers of attached lice on infested wild salmonids. For further information on variables see Table 1

	Estimate	SE	*z*	p
Intercept	2.11927	0.19	11.21	<0.001
Fish length	−0.05375	0.06	−0.93	0.351
Temperature	0.52905	0.09	6.07	<0.001
Infestation pressure	1.18889	0.19	6.26	<0.001
Year 2011	0.11261	0.13	0.90	0.371
Fish length × Infestation pressure	0.00915	0.04	0.22	0.830
Temperature × Infestation pressure	0.08006	0.08	1.02	0.306
Infestation pressure × Year 2011	−0.67073	0.18	−3.75	<0.001

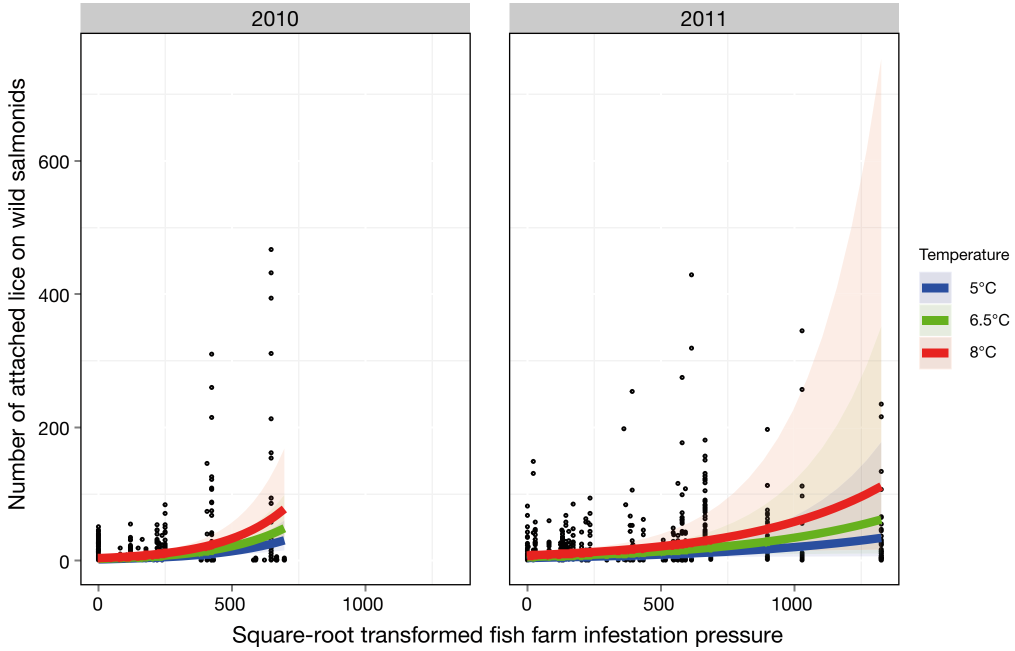

Fig. 3. Fitted values (with 95% CI) from the zero-truncated negative binomial generalized linear mixed model showing predicted attached lice counts on wild salmonids as a function of the lice infestation pressure imposed by nearby farms (<30 km) for 3 different temperature values. Fish farm pressure is expressed as the total number of female lice on nearby farms, linearly corrected by distance as explained in Serra-Llinares et al. (2014). Black dots show actual observed number of attached lice

Concluding remarks

Water temperature is an important modulator of salmon lice dynamics, as a regulator of the *per capita* reproductive rate of female lice (Stien et al. 2005). Therefore, it is natural to observe seasonal patterns in lice counts both on wild and farmed fish, with higher counts in summer compared to spring. However, water temperature has little influence on the total number of lice found at a particular location and time unless it is combined with a lice source (number of host fish and female lice per fish), irrespective whether they originate from wild or farmed fish. Norway is the world's largest producer of farmed Atlantic salmon, and farmed salmon production can exceed 10 000 times the production of wild Atlantic salmon in some fjords (Skaala et al. 2014). Consequently, salmon farms ought to represent an important source of lice for wild salmonids, even when the numbers of female lice per farmed salmon may remain low.

In this Reply Comment to Jansen et al. (2016), we revisited the data published in Serra-Llinares et al. (2014), with a special focus on ruling out the potential confounding effect of temperature on the correlation between lice abundances on farmed and wild fish. First, we re-fitted the linear regression at issue (Fig. 3 in Serra-Llinares et al. 2014) including temperature as a covariate in the model. Second, we re-analyzed the data set using GLMMs to compare our results with those from Helland et al. (2015). Jansen et al. (2016, p. 2) conclude that 'if temperature is a confounder and there is no additional effect of farm origin lice as a factor that affects lice numbers in wild fish, then we would argue that there is no support in Serra Llinares et al. (2014) for the notion that lice of farm origin are a main source of infection in wild salmonids'. Results provided in this Reply Comment show that, even though Jansen et al. (2016) were correct in attributing part of the correlation between lice abundances on farmed and wild fish to the effect of temperature, there is strong evidence of a significant influence of lice originating from nearby farms on lice abundances on wild salmonids while accounting for the effect of temperature. In summary, our results from the analyses presented here and the results by e.g. Helland et al. (2015) and Thorstad et al. (2015) strongly suggest the transmission of lice from farm salmon to wild salmonids in systems where the fish occur in close vicinity. Future similar analyses should use available hydrodynamic-biological dispersal models (Asplin et al. 2014, Johnsen et al. 2016) to get a better estimate of the lice infestation pressure imposed by fish farms in different parts of the fjord systems along the Norwegian coast.

Acknowledgements. We thank Alain F. Zuur (Highland Statistics) for carrying out the zero-altered negative binomial mixed model analysis.

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Oban, UK

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The following supplement accompanies the article

Nearby farms are a source of lice for wild salmonids: a reply to Jansen et al. (2016)

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Aquacuture Environment Interactions 8: 351–356 (2016)

This Supplement provides further details regarding the re-analysis of the data from Serra-Llinares et al. (2014) using zero-altered negative-binomial (ZANB) mixed models. The aim of this re-analysis was to model the observed number of attached stages (copepodites and chalimi, hereafter referred to as "attached lice") of salmon lice (*Lepeoptheirus salmonis* Kröyer) on wild salmonids (sea trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* L.) using fish length, temperature, infestation pressure from nearby fish farms and year (categorical variable with the levels 2010 and 2011) as explanatory variables.

Methods

Prior to the analysis, the infestation pressure data used in Serra-Llinares et al (2014) was quality-controlled against analogous data currently available. The reason for this is that, at the time these data were gathered, we only had access to biomass and lice reports from fish farms separately; due to the reporting system at the time, some of the information required for merging both data sets (biomass and lice reports) was occasionally lacking, and thus some of the data was at times lost. Currently, we have access to biomass and lice data (included historical data) from fish farms through the Norwegian Marine Data Center at the Institute of Marine Research, where biomass and lice reports from all Norwegian fish farms are collected and merged together, the resulting data being of presumably higher quality than what was previously available. A comparison with analogous data currently available through the Norwegian Marine Data Center showed that our original data set tended to underestimate the amount of in-farm lice in some areas (data not shown), and thus we decided to use the newest data for this re-analysis. No changes were made on the data regarding lice counts on wild salmonids from the original data set.

As described in Serra-Llinares et al. (2014), water temperatures reported by the fish farms on a monthly basis were used in the study. However, the re-analysis of the data set following the approach by Helland et al. (2015) required gathering water temperature data also for reference locations, i.e. those locations without any active farms within 30 km. For those locations, water temperatures at 3 m depth were extracted from the results of the NorKyst800 numerical current model (Albretsen et al. 2011). The NorKyst800 has a grid resolution of 800 m, and comparison of model results with observations exhibits a typical error less than 1^oC. Mean monthly temperatures were obtained from the model grid (Albretsen et al. 2011) closest to the sampling sites, for the period May-August in the years 2010 and 2011. For each wild fish sampling occasion, mean water temperature corresponding to the actual sampling month was used. It was not possible to verify the robustness of model predictions for two locations (Handelsbukt in Porsangerfjord and Løksa in Salangen), and thus these locations were excluded from the analyses (137 fish of 2959 fish were removed).

Prior to the analyses, data exploration was applied following the protocol in Zuur et al. (2010). In brief, Cleveland dotplots were used for outlier detection; pair plots, Pearson's correlations and variance inflation factors (VIF) were used to detect collinearity.

As a first step, the number of attached salmon lice observed on wild salmonids was analyzed using a generalized linear mixed model (GLMM) with a Poisson distribution. Fish length (FL), temperature (Temp), infestation pressure from nearby fish farm (Pres) and year (categorical variable with the levels 2010 and 2011) were used as covariates, together with the interactions between each covariate and fish farm pressure. Infestation pressure was calculated as the number of female lice in nearby farms (<30 km), linearly down-weighted according to distance as described in Serra-Llinares et al. (2014). To account for repeated measurements at the same location, location was used as a random effect. The fixed covariates in the Poisson GLMM explained 25% of the variation but the model was over-dispersed. Model validation techniques (comparison of the percentage of zeros from simulated vs. observed data) indicated that the main reason for over-dispersion was zero inflation.

Zero-inflated and zero-altered models have been developed to cope with data sets presenting an excess of zeros (as is often the case with parasitological data). One limitation of standard count models is that the zeros and the non-zeros (positives) are assumed to come from the same data-generating process. With zero-altered models (also called “hurdle models”), these two processes are not constrained to be the same. The basic idea is that a Bernoulli probability governs the binary outcome of whether a count variable has a zero or positive realization. If the realization is positive, the hurdle is crossed, and the conditional distribution of the positives is governed by a truncated-at-zero count data model. With zero-inflated models, the response variable is modeled as a mixture of a Bernoulli distribution and a Poisson distribution (or any other count distribution supported on non-negative integers). Thus, the main difference between the zero-inflated negative binomial (ZINB) GLMM used by Helland et al. (2015) and the ZANB GLMM used for the analysis presented here is the way they interpret and analyze zero counts. For more detail and formulae, see, for example, Gurmu & Trivedi (1996) or Dalrymple et al. (2003).

The advantage of using a zero-altered GLMM is that it can be used to simultaneously investigate the following two questions: What is driving the absence and presence of lice? And when lice are present, what is driving their numbers? Thus, a zero-altered negative binomial (ZANB) GLMM was used for this analysis, with a full model specification as follows:

$$\begin{aligned}
&Ch_{ij} \sim ZANB(\mu_{ij}, \pi_{ij}, k) \\
&E\left(Ch_{ij}\right) = \frac{1-\pi_{ij}}{1-P_0} \times \mu_{ij} \qquad \text{where } P_0 = \left(\frac{k}{\mu_{ij}-k}\right)^{k} \qquad (1) \\
&\log\left(\mu_{ij}\right) = FL + Temp + \text{Pres} + Year + FL \times \text{Pres} + Temp \times \text{Pres} + Year \times \text{Pres} + \text{a}_{\text{i}} \\
&\text{logit}\left(\pi_{ij}\right) = FL + Temp + \text{Pres} + Year + FL \times \text{Pres} + Temp \times \text{Pres} + Year \times \text{Pres} + \text{b}_{\text{i}}
\end{aligned}$$

where Ch_{ij} is the number of attached lice for the jth observation at location i (n = 30). The random effects a_i and b_i capture the within-location dependency, and are assumed to be normally distributed. Prior to analysis, all continuous covariates were standardized by subtracting the mean and dividing by the standard deviation, which is common practice when fitting GLMMs.

The ZANB GLMM was fitted in two separate steps (Zuur et al. 2012). First a Bernoulli GLMM was fitted to the absence and presence data. In the second step, the presence-only data (i.e. all the non-zero Ch_{ij} data) were analyzed using a zero-truncated negative binomial GLMM (NB GLMM). Both models were fitted using the glmmADMB package (Skaug et al. 2015) in R (R Development Core Team 2015). Once the 2 separate models were fitted, we combined the 2 components to calculate the fitted values and Pearson residuals for the combined model (ZANB GLMM).

Model validation was performed by plotting the Pearson residuals from the ZANB GLMM vs. fitted values, each covariate in the model, and each covariate not in the model. The percentage of zeros obtained by simulating data using the ZANB GLMM was compared with the percentage of zeros in the original data set. Comparison was also made between the sum of squared Pearson residuals for simulated and original data, and between maximum values of simulated and original data.

RESULTS

Based on data exploration results, 32 fish with questionable length values (based on condition factor) were removed, and fish farm pressure was square-root transformed to deal with its high dispersion. Scatterplots, Pearson's correlation and VIF values did not indicate any collinearity problems, not even between temperature and infestation pressure (VIF <2). Further, modeling fish farm pressure as a function of temperature using a linear mixed effects model (using location as random effect) identified a temperature effect, but the effect size was small, explaining only 8% of the variation.

The ZANB GLMM was fitted and successfully validated. According to the results from the first step (i.e. the Bernoulli GLMM) (Table 1), the fitted model for the years 2010 and 2011 can be written as follows:

$$\begin{aligned} \text{logit}(\pi_{ij}) &= -0.59 + 0.09 \times FL + 0.81 \times Temp + 0.26 \times Pres - 0.18 \times FL \times Pres + 0.30 \times Temp \times Pres \quad (2010) \\ \text{logit}(\pi_{ij}) &= 0.02 + 0.09 \times FL + 0.81 \times Temp + 0.85 \times Pres - 0.18 \times FL \times Pres + 0.30 \times Temp \times Pres \quad (2011) \end{aligned} \tag{2}$$

For a graphical representation of these results, the numerical output of the Bernoulli GLMM was used to predict the probability of presence of attached lice on wild salmonids for increasing fish farm pressure values at 3 different temperatures (Fig. 2 in the main article). These results suggest that increasing values of length, temperature and fish farm pressure lead to an increase in the probability of presence of lice larvae on wild salmonids, the fish farm pressure effect being considerably stronger in 2011.

Results from zero-truncated NB GLMM model (Table 2 & Fig. 3 in the main article) suggest a significant effect of both fish farm pressure and temperature also on the actual lice counts on infested wild salmonids, but there is a large variation around the fitted values. In this case, the underlying equations can be specified as follows:

$$\begin{aligned} \text{logit}(\mu_{ij}) &= 2.11 - 0.05 \times FL + 0.52 \times Temp + 1.18 \times Pres + 0.00 \times FL \times Pres + 0.08 \times Temp \times Pres \quad (2010) \\ \text{logit}(\mu_{ij}) &= 2.23 - 0.05 \times FL + 0.52 \times Temp + 0.51 \times Pres + 0.00 \times FL \times Pres + 0.08 \times Temp \times Pres \quad (2011) \end{aligned} \tag{3}$$

Note that, in this case, the effect of fish farm pressure is weaker in 2011 compared to 2010.

Model validation tools indicated that the ZANB GLMM produced simulated data sets with similar percentages of zeros as the original data, and the sum of squared Pearson residuals for the simulated data sets are comparable to the sum of squared Pearson residuals for the observed data, indicating that there are no major issues with the model.

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Paper 3

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Towards direct evidence of the effects of salmon lice (*Lepeophtheirus salmonis* Krøyer) on sea trout (*Salmo trutta* L.) in their natural habitat: proof of concept for a new combination of methods

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Abstract Studies addressing the impact of salmon lice (*Lepeophtheirus salmonis* Krøyer) on sea trout in their natural habitat are scarce and mostly limited to prophylaxis-based experiments. The main drawbacks with this approach are that lice infestations on control fish are not known and the anti-parasitic treatment is of unknown efficacy and may have unwanted side-effects. We tested an innovative approach where prophylaxis is replaced with artificial infestation of the fish. Twenty-nine sea trout post-smolts were caught in a farming-free area in southern Norway and half the fish were artificially infested with lice. Survival and behavior of individual fish was investigated using acoustic telemetry. Furthermore, salinity values were extracted from an hydrodynamical model simulation and connected to individual 3D positions. Results from this pilot study show consistent trends in behavioral differences between artificially infested and control fish. All fish that died or prematurely returned to freshwater were artificially infested fish, although results were not statistically significant. Besides, power analysis confirmed the limitations of this small pilot study for delivering statistically significant inferences. We found also indications of artificially infested fish remaining in shallower waters and within shorter distance to low salinity habitats, but only differences in modelled salinity values were statistically significant. Methodological progress and limitations with this original approach are discussed, and we recommend further studies using this combination of methods and the lessons learnt from this pilot study to provide better quantitative evidence on the effect of salmon lice on sea trout in the wild.

Keywords Salmon lice · Sea trout · Artificial infestation · Survival · Behavior · Acoustic telemetry

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Introduction

Salmon farming has experienced a very rapid development in Norway, from a production of a few hundred tons in the early 70's to more than 1.3 million tons in 2015 (Statistics Norway, https://www.ssb.no). There are currently close to 1000 salmon farms scattered along most of the Norwegian coastline, and only a few areas remain free from intensive farming (Directorate of Fisheries, www.fiskeridir.no). The growth of this industry has not been without environmental concerns. Together with escaped salmon, the spread of the parasitic copepod salmon louse (*Lepeophtheirus salmonis*, Krøyer) from fish farms has been identified as a major threat for wild salmonids in Norway (Costello 2009; Taranger et al. 2015; Forseth et al. 2017). Amongst salmonids, sea trout may be particularly vulnerable to salmon lice infestation because most sea trout remain in fjords and coastal waters, where salmon farms -and thus salmon lice- concentrate, during most part of their marine migration. Although the causal relationship between salmon farming and lice epizootics on sea trout has been controversial over the last decade, there is extensive evidence linking high lice infestations on wild sea trout to farm-intensive areas (Bjørn et al. 2001; Bjørn et al. 2011; Middlemas et al. 2013; Serra-Llinares et al. 2014; Serra-Llinares et al. 2016; Shephard et al. 2016). Furthermore, it has recently been documented that lice collected from wild salmonids in farm-dense areas often carry resistance to organophosphates in a frequency that mimics those in nearby farms, unequivocally demonstrating that salmon farms are a primary driver of the salmon louse infection dynamics (Fjørtoft et al. 2017).

