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Development of the salmon louse *Lepeophtheirus salmonis* parasitic stages in temperatures ranging from 3 to 24°C

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ABSTRACT: The development rate of the salmon louse *Lepeophtheirus salmonis* is greatly influenced by seawater temperature. This study describes how the growth rate of *L. salmonis* changes with temperature and identifies the extreme high and low temperatures at which development to the adult stage is compromised. Atlantic salmon *Salmo salar* were infected with copepodids and development was monitored in 8 temperature groups spanning 3 to 24°C until the lice were adults. Development was severely compromised at 3 and 24°C, while the lice developed normally in the temperature range from 6 to 21°C. At 6°C, most female lice had become adults at 72 d post infection (432 degree-days). At 21°C, development was significantly faster and most females were adults after 13 d, at only 271 degree-days. After infection, lice grew through 5 stages before reaching the adult stage, all of which, with a few exceptions, appeared to last approximately equally long. Thus, a simple model describing the mean daily growth rate (stages per day) as a function of temperature was made for each sex. The relationship between mean daily growth rate and temperature was best described by a second-order polynomial. The term relative age is introduced and used to describe the pattern of development in terms of percent of total development time to the adult stage. This was applied to calculate the timing of developmental events as a function of temperature. Photoperiod and development under rising or decreasing temperatures had minor effects on development rate.

KEY WORDS: *Salmo salar* · Molt rate · Thermal tolerance · Reproduction · *Lepeophtheirus salmonis* · Poikilotherm · Ecdysis

1. INTRODUCTION

The salmon louse *Lepeophtheirus salmonis* (Copepoda) is an ectoparasite on salmonid fishes with a direct life cycle comprising 8 stages, each separated by a molt. The first 2 stages are planktonic nauplius larvae preceding the third, infective copepodid stage. The remaining 5 stages (chalimus I and II, preadult I and II, and adult) develop on the host (Johnson & Albright 1991b, Schram 1993, Hamre et al. 2013). Mature adult females produce eggs that are deposited in batches within paired strings containing up to 1000 eggs (Brooker et al. 2018). The eggs are carried by the female until hatching, whereby larvae are released into the water to drift during their planktonic stages. The sex ratio in *L. salmonis* is 1:1, but females develop slower than males. Sexual dimorphism is discernible from the chalimus II stage, but only easily detected from the preadult I stage (Eichner et al. 2015). Salmon lice infections can negatively affect the welfare of the host fish as the parasite feeds on their skin and blood, leading to general stress, wounds,

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osmotic stress and higher susceptibility to secondary infections (Grimnes & Jakobsen 1996, Wagner et al. 2008). The pathology caused by salmon lice is mainly associated with the mobile preadult and adult stages (Jones et al. 1990, Jonsdottir et al. 1992, Grimnes & Jakobsen 1996).

Salmon lice are poikilotherms, and temperature is a strong regulator of their development and reproductive output (Johnson & Albright 1991a, Bjorn & Finstad 1998, Finstad et al. 2000, Heuch et al. 2000, Tucker et al. 2000a), with larger egg batches produced at lower temperatures (Samsing et al. 2016). Most previous studies have been limited to 1 or 2 intermediate temperatures between 7 and 13°C (Stien et al. 2005), except for a recent study where the rate of development for planktonic larvae was described over a wider temperature range (3 to 20°C) (Samsing et al. 2016). For a parasite with a substantial impact on an expanding salmon farming industry (Torrissen et al. 2013), more detailed knowledge of their temperature-dependent development rate across realistic ranges is required.

Although salmon lice populations are perpetuated by both wild and farmed fish (Fjortoft et al. 2017), the abundance of farmed Atlantic salmon in the Atlantic ocean exceeds wild salmonid abundance by several hundred-fold (Taranger et al. 2015); thus, this overwhelming bias in farmed host density is thought to disproportionately contribute to and propagate lice populations. However, the temperature experiences of host fish differ in relation to their life cycle. Wild salmon migrate from rivers through brackish fjords to reach the open ocean in a matter of days or weeks, and return to the rivers as maturing adults years later. Atlantic salmon naturally distribute from northern Spain (42° to 43° N) to Svalbard, Norway (78° N) and thus experience water temperatures from below 3°C to above 20°C (Horreo et al. 2011, Jensen et al. 2014). Other salmonid hosts, including sea trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*, mainly occupy coastal waters in their lifetime and are likely to experience a range of temperatures similar to those experienced by Atlantic salmon. In contrast, farmed salmon are constrained by their net pens, and involuntarily experience the small spatial scale of environmental conditions within. These can be extreme, from frozen surface waters (e.g. in Atlantic Canada and Northern Scandinavia) to temperatures of more than 20°C in southern Norway. The most extreme record for salmon is 24°C in Tasmania, Australia (Stehfest et al. 2017), although not relevant for *L. salmonis*, which are not naturally present in the southern hemisphere. In sea cages, and likely in the wild, salmon avoid sub-optimally high temperatures, characterized in previous studies as >18°C (Johansson et al. 2007) and >20°C (Stehfest et al. 2017), with preferences around 15 to 17.5°C. When post-smolt salmon are maintained over time at temperatures of 23°C and above, mortality can occur as they are close to their upper tolerance threshold (Hvas et al. 2017). In contrast, acclimatized salmon can grow and double their size over 220 d at 3°C (Bogevik et al. 2010).

In Norway, sea temperatures generally start to rise in March and decrease in August, which somewhat lags behind photoperiod, whereby the minimum is in December and maximum in June. Photoperiod is a strong regulator of swimming behavior, growth and sexual maturation in the host fish (Taranger et al. 2010, Hansen et al. 2017). While a few studies have explored the effect of light on hatching, swimming behavior and infection success in salmon lice (Boxaspen & Naess 2000, Flamarique et al. 2000, Browman et al. 2004), little is known about the effects of photoperiod on development. In arthropods, fluctuations or gradients in environmental conditions have been shown to affect development time (Fischer et al. 2011, Carrington et al. 2013, Singh et al. 2018).

The aim of the present study was to describe the full temperature range that permits development of the post-infection stages of *L. salmonis* and the effect of temperature on the rate of development. Furthermore, we determined the effects of a changing environment on the development rate of salmon lice (with respect to temperature and photoperiod) and the relationship between temperature and egg string extrusion frequency.

2. MATERIALS AND METHODS

2.1. Temperature and development rate experiment

2.1.1. Experimental animals

Atlantic salmon lice *Lepeophtheirus salmonis salmonis* (Skern-Mauritzen et al. 2014) were used for this study. Salmon lice eggs used to initiate a culture of lice were collected in June 2016 from an operating salmon farm (60° 87′ N, 05° 55′ E) on the southwest coast of Norway. In August 2016, eggs from this culture were collected and were allowed to hatch and develop to copepodids in incubators (Hamre et al. 2009) kept at 12°C.

All experiments were conducted in accordance with the Norwegian legislation for animal welfare at the

Matre Research Station of the Institute of Marine Research, Norway (Ethics approval #9192). Atlantic salmon *Salmo salar* postsmolts (farmed strain, Aquagen) from a single cohort were used for propagation of lice and ranged from 200 to 450 g (fork length 28 to 36 cm). Fish were fed to satiation (Skretting Spirit S, pellet size 75 and 150) and kept in tanks with a continuous flow-through of filtered and UV-treated seawater (34.5 ppt) pumped from 90 m depth in the adjacent fjord. Salmon lice unable to remain on their host were lost from the system. Very low levels of mortality of fish were observed throughout the trial, except in the 24°C group, where daily mortalities were observed from 5 d post infection (dpi) and reached an accumulated mortality of 20 to 35% among replicate tanks at 8 to 16 dpi (200 to 400 degree-days). Mortality was likely caused by high temperature alone and resulted in early termination of the 24°C group due to fish welfare considerations. In parallel to mortality, a behavioral change indicating extremely high metabolism was observed, with 75 to 95% of fish ram ventilating at 24°C in contrast to 0 to 5% ram ventilating at 21°C or lower, despite oxygen saturation levels >120%.

