



Disentangling the key drivers of salmon louse *Lepeophtheirus salmonis* fecundity using multiyear field samples

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ABSTRACT: Planktonic salmon louse *Lepeophtheirus salmonis salmonis* larvae produced at salmon farms spread to and infect both wild and farmed salmonids. Understanding and forecasting the production and distribution of these larval stages from farms is critical to aquaculture management. However, model forecasts are based on available data and therefore include parameters with limited empirical support. This investigation examined salmon louse fecundity with a focus on batch egg clutch size by collecting lice from farmed Atlantic salmon *Salmo salar* at multiple farms and from wild Atlantic salmon and sea trout *S. trutta* captured at field sites throughout Norway. The data were analyzed with mixed effects models and total length of female lice was identified as the primary determinant of clutch size. Further analysis revealed that female louse total length is partially explained by temperature at sampling. However, if the temperature at sampling is spatially or temporally disconnected from rearing temperature, it may not be possible to predict the total length of a louse using temperature. The fecundity investigation further found that 66% of female lice on farmed salmon were sexually mature, and 10% of these were not egg-bearing. In comparison, 73% of adult female lice on sea trout were sexually mature, and 40% of these were not egg-bearing. Our results indicate that salmon louse production forecasts would be improved by incorporating female louse sexual maturity and a clutch size parameter that is related to total length of female lice.

KEY WORDS: Aquaculture · Salmon farming · Sea lice · Parasite · Copepod · Reproduction · Temperature · Salinity

1. INTRODUCTION

The copepod salmon louse *Lepeophtheirus salmonis salmonis* (Krøyer, 1837), is an obligate ectoparasite of salmonids (Skern-Mauritzen et al. 2014). It consumes the skin, blood and mucus of its host, causing sores, osmotic imbalance, immunosuppression, and reduced growth (Bowers et al. 2000, Finstad et al. 2000, Fjell-dal et al. 2020). It is a persistent challenge for farmed fish, and its spread from salmon farms to wild salmon poses an environmental threat to those stocks (Torrissen et al. 2013, Vollset et al. 2018). From 1979 to

2019, aquaculture production of salmon increased from around 5000 to over 2.6 million t world-wide, with Norway accounting for 52% of the total (FAO-FIGIS 2021). The economic loss in Norway caused by salmon lice due to reduced farm production and treatment costs is estimated to be 9% of total revenue, which reached over 7.7 billion USD in 2019 (Abolofia et al. 2017, FAO-FIGIS 2021). Norwegian regulations mandate that farms monitor the number of lice on their fish, and if they exceed an intensity threshold, treatments must be applied (Heuch et al. 2005, Nærings- og fiskeridepartementet 2012). Along

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with rules covering the operations at salmon farms, regulations stipulate that future expansion be linked directly to the threat posed by salmon lice to the wild stocks. Under a 'traffic light system', the risk of lice-induced mortality is determined for 13 production zones and governs whether production at farms must be cut, may continue as is, or can expand (Nærings- og fiskeridepartementet 2020). Making that assessment involves an expert group evaluating several sources of information including monitoring data from farms (Jansen et al. 2012) and wild-caught salmonids (Serra-Llinares et al. 2014), from sentinel cages with fish (Bjørn et al. 2011), and using various models (Sandvik et al. 2016).

The health of wild populations and the future growth of the salmonid aquaculture industry is reliant on accurate parameterization of salmon louse life histories. Such information is vital for creating reliable estimates of louse production from farms and for understanding how best to treat and prevent louse epidemics. Egg production and release of newly hatched nauplii is the starting point for models of salmon louse infestation pressure and the risk of mortality to wild salmon (Sandvik et al. 2019). The estimation of egg production is based on the work of Stien et al. (2005), with egg production per fish being a function of the number of adult females, temperature, and a constant number of eggs per female (Ådlandsvik et al. 2017). This formulation, which is central to the 'traffic light assessment', is based on an incomplete understanding of fecundity, which was acknowledged by Stien et al. (2005). Given how important the reproductive output of female lice on farms is to the monitoring and management of the industry, it is surprising how fecundity remains understudied (Brooker et al. 2018). Following Stien et al. (2005), fecundity here refers to batch fecundity rather than lifetime fecundity and is defined as a function of the rate of egg batch production, the proportion of eggs that are viable, and the number of eggs produced per batch.

After hatching from an egg, the salmon louse starts its lifecycle as a planktonic free-living larva which features 2 short-lived nauplius stages followed by a molt into the infectious copepodid stage (Johnson & Albright 1991). These are non-feeding stages, surviving on maternally derived lipids; the copepodid can survive 150 degree-days or more at low temperatures before it finds and attaches to a host or ultimately dies of starvation (Samsing et al. 2016, Thompson et al. 2019). Once attached to the host fish, the louse proceeds through 2 attached chalimus stages, 2 mobile preadult stages, and the final adult stage in which the female attains approximately twice the size of the

male (Pike & Wadsworth 1999, Hamre et al. 2013, Eichner et al. 2015). Salmon lice follow the typical pattern of caligid copepod mating, with adult males attaching to preadult females and copulation occurring after the females develop into the final adult stage (Ritchie et al. 1996). After the final molt to the adult stage, the female must undergo further sexual maturation before egg production can occur. This process is marked by transcription of egg yolk proteins and an expansion and lengthening of the genital segment while the cephalothorax remains unchanged (Eichner et al. 2008). The oviducts inside the genital complex become filled with eggs that are then extruded and enveloped with a secretion from the cement gland, leading to the formation of 2 egg strings (Pike & Wadsworth 1999). The clutch of eggs, egg strings, or egg sacs, are eggs stacked within a transparent casing that remain attached to the female but enclosed with a layer of the cement secretion and at this stage are functionally isolated from the female as they trail loosely behind (Pike & Wadsworth 1999). It has been regularly observed that portions of the egg strings are not differentiated into eggs, and not all the eggs are viable, whereby some of them never develop and hatch (Brooker et al. 2018, Heuch et al. 2000).

Egg strings reared in low-salinity waters have reduced hatching success (Gravil 1996), and the survival of the planktonic stages is compromised at levels below 29 ppt (Bricknell et al. 2006), but the parasitic stages have greater tolerance and adult females are capable of surviving for 13 d at 15 ppt (Johnson & Albright 1991). Likewise, low temperatures decrease the hatching success of salmon lice, but studies differ on the reported low-temperature threshold (Gravil 1996, Samsing et al. 2016, Boxaspen & Naess 2000, Heuch et al. 2000). Brooker et al. (2018) suggests the temperature acclimation of the adult female might be important in determining the temperature tolerance of the eggs and subsequent nauplii, and thus the examination of unacclimated females may not be appropriate. Supporting that suggestion, in other copepod species thermal tolerance over multiple generations has a genotypic dependence (Sasaki & Dam 2020).

Duration of the embryonic development and further developmental progression to the adult stage has a negative relationship with temperature in salmon lice (Samsing et al. 2016, Hamre et al. 2019). This follows the general pattern seen in ectotherms, with organisms growing slower at colder temperatures and faster at warmer temperatures (Angilletta et al. 2004). Temperature-dependent development rates have been well described in some copepod species and often fit to the Bělehrádek function (Camp-

bell et al. 2001, Leandro et al. 2006, Samsing et al. 2016), though Quinn (2017) argues that, depending on the taxa, other functions would fit the relationship better. In salmon lice, egg development and hatching time has been observed to take as much as 45 d at 2°C (Boxaspen & Naess 2000), and as little as 1.8 d at 20°C (Samsing et al. 2016), a 25-fold difference. At 5°C it takes nearly 12 d to develop through the nauplius stages, but at 20°C it takes less than 2 d (Samsing et al. 2016). Once the louse copepodids attach to the host, it takes 72 d at 6°C for females to reach the adult stage and just 13 d at 21°C (Hamre et al. 2019). As demonstrated by these studies, development time is very responsive to temperature. However, the accumulation of mass and increase in body size does not follow the same relationship with temperature in ectotherms (Atkinson 1994).