Salmon lice feeds on skin, blood and mucus of salmonid fish and their harmful effects on individual sea trout have been widely documented. These include osmoregulatory problems and physiological stress responses, secondary infections, reduced fitness and in worst cases a complete physiological breakdown and death of the salmonid host (Birkeland and Jakobsen 1997; Bjorn and Finstad 1997; Dawson 1998; Poole et al. 2000; Bjørn et al. 2001). Changes in migratory behavior (Birkeland 1996; Birkeland and Jakobsen 1997; Bjørn et al. 2001; Gjelland et al. 2014; Hatton-Ellis et al. 2006; Pert et al. 2009) and reduction of marine growth of individual fish (Birkeland 1996; Poole et al. 1996; Butler and Walker 2006; Fjørtoft et al. 2014) have also been documented. Salmon lice in areas with high farming intensity may also negatively impact sea trout on the population level, as shown in Ireland (Tully and Whelan 1993; Tully et al. 1999; Gargan et al. 2003, 2006, 2016), Scotland (Butler and Walker 2006) and Norway (Bjørn et al. 2001; Skaala et al. 2014). Most of this evidence is, however, circumstantial. Direct and quantitative evidence of the relative role of salmon lice infestation on these population declines is still missing. In a recent and extensive review of available scientific literature on the impact of salmon lice on sea trout, Thorstad et al. (2015) concluded that “the most important knowledge gaps are related to salmon lice impacts at the population level and in quantifying the reduction in wild sea trout populations arising from increased mortality and reduced growth attributable to salmon lice”.

Disentangling the relative role of salmon lice from other factors regulating mortality and fitness on wild fish, such as food availability, predation, water quality and/or other parasites, is a challenging task, especially since these factors may affect each other in many possible ways. Additionally, free-swimming sea trout may modify their natural behavior to mitigate the effect of the parasites by prematurely returning to freshwater. This behavioral adaptation may mask, delay or prevent direct mortality, but at the cost of reduced marine growth, reduced fecundity and/or reduced spawning success (Birkeland 1996; Birkeland and Jakobsen 1997; Dawson 1998; Gjelland et al. 2014; Shephard et al. 2016; Halttunen et al. 2018), which may ultimately have an impact on the population level. Due to this complexity, field experiments where lice-infested fish can interact with their natural environments are needed to properly measure the relative impact of salmon lice on sea trout at both individual and population levels.

Field studies performing paired releases of control groups and groups treated with an anti-parasitic agent, i.e. prophylaxis-based “randomized control trials”, (RCTs), have become a widespread method to disentangle the effect of salmon lice from other factors regulating mortality and growth of wild fish. This approach has been extensively used in recent years to study the impact of salmon lice on the marine survival of Atlantic salmon, estimated through recapture of returning fish (Jackson et al. 2011; Gargan et al. 2012; Krkošek et al. 2013; Skilbrei et al. 2013; Vollset et al. 2014, 2016), whilst very few similar studies have addressed the effect of salmon lice on sea trout (Gjelland et al. 2014; Skaala et al. 2014; Halttunen et al. 2018). However, and despite the potential of RCTs to provide a more accurate picture of the effect of salmon lice on wild fish compared to laboratory studies, there are some caveats associated with the prophylaxis-based approach. Firstly, there may be limitations in the protection provided by the treatment (in terms of efficacy, variation and duration). Secondly, there may be negative effects associated with the use of the prophylaxis itself (e.g., toxicity). Finally, prophylaxis-based studies rely on the "natural" lice infestation pressure in the study area which, in most intensively-farmed areas, has been proved to be highly variable and difficult to predict (Serra-Llinares et al. 2014; Helland et al. 2015; Serra-Llinares et al. 2016). This, in combination with the typically skewed distribution of lice between wild hosts, makes it very difficult to make inference on the actual lice load in non-protected (control) fish. A more detailed discussion on the limitations of prophylaxis-based RCTs can be found at Vollset et al. (2018) and at Thorstad et al. (2015).

An alternative approach to prophylaxis-based RCTs are studies based on artificial infestation of individual fish with salmon lice and comparison to an un-infested control group in a lice-free area. Such "infestation-based RCTs" may be more suitable for assessing the effect of salmon lice on wild salmonids, as they 1) don't rely on the sustained effect of a prophylactic drug, 2) allow for a better control on the actual lice load on the fish, and 3) no other secondary effects are expected associated with the treatment (artificial infestation). Furthermore, in areas where the lice density is low, this method may also be the only way to conduct a treatment-control experiment.

In this pilot study, we tested the feasibility of an infestation-based RCT to study the effects of salmon lice on the survival and behavior of wild sea trout in their natural habitat. We combined this approach with the use of acoustic telemetry, which allows direct observation of survival of tagged individuals (thus reducing the dependency on the recapture of returning fish) as well as other potential behavioral changes motivated by lice. Finally, we combined 3D fish positions provided by acoustic telemetry with salinity estimates provided by a hydrodynamical model, so that differences in salinity preferences other than premature return to freshwater could be studied. Although we tested this new approach in a pilot study, with a limited number of fish included, we hypothesized that artificially infested fish would be stressed by their parasite load (compared to the control group) and alter their habitat choice seeking for lower salinities. We also hypothesize that the stress caused by the lice would lead to premature returns to freshwater and/or to an increased mortality risk. However, the main goal of this pilot study was to test 1) whether this combination of methods was feasible for a field study, and 2) whether there was indication of an added value on this shift in method (from prophylaxis- to infestation-based studies).

Methods

Study area

The study took place in Sandnesfjord, Southern Norway (58.6943°N, 9.1488°E) (Fig. 1) from June to September 2016. The closest active salmon farm is situated more than 85 km away (sea-way distance) from Sandnesfjord, and sustained low lice levels have been recorded on wild sea trout in the area in recent years (Serra-Llinares et al. 2014). The river Storelva runs out to Sandnesfjord, and provides a 20 km river stretch for anadromous fish. The salinity in Sandnesfjord is commonly above 20, but the inner part of the fjord can be influenced by a shallow layer of fresh or brackish water. The transition areas between Sandnesfjord and the river Storelva, i.e. the areas known as Songevatn and Nævestadfjord, (Fig. 1) are characterized by low salinities, which vary between 0 and 15 depending on depth and the volume of inland freshwater inflow from the Skagerrak and the coastal current (Tjomsland and Kroglund 2010). The whole area between the Nævestadfjord outlet and all the way up to the river Storelva will be referred to as "freshwater" from this point for simplicity.

Fish tagging and artificial infestation

Sea trout post-smolts ($n = 36$, size range 40–156 g) were caught in mid-June in Sandnesfjord using fishing traps designed for live sampling of sea trout (Barlaup et al. 2013) (Table 1). Of these, 29 fish were tagged using acoustic tags from Thelma Biotel (ADT-LP-7.3 model, size of 22 × 7.3 mm; 1.1 g in water; typical battery life of 5 months) following standard chirurgical procedure, as described in Serra-Llinares et al. (2013). Tags were built with a depth sensor (0.2 m resolution and 51 m maximum depth) and programmed to emit a unique identification code at random intervals every 30 to 90 s. The fish were inspected

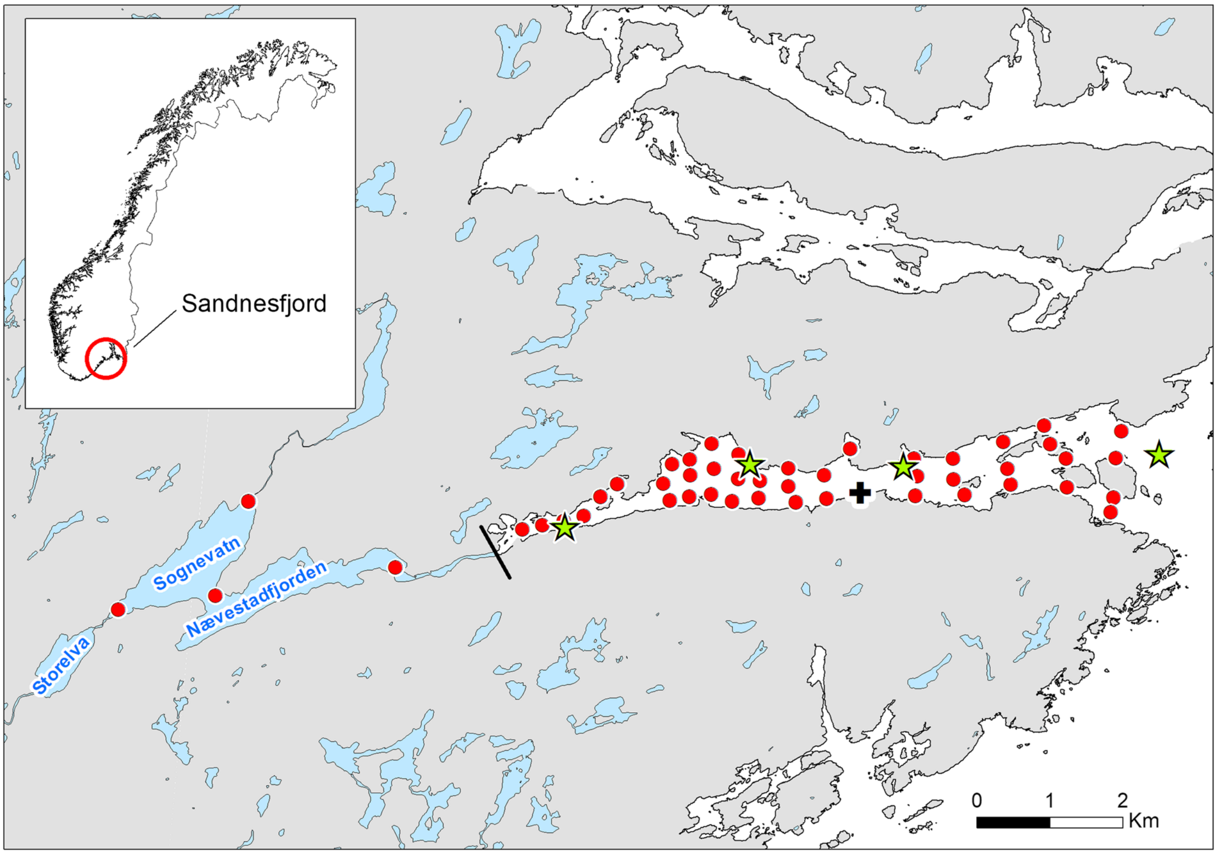

Fig. 1 Sandnesfjord, Southern Norway. Red dots show the configuration of the acoustic hydrophones array. Green stars indicate the position of the DST tags (1 to 4 from East to West). Black cross indicates the position of the release site. The solid black line indicates the limit between sea water (white) and freshwater (light-blue) areas

for lice (counts) and randomly assigned to group: "*control*" ($n = 14$) or "*infested*" ($n = 15$). The remaining seven fish were tagged with passive dummy tags of the same size and weight as the ADT-LP-7.3 tags. This "*reference*" group was used to evaluate the success of the artificial lice infestation and the recovery after tagging.

Salmon lice copepodids used for artificial infestation were supplied by the Institute of Marine Research in Bergen. First, 40 mature egg strings were collected from female lice ("LsGulen" family, 30/31 generation in the laboratory). After incubation at 9.5 °C for 10 days, approximately 4000 copepodids were collected and transferred in a 2000 ml thermos bottle to the field site in Sandnesfjord. The procedure of incubation and quantitative assessment is described in Hamre et al. (2009).

For artificial infestation, sea water (salinity 30, 15 °C) from 15 m depth was pumped and circulated into the infestation tank, where fish from both the artificially infested and reference groups were exposed to approximately 180 newly hatched copepodids each. During infestation, water circulation in the tank was stopped and the level kept at approximately 10 cm for 30 min; oxygen content was continuously monitored and regulated during the process. After infestation, the water circulation was re-established in the tanks, where the fish could recover for 5 h. To avoid a handling effect, the "*control*" group received a sham infestation following the same procedure as infested fish. After recovery, all fish in the artificially infested and control groups where simultaneously released in the middle part of the fjord (see Fig. 1 for exact position of the release site). Fish in the reference group were transferred to a nylon net recovery cage (volume 0.5 m^3) and kept for another 48 h prior to sampling.

Table 1 Sea trout individuals tagged and released in the study

Acoustic ID	Group	Weight (g)	Fork length (mm)	Lice abundance	Relative intensity (lice gr^{-1})	Final rel int (lice gr^{-1})	Fate	Fate date
ST_076	Control	40	170	3	0,075	–	S	25.08.2016
ST_078	Control	41	163	8	0,195	–	S	25.08.2016
ST_080	Control	92	209	8	0,087	–	D	02.07.2016
ST_082	Control	79	199	6	0,076	–	D	22.06.2016
ST_083	Control	69	189	11	0,159	–	S	25.08.2016
ST_085	Control	70	190	6	0,086	–	S	25.08.2016
ST_087	Control	90	198	8	0,089	–	S	25.08.2016
ST_089	Control	88	195	11	0,125	–	D	22.06.2016
ST_092	Control	70	190	3	0,043	–	S	25.08.2016
ST_093	Control	43	152	0	0,000	–	S	25.08.2016
ST_096	Control	70	187	22	0,314	–	S	25.08.2016
ST_098	Control	87	201	16	0,184	–	D	21.06.2016
ST_100	Control	52	163	5	0,096	–	S	25.08.2016
ST_102	Control	79	198	24	0,304	–	D	27.06.2016
ST_075	Infested	40	155	9	0,225	0,700	R	25.06.2016
ST_077	Infested	75	194	16	0,213	0,467	M	15.07.2016
ST_079	Infested	45	165	9	0,200	0,622	S	25.08.2016
ST_081	Infested	57	172	8	0,140	0,474	M	23.07.2016
ST_084	Infested	44	156	9	0,205	0,636	D	20.06.2016
ST_086	Infested	52	176	2	0,038	0,404	R, M	22.06, 08.07.2016
ST_088	Infested	94	205	14	0,149	0,351	S	25.08.2016
ST_090	Infested	84	195	15	0,179	0,405	D	27.06.2016
ST_091	Infested	73	189	22	0,301	0,562	S	25.08.2016
ST_094	Infested	122	223	10	0,082	0,238	D	21.06.2016
ST_095	Infested	69	183	6	0,087	0,362	S	25.08.2016
ST_097	Infested	93	210	42	0,452	0,656	Did not provide data	
ST_099	Infested	47	170	16	0,340	0,745	S	25.08.2016
ST_101	Infested	62	187	25	0,403	0,710	D	29.06.2016
ST_103	Infested	65	188	10	0,154	0,446	D	29.06.2016

All fish were released on 20.06.2016. “Relative intensity” indicates observed lice loads prior to tagging. “Final rel int” indicates minimum estimated lice loads after artificial infestation. Fate abbreviations: S, survived inside the fjord; D, dispersed towards the ocean; M, died; R, returned to freshwater

Acoustic tracking of the fish

Tagged fish were monitored from release (20th June) to the end of the study (25th August) through an array of hydrophones (Vemco VR2W) covering the study area both at sea and in freshwater. Range tests indicated an optimal detection range of 150–200 m in sea water (85% of the signals were still picked up by the hydrophones at this distance). Based on this, we designed a hydrophone array consisting of 56 receivers, of which 51 were placed at sea water and five in freshwater (Fig. 1). The array was designed so that it would provide the best possible coverage of the inner part of the fjord, covering the possibility that infested fish would search for a freshwater refuge for delousing. Fish movements in the fjord were monitored using parallel lines of receivers, so that we could assess which part of the fjord (inner/middle/outer) the fish were at any time. Migration out of the fjord was monitored by a double fence of receivers at the fjord mouth, so that the direction of the fish passing by this area could be assessed. Last, receivers situated in freshwater provided information on the timing and frequency of returns.

The hydrophones recorded the depth and ID information emitted by the fish tags. Data were downloaded from the hydrophones at the end of the study and imported to Vemco VUE software (version 2.2.2). Data were corrected for temporal drift using VUE, and then exported to R software (R Core team 2015) where all analyses were performed. Any single isolated detection occurring in a 24 h period was removed as potentially spurious. Fish positions (1 h intervals) were estimated using center-of-activity locations (Simpfendorfer et al. 2002). The center-of-activity location for a given time interval t is the mean position of the hydrophones that detected the animal at that time interval, weighted by the number of times the animal was detected at each hydrophone (see Simpfendorfer et al. 2002). A mean depth value was also associated to each center-of-activity position, so that they consisted of a 3D position with latitude, longitude and depth.

Environmental monitoring

Salinity data were obtained for each 3D fish position from a hydro-dynamical model simulation using ROMS (Regional Ocean Modeling System, http://myroms.org) with 50 m horizontal resolution based on output from NorKyst800 (Albretsen et al. 2011) and a 160 m model for the southeastern Norwegian coast (similar set ups as conducted in Asplin et al. (2014) and Espeland et al. (2015). All three models applied high-resolution atmospheric forcing from the non-hydrostatic 2.5 km AROME MetCoOp regional atmospheric model (Müller et al. 2015) provided by the Norwegian Meteorological Institute. The freshwater discharge from Storelva was supplied outside the narrow strait between the Nævestadfjord and Sandnesfjord, and volume fluxes for all rivers were based on daily estimates from the Norwegian Water Resources and Energy Directorate. For model validation purposes, salinity was continuously monitored during the study at four different points along the fjord using DST tags (Star-Oddi, Iceland) (see Fig. 1 for exact positions of the loggers). Measurements from repeated CTD casts were also used to correct the model output for bias. The DST tags deployed near the surface along the fjord revealed that the hydro-dynamic model predicted higher salinity than direct measurements (positive bias) in the surface layer. This is probably attributed to how well defined the river outflow from Storelva is and/or how the vertical river outflow is implemented in the model. A similar bias was found for surface waters, also along the fjord axis (Fig. S1, electronic appendix). However, the model error in salinity was reduced linearly and were close to zero by 22 m depth. To estimate a more precise salinity level for all tagged fish in the entire Sandnesfjord, we applied a linear relation for salinity bias correction with depth:

$$S_{\text{corrected}} = S_{\text{model}} - \left(-0.1776^{*}D + 4.0903\right), \text{ for depths } (D) \text{ above or equal to 22 m}$$
$$S_{\text{corrected}} = S_{\text{model}}, \text{ for depths below 22 m}$$

The corrected salinity values were used in all further statistical analysis.

Data interpretation and statistical analysis

Fate assessment

The fate of each individual fish was assessed from their depth profiles and positions. Individuals were classified as: 1) *returned to freshwater* (moved beyond the Nævestadfjord outlet and did not re-enter the fjord), 2) *dispersed* (last detection crossing the outer double-fence of receivers), 3) *dead* (either stopped transmitting while inside the study area or started transmitting continuously at the same depth), or 4) *survived* (detections indicating normal vertical and horizontal swimming activity in the fjord by the end of the study).

Survival and premature return to freshwater

Cox proportional hazard models (Coxph) were used to analyse potential differences in the probability of survival and of premature return to freshwater between control and infested fish. Coxph models estimate the likelihood that an event will occur at time t. When modelling survival and premature return to freshwater, the fate/status was set as 0 (survived/did not return to river), or 1 (died/returned to river). Fate time was the number of days after release. For fish with fate/status = 0 (i.e. survived/did not return to river) fate date (t) was defined by the last observation and specified as right-censored data. *Group* (infested/control) and fish *length* (fork length) were used as explanatory variables. Models were fitted using the R library *survival*.