2.1.2. Experimental design

The growth of *L. salmonis* was measured at 8 different temperatures ranging from 3 to 24°C, at 3 degree intervals. Each temperature group consisted of 160 fish distributed among 4 tanks (0.9 × 0.9 × 0.4 m deep; volume ca. 0.32 m^3). The fish tanks were provided with continuous light and supplied with 34 ppt seawater (12 l min^{-1}) at temperatures held at a stable 3, 6, 9, 12, 15, 18, 21 or 24 ± 0.1°C. Temperature was measured continuously using digital thermometers within header tanks supplying the experimental tanks (1 header tank per 4 experimental tanks). These header tanks were temperature-adjusted manually; however, once set, did not deviate by more than 0.1°C. Temperatures were monitored with a management system provided by Normatic (www.normatic.no) that sends an alarm when conditions outside the set thresholds are sensed. The host fish were acclimated to their respective temperature groups (9 to 21°C) for at least 14 d, except for the extreme temperature groups 3 and 6°C, which were acclimated to 9°C, and the 24°C group, which was acclimated to 21°C (see below). Fish followed a standard infection procedure: tank water level was reduced to 1/3 of the normal volume and inflow adjusted to 6 l min^{-1} before copepodids were added. Tank outlets were blocked until normal tank levels had been reached (45 min), and thereafter normal water flow (12 l min^{-1}) was re-established. Oxygen levels were monitored to ensure saturation did not fall below 60%. Due to logistics, the 18, 21 and 24°C groups were infected with 30 copepodids per fish 10 d prior to the 3, 6, 9, 12, 15°C groups, which were infected with 28 copepodids per fish. To maintain infection success at the extreme temperatures (Samsing et al. 2016), the 3 and 6°C groups were infected at 9°C and set to 6°C after 3 h, and further down to 3°C the following day (24 h). Similarly, the 24°C group was infected at 21°C, and then set to 24°C after 3 h.

In each temperature group, 24 consecutive samples were obtained approximately 20 degree-days apart during development from the copepodid to the adult stage. Consequently, the sampling of lice in the 3°C group was distributed over 5 mo, whereas the 24°C group was sampled within 20 d. Due to an error, the 9°C group was sampled too frequently in the first period of the experiment, leading to a lack of fish and a slightly earlier termination of the group (at 421 degree-days), whereas the 12°C group was sampled less frequently in the first period of the experiment. An overview of all sampling events and lice counts can be found in Table S1 in the Supplement at www.int-res.com/articles/suppl/q011p429_supp.xlsx. For each sampling, the water level in the tank was lowered, and a small dose of sedative (metomidate hydrochloride, 0.2 g at ≤6°C or 0.4 g at ≥7°C) was added to calm the fish. Five fish were then carefully removed by hand and euthanized in an overdose sedative bath (1 g of metomidate hydrochloride in 10 l). Lice assessments were conducted immediately afterwards. Sampling events were planned on a rotating scheme so that each tank was only disturbed at every fourth sampling to minimize disturbance to the remaining fish in the tanks and potential loss of lice through too-frequent handling. The last samples obtained at 3°C comprised lice from 7 to 8 fish per sample in order to obtain enough lice to evaluate stage composition (Table S1).

All lice were counted and staged by careful inspection by trained personnel. All mobile stages were photographed for later re-confirmation of stage in cases of doubt. Staging was performed using a combination of a published description of salmon lice (Schram 1993) and identification of groups based on morphometric data. Using this method, all stages could easily be distinguished, except for preadult II and adult males at 24°C, where the number of males per sampling was less than 4 and no distinct size cohorts could be assigned. Four males were assigned as adults based on the size of adult males in the 21°C group.

2.1.3. Growth rate estimates

Within male and female populations, lice were generally either at the same stage or distributed among 2 stages in phases of molting. Occasionally, lice in samples were 2 stages behind the most advanced-developed individuals, and these are hereafter defined as late abnormal developers. Lice that developed within the normal range are referred to as early, average and late developers. As a measure of development, the mean number of molts undertaken per louse since infection (MnM) was calculated for each sample, excluding the very few late abnormal developers. As *L. salmonis* grow through a series of 5 molts on the fish, MnM is a continuous variable ranging from 0 at infection (all individuals are copepodids) to 5 (all individuals have become adults). Males develop faster than females, hence MnM was calculated separately for each sex at each sampling point (see Section 2.1.4) to explore the relationship between temperature and growth rate. On a wider temporal scale, there was an overall linear relationship between MnM and dpi at each of the temperatures tested, and the linear regression slopes thus represented an estimate of the mean daily molt rate (rM). The relationship between rM and temperature (T) was explored by fitting a linear function, a power function, an exponential function and a second-order polynomial to the data. The model explaining the largest proportion of variation was a second order polynomial, thus the full model fitted to the data was $\mathrm{MnM}(T) = a + (bT^2 + cT + d)\mathrm{dpi}$, where $\mathrm{rM}(T) = bT^2 + cT + d$. Note that $\mathrm{rM}(T)$ represents the mean daily growth rate across the 5 developmental stages prior to adult, and that it estimates the growth rate for the late developers, since the entire population is adult when MnM = 5. This way the term $5/\mathrm{rM}(T)$ estimates the point in time when the majority of the population has become adult. Only the development phase from infection until the point where the majority of the population (>85%) had reached the adult stage was included in the regression analysis. The 85% cut off was chosen to ensure that only the growth phase was included and to exclude the random effect of late abnormal developers in small samples. The constants a, b, c and d were estimated using the non-linear estimation module in Statistica v. 13 (TIBCO Software, http://statistica.io).

2.1.4. Sex determination

The workload involved in sex determination of chalimi is onerous (Eichner et al. 2015) and could not be achieved within the frames of this project. Thus, depending on the phase of development, different methods were used to determine gender. While the sex of preadults and adults could be determined visually, an indirect approach had to be applied for copepodids and chalimi. Counts of preadult and adult males and females in the present dataset and reports in literature (Carmichael et al. 2013) show that *L. salmonis* has a 1:1 sex ratio. Thus, when the entire population was either copepodids or had molted to chalimus I (ch1), a 1:1 sex ratio was assumed. In the period from the first appearance of chalimus II (ch2) and until the first appearance of preadult I (pa1), the population was either ch1 or ch2. Since the males develop faster than the females, the first ch2 to appear are males (Eichner et al. 2015). However, as the females gradually molt, the initially skewed ch2 sex ratio approaches a 1:1 distribution as all the lice enter the ch2 stage (Fig. 1). Accordingly, the ch1 sex ratio changes gradually from 1:1 at the beginning to almost 100% females. Under the assumption that there is a 1:1 sex distribution and that the male:female growth rate ratio is temperature independent (see Sections 3 & 4), data from experiments carried out at 10°C (Eichner et al. 2018) allowed us to estimate the MnM of males and females based on the proportion of chalimi that had entered the ch2 stage (Table 1, Fig. 1). In the period from the first appearance of preadults and until the majority of the population had become preadults, the sex of preadults was determined directly, and the number of ch2 males was estimated according to $\mathrm{ch2}_{\mathrm{males}} = (\mathrm{ch2} + \mathrm{pa1}) / 2 - \mathrm{pa1}_{\mathrm{males}}$, and likewise for the ch2 females. When all individuals were either preadults or adults, sex was determined directly.

