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ARTICLE

# Salmon lice (*Lepeophtheirus salmonis*) development times, body size, and reproductive outputs follow universal models of temperature dependence

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**Abstract:** Temperatures regulate metabolism of marine ectotherms and thereby influence development, reproduction, and, as a consequence, dispersal. Despite the importance of water temperatures in the epidemiology of marine diseases, for the parasitic copepod *Lepeophtheirus salmonis*, the effect of high and low temperatures has not been methodically investigated. Here, we examined the effects of a wide temperature range (3–20 °C) on *L. salmonis* larval development, adult body size, reproductive outputs, and infestation success. Further, we tested if dispersal of salmon lice differed with two temperature-dependent development times to the infective stage (30 and 60 degree-days) using an individual-based dispersal model. Development times followed universal models of temperature dependence described for other marine ectotherms. Water temperatures had a negative relationship with development times, adult body size, and reproductive outputs, except at 3 °C, where larvae failed to reach the infective stage and all parameters were decreased, indicating low temperatures are more detrimental than high temperatures. The predictable effect of temperatures on lice development and reproduction will have important applications, such as predicting dispersal and population connectivity, to assist in controlling lice epidemics.

**Résumé :** La température régule le métabolisme des ectothermes marins, influençant ainsi leur développement, leur reproduction et, par conséquent, leur dispersion. Malgré l'importance de la température de l'eau dans l'épidémiologie des maladies marines, pour le copépode parasitique *Lepeophtheirus salmonis*, l'effet de températures élevées ou basses n'a pas fait l'objet d'un examen méthodique. Nous examinons les effets d'une grande fourchette de températures (3–20 °C) sur le développement des larves, la taille du corps des adultes, l'efficacité de la reproduction et le succès des infestations de *L. salmonis*. Nous vérifions également si la dispersion du pou du poisson est différente pour deux temps de développement dépendant de la température avant le stade infectieux (30 et 60 degrés-jours) en utilisant un modèle de dispersion basé sur l'individu. Les temps de développement suivent des modèles universels de dépendance de la température décrits pour d'autres ectothermes marins. La température de l'eau est négativement reliée au temps de développement, à la taille du corps des adultes et à l'efficacité de la reproduction, sauf à 3 °C, température à laquelle les larves n'atteignent pas le stade infectieux et tous les paramètres diminuent, indiquant que les basses températures sont plus néfastes que les températures élevées. L'effet prévisible de la température sur le développement et la reproduction des poux aura d'importantes applications, notamment dans la prédiction de la dispersion et la connectivité des populations, pour aider à maîtriser les épidémies de poux. [Traduit par la Rédaction]

## Introduction

Environmental conditions are critical in the dispersal and epidemiology of marine pathogens (McCallum et al. 2004; Murray 2009; Salama and Rabe 2013). Water temperatures control the metabolic rate of fundamental biochemical processes (Gillooly et al. 2001) and thereby regulate growth and development rates of marine organisms. For marine animals that develop in the water column, larval stage duration determines the length of time that larvae are subject to transport by currents and exposed to sources of mortality (O'Connor et al. 2007; Treml et al. 2008). Therefore, predicting disease transmission of marine pathogens requires a considerable understanding of the biology of the agent and its interaction with the surrounding physical environment (Murray 2009; Asplin et al. 2014; Johnsen et al. 2014).

Salmon lice are external parasites that cause substantial economic losses to the salmon industry (Costello 2009*a*), and farm-assisted parasitic outbreaks can reduce return rates of spawning salmon in sensitive rivers (Vollset et al. 2014, 2015), posing a potential threat to wild salmonid populations (Costello 2009*b*; Krkošek et al. 2011, 2013). The salmon louse has a direct life cycle that involves three planktonic larval stages that hatch from a pair of egg strings produced by an adult female: two noninfective naupliar stages and an infective copepodid stage (Hamre et al. 2013). Water temperature is a key regulator of the development times of all lice stages (Johnson and Albright 1991), including both planktonic and parasitic stages. However, temperature dependence is particularly critical for nonfeeding (lecithotrophic) planktonic larvae, which rely on energy reserves to survive and find a host (Tucker et al. 2000). Water temperatures have a negative relationship with development times in most ectothermal organisms (Angilletta et al. 2004). Accordingly, salmon lice in warmer waters develop faster to the infective copepodid stage, but are viable for a shorter period of time, as they consume their energy reserves faster (Pike and Wadsworth 1999). Conversely, in colder environments, lice larvae have a longer development

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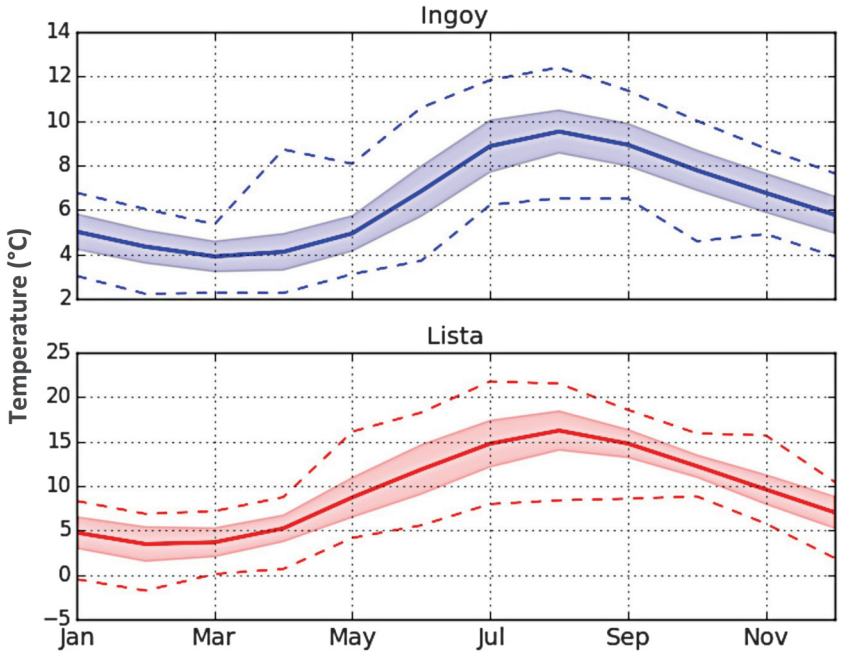
**Fig. 1.** Mean water temperatures in Norway. Figure shows mean ± SD (shaded area) and minimum and maximum (dashed lines) water temperatures recorded at two meteorological stations located in the southern (Lista: 58°N, 1°E) and northern (Ingøy: 71°N, 8°E) coast of Norway. Measurements were taken every 14 days since 1936 (Ingøy) and 1942 (Lista). [Colour online.]




period and can potentially be transported longer distances by ocean currents, depending on local hydrographical conditions, but are exposed to a higher risk of mortality. Therefore, the duration of lice pre-infective and infective larval stages are key factors in the potential dispersal (Asplin et al. 2011) and survival of this parasite (Johnsen et al. 2014).