To further investigate the ability of our approach to detect between-groups differences in survival and premature return under different scenarios, we performed power analysis using the function *ssizeCT* from the R library *powerSurvEpi.* This function allows for sample size calculation for the comparison of survival curves between two groups under the Cox Proportional-Hazards Model. Some parameters, such as postulated hazard ratio (*RR*), *power* (i.e. power to detect the magnitude of the hazard ratio as small as that specified by *RR*), *alpha* (i.e. type I error rate) or the ratio of participants in each group (*k*) can be manually adjusted in the function. Other parameters, such as the expected total number of events in each group, are estimated based on a pilot data set. We performed scenario testing to estimate the minimum sample required to detected different hazard ratios (*RR*) under the premise of three fixed parameters: $power = 0.8$, $alpha = 0.05$ and $k = 1$ (i.e. minimum sample size to have an 80% probability of detecting a survival ratio *RR* with a confidence of 95%, and given that there are the same number of fish in both groups).

Habitat choice

Both distance to freshwater and swimming depth were used as indirect indicators of salinity. The distance to freshwater were estimated as the linear distance between the center of activity (for each individual fish) and the Nævestadfjorden outlet (Fig. 1). Negative distances were assigned to positions beyond this point. Daily mean distance to freshwater was then calculated for each fish. Mean daily swimming depth and mean daily salinity (obtained from the hydro-dynamical model) were also obtained for each fish.

Differences in distance to freshwater, swimming depth and salinity preferences between control and infested fish were tested using linear mixed models. Daily means were used as response variables, while *group* and fork *length* were used as explanatory variables. Individual identification was used as random intercept to account for random variation between individuals. An autoregressive process of order 1, corAR1 (see Zuur et al. 2009), was added to the random structure of the model to take temporal autocorrelation into account. Since the hydro-dynamical model did not cover the locations in low salinity areas, i.e. in Songevatn and Nævestadfjord, we fitted two different models for salinity: one model using all fish, where fish in freshwater locations were given a salinity value of zero (to test differences in salinity used in general), and one model where fish in freshwater locations were taken out of the data set (to look at differences in salinity used while inside the fjord only). Models were fitted using the R library nlme. All models were validated following Zuur et al. (2009) to verify that the underlying statistical assumptions were not violated.

Results

Initial and artificial lice infestation

Initial inspection of the sea trout post-smolts used in this study, i.e. directly after capture at sea, showed that 96% of the fish were infested with lice, with infestations ranging from two to 42 lice per fish, corresponding to 0.038 to 0.45 lice per gram fish weight (Table 1). Chalimus II and pre-adult were the predominant stages. After artificial infestation, fish in the "*reference*" group (artificially infested) had acquired new copepodites, with a mean added intensity of 33 lice per fish. The minimum increase in observed lice on reference fish was 19 copepodites; we used this number to conservatively estimate the minimum final infestation on artificially infested fish ("infested" group), which were then estimated to have post-infestation lice loads ranging from 21 to 61 lice, corresponding to final relative intensities of 0.24 to 0.74 lice gr^{-1} (Table 1).

Acoustic tracking and fate assessment

A total of 28 fish (14 control and 14 infected) provided depth and movement data (one fish did not provide any data) (Table 1). Most individuals were observed inside the fjord or dispersed towards the ocean. Approximately 1/3 (nine of 28) of the fish left the study area within the first week after release and were never observed again, thus providing limited data for posterior analysis.

Return to freshwater

All sea trout that returned to freshwater during the study period (two of 28 individuals) were artificially infested fish, but the difference between the groups was not statistically significant (p = 0,999; Table 2). Returning fish tended to be small sized (p = 0,145; Table 2).

Survival

Three individuals died during the study period, all belonging to the infested group. However, survival analysis showed no statistically significant difference between the groups (p = 0,999; Table 2, Fig. 2). Fish length did not significantly affect the probability of death (p = 0,896; Table 2).

Power analysis

Results from the power analysis showed, based on the structure of the data provided by our pilot study (i.e. frequency of events and distribution of censoring points), that a sample size as low as that in the present study (14 fish in each group) would only have the sufficient power to detect significant differences in survival and/or premature return to freshwater between control and artificially infested fish given the survival (or return) ratio (*RR*) was higher than 10 (i.e. if fish in the control group had a 10 times higher probability of survival/premature return than artificially infested fish) (Table 3). Conversely, to detect small differences in survival/premature return, such as $RR = 1.5$, the minimum required sample size would be as high as 2198 fish (1099 fish in each group).

Distance to freshwater

Sea trout made use of the entire fjord, and were also located up to 5.3 km up from the Nævestadfjorden outlet (Fig. 3). The distance to freshwater (defined as the distance to the Nævestadfjorden outlet; Fig. 1) increased significantly with body size ($p < 0.001$, Fig. 4, Table 4). The mean daily distance to freshwater was shorter for infested fish (4.0 km) compared to the control group (4.9 km) but the difference was not significant (p = 0.136; Table 4, Figs. 3 and 4).

Depth use

Sea trout were in general located close to the surface. Average daily depth ranged between 0.2 and 3.8 m (Fig. 4). Artificially infested fish were located marginally closer to the surface (average daily depth = 1.1 m) compared to non-infected fish (average daily depth = 1.3 m) (p = 0.053; Fig. 4, Table 4). Average daily depth increased significantly with body size for both groups, showing that larger fish in general used deeper waters (p = 0,013; Fig. 4, Table 4).

Salinity

Average daily salinity used by sea trout varied between 0 and 27.6 (Fig. 4). Results from linear mixed models showed that, while in the Sandnesfjord, trout in the artificially infested group remained in water with significantly lower salinity compared to their counterparts in

Table 2 Results of the Cox proportional hazard models, modelling the probability of returning to freshwater and the probability of dying at time *t* as a function of treatment group (untreated vs artificially infested) and body size (fork length, FL)

Event	Covariate	β	exp(β)	se(β)	z	*P*
Returnal to freswater	Group [Infested]	21,24	1,676,000,000	26,050	0.00	0.999
	Length	−0.09	0.92	0,06	−1.46	0.145
Mortality	Group [Infested]	21.52	2,220,000,000	23,570	0.00	0.999
	Length	−0.01	0.99	0,04	−0.13	0.896

the control group, although the difference was small (salinity 20 versus 21, $p = 0.009$; Table 4, Fig. 4). The preference for higher salinity increased significantly with body size ($p = 0.002$; Table 4).

Discussion

Salmon lice and its impact on wild fish populations are sources of intense debate in all salmon producing countries, and it is currently of high political and economic relevance in Norway. Solid science-based advice is therefore needed by decision makers to be able to apply sound management strategies. Given the complexity of the systems, coupled with methodological challenges, direct quantitative evidence of the impact of salmon lice on the survival and growth of sea trout in their natural habitat have been proved difficult to obtain. A study by Skaala et al. (2014) in an intensively farmed fjord in western Norway showed that the survival of sea trout smolts treated with an anti-parasitic drug was nearly double compared to a control group. However, overall survival in this study was very low, and the number of surviving fish were limited. In the same fjord, Gjelland et al. (2014) and Halttunen et al. (2018) combined the administration of anti-parasitic treatments with the use of acoustic telemetry to study the survival and migratory behavior of wild, free-swimming sea trout. Despite most of the sea trout tracked in these studies adopted a movement pattern expected to suppress or alleviate salmon

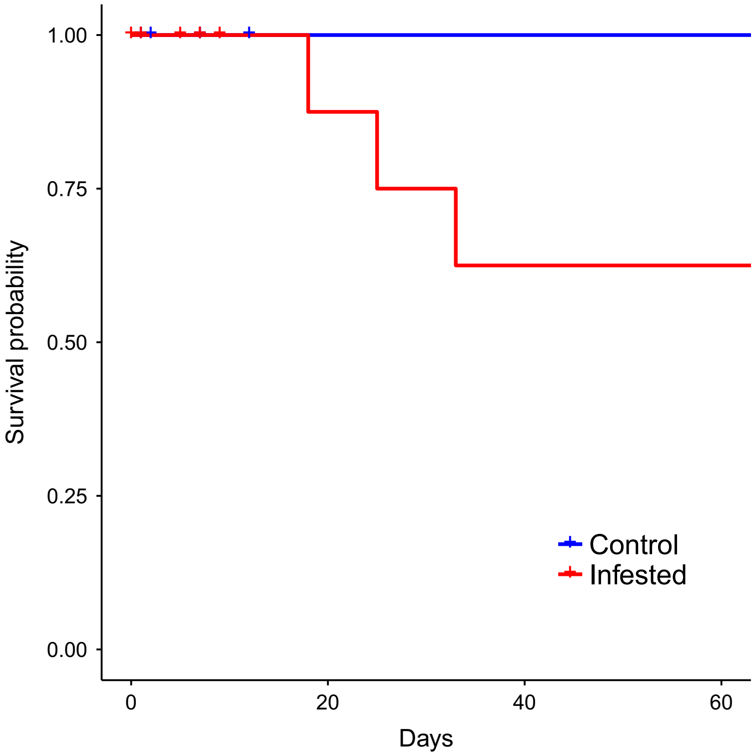

Fig. 2 Survival curves, showing the probability of survival of sea trout (*Salmo trutta*) post-smolts as a function of treatment group (control, $n = 14$ vs infested, $n = 14$)

Table 3 Estimated minimum sample sizes (control + experimental group) needed to have an 80% probability (i.e. power = 0.8) to detect statistical differences in survival/premature return between infested and control fish (survival ratio, RR), with a confidence of 95% (i.e. alpha = 0.05), given that there are the same number of fish in both groups (*k* = 1)
All estimates are based on results from our pilot study and performed using the function ssizeCT from the R library powerSurvEpi

RR	Minimum Sample Size
1.5	2198
2	660
5	84
10	30

lice infestation, showing a strong preference for fresh and brackish water especially in high exposure years (Gjelland et al. 2014; Halttunen et al. 2018), no significant differences in behavior nor in survival were found between treated and control groups (Gjelland et al. 2014; Halttunen et al. 2018), making it difficult to draw firm conclusions about the relative role of salmon lice itself. Nevertheless, all these studies are based on the use of anti-parasitic drugs. Due to the earlier stated caveats associated with this method, the lack of decisive results in these studies could arguably be related to methodological limitations. Furthermore, this kind of studies only test the effect of treatment against unknown lice intensities on control fish, and thus are not suited to investigate the shape of the relationship between lice and their impact on the host fish. Consequently, it has been suggested that moving from treatment-based to exposure-based studies may be a more suited approach (Vollset et al. 2018).

In this pilot study, we have tested an infestation-based randomized control trial (RCT) as an alternative to the more extended prophylaxis-based approach to investigate the effect of salmon lice on wild, free-swimming sea trout. We have compared survival and habitat preferences between wild sea trout post-smolts either from a control group or from a group artificially infested with salmon lice. The combination of methods presented here (i.e. artificial infestation of wild fish in an area with natural low lice infestation pressure, combined with acoustic telemetry and hydrodynamical modeling) is, to our knowledge, novel in the pursue of quantitative evidence of the impact of salmon lice on sea trout in their natural habitat.

Only one of the end-points analyzed in this pilot study, i.e. differences in salinity preferences as determined by model simulations, showed statistically significant differences between control and artificially infested fish. Besides, limitations on the statistical

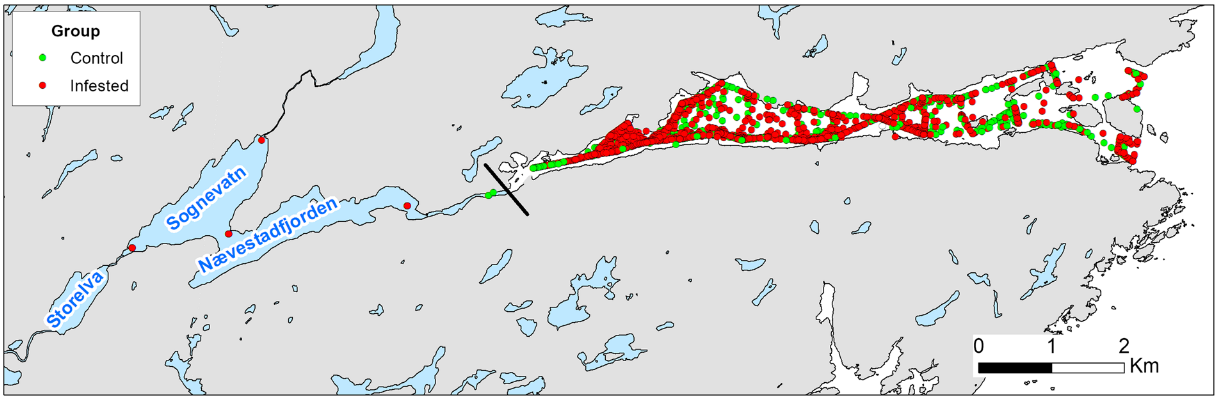

Fig. 3 Trout positions (centres of activity), coloured per group (control and infested). Distance to freshwater was calculated as the linear distance to the black solid line. Positions in freshwater (light-blue areas) were attributed negative distances

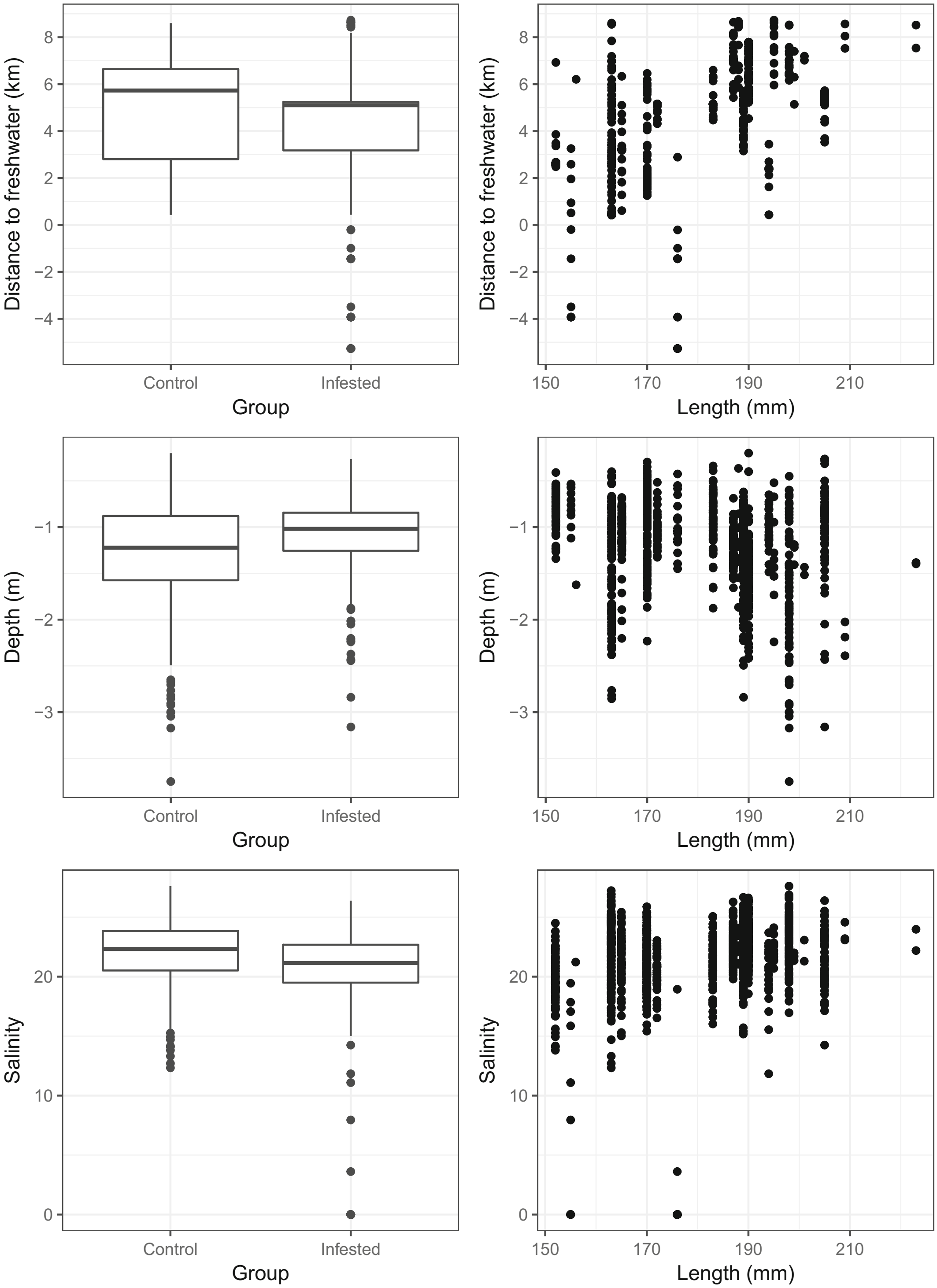

Fig. 4 Mean daily distance to freshwater, mean daily depth and mean daily salinity used of sea trout (*Salmo trutta*) post-smolts, as a function of group (control, *n* = 14 vs infested, *n* = 14) and body length

Table 4 Model coefficients (β) and corresponding standard errors (SE), t values and significance levels (*P*), describing the effect of group and body size on mean daily distance to freshwater, mean depth and mean salinity used by sea trout in Sandnesfjord

Response	Term	β	SE	DF	t	*P*
Distance to freshwater	Intercept	−11.011	4.031	1021	−2.732	0.006
	Group [Infested]	−1.139	0.740	25	−1.539	0.136
	Length	0.090	0.022	25	4.184	< 0.001
Depth	Intercept	−0.305	0.618	1021	−0.494	0.622
	Group [Infested]	−0.222	0.109	25	−2.029	0.053
	Length	0.009	0.003	25	2.676	0.013
Salinity (river and fjord)	Intercept	4.174	8.342	1021	0.500	0.617
	Group [Infected]	−2.900	1.523	25	−1.903	0.069
	Length	0.097	0.045	25	2.172	0.040
Salinity (only fjord)	Intercept	13.439	2.500	1000	5.376	0.000
	Group [Infested]	−1.243	0.436	25	−2.854	0.009
	Length	0.048	0.014	25	3.479	0.002

power of such a small study (with only 14 fish in each group) were somehow expected. Furthermore, almost one third of the fish was lost from the study area after only 1 week post-release, which severely limited the amount of data available for posterior analyses. Power analysis further confirmed that the limited statistical power of our pilot study was not robust enough to unequivocally demonstrate whether the lack of differences between the groups reflected a real lack of impact from the lice burden, or was rather the result of the low statistical power. Thus, the lack of support for our hypothesis of increased risk of mortality and premature return for artificially infested fish cannot be seen as a proof for the hypothesis being refuted. Regardless, all our results consistently point at the same direction, i.e. towards artificially infested fish showing an altered behavior compared to their control counterparts, which may ultimately result in reduced survival and/or fitness. We see these results as a support for our idea that this shift in method is the way forward for disentangling and finally quantifying the impact of salmon lice on wild sea trout. However, in order to provide solid quantitative proof of this impact (or lack of), robust study designs which will deliver the necessary statistical power to unequivocally support (or refute) these hypotheses are urgently needed. In the following paragraphs, we share some thoughts about the strengths and limitations of the study design presented here, and further make some recommendations on how to increase the statistical power of future study designs following this approach.