2.1.5. Analysis of development pattern

The post-infection development pattern is defined as how the development time is distributed between the 5 stages before adult. In each separate temperature group, the frequency of sampling was insufficient to accurately resolve the development pattern. In order to describe the development pattern, the relative age (RA) of lice was calculated for each sampling point at each temperature tested. The results were then compared across all temperature groups by plotting the RA of lice in all samples against MnM. Developmental events, such as beginning and end of molt phases, were identified graphically from this plot and described in terms of RA. The RA is the age of lice given as a percentage of total development time required until the majority of the population has

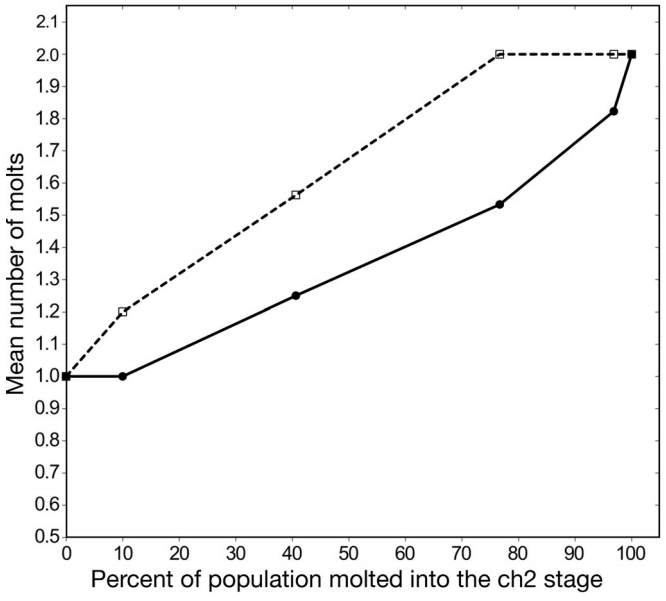

Fig. 1. Mean number of molts for male (□) and female (■) *Lepeophtheirus salmonis* as a function of the percent of the population that has entered the chalimus II stage. Graph is based on data presented in Table 1

become adult. To calculate the relative age, the term 5/rM(T) was used to estimate the total development time until the majority of the population had become adult in each of the temperature groups, thus $RA_{\%}$ = rM(T)(dpi/5)100. The mean daily growth rate in terms of relative age as a function of temperature is given as $\mathrm{rRA}_{\%}(T) = 100 \times \left[1 / \left(\frac{5}{\mathrm{rM}(T)}\right)\right]$. Alternatively, RA can also be given as a fraction of total development time: RA_{frac} = rM(T)(dpi/5), and thus the $\mathrm{rRA}_{\mathrm{frac}}(T) = 1 / \left(\frac{5}{\mathrm{rM}(T)}\right)$

Table 1. Distribution of male and female *Lepeophtheirus salmonis* among the chalimus (ch) stages during the phase when the population molts from ch I (ch1) into the ch II (ch2) stage at 10°C (unpublished details from experiments reported by Eichner et al. 2018). The table shows the number and stage of lice counted and sex of the ch2. The overall number of males and females present in the samples is estimated under the assumption that there is a 1:1 male:female distribution. Mean number of molts (MnM) is estimated by MnM_{males} = 1 + ($ch2_{males}$/males) and $MnM_{females}$ = 1 + ($ch2_{females}$/females) for males and females, respectively. dpi: days post infection

dpi	Lice counted (n)			Estimated		Ch2	MnM_{males}	$MnM_{females}$
	Ch1	Ch2 males	Ch2 females	Males (n)	Females (n)	(%)		
9						0	1.0	1.0
10	45	5	0	25	25	10	1.2	1.0
11	38	18	8	32	32	41	1.6	1.3
12	14	29	17	30	30	77	2.0	1.5
13	5	88	65	79	79	97	2.0	1.8
≥14						100	2.0	2.0

2.1.6. Egg batch production

L. salmonis eggs are produced in batches and deposited in external egg strings. The frequency of egg batch production was measured by observing the time of hatching in 2 subsequent batches of eggs. Egg strings were carefully removed from adult females in the 6, 12 and 18°C groups and held individually in continuous flow incubators (Hamre et al. 2009). The genital segments of the females were colored with marker pens in unique combinations (e.g. red:green, red:black, green:black), thus linking the females to their respective incubated egg strings before reattaching the lice to their hosts. The incubators were provided with water from the same source as the respective host fish tanks. When the next set of egg strings emerged, the lice were again removed from the fish, identified, and the new set of egg strings was incubated. Incubators were checked twice a day and hatching frequency was calculated as the average time between hatching of the first and the second batches of egg strings from individual females. Egg production was calculated as the average frequency of egg hatching multiplied by the average number of eggs obtained at the given temperature using earlier published data (Samsing et al. 2016).

2.2. Photoperiod and changing temperature experiment

The effect of photoperiod and changing temperature on development rate was investigated in a separate trial. The culture of lice used for this experiment was initiated by collecting *L. salmonis* eggs at the sea cage facilities of the Austevoll Research Station (Institute of Marine Research, Norway; 60° 05′ N, 05° 16′ E) in December 2016. F1 copepodids were reared in incubators (9°C) and fish were infected with lice (26 copepodids per fish) as described in Section 2.1.2. All infections were performed at 9°C with continuous light. The following day, tank environments were adjusted to the respective treatments. Atlantic salmon postsmolts (Aquagen strain) from a single cohort were used for propagation of lice and ranged from 300 g at start to 454 g at termination. Four experimental groups were exposed to long (20 h light:4 h dark cycle) or short

days (8 h light:16 h dark cycle) with either increasing (6.0 to 11.4°C) or decreasing (11.4 to 6.0°C) temperatures in a factorial design. The temperature was changed at a rate of 0.2°C d^{-1}. Treatments spanned over 244 degree-days for all groups and were terminated at 28 dpi. Each group contained 40 fish distributed in 4 replicate tanks. When sampled, fish were euthanized by an overdose of anesthetic as described in Section 2.1.2, and lice were collected and staged. When determining the effect of temperature and photoperiod, male and female populations were analyzed separately. At termination, lice were either pa1 or pa2, and therefore the inverse logit transformed proportion of males and of females that had reached the pa2 stage was used as the response variable in a generalized linear mixed model (Crawley 2007, Warton & Hui 2011). Predictor variables were temperature (increasing or decreasing) and light (long or short days), and tank number was a random effect. Initially, the model included the interaction of the predictor variables; however, the interaction was removed if found to be non-significant. Analyses were conducted in the R Core Environment (R Core Team 2014) using the *glmmTMB* function (package 'glmmTMB').

The observed development in this experiment was compared to the predictions made by the model derived in the temperature and development rate experiment (see Section 3 for model details). This was achieved by adding the day-by-day growth to provide an estimate of the relative age at sampling in each of the groups (see Section 3.5), using the following formula $\mathrm{RA\%} = 100 \times \sum_{d=0}^{28} 1 / \left(\frac{5}{\mathrm{rM}(T_d)} \right)$. The observed MnM at sampling was graphically translated to RA (see Figs. 4 & 5).

3. RESULTS

3.1. Development of *Lepeophtheirus salmonis* from infection to the adult stage at 8 different temperatures

Lepeophtheirus salmonis males developed faster than females; however, both sexes developed rather synchronously. Survival of *L. salmonis* was compromised in the lowest and the highest temperature groups (3 and 24°C), and highest loss rates were observed between the attached ch2 and the mobile pa1 stage. At 24°C, a total of only 4 adult males and no adult females were observed. At 3°C, the first adult females appeared after 135 d, but only 5 adult females were found in total. Consequently, samples from the 3 and 24°C groups were not included in further analysis.

Development rate increased with temperature. Time from infection until the majority (≥85%) of the females had become adults decreased from 71.9 d at 6°C to 12.9 d at 21°C (Table 2). Correspondingly, the number of degree-days required to reach the adult stage decreased with increasing temperature. There was random, but not systematic, variation in the relative relationship between male and female growth rates among the temperature groups (Table 2). However, the overall pattern of male and female growth rates among temperature groups was comparatively similar, indicating that temperature had the same effects on males and females. Independent of temperature, males consistently developed faster and became adults at around 80% of the total development time for females (Table 2).

It should be noted that the 24°C group was infected at 21°C, and the 3 and 6°C groups were infected at 9°C. The development at the infection temperature, before adjusting to target temperature, amounted to 1 and 2% of total development time to the adult stage (female lice), respectively, and hence introduced only minor effects on the measured development.