Despite the importance of water temperatures on salmon lice development, studies that cover the entire temperature range of salmon lice host fishes are still missing. Host fishes include Atlantic salmon (*Salmo salar*), sea trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*) and have a large geographical range that span from Greenland to Portugal and to Connecticut in the Atlantic Ocean (Froese and Pauly 2009). Therefore, salmon lice experience a wide water temperature range from approximately 0 to 20 °C. In a study on wild sea trout, fish infested with salmon lice were caught when the water temperatures in winter were 2 to 3 °C (Heuch et al. 2002). In Norway, surface water temperatures in fjord–coastal areas fluctuate seasonally, averaging between 2 and 5 °C during winter and between 12 and 20 °C in the warmest months of the year at some southern locations (Fig. 1). The absolute effect of water temperatures on salmon lice biology and epidemiology remains unclear; individual louse will grow faster at higher temperatures (Johnson and Albright 1991; Nordhagen et al. 2000), but population-level effects of ocean temperature are uncertain (Ritchie et al. 1993; Saksida et al. 2007).

Water temperatures also influence the body size and reproductive output of marine ectotherms (Atkinson 1994; Angilletta et al. 2004). A temperature–size rule proposes that most ectotherms grow faster and mature at a smaller adult size at higher temperatures, with the opposite effect at lower temperatures. However, empirical studies of the effects of low or high temperatures on *L. salmonis* viability and infestivity show contradictory results. In a study using egg strings collected from an autumn lice population from the west coast of Scotland, salmon lice naupliar stages failed to moult to the infective copepodid stage (Gravil 1996). However, a cold-acclimatized (6.8 °C) salmon lice population from Norway hatched and developed to the infective copepodid stage at temperatures as low as 2 °C (Boxaspen and Næss 2000). Therefore, temperature-induced effects on body size and reproductive outputs require further investigation across the entire temperature range encountered by *L. salmonis*.

Salmon lice copepodids complete their transmission by locating and infesting a suitable host. Successful infestation and settlement of *L. salmonis* encompasses three phases: initial attachment, exploration, and fixation to the host (Bron et al. 1991). The final phase of fixation is completed by the production of a frontal filament that anchors the larvae to the host (Pike et al. 1993). All these phases require energy to be completed, and energy depletion prior to infestation could result in the loss of infectivity (Tucker et al. 2000). Water temperature and the infestation success of *L. salmonis* are positively correlated (Costello 2006). However, none of these studies have been conducted at low (≤5 °C) or high (≥15 °C) temperatures; the effect of water temperatures at the top and bottom of the range that lice naturally experience on infestation success remains to be determined (Gravil 1996; Stien et al. 2005; Groner et al. 2014).

Even though the effect of high and low water temperatures on the early stages of *L. salmonis* is uncertain, temperature dependence of marine ectotherms is conserved across taxa and geographic areas (O'Connor et al. 2007). Universal models describe the effect of temperature on size and metabolic rates of a wide variety of organisms (Gillooly et al. 2001). We predicted that salmon lice larvae would have longer development times at colder temperatures and that this relationship would fit universal theoretical models of temperature dependence. Furthermore, we predicted that water temperatures would have a negative relationship with adult body size and reproductive outputs; colder water temperatures would produce larger females, with longer egg strings and more eggs per string. However, low temperatures (<5 °C) could have a detrimental effect on the viability and infestivity of salmon lice copepodids, reducing infestation success. Finally, we predicted that differences in temperature-dependent development times would generate distinctive dispersal patterns of *L. salmonis* copepodids.

Therefore, in our study we tested the effect of a wide range of environmentally relevant water temperatures (3–20 °C) on (*i*) hatching and development times of salmon lice (*L. salmonis*) larvae; (*ii*) adult size of male and female salmon lice; (*iii*) reproductive outputs (egg

string length, number of eggs per string, and egg size); and (*iv*) infestation success of lice copepodids. In addition, using an existing coupled biological–physical model, we simulated the dispersal of lice planktonic stages using two different temperature-dependent development times from hatching to the infective copepodid stage (i.e., total duration of the naupliar stages) to assess differences in dispersal patterns.

## Materials and methods

### Location and experimental setup

We conducted the experiment at the Matre research station of the Institute of Marine Research, Norway. We used a set of six holding tanks (3.0 m diameter × 0.75 m deep; volume ≈ 5 $m^3$) to produce lice and eggs strings on Atlantic salmon (*S. salar*) at different temperatures and 12 smaller tanks (3 treatments × 4 replicates; 0.9 m × 0.9 m × 0.4 m deep; volume ≈ 0.32 $m^3$) for the infestation success trials. A silicon tube connected to the inlet of each holding tank provided the water at the different experimental temperatures to the incubators used in the experiment. Salinity was 34‰ in all tanks, which were continuously illuminated (24 h light : 0 h dark).

### Experimental fish

We used Atlantic salmon postsmolts (Aquagen strain) as host fish and for infestation success trials. Host fish used to produce lice at different temperatures ranged from 200 to 300 g. Fish used for the infestation success trials had a mean (±SE) fork length of 25.4 ± 0.2 cm and mass of 195.3 ± 4.6 g. All fish were continuously fed to satiation (2–3 mm pellets, Skretting, Norway), except 24 h before anaesthesia.

### Salmon lice origin and production of eggs at the experimental temperatures

Salmon lice egg strings used to initiate the culture of lice for our experiments were collected in late March 2015 from the operating salmon farm at the Austevoll Aquaculture Research Station (60°05′N, 05°16′E) on the southwest coast of Norway. The copepodids that hatched from collected egg strings were then used to infect fish and produce lice at the different experimental temperatures (20, 15, 10, 7, 5, and 3 °C). Temperatures in the holding tanks were recorded daily and were stable at 19.6, 15.0, 10.0, 7.0, 5.0, and 2.9 ± 0.1 °C (mean ± SD). We infected six groups of 50 fish each by incorporating ~1200 lice larvae to each holding tank after stopping the incoming flow for 1 h and reducing the level of the tank to one-third of its total volume. All fish were infected at 10.0 °C, and 15 days postinfestation (DPI), when lice were at the late chalimus II stage (Stien et al. 2005), water in the tanks was adjusted (i.e., increased or decreased) for salmon lice to mature and produce eggs at the different experimental temperatures.

Adult females with egg strings were collected from anesthetized fish (metomidate 10 $mg \cdot L^{-1}$) when all females had extruded their second batch of egg strings. All egg strings pairs produced after the second batch are less variable in length and egg size than the first one (Heuch et al. 2000). After egg string collection, female lice were placed back on the fish to produce more egg strings for the infestation success trials. Egg strings were gently removed from the females using fine forceps after examination under a dissecting microscope. During sampling we also collected five male lice from each temperature to include in morphometric analyses.

### Incubation of egg strings at the experimental temperatures

Egg string pairs and planktonic larval stages (nauplii I and II and copepodids) were incubated in small flow-through incubators, as described by Hamre et al. (2009), suitable for single pairs of egg strings in each well (mesh 150 μm). For each temperature, two incubators with 16 wells were connected to the same water supply from the holding tank (2 × 16 = 32 egg string pairs in each temperature). Temperature in the incubators was measured daily from four random wells from each incubator with an external electric thermometer (Testo 176T2 Temperature Data Logger, Thermon South Africa Ltd.) until stable and then monitored every second day. Mean (±SD) temperatures in the incubators were 19.5 ± 0.1, 14.7 ± 0.1, 10.0 ± 0.1, 6.9 ± 0.1, 5.1 ± 0.2, and 3.1 ± 0.2 °C. Hereinafter, we refer to these experimental temperatures as 20, 15, 10, 7, 5, and 3 °C.