Two important premises for an infestation-based approach to causally explain the effects of salmon lice per se is that *i*) all fish should be free for lice by the start of the study, and *ii*) control fish should remain free from lice during the rest of the study. Lice burdens on wild fish typically remain very low during the whole sea migration period in farm-free areas (Tingley et al. 1997; Schram et al. 1998; Heuch et al. 2002; Rikardsen 2004; Urquhart et al. 2009; Serra-Llinares et al. 2014). We consequently selected Sandnesfjord, situated more than 80 km away from the closest salmon farm, to conduct our study. Unfortunately, most wild sea trout used in our study carried unexpectedly high lice loads at the moment of capture, circumstance that may have obscured the differences between groups and reduced the effect size (and thus statistical power) of our analysis. Occasional lice outbreaks have previously been described in farm-free areas (Serra-Llinares et al. 2014), probably related to infrequent hydrodynamical conditions, even though these tend to be isolated peaks and to occur only rarely. One possible and safe alternative to find lice-free sea trout by the start of the study could be to capture out-migrating sea trout smolts right before they leave the river on their way out to the sea. Moreover, control fish could be further shielded from lice infestation by use of antiparasitic treatments. However, chemical prophylaxis may have unwelcome side-effects on the fish as well as affect other parasites than sea lice, which may interfere with the results of the study. Also, it has been suggested that some physiological and behavioral responses to high salmon lice infestation pressure may be present even before the lice impose osmoregulatory problems (Birkeland and Jakobsen 1997; Gjelland et al. 2014). Thus, prophylaxis treatments preventing the molting of the lice but not the attachment (such as substance EX, Pharmaq, Norway) may still fail to prevent lice-induced behavioral changes (Birkeland and Jakobsen 1997; Gjelland et al. 2014). Sivertsgård et al. (2007) evaluated the effect of salmon lice on i) artificially infested, ii) control and iii) lice-protected (substance EX, Pharmaq, Norway) hatchery-reared sea trout and Atlantic salmon smolts equipped with acoustic transmitters. In their study, no differences in mortality where observed between the three groups during fjord migration for neither species. However, the study extended only over a short time period, and during which period the salmon lice could develop only to the chalimus stage of the life cycle. Independently of whether the chosen design includes or not the treatment of some fish with antiparasitic medication, a proper monitoring of the lice infestation pressure in the study area should be mandatory to detect the potential exposure of the experimental fish to additional lice.

In this pilot study we did not perform individual lice counts on the fish prior to release but rather followed a group-level approach, where the final lice burden on the artificially infested fish was estimated based on lice counts from a reference group. We estimated a final artificial infestation of more than 0.3 lice/g fish for lice in the infested group. This is regarded as a heavy load with severe consequences for the host (Taranger et al. 2015; Thorstad et al. 2015) and at the same time it is not uncommon to find such infestation levels on wild fish (MacKenzie et al. 1998; Tully et al. 1999; Bjørn et al. 2001; Serra-Llinares et al. 2014; Gargan et al. 2017). The advantage with group-based studies is that only a portion of the fish needs to be handled and inspected for lice after artificial infestation. The drawback, on the other hand, is that any differences observed between infested and control fish can only be attributed to the particular lice burden estimated for the infested group. Alternatively, individual-based studies where each individual fish is examined for lice prior to release and where the whole range of possible lice burdens is represented would be a more suitable approach for describing the shape of the relationship between salmon lice and sea trout mortality (or other life history traits). In this case, especial care should be taken during the manual examination of the fish post-infestation, in order to minimize the risk of removing the delicate newly attached copepodites. Also, little is known about the effect of fish anesthetics on sea lice, and thus potential extra loses of lice due to the sedation of the fish should not be ignored.

The use of acoustic telemetry in this study allowed direct observation of mortality, thus avoiding a total dependence on returns/recaptures, which are often limited and hard to obtain (Skilbrei et al. 2013; Skaala et al. 2014; Vollset et al. 2016). However, nearly 1/3 of the fish in this study rapidly left the study area and were never observed again, severely reducing the amount of data available for analysis and thus limiting the ability of the study to reach statistically significant inferences. Increasing the number of fish released would be the first and most obvious way to bust the statistical power of the study, but it would also entail elevated costs related to the tagging and lice-counting of an elevated number of individuals. Besides, the release of a large number of artificially infested sea trout in an otherwise relatively lice-free area may have ecological and ethical implications that, in any case, should be given proper consideration. Another way to increase our chances of reaching statistically significant inferences would be to expand the temporal and/or spatial coverage of the study. A common caveat of telemetry studies is the limited life-span of acoustic tags due to battery capacity, which may not allow for the detection of returning fish later in the season or even in following years. Other tagging techniques such as PIT tags can unlimitedly extend the duration of the study but are dependent on number or returns/recaptures. A combination of both techniques could be a good trade-off, so that additional detections gained from PIT tags can add robustness to survival analyses based on telemetry data. Additionally, expanding the spatial coverage of the study would increase the chances of detecting tagged fish and thus increase the amount of information available for analysis. One could, for instance, add additional hydrophones in more remote areas which the fish may be sporadically visiting or even add PIT antennas in other rivers in the system, this way increasing both the temporal and the spatial coverage of the study simultaneously.

Last, the high acoustic coverage of the study area allowed us to observe habitat choice preferences, including depth and salinity, in this pilot study. Indeed, infested and smaller fish were found in shallower waters, as well as closer to the estuary area, compared to control fish. By combining 3D fish positioning with a state-of-the-art hydrodynamic model, we were able to further demonstrate that the observed differences in habitat use reflect a preference for lower salinities for infested fish, illustrating that this combination of methods can be of excellent value for understanding more detailed behavioral responses, e.g. beyond the mere observation of premature return to freshwater. Behavioral restrictions caused by high parasite load, such as dependency for low salinity waters, can result in the loss of foraging opportunity and efficiency (Birkeland 1996), which, in turn, may entail a reduction in growth, survival, and reproductive potential for the host fish (Birkeland 1996; Wells et al. 2007; Fjørtoft et al. 2014).

The impacts of sea lice on wild salmonids is and will continue to be an important constrain for the development of a sustainable marine aquaculture industry. Thus, studies that aim to quantify the impact of salmon lice in the wild are urgently needed. Our study showed promising results from the combination of an infestation-based RCT in a fjord with low density of salmon lice, the use of acoustic telemetry and the use of detailed 3D hydrodynamical modeling for analyzing the impact of salmon lice on wild, free swimming sea trout. We recommend further studies with larger data sets over a number of years and under different environmental conditions following this innovative approach. Some suggested improvements to increase the statistical power of future studies would be to *i*) increase the number of tagged fish to reach a minimum required sample size, *ii*) catch out-migrating sea trout smolts before they enter the sea, and *iii*) expand the spatial and/or temporal coverage of the study, for instance by combining acoustic telemetry with PIT tagging. This is likely to provide new and valuable quantitative evidence on the effect of salmon lice on sea trout in the wild.

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Impacts of salmon lice on mortality, marine migration distance and premature return in sea trout

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ABSTRACT: Brown trout *Salmo trutta* (L.) is a facultative anadromous species, where a portion of individuals in populations with access to the sea perform migrations to use the richer feeding resources. We investigated the effect of salmon lice *Lepeophtheirus salmonis* (Krøyer 1837) infestation on the survival and behaviour of wild trout post-smolts (average fork length = 180 mm) during their marine migration. Comparisons of the marine migratory behaviour were made between an artificially infested group (n = 74) and a control group (n = 71) in an area with low natural lice infestation pressure. Artificial infestation was estimated to cause 100% prevalence and a mean intensity of 65 lice fish^{-1} (mean relative intensity of 2.4 lice g^{-1} fish). Survival analysis showed limited statistical power but revealed lice-induced mortality, with an estimated hazard ratio of 2.73 (95% CI = 1.04–7.13) compared to the control group, when data from a previous pilot study were included. Surviving individuals in the infested group additionally responded by residing closer to fresh water while at sea, and by prematurely returning to fresh water. On average, infested fish returned to fresh water after only 18 d at sea, while control fish spent on average 100 d at sea. The residency in the inner part of the fjord and the premature return to fresh water represent an adaptive behavioural response to survive the infestation, at the probable cost of reduced growth opportunities and compromised future fitness.

KEY WORDS: Parasite · Salmon louse · *Lepeophtheirus salmonis* · Sea trout · *Salmo trutta* · Epizootic · Acoustic telemetry · Mortality · Behaviour · Field experiment

1. INTRODUCTION

Migration is a behavioural adaptation that occurs in many animal taxa, enabling individuals to use the best-suited habitat during different life stages and seasons and thereby leading to an increase in individual fitness (Lucas & Baras 2001, Dingle & Drake 2007). Partial migration, where only a fraction of the individuals in a population perform migrations, is common, and the balance between the costs and benefits of migration versus residency is thought to affect the tendency to migrate (Chapman et al. 2012, Sahashi & Morita 2018). Salmonid fish spawn in fresh water, but in many of the species, some or all individuals perform migrations to use the richer feeding resources at sea (Gross et al. 1988). Among salmonids, brown trout *Salmo trutta* is an especially adaptable and flexible species, showing considerable life-history variation within and among populations (Klemetsen et al. 2003), including partial migration. For sea trout (i.e. the anadromous form of brown trout), the advantages of marine migration include

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the opportunity to access more food, which in turn enhances growth and fecundity. On the other hand, costs may be related to increased predation risk and physiological adjustment to different salinities. The balance of costs and benefits associated with freshwater residency and anadromy may result in different life-history strategies coexisting within the same water system (Jonsson & Jonsson 1993, 2006, Ferguson 2006). Females tend to adopt the anadromous life-history strategy more than males (Jensen 1968, Pratten & Shearer 1983, Euzenat et al. 1999, Knutsen et al. 2004), arguably because females have more to gain by increasing body size in terms of higher fecundity (Thorstad et al. 2016). Anthropogenic impacts that reduce the benefits or increase the costs of migration may result in selection against migration, altered life-history traits, reduced body size of individuals and reduced recruitment on a population level (Chapman et al. 2012, Shaw 2016). Studies of how human activities influence animals during their migration are therefore necessary to evaluate consequences for wildlife and biodiversity, both at the individual and population levels, and for the consideration of management measures.

Atlantic salmon *Salmo salar* (L.) farming has become a large industry, negatively impacting wild salmonids through the occurrence of farmed salmon escapees and the spread of salmon lice *Lepeophtheirus salmonis* (Krøyer,1837) and infectious diseases (Taranger et al. 2015, Forseth et al. 2017). Salmon lice are marine parasites that occur naturally on wild salmonids, but salmon farming has increased the number of potential hosts for lice in coastal areas, resulting in an increased infestation pressure on wild salmonids (Heuch & Mo 2001, Krkošek et al. 2005, Jansen et al. 2012). Sea trout are particularly at risk of experiencing unnaturally high infestation rates as a result of salmon farming, because they typically remain inside the fjords or in coastal waters during their entire marine migration, where most salmon farms are situated.

Salmon lice are ectoparasites that feed on the mucus, skin, muscle and blood of the host fish, causing tissue erosion, osmoregulatory dysfunction, physiological stress, reduced feeding and growth, and increased mortality (Birkeland & Jakobsen 1997, Bjørn & Finstad 1997, Dawson 1998, Bjørn et al. 2001, Wells et al. 2006). Salmon lice-induced stress responses and mortality have been documented for both hatchery-reared and wild trout post-smolts under laboratory conditions (Bjørn & Finstad 1997, 1998, Wells et al. 2006, 2007). Equivalent physiological disturbances, including an integrated stress response and osmoregulatory disfunctions, have been shown on free-swimming wild trout post-smolts both feeding at sea and prematurely returned to fresh water (Poole et al. 2000, Bjørn et al. 2001). Based on threshold levels shown to induce mortality in laboratory experiments, wild trout carrying potentially deadly lice loads are at times reported in Scotland, Ireland and Norway (Thorstad et al. 2015). However, conclusive results from field experiments are still scarce, and quantitative knowledge on the effects of lice on sea trout in their natural habitat is still limited. Moreover, free-ranging sea trout have the capacity to modify their behaviour to mitigate physiological stress and osmoregulatory dysfunction by prematurely returning to fresh water (Birkeland 1996, Bjørn et al. 2001, Gjelland et al. 2014). This would enable infested fish to regain osmotic balance and increase chance of survival at the probable cost of reduced growth.

Knowledge of the impacts of lice on trout is extensive, but field studies on wild fish that examine population-level effects are still needed (Thorstad et al. 2015). Disentangling the relative role of lice from other factors impacting mortality and fitness of wild fish in nature is challenging. Population-level effects have been quantified in Atlantic salmon in large-scale field studies by comparing the survival of fish chemically protected against lice to that of untreated control fish (Gargan et al. 2012, Vollset et al. 2014). Few such studies have been performed on trout (Skaala et al. 2014). These studies have primarily relied on the natural lice infestation level in the study area, which can be highly variable in intensively farmed areas (Serra-Llinares et al. 2014, 2016, Helland et al. 2015). This, in combination with the typically skewed distribution of lice among individuals and the limitations of the prophylactic treatment, make it difficult to deduce the actual infestation level of the experimental fish. An alternative approach is to compare artificially infested fish with non-infested control fish in a farm-free area with low natural lice levels. This approach does not rely on the limited duration of the effect from the prophylactic drug and allows for better control of the levels of lice on the studied fish. Moreover, other secondary effects associated with the treatment are not expected. Serra-Llinares et al. (2018) performed a pilot study to test this new method using wild trout post-smolts (n = 29) caught in bag nets shortly after they entered the sea in Sandnesfjord, southern Norway. The authors concluded that, despite limitations in the statistical power rendered by the study, results indicated consistent trends in behavioural differences between control and artificially infested fish, suggesting that this shift in method (i.e. using artificial infestation instead of prophylaxis) is a promising novel approach to study impacts from lice on wild free-swimming sea trout. Furthermore, the authors made recommendations on how to increase the power of an eventual follow-up study by (1) increasing the number of individuals tagged, (2) capturing the fish before they enter the sea, to ensure lice-free individuals and (3) expanding the spatial/temporal coverage of the study by use, for instance, of a combination of acoustic telemetry and PIT-tagging. This approach with suggested improvements is followed up in the present study.

Here, we used acoustic telemetry methods to investigate the survival and habitat use of wild trout post-smolts during their marine migration in an area without fish farms and with a low lice infestation pressure. The main aim was to study the impacts of lice on (1) survival, (2) rate and timing of freshwater returns and (3) migration behaviour (in terms of horizontal and depth use of the fjord) by comparing the movements of trout artificially infested with lice and non-infested control fish. Fish in both groups were tagged with acoustic transmitters with depth sensors, enabling recording of both horizontal and vertical fish movements. We hypothesized that (1) lice-infested fish would have a lower survival probability during the first summer at sea compared to control fish; (2) infested fish would return to fresh water earlier than control fish, to regain osmotic balance and de-louse, and (3) infested fish would remain closer to the river and/or swim higher up in the water column, seeking areas/layers with lower salinity.

2. MATERIALS AND METHODS

2.1. Study area

The study was performed in Sandnesfjord, southern Norway (58.6943° N, 9.1488° E, Fig. 1) from June to November 2017. There is no salmon farming activity in this fjord, with the nearest active salmon farm located more than 85 km (sea-way distance) away. Consistently low lice levels were recorded on wild sea trout in Sandnesfjord in the period 2010–2017, with a mean prevalence of 30% (range 0–98%) and a mean intensity of 2.5 lice per infested fish (range 1–13) (Karlsen et al. 2018).

The river Storelva flows into Sandnesfjord through a transition area (Songevatn estuary and Nævestadfjord, Fig. 1). The salinity in Sandnesfjord is commonly above 20 psu, but the inner part can have a shallow layer of fresh or more brackish water. The transition area is characterized by a strong salinity gradient, increasing from close to 0 psu at the surface to over 30 psu at 50 m depth. In the upper 3 m, salinity is commonly between 0 and 15 psu, depending on depth, freshwater supply and the hydrodynamic properties of the coastal current (Tjomsland & Kroglund 2010, Haraldstad et al. 2016).

Atlantic salmon and brown trout use the lowermost 20 km of the river Storelva as spawning and nursery habitats. The catchment area is 409 km^2, with an average annual water discharge of 12 m^3 s^{-1} measured at the outlet of Lake Lundevatn (Norwegian Water Resources and Energy Directorate ID: 18.4.0, HYDRA database NVE, www.nve.no/hydrologi/hydrologiske-data/historiske-data/data-i-hydra-ii-databasen/). The local trout population has been monitored for several years. The smolts descend during April and May, with an average annual total length ranging from 150 – 190 mm (Haraldstad et al. 2017). Sea trout in this system usually spend 2 growth seasons at sea before first returning to the river (Haraldstad & Güttrup 2015). Annual sea survival from smolt to first-time spawning ranges from 14–18%, while survival from one spawning to another ranges from 30 – 60% (Haraldstad et al. 2018). During a study by Olsen et al. (2006), the age of the sea trout caught in the Skagerrak coastal area, to which the Storelva catchment belongs, between 1998 and 2003 ranged from 2–8 yr, with more females (64%) than males. The average growth during the first season at sea is about 150 mm (K. Bleeker pers. comm.).