Ectotherms, including copepods, follow the so-called 'temperature-size' rule, where animals reared at colder temperatures grow more slowly but achieve a greater body size than animals grown at warmer temperatures, which grow faster but reach a smaller size at maturity (Atkinson 1994, Angilletta et al. 2004). The rule is based on a lesser response of growth rate to temperature change than development rate. Long development times at colder temperatures grant more time for growth before the mature stage is reached and growth ceases. The rule has broad empirical support, and across taxa the relationship follows a 5% decrease in body size (mass) for every 1°C increase in aquatic species (Forster et al. 2012). This pattern is also observed for copepod species with animals maturing in seasonally warmer water reaching a smaller size than those that developed in seasonally colder water (Horne et al. 2016). Nevertheless, there are exceptions to the rule, and it may only apply when the animal is experiencing non-stressful temperatures for that genotype (Kingsolver & Huey 2008).

The seasonal pattern of animals growing larger during colder times of year has been frequently reported in salmon lice (Ritchie et al. 1993, Gravil 1996, Pike & Wadsworth 1999), and further investigations have revealed the underlying temperature-size relationship (Heuch et al. 2000, Nordhagen et al. 2000). Similarly, a study by Bravo et al. (2013) demonstrated a relationship between season, sea temperature, and size in *Caligus rogercresseyi*, another sea louse species which impacts salmon farming in Chile. While previous investigations have also established a positive (Ritchie et al. 1993) and a negative (Gravil 1996) relationship between salmon louse size and photoperiod, their statistical models did not account for covariates such as temperature. In the case

of size, temperature could be the sole responsible mechanism, but, without experimental control or complex statistical models, it is difficult to disentangle it from collinear variables such as season, photoperiod, and host influence. Samsing et al. (2016) completed the most extensive examination of salmon louse size in relation to rearing temperatures in a laboratory setting, the results of which conform to the temperature-size rule. However, at the lowest temperature treatment of 2.9°C, a slight decrease in size was found (Samsing et al. 2016), indicating the temperature was outside the non-stressful thermal limits of the animal (Kingsolver & Huey 2008, Brooker et al. 2018).

The size of an organism affects nearly all aspects of an organism's biology, and within a population, being bigger can grant higher fitness, in part because of the increased fecundity of the organism (Kingsolver & Huey 2008). In several studies, the positive relationship between body size and clutch size has been reported for salmon louse (Tully 1989, Jackson & Minchin 1992, Ritchie et al. 1993, Gravil 1996, Heuch & Mo 2001) and for *C. rogercresseyi* (González et al. 2012, Bravo et al. 2013). In the laboratory investigation by Samsing et al. (2016), clutch size was fit to a temperature function which showed a parabolic relationship, with an increasing number of eggs produced per batch from the lowest temperatures to a maximum at 8°C and egg production subsequently decreasing as temperature continued to increase. However, the measured clutch size differed somewhat from their modeled results. The greatest mean output of 295 eggs per egg string was found at 5°C, which corresponds to largest female lice; then as the experimental temperature increased, the observed egg clutch size and size of the female decreased (Samsing et al. 2016). Apart from body size, the reproductive output of free-living copepods may be negatively impacted by a lack of available food (Brun et al. 2016, Horne et al. 2016). While salmon lice may not face the same explicit food limitation, a similar density-dependent relationship has been demonstrated with higher numbers of female lice per fish yielding smaller egg clutch sizes (Ugelvik et al. 2017).

In this study we evaluated the relationship between salmon lice fecundity, body size, and environment and we compared those results to previously derived measures of fecundity. The fecundity investigation specifically focused on the number of eggs produced per batch or female, also referred to as clutch size. The literature suggests that temperature drives body size, and larger body sizes result in greater clutch sizes in salmon louse, but this relationship may be disrupted by a stressful environment

which cannot be controlled for using *in situ* samples (Angilletta et al. 2004, Kingsolver & Huey 2008, Brooker et al. 2018). Nevertheless, the aim of this study was to (1) better parameterize models of salmon louse egg production used in the traffic light assessment by identifying which variables best predict clutch size. Further aims were to (2) model salmon louse body size as a function of environmental and host variables and (3) describe the pattern of female sexual maturity and egg-bearing status.

2. MATERIALS AND METHODS

To evaluate salmon louse fecundity *in situ*, samples were collected from both wild and farmed salmonids at multiple localities throughout Norway over a 4 yr period. The fecundity of sampled salmon louse females was assessed through image analysis, and then statistical models were used to investigate clutch size, louse body size, sexual maturity, environmental conditions, and other host- or parasite-related attributes of the sample.

2.1. Sample collection

Fish handling procedures and data collected on the fish at time of sample collection differed depending upon the source (details below). Samples were sourced from: farmed Atlantic salmon *Salmo salar* Linnaeus, 1758 in commercial farms, wild Atlantic salmon and sea trout *S. trutta* Linnaeus, 1758 captured through a national salmon lice surveillance program for wild salmonids (NALO) (Nilsen et al. 2022), and wild Atlantic salmon and sea trout captured in a salmonid trap located in the Etne River, Norway.

Prior to handling, fish sampled at the farm and at NALO field sites were fully anaesthetized with tricaine methanesulfonate (Finquel: 10 g 100 l⁻¹); no anesthetic was used for handling of the river-caught fish. In all cases, salmon lice were removed from the host fish with forceps and placed in a single sample vial with ethanol before being sent to the Institute of Marine Research (IMR) facilities in Bergen, Norway, for further processing. At the farm and field sites, all mobile stages of lice were collected from the host fish, but at the river trap, only female lice with egg strings were removed.

In accordance with the Norwegian Food Safety Authorities regulation over the control of lice, all farmers must make counts of sea lice on fish in all cages and report the averages. Depending on the

time of year, temperature, and location, the counts are made weekly or bi-weekly on 10 or 20 fish per cage (Nærings- og fiskeridepartementet 2012). During those counts, commercial partners collect the lice samples from the farm sites. The salmon farm samples were collected from 12 commercial sites and from the experimental facilities at the IMR research station at Austevoll, south of Bergen, between May 2018 and February 2020. Corresponding metadata was collated for each farm site from the Norwegian Coastal Administration's open data portal Barents-Watch (www.barentswatch.no).

Several methodologies are used by NALO for capturing fish and monitoring infection pressure, with the samples used in this study coming from salmonids caught in fyke nets. The nets are deployed shortly after the expected date of smolt migration, and they extend 30–50 m perpendicular to the shoreline and down to a depth of 1–3 m. Fish swimming along the shore are caught in the nets, which are checked daily. The fish are removed, and salmonids undergo a thorough examination that includes a lice count, size measurements, and optional tagging and tissue sampling (Nilsen et al. 2022). The monitoring program primarily targets sea trout, which are more abundant in the nets than wild salmon. Due to welfare concerns, larger wild salmon are examined first and released as soon as possible. Thus, the collection of female lice with egg strings was prioritized with some non-egg-bearing females and all other stages remaining on the host fish. All NALO field samples were collected during 2 wk in June 2018.

The Etne River is designated as a national salmon watercourse, which gives the salmon stock therein special protection against certain threats including introgression from farmed fish. The salmon trap is part of a field platform designed to collect data on the salmonids migrating upriver and to remove farmed fish from the stock (Madhun et al. 2017, Glover et al. 2019). The trap spans the 40 m width of the river and is located in freshwater 1 km from the mouth with the fjord. Salmonids entering the trap were handled and released within 24 h: they were weighed and measured without removing them from the water, and any observed female salmon lice with egg strings were collected. All river-trap samples used in this investigation were collected during 8 wk in the summer of 2021.

2.2. Imaging and measurement

Two sets of images were taken for fecundity and size measurements: a macro-image of whole louse in

the sample and a micro-image of the eggs (Fig. 1). The macro-image was taken with an Olympus OM-D-E-M5 II equipped with a 50 mm macro-lens at a distance of 35–50 cm. Anytime the camera was moved or adjusted, a scale image was taken. A glass slide was placed on top of the females and egg strings to prevent curling of the animals, which occurs in ethanol samples. Several images were taken for a single sample when all the animals could not be captured in one image. After taking the macro-image, the egg-bearing females were moved to a microscope and an image was taken of a portion of the egg strings. For consistency, the portion of the egg string selected for imaging was preferably the left one at the proximal end. Due to the preservation, the egg strings were often occluded and would not lay flat on the dish; it was then necessary to take the image where the clearest view could be found. A Nikon SMZ 18 stereomicroscope equipped with a DS-Qi2 monochrome camera and an Olympus SZX-10 stereomicroscope with a DP12 color camera were used to take the micro-images of egg strings, and scale images were taken with equivalent settings.