3.2. Growth rate is dependent on temperature and sex

Linear regressions encompassing all the sampling points (Fig. 2) were assumed to provide a more reliable measure of mean daily molt rates (rM)

Table 2. Time until the first sampling point at which the majority (≥85%) of *Lepeophtheirus salmonis* males and females were adults. The total number of males and females in the respective samples is given in brackets. Average daily molt rate (rM) = 5/days to adult; male:female = $\mathrm{rM_{male}}$:$\mathrm{rM_{female}}$; na: not applicable

Temp (°C)	Days to adult		Degree-days to adult		Molt rate		Male:female
	Male	Female	Male	Female	$\mathrm{rM_{male}}$	$\mathrm{rM_{female}}$	
3	109.8 (1)	135.0 (1)	329	405	0.05	0.04	0.80
6	54.8 (15)	71.9 (15)	329	431	0.09	0.07	0.78
9	36.8 (53)	43.1 (29)	332	387	0.14	0.12	0.86
12	22.8 (43)	29.1 (32)	273	349	0.22	0.17	0.77
15	16.0 (63)	20.9 (38)	240	314	0.31	0.24	0.77
18	13.2 (42)	16.0 (26)	237	289	0.38	0.31	0.82
21	11.1 (42)	12.9 (29)	234	271	0.45	0.39	0.87
24	na	na	na	na	na	na	na

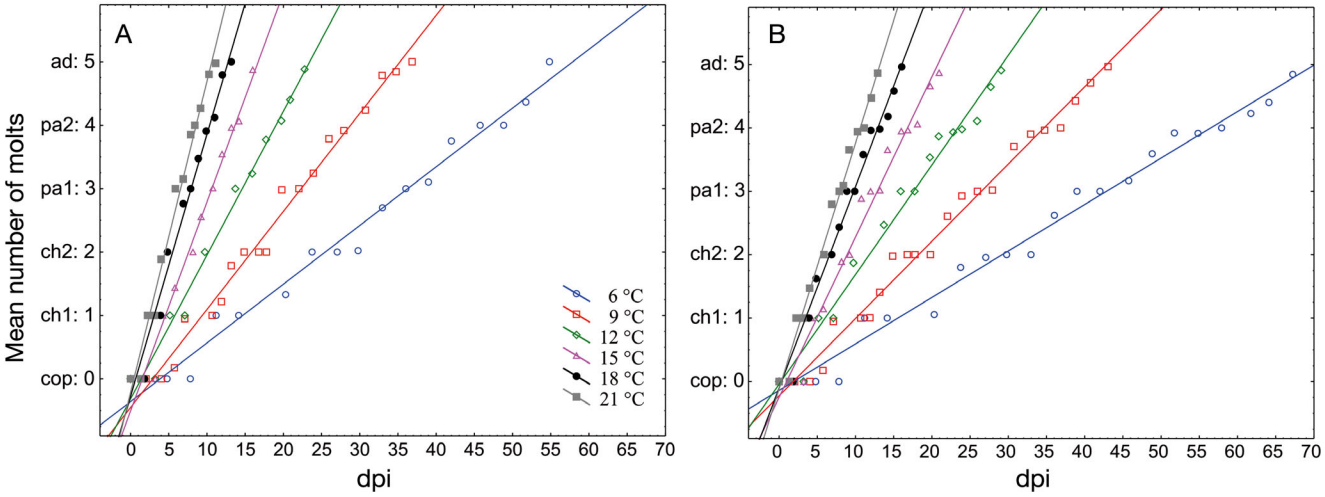

Fig. 2. Mean number of molts (MnM) vs. days post infection (dpi) for *Lepeophtheirus salmonis* (A) males and (B) females. Lines represent the predicted MnM as a function of dpi at each of the temperatures tested: $\mathrm{MnM}(T) = a + \mathrm{rM}(T)\mathrm{dpi}$, where the mean daily growth rate is $\mathrm{rM}(T) = bT^2 + cT + d$. The values of the constants a, b, c and d are listed in Table 3

rather than the estimates based on the observed appearance of adults only (Table 2). The values of the constants of the model $\mathrm{MnM}(T) = a + (bT^2 + cT + d)\mathrm{dpi}$, where $\mathrm{rM}(T) = bT^2 + cT + d$, is given in Table 3. The observed and predicted linear relationship between MnM and dpi at each of the temperatures tested is shown in Fig. 2, whereas the predicted relationship between rM and T is shown in Fig. 3. The overall linear nature of the relationship between MnM and dpi suggests that the copepodid stage, the 2 chalimus stages and the 2 preadult stages are all of approximately equal duration, implying that each of the stages lasts about 20% of the total development time to the adult stage. The molt rate (rM) for males and females changed with temperature according to a pattern best described by a second-order polynomial (Fig. 3).

Table 3. Growth model parameters for male and female *Lepeophtheirus salmonis* estimated using nonlinear estimation (Statistica). The growth model fitted was $\mathrm{MnM} = a + (bT^2 + cT + d)\mathrm{dpi}$, where MnM is mean number of molts, T is temperature, and dpi is days post infection. The model assumes a constant molt rate during development from copepodid to adult at temperature (T), and a polynomial relationship between growth rate and T. The constants a, b, c and d were estimated for each sex and the model was parameterized using data for temperatures from 6 to 21°C. The proportion of variance accounted for by the model was 0.98 for both males and females. The mean molt rate at temperature T is $\mathrm{rM}(T) = bT^2 + cT + d$

Parameter	Estimate	SE	t (df = 82)	p
Male				
a	−0.354753	0.051165	−6.93352	<0.0001
b	0.000677	0.000086	7.88884	<0.0001
c	0.010294	0.001997	5.15432	<0.0001
d	0.005729	0.009898	0.57879	0.564
Female				
a	−0.152008	0.042580	−3.56992	<0.0001
b	0.000485	0.000060	8.12457	<0.0001
c	0.008667	0.001360	6.37421	<0.0001
d	0.003750	0.006596	0.56859	0.571

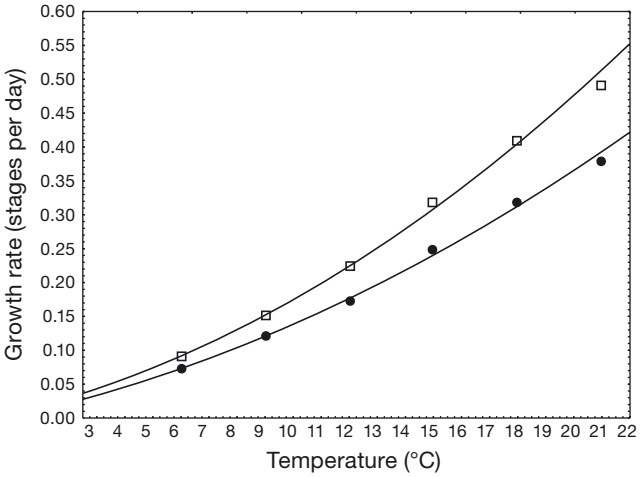

Fig. 3. Mean daily growth rate (rM) for male (□) and female (●) *Lepeophtheirus salmonis* vs. temperature (T) over the 5 post infection development stages calculated based on individual regression lines for each temperature group. The lines are the model predictions of the mean daily growth rate as a function of temperature (T) for males and females respectively given by the term $\mathrm{rM}(T) = bT^2 + cT + d$ in the overall growth model $\mathrm{MnM}(T) = a + \mathrm{rM}(T)\mathrm{dpi}$. The values of the constants a, b, c and d are listed in Table 3

