Parasite development affect dispersal dynamics; infectivity, activity and energetic status in cohorts of salmon louse copepodids

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ABSTRACT

The salmon louse, Lepeophtheirus salmonis, is a parasitic copepod infecting wild and farmed salmonid fishes in the northern hemisphere. It has a direct lifecycle with a planktonic dispersal phase and an infective copepodid stage preceding five host bound stages. Several models predicting the dispersal of this ecologically and economically important pathogen have been developed, but none include variability in capability to infect. Therefore, the effect of age and temperature on infectivity and lipid metabolism was investigated experimentally using seven synchronized cohorts of copepodids at 5, 10 and 15 °C. In newly molted copepodids infectivity initially increase and then decrease with senescence. Within the experimental temperature range, peak infectivity was higher and occurred earlier at higher temperatures. While degree-days may serve as a useful crude descriptor of developmental age, it did not allow accurate prediction of infectivity peak timing and magnitude unless temperature was included as a separate factor in the derived infectivity model. Senescence was reflected in lipid store depletion and a temperature dependent variability in membrane lipid composition was evident. Interestingly, copepodids developing at 5 °C had approximately 50% less storage lipids when they molted into the parasitic stage than those developing at 10 and 15 °C. The declines in infectivity and storage energy were mirrored in decreasing copepodid swimming activity. When incorporating the copepodid infectivity results from this study into salmon louse dispersal model parameterization, the predictions suggest that earlier models may have underestimated the seasonal differences in salmon louse infection risk.

1. Introduction

Predicting the influence of parasites on their hosts is a key epidemiological challenge that requires accurate knowledge of both pest and host biology (Franceschi et al., 2008; Macnab and Barber, 2012; Mouritsen and Jensen, 1997). Increasing complexity of parasite lifecycle and host-parasite interactions creates more challenges in prediction of epidemiological dynamics. In this landscape, the salmon louse (Lepeophtheirus salmonis) emerges as a “species in the middle”; it has a simple transmission requiring no intermediate hosts, but has a relatively complicated lifecycle including stages within both a planktonic and a parasitic niche. While the louse – salmonid host relationship has been the focus of a large number of studies, the planktonic stages have historically attracted less scientific attention (Brooker et al., 2018).

The salmon louse is a copepod ectoparasite of salmonid fishes belonging to the order Caligidae, commonly known as sea lice. It has a lifecycle encompassing eight stages; two planktonic nauplii stages, one infective copepodid stage where the larvae exhibit host-searching behaviours, fueled by a finite energy resource that dictates survival time if a host is not found. If a host is successfully infected, the copepodid stage is followed by two chalimus stages, two mobile preadult stages and lastly, the reproductive adult stage (Hamre et al., 2013). Reproductive females produce eggs organized in eggstrings that remain attached to the female during embryonic development, until hatching as nauplius.
larvae (Pike and Wadsworth, 1999).

Farming of Atlantic salmon (Salmo salar) in the Northern Atlantic has changed the salmon louse host availability drastically: Over a period of a few decades farming has supplied a stable and increasing supply of hosts, in many locations outnumbering wild salmonids by 2–3 orders of magnitude, as illustrated by the Norwegian farmed standing stock of around 400 million Atlantic salmon (www.ssb.no) in contrast to an estimated < 0.5 million wild Atlantic salmon returning to spawn (Aan, 2019). The salmon louse has responded with exploding populations sizes in parallel, and now represent a formidable challenge for marine salmonid aquaculture and wild salmonid populations in the North Atlantic Ocean (Bjørn et al., 2001; Costello, 2009; Finstad and Bjørn, 2011; Fjelldal et al., 2019; Fjørtoft et al., 2019; Forseth et al., 2017; Halttunen et al., 2018; Skern-Mauritzen et al., 2014; Torrissen et al., 2013). Formerly, sea lice infections in farmed salmonids were controlled mainly by administration of antiparasitic pharmaceuticals (Burridge et al., 2010). Since the late 2000’s, resistance and decreased sensitivity towards the compounds used have resulted in the emergence of a number of non-chemical delousing strategies including shielding skirts, warm water treatments and use of cleaner fish (Aan et al., 2015; Gonzalez and de Boer, 2017; Grønvedt et al., 2018; Oppedal et al., 2017; Stien et al., 2018). Despite the efforts, lice abundances regularly exceed management aims (Myksvoll et al., 2018; Taranger et al., 2015). Lice infection levels are monitored through compulsory routine counts at farms (e.g. Ireland: Monitoring Protocol No. 3 for Offshore Finnfish Farms - Sea Lice Monitoring and Control. https://www.agriculture.gov.ie, Scotland: The regulation of sea lice in Scotland 2019. Topic Sheet 71 (v3). https://www2.gov.scot/, Norway: Forskrift om bekjempelse av lakselus i akvakulturanlegg. 2018. https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140) and through field surveys of wild populations (Myksvoll et al., 2018). Additionally, modelling allows extrapolation of infection risk in areas without observations (Greidsrud et al., 2018; Myksvoll et al., 2018; Sandvik et al., 2020).