All images were analyzed in ImageJ using the segmented line tool to measure lengths in pixels which were converted with the scale measures to mm (Rasband 2011). Measurements included total animal length, genital segment length, cephalothorax width, and length of egg strings. Adult males as well as females were measured, and a total count was made of all stages found in the samples (data available in the Supplement at www.int-res.com/articles/suppl/q015p161_supp.xlsx). Cephalothorax length was calculated as the difference between the total length and genital segment length, and the ratio of cephalothorax to genital segment length (CT/GS) was calculated following Eichner et al. (2008). Status of females as sexually mature and capable of producing egg strings was determined by the presence of egg strings or by the presence of an empty egg-string case. Short non-viable egg strings, 'stubs', were occasionally observed on females; these lice were considered sexually mature, but not to have any eggs. Similarly classified, the egg strings on some lice were in the process of hatching with empty segments of the egg string visible towards the distal end and eggs

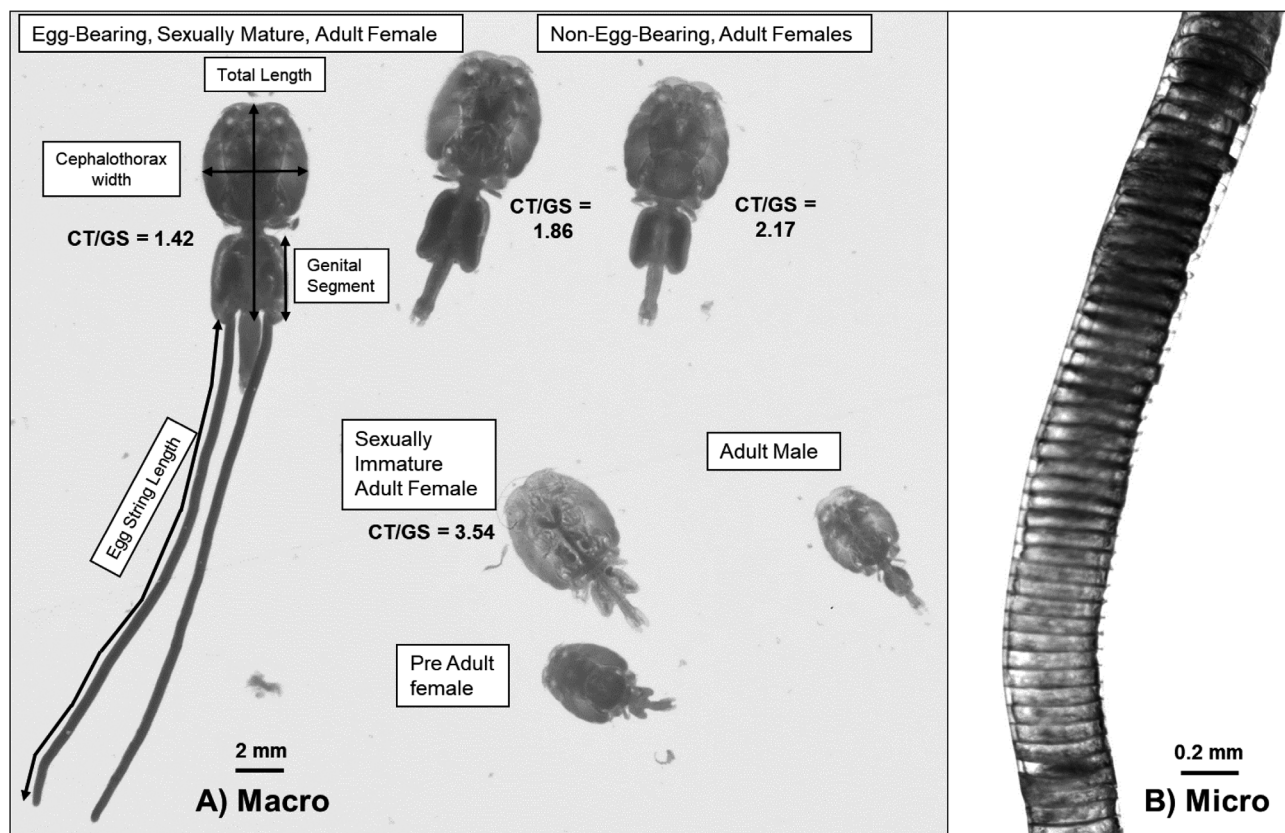


Fig. 1. (A) Macro- and (B) micro-images of fecundity samples of salmon louse. (A) Measurements taken with ImageJ are shown along with identification of stages present and the cephalothorax to genital segment length ratio (CT/GS)

no longer exhibiting the compact stacked arrangement. While the goal was to take a micro-image for all egg-bearing females to measure egg size, there were instances in which the images could not be taken or they were not useful for making the measurement (e.g. occluded egg strings, unfocused images).

2.3. Environmental data

All temperature and salinity data were generated using the 160 × 160 m grid, hydrodynamic fjord model (Dalsøren et al. 2020), which has a higher resolution than the 800 × 800 m grid NorKyst800 model (Asplin et al. 2020). The latter model is utilized for dispersion modeling of salmon lice throughout the Norwegian coast and, although the model dynamics differ, the temperature outputs of both models are similar. Based on a review of the literature, a depth of 3 m was chosen for extracting the salinity and temperature data. Wild Atlantic salmon have been observed swimming at mean depths of 0.5–2.5 m with occasional dives to 30 m (Davidsen et al. 2013). Sea trout have mean swimming depths between 2.0 and 2.5 m nearshore with dives down to 24 m (Eldøy et al. 2017). Farmed salmon spend the majority of their time between depths of 2 and 4 m, with those having a higher parasite load exhibiting a preference for deeper waters (Bui et al. 2016). Thus, regardless of their origin, all the host fish exhibit a shallow-water preference and a depth of 3 m was regarded as representative of the environmental conditions experienced by the fish across circumstances, although sharp haloclines may occur. However, further assumptions needed to be made for the samples obtained from the river trap. Here we assumed that all fish were in the process of migrating upriver and had come directly from the fjord. A point 500 m from the mouth of the Etne River in the fjord was chosen as representative of the environmental conditions experienced by the salmon lice and host fish, and data was extracted from the model for the day they were sampled.

2.4. Data analysis

Clutch size, female salmon louse size, and the presence of eggs on females were examined in statistical models as response variables. Since the sampling design is hierarchical, with observations nested within a sample set from a single fish that is nested within a sampling event, a mixed effects model approach was utilized. The models were fit using the 'glmmTMB'

function from the same-named package (Brooks et al. 2017) within the R software environment for statistical computing (R Core Team 2020). All generalized linear mixed models (GLMMs) included the random effects 'fish sampled' nested within 'sampling event'. Where the models are used to make predictive plots, the intercepts do not account for differences due to random effects and instead represent the mean. Standard procedures for data exploration and model validation were used to identify statistical problems arising from outliers, heterogeneity of variance, collinearity, dependence, and interactions (Zuur et al. 2010). In addition, diagnostic functions in the R package DHARMA (Hartig 2019) were used to evaluate dispersion, dependency, and normality of model residuals. Presentation of results from statistical analysis and the selected models follows standard protocols described by Zuur & Ieno (2016). Evaluation of the models is further accomplished through calculation of the coefficient of determination R^2 as calculated by the 'r.squaredGLMM' function from the MuMIn package (Barton & Barton 2015). The calculation quantifies the proportion of variance which is explained by the fixed effects in the model and the variance explained by the full model including fixed and random effects (Nakagawa et al. 2013, 2017).

The models fitting clutch size and female louse size used a Gaussian distribution, and all possible models using the included variables were evaluated by the Akaike information criterion (AIC). The model with the lowest AIC was selected unless another model had a lower number of parameters within 2 points of the lowest AIC, in which case the latter model was chosen. Due to the fragmented sampling of the different host fish across latitudes and season, the datasets for modeling clutch size and louse size are partitioned accordingly. A variable of latitudinal zone was created to divide the distribution of samples into 3 zones: the northern latitudinal zone is above 68° N, the southern zone is below 62° N, and the middle zone includes the latitudes between those boundaries. Due to its skewed distribution, the continuous variable latitude was replaced in the statistical models with the categorical variable of latitudinal zone, which was also used as a means of partitioning the dataset.