**Table 1.** Akaike's information criteria (AIC) to select for the best theoretical model fitted to the effect of water temperatures on development times of salmon lice (*Lepeophtheirus salmonis*) planktonic stages.

| Models | $LL_i$ | AIC | $\Delta_i$ (AIC) | $>\omega_i$ |
|---|---|---|---|---|
| **Linearized power law model:** $\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c)$ | | | | |
| Development time | | | | |
| Duration of naupliar stages | −8.4 | **22.9** | 0 | 0.57 |
| Infective window | −33.1 | 72.1 | 59.2 | $1.3\times10^{-13}$ |
| PLD | 64.5 | −123.0 | 56.3 | $5.9\times10^{-13}$ |
| Development in degree-days | | | | |
| Duration of naupliar stages | −8.3 | **22.6** | 0 | 0.53 |
| Infective window | −32.4 | 70.8 | 58.0 | $2.6\times10^{-13}$ |
| PLD | 65.2 | −124.4 | 53 | $3.09\times10^{-12}$ |
| **Exponential–quadratic model:** $\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c) + \beta_2 \times [\ln(T/T_c)]^2$ | | | | |
| Development time | | | | |
| Duration of naupliar stages | −7.7 | 23.4 | 0.5 | 0.4 |
| Infective window | −2.4 | **12.8** | 0 | 1.0 |
| PLD | 93.7 | **−179.3** | 0 | 1.0 |
| Development in degree-days | | | | |
| Duration of naupliar stages | −7.4 | 22.8 | 0.2 | 0.5 |
| Infective window | −2.4 | **12.8** | 0 | 1.0 |
| PLD | 92.7 | **−177.4** | 0 | 1.0 |
| **UTD (universal temperature dependence) equation:** $\ln(Y) = \beta_0 + \beta_1/[k \times (T + 273)]$ | | | | |
| Development time | | | | |
| Duration of naupliar stages | −8.4 | 61.5 | 38.6 | $2.3\times10^{-9}$ |
| Infective window | −26.9 | 59.7 | 46.9 | $6.6\times10^{-11}$ |
| PLD | 89.7 | −173.4 | 5.9 | 0.05 |
| Development in degree-days | | | | |
| Duration of naupliar stages | −12.5 | 30.9 | 8.4 | $7.9\times10^{-3}$ |
| Infective window | −46.8 | 99.6 | 86.8 | $1.4\times10^{-19}$ |
| PLD | 52.3 | −98.0 | 78.8 | $7.8\times10^{-18}$ |

**Note:** In each model, $Y$ is the response variable, presented on the left column on the table, $T$ is temperature (°C), and $T_c$ = 10 °C. $LL_i$, log-likelihood for the model; $\Delta_i$ (AIC), change in AIC; $\omega_i$, Akaike's weights; and PLD, pelagic larval development. Numbers in bold indicate the lowest AIC values used for model selection.

### Development times of salmon lice planktonic larval stages

Egg strings pairs were monitored daily to record (*i*) hatching date, (*ii*) hatching success, (*iii*) copepodid date or day when >80% of nauplii had moulted to the copepodid stage, and (*iv*) day when >80% of copepodids died. Hatching success was the fraction of eggs that successfully hatched and was estimated visually as 0%, 25%, 50%, 75%, and 100% for each individual well (Espedal et al. 2013). Copepodids were considered dead when >80% of them become immobile and were lying on the bottom of the plankton mesh. Salmon lice are nonfeeding larvae that rely on yolk reserves to survive and starve to death if they are unable to swim to find a host (Tucker et al. 2000). We collected between 16 and 32 egg string pairs for each temperature treatment (Table 1). With these data, we calculated the following: (*i*) mean duration of lice naupliar stages; (*ii*) mean duration of the infective window; and (*iii*) mean planktonic larval duration (PLD). The duration of naupliar stages was the total time from hatching until lice moulted to the copepodid stage. Infective window was the time between when lice moulted to the copepodid stage and the day when >80% of the copepodids died. PLD was the mean total life span of salmon lice planktonic stages (PLD = duration of lice naupliar stages ± infective window).

Development times are crucial biological parameters in larval dispersal (Treml et al. 2008), since larval durations influence transport distances, distribution patterns, and connectivity of planktonic

organisms. Here, we calculated development times in degree-days for all salmon lice larval stages (duration of naupliar stages, infective window, and PLD). A degree-day is the product of time and temperature (e.g., 30 days at 10 °C = 300 degree-days), and it is a useful index of "physiological age" or the temperature required for growth and development of ectotherms within their range of tolerance (Costello 2006). Moreover, this index is used as a temperature-dependent development time in salmon lice dispersal models (Asplin et al. 2014; Johnsen et al. 2014). Here, we calculated degree-days by multiplying the mean temperature of the incubators by development time in days.

### Size and reproductive outputs at different temperatures

Adult males, females, and egg strings were examined and photographed using a stereomicroscope (Leica MZ8, Leica Microsystems, UK). Digital images were taken using a camera (Toupcam U3CMOS 14 MP 1/2.3") mounted on a stereomicroscope coupled to a computer. Males and gravid females were photographed at a magnification of 6.3× and egg strings at 50×. All morphometric measurements were done in ImageJ 1.48v (W. Rasband, National Institutes of Health, USA). We measured adult female total length, cephalothorax length, and reproductive outputs (egg string length, egg size, and number of eggs per string). Egg string length was measured directly from the microscope images and used to estimate egg size (i.e., mean length of a single egg) by measuring the length of two randomly chosen 10-egg sections in the egg string and dividing the mean length of these 10-egg sections by 10 (Heuch et al. 2000). Total number of eggs per string was calculated by dividing the total egg string length by the mean length of a single egg. All measurements were done on one string per pair. We also examined the effect of temperature on adult male size by measuring cephalothorax length.

### Infestation success at different temperatures

We tested salmon lice infestation success at 20, 10, and 5 °C for larvae produced at these temperatures. Infestation success is the proportion of parasites that successfully infect a host (i.e., infestation intensity) out of the total number of parasites available per host during infestation (i.e., infective dose) and reflects parasite performance (Poulin and FitzGerald 1989; Samsing et al. 2014). At the time of infestation, all lice copepodids had approximately the same physiological age (mean ± SE: 30.84 ± 2.04 degree-days; one-way ANOVA $F_{[3,42]} = 2.02$, $P = 0.14$). The total number of copepodids in the suspension was calculated indirectly by counting them in 3 × 10 mL aliquots. Each temperature treatment had four replicate tanks with 15 fish in each, and 450 infective copepodids were used to infect each tank (i.e., infective dose: 30 lice·fish$^{-1}$). During infestation, water level in the tanks was reduced to one-third of its total volume, and the inflow of water was reduced (4.5 L·min$^{-1}$) for 1 h. After the first hour, normal inflow (18 L·min$^{-1}$) was restored until sampling. Fish were sampled the day lice were estimated to be in the chalimus stages to account for successful settlers (Bron et al. 1991). Sampling times were estimated with the formula in Stien et al. (2005), and accordingly, fish were sampled 5, 13, and 28 DPI at 20, 10, and 5 °C, respectively. During sampling, 10 random individuals were collected from each replicate tank with a hand net, culled with a rapid blow to the head, and placed in individual plastic bags to be weighed, measured, and analyzed for the presence of lice. We recorded total number of lice per fish and their location on the body surface (excluding gills surfaces) and the fins.