2. Materials and methods

2.1. Experimental overview

Cohorts of enumerated Lepeophtheirus salmonis salmonis (Skern-Mauritzen et al., 2014) copepodids with defined ages were used to infect four replicate tanks containing ten Atlantic salmon at seven time-points spanning the copepodid stage. The experiment was performed at 5, 10 and 15 °C. At the time of infection, copepodids were counted, activity levels were assessed, and samples were taken for later lipid analysis. The individual salmon groups were terminated when copepodids had molted to the chalimus I stage (estimated according to (Stien et al., 2005), and the infection levels were determined by counting attached larvae.

2.2. Experimental fish, rearing conditions and husbandry

The experiment comprised of 840 Atlantic salmon (Salmo salar), and additional salmon used to maintain a population of salmon lice (see below). The salmon were supplied by AquaGen, originating from their Atlantic QTL-inOva PRIME strain, and were vaccinated with AlphaJect micro 6 on September 12th, 2017 with an average weight of 55 g. Upon completed smoltification, the fish were transferred to seawater on November 2nd.

The experimental fish were introduced in groups of ten to separate 500 l tanks with running sea water at the experimental temperature, at least 5 days prior to infection to allow for acclimatization. Fish were provided a commercial diet using a standard feed regime throughout the experiment. At each experimental temperature, the fish were infected with enumerated cohorts of copepodids at seven defined ages in four replicate tanks; a total of 28 tanks with 280 fish were used at each temperature. The experiment was conducted at 15 °C in November–December 2017, and at 5 °C and 10 °C in February and March 2018. At the experiment conclusion, all fish were euthanized by an overdose of anaesthesia (0.1 g/l metomidate hydrochloride, Aquacalm®). In some instances, fish died during the experiment; these were removed from the tanks during the daily inspections and excluded from the dataset. The experiments were conducted at the Matre research station of the Institute of Marine Research, Norway, in accordance with Norwegian legislation for animal welfare, under permit ID 14047 from the Norwegian Food Safety Authority.

2.3. Salmon louse culturing

Adult female salmon lice were collected from farmed fish in Masfjorden on October 6 and 12 2017, and their eggstrings transferred to an incubator with running seawater (Hamre et al., 2009). Once hatched, the resulting copepodids were used to infect 30 adult salmon kept in three 500 l tanks at 10 °C (under permit ID 12935 from the Norwegian Food Safety Authority). Once these lice had developed to adults, the resulting eggstrings were used to produce the experimental copepodid cohorts for the infectivity experiments. Adult female lice produce eggs where embryonic development occur within the eggstring, until nauplius larvae are released during hatching. Almost immediately after hatching, the female extrudes a new pair of eggstrings. For each experimental temperature (5, 10 and 15 °C), the production lice and their hosts were kept at the test temperature for at least one
Table 1
Details of the experimental groups of salmon lice cohorts: across the test temperatures, the ages of copepodids used to infect hosts (days post hatching, DPH), the duration of the naupliar stages and expected survival time for copepodids (estimated from (Samsing, 2005)). The cohort ages and additional details at the different temperatures are shown in Table S1 and Table S2. The duration of the naupliar stages and window varied from 1 to 12 h and did not exceed 8 h for the four youngest ages at each temperature. Details on cohort production are given in Table 1 and Table S1. The duration of the naupliar stages and the earliest ages at each temperature. Details on cohort production are given in Table 1 and Table S1. The duration of the naupliar stages and window varied from 1 to 12 h and did not exceed 8 h for the four youngest ages at each temperature. Details on cohort production are given in Table 1 and Table S1.