3.3. StageAge and pattern of development

The growth rate, here given by the mean daily molt rate, is equivalent to the mean number of stages that the louse grows per day over the time from infection to the adult stage. As the copepodid ch1 and 2 and pa1 and 2 stages are of approximately equal duration (isochronous), a simple growth model can be established that describes the age of lice in terms of number of stages, StageAge = rM(T)dpi, thus redefining the term 'stage age' (Eichner et al. 2015). While 'stage age' formerly described the number of days spent in a stage, the extended definition, hereafter termed StageAge, is a linear continuous measure of lice age denoting how many molts a louse has undertaken since infection plus the elapsed proportion of the present stage, i.e. if a ch1 is halfway to its next molt, its StageAge is 1.5. A simple StageAge model represents an easy way to calculate time to reach any stage in question, or the time remaining to adult from any stage, at a given temperature. However, a StageAge growth model is only valid if the pattern of development is stable across temperatures, and if all the stages are of similar duration. Fig. 4 shows that the pattern of development was stable across temperatures ranging from 6 to 21°C, and that all the stages, with a few exceptions, had a similar duration. For males, the ch1 stage was reached at RA = 20%, ch2 at RA = 40%, pa1 at RA = 60%, pa2 at RA = 80%, and adult at RA = 100%. Thus all post-infection stages lasted each approximately 20% of the total development time to the adult stage. In females, the pattern of development was different: the copepodid stage lasted only about 16%, and the pa2 stage 24%, of the total development time to adult, while both chalimus stages and pa1 each lasted 20%. Hence, a simple StageAge growth model works well for males, but underestimates the growth rate of female copepodids and overestimates the growth rate of female pa2 (see red dashed line, Fig. 4). The StageAge growth rate model predicts the average growth rate over the 5 stages from infection to adult correctly, but for individual stages, it is strictly only correct for stages that last 20% of the total development time. The corrected growth rate for female copepodids is thus (20/16) × rM(T)$_{females}$ and for pa2 females it is (20/24) × rM(T)$_{females}$. Furthermore, by using the present growth rate model rM(T), which predicts the growth rate of the late developers, a simple StageAge model would underestimate the growth of the average louse. However, the relative age can be used to calculate the timing of events in terms of dpi accurately, since the pattern of *L. salmonis* development is invariant. The term 5/rM(T) correctly estimates time until the majority of the population has become adult (i.e. when the late developers become adult) at a given temperature, and the timing of any other developmental event can then be calculated based on the relative age. For example, at 10°C, the majority of females have entered the adult stage at 36.1 dpi (5/(rM(10°C)$_{females}$). From Fig. 4, we observe that the majority of females have become ch2 at RA ≈36%, thus at 10°C, the majority of females are ch2 by 0.36 × 36.1 = 13 dpi. The first adult females always appear at RA ≈ 87%, thus at 10°C: 0.87 × 36.1 = 31.3 dpi (Table 4). Furthermore, the relative age at which events occur can be used to derive a growth rate model predicting the growth rate for the average

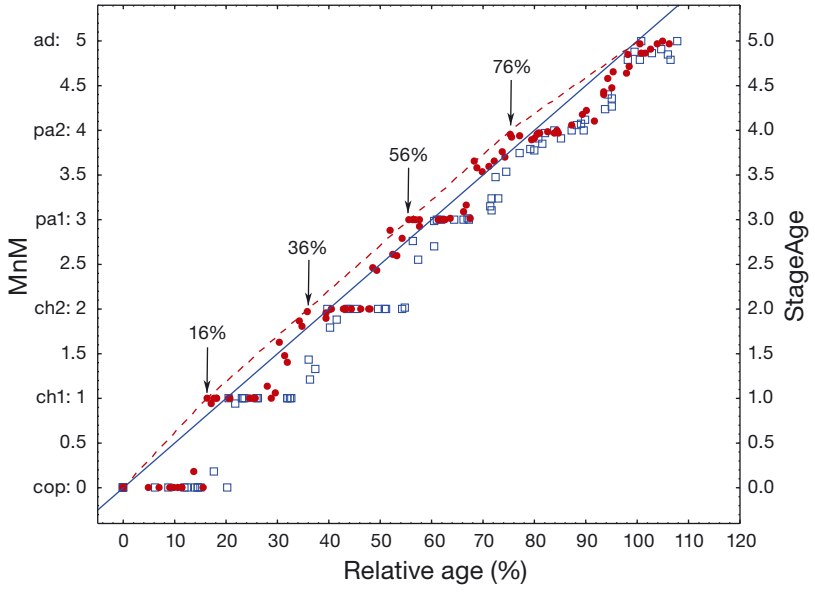

Fig. 4. Mean number of molts (MnM) for male (□) and female (●) *Lepeophtheirus salmonis* vs. relative age (RA = % of days post infection until adult) for all samples in the range from 6 to 21°C. Arrows indicate the relative age at which the majority of females reach ch1, ch2, pa1 and pa2 stages respectively. Predicted StageAge of the slow developers (solid blue line); corrected StageAge for females (red dashed line). These lines provide the means to graphically translate an observed MnM value to RA and vice versa

Table 4. The *Lepeophtheirus salmonis* pattern of development described in terms of relative age (RA_{frac}) and the timing of development events in terms of dpi at temperatures from 3 to 21°C. The table shows when the fastest developers start molting to the next stage (early) and when the majority of males and females reach a certain stage (all). The growth model estimates the number of days until the majority of the population has become adult (DTA) at each temperature using the term $5/rM(T)$, and the timing of developmental events in terms of dpi is calculated by $RA_{frac} \times DTA$. The timing of extrusion of first egg string in terms of relative age is based on data from this study and L. A. Hamre (unpubl. data). Development from hatching to copepodid was translated to relative age based on data from the literature. The rationale behind this estimate is given in Section 3.3

Males	RA_{frac}	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ch1 all	0.2	23.4	17.3	13.5	10.9	9	7.6	6.5	5.7	5	4.4	3.9	3.5	3.2	2.9	2.7	2.4	2.2	2.1	1.9
Ch2 early	0.35	41	30.3	23.6	19.1	15.8	13.3	11.4	9.9	8.7	7.7	6.9	6.2	5.6	5.1	4.6	4.3	3.9	3.6	3.4
Ch2 all	0.4	46.8	34.6	27	21.8	18	15.2	13.1	11.3	10	8.8	7.9	7.1	6.4	5.8	5.3	4.9	4.5	4.1	3.8
Pa1 early	0.55	64.4	47.6	37.1	29.9	24.8	20.9	17.9	15.6	13.7	12.1	10.8	9.7	8.8	8	7.3	6.7	6.2	5.7	5.3
Pa1 all	0.6	70.3	52	40.5	32.7	27	22.8	19.6	17	14.9	13.2	11.8	10.6	9.6	8.7	8	7.3	6.7	6.2	5.8
Pa2 early	0.72	82	60.6	47.2	38.1	31.5	26.6	22.8	19.8	17.4	15.4	13.8	12.4	11.2	10.2	9.3	8.5	7.9	7.3	6.7
Pa2 all	0.8	93.7	69.3	54	43.5	36.1	30.4	26.1	22.7	19.9	17.6	15.8	14.2	12.8	11.6	10.6	9.7	9	8.3	7.7
Adult early	0.87	101.9	75.3	58.7	47.4	39.2	33.1	28.4	24.7	21.7	19.2	17.1	15.4	13.9	12.7	11.6	10.6	9.8	9	8.4
Adult all	1	117.1	86.6	67.5	54.4	45.1	38.1	32.6	28.4	24.9	22.1	19.7	17.7	16	14.5	13.3	12.2	11.2	10.4	9.6
Planktonic development: N1+N2	0.134	15.7	11.6	9	7.3	6	5.1	4.4	3.8	3.3	2.9	2.6	2.4	2.1	1.9	1.8	1.6	1.5	1.4	1.3
Females	RA_{frac}	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ch1 all	0.16	23.5	17.3	13.5	10.9	9.1	7.7	6.6	5.8	5.1	4.5	4	3.6	3.3	3	2.7	2.5	2.3	2.2	2
Ch2 early	0.29	42.5	31.4	24.5	19.8	16.4	13.9	12	10.4	9.2	8.2	7.3	6.6	6	5.4	5	4.6	4.2	3.9	3.6
Ch2 all	0.36	52.8	39	30.4	24.6	20.4	17.3	14.9	13	11.4	10.1	9.1	8.2	7.4	6.8	6.2	5.7	5.2	4.9	4.5
Pa1 early	0.48	70.4	52	40.5	32.8	27.2	23.1	19.8	17.3	15.2	13.5	12.1	10.9	9.9	9	8.2	7.6	7	6.5	6
Pa1 all	0.56	82.1	60.6	47.3	38.2	31.8	26.9	23.1	20.2	17.8	15.8	14.1	12.7	11.5	10.5	9.6	8.8	8.2	7.5	7
Pa2 early	0.66	96.7	71.5	55.7	45.1	37.4	31.7	27.3	23.8	20.9	18.6	16.6	15	13.6	12.4	11.3	10.4	9.6	8.9	8.3
Pa2 all	0.76	111.4	82.3	64.2	51.9	43.1	36.5	31.4	27.4	24.1	21.4	19.2	17.3	15.6	14.3	13.1	12	11.1	10.2	9.5
Adult early	0.87	127.5	94.2	73.5	59.4	49.3	41.8	35.9	31.3	27.6	24.5	21.9	19.8	17.9	16.3	14.9	13.7	12.7	11.7	10.9
Adult all	1	146.6	108.3	84.5	68.3	56.7	48	41.3	36	31.7	28.2	25.2	22.7	20.6	18.8	17.2	15.8	14.6	13.5	12.5
First egg string all	1.3	190.5	140.8	109.8	88.8	73.7	62.4	53.7	46.8	41.2	36.6	32.8	29.5	26.8	24.4	22.3	20.5	18.9	17.5	16.3
Days between egg batches	0.25	36.6	27.1	21.1	17.1	14.2	12	10.3	9	7.9	7	6.3	5.7	5.1	4.7	4.3	3.9	3.6	3.4	3.1
Planktonic development: N1+N2	0.106	15.5	11.5	9	7.2	6	5.1	4.4	3.8	3.4	3	2.7	2.4	2.2	2	1.8	1.7	1.5	1.4	1.3