### Simulated salmon lice dispersal patterns

To test for differences in distribution patterns due to variations in development times to the infective stage, we used simulations from an individual-based coupled biological–physical dispersal model (described in Johnsen et al. (2014). In our simulation, 10 particles, representing salmon lice during its planktonic stages, were released every hour from two sites (coastal and fjord site) around the Hardangerfjord area (60°N, 5.5°E) on the west coast of Norway for a winter month in January 2015. All environmental conditions (water currents, temperature, salinity) or forcing parameters in the model were provided by a fjord hydrodynamic model (Albretsen et al. 2011). After hatching, the modeled particles were set to become infective at either 30 or 60 degree-days, which were the development times to the infective stage at 20 and 5 °C, respectively. Further, they were set to have the same total life span of 150 degree-days. We chose the same life span value for both groups to compare differences in dispersal patterns caused by the development time to the infective stage. The value itself has been used in previous modeling studies (Asplin et al. 2014, Johnsen et al. 2014) and was the mean life span of lice in our experiment for all temperatures. Past this time, all lice particles were assume to have died of starvation or senescence. Mortality was parameterized at a constant rate of 17% per day. Model outputs were aggregated number of infective copepodids per grid cell (160 m × 160 m grid cells).

**Table 2.** Model parameter estimates $\beta_0$, $\beta_1$, and $\beta_2$ (±SE) of the theoretical model that best fitted the effect of water temperature on development times of salmon lice (*Lepeophteirus salmonis*) larvae and body size and reproductive outputs of adult lice.

| | $\beta_0$ | $\beta_1$ | $\beta_2$ |
|---|---|---|---|
| **Development time** | | | |
| Duration of naupliar stages | 1.4 (±0.02) | −1.48 (±0.04) | NA[a] |
| Infective window | 2.6 (±0.04) | −0.26 (±0.04) | −1.03 (±0.12) |
| PLD | 2.8 (±0.02) | −0.67 (±0.02) | −0.47 (±0.06) |
| **Size and reproductive outputs** | | | |
| Female total length | 2.4 (±0.01) | 0.22 (±0.01) | −0.24 (±0.01) |
| Female cephalothorax length | 1.5 (±0.01) | −0.15 (±0.01) | −0.14 (±0.01) |
| Male cephalothorax length | 1.2 (±0.02) | −0.14 (±0.02) | −1.13 (±0.03) |
| Egg string length | 2.8 (±0.02) | −0.39 (±0.03) | −0.69 (±0.04) |
| Egg size | 4.1 (±0.01) | 0.04 (±0.01) | 0.09 (±0.02) |
| Number of eggs per string | 5.6 (±0.03) | −0.43 (±0.04) | −0.78 (±0.05) |
| **Development in degree-days** | | | |
| Duration of naupliar stages | 2.0 (±0.14) | −1.1 (±0.09) | NA[a] |
| Infective window | 4.8 (±0.04) | 0.72 (±0.04) | −1.02 (±0.12) |
| PLD | 5.1 (±0.02) | 0.30 (±0.02) | −0.46 (±0.06) |

**Note:** Models were chosen based on Akaike's information criteria (AIC), presented in Table 1 and Table S2[1]. All data fitted an exponential–quadratic model ($\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c) + \beta_2 \times [\ln(T/T_c)]^2$), except for the duration of naupliar stages that fitted a linearized power law model ($\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c)$). $Y$ is the response variable, presented on the left column of the table, $T$ is temperature (°C), and $T_c = 10$ °C is a centering parameter. PLD, pelagic larval development.

[a]Linearized power law model (Materials and methods, eq. 1) that best fitted duration of naupliar stages only had an intercept ($\beta_0$) and a linear scaling parameter ($\beta_1$).

### Data analyses

All statistical calculations were carried out in R statistical package version 3.2.1 (R Development Core Team 2009). We fitted theoretical models of the effect of water temperatures on development times of marine ectotherms to the salmon lice (*L. salmonis*) data. All data were log-transformed (natural logarithm) to linearize the models and satisfy statistical assumptions. The theoretical models we fitted to our data were described by O'Connor et al. (2007); these models fit the development time data of 69 out of 72 marine ectotherms with planktonic larval life-history stages. Models included the following:

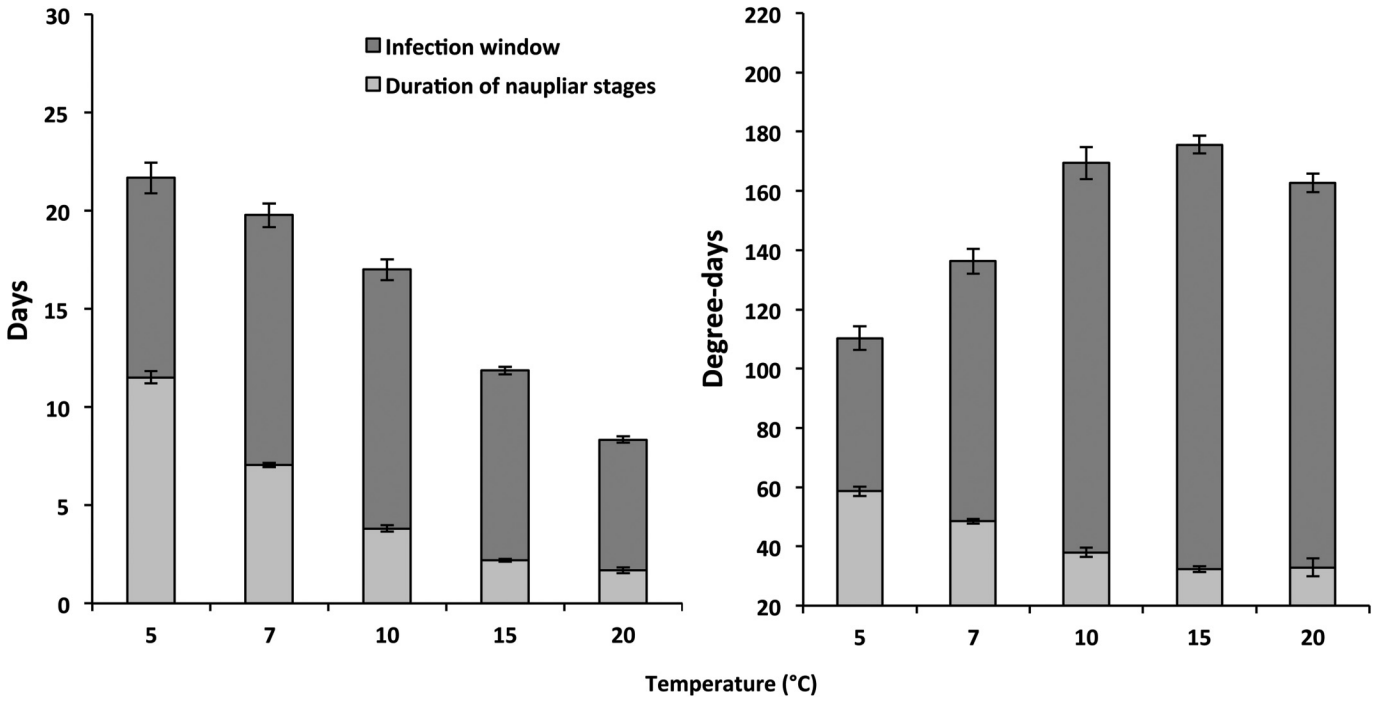
(*i*) A linearized power law model (Běhrádek 1930):

$$\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c) \quad (1)$$

(*ii*) A linearized power law model quadratic in temperature (McKinney 1984); the exponential–quadratic model:

$$\ln(Y) = \beta_0 + \beta_1 \times \log(T/T_c) + \beta_2 \times [\ln(T/T_c)]^2 \quad (2)$$

**Fig. 2.** Effect of water temperatures on the development times in days and degree-days of salmon lice (*Lepeophtheirus salmonis*) larval stages. Bars show mean pelagic larval duration (PLD) divided into mean duration of lice naupliar stages (nauplius I and II; light grey) and infective window (dark grey). Error bars are SE.