| Temp. | Age (DPH) | Cohort window | Infection date | Termination date | #Fish | #Fish lost | Infection dose | #Replicate tanks |
|-------|-----------|---------------|----------------|------------------|-------|-----------|---------------|------------------|
| 5     | 10.51     | 0.29          | 06.02.2018     | 20.02.2018       | 39    | 0         | 991           | 4                |
| 5     | 11.95     | 0.25          | 09.02.2018     | 20.02.2018       | 67    | 2         | 1232          | 4                |
| 5     | 13.03     | 0.25          | 08.02.2018     | 20.02.2018       | 35    | 0         | 1209          | 4                |
| 5     | 14.96     | 0.25          | 08.02.2018     | 20.02.2018       | 37    | 0         | 1269          | 4                |
| 5     | 17.84     | 0.25          | 09.02.2018     | 20.02.2018       | 40    | 3         | 811           | 4                |
| 5     | 20.93     | 0.25          | 13.02.2018     | 20.02.2018       | 40    | 3         | 1173          | 4                |
| 5     | 23.34     | 0.25          | 16.02.2018     | 20.02.2018       | 40    | 9         | 742           | 4                |
| 10    | 4.39      | 0.26          | 02.03.2018     | 08.03.2018       | 40    | 0         | 1217          | 4                |
| 10    | 5.00      | 0.21          | 02.03.2018     | 08.03.2018       | 40    | 0         | 1247          | 4                |
| 10    | 6.33      | 0.33          | 05.03.2018     | 08.03.2018       | 40    | 0         | 1223          | 4                |
| 10    | 7.94      | 0.16          | 01.03.2018     | 08.03.2018       | 40    | 0         | 1190          | 4                |
| 10    | 11.05     | 0.21          | 05.03.2018     | 12.03.2018       | 40    | 1         | 1232          | 4                |
| 10    | 14.06     | 0.16          | 07.03.2018     | 12.03.2018       | 40    | 1         | 1223          | 4                |
| 10    | 16.41     | 0.27          | 09.03.2018     | 16.03.2018       | 40    | 0         | 1477          | 4                |
| 15    | 2.60      | 0.07          | 06.12.2017     | 15.12.2017       | 40    | 0         | 878           | 4                |
| 15    | 2.96      | 0.13          | 04.12.2017     | 14.12.2017       | 40    | 0         | 1201          | 4                |
| 15    | 3.84      | 0.14          | 07.12.2017     | 17.12.2017       | 40    | 0         | 1218          | 4                |
| 15    | 6.32      | 0.31          | 08.12.2017     | 17.12.2017       | 40    | 0         | 1287          | 4                |
| 15    | 7.51      | 0.34          | 23.11.2017     | 03.12.2017       | 40    | 0         | 1200          | 4                |
| 15    | 10.34     | 0.49          | 27.11.2017     | 06.12.2017       | 40    | 0         | 803           | 4                |
| 15    | 12.67     | 0.50          | 28.11.2017     | 07.12.2017       | 40    | 0         | 664           | 4                |

2.5. Infection procedure

During the infections, water flow was stopped in the tanks and the water level was reduced to 50% before copepodids were introduced into the water (for exact numbers see Table S1). After 20 min, flow was increased to 3 l min⁻¹, and after a further 25 min, normal flow of 10 l min⁻¹ was reinstated. Oxygen condition was monitored in the tanks throughout, ensuring that levels higher than 20% were maintained.

2.6. Experiment termination

The experiments were terminated at the time lice were expected to have developed into chalimus I (Table 1 and Table S1), based on the temperature and development rate according to Stien et al., (2005). The fish were euthanized by an overdose anesthetic as described above. All attached salmon lice were counted under a dissecting microscope and the euthanization vessel was scrutinized for detached lice, by staff having passed the research institute’s internal salmon louse larvae detection tests (passing requires detection and identification of all stages, including copepodids, present on experimentally infected fish). The observed infectivity (probability of attachment success) was calculated by dividing the total number of lice found on the fish and in the euthanization vessel with the number of copepodids used to infect the fish. Fish that died during the experimental period were routinely removed in collective lots not allowing lice numbers to be reliably enumerated. Thus, in tanks with mortality, the fraction of lice recovered was calculated by excluding a proportion of the copepodids scaled to the proportion of fish lost (i.e. assuming that infection levels on fish were uniform). At 5°C 17 out of 280 fish died, at 10°C two out of 280 fish died, while there were no mortality at 15°C.

2.7. Lipid analysis

Samples of 41–87 copepodids from all temperatures and timepoints were counted and placed on a small piece of glass-filter (Whatman™ 1001–090 Grade 1 Qualitative Filter Paper, Pore Size: 11 μm) and frozen in 1.5 ml glass vials at –80 °C for lipid analysis. The samples were extracted with 2 × 0.5 ml of chloroform:methanol (2:1). The extract was filtered through a Pasteur pipette with glass wool, to remove tissue residue, in to a 16 ml glass tube.
The extract was evaporated to dryness by N2 (g) and the lipid was dissolved in 30 μl chloroform:methanol. 15 μl was used for separation of lipid class analysis followed by fatty acid analysis and the remaining 15 μl was used for analysis of total fatty acids in the samples. These were added 10 μg of nonadecanoic acid (19:0) as an internal standard.