The analysis of clutch size using statistical models was limited to the observations of farm-collected samples and included the following 6 explanatory variables: temperature, salinity, day length, females per fish, total length of salmon louse, and latitudinal zone (Table 1). Where both females per fish and latitudinal zone were included in the models, an interaction effect was included between them. The response

variable clutch size is calculated from the total length of both egg strings divided by the observed egg size of that egg bearing female. Females with a single egg string were removed, as were females with first 'stubs', and those whose eggs were not stacked within the egg case. Less than 1% of the egg string pairs featured damaged sections. If it was determined that the damage was due to handling, then the section was included in the calculation of clutch size, otherwise the length of the damaged section was subtracted from the total length.

Female louse size was measured by total length from the anterior of the cephalothorax to the posterior of the genital segment (Fig. 1). Due to limited distribution of observations from wild salmon and sea trout over latitudinal zones and throughout the year (Fig. 2), female total length was modeled twice using differing datasets and variables. The first analysis was restricted to observations from farm samples and included the explanatory variables: temperature, salinity, latitudinal zone, day length, and females per fish. Where both temperature and latitudinal zone were included in the models, an interaction effect was included between them. In the second analysis, the data set was restricted to the southern latitudinal zone but included observations from farm, river, and field samples. The explanatory variables included temperature, day length, and host fish origin. Salinity was removed, since it is collinear with host fish origin, and females per fish was removed because of bias from the river samples. Complete lice counts

were not done on river-caught fish, and the samples did not contain all females found on the fish; only those with egg strings.

For both the salmon farm observations and those from sea trout collected in the field, CT/GS of sexually mature females was fit to a gamma distribution using the 'fitdist' function found in the fitdistrplus package (Delignette-Muller & Dutang 2015). The 95% quantile of that distribution was then found so that females with a ratio below had a 95% probability of being sexually mature and capable of bearing eggs. Those females were then selected for inclusion into a model that examined egg-bearing status of the females in relation to host fish origin and temperature. A binomial distribution was used to fit the mixed effects model of egg presence on female salmon lice. Rather than use an AIC selection approach, hypothesis testing with ANOVA was done to determine if the null model was statistically different from the model with the explanatory variable host fish origin and to determine if that model was different from one which included the variable temperature.

3. RESULTS

3.1. Description of samples collected

A total of 729 host fish were sampled, and 2650 female salmon lice were collected, with 59% of them bearing eggs. While more than 78% of the sampled

Table 1. List and description of covariates used in the statistical models

Variable	Category	Description
Temperature (°C) at 3 m depth	Continuous	Data generated at specified coordinates and date from 160 m grid Fjord model
Salinity at 3 m depth	Continuous	Data generated at specified coordinates and date from 160 m grid Fjord model
Day length (centered)	Continuous	Day length calculated from latitude and date, centered to the mean value
Latitudinal zone of site	Categorical, 3 levels	Northern: above 68°; middle: between 68° and 62°; southern: below 62°
Females per fish	Continuous	Natural log transformed number of adult female lice found on host fish
Total length (mm)	Continuous	Total length of female salmon louse from anterior cephalothorax to posterior of genital segment, excluding abdomen
Clutch size	Continuous	Total number of eggs produced by louse, calculated from length of both egg strings and observed number of eggs per mm of egg string
Host fish species and origin	Categorical, 3 levels	Farmed salmon (<i>Salmo salar</i>); wild salmon (<i>S. salar</i>); sea trout (<i>S. trutta</i>)
Egg presence	Categorical, 2 levels	Presence of egg strings or empty egg string case: yes or no
Sampled fish	Random effect	Observations of female lice which all come from the same sampled host fish
Sampling event	Random effect	The date and location from where samples were collected; the random effect 'sampled fish' is nested within

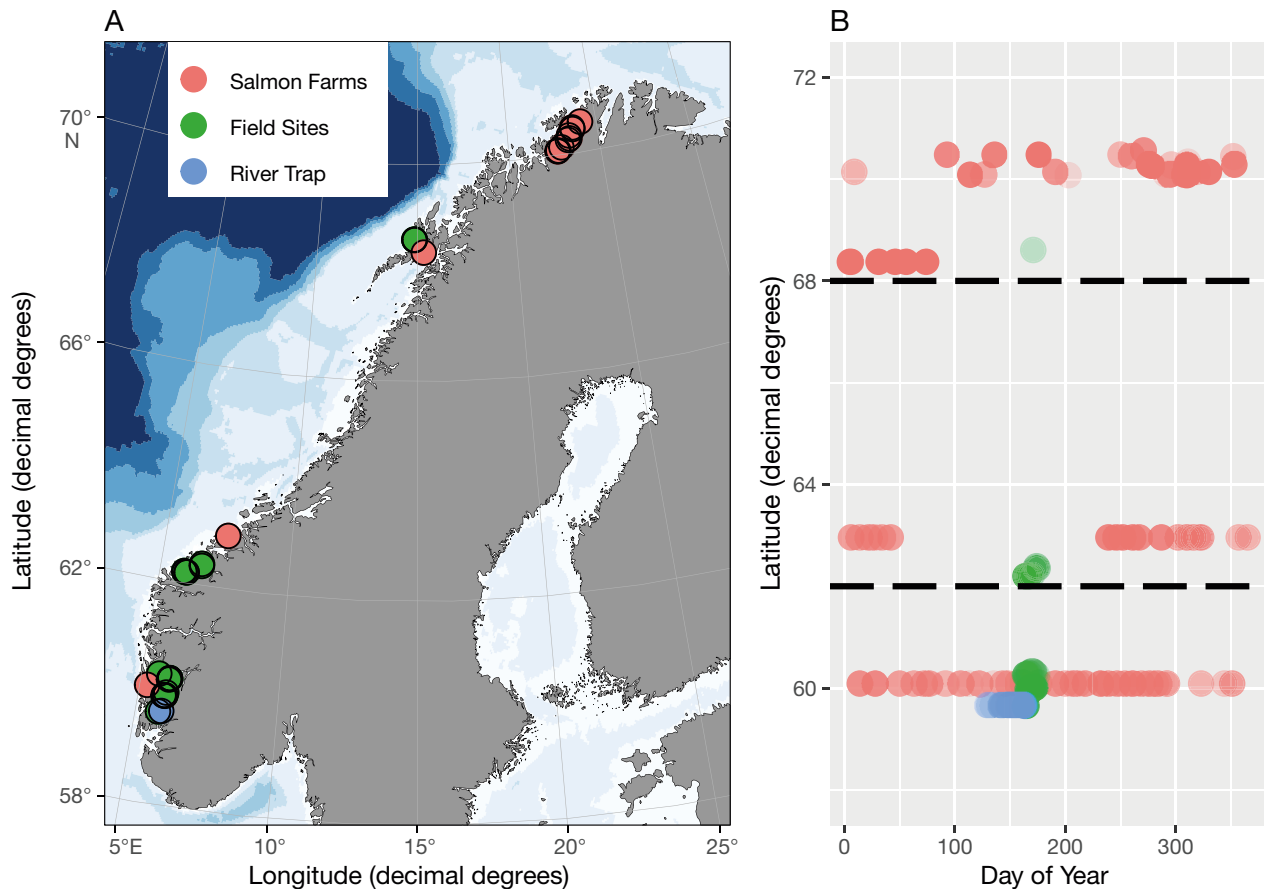


Fig. 2. (A) Distribution of sampling sites across the Norwegian coast and (B) sampling events throughout the year, with dashed lines demarcating the latitudinal zones. The different types of sample collections are indicated by the marker colors with darker shades indicating multiple samples collected at that time

fish came from farms, just over 57% of the female lice were sourced from those samples due to the relatively lower number of females hosted per fish (Table 2). The river-sourced and field-site samples were concentrated in the southern latitudes during the summer, while farm-sourced samples had a broader spatial and temporal distribution (Fig. 2). The field sites had much lower mean salinities than the farms, while also having higher mean temperatures (Table 2). The mean weight of sea trout and wild salmon was 0.32 and 3.54 kg, respectively, at field sites and 1.7 and 5.4 kg, respectively, at the river trap. The weights of farmed fish were not measured. The smallest salmon lice eggs measured were collected from wild salmon caught in the river trap, but it is unclear if freshwater lysis distorted the egg size, since those sourced from wild salmon collected at field sites were 9% larger than those collected at the river trap (Table 2). Overall, female lice sampled from wild salmon in the river trap had the largest clutch size with a mean of 837 eggs, followed by those from wild salmon captured at the field sites with 627 eggs. Females from farm sites had the next greatest clutch size with 570 eggs,

followed by those from sea trout captured at field sites (294 eggs) and those from sea trout caught in the river trap (316 eggs) (Table 2). These statistics of clutch size do not account for spatio-temporal differences in sampling, and the range of observations was considerable, with farm-sourced females having clutch sizes ranging from 48 to 1165 eggs.