(*iii*) The universal temperature dependence (UTD) equation (Gillooly et al. 2001), where $k$ is the Boltzmann constant ($8.62 \times 10^{-5}$ eV·K$^{-1}$), and ($T$ (°C) + 273) is absolute temperature (K):

$$(3) \qquad \ln(Y) = \beta_0 + \beta_1/[k \times (T + 273)]$$

In each model, $Y$ is the response variable; eq. 1: development times of lice larvae (duration of naupliar stages, infective window, and PLD; in days and degree-days); eq. 2: adult size (female total length, male and female cephalothorax length); and eq. 3: reproductive outputs (egg string length, egg size, and total number of eggs per string). Further, $\beta_0$ is the intercept, $\beta_1$ and $\beta_2$ are linear and quadratic scaling parameters, respectively, $T$ = temperature (°C), and $T_c$ = 10 °C (a centering parameter: the median temperature value in our study). In polynomial regressions (i.e., eq. 2), polynomial terms are always correlated with lower-order terms in the model, so collinearity can be a problem (Quinn and Keough 2002). Thus, dividing each temperature observation ($T$) by $T_c$ = 10 °C on a log scale centers the data to reduce the degree of collinearity. Centered data generates more reliable parameter estimates for the lower terms (i.e., smaller standard errors) without affecting both the estimate of the highest term or the partitioning of the sum-of-squares. The best model was selected using Akaike's information criteria (AIC). Low AIC values indicate higher degree of model parsimony, and it penalizes models for the addition of extra parameters (Quinn and Keough 2002).

Differences among temperature treatments in percentage of infestation success (= infestation intensity or mean lice per fish/infective dose × 100) and the proportion of lice attached to the body surface and the fins were compared using one-way ANOVAs, and post hoc multiple comparison tests were performed using Tukey's honestly significant difference (HSD) test. Assumptions of normality and homogeneity of variance were evaluated by assessing boxplots and plots of model residuals. Hereinafter, all statistical means are presented as mean ± SE, unless otherwise stated.

## Results

### Hatching and development times of salmon lice planktonic larval stages

Hatching and development of salmon lice larvae was strongly influenced by water temperatures. The fraction of eggs that successfully hatched was 100% at 20 and 15 °C, 87% ± 3% at 10 °C, 90% ± 4% at 7 °C, 85% ± 4% at 5 °C, and 28% ± 4% at 3 °C. Average time before hatching increased with temperature and was 11.7 times longer at 3 °C (20.8 ± 1.5 days) compared with 20 °C (1.8 ± 0.1 days; refer to online Supplmentary Material[1]). All larvae successfully developed to the copepodid stage except for those incubated at 3 °C. At this temperature, larvae were monitored for approximately 41 days, but all nauplii died without moulting to the copepodid stage. However, the presence of exuvia in some of the wells at 3 °C indicated some larvae moulted to the second naupliar stage. The duration of lice naupliar stages and the infective window had a significant negative relationship with temperature (Table 2) and were 6.8 and 1.5 times longer at 5 °C compared with 20 °C, respectively. When transformed to degree-days, the opposite was observed for the infective window (Fig. 2); it increased with temperature until 15 °C (176 ± 2.9 and 143 ± 2.9 degree-days, respectively) and then decreased at 20 °C (163 ± 2.1 and 130 ± 3.1 degree-days, respectively). The duration of naupliar stages, though, still had a negative relationship with temperature (33 ± 3.1 degree-days at 20 °C, 59 ± 3.2 degree-days at 5 °C).

### Size and reproductive outputs at different temperatures

Water temperature also influenced body size and reproductive outputs of adult lice. All morphometric measurements, except for egg size, increased with decreasing temperatures from 20 to 5 °C and decreased at 3 °C (Figs. 3 and 4). Egg size was largest at 20 °C (67 ± 1.3 μm) and 3 °C (68 ± 0.9 μm) and averaged 62 ± 0.8 μm at all other temperatures (Fig. 4). Adult male size was highest at colder temperatures ($R^2$ = 0.28; Fig. 3), but greater variation in the obser-

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2016-0050.

**Fig. 3.** Effect of water temperatures on adult salmon lice (*Lepeophtheirus salmonis*) body size: female total length (mm) and female and male cephalothorax length (mm). Error bars are 95% CI.

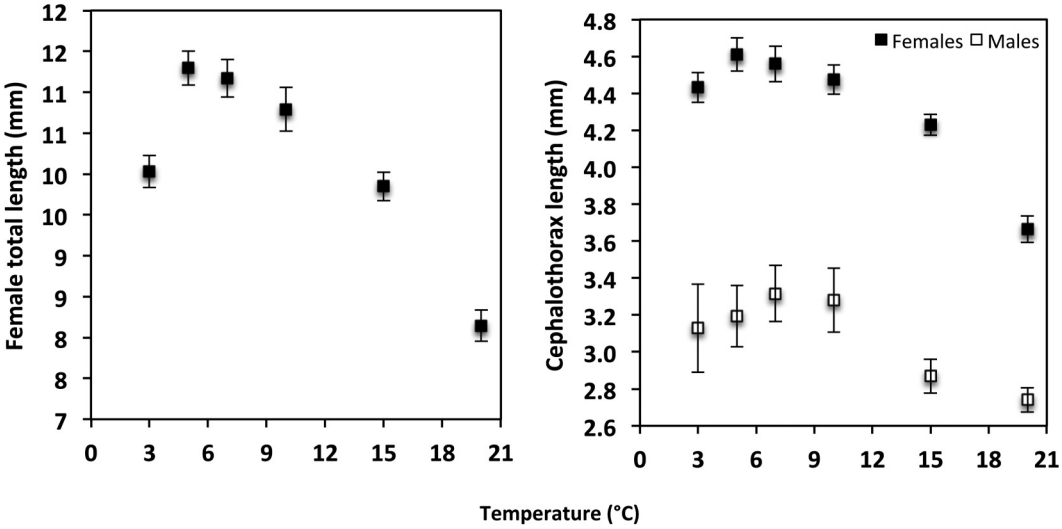
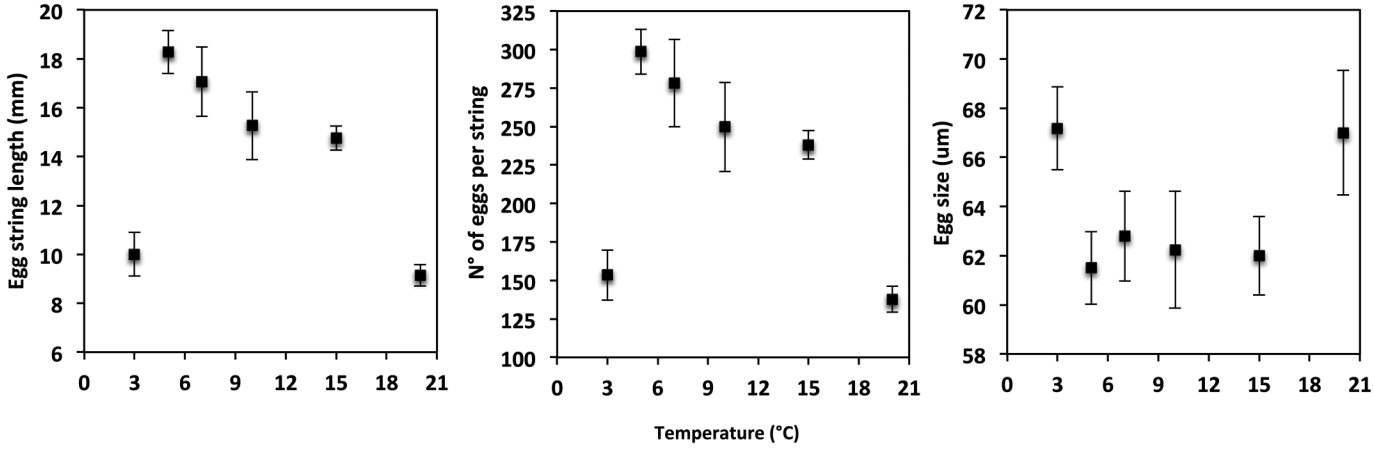