Three lipid classes - polar lipid (mixture of phospholipids, PL), neutral lipid (mainly triacylglycerol, TAG) and free fatty acid (FFA) - were separated on high-performance thin-layer chromatography (HPTLC) (10 × 10 cm TLC plates from Merck) according to Olsen and Henderson, (1989). 15 μl of each sample was loaded as a spot to the lower part of the TLC plate using a glass micro-syringe. The TLC plates were developed with a single system (methyl acetate: isopropanol: chloroform: methanol: 0.25% KCl (aq) (25:25:25:10:9 by volume)) until solvent had been absorbed 9 cm up the plate. The three lipid classes were visualized by dichlorofluorescein and UV, carefully scraped off the plate with a razor blade and placed into thick walled glass tubes prepared with 10 μg of internal standard 19:0.

All samples (both the three lipid classes and the unseparated lipid extract) were methylated, and the respective fatty acid methyl esters (FAME) were analysed on a HP-7890A gas chromatograph (Agilent, USA) with a flame ionization detector (GC-FID) according to Meier et al., (2006). As a methylation reagent 2.5 M dry HCl in methanol was used. The FAMEs were extracted using 2 × 2 ml of hexane. Due to the very small samples sizes, the extracted hexane samples were evaporated down to a minimum volume of 50 μl to obtain a suitable chromatographic response. Ten μl were injected splitless (the split was open after 2 min), the injection temperature was set to 280 °C. The column was a 25 m × 0.25 mm fused silica capillary, coated with polyethylene-glycol of 0.25 μm film thickness, CP-Wax 52 CB (Varian-Chrompack, Middelburg, The Netherlands). Helium (99.9999%) was used as mobile phase at 1 ml min⁻¹ for 45 min and then increased to 3 ml min⁻¹ for 30 min. The temperature of the flame ionization detector was set at 300 °C. The oven temperature was programmed to hold at 90 °C for 2 min, then from 90 °C to 165 °C at 30 °C min⁻¹ and then to 240 °C at 2.5 °C min⁻¹ and held there for 35 min. Total analysis time was 75 min. 29 peaks in the chromatogram were selected, and identified by comparing retention times with a FAME standard (GLC-463 from Nu-Chek Prep, Elysian, MN, USA) and retention index maps and mass spectral libraries (GC–MS) (http://www.chrombox.org/index.html) performed under the same chromatographic conditions as the GC-FID (Wasta and Mjøs, 2013). Chromatographic peak areas were corrected by empirical response factors calculated from the areas of the GLC-463 mixture. The chromatograms were integrated using the EZChrom Elite software (Agilent Technologies).

Possible background contamination: Due to the small size and low fatty acid content (0.14–0.7 μg/individ) of the copepodids, pooled samples of a large number of individuals were used. Even so, the total amount of fatty acids in the extracts ranged from 7 to 37 μg, making the analysis vulnerable for background contamination. Analysis of blank samples that have gone through the whole analytical procedure (Lipid extraction, TLC lipid classes separation, methanolysis and GC-FID analysis) revealed a background of the two saturated FAs; 16:0 and 18:0 in the FFA fraction. To avoid introducing bias in the results, data from the FFA samples were not used in data analysis. Details of the background analysis are given in the supplementary results.

### 2.8. Statistical analyses

The aim of the study was to improve our understanding of how infectivity and lipid metabolism varies with copepodid age at different temperatures.

To derive an infectivity model, we performed logistic regression on the binary infestation results (successful or unsuccessful infestation judged from counting lice on the fish at experiment termination) from the ageing cohorts of copepodids using age and experimental temperatures as predictors. Developmental age was recorded as days post hatching (DPH) and degree-days post hatching (DD: temperature x DPH). Degree-days is a commonly used parameter in biological-hydrodynamic coupled models and we therefore include DPH and DD when performing the logistic regressions. Since the nauplius II stage preceding the copepodid stage is unable to infect, datapoints with zero infection at the estimated time for molting (Stien et al., 2005) were added to the analysis. Likewise, since dead or moribund copepodids are unable to infect hosts, datapoints with zero infection at 200 DD were added to the analysis. Initial plots suggested curve-linear relationship between infectivity and age that differed depending on temperature. Thus, we fitted polynomial logistic regressions of the form:

\[
\text{Log (infection odds)} = \alpha + \beta_{\text{Temp}} + \beta_{\text{Age}} + \beta_{\text{Temp} \times \text{Age}} + \beta_{\text{Temp}^2} + \beta_{\text{Age}^2} + \beta_{\text{Temp}^2 \times \text{Age}} + \beta_{\text{Age}^2 \times \text{Temp}}
\]

using a logit link function and assuming a binomial distribution estimating the impact of temperature (βT) and age (βA as DPH or DD) as main effects, as well as interaction effects for the different polynomials of age and temperature (βAT). To identify functions reflecting the general trends in infectivity, we included polynomials of age (1 ≤ Na ≤ 4) and polynomials of temperature (1 ≤ Nt ≤ 2) in the numerical model. Hence, the most complex model included a 4th order polynomial of age, a 2nd order polynomial of temperature and interactions between polynomial age and temperature, treated as a linear, continuous variables. To identify the most appropriate model we included all possible models nested within Eq. (1) in the comparison (selected models shown in Table 2). We ran all the models and selected the best model based on topographic consideration and model comparison using AIC. The chosen model was further assessed using diagnostics including trends in residuals, outliers, dispersion and deviance explained. All statistical analyses were performed in RStudio v. 3.5.3 using the “rms” and “mgcv” packages.