3.2. Clutch size of salmon louse females from salmon farms

The selected model for clutch size (Eq. 1; Table 3) had an R^2 of 0.470, of which 0.269 was due to the fixed effects with 730 observations on 399 sampled fish within 79 sampling events. The 6 explanatory variables with the interaction between 2 of them yielded 63 alternative models to select from, and ΔAIC from the null to the chosen model was 173.9. The dispersion test statistic of the chosen model had a p-value of 0.912, thus the null hypothesis of no over- or underdispersion was accepted. Although the number of females per fish was not a significant parameter

Table 2. Summary of samples taken and observations made of female salmon lice fecundity, separated by host fish and sample source. Temperature, salinity, egg size, and clutch size are mean \pm SE

	Farms	Field Sites			River Trap	
Temp. ($^{\circ}$ C)	8.7 \pm 0.41	12.6 \pm 0.31			11.2 \pm 0.26	
Salinity (ppt)	31.7 \pm 0.19	26.2 \pm 0.57			28.6 \pm 0.34	
Sampling events	83	35			22	
	Farmed salmon	Wild salmon	Sea trout	Wild salmon	Sea trout	
Sampled fish	575	9	82	33	30	
Female lice	1520	149	631	176	174	
Egg-bearing	878	276	90	165	162	
Egg measurements	786	197	51	118	119	
Median females per host	5	26	14	7	8	
Egg size (μ m)	62.5 \pm 0.27	65.3 \pm 1.41	65.8 \pm 0.69	59.9 \pm 0.86	64.3 \pm 0.90	
Clutch size	570 \pm 8.2	627 \pm 54.4	294 \pm 10.9	837 \pm 34.7	316 \pm 16.4	

($p = 0.213$), it was included in the model because it produced a better overall fit as indicated by a lower AIC.

$$\begin{aligned} \text{Clutch Size}_{ij} = & \alpha + \beta_1 \text{Temperature}_{ife} \\ & + \beta_2 \text{Females Per Fish}_{ife} + \beta_3 \text{Total Length}_{ife} \quad (1) \\ & + \varepsilon_{ife} + \varepsilon_{fle} + \varepsilon_e \end{aligned}$$

where ε_{ife} is error on each observation, ε_{fle} is the variation due to different levels of sampled fish within a common sampling event, and ε_e is the variation due to different sampling events.

According to the GLMM, salmon louse clutch size increases with greater temperature, size of the female louse, and number of females per fish. The influence of the different explanatory variables on the modeled clutch size varies considerably across the observed range of values, with some variables responsible for greater changes than others. The number of females had the least impact, followed by temperature; size caused the greatest change in clutch size between the smallest and largest female lice (Fig. 3). When setting the number of females per host to a median of 5 and temperature to an average of 8.5 $^{\circ}$ C (Table 2), the model (Eq. 1; Table 3) predicts that clutch size will increase from 201 to 874 eggs

Table 3. Estimated regression parameters, SE, Z-values, and p-values for the GLMM presented in Eq. (1)

	Estimate	SE	Z	p
Intercept	-1084.7	126.9	-8.55	<0.001
Temperature ($^{\circ}$ C)	11.2	3.4	3.25	0.001
Females per host	13	10.4	1.24	0.213
Total length (mm)	168.4	11.8	14.21	<0.001

when the total length of the females increases from 7 to 11 mm. Thus, over the nominal range of observed female sizes (Fig. 3) there is a 4.4-fold predicted difference in clutch size.

3.3. Female size

3.3.1. Model-fitting to observations from salmon farms

Using the farm-sourced data set, a model of female louse size was selected from 31 possible models that were formed from 5 explanatory variables including an interaction between 2 of them. The selected model (Eq. 2; Table 4) had a Δ AIC of 69 from the null model and an R^2 of 0.511 of which 0.350 was due to the fixed effects with 1483 observations on 574 fish sampled within 80 sampling events. The dispersion test statistic of the chosen model had a p-value of 0.912, and the null hypothesis of no over- or under-dispersion was accepted.

$$\begin{aligned} \text{Total Length}_{ife} = & \alpha + \beta_1 \text{Temperature}_{ife} \times \beta_2 \text{Latitudinal Zone}_{ife} \quad (2) \\ & + \varepsilon_{ife} + \varepsilon_{fle} + \varepsilon_e \end{aligned}$$

The selected GLMM of female size at farm sites only includes the interaction between temperature and latitudinal zone (Eq. 2). Under the GLMM framework, the northern zone is the reference condition to which the others are compared, and it was the only zone to have significant effect on the model ($p < 0.001$). However, if a different reference zone had been chosen, then that one would have been significant while the others would not have been signifi-

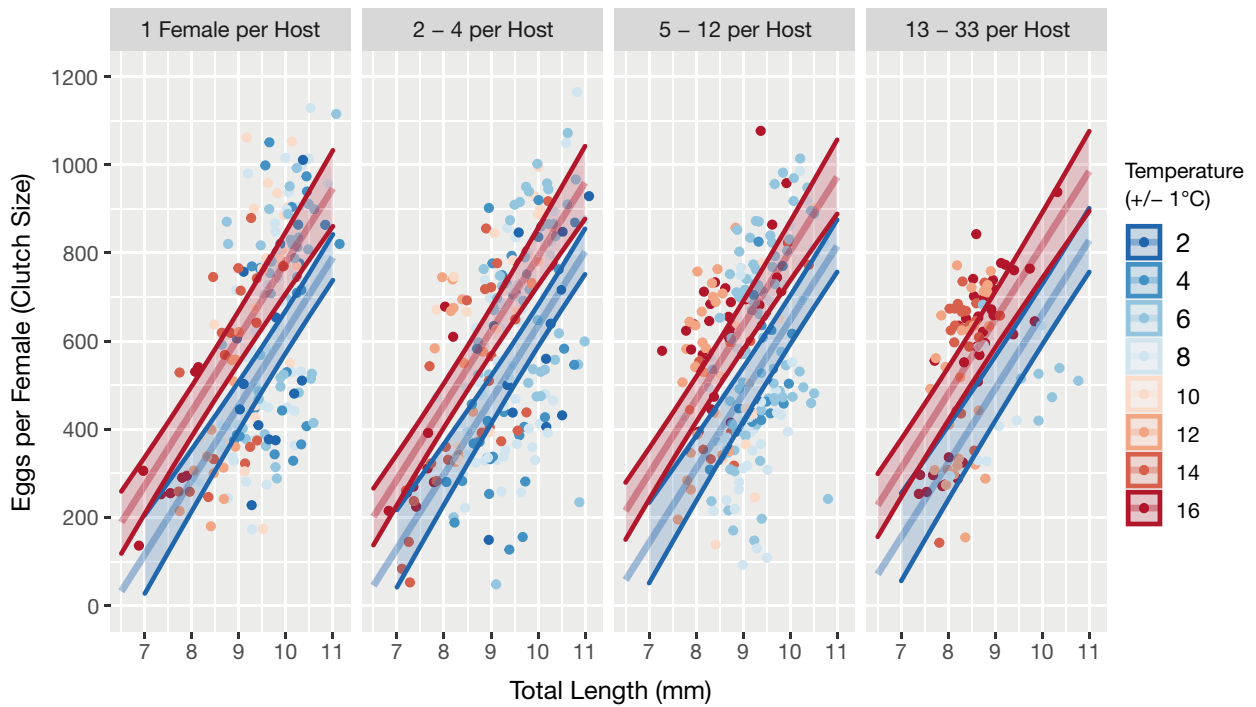


Fig. 3. Clutch size (eggs per female louse), as predicted by the model (Eq. 1; Table 3). The observed data is included as the colored data points, with the color indicating temperature $\pm 1^\circ\text{C}$. Model predicted clutch size is depicted by the lines for the lowest (2°C) and highest (16°C) temperatures, with 95% confidence intervals represented by the shaded region. Plots are subdivided by log number of females (0–3), with the number transformed back for subplot titles

cant. Thus, the model indicates that while zone does not significantly affect the response variable, the effect of temperature interacts with the zone. Temperature was only significant in the southern zone ($p = 0.032$). A 1° increase in temperature in the southern zone would decrease total length by 0.104 mm. The middle zone had a similar coefficient to the southern zone, but it was not significantly different than the northern zone ($p = 0.087$). In the northern zone the temperature was not significant, and the model coefficient had little effect on total length across the range of observed values (Table 4; Fig. 4). The distribution of observations shows that in the northern zone, temperature was skewed towards lower values, and there was an absence of observations

greater than 10°C , thus the 95% confidence intervals of the model prediction increased at higher temperatures. Similarly, the middle zone had fewer observations overall, especially towards the lower and higher temperature limits which resulted in wider 95% confidence intervals there (Fig. 4).