**Fig. 4.** Effect of water temperatures on salmon lice (*Lepeophtheirus salmonis*) reproductive outputs: egg string length (mm), number of eggs per string, and egg size (μm). Error bars are 95% CI.




vations due to a smaller sample size ($n$ = 5) perhaps masked any stronger correlation.

### Theoretical models of the effect of water temperatures on development times

Using AIC for model selection, we determined that PLD and infective window in days and degree-days fitted the exponential–quadratic model (Materials and methods, eq. 2), whereas the duration of lice naupliar stages fitted the linearized power law model (eq. 1). Adult size and reproductive outputs all fitted the exponential–quadratic model (Fig. 5). Model intercepts ($\beta_0$) in O'Connor et al. (2007) were highly variable and species-specific and on average lower for lecithotrophic (nonfeeding) larvae, whereas model coefficients were constant for most species. Model intercept for salmon lice PLD matched the mean for lecithotrophic larvae ($\beta_0$ = 2.8; PLD in days; Table 2), and model coefficients ($\beta_1$ = –0.67 and $\beta_2$ = –0.44; Table 2) had the same sign and similar values to the meta-analysis ($\beta_1$ = –1.34 and $\beta_2$ = –0.28). The difference in the linear scaling coefficient ($\beta_1$) was due to a different centering parameter; we used $T_c$ = 10 °C (median temperature value in our study), while O'Connor et al. (2007) used $T_c$ = 15 °C. The absolute value of our scaling parameter ($\beta_2$) was 1.6 times higher than the meta-analysis, but still within the observed variation for the 69 species in their study.

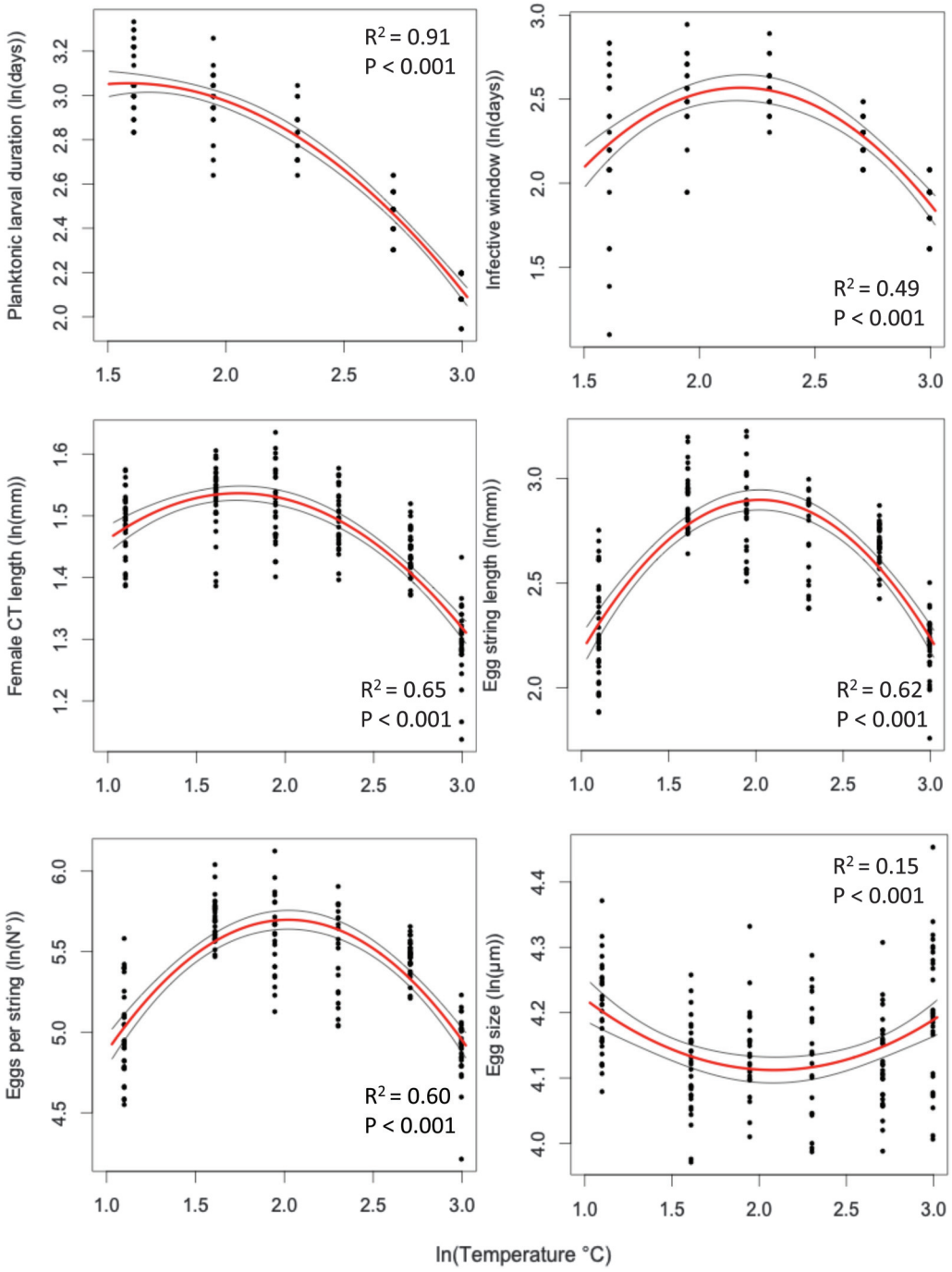
### Infestation success

Infestation success differed among the three temperature treatments ($F_{[2,9]}$ = 230, $P$ < 0.001). Fish infested at 10 °C (16.0 ± 0.6 lice·fish$^{-1}$; 53.2% ± 2.3% infestation success) had 1.2 times more lice than fish infested at 20 °C (13.3 ± 0.6 lice·fish$^{-1}$; 41.6% ± 2.0% infestation success) and 25 times more lice than fish infested at 5 °C (0.62 ± 0.12 lice·fish$^{-1}$; 2.1% ± 0.4% infestation success). All differences in lice infestation success were significant (Fig. 6; post hoc Tukey HSD: 20 versus 5, 10 versus 5, $P$ < 0.001; 20 versus 10, $P$ = 0.02). However, there were no differences in the proportion of salmon lice attached to the body ($F_{[2,9]}$ = 0.81, $P$ = 0.47) and the fin ($F_{[2,9]}$ = 0.85, $P$ = 0.46) among temperature treatments. Lice on all sampled fish had moulted to the chalimus stage.