To better understand copepodid lipid metabolism, we performed principal component analysis (PCA) and linear regression on the PL, FFA and TAG lipid composition. The PCAs was carried out in Sirirus (version 11.0, PRS, Norway). To make the systematic variation in the PCA independent of variable “size” each variable was weighted divided by its mean before PCA. The linear regressions were performed in XLSTAT, 2019 (Addinsoft, US).

### 2.9. Modelling infection risk in time and space

We incorporated the age- and temperature-dependent infectivity model derived in section 2.8 into a biophysical salmon lice dispersion model used by the Norwegian national salmon lice monitoring system (Myksvoll et al., 2018; Sandvik et al., 2020). In this model, lice larvae develop as a function of temperature and have a vertical behavior with response to light and salinity. Previously, lice were assigned a fixed infectivity rate inside the range 40 to 170 DD, and zero outside this range. The predictive ability of the old and new formulation was assessed using the relative operating characteristic (ROC) method.

#### Table 2

| Model DF AIC DE    |
|--------------------|
| A) DD + DD² 3 5792 26.3 |
| B) Temp + Temp² + DD + DD² + DD Temp + DD³ + Temp 7 1913 79.5 |
| C) Temp + Temp² + Age + Age² + Age³ + Age four + Temp + Age + Temp² + Age + Temp² 9 1841 80.5 |
| D) Temp + Temp² + DD + DD² + DD³ + DD Temp + DD² + Temp + DD³ + Temp + DD² + Temp + DD³ + Temp + DD⁴ + Temp 9 1654 83.1 |
| E) Temp + Temp² + DD + DD² + DD³ + DD Temp + DD³ + Temp + DD² + Temp + DD³ + Temp + DD⁴ + Temp 11 1258 88.6 |
described in Sandvik et al. (Sandvik et al., 2016; Sandvik et al., 2020). The ROC is a graph of the hit rate, H, against the false alarm rate, F, for different decision thresholds (Mason, 1982), developed based on observations from sentinel cages in the Hardangerfjord for the years 2012–2017. The sentinel cage data and ROC-output uses three categorical events (high, medium, and low) for the lice infestation pressure, and is at present in use as components of the “Traffic-light system” for a sustainable management of Norwegian salmon farming (Vollset et al., 2019). Forcing for the dispersion model (currents, temperature and salinity) was provided by a hydrodynamic fjord model with horizontal resolution of 160 m × 160 m, and 35 vertical sigma layers (Albretsen et al., 2011; Skarðhamar et al., 2018).

In addition to the ROC evaluation based on realistic parameterization, we also performed idealized simulation model experiments to visualize the impact of temperature on the infection risk. In our model experiment, 5 virtual sea lice particles (each representing 100,000 eggs) were released per hour from a source in the Hardangerfjord over the period March 2 to August 15, 2019. We then computed the mean infection pressure on a regular 500 m × 500 m grid, under three constant water temperature scenarios (5, 10 and 15 °C), for both the old and new infectivity model. Infection risk was defined as the infectivity-scaled particle density. To make the results comparable, the new infectivity formulation was scaled by parameters obtained from the ROC assessment.

3. Results

3.1. Copepodid activity and infectivity

The results showed that infectivity initially increased after molting into copepodids and subsequently declined with senescence, at all investigated temperatures (Fig. 1A). The observed infectivity was positively correlated with temperature and the peak values observed were 65% at 3 DPH and 15 °C, 43% at 11 DPH and 10 °C, and 24% at 21 DPH and 5 °C. Peak infectivity were several times greater than infectivity observed for preceding or succeeding ages. Furthermore, the peaks occurred in younger age cohorts at higher temperatures than at colder temperatures. Activity of the copepodids declined with time and thus mirrored the infectivity and storage lipid declines (see below). Interestingly, activity appeared stable until a point where after it dropped abruptly at a time coinciding with declining infectivity (Fig. 1B and C). Furthermore, there was no initial increase in activity that mirrored the initial increase in infectivity (Fig. 1B).

3.2. Infectivity model

Several statistical models including those shown in Table 2 were evaluated. Including polynomial temperature and age (as DPH or DD) as predictors increased the explanatory power and improved the model fit relative to simpler models. Models including age as a 4th-order polynomial had higher explanatory power and better AIC scores than the simpler models. However, examination revealed that such models were overfitted and exhibited unrealistic topologies (e.g. > 1 infestation peak) compared to lower order models.