2.2. Hydrographic conditions

Estimates of temperature and salinity to describe the hydrographic properties in Sandnesfjord were retrieved from a hydro-dynamical model simulation using the Regional Ocean Modeling System (ROMS, http://myroms.org; Shchepetkin & McWilliams 2005, Haidvogel et al. 2008) applying 32 m × 32 m horizontal resolution. The highest-resolution model was based on a four-fold nested model system where the horizontal grid was refined from 4 km (the operational forecast model for the Nordic Seas provided by the Norwegian Meteorological Institute and accessible at http://thredds.met.no) to 800 m (see e.g. Albretsen et al. 2011) and 160 m, all model systems using ROMS. Tides were added to the boundaries of the 800 m model and interpolated from the global TPXO7.2 (Egbert & Erofeeva 2002). Applications evaluating and using 160 m model systems are

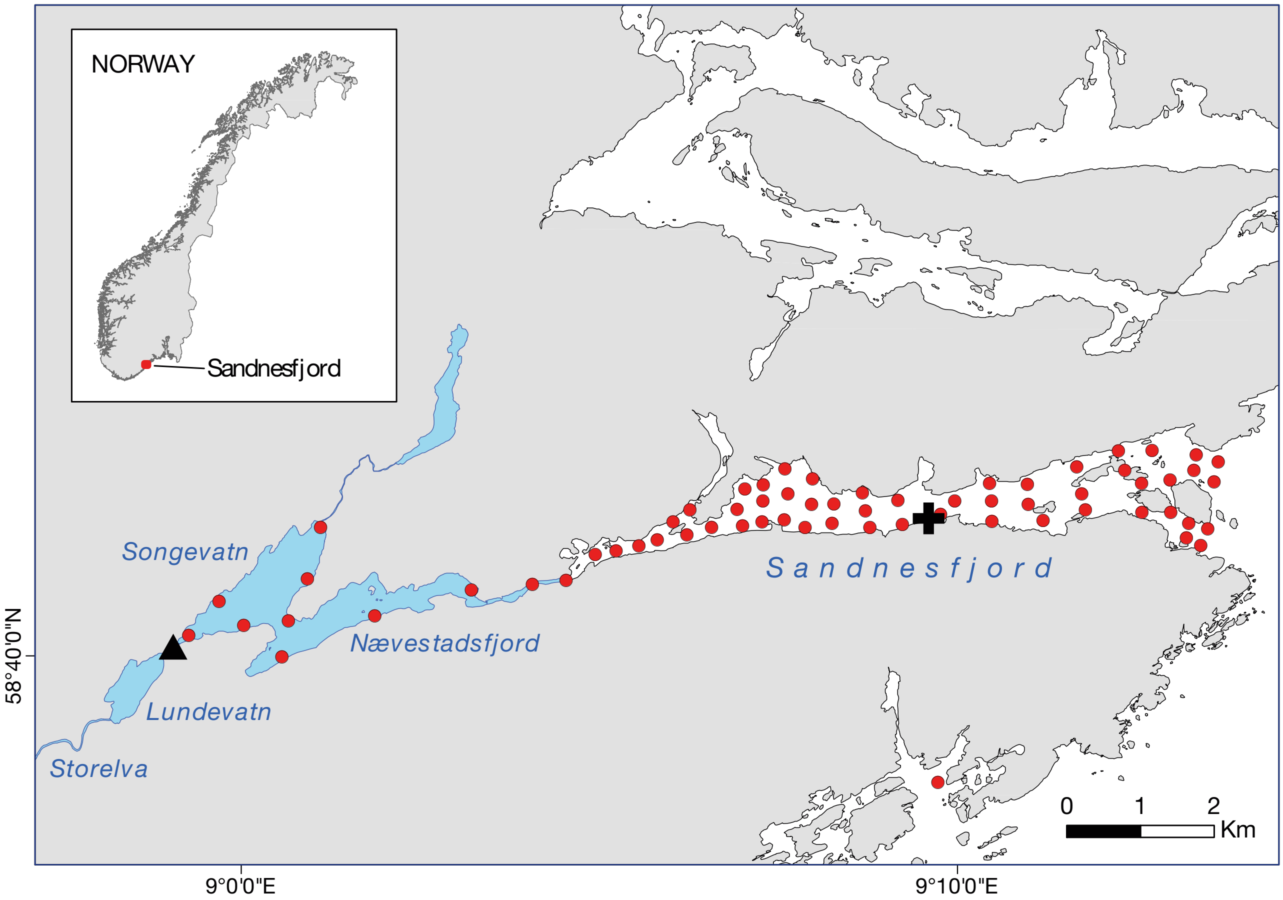


Fig. 1. Study area. Freshwater and transition areas are indicated in blue. The positions for the acoustic receivers (red dots), the fish trap and PIT antenna (black triangle) and the tagging and release location (black cross) are indicated. Note: the farthest receiver, situated approximately 10 km further south from the outermost receiver in the map, is not shown

described by Espeland et al. (2015) and Huserbråten et al. (2018). The 800, 160 and 32 m models applied high-resolution atmospheric forcing from the non-hydrostatic 2.5 km AROME MetCoOp regional atmospheric model (Müller et al. 2017) provided by the Norwegian Meteorological Institute and accessible at http://thredds.met.no. The freshwater runoff from Storelva was inputted in the model downstream of the narrow strait of Lagstrømmen (the transition zone denoted with a change from blue to white in Fig. 1), and volume fluxes for all rivers applied in the ROMS models were based on daily measurements from the Norwegian Water Resources and Energy Directorate.

2.3. Fish tagging and artificial infestation

Wild trout post-smolts (n = 145; average ± SD fork length [FL] = 180 ± 14 mm; range 155–226 mm) were captured in a rotary screw fish trap located in the river mouth (Haraldstad et al. 2017) (Fig. 1) between 18 and 24 May 2017. A rotary screw trap is a sampling gear that takes advantage of flowing water to gently capture and retain downstream migrating fish (Chaput & Jones 2004). The trap was monitored once or twice a day, depending on capture rates. Captured fish were identified to species, and trout post-smolts were held for a maximum of 48 h before further handling.

Early-stage lice are highly sensitive to fresh water (Wright et al. 2016). Consequently, the fish were transported by boat in a tank with oxygenated water from the capture site in fresh water to the more saline central fjord prior to tagging and artificial infestation (Fig. 1). First, the fish were moved into a large tank with circulating fjord water, where they could recover and acclimatize for several hours prior to tagging. The fish were then tagged with individually coded acoustic tags with depth sensors produced by

Thelma Biotel (model ADT-LP-7.3, size: 22 × 7.3 mm; 1.1 g in water; depth sensor with 0.2 m resolution and maximum depth of 51 m, random pulse repetition intervals of 30–90 s; typical battery life of 5 mo). We anaesthetized the fish by a 3 min immersion in an aqueous solution of benzocaine (0.1–0.2 ml Benzoak® l^{-1}). We then made a small incision on the ventral surface posterior to the pelvic girdle, through which we inserted the acoustic transmitter. Additionally, a PIT tag (23 mm, half duplex, Oregon RFID) was also inserted into the body cavity via the same incision, which was closed using a single silk suture (4/0 Ethicon). After tagging, the fish were randomly assigned to 1 of 2 groups: a control group (n = 71, average ± SD FL: 181 ± 14 mm; range 156–220 mm) or an infestation group (n = 74, FL: 180 ± 14 mm; range 155–226 mm) and placed into a small net-pen at sea with 2 separated compartments for a recovery period prior to infestation (1–5 d).

Salmon lice copepodids ('LsGulen' family, 30/31 generation in the laboratory) used for artificial infestation were provided by the Institute of Marine Research in Bergen. Approximately 35 000 copepodids were collected in the laboratory and sent to the field site in Sandnesfjord. Incubation and quantitative assessment were performed as described by Hamre et al. (2009).

For artificial infestation, all fish in the infested group were placed in an infestation tank (1 × 1 × 0.8 m fibreglass tank) with circulating fjord water (13°C, 25 psu), where they were exposed to approximately 200 copepodids each (i.e. approximately 14 800 copepodids were added to the infestation tank). During infestation, water circulation in the tank was stopped and the level was kept at approximately 10 cm for 30 min. The oxygen content was continuously monitored and regulated during the process. After infestation, the water circulation was re-established in the tanks, where the fish could recover for 12 h prior to release. To reduce the risk of a potential handling effect impacting our results, the control group received a parallel sham infestation following the same procedure as infested fish in a separate tank. After recovery, all control and infested fish were released in the middle part of the fjord (Fig. 1).

To evaluate the success of the artificial lice infestation, additional 30 post-smolts (FL: 151 ± 18 mm; range 119–196 mm) were used as laboratory reference groups. These were not tagged with acoustic transmitters. Half of them were infested with lice together with the infested fish which were released, as described above. After infestation, both infested and non-infested reference groups were transported by boat in a tank with 2 compartments to the nearby research facilities in Flødevigen, where they were placed in 2 separate tanks (1 per group). After 1 wk, their lice levels were recorded.

2.4. Fish tracking

The movements of tagged fish were monitored from release (25 May 2017) to the end of the study (29 October 2017) through an array of receivers (Vemco VR2W, which recorded depth, ID and the time when tags were within receiver range) covering the study area both in the fjord and in the transition areas. Range tests indicated an optimal detection range of up to at least 150–200 m (85 % of the signals were still recorded at this distance). Based on this, we designed a hydrophone array consisting of 66 receivers, of which 55 were placed in sea water and 11 in the transition area (Fig. 1). Migration out of the fjord was monitored by a double array of receivers at the fjord mouth, enabling recording of the movement direction of the fish in this area.

Movements of fish in the river were detected using 2 swim-through PIT-antennas located 150 m upstream of the river mouth (Haraldstad et al. 2017). At this location, the river is 9 m wide and 0.9 m deep. The 2 antennas were installed 2.5 m apart and wired to 2 remote tuner boards, one for each antenna. The tuner boards were connected to an antenna reader box (TIRIS RI-CTL MB2A; Oregon RFID) and supplied with a 12V battery. When a tagged fish passed through the antenna loop, the PIT-tag number, antenna number, date and time were recorded and logged by the reader box.

2.5. Fish fate assessment

Individual fish fates were assessed by examination of individual detection plots. Based on their vertical profile and horizontal movements, the fish were classified as:

(1) Dead, when the vertical profile indicated that a tag became stationary. When a diving pattern inconsistent with trout vertical swimming behaviour was observed prior to the tag becoming stationary, the fish was considered to have been eaten by a predator (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m635p151_supp.pdf). The final individual trout record was defined as the last transmitter detection before the tag became stationary or before the predation event. Transmitter detections after this time were removed before further analyses.

(2) Returned, for fish swimming into the transition area and/or the river (hereafter referred to as fresh water) and remaining there for the rest of the study (i.e. never observed in the main fjord again). Returns before 1 August 2017 were further categorized as early returns, as less than 25% of first-time migrants return to the river Storelva before that date (Haraldstad & Güttrup 2015).

(3) Migrated, when last detections occurred in the outer double receiver array in an outwards direction.

(4) Alive at sea, for fish detected alive in the main fjord by 1 October 2017 unless another fate could be assigned after that date. This date was chosen based on the expected life span of the acoustic tags.

(5) Unknown, for fish disappearing inside the study area before the end of the study and before the end of the expected lifetime of the tag.

2.6. Statistical analyses

All analyses were performed using the R software version 3.6.0. The receivers recorded 2 166 380 detections with IDs matching those from tagged trout, and 1601 detections with IDs not corresponding to any tagged fish (which were consequently discarded). A single receiver accumulated 27% of these unidentified detections. To filter out additional false detections with IDs matching those from fish tags, single detections within a 24 h window were considered as spurious and discarded. A more restrictive 2 h filter was applied to the receiver with the most false detections. In total, 1242 spurious detections were removed following this method. Finally, we examined individual vertical and horizontal plots to detect and remove remaining false detections, based on spatial inconsistency.

Fish positions (1 h intervals) were estimated using center-of-activity locations (Simpfendorfer et al. 2002). The center-of-activity location for a given time interval is the mean position of the hydrophones that detected the animal at that time interval, weighted by the number of times the animal was detected at each hydrophone (Simpfendorfer et al. 2002). A mean depth value was also associated with each center-of-activity position, so that they consisted of a 3D position with latitude, longitude and depth. In addition, we calculated sea-way distance to fresh water (i.e. to the Nævestadfjord outlet) for each center-of-activity position using the 'Spatial Analyst' package from ArcGIS.

Differences in mortality and return to fresh water between control and infested fish were analysed using Cox proportional hazard (Coxph) models, fitted with the 'survival' package (Therneau 2014) in R. For analysis of mortality, the fate/status of each individual fish was set as 1 (died) for fish having a vertical profile indicating mortality, and as 0 (alive) for fish for which mortality could not be identified. Fate time (t) was specified as the number of days between tagging and the observed fate and specified as right-censored data. By using right-censored data, we could use information on all fish, including fish that migrated out of the study area, which were alive at least until they left. For fish that died by fishing, the fate was set as 0 and the fishing date was used as the fate date. The following Coxph model was used to model the hazard of death at time t, as a function of group (control/ infested) and fish length (FL):

$$h(t) = h_0(t) \times \mathrm{e}^{(\beta_1 Group \, + \, \beta_2 \, Fish\ Length \, + \, \beta_3 \, Group \, \times \, Fish\ Length)} \quad (1)$$

where $h(t)$ is the hazard of the event (death) at time t, and $h_0(t)$ is the baseline hazard (i.e. the value of the hazard when all exposure variables are equal to 0).

Power analysis using the function 'ssizeCT' from the R library 'powerSurvEpi' (Qiu et al. 2012) was used to assess the ability of the fitted Coxph model to detect differences between infested and control fish in this study. We performed scenario testing to estimate the minimum sample size required to detect different hazard ratios (HR) under the premise of 3 fixed parameters: power (i.e. power to detect the magnitude of the hazard ratio as small as that specified by HR) = 0.8, alpha (i.e. type I error rate) = 0.05, and k (ratio of participants in each group) = 1. In other words, we calculated the minimum sample size required to have an 80% probability of detecting a HR with a confidence of 95%, given the same number of fish in both groups, and using our own data to estimate the remaining parameters such as the expected total number of events in each group.

In anticipation of power analysis indicating insufficient statistical power for the survival analysis, a second Coxph model was specified including data from both the current study (n = 143) and data from the pilot study (n = 29; Serra-Llinares et al. 2018). Both studies were performed in the same fjord in consecutive years and are to a high degree comparable when it comes to methods and study design. However, there are some small differences that must be accounted for when results from both studies are to be combined. First, fish used in the pilot study were caught at sea in mid-June and therefore (1) they were slightly bigger (mean FL = 185 ± 18 mm) than fish in the present study, (2) they could have spent different amounts of time at sea prior to capture, and

(3) initial mortality immediately after migration to sea, when the fish are the smallest and thus most vulnerable to predation, was not properly captured in the pilot study. Second, fish in the pilot study were not completely lice free prior to tagging: 96% of the fish were infested with 1 or more lice, with intensities ranging from 2–42 lice fish^{-1}. Thus, control fish in the pilot study could arguably be affected to some degree by the lice they carried, possibly obscuring the differences between treatment groups. Last, artificial infestation in the pilot study resulted in more moderate lice loads (estimated relative intensities after artificial infestation between 0.24 and 0.74 lice g^{-1} fish) compared to the present study, and thus differences in survival between control and infested fish can also be expected to be smaller. Based on all of the above, and to account for potential differences between the 2 studies, Year was added as a covariate in the model, which was finally expressed as:

$$h(t) = h_0(t) \times e^{(\beta_1\, Group + \beta_2\, Fish\ Length + \beta_3\, Group \times Fish\ Length + \beta_4\, Year)} \quad (2)$$

For analysis of return to fresh water, fate/status was set to 1 (returned) for fish that returned to fresh water and were never observed at sea again afterwards, and to 0 (not returned) for fish having their last detection at sea. Fate time t of returned fish was set as the number of days between tagging and fate date. Fate time t for fish that did not return to fresh water was defined by the last observation and specified as right-censored data. Analysis of the Schoefeld residuals showed that the effect of Group (control/infested) shifted after approximately 60 d, showing a time dependency. We therefore applied a stratified analysis before and after this 60 d threshold (Period) using the formula:

$$h(t) = h_0\,(t) \times e^{(\beta_1\, Group + \beta_2\, Group \times Strata\ (Period) + \beta_3\, Fish\ Length)} \quad (3)$$

Here, the term $h(t)$ denotes the hazard of returning to fresh water at time t.

Differences in distance to fresh water and depth use during the marine migration (i.e. before final return to fresh water) were investigated using mixed models. Daily values (calculated as averages of hourly positions) were used to avoid severe autocorrelation in model residuals. Group (control/infested), fish length (FL in mm) and their interaction term were used as covariates in all models, while time of day (day/night) was additionally used for analysis of swimming depths. For this, daytime was defined as the time between sunrise and sunset using data from the Astronomical Applications Department of the US Naval Observatory (www.usno.navy.mil) for the coordinates 58° 41′ N, 9° 07′ E. Night was defined as the time between sunset and sunrise. Individual daily mean swimming depths were calculated for day and night separately. Fish ID was used as a random effect in all models, as well as a correlation structure (auto-regressive model of order 1) to account for the temporal correlation between consecutive daily data from the same fish.

Distance to fresh water was first modelled using the above described linear mixed model. Exploration of the residuals showed a strong non-linear temporal pattern for infested fish, and consequently week was included as a smoother, which was allowed to differ between experimental groups. The final generalised additive mixed-effect model (GAMM) used was fitted according to the formula (full model):

$$Dist_{ij} = \alpha + \beta_1\, Group_i + \beta_2\, Fish\ Length_i + \beta_3\, Group_i \times Fish\ Length_i + f\,(Week\_\ Group_i) + ID_i \quad (4)$$

where $Dist_{ij}$ denotes the mean distance to fresh water for individual i on day j, $Group_i$ is the experimental group (control or infested) of individual i, $Fish\ Length_i$ is FL and $f\,(Week\_Group_i)$ is the week-effect smoother for the corresponding experimental group. ID_i is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2.

For analysis of daily mean swimming depth, the variable was first log-transformed in order to normalize the residuals (prior to log-transformation, 0.2 m was added to have all observations above 0; this was subtracted when back-calculating model coefficients). Daily mean swimming depth showed a nonlinear temporal pattern, and therefore week was included as a smoother, which was allowed to differ between day and night. The final GAMM used was fitted according to the formula (full model):

$$Log\,(Depth_{ij} + 0.2) = \alpha + \beta_1\, Group_i + \beta_2\, Fish\ Length_i + \beta_3\, Time\ Of\ Day_{ij} + \beta_4\, Group_i \times Fish\ Length_i + \beta_5\, Group_i \times Time\ Of\ Day_{ij} + f\,(Week\_\ Time\ Of\ Day_{ij}) + ID_i \quad (5)$$

The term $Depth_{ij}$ denotes the mean depth of individual i on day j, $Group_i$ is the experimental group (control or infested) of individual i, $Fish\ Length_i$ is FL of individual i, $Time\ Of\ Day_{ij}$ denotes the time of the day (day/night) of individual i on day j, and $f\,(Week\_\ Time\ Of\ Day_{ij})$ is the week-effect smoother for the corresponding time of day. ID_i is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2.