### Simulated salmon lice dispersal patterns

The distribution of lice copepodids differed with both release location and development time to the infective stage. Particles released from the coastal site were transported northwards along the coast, with only a few particles entering the main fjords (Fig. 7). Particles released from the fjord site were transported northwards, but they were also transported into the inner fjord areas. Further, irrespective of release location, simulated lice particles with a

**Fig. 5.** Theoretical models fitted to the effect of water temperatures on development times of salmon lice (*Lepeophteirus salmonis*) larvae (planktonic larval development and infective window) and body size (female cephalothorax (CT) length) and reproductive outputs (egg string length, eggs per string, egg size) of adult lice. Models were chosen based on Akaike's information criteria (AIC; Table 1 and Supplementary Table S2[1]). All data fitted an exponential–quadratic model ($\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c) + \beta_2 \times [\ln(T/T_c)]^2$), except for the duration of naupliar stages that fitted a linearized power law model ($\ln(Y) = \beta_0 + \beta_1 \times \ln(T/T_c)$). $Y$ is the response variable, presented on the left column of the table, $T$ is temperature (°C), and $T_c$ = 10 °C is a centering parameter. [Colour online.]

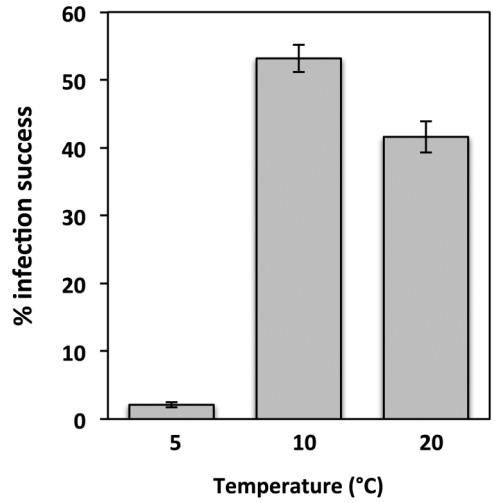



shorter development time to the infective stage (30 degree-days) were transported shorter distances, and higher retention of lice copepodids was observed around the release locations. Conversely, in the model simulation where lice particles had a longer development time to the infective stage (60 degree-days), particles showed less retention around the release locations, and most of them died before entering the copepodid stage.

## Discussion

Our results demonstrate a strong, negative, and predictable effect of water temperature on the development times and reproductive outputs of *L. salmonis* planktonic stages. A single regression model, the quadratic–exponential model, described the temperature dependence of most lice larval development, and the same model fit all data on adult body size and reproductive outputs. The fit to universal

**Fig. 6.** Salmon lice (*Lepeophtheirus salmonis*) infestation success (percent infestation intensity = infestation intensity or mean number of lice per fish/infective dose × 100) of Atlantic salmon (*Salmo salar*) postsmolts infected at 20, 10, and 5 °C. Error bars are SE.




theoretical models of temperature dependence indicates that the effects of water temperatures on larval duration and egg production of *L. salmonis* are predictable. This has important ecological implications and applications in modelling to inform management measures to prevent lice outbreaks.

Survival of planktonic larvae is generally very low (Thorson 1950) and decreases progressively with time (Graham et al. 2008). By influencing larval duration, water temperatures mediate the exposure of these organisms to sources of mortality (O'Connor et al. 2007). Reduced survival rates over longer larval periods in colder waters may select for shorter larval durations (Pearse et al. 1991), and therefore shorter PLDs may be the expression of a plastic phenotypic response to cold temperatures (Nylin and Gotthard 1998). In most situations, faster larval developments are adaptive because they decrease mortality before reproductive age (Nylin and Gotthard 1998). However, free-swimming salmon lice larvae must reach the copepodid stage to infest a host, grow, and reproduce. Therefore, a shorter duration of the naupliar stages, with the resulting decrease in pre-infective mortality, would be adaptive. In contrast, a shorter infective window would not be adaptive. Recent studies suggest that salmon lice naupliar stages may actively migrate to warmer temperatures in the water column to accelerate development times (á Norði et al. 2015) and decrease pre-infective mortality (Johnsen et al. 2014). However, salmon lice had a shorter physiological PLD (i.e., in degree-days) at colder temperatures owing to a shorter infective window, suggesting a nonadaptive response to these conditions. In addition, the poor larval survival at 3 °C, the low infestation success at 5 °C, and the contrasting exceptional larval survival and infestation success at 20 °C suggest that the salmon lice population we tested was better adapted to a higher temperature range because of their origin, the warmer waters of the southwest coast of Norway (Heuch et al. 2009).

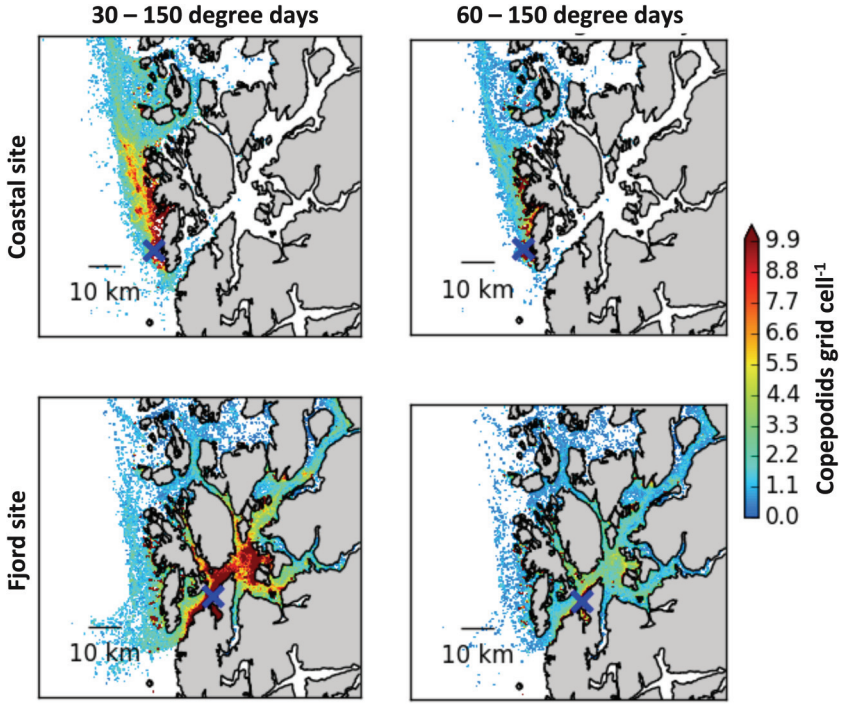
Parasites can adapt to intensive farming regimes, and these human-altered environments may also select for faster development (Mennerat et al. 2010). In salmon aquaculture, fish are grown at high densities in concentrated farming areas. Higher host availabilities select for faster life histories of parasites because of the trade-off between current reproduction and future survival (Mennerat et al. 2010). If hosts are easier to encounter, it is more efficient to invest in early reproduction, or early development, than in future survival. Accordingly, in salmon lice early life history stages, any potential human-induced selection for faster development rates would act on the duration of the naupliar stages. This phenomenon is not new and has been described for other parasites. Over generations, larvae of the nematode parasite *Steinernema feltiae* became infective earlier when host availabilities were experimentally increased (Crossan et al. 2007).