3.3.2. Model-fitting to all observations from the southern latitudinal zone

An additional analysis of female louse size was conducted using only the observations from the southern latitudinal zone, which included samples sourced from farms, field sites, and the river trap.

Table 4. Estimated regression parameters, SE, Z-values, and p-values for the GLMM presented in Eq. (2)

	Intercept				Temperature			
	Estimate	SE	Z	p	Estimate	SE	Z	p
Northern	9.45	0.244	38.6	<0.001	-0.012	0.037	-0.34	0.737
Middle	10.14	0.498	1.38	0.167	-0.111	0.057	-1.71	0.087
Southern	9.33	0.34	-0.37	0.715	-0.104	0.043	-2.15	0.032

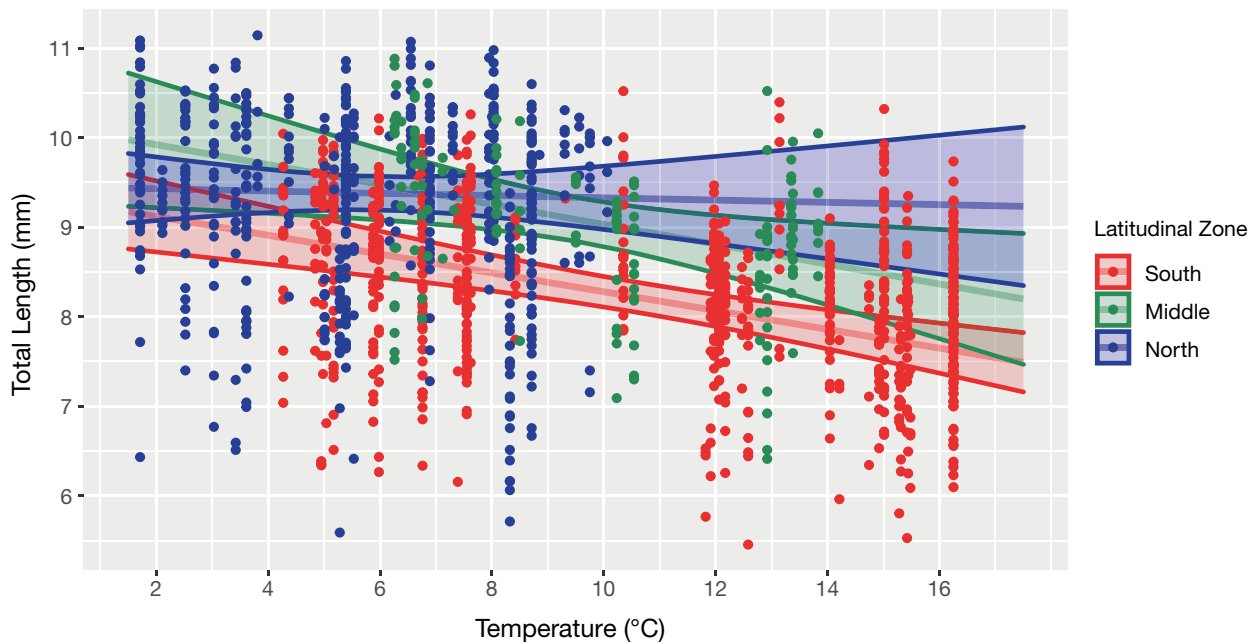


Fig. 4. Total length of female louse from farmed salmon, as predicted by the model (Eq. 2; Table 4). The observed data is included as colored data points, with the color indicating latitudinal zone. Model-predicted total length of females is depicted by the lines with 95% confidence intervals represented by the shaded region

The dataset included 1633 observations which came from 267 sampled fish nested in 83 sampling events. Stemming from the 3 explanatory variables, there were 7 alternative models to select from. The selected model (Eq. 3; Table 5) had a ΔAIC of 277 from the null model and an R^2 of 0.739 of which 0.581 was due to the fixed effects. The dispersion test statistic of the chosen model had a p-value of 0.84, and the null hypothesis of no over- or under-dispersion was accepted.

$$\text{Total Length}_{ife} = \alpha + \beta_1 \text{Temperature}_{ife} + \beta_2 \text{Host Fish Origin}_{ife} + \varepsilon_{ife} + \varepsilon_{fle} + \varepsilon_e \quad (3)$$

According to the model, both sea trout and wild salmon were significantly different from the reference category of farmed salmon with respective p-values of 0.035 and <0.001 (Table 5). As categorical variables, they shifted the intercept coefficient so that wild salmon hosted the largest salmon lice followed by farmed salmon and sea trout (Fig. 5). All categories were then affected by temperature with a 0.164 mm decrease in total female louse length for every degree increase. Since farm-sourced samples were collected year-round, there is greater distribution of observations across temperatures than among the field and river samples, which were only collected during a few weeks in summer. Consequently,

there is a lack of observations with temperatures below 9°C from wild salmon and sea trout.

3.4. Egg-bearing females and maturity status

3.4.1. Distribution of observations from salmon farms and field sites

There were 1483 female lice collected from farm sites, 58.9% of those were egg-bearing, and a gamma distribution was fit to CT/GS, which gave a shape of 106.7 with a rate of 72.1. Of the 625 female lice collected from sea trout, 44.2% were egg-bearing, and the gamma distribution fit to their CT/GS had a shape of 81.1 and a rate of 47.7. The 95th percentile of both gamma distributions was found, and all female lice with a CT/GS below it were considered to be sexually mature. Of the 978 females on farmed salmon expected to be egg-bearing (66% of the total), 104 were not (10.6%). Of the 457 females on sea trout expected to be egg-bearing (73% of the total), 181 were not (39.6%) (Fig. 6; Table 6). The 95th percentile CT/GS for female lice on farmed fish and sea trout was 1.72 and 2.02, respectively. Thus, the genital segments of the female lice on sea trout were relatively smaller at sexual maturity, and fewer mature female lice on sea trout were egg-bearing in comparison to those on farmed salmon.

Table 5. Estimated regression parameters, SE, Z-values and p-values for the GLMM presented in Eq. (3)

	Intercept				Temperature			
	Estimate	SE	Z	p	Estimate	SE	Z	p
Farmed salmon	9.95	0.278	35.8	<0.001	-0.164	0.024	-6.83	<0.001
Sea trout	9.63	0.152	-2.1	0.035				
Wild salmon	12.53	0.173	14.9	<0.001				

3.4.2. Binomial model-fitting to observations of egg-bearing in mature female lice

A GLMM with a binomial distribution was fit to the dataset of sexually mature females from both farmed fish and sea trout. Following the previous section, sexual maturity was determined by the 95 % quantile of CT/GS. Observed egg-bearing status was the response variable. An ANOVA was performed and the Wald test statistic indicated a significant difference ($W_1 = 24.9, p < 0.001$) between the null model and one with host fish origin as an explanatory variable. The latter model was kept and another ANOVA was performed between it and a model with the additional explanatory variable of temperature; this final model (Eq. 4) was also significant ($W_1 = 12.2, p < 0.001$). The binomial GLMM included 1435 observations from 564 fish within 115 sampling events. The R^2 was

0.342, with 0.171 of that due to the fixed effects. The dispersion statistic gave a p-value of 0.456, and the null hypothesis of no over- or underdispersion was accepted.

$$\text{Eggs Present}_{ij} = \alpha + \beta_1 \text{Temperature}_{ife} + \beta_2 \text{Host Fish Origin}_{ife} + \varepsilon_{ife} + \varepsilon_{fle} + \varepsilon_e \quad (4)$$

Farmed salmon was the reference category within the binomial GLMM of egg-bearing status. The coefficient of -1.10 for sea trout ($p < 0.001$) indicates that mature female lice on the sea trout hosts had a lower probability of bearing eggs than those on farmed salmon. The probability of egg-bearing also significantly ($p < 0.001$) increased with increased temperature, as indicated by the model (Table 7).