louse, and not only for the late developers. The earliest developers become adult at 87% of the time it takes for the late developers to become adult, and under the assumption that 50% of the population has become adult halfway between RA = 87% and RA = 100%, the average lice become adult at RA = 93.5%; hence $rM(T)_{average} = rM(T)(100/93.5)$. The growth rate for the fastest-developing louse is $rM(T)_{fast} = rM(T)(100/87)$. Furthermore, by estimating when molt phases start and relating this graphically to the StageAge of the slow developers in Fig. 4, the following can be observed: the fastest males start molting to ch2 when the slow males have about 30% of their instar period left. At the last molt, the late developers have 60% of their instar period left when the early developers become adult.

The females developed at a rate about 20% slower than the males. Thus, according to Fig. 5, the relationship between male and female development could be characterized, independent of temperature, according to the following pattern: male and female copepodids molt to the ch1 stage simultaneously (assumedly). The first ch2 appearing are all males, and until the first pa1 appear, there is a skewed sex ratio within each of the chalimus stages. The majority of males become pa1 before the first pa1 females start emerging, and at this point the majority of ch2 left are females. The first adult males appear when approximately 50% of the females have become pa2.

3.4. Egg hatching frequency

The frequency of hatching increased substantially with temperature and was 4.2 times faster at 18°C than at 6°C (Table 5). The number of degree-days between each hatching of subsequent egg batches was ca. 75 at

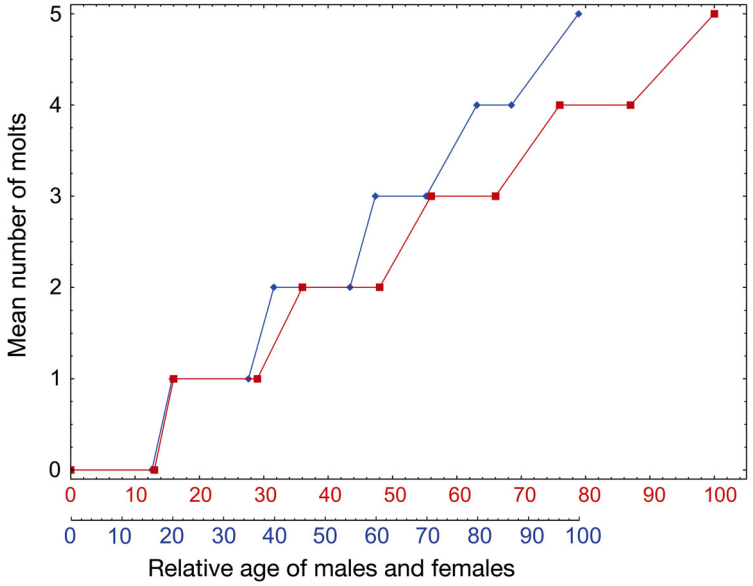

Fig. 5. Mean number of molts (MnM) against the relative age (RA) of male (blue) and female (red) *Lepeophtheirus salmonis*. Males develop faster than females and reach the adult stage (RA = 100 % and MnM = 5.0) when the female RA is about 80 %. To illustrate this, the RA is given in separate *x*-axes for males and females. The graph shows the development pattern and indicates when the male and female populations are in phases of instar growth (stable MnM) and the phases when the populations are molting (increasing MnM). At MnM = 1.0, the majority of the population is ch1, and at MnM = 1.5, 50 % of the population has become ch2. The beginning and end of the population molt phases were identified from Fig. 4

Table 5. Effect of temperature on egg production by *Lepeophtheirus salmonis*. Frequency of egg batch hatching is given in days and degree-days (dd). N: number of egg batches (egg strings) observed; Egg prod.: calculated mean daily egg production

Temperature (°C)	Hatch freq. (d)	Hatch freq. (dd)	N	Egg prod. (d^{-1})
6	16.8	100.8	37	28.9
12	6.3	75.6	41	80.9
18	4.0	72	13	90.8

12 and 18°C, and 100 degree-days at 6°C. Average daily production of eggs was estimated by combining this hatching frequency with number of eggs per string from similar temperatures (Samsing et al. 2016). Egg production was highest at 18°C where each female produced 90 eggs d^{-1}, which was approximately 3 times more than production at 6°C (Table 5).

3.5. The effect of day length and changing temperature on development rate

Lice experiencing a decrease in temperature from 11.4 to 6°C while developing to preadults displayed a slightly higher growth rate than those developing under a regime with rising temperatures (6 to 11.4°C). This pattern was observed in both the long-day group and the short-day group, but there was no evident effect of photoperiod (Table 6). The generalized linear mixed model revealed temperature, but not light, to be a significant factor influencing the proportion of pa2 males in the male population (Table S2), with no interaction between temperature and light. For females, both light and temperature significantly affected the proportion of pa2 in the population (Table S2). Furthermore, there was a significant interaction between the 2 factors which was likely to be from the slight increase in proportion of pa2 females in short day length (8 h light:16 h dark cycle group) compared to long day length (20 h light:4 h dark cycle).

Table 6. Effects of temperature gradient and light regime on *Lepeophtheirus salmonis* development. The table shows the mean number of molts undertaken by males and females when temperatures increase from 6 to 11.4°C during development to preadults in a long-day regime and a short-day regime, likewise when temperatures decrease from 11.4 to 6°C. The observed relative age (%) is given in brackets. The growth model used to calculate the predicted relative age was derived from data obtained at 24 h daylight and stable temperatures. Note that the lice were sampled in a period when the female population was in a phase of molting from preadult 1 to preadult 2, whereas the male population was close to (increasing temperature group) or in in a phase when no molts take place (decreasing temperature group). Thus, for males MnM ≈ 4 in the period from about 79 to 87 % relative age (see Fig. 5)

Photoperiod (h daylight)	Method	Male		Female	
		Increasing temp.	Decreasing temp.	Increasing temp.	Decreasing temp.
18	Observed	3.88 (78 %)	3.98 (79–87 %)	3.05 (66 %)	3.38 (69 %)
6	Observed	3.91 (78 %)	3.99 (79–87 %)	3.03 (66 %)	3.53 (70 %)
24	Predicted	86 %		68 %	

4. DISCUSSION

The Atlantic population of *Lepeophtheirus salmonis* is considered as one population (Glover et al. 2011). Due to a wide geographical distribution and migration of the host fish in the North Atlantic Ocean, the salmon louse can experience large differences in sea temperature and day length. Being attached to the host, salmon lice are exposed to the same temperature, salinity and light regime as their hosts and must adapt to handle a wide range of environmental conditions. In this study, development of salmon lice was monitored by repeated sampling throughout the life cycle on fish kept at a range of temperatures corresponding to sea temperatures within the geographic range of the host fish. At the highest temperature tested (24°C), the growth rate among the young stages of *L. salmonis* was lower compared to development at 21°C, and the lice were lost from the host as they progressed from the attached chalimus stages to free-living preadults. The fastest development to the adult stage without severe mortality took place at 21°C. This suggests that the maximum temperature limit for *L. salmonis* development is somewhere between 21 and 24°C. It should, however, be noted that a few adult males did develop at 24°C, highlighting that this limit is not absolute; rather, there is a continuous interval with increasing restraints on development, ranging from the temperature at which the highest molt rate is achieved through all stages to the temperature where no individuals reach the adult stage. At the lowest temperature tested (3°C), only a few males and females developed into adults, demonstrating that development is severely constrained at 3°C and presumably close to the absolute minimum temperature for development to adults. It should, however, be noted that the present results were derived from *L. salmonis* collected in Norway, and that isolates from other parts of the Atlantic or Pacific Oceans may potentially display variations in temperature tolerance limits, either due to genetic (Atlantic vs. Pacific) or epigenetic effects.