Both models C and D reflected the data quite accurately, and ultimately model D (Fig. 1C) was selected based on its lower AIC score, higher explanatory power and easier implementation in biological-hydrodynamical coupled models. An ANOVA test on model parameters demonstrated that all terms were highly statistically significant (p < 10^-10). Retrieving parameters from model D yielded Eq. (1), for estimating log infectivity (Ln(I)) under the present experimental conditions.

\[
\text{Ln(I)} = -34.660 + 2.306 T - 2.585 e^{-2 T} + 7.156 e^{1 A} + 5.354 e^{3 A} + 1.191 e^{5 A} - 3.577 e^{-2 AT} + 2.526 e^{4 AT} - 5.541 e^{-7 AT}
\]

(1)

Where T is temperature in °C and A is age as DD. The infectivity (probability of attachment success, I) can be calculated from:

\[
I = e^{\text{Ln(I)}} / (1 + e^{\text{Ln(I)}})
\]

(2)

If the age is desired as time since hatching into copepodids, the age will have to be offset by the developmental time for the nauplius stages which can be estimated according to Stien et al. (Stien et al., 2005):

\[
\text{Development time (days)} = \tau (T) = (\beta_1/(T-10+\beta_2))^2
\]

(3)

Where T is temperature in °C, \(\beta_1 = 24.79\) and \(\beta_2 = 0.525\).

3.3. Energy depletion and lipid metabolism

Total lipid content was reduced over time in all groups, driven by a

Fig. 1. Copepodid activity and infectivity, at 5 °C (black), 10 °C (red) and 15 °C (green). Vertical lines indicate calculated first appearance of copepodids (Stien et al., 2005). Model results are shown as a solid line. Panel A: Infectivity, as a function of age as days post hatch (DPH, indicated on the x axis). Panel B: Mean activity score as a function of age as degree-days (DD, indicated at the x-axis in panel C). Panel C: Infectivity as a function of DD (indicated on the x axis). Datapoints are denoted with “x” or solid dot “●”; “x” denotes that copepodids were counted on all infected fish, “●” denotes that fish were lost and that copepodid numbers were derived from the remaining infected fish (see materials and methods for further details). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
fast reduction of the amount of TAG, while the amount of the polar membrane lipids remained relatively stable over time (Fig. 2A–C). The linear regression equations show that the reduction of TAG was temperature dependent and faster in the 15 °C group than in the 5 °C and 10 °C groups (Fig. 2B, Table 3). At all temperatures, the FA levels were stable for the first 2 days and then started declining thereafter. From the youngest to the oldest copepodids there was a TAG reduction of 87% for the 5 °C group, 97% for the 10 °C group and 89% for the 15 °C group.

With the polar lipids, the amount of FAs was relatively stable in the 5 °C and 10 °C groups (only a 28% and 11% reduction, respectively, with the slope of the regressions not being significantly different from zero, Table 3), while in the 15 °C group there was a reduction of 57% (small significant negative slope, Table 3). Remarkably, there were large differences in the lipid amounts in newly molted copepodids between temperature groups. Young copepodids at 5 °C had ≈50% less storage TAG FAs compared with the 10 °C group (Fig. 2A). Interestingly the 5 °C group had slightly higher levels of FAs in the polar fraction (Fig. 2C).

The relative composition of the fatty acid profile of TAG changed over time at all temperatures (Fig. 3A–F). While all fatty acids were depleted over time, PUFA (: polyunsaturated fatty acids) and MUFA (monounsaturated fatty acids) were depleted at a higher rate than SFA, leaving a higher relative content of SFA at the later time points (Fig. 3). This was also supported by principal component analysis where the score plots showed a clustering of the early sampling points (0.5–4 days) separate from the late sampling points (7–13 days), and the loading plot showing that the late samplings points have relatively higher levels of all SFA (from 14:0 to 24:0), while the early samplings points have relatively more MUFA and PUFA (see supplementary data, Fig. S2).

In the polar lipid fraction, the changes in the FA profiles were much less pronounced than for the TAG fraction. The only clear changes in FA profile were a marked decline in PUFA and corresponding increase in SFA seen in the PL fraction at 15 °C (Fig. 3).