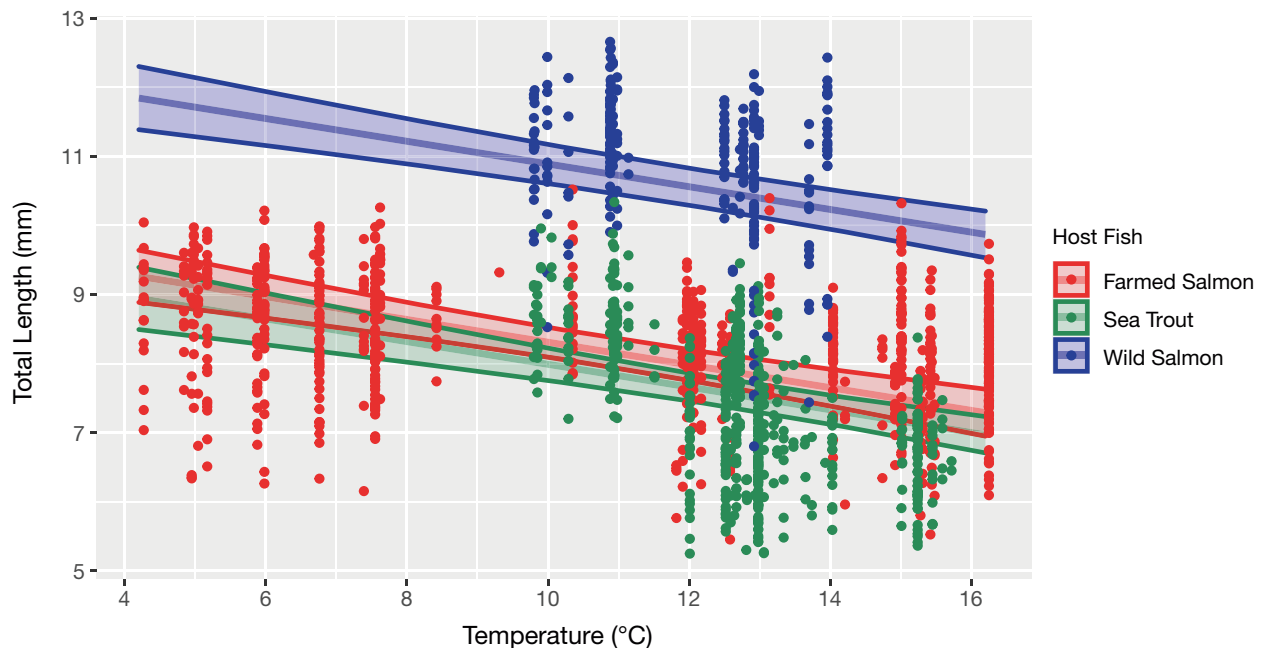


Fig. 5. Total length of female louse from southern latitudinal zone including samples from farm, field, and river sites. Prediction is from the model in Eq. 3; Table 5. The observed data is included as the colored data points, with the color indicating host fish. Model predicted total length of females is depicted by the lines with 95 % confidence intervals represented by the shaded region

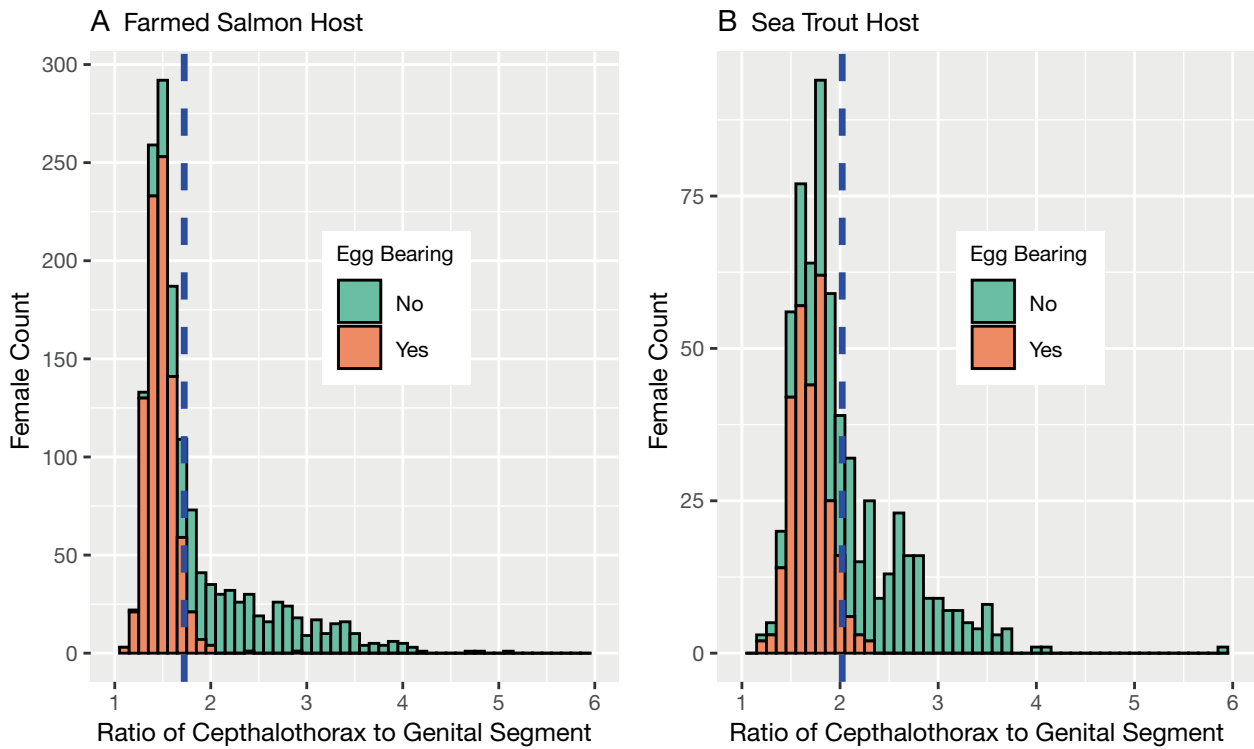


Fig. 6. Female louse egg-bearing status and ratio of cephalothorax to genital segment length (CT/GS), separated by host fish origin. (A) farmed salmon hosts and (B) sea trout hosts collected at field sites. Blue dashed lines indicate the 95th percentile of the CT/GS is in egg-bearing females

4. DISCUSSION

4.1. Clutch size

Clutch size is primarily driven by the size of the louse, with larger females producing more eggs, and according to the model, higher temperatures and a greater number of lice on the host fish leads to larger clutches. The size effect is unsurprising, as it conforms to previous observations of sea lice (Gravil 1996, Ritchie et al. 1996, Bravo et al. 2013) and follows the ecological understanding of size as a master trait (Kingsolver & Huey 2008, Horne et al. 2016), but the effect of increased temperature and number of females contradict the literature (Samsing et al. 2016, Ugelvik et al. 2017).

The model coefficient and range of observed values for number of females indicates a relatively small effect on clutch size compared to the other variables, and it was not significant in the model. It is unlikely that the parameter here was capturing the density-dependent effect of parasite load described by Ugelvik et al. (2017). The variable may instead be collinear with another undescribed variable which causes an increase in clutch size. This result exemplifies the

challenge of using AIC to select the best-performing model from multiple regression models fit to field data. The effects of some explanatory variables included in the selected model are not readily explained, yet they contribute to the superior fit. Leaving such a variable out of the model would make the interpretation simpler and increase the amount of variance explained by the other variables left in the model. It may also be tempting and practical to reduce the model to a single variable, e.g. use either

Table 6. Summary statistics of female salmon lice collected from both farmed salmon and sea trout hosts. Modeled ratio of cephalothorax (CT) to genital segment (GS) length, at which there is a 95% probability of observing a female bearing eggs, was used to calculate expected number of observations

Egg-bearing	Farmed salmon		Sea trout	
	No	Yes	No	Yes
Mean total length (mm)	8.15	9.10	6.75	7.58
Median CT/GS ratio	2.12	1.47	2.14	1.69
Count	609	874	349	276
95% quantile of CT/GS Ratio		1.72		2.02
Females predicted to have egg strings		978		457

Table 7. Estimated regression parameters, SE, Z-values and p-values for the binomial GLMM presented in Eq. (4)

	Intercept				Temperature			
	Estimate	SE	Z	p	Estimate	SE	Z	p
Farmed salmon	1.00	0.34	3.0	0.003	0.13	0.04	3.6	<0.001
Sea trout	-1.10	0.32	-6.6	<0.001				

temperature or total length, but it would not be the best model as judged by AIC and its ability to make predictions.