Each global model (as specified above) was used to generate a model set of all possible sub models, using the function 'dredge' in the R package MuMIn (Bartón 2007). Models were ranked by Akaike's information criterion corrected for small sample size (AICc). The model with the lowest AICc value was considered as 'best fit' and was used to estimate model coefficients. Models with ΔAICc < 2 were considered competing models. Complete model selection tables are presented in the Supplement (Tables S2–S5).

3. RESULTS

3.1. Artificial infestation

Examination of the laboratory reference groups (Table 1) revealed significant differences in lice loads between control and artificially infested fish. Control fish (n = 14) showed a 78% prevalence (i.e. number of fish carrying 1 or more lice divided by the number of fish examined), indicating either that the fish had time to get infested with lice during the short acclimation period at sea, or that there was a cross-infestation between the 2 tank compartments during the transport by boat to our lab facilities. However, mean intensity (i.e. average number of lice on infested fish) and mean relative intensity (i.e. average number of lice per g of fish weight) remained low in this group (mean ± SD intensity = 1.9 ± 1.3 lice fish^{-1}; mean relative intensity = 0.05 ± 0.05 lice g^{-1}). Prevalence in the infested group (n = 16) was 100%, with a mean intensity of 65.4 ± 30.6 lice fish^{-1} and a mean relative intensity of 2.4 ± 0.9 lice g^{-1}.

3.2. Hydrography

According to model simulations, surface salinity in Sandnesfjord was lowest in the inner part and increased progressively towards the ocean (Fig. 2). There was a surface layer of brackish water in the inner fjord, with salinities remaining under 15 psu during most of May and June whilst increasing to above 20 psu in July. In the middle fjord, surface salinity remained between 15 and 25 psu during May and June and increased to 30 psu in July. Surface salinity in the outer part of the fjord was between 25 and 30 psu from May to September. Surface temperature was relatively homogeneous throughout the whole length of the fjord and increased progressively from 12°C on the release day to a maximum of 19°C in late July (Fig. 2).

3.3. Acoustic tracking and fate assessment

A total of 143 fish (70 control and 73 infested) provided depth and movement data (2 fish did not provide any data) (Table S1). A substantial proportion of the individuals (approximately 60% in both groups) moved towards the ocean within the first 2 wk after release and were never observed again in the study area (Fig. 3). We found that 35 fish visited fresh water at least once during the study; of these, 18 individuals (6 control and 12 infested) had the last detection in fresh water and were therefore classified as returns. Twelve of the returns (1 control and 11 infested) returned before 1 August and were therefore classified as early returns. Among early returning fish from the infested group, 6 (of 11) individuals remained in the transition area at least until August 1st, 2 of which were later detected migrating back to the river (the remaining 4 individuals had the last detection as living fish in the transition area); the remaining 5 indi-

Table 1. Size and lice counts from sea trout in the reference groups after artificial infestation. FL: fork length; W: weight; N lice: number of lice; Rel. int: relative intensity of infestation (number of lice per g fish weight)

	FL (mm)	W (g)	N lice	Rel. int.
Control	167	45	1	0.02
	153	37	0	0.00
	145	32	1	0.03
	141	25	5	0.20
	139	25	1	0.04
	180	45	1	0.02
	137	23	1	0.04
	158	34	0	0.00
	187	68	1	0.01
	132	23	0	0.00
	184	60	2	0.03
	128	21	2	0.10
	140	29	4	0.14
	148	35	2	0.06
Infested	132	22	46	2.09
	153	31	74	2.39
	146	28	67	2.39
	150	29	38	1.31
	143	27	66	2.44
	125	23	34	1.48
	114	14	28	2.00
	137	24	118	4.92
	140	25	24	0.96
	128	18	47	2.61
	118	15	31	2.07
	136	25	97	3.88
	160	41	92	2.24
	163	37	102	2.76
	170	46	89	1.93
	154	35	94	2.69

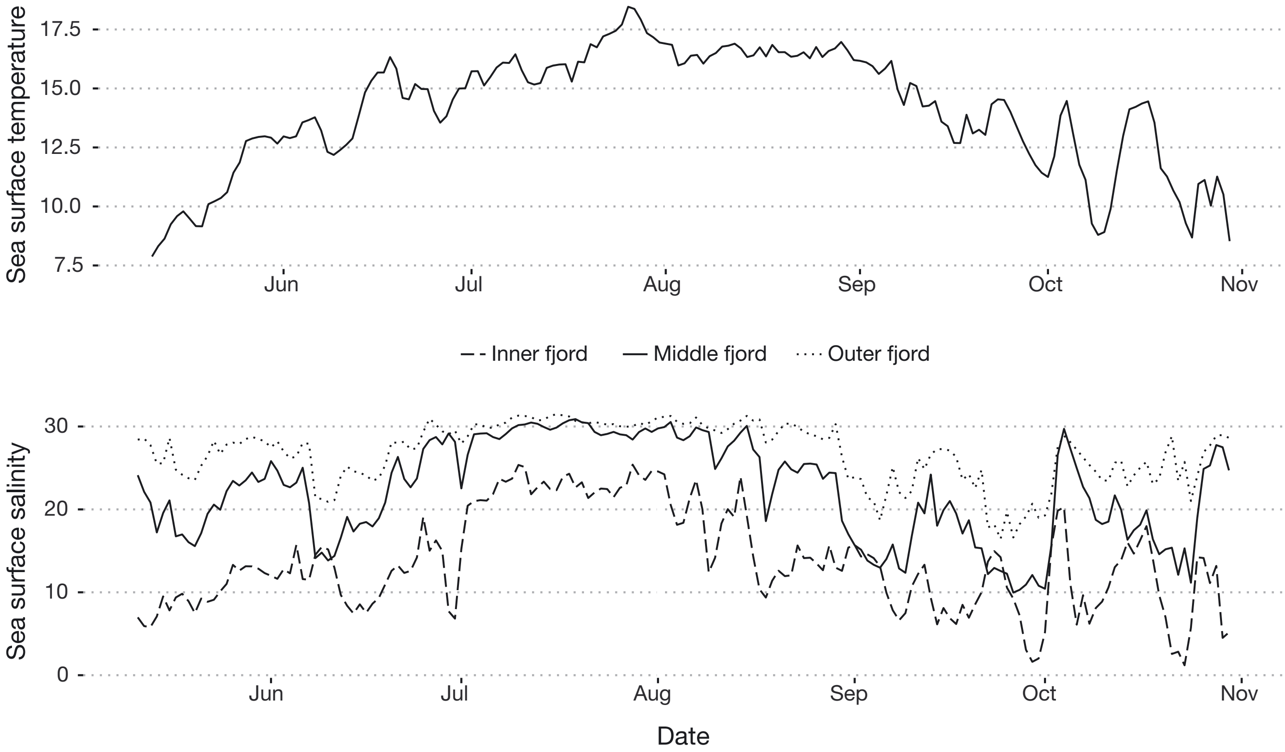


Fig. 2. Modelled sea surface temperatures and salinities in the inner, middle and outer part of Sandnesfjord. Only middle-fjord data are shown for temperature due to little variation along the fjord length

viduals either died (4) or disappeared (1) shortly after premature return to freshwater. A total of 19 individuals, 7 control and 12 infested, were classified as dead. Eleven fish (9 control and 2 infested) were observed alive at sea by 1 October. Nine fish (6 infested and 3 control) were detected at the first and closest receiver outside Sandnesfjord, situated approximately 16 km (sea-way distance) from the innermost part of the fjord. Another 6 fish (4 infested and 2 control) were further detected at the most distant outer receiver, situated another 9 km farther south along the coast.

All fish detected in the PIT antenna returning during the first season at sea could also be positioned at the river mouth through detections by the corresponding acoustic receiver. The latest of these detections were used for analysis. None of the fish that disappeared during the study period was later detected in the PIT antenna.

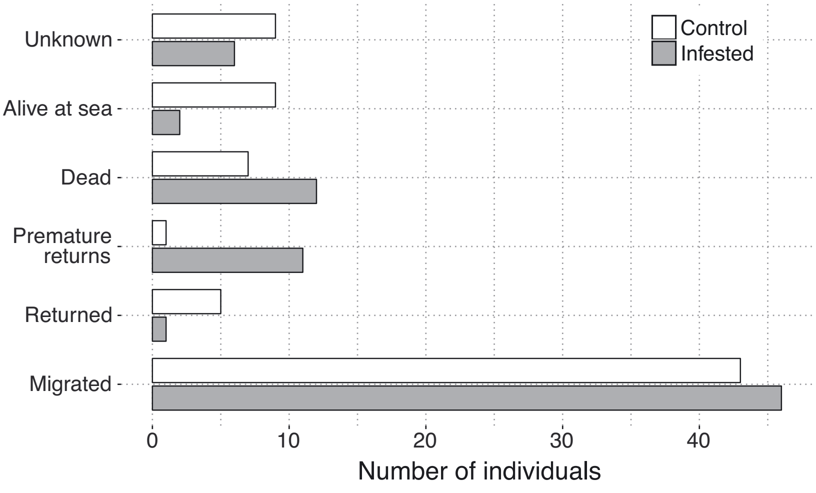


Fig. 3. Summary of fish fates. See Section 2 for further details on fate assessment and Table S1 in the Supplement for details on individual fates

3.4. Survival

None of the covariates tested had a statistical effect on the mortality of the fish in 2017 according to the 'best fit' model, which included only the intercept (Table S2a). The top competing model included the variable 'group,' and was practically indistinguishable from the 'best fit' model (ΔAICc = 0.22). This competing model showed higher, albeit statistically non-significant, mortality for lice-infested fish compared to controls (Coxph, 0.70 ± 0.51 SE; hazard ratio = 2.01, 95% CI = 0.74–5.46, z =

1.37, p = 0.17; Figs. 4 & 5a). Power analysis revealed that, given the structure of the data in this study, a sample size of 362 fish (181 in each group) would be needed to detect an HR between infested and control fish of 2 (as suggested by our results) with an 80% probability and a confidence of 95%. Furthermore, our actual sample size (~70 fish per group) would only be able to provide statistical significance given an HR between infested and control fish of 2.9 or higher.

Survival analysis on the merged dataset (2016 and 2017 data) revealed a higher mortality risk for lice-infested trout compared to control individuals (Coxph, 1.00 ± 0.49 SE; hazard ratio = 2.73, 95%

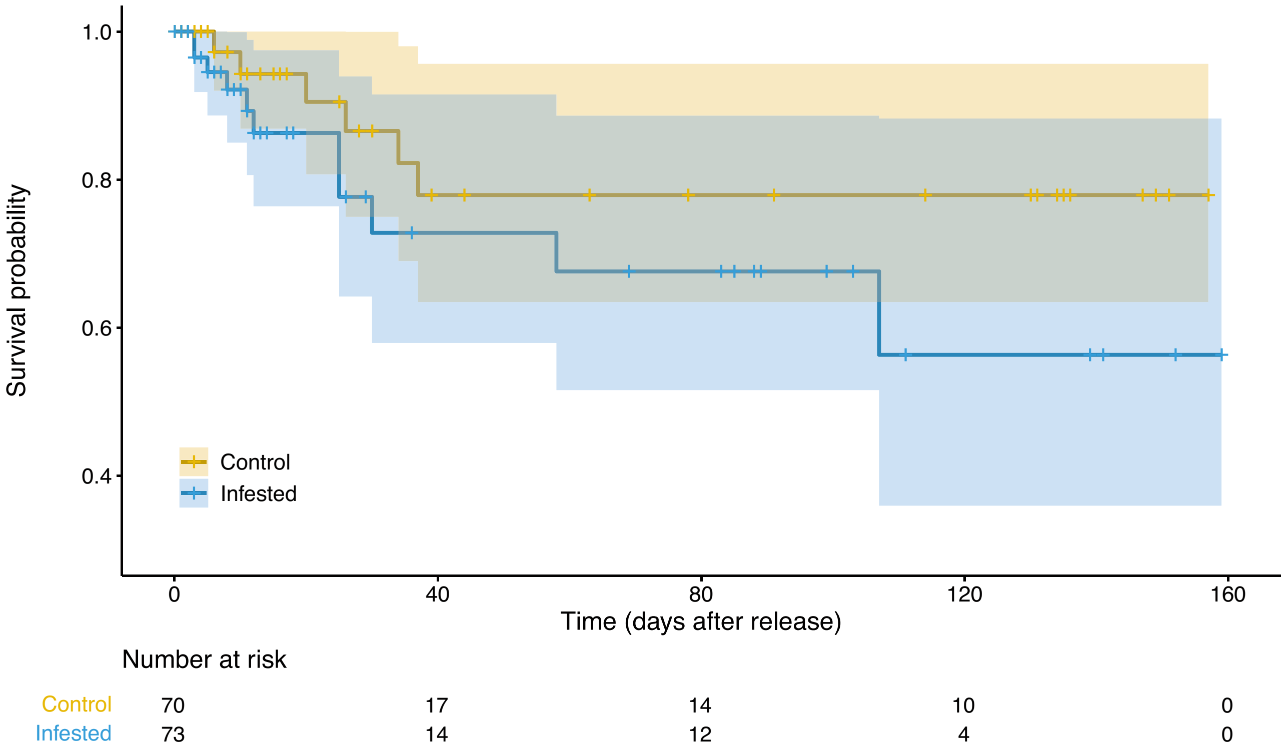


Fig. 4. Survival curves with 95% confidence bands for control (yellow) and artificially infested (blue) sea trout post-smolts in 2017. Crosses represent censoring points. The table under the graphic shows the number of individuals at risk (not censored) at each time step

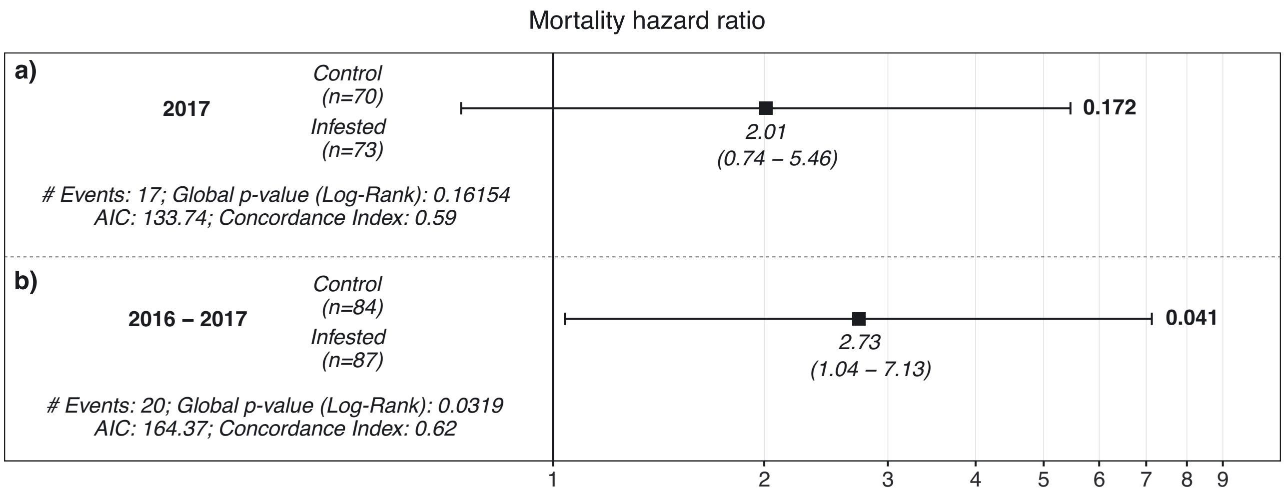


Fig. 5. Mortality hazard ratio between control (reference level) and artificially infested sea trout in (a) the present study (2017 data) and (b) the merged dataset including data from the pilot study. Solid squares and error bars show estimates and 95% CI, respectively (also noted under the corresponding symbols). Associated p-values are given as annotations on the right side of each graphic. AIC: Akaike's information criterion

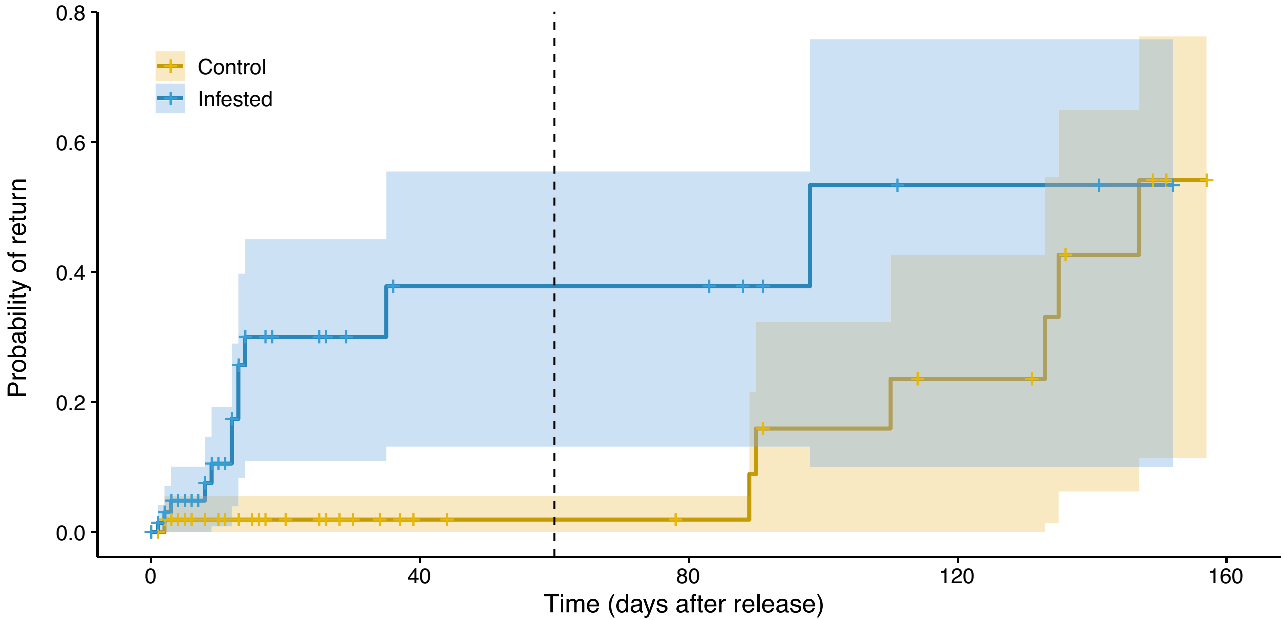
CI = 1.04–7.13, *z* = 2.04, p = 0.041; Fig. 5b). Competing models included the effect of year on the mortality hazard ratio between control and infested fish (Table S2b) in the direction of a bigger (although not statistically significant; p > 0.05) difference between groups in 2017. Neither the 'best fit' model nor competing models included fish length as a covariate.