The present data suggest that development is not constrained in the temperature range from 6 to 21°C. Within this range, molt rate increases with temperature, and the relationship between molt rate and temperature is well described by a second-order polynomial. Furthermore, irrespective of temperature, all the 5 developmental stages prior to adult are of approximately equal duration (isochronal). Thus, the post-infection age of a louse can be described by a linear function of time at any given temperature from 6 to 21°C and expressed in terms of StageAge. However, as the female copepodids develop somewhat faster than the other post-infection stages, the simple growth model underestimates the StageAge of females by about 0.25 stages in the period between copepodid and pa2 (Fig. 4); but, since the female pa2 stage lasts equivalently longer than average, the model predicts the time until the adult stage without bias (Fig. 4). Although the 3°C group was not included when fitting the model (due to lice mortality and low sample sizes), the growth model predicted the development at 3°C well (comparison not shown), thus suggesting that the model predicts growth rate satisfactorily in the temperature range from 3 to 21°C.

The number of degree-days required by *L. salmonis* to reach the adult stage decreased considerably with increasing temperature (Table 2) and was thus not a good metric to describe the timing of developmental events. The RA, however, allowed data from all the temperature groups to be assembled along a temperature-independent *x*-axis. This enabled comparisons of developmental events across temperatures and sex. When pooling all temperature groups and plotting RA against MnM, the data points formed an orderly pattern (Fig. 4). Due to the sample size and method for sex determination, the quality of the MnM estimates varied among the individual data points. Also, only 1 tank per temperature group was sampled at each time point; hence there is a potential risk that tank effects could have introduced increased variability or systematic errors in the data. However, the densely assembled data points suggest (1) there were no detectable tank effects on lice development rate and (2) the relative duration of the 5 development stages was constant. Thus, the overall pattern of development was consistent over the range of temperatures tested.

Much of the growth data for *L. salmonis* available in the literature has been summarized by Stien et al. (2005) who developed a model encompassing growth rates and demographic rates for temperatures in the range from 7 to 15°C based on published data (Johannessen 1977, Wootten et al. 1982, Johnson & Albright 1991a, Johnson 1993, Ritchie 1993, Grimnes & Jakobsen 1996, Grimnes et al. 1996, Dawson et al. 1997, 1998, 1999, Bjorn & Finstad 1998, Boxaspen & Naess 2000, Finstad et al. 2000, Heuch et al. 2000, Tucker et al. 2000a,b, 2002). Stien et al. (2005) provided model predictions on time from infection to 'the presence of' (interpreted here as 'first observation of') adult males and females at temperatures ranging from 7 to 15°C. Comparing these predictions to the present study's

model shows that both models predict similar development times; however, it appears that the growth model of Stien et al. (2005) predicts a slightly steeper change in growth rate with increasing temperature for females (Fig. 6).

Few experiments have been performed with salmon lice at temperatures above 12°C, but rates of development similar to those observed in the present study have been observed in the subtropical sea louse *L. simplex* that developed from copepodids to first adult females in 8 d at 22°C (Morales-Serna et al. 2015).

Reported growth rates of *Caligus rogercresseyi* (Gonzalez & Carvajal 2003) appear similar to the growth rates observed for female *L. salmonis* in this study; however, the *C. rogercresseyi* growth data were not obtained at stable temperatures, rather days to adult was related to the means of wide ranges in temperature during development. The *L. salmonis* growth rate increased more than linearly with increasing temperature and this is most likely true for *C. rogercresseyi* as well; hence, the estimates related to mean temperature could not be directly compared to the present results.

C. elongatus develop to adults in 24.7 dpi at 10°C (Piasecki & Mackinnon 1995). Post-infection, they develop through the copepodid stage and 4 chalimus stages before reaching the adult stage, and the sexes display no difference in development rates. Interestingly, the development pattern of *C. elongatus*, described in relative age units ($RAU_{\%}$) based on data for average development rate (Piasecki & Mackinnon 1995), is approximately 29:17:11:15:29. The comparable numbers for *L. salmonis* observed in this study

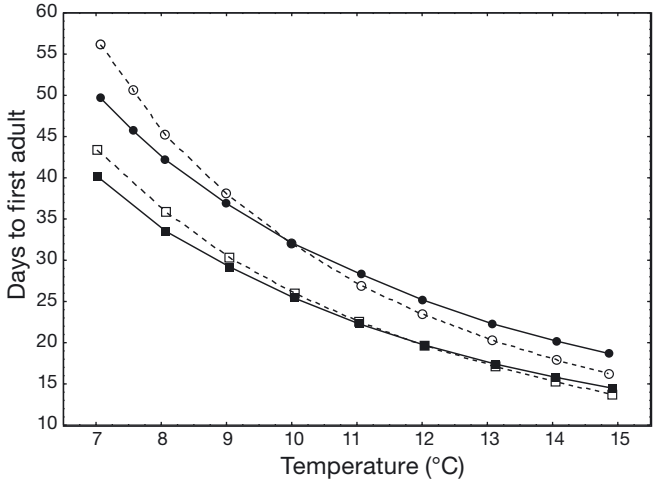

Fig. 6. Comparison of the predictions made by the Stien et al. (2005) model (dashed line) vs. the present model (solid line) for male (□) and female (○) *Lepeophtheirus salmonis*

are 20:20:20:20:20 (males) and 16:20:20:20:24 (females). Thus, the *C. elongatus* development pattern appear to deviate significantly from that of *L. salmonis*, as the copepodid stage takes up a considerably larger proportion of the total development time to the adult stage, but also the ch4 stage, equivalent to the pa2 stage in *L. salmonis*, appears to last substantially longer than the intermediate stages, even longer than observed for *L. salmonis* females. However, in contrast to the rather synchronous development of *L. salmonis*, Piasecki & Mackinnon (1995) observed a far less synchronous development of *C. elongatus*. Whether this reflects the true biology of *C. elongatus* or experimental artifacts is unknown.

Salmon lice are exposed to large variations in temperature and light regime driven by the spatial behaviors of their host. Changing environmental conditions over the course of development is likely to impact growth and thus limit the performance of the present growth model, parameterized under a 24 h daylight regime and stable temperatures. When exposed to long or short days and changing temperature conditions, lice development was slightly faster in the fall-to-winter scenario (decreasing temperatures) compared to a spring-to-summer scenario (increasing temperatures) for both daylight regimes (Table 6). The lice were sampled in a phase where the majority of the males were pa2 and thus in an instar growth phase between molts, where MnM remains stable over a long period (Figs. 4 & 5). The females were in the molting phase from pa1 to pa2 where MnM changes rapidly. Consequently, the female data provided a substantially higher resolution for comparing the growth rate among the experimental groups than the male data. It should be noted that this does not indicate that males react differently to changes in environmental conditions, but rather that the time of sampling happened to take place when one sex was in a molt phase whereas the other was not. The females in the 2 groups with decreasing temperature had molted on average 3.5 times and had developed faster than the 2 groups with increasing temperature, which had just started molting (MnM = 3.04). However, as mentioned above, while the molt rate can be regarded as stable on a wider temporal scale along development to the adult stage (all stages last approximately equally long), the molt rate displays a huge variation on a narrower time scale, from zero in phases between molts to high rates in phases of molting. It is thus difficult to evaluate the true biological significance and magnitude of the difference observed in this experiment and how it translates to units of time. Fig. 4 graphically links RA

and MnM. By reading the RA at sampling for the 2 temperature regime groups (comparing first observation of pa2 females vs. the point where 50% of the female population has become pa2), there is only about 4% difference in relative age. This translates, for instance, to a time difference equal to approximately 1.4 d at 10°C, or alternatively, to a temperature increase from 10.0 to 10.3°C. The practical significance of a 0.3°C difference is negligible, as temperature readings can often vary among thermometers by more than this.