4.2. Female louse body size

Generally, temperature and female louse size at the farm sites follows a north–south gradient, with the coldest temperatures and largest females in the north, but the model fit poorly except in the southern zone. The relatively few data points in the middle zone and temporal gaps in sampling may have contributed to the poor fit there. In the north, the lack of a temperature–size relationship was likely caused by the temporal disconnection between sampling temperature and rearing temperature that becomes greater as temperatures decrease and development times increase. At higher temperatures, development to the adult stage is relatively short (Hamre et al. 2019), and the temperature recorded at time of sampling will be more similar to the rearing temperature, assuming females are sampled soon after maturing. Complicating matters further, it has been shown that the temperature–size rule is dependent on ontogeny in copepods, with the relationship between temperature and size strongest when the animals are in the middle stages of their development (Horne et al. 2019). Thus, the water temperature at time of sampling cannot be assumed to be equivalent to the rearing temperature, and experimentally derived temperature–size models (e.g. Samsing et al. 2016) cannot naively be used to estimate body size. Nevertheless, the results here show that the variables are related, and sampling temperature can be predictive of size under certain circumstances.

When the analysis is restricted to the southern zone, where most observations were made and where temperatures are higher, a clear relationship can be seen between female size and temperature. According to the model fit (Eq. 3; Table 5), female lice parasitizing farmed Atlantic salmon would be predicted to have total lengths of 9.13 mm at 5°C and 6.75 mm at 19.5°C. At the same temperatures, female lice reared under

laboratory conditions had total respective lengths of 11.75 mm and 8.2 mm (Samsing et al. 2016). The total lengths here are much smaller than would be expected from the laboratory experiments and less responsive to temperature changes. These results further demonstrate that that laboratory-based formulations (e.g. Samsing et al. 2016) may not accurately predict *in situ* total length, and since the model fit is unlikely to be reflecting the temperature–size rule, it should not be applied beyond the conditions described for this dataset.

Salmon lice from wild salmon were much larger than those from farms, which follows previously reported observation (Ritchie et al. 1993, 1996, Pike & Wadsworth 1999). The reliable size difference was investigated by Nordhagen et al. (2000) as a potential means of identifying the origin of the louse, and no difference in size was found between progeny of salmon lice from different origins reared at the same temperature. However, the lice, regardless of origin, followed the temperature–size rule with those reared in the colder treatment achieving a greater size (Nordhagen et al. 2000). In this study, there were fewer observations of lice from wild salmon, and the range of water temperatures they were found in was narrow compared to those sourced from farmed salmon and sea trout. Perhaps no temperature–size relationship should have been fit to the wild salmon dataset because, like the lice from the northern latitudinal zone, the temperature at sampling can differ greatly from the rearing temperature. Homing behavior of salmonids remains understudied due to the difficulty of sampling the migrating adults. Davidsen et al. (2013) assume a direct migration of Atlantic salmon to the natal river following entry into the fjord, while Mahlum et al. (2018) show them residing in a fjord system for a mean of 30 d prior to entering the river. A relatively direct migration implies that the lice developed in the open ocean where temperatures are relatively low, which could account for the large size.

Following the salinity effects seen on other aspects of salmon louse life history (Johnson & Albright 1991, Gravid 1996, Bricknell et al. 2006), the lower size in

the sea trout-sourced lice is possibly due to added stress from the lower-salinity waters from which they were sampled. No investigations have previously been done on the effect of salinity stress on body size or egg production in salmon lice. A salinity relationship has been observed for the sea louse *Caligus rogercresseyi* in a study by Bravo et al. (2009), but their analysis did not account for a size or temperature effect. In another investigation of *C. rogercresseyi*, it was found that the body size and fecundity of lice was related to the host fish species (González et al. 2012). Thus, both salinity and host fish may separately influence salmon louse body size, but in this study, the 2 variables were highly collinear, and their relative importance could therefore not be discerned by including them together in the model.

4.3. Female egg-bearing status and maturity

A third of adult female lice on farmed salmon in this study were not sexually mature and not expected to produce egg strings. This finding contradicts the assumption made by Stien et al.'s (2005) egg-production calculation, which is utilized in salmon louse infestation pressure models (Ådlandsvik et al. 2017, Sandvik et al. 2019), that all adult females produce eggs. Nevertheless, it is unclear how such a distinction will affect the interpretation of model projections, since the focus is on the relative difference in infestation pressure rather than the absolute number of planktonic lice in the water column (Vollset et al. 2019). The further finding from farm-sourced lice that a tenth of mature females are not egg-bearing reasonably follows the previous observation that female lice will extrude and replace a hatched set of egg strings with a new set within 24 h (Johannessen 1977 as cited in Stien et al. 2005). The increased probability of egg-bearing with increased temperature also conforms with the general understanding of increased rates of physiological mechanisms with temperature (Atkinson 1994).

Farmed salmon are responsible for producing 99% of the adult female lice in Norwegian waters and thus produce the preponderance of the infectious planktonic stages (Dempster et al. 2021). Here it has been shown that female lice on sea trout produce fewer eggs than those on farmed and wild salmon. Therefore, the relative contribution of infectious planktonic stages from sea trout is still lower. Determining the responsible mechanism for that difference calls for further investigation since this study

was unable to discern whether it is a host effect, an environmental response, or due to some other cause. Low-salinity waters traversed by the host fish could have stressed the females and reduced their egg production. Salmon louse salinity sensitivity has been identified as the ultimate reason for the premature return of sea trout to freshwater rivers, where they can rid themselves of much of their lice load (Birkeland 1996, Birkeland & Jakobsen 1997). However, the adult stages of lice have greater tolerance to freshwater and could have survived while the eggs did not (Wright et al. 2016), and it is unclear how long the egg strings would remain attached. Alternatively, the effect could be due to a pathogen which is more likely to impact female lice on sea trout than those on farmed fish. Under laboratory conditions, female lice have been observed with unknown infections that visibly affect their genital segments and obstruct their ability to produce egg strings (L. Hamre pers. comm.). Regardless, lower salinity could reduce the rate of egg extrusion and substantially reduce the overall fecundity of a salmon louse, and that possibility calls for greater study.

4.4. Conclusion

This investigation focused on one aspect of salmon louse fecundity—the number of eggs produced by each female per clutch—and found that louse body size is the major driver of clutch size. The estimation of clutch size from temperature and size would be superior to a temperature-only clutch size formulation, but determining size of the salmon lice presents its own challenge. While salmon lice follow the temperature–size rule and reach larger sizes at colder temperatures, this study demonstrated that the *in situ* relationship between temperature and total length is complicated by the disconnection between sampling and rearing temperature. Therefore, experimentally derived temperature functions, such as those by Samsing et al. (2016), cannot be readily used to forecast female body size, clutch size, and the resulting egg production at salmon farms.

Accurately estimating salmon louse clutch size would require further modeling of life histories that incorporates rearing temperature or the direct observation of female total lengths. The modeling would add uncertainty, while manual observation would be too cumbersome for routine application. However, an innovation in salmon lice imaging suggests a pathway towards automation. Hyperspectral imaging is capable of monitoring and staging salmon lice on

their host fish without sampling and handling (Petersen et al. 2019). Thus, technology exists which could image and measure the size of adult female lice on fish and those measurements could be used in calculations of clutch sizes.

Regardless of whether clutch sizes are better parameterized in models, temperature remains the predominant determinant of the rate of egg string production and hatching. While mean clutch size may differ by a factor of 1.5 to 4.4 (Ritchie et al. 1993, Gravil 1996, Samsing et al. 2016, present study) over the range of temperatures and female sizes observed, the rate of egg production can vary 25-fold (Boxaspen & Naess 2000, Samsing et al. 2016). Thus, fecundity of a female salmon louse is affected more by a difference in temperature that changes those rates than by the total number of eggs produced at one time (see Kingsolver & Huey 2008). In that context and in consideration of the great variance observed in egg production here, the infestation pressure models (Sandvik et al. 2016) can assume a constant number of eggs per female and still fairly accurately reflect temporal and spatial differences in salmon louse fecundity. Nevertheless, improved forecasts can be made if the models accounted for variable clutch sizes and the proportion of female lice likely to be sexually mature.

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