3.5. Return to fresh water

Eighteen fish (6 control, 12 infested) returned to fresh water. The timing of the returns was significantly earlier for infested (18 ± 26 d after release) than for control fish (100 ± 49 days; *t*-test, p = 0.003), with 12 individuals (1 control and 11 infested) returning before 1 August. The 'best fit' model included both fish length and the interaction group:strata (period) (Table S3), both having a significant effect on the probability of return. During the first 60 d of the migration (period 1), infested fish had a higher probability of returning to fresh water compared to control fish of the same size (Coxph, 2.43 ± 1.05 SE; hazard ratio = 11.33, 95% CI = 1.45–88.4, *z* = 2.32, p = 0.021; Figs. 6 & 7). After 60 d (period 2), the return probability for infested fish decreased until becoming lower, although not statistically significant, than that of their control counterparts (Coxph, −1.16 ± 1.12 SE; hazard ratio = 0.31, 95% CI = 0.03–2.84, *z* = 1.03, p = 0.30; Figs. 6 & 7). In general (both periods), bigger fish had a higher probability of returning to fresh water (Coxph, 0.03 ± 0.02 SE; hazard ratio = 1.04, 95% CI = 1.01–1.07, *z* = 2.31, p = 0.021).

3.6. Distance to fresh water

Lice-infested trout remained closer to the estuary compared to control fish while at sea (i.e. before they eventually terminated their sea migration and moved back to fresh water or into the transition area) (Table 2). This difference was especially noticeable after the first 3 wk post release and remained a consistent pattern for the rest of the study period (Fig. 8). There was a significant interaction between group and fish length, with fish length having a small positive effect for control fish and a stronger and negative effect for infested fish. The average distance to fresh water for a control fish of average size (FL = 180 mm) was approximately 6 km, with smaller fish staying slightly closer (approximately 80 m for each 1 cm increase in body length). For an infested fish of the



Number at risk

Control	70	16	14	9	0
Infested	73	7	7	2	0

Fig. 6. Probability of return to fresh water with 95% confidence bands for control (yellow) and artificially infested (blue) sea trout post-smolts. Crosses represent censoring points. Dotted line indicates Day 60 post release, separating period 1 from period 2 in the stratified analysis. The table under the graphic shows the number of individuals at risk (not censored) at each time step

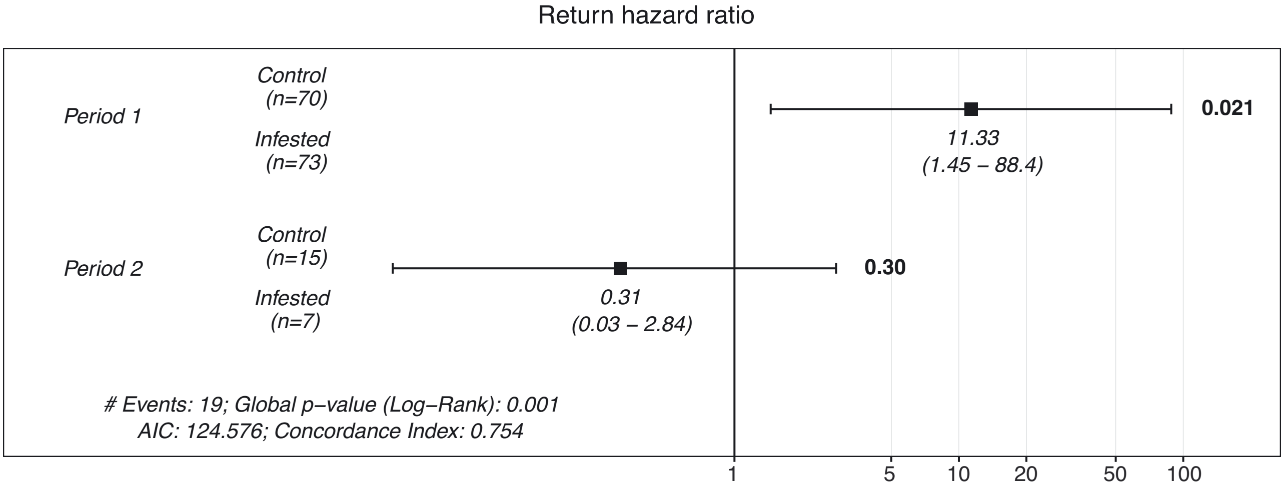


Fig. 7. Hazard ratio for freshwater return between control (reference level) and artificially infested sea trout before (period 1) and after (period 2) 60 d post release. Solid squares and error bars show estimates and 95% CI, respectively (also noted under the corresponding symbols). Associated p-values are given as annotations on the right side of each graphic. AIC: Akaike's information criterion

same size, the corresponding distance was estimated at approximately 2.5 km, with larger fish remaining closer than smaller fish (approximately 565 m closer for each 1 cm increase in body length). The model explained 46% of the variance. All candidate models evaluated are shown in Table S4.

3.7. Depth use

Trout resided close to the surface during the whole marine migration, with more than 98% of all detections recorded at depths shallower than 3 m. Only 8 fish (6%) were detected performing 1 or more dives >15 m during the whole study. Mean swimming depth during the whole marine migration was 0.78 m (range of means for individuals: 0.04–2.54 m), with individuals staying shallower at night than during the day (mean swimming depths were 1.01 and 0.55 m for day and night time, respectively). Infested fish tended to swim slightly shallower (approximately 15 cm) than control fish during the day, whilst both groups occupied approximately the same depth at night (Table 2, Fig. 9). Fish size also had an effect on swimming depth, with bigger fish swimming slightly deeper than smaller fish (approximately 7 cm deeper for each 1 cm increase in fish length) independently of lice infestation. All candidate models evaluated are shown in Table S5.

Table 2. Results from generalised additive mixed-effects models (GAMMs) for distance to fresh water and swimming depth, modelled as a function of fish length (fork length in mm), group (control/infested) and time of day (day/night; included only in the analysis of swimming depth). *Significant values ($p < 0.05$)

	Estimate	SE	*t*	p(>|*t*|)
Distance to fresh water				
(Intercept)	4781.07	2414.21	1.980	0.048*
Fish length	8.08	13.46	0.600	0.548
Group (infested)	7481.47	3306.21	2.263	0.024*
Fish length: Group (infested)	−56.51	18.30	−3.088	0.002*
Log (Swimming depth + 0.2)				
(Intercept)	−0.746	0.331	−2.256	0.024*
Fish length	0.005	0.002	3.010	0.003*
Group (infested)	−0.134	0.052	−2.576	0.011*
Time of day (night)	−0.656	0.011	−59.809	0.000*
Group (infested): Time of day (night)	0.122	0.019	6.510	0.000*

4. DISCUSSION

In this study, we documented parasite-induced mortality and significant behavioural changes in trout post-smolts as a consequence of a heavy lice infestation. The lice levels artificially imposed on experimental fish in our study were estimated at 65 lice fish^{-1} on average, or a relative intensity of 2.4 lice g^{-1}. This is well above the expected mortality-inducing threshold for trout, estimated at 0.3 lice g^{-1} (see Taranger et al. 2015), and individuals carrying such high lice loads represent only a small fraction (between 4 and 5%) of all trout sampled in the Norwegian Salmon Lice Surveillance Program (R. Nilsen pers. obs.). However, in some areas with intensive fish farming, the percentage of fish carrying

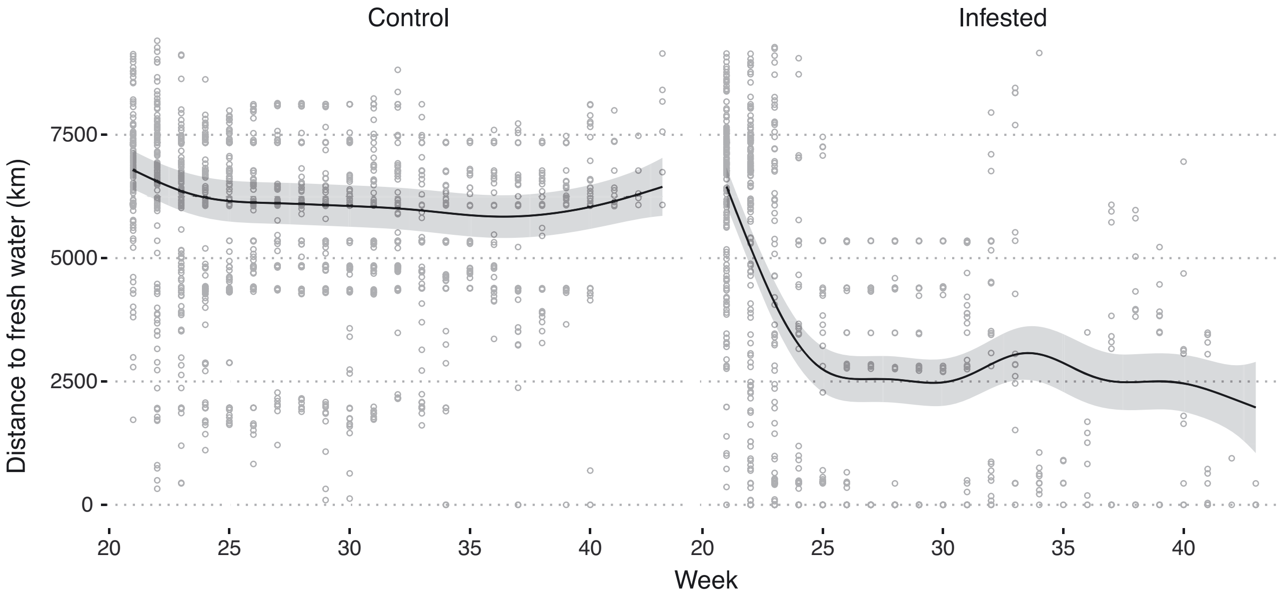


Fig. 8. Observed (points) and predicted (lines and shaded areas, representing estimates with 95 % CI) distance to fresh water for individual fish by experimental group (control or infested). Predictions are made for a fish of average size (180 mm fork length)

>65 lice (or relative intensities >2 lice g^{-1}) has occasionally been registered to be as high as 70 % of all fish sampled (R. Nilsen pers. obs.). Thus, lice loads such as that in this study may be extreme and represent perhaps only a 'worst-case' situation, but they are not implausible under the current scenario in Norway.

4.1. Role of lice in post-smolt mortality

Dead and moribund trout observed in estuaries have been linked to lice infestations (Tully et al. 1993a,b, Birkeland 1996), but proof of a causal relationship remains elusive (Thorstad et al. 2015). Direct observation of mortality in free-ranging fish at sea is

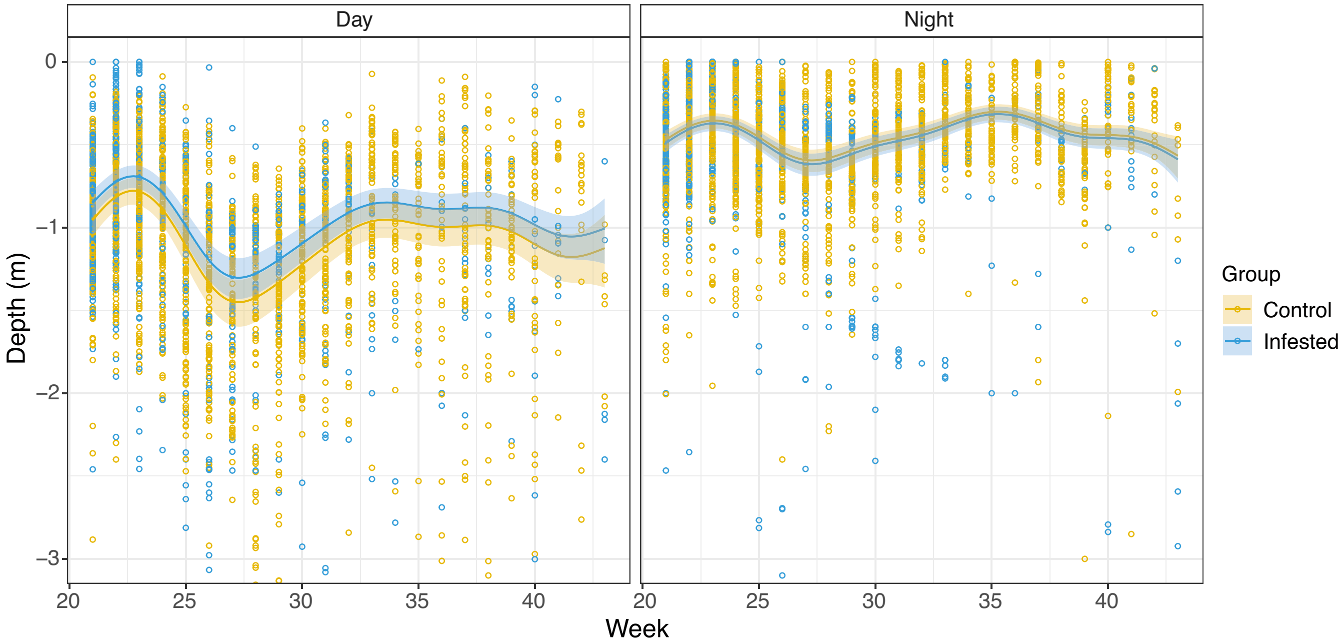


Fig. 9. Observed swimming depths for control (yellow dots) and infested (blue dots) sea trout post-smolts during day and night. Lines and shaded areas represent predicted swimming depths and 95 % CI, respectively, for a fish of average size (180 mm fork length)

difficult, and studies are often limited by low numbers of returning individuals and large natural variation in mortality. In this study, we document a higher mortality of lice-infested trout post-smolts compared to non-infested control individuals, with an estimated HR of 2.7 (95% CI = 1.04–7.13). In other words, the probability of a trout post-smolt to survive the first summer at sea was reduced by almost two-thirds when infested with a heavy lice load. These results are in agreement with Skaala et al. (2014), who showed that the survival of sea trout protected against lice was nearly doubled compared to non-treated fish, in a large-scale field experiment involving PIT-tagging of 2000 fish over 2 yr in a fjord with intensive salmon farming in Norway. To our knowledge, this is the only other study showing the impact of lice on the survival of wild sea trout in their natural environment. Other studies have not found a correlation between prophylactic treatment and marine survival of trout post-smolts, but these are (as stated by the authors of those studies) likely constrained by methodological caveats and low statistical power (Gjelland et al. 2014, Halttunen et al. 2018).

4.2. Salmon lice as a causal agent for premature return

In addition to an increase in mortality for lice-infested individuals, lice infestation triggered the almost immediate return of the fish to fresh water in our study. Most of the infested fish that did not rapidly disperse from the study area actively sought fresh or brackish waters within the first few days post release. Of these, almost half were never detected again at sea, having spent on average only 18 d feeding in the marine environment. Given a water temperature of around 12–13°C during that period, the attached copepodites had presumably reached the preadult stage around that time (Stien et al. 2005). Premature return to fresh water of lice-infested trout has been previously documented in Ireland, Scotland and Norway (Tully et al. 1993a, Birkeland & Jakobsen 1997, Pert et al. 2009). This return to fresh water is suggested to allow the fish to delouse and regain osmotic balance in order to survive the infestation (Birkeland & Jakobsen 1997, Bjørn et al. 2001). Even though preadult and adult lice are the most detrimental stages for the host fish (Bjørn & Finstad 1998), heavy infestations with copepodites and chalimus stages can also cause skin damage and hydromineral imbalance and can trigger a stress response in trout (Bjørn & Finstad 1997, Poole et al. 2000, Bjørn et al. 2001). Thus, heavily infested individuals may return to fresh water even before the offset of severe lice-induced physiological effects. Additionally, larger fish had a higher probability than smaller fish of returning to fresh water in both groups throughout our study. Higher return rates for larger trout post-smolts can be expected due to size-dependent mortality (Thorstad et al. 2015, 2016, Flaten et al. 2016). According to results from our survival analysis, the effect of fish size on the probability of return to fresh water cannot be attributed to size-dependent mortality, since no effect of fish size was detected on the probability of surviving the sea journey. However, this could be an artifact of low statistical power combined with large inter-individual variability.

4.3. Other behavioural adaptations to lice infestation

Of the 11 lice-infested fish that returned prematurely to fresh water in our study, 7 remained in the transition area for the rest of the migration. This is likely because the costs of ionic regulation are lower in brackish water than in both fresh and sea water (Rao 1968, Otto 1971, Brett & Groves 1979). Lice-infested individuals that did not prematurely return to fresh water spent most of the feeding migration in the innermost part of the fjord, characterized by lower surface salinities and shorter distance to the native river. Unlike lice-infested individuals, most control fish exploited the feeding grounds in the outer fjord and spent on average 100 d feeding at sea before returning to fresh water.

The vertical profiles of the trout movements showed a strong preference for the upper 3 m of the water column. Average swimming depths were slightly shallower at night than in the daytime, indicating small-scale diel vertical movements. These results agree with other studies investigating the behaviour of wild trout in the marine environment (Lyse et al. 1998, Eldøy et al. 2017, Kristensen et al. 2018). However, most of these studies have focused on veteran migrants, mainly due to technological constraints such as tag size for telemetry studies. Thus, our findings provide a new insight into the behaviour and habitat use of trout post-smolts during their sea journey. Lice-infested fish resided slightly closer to the surface compared to control fish during daytime in our study. Lice-infested trout have been previously reported to swim closer to the surface (Gjelland et al. 2014, A. Mohn unpublished data). Because the upper water layers are typically more influenced by rivers and other freshwater sources, this can be seen as a behavioural adaptation to counteract the osmoregulatory problems derived from the parasitic infestation. All in all, it seems likely that lice-infested individuals in our study were seeking fresh and brackish water by adjusting both their horizontal and vertical use of the fjord.

4.4. Indirect and delayed impacts

Growth in the sea is generally better than in fresh water (L'Abee-Lund et al. 1989, Jonsson & L'Abée Lund 1993, Frier 1994), and growth is positively correlated with the duration of the sea sojourn (Berg & Jonsson 1990). Thus, an abrupt interruption of the feeding migration after just a few weeks at sea, as reported in this study, may result in a significant loss of feeding and growth opportunities. Birkeland (1996) observed prematurely returned, lice-infested trout re-entering the sea after a median stay of 38 d in fresh water. By that time, they had lost 23.5 % of their body mass. Similarly, Fjørtoft et al. (2014) calculated a 20–40 % reduction in summer growth in the Etne River, western Norway, during a period of intensive farming, and suggested lice as a possible cause. In Ireland, proximity to salmon aquaculture and associated louse infestation pressure have been demonstrated to reduce weight in wild trout post-smolts by up to 9 g (at an average length of 18 cm), thus reducing Fulton's condition factor by ca. 0.15 (Shephard et al. 2016). Reduced summer growth may additionally reduce the probability of surviving through the following winter, since individuals that have not attained a critical size or sufficient energy stores may be unable to meet minimum metabolic requirements during winter and die (Jensen et al. 2018). Also, as fecundity increases with body size (Jonsson 1985, L'Abée Lund & Hindar 1990) and age at first maturity is influenced by growth rate at sea (L'Abée-Lund 1994, Vollset et al. 2014), reduced growth and energy reserves as a result of louse infestation may reduce fecundity and reproductive success.