### Table 3

Linear regression of FA amount plotted against days after hatching for total lipid, triacylglycerol (TAG) and polar lipid (PL). Significant p-values are in bold.

|                | Linear regression | R-squared value | P-value |
|----------------|-------------------|-----------------|---------|
| Total lipid    |                   |                 |         |
| 5 °C           | $y = -0.013x + 0.399$ | 0.71            | 0.018   |
| 10 °C          | $y = -0.027x + 0.481$ | 0.88            | 0.002   |
| 15 °C          | $y = -0.029x + 0.408$ | 0.84            | 0.003   |
| TAG            |                   |                 |         |
| 5 °C           | $y = -0.011x + 0.260$ | 0.84            | 0.004   |
| 10 °C          | $y = -0.026x + 0.393$ | 0.90            | 0.001   |
| 15 °C          | $y = -0.025x + 0.297$ | 0.78            | 0.009   |
| PL             |                   |                 |         |
| 5 °C           | $y = -0.002x + 0.139$ | 0.15            | 0.397   |
| 10 °C          | $y = -0.001x + 0.088$ | 0.11            | 0.469   |
| 15 °C          | $y = -0.005x + 0.111$ | 0.75            | 0.011   |

In the salmon lice dispersal model, we used Eq. (3) to determine the age at which the larvae become infective and Eq. (1) to determine the infectivity after this age. The resulting infectivity profile must be multiplied by a scaling factor of 3.53 to be comparable with the previously used step-function infectivity (Fig. 4). The scaling factor is the ratio of threshold levels (1.8 / 0.51) obtained by the relative operating characteristic (ROC) method (Sandvik et al., 2020) when calibrating the model against lice counts from in-situ observation sentinel cages.

The realistically parameterized ROC analysis revealed that the match between model results and observational data did not change significantly when compared with the infectivity model used previously. The match was slightly improved in low-temperature scenarios and slightly worsened in the higher temperature scenarios. As discussed by Sandvik et al., (2020), the available dataset used to calibrate the model represents a relatively narrow temperature range (8.5 to 13 °C), and minor changes in model fit is therefore to be expected.

The idealized dispersal model experiments with fixed sea water temperature demonstrated that the new infectivity formulation was more sensitive to temperature variations (Fig. 5D–F) than the model presently in use (Fig. 5A–C). In the new formulation, low temperatures give the lice a longer life span (increasing the area of dispersion), but also decreased their infectivity. The old formulation included the first
effect of increased dispersal range, but not the second effect of declining infectivity. Moreover, lice in the new model have a narrower time window of high infectivity, which gives somewhat less dispersive infectivity patterns over short time periods (hours). This effect is not evident from Fig. 5 as the data are averaged over a longer period.

4. Discussion

Planktonic salmon lice are lecithotrophic and have an infection period limited by depletion of energy stores, and an infectivity that varies with age and temperature (Brooker et al., 2018; Frenzl, 2014; Gravil, 1996; Tucker et al., 2000). However, previous data were
insufficient to allow the effect of age and temperature on infectivity to be modelled. Similarly, previous studies on lipid metabolism underlying energy storage depletion have focused on gross energetic patterns, ignoring fine-scale temporal changes and the role of temperature. In the present study we 1) describe the temporal patterns in infectivity at 5, 10 and 15 °C, 2) investigate the explanatory role of copepodid lipid metabolism, and 3) explore the impact of variable infectivity on spatial patterns in salmon louse infection risk.

4.1. Infectivity in ageing cohorts of copepodids at three temperatures

It has earlier been described that copepodid infectivity decreases with senescence and that the newly molted copepodids have limited capability to infect (Frenzl, 2014; Gravil, 1996; Tucker et al., 2000). Only one previous study (Frenzl, 2014) allow tentative identification of the time of peak infectivity; 4 days after molting into copepodids at 14 °C. This equals ≈ 85 DD post hatching, which is close to the 82 DD peak predicted using Eq. (1) at 14 °C. While DD is a useful crude indicator of copepodid maturation and senescence, precise estimation of the time of peak infectivity requires that temperature is considered as a separate model factor, since peak infectivity occurs at a lower DD age at higher temperatures. Whereas maturation and senescence govern the timing of the infectivity peak, the amplitude of the peak appears greater with increasing temperatures until 10–15 °C, and then declines at higher temperatures (Hamre et al., 2019; Samsing et al., 2016). We found both peak and integrated infectivity to increase with warmer temperatures throughout the experimental range (5–15 °C). The peak infectivity was almost 3 times greater at 15 °C compared to 5 °C, providing further support for keeping temperature as a separate model factor. Nevertheless, DD appear to be a useful crude indicator of the timing of copepodid maturation and senescence. Although trends in infectivity are comparable between studies, it is challenging to compare actual infestation levels since ‘peak’ infestations values from the literature vary enormously. For instance, Frenzl, (2014) reported a peak infectivity of 0.5%, while in Tucker et al., (2000) > 80% of the copepodids successfully attached to a host. These disparities most likely reflect differences in experimental design or conditions, rather than biological variability; while Frenzl, (2014) conducted trials using a flume chamber allowing copepodids to pass the fish only once, Tucker et al., (2000) allowed the copepodids to cohabitate with hosts for 8 h in stagnant aerated water. In the present study, the infection protocol included a 60-min infection period in semi-stagnant water and obtained intermediate peak infection rates relative to the beforementioned studies; 12–56% depending on temperature. Consequently, comparing infection levels between studies with different infection protocols should be done with great caution.