With our experimental design, we could not demonstrate if *L. salmonis* have a shorter development time to the infective stage than prefarming lice populations, and previous research provides no clear evidence of this phenomenon. Gravil (1996) observed longer development times from hatching to the copepodid stage than our study (7.8 and 5 days at 10 °C, respectively, compared with 3.8 days in this study). Others observed similar, or slightly shorter, development times from hatching to the copepodid stage: Boxaspen and Naess (2000): 4 days at 10 °C; Wootten et al. (1982): 2.7 days at 12 °C; Johannessen (1978): 2.6 days at 11 °C; and Tucker et al. (2002): 3 days at 8.8 °C. However, most of these studies estimated minimum development times to stage, and methodologies varied (e.g., temperature monitoring procedures, incubation technique, temperature at which adult females were acclimatized to produce egg strings, monitoring frequency, etc.). In addition, there is a lack of data on distributions of development times after initial minimum development times, particularly at high and low temperatures (Stien et al. 2005). On the other hand, the fit of our data to universal models of temperature dependence suggests that faster than expected development times to the infective stage may not have occurred yet, or the genetic change has not spread to all lice populations yet. However, the salmon louse has a huge spread potential, and evidence suggests a panmictic population (Glover et al. 2011). Therefore, any genetic changes would spread within a few years (Besnier et al. 2014). Further evidence is needed to conclude that present day salmon lice develop faster to the infective stage than prefarming populations. However, epidemiological theory (Mennerat et al. 2010) and evidence from other host–parasite systems (Crossan et al. 2007) strongly suggest that current farming conditions will induce selection for faster development rates.

The same universal models of temperature dependence that describe development times of marine ectotherms explained the response to temperature of *L. salmonis* body size and reproductive outputs. Body size and reproductive outputs (egg string length and number of eggs per string) followed the temperature–size rule between 15 and 5 °C, increasing in size, length, and number, respectively, as temperatures became colder, but decreasing at the extremes. Studies that have investigated the effect of seasonal changes in water temperatures on *L. salmonis* and other parasitic copepods have found similar results (Ritchie et al. 1993; Heuch et al. 2000); females are bigger in colder waters, and these larger specimens produce more eggs per clutch (Poulin 1995; Cavaleiro and Santos 2014). Larger clutches are, most likely, a compensatory response to longer generation times at lower temperatures. However, unlike other animals, larger body sizes in parasitic copepods are generally not correlated with larger eggs (reviewed in Poulin 1995), and this was observed for *L. salmonis* in our study.

Negative relationships between egg size and egg number are commonly seen in animal taxa (e.g., Christians 2000; Kinnison et al. 2001; Brown 2003) and are associated with a phenotypic trade-off that results from the allocation of resources to either one or the other reproductive strategy. In parasitic species though, where resources are generally high, there are less constraints on allocation strategies, and this reproductive trade-off may be weakened (Timi et al. 2005). In our study, within an optimal temperature range, this trade-off was not observed; the number of eggs decreased with temperature while egg size remained constant, and size only increased at high and low temperatures. The optimal allocation between egg number and egg size depends on the environmental conditions that the female encounters during reproduction (Poulin 1995). Therefore, larger eggs in *L. salmonis* could be an adaptation to extreme temperatures, since egg size is related to the amount of energy reserves (Levitan 2000; Tucker et al. 2000), critical for the survival of nonfeeding larvae. At 20 °C, larger eggs could increase dispersal time and the

**Fig. 7.** Simulated salmon lice (*Lepeophtheirus salmonis*) dispersal patterns. We used a coupled biological–physical dispersal model described in Asplin et al. (2014) and Johnsen et al. (2014). In the simulation, 10 particles, representing salmon lice during the planktonic stages, were released every hour from two sites (coastal and fjord site) around the Hardangerfjord area (60°N, 5.5°E) in the west coast of Norway for a winter month in January 2015. After hatching, the modeled particles became infective at either 30 or 60 degree-days and had a total life span or pelagic larval duration (PLD) of 150 degree-days. The map was created using the Basemap Matplotlib Toolkit for Python. [Colour online.]




chances of finding a suitable host. At cold temperatures, larger eggs could help naupliar larvae survive for longer periods while they develop to the infective stage. However, overly long periods in the ocean increase pre-infective mortality and may therefore be a non-adaptive response to cold conditions. Moreover, at 3 °C all lice failed to reach the copepodid, indicating this temperatures may be close to the biological tolerance of *L. salmonis*, at least for the population tested from southern Norway, which rarely experiences such cold winter temperatures (Fig. 1). Results from our study suggest that within a physiological optimal temperature range, sea lice adjust reproductive outputs mainly by changing the number of eggs produced per clutch and not the size of the eggs. However, further evidence is needed to confirm that this pattern holds for northern, cold-adapted lice populations.

Model simulations showed a strong effect of development times on the dispersal patterns of salmon lice copepodids. In the simulation where lice particles had longer development times, an important proportion of them died before reaching the copepodid stage. This suggests that the decrease in salmon lice abundances observed in farms after the coldest months of the year (Heuch et al. 2009) could be driven by a high mortality of larvae in the pre-infective stages, which hatch during those cold periods. In addition, observed lower lice levels on wild and farmed fish in the colder northern waters of Norway (Heuch et al. 2005) could be the result of slower generation and development times and lower infestation success. However, the topology of this area, typically with shorter and more open fjords than found on the southwest coast of Norway, would also cause a lower retention of lice pre-infective stages, flushing them offshore before they become infective. This, in addition to lower farmed fish biomasses (Norwegian Directorate of Fisheries 2014) in the north, also reduces lice abundance. In contrast, areas with higher water temperatures and topologies that generate greater lice retention, such as the long and narrow fjords of the Hardangerfjord on the southwest coast of Norway, probably aggravate salmon lice outbreaks. Overall, water temperatures are a key component of salmon lice dispersal models because of their influence on larval duration and mortality, and these models are key to determining the geographic patterns of lice dispersal.

The effects of water temperatures on salmon lice development and reproductive outputs follow universal models that describe the temperature dependence of a vast group of marine ectotherms. The predictable effect of water temperatures on lice development has important management applications, such as ensuring predictions of larval dispersal, mortality, and population connectivity are accurate and inform aquaculture planning and management of salmon lice outbreaks. Recognizing that the effects of temperature extend to the salmon louse will also improve our ability to predict the effects of global climate change on the ontogeny and dispersal of this marine parasite. In addition, low temperatures have a more detrimental effect on salmon lice survival and infestivity than high temperatures and may thus partially explain the lower occurrence of lice outbreaks in the northern parts of Norway. However, a cold-adapted lice population could perform better in colder waters (e.g., show a shorter development time to the infective stage or higher infestation success), but this remains to be determined. Overall, our study shows that water temperatures are a critical factor of salmon lice epidemiology, and the use of correctly parameterized dispersal models is essential to predict transmission for better management of lice outbreaks.

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