4.5. Consequences for populations

It has been suggested that an increase in marine mortality and a reduction in sea growth due to lice or other factors affecting trout in the marine environment can result in a population shift in life-history strategy (Thorstad et al. 2015). Gargan et al. (2016) showed significant changes in quantitative life-history traits in the trout population in the Erriff River, western Ireland, after the establishment of salmon farming in the local estuary. These changes included a reduction in the number and size of trout kelts, the estimated number of eggs deposited, the sea trout rod catch, the proportion of older (1+ and 2+ sea age) fish and the frequency of repeat spawners. Similarly, Butler & Walker (2006) recorded a collapse in sea trout rod catch in the River Ewe/Loch Maree system, Scotland, in 1988. This collapse was related to an apparently unprecedented reduction in marine growth and survival, changes that were at least partly attributable to lice epizootics from nearby salmon farms. Reduced marine survival and growth as a result of lice epizootics may also shift the balance between costs and profits of the marine migration and ultimately lead to a decrease in the frequency of sea-run brown trout (Gargan et al. 2006).

4.6. Concluding remarks

In summary, our study reports a reduced survival of trout post-smolts as a direct consequence of a heavy lice infestation, suggesting that in areas with high lice infestation pressure, the number of trout post-smolts surviving the first season at sea may be significantly reduced. Furthermore, we document an altered migration behaviour of lice-infested individuals, including the abrupt interruption of the feeding migration after just a few weeks at sea. This interruption could result in a significant loss of feeding and growth opportunities for migrating fish, further affecting later survival and fecundity and ultimately having a potential impact at the population level. Despite trout being a culturally, socially and economically important species in Norway and elsewhere, there are still important knowledge gaps on its population biology and potential responses to parasites. This is partly explained by a complex and flexible life history with a wide range of strategies within and between populations. Notwithstanding the challenges, this information is urgently needed. Salmon lice epizootics are currently regarded as a major threat for wild salmonids in Norway (Costello 2009, Taranger et al. 2015, Forseth et al. 2017) and other salmon-producing countries, and robust scientific knowledge on the impact of lice on individuals and populations is key to the implementation of sound conservation strategies.

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Impacts of salmon lice on mortality, marine migration distance and premature return in sea trout

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Fig. S1. Example plot showing the vertical profile of a fish classified as dead and suspected to be eaten by a predator. Detections before the red dotted line are regarded as normal sea trout swimming activity. Detections between the red and blue dotted lines are suspected to correspond to a predator. Detections after the blue dotted line indicate the tag becoming stationary.

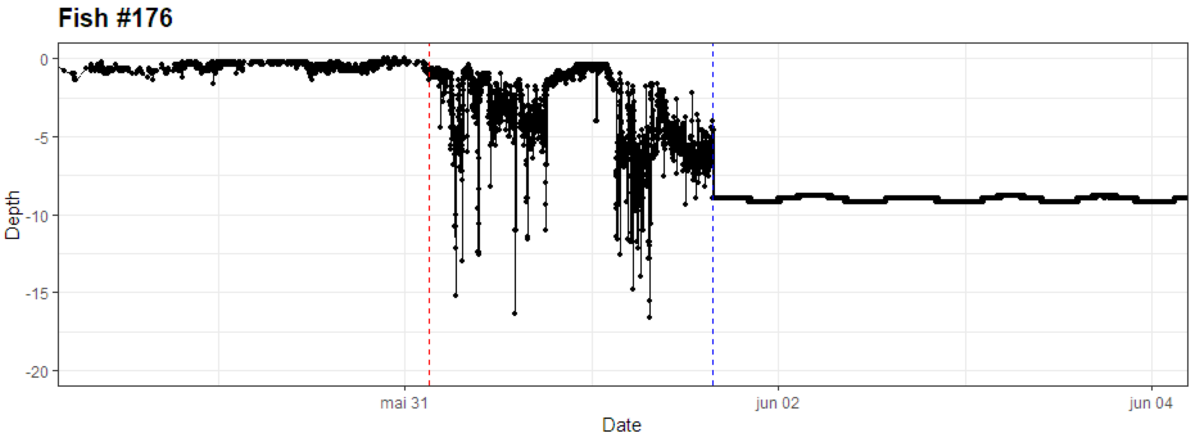

Table S1: Individual information on tagged sea trout post-smolts. Fate codes are as follows: M = migrated; D = dead; D_f = dead by fishing; R = returned to freshwater; R_P= prematurely returned to freshwater (before August 1st); AS = alive at sea (by October 1st).

ID	Group	Weight (g)	Fork length (mm)	Fate	Fate date	Last trout detection
25	Infested	64	191	M	29.05.2017	
26	Control	46	165	D	20.06.2017	
27	Infested	66	194	M	25.05.2017	
28	Control	76	201	R_P	27.05.2017	29.05.2017
29	Infested	51	173	Unknown	27.05.2017	
30	Control	65	195	M	25.05.2017	
31	Infested	52	171	M	28.05.2017	01.06.2017
32	Control	51	178	M	26.05.2017	
33	Infested	47	174	M	02.06.2017	
34	Control	60	188	M	10.06.2017	
35	Infested	57	184	D	19.06.2017	
36	Control	54	179	M	30.05.2017	
37	Infested	45	171	R_P, D	06.06.2017	19.06.2017
104	Control	91	205	M, D_f	30.05.2017, 27.07.2017	
105	Infested	54	170	M	26.05.2017	05.06.2017
106	Control	59	180	M	26.05.2017	
107	Infested	46	169	R_P	02.06.2017	05.09.2017
109	Infested	55	182	D	30.05.2017	
110	Control	50	171	M	04.06.2017	
111	Infested	59	186	M	04.06.2017	
112	Control	51	176	M	26.05.2017	07.06.2017
113	Infested	46	183	R_P, D	28.05.2017, 24.06.2017	
114	Control	75	200	M	25.05.2017	05.06.2017
115	Infested	57	178	M	04.06.2017	
116	Control	57	179	M	27.05.2017	
117	Infested	45	168	R_P	08.06.2017	02.08.2017
118	Control	59	185	M	26.05.2017	
119	Infested	43	167	Unknown	16.08.2017	
120	Control	51	174	M	31.05.2017	
121	Infested	70	186	M	01.06.2017	06.06.2017
122	Control	67	185	M	19.06.2017	
123	Infested	44	161	AS	13.10.2017	
124	Control	40	162	Unknown	04.06.2017	
125	Infested	70	198	M	21.08.2017	
126	Control	50	170	AS	08.10.2017	
129	Control	40	161	M	30.05.2017	
130	Infested	50	175	M	27.05.2017	31.05.2017
132	Control	64	186	M	26.05.2017	
133	Infested	52	181	M	03.06.2017	
134	Control	54	179	M	28.05.2017	
140	Control	55	180	M	26.05.2017	
141	Infested	64	190	R	31.08.2017	01.09.2017
142	Control	55	178	Unknown	03.07.2017	
143	Infested	72	198	M	27.05.2017	
144	Control	44	165	D	04.06.2017	
145	Infested	44	172	M	08.06.2017	
147	Infested	47	167	M	08.06.2017	
148	Control	46	168	M	26.05.2017	
149	Infested	61	187	M	09.06.2017	23.06.2017
150	Control	40	165	Unknown	08.07.2017	
151	Infested	52	172	M	06.06.2017	
152	Control	48	170	M	26.05.2017	
153	Infested	76	200	M	25.05.2017	
154	Control	56	176	R	23.08.2017	24.08.2017
155	Infested	65	190	M	26.05.2017	

156	Control	50	166	M	27.05.2017	
157	Infested	67	187	R_P	06.06.2017	11.10.2017
158	Control	41	162	R	06.10.2017	
159	Infested	50	175	M	26.05.2017	
160	Control	45	163	AS	03.10.2017	
161	Infested	75	202	M	26.05.2017	
162	Control	71	186	Unknown	28.05.2017	
163	Infested	93	212	M	02.06.2017	
164	Control	46	166	AS	23.10.2017	
165	Infested	48	166	M	12.06.2017	
166	Control	55	175	M	25.05.2017	
167	Infested	45	169	M	02.06.2017	
168	Control	70	197	M	30.05.2017	
169	Infested	92	217	M	30.05.2017	
170	Control	46	168	R	12.09.2017	02.10.2017
171	Infested	52	179	R_P	03.06.2017	31.10.2017
172	Control	89	210	M	09.06.2017	
173	Infested	40	162	Unknown	11.06.2017	
174	Control	60	185	M	27.05.2017	
175	Infested	65	188	M	26.05.2017	
176	Control	59	195	D	31.05.2017	
177	Infested	55	180	R_P, D	30.05.2017, 05.06.2017	
178	Control	71	198	M	27.05.2017	
179	Infested	68	194	M	28.05.2017	
180	Control	46	168	Unknown	24.08.2017	
181	Infested	93	220	R_P, D	29.06.2017, 22.07.2017	
182	Control	66	188	M	26.05.2017	
183	Infested	66	186	M	26.05.2017	
184	Control	78	194	M	27.05.2017	
185	Infested	43	165	M	27.05.2017	
186	Control	50	176	M	08.06.2017	22.06.2017
187	Infested	47	172	M, D	05.06.2017, 24.08.2017	
188	Control	58	183	AS	19.10.2017	
189	Infested	43	160	Unknown	07.06.2017	
190	Control	42	170	AS	21.10.2017	
191	Infested	42	161	D	28.05.2017	
192	Control	65	191	M	25.05.2017	
193	Infested	61	185	M	26.05.2017	
194	Control	60	187	M	26.05.2017	
195	Infested	49	167	D_f	13.09.2017	
196	Control	60	184	M	02.06.2017	
197	Infested	85	205	R_P	07.06.2017	18.08.2017
198	Control	47	176	M	26.05.2017	
200	Control	43	158	M	28.05.2017	
201	Infested	49	166	M	28.05.2017	
203	Infested	52	175	D	30.05.2017	
204	Control	62	188	M	26.05.2017	
205	Infested	54	183	M	26.05.2017	
207	Control	60	180	R	07.10.2017	
208	Infested	62	187	D	28.05.2017	
209	Control	58	180	M	31.05.2017	11.06.2017
210	Infested	57	176	Unknown	20.06.2017	
211	Control	70	195	D	28.06.2017	
212	Infested	56	175	M	30.05.2017	01.06.2017
214	Infested	69	190	R_P	07.06.2017	22.08.2017
215	Control	57	180	D	01.07.2017	
216	Infested	70	192	M	25.05.2017	03.06.2017
217	Control	45	163	M	30.05.2017	16.09.2017
218	Infested	66	192	M	26.05.2017	28.05.2017
219	Control	56	185	M	26.05.2017	
220	Infested	56	180	M	28.05.2017	
221	Control	42	155	M	30.05.2017	
223	Control	42	162	AS	29.10.2017	

224	Infested	75	203	AS	24.10.2017	
225	Control	77	201	M, D	29.05.2017, 14.06.2017	
226	Infested	46	165	M	30.05.2017	
227	Control	75	195	M	30.05.2017	
228	Infested	42	158	M	04.06.2017	06.06.2017
229	Control	74	195	R	22.08.2017	24.08.2017
230	Infested	50	167	M	29.05.2017	
231	Control	74	198	Unknown	11.08.2017	
232	Infested	72	200	M	30.05.2017	
233	Control	76	199	M	26.05.2017	
234	Infested	53	171	M	06.06.2017	
236	Infested	50	178	D	28.05.2017	
238	Infested	64	187	R_P	27.05.2017	01.06.2017
239	Control	41	163	M	25.05.2017	
240	Infested	53	178	M	31.05.2017	
242	Infested	63	190	Unknown	27.05.2017	
243	Control	50	171	AS	23.10.2017	
244	Infested	66	190	*Did not provide data*	-	
245	Control	100	226	M	29.05.2017	
246	Infested	48	168	M	26.05.2017	
247	Control	62	185	Unknown	26.05.2017	
248	Infested	59	180	M	16.06.2017	30.06.2017
249	Control	46	173	Unknown	28.05.2017	
250	Infested	72	196	M	27.05.2017	
253	Control	46	167	Unknown	24.06.2017	
255	Control	46	169	*Did not provide data*	-	
256	Infested	42	156	M	27.05.2017	

Table S2. Candidate models evaluated for survival analysis using Coxph models including (a) only 2017 data and (b) including data from the pilot study (2016). *Int*: intercept; *F*L: fork length; *Gr*: group; *Y*: year. Models are sorted by corrected Akaike information criteria (AICc) value, with logLik, AICc, difference in AICc from the best model (delta) and weight values of models also indicated.

a)

Candidate models					**df**	**logLik**	**AICc**	**delta**	**weight**
	Int	*FL*	*Gr*	*FL:Gr*					
1	+				0	-66.998	134.0	0.00	0.344
2	+		+		1	-65.975	134.2	0.22	0.308
3	+	+			1	-66.470	135.2	1.21	0.187
4	+	+	+		2	-65.651	136.2	2.16	0.116
5	+	+	+	+	3	-65.107	138.1	4.07	0.045

b)

Candidate models						df	logLik	AICc	delta	weight
	Int	*FL*	*Gr*	*Y*	*Gr:Y*					
1	+		+			1	-81.271	164.8	0.00	0.326
2	+		+	+		2	-80.866	166.4	1.67	0.141
3	+		+	+	+	3	-79.542	166.6	1.82	0.131
4	+	+	+			2	-81.092	166.9	2.13	0.113
5	+					0	-83.629	167.3	2.49	0.094
6	+			+		1	-83.163	168.5	3.78	0.049
7	+	+				1	-83.212	168.6	3.88	0.047
8	+	+	+	+		3	-80.671	168.8	4.08	0.042
9	+	+	+	+	+	4	-79.320	169.3	4.54	0.034
10	+	+		+		2	-82.705	170.1	5.35	0.022

Table S3. Candidate models evaluated for the analysis of return to freshwater using Coxph. *Int*: intercept; *FL*: fork length; *Gr*: group. Models are sorted by corrected Akaike information criteria (AICc) value, with logLik, AICc, difference in AICc from the best model (delta) and weight values of models also indicated.

Candidate models					df	logLik	AICc	delta	weight
	Int	*FL*	*Gr*	*Gr:strata*					
1	+	+	+	+	3	-59.288	126.2	0.00	0.659
2	+		+	+	2	-61.795	128.3	2.16	0.223
3	+	+	+		2	-63.398	131.5	5.37	0.045
4	+		+		1	-64.936	132.1	5.93	0.034
5	+	+			1	-65.023	132.3	6.11	0.031
6	+				0	-67.560	135.1	8.94	0.008

Table S4. Candidate models evaluated for the analysis of distance to freshwater using Generalized Additive Mixed Models (GAMM's). *Int*: intercept; *FL*: fork length; *Gr*: group. Models are sorted by corrected Akaike information criteria (AICc) value, with logLik, AICc, difference in AICc from the best model (delta) and weight values of models also indicated.

Candidate models						df	logLik	AICc	delta	weight
	Int	*FL*	*Gr*	*FL:Gr*	*s(Week,by = Group)*					
1	+	+	+	+	+	10	-24221.02	48462.1	0.00	0.967
2	+	+	+		+	9	-24225.59	48469.2	7.12	0.027
3	+		+		+	8	-24228.26	48472.6	10.46	0.005
4	+	+			+	8	-24258.23	48532.5	70.40	0.000
5	+				+	7	-24260.87	48535.8	73.65	0.000
6	+	+	+	+		6	-24531.20	49074.4	612.31	0.000
7	+	+	+			5	-24535.24	49080.5	618.38	0.000
8	+		+			4	-24536.99	49082.0	619.88	0.000
9	+	+				4	-24539.87	49087.7	625.63	0.000
10	+					3	-24541.91	49089.8	627.70	0.000

Table S5. Candidate models evaluated for the analysis of swimming depth using Generalized Additive Mixed Models (GAMM's). *Int*: intercept; *FL*: fork length; *Gr*: group; DyN: time of day (day/night). Models are sorted by corrected Akaike information criteria (AICc) value, with logLik, AICc, difference in AICc from the best model (delta) and weight values of models also indicated.

Candidate models								**df**	**logLik**	**AICc**	**delta**	**weight**
	Int	*FL*	*Gr*	*DyN*	*DyN:Grp*	*FL:Grp*	*s(week, by=DyN)*					
1	+	+	+	+	+		+	11	-2070.682	4163.4	0.00	0.708
2	+	+	+	+	+	+	+	12	-2070.644	4165.3	1.93	0.269
3	+		+	+	+		+	10	-2075.102	4170.2	6.83	0.023
4	+	+	+	+			+	10	-2091.805	4203.6	40.24	0.000
5	+	+		+			+	9	-2092.922	4203.9	40.47	0.000
6	+	+	+	+		+	+	11	-2091.764	4205.6	42.16	0.000
7	+			+			+	8	-2097.200	4210.4	47.02	0.000
8	+		+	+			+	9	-2096.231	4210.5	47.08	0.000
9	+	+	+	+	+			7	-2459.740	4933.5	770.09	0.000
10	+	+	+	+	+	+		8	-2459.608	4935.2	771.83	0.000
11	+		+	+	+			6	-2464.526	4941.1	777.66	0.000
12	+	+	+	+				6	-2494.446	5000.9	837.50	0.000
13	+	+		+				5	-2495.904	5001.8	838.41	0.000
14	+	+	+	+		+		7	-2494.308	5002.6	839.23	0.000
15	+		+	+				5	-2499.256	5008.5	845.11	0.000
16	+			+				4	-2500.518	5009.0	845.63	0.000
17	+	+					+	8	-3872.034	7760.1	3596.68	0.000
18	+	+	+				+	9	-3871.270	7760.6	3597.16	0.000
19	+	+	+			+	+	10	-3871.248	7762.5	3599.13	0.000
20	+						+	7	-3875.793	7765.6	3602.20	0.000
21	+		+				+	8	-3875.159	7766.3	3602.93	0.000
22	+	+	+					5	-4077.487	8165.0	4001.57	0.000
23	+	+						4	-4078.570	8165.1	4001.74	0.000
24	+	+	+			+		6	-4077.377	8166.8	4003.36	0.000
25	+							3	-4082.583	8171.2	4007.76	0.000
26	+		+					4	-4081.677	8171.4	4007.95	0.000