4.2. Lipid composition and metabolism in ageing cohorts of copepodids at three temperatures

The progression of maturation and subsequent senescence is reflected in the linear decrease in total lipid and TAG content in the copepodids over time, which corresponds well with previous findings (Cook et al., 2010; Gravil, 1996; Khan et al., 2018; Tucker et al., 2000). This is a natural result of the copepodids not feeding during the planktonic phase, thus relying on stored energy for development, basic metabolism, and locomotion until a host is located. Lipids constitute the major energy reserve, and it is logical to expect that metabolism and size of the energy reserves will impact on the lifespan, swimming activity and infectivity of the copepodids. Salmon lice kept at 5 °C had far less remaining energy reserves when they molted into copepodids, with 50% of that found in copepodids reared at 10 °C. This may be one contributing factor explaining the reduced infectivity of lice at low temperatures observed here and in earlier studies (Samsing et al., 2016).
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4.3. The effect of temperature and ageing on infection risk

The main effect of including variable infectivity in models is an increased infection risk at higher temperatures. While existing models already predict higher infection risk at warmer temperatures due to increased larval production and accelerated development (Brooker et al., 2018), the addition of the variability in infectivity exacerbates the predicted infection risk at higher temperatures. This finding is in accordance with the general increase in infection levels observed on sea trout during spring and early summer (Grefsrud et al., 2019; Nilsen et al., 2019) and illustrates that the number of copepods alone does not accurately reflect the infection risk. While the results suggest that the relative geographic infestation risk pattern remains similar, regardless of temperature when simulations are run over a prolonged period, the situation would be different if the scenario were run for periods characterized by diverging current and temperature regimes. It should be noted that the simulations were run with constant temperature in the entire water column over a long period – a highly unrealistic scenario that served to highlight the possible effects of the found infection variability.

Under the realistic setting with variable temperatures in the ROC analysis, the temperature dependent infectivity improved the results at low temperatures while the performance was slightly worsened at the higher ones. In our biological-hydrodynamic coupled model, we have assumed the polyomnic infectivity model to be valid across both temperatures and ages. However, the experiments were conducted at constant temperatures, and lice realistically move through micro-environments whereby their temperature history might have a more complex impact on infectivity than the model predicts. Controlled experiments with variable temperatures are needed to further investigate this effect. Validating models is an inherent challenge in model development, and empirical data for model validation may be obtained by, for example, deploying uninfected fish in sentinel cages or by monitoring wild fish and their infection levels (Myksvoll et al., 2018; Sandvik et al., 2016). Unfortunately, the available data obtained using these approaches share a narrow and unmonitored temperature range (sampled during a restricted time window without logging temperature) and hence does not allow for rigorous testing of the modelled temperature effect presented here. However, even though existing observations are insufficient to confirm an improved model performance, we have shown that temperature and age have a large impact on infectivity, and therefore this should be included in biological-hydrodynamic coupled models based on the predicted improvement in performance outside the validated temperature range. Future sentinel cage surveillance efforts should spatiotemporally focus on areas with high infestation pressures and high temperatures.

5. Conclusions

Copepod infectivity is highly influenced by temperature, development and senescence and the use of degree-days as an approximation of biological age is justified albeit imprecise. Copepod infectivity increase with higher temperatures within the experimental range. Copepod infectivity increased rapidly after molting into copepods and subsequently declined with senescence, a pattern that should be carefully considered when conducting experiments involving infections with parasitic copepods. Copepod activity decreased with time and hence reflected the observed declining TAG energy stores. Temperature regimes affected not only infectivity, but also the membrane composition as manifested by the differences in phospholipid composition. This illustrates the importance of acclimatizing the experimental animals in experiments involving different or changing temperatures. Model simulations using the derived infectivity profiles indicated that present models simulating salmon louse dispersal and infection risk may underestimate risks at higher temperatures.
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Authors’ Contributions
RSM, SB and SD conceptualized the study. TV, SB, RSM and SD performed the experimental investigation. MSM and RSM performed the formal analyses of the experimental data. NS and SM performed the lipid investigation and formal analyses of the lipid investigation results. ADS, MDS and PNS performed the biological-hydrodynamic coupled model analyses. RSM, SB, NS, SM, ADS and PNS wrote the manuscript. RSM finalized the manuscript. All authors revised and approved the final manuscript.

Declaration of Competing Interest
The authors declare that they have no competing financial, personal, or political interests likely to influence the work reported in this paper.

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Appendix A Supplementary data
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