Furthermore, although there was no significant effect of photoperiod, the interaction between light regime and temperature was statistically significant. This effect translates into a change in development time of merely 1%, a difference likely to be even less significant in practical terms. In conclusion, the light regime appears to have limited effect on *L. salmonis* post-infection development rate, similar to what has been observed for embryo development (Boxaspen & Naess 2000).

While the growth rate model was parameterized under a 24 h photoperiod and stable temperatures, *L. salmonis* in nature or fish farms usually develops in a constantly changing environment. Comparing the development in the respective groups to the predictions of the growth model reveals how well the model predicts lice growth under variable conditions (Table 6). The growth under long and short days and changing temperatures was similar to the growth predicted by the model, thus suggesting that (1) the observed effects of a changing environment compared to a stable environment are relatively small and (2) the model describes development in a changing environment, but potentially with a minor error.

Understanding the dynamics of egg production and the development and survival of planktonic stages is crucial for estimating the net production of infective salmon lice copepodids, and thus the potential infection pressure on wild and farmed fish. Here, the frequency of egg batch production was approximated by measuring the hatching frequency of subsequent egg strings. Estimates of the mean daily egg production, calculated by combining data from Samsing et al. (2016) with the present data, suggest that the estimated daily reproductive output is approximately 3 times higher at 18°C compared to 6°C. This effect size of warmer temperature has important consequences for parasite management. High temperatures result in higher egg production but also a decrease in the copepodid lifespan (Samsing et al. 2016), thus suggesting that high temperatures may lead to higher local infection pressures, whereas lower temperatures result in lower infection pressures spread over a wider area. However, the relationship between temperature, copepodid production and survival time of the infective copepodid in plankton must be modelled to fully understand the impact of temperature on overall infection pressure.

The time between hatching of 2 subsequent sets of egg strings reflects the total embryo development time, from fertilization to hatching, since females fertilize and extrude a new set of egg strings shortly after the previous egg strings hatch (L. A. Hamre pers. obs.). The present data thus allows a comparison of the effect of temperature on embryo development rate and the post-infection development rate, which in both cases progressed 4.2 times faster at 18°C than at 6°C. A recent study shows that the development rate of the planktonic stages (nauplius I and II) is similarly affected by temperature, where development from hatching to copepodid is approximately 4.9 times faster at 18°C than at 6°C (calculated from supplementary data from Samsing et al. 2016). Interestingly, development of *C. rogercresseyi* nauplii from hatching to copepodid also progressed 4.2 times faster at 18°C than at 6°C (Montory et al. 2018). The magnitude of change in growth rate in response to temperature thus appears similar for embryonic development, planktonic development and development of the post-infection life stages on the host fish. However, the survival time of the planktonic copepodid did not change in a similar manner with temperature (Samsing et al. 2016). This is perhaps not surprising, since copepodid lifespan is dependent on a finite energetic resource (yolk) and development is most likely put on hold to optimize survival and infection success. We thus hypothesize that the rate of all developmental processes in *L. salmonis*, including embryo development, development of the planktonic stages and post-infection development is similarly affected by temperature and can be expressed in terms of relative age units and translated to units of time by means of the present growth rate model (Table 4). We also consider it likely that this general principle applies to most caligid copepods.

The present study provides fundamental information required to better parameterize present and future infection pressure models (Groner et al. 2016, Rittenhouse et al. 2016, Sandvik et al. 2016). The results herein also enable precise back-calculation of louse settlement time to study the impact of host behavior and experience of environmental conditions (e.g. Oppedal et al. 2011, Wright et al. 2017), as well as models for lice mitigation management (Stien

et al. 2018). Furthermore, in the literature on caligid life cycles, data has been acquired using a variety of measures and temperatures, and therefore comparing development patterns is not straightforward. Here, the introductions of the term relative age allowed us to pool data and extract more details on *L. salmonis* development, but it is evident that RA also represents a measure that enables the establishment of temperature-independent descriptions of the life cycle patterns of caligid copepods, a description that is directly comparable among species and genera.

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LITERATURE CITED

Bjorn PA, Finstad B (1998) The development of salmon lice (*Lepeophtheirus salmonis*) on artificially infected post smolts of sea trout (*Salmo trutta*). Can J Zool 76:970–977

Bogevik AS, Henderson RJ, Mundheim H, Waagbo R, Tocher DR, Olsen RE (2010) The influence of temperature on the apparent lipid digestibility in Atlantic salmon (*Salmo salar*) fed *Calanus finmarchicus* oil at two dietary levels. Aquaculture 309:143–151

Boxaspen K, Naess T (2000) Development of eggs and the planktonic stages of salmon lice (*Lepeophtheirus salmonis*) at low temperatures. Contrib Zool 69:51–55

Brooker AJ, Skern-Mauritzen R, Bron JE (2018) Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Kroyer, 1837): current knowledge and implications for epidemiological modelling. ICES J Mar Sci 75:1214–1234

Browman HI, Boxaspen K, Kuhn P (2004) The effect of light on the settlement of the salmon louse, *Lepeophtheirus salmonis*, on Atlantic salmon, *Salmo salar* L. J Fish Dis 27:701–708

Carmichael SN, Bekaert M, Taggart JB, Christie HRL and others (2013) Identification of a sex-linked SNP marker in the salmon louse (*Lepeophtheirus salmonis*) using RAD sequencing. PLoS One 8:e77832

Carrington LB, Armijos MV, Lambrechts L, Barker CM, Scott TW (2013) Effects of fluctuating daily temperatures at critical thermal extremes on *Aedes aegypti* life-history traits. PLOS ONE 8:e58824

Crawley MJ (2007) The R book. Wiley, Chichester

Dawson LHJ, Pike AW, Houlihan DF, McVicar AH (1997) Comparison of the susceptibility of sea trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) to sea lice (*Lepeophtheirus salmonis* (Kroyer, 1837) infections. ICES J Mar Sci 54:1129–1139

Dawson LHJ, Pike AW, Houlihan DF, McVicar AH (1998) Effects of salmon lice *Lepeophtheirus salmonis* on sea trout *Salmo trutta* at different times after seawater transfer. Dis Aquat Org 33:179–186

Dawson LHJ, Pike AW, Houlihan DF, McVicar AH (1999) Changes in physiological parameters and feeding behaviour of Atlantic salmon *Salmo salar* infected with sea lice *Lepeophtheirus salmonis.* Dis Aquat Org 35:89–99

Eichner C, Hamre LA, Nilsen F (2015) Instar growth and molt increments in *Lepeophtheirus salmonis* (Copepoda: Caligidae) chalimus larvae. Parasitol Int 64:86–96

Eichner C, Dondrup M, Nilsen F (2018) RNA sequencing reveals distinct gene expression patterns during the development of parasitic larval stages of the salmon louse (*Lepeophtheirus salmonis*). J Fish Dis 41:1005–1029

Finstad B, Bjorn PA, Grimnes A, Hvidsten NA (2000) Laboratory and field investigations of salmon lice [*Lepeophtheirus salmonis* (Kroyer)] infestation on Atlantic salmon (*Salmo salar* L.) post-smolts. Aquacult Res 31:795–803

Fischer K, Kolzow N, Holtje H, Karl I (2011) Assay conditions in laboratory experiments: Is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? Oecologia 166:23–33

Fjørtoft HB, Besnier F, Stene A, Nilsen F and others (2017) The *Phe362Tyr* mutation conveying resistance to organophosphates occurs in high frequencies in salmon lice collected from wild salmon and trout. Sci Rep 7:14258

Glover KA, Stølen ÅB, Messmer A, Koop BF, Torrisen O, Nilsen F (2011) Population genetic structure of the parasitic copepod *Lepeophtheirus salmonis* throughout the Atlantic. Mar Ecol Prog Series 427:161–172

Gonzalez L, Carvajal J (2003) Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. Aquaculture 220:101–117

Grimnes A, Jakobsen PJ (1996) The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. J Fish Biol 48:1179–1194

Grimnes A, Finstad B, Bjorn PA (1996) Økologiske og fysiologiske konsekvenser av lus på laksefisk i fjordsystem. Report No. 381. Norwegian Institute of Nature Research, Trondheim

Groner ML, Rogers LA, Bateman AW, Connors BM and others (2016) Lessons from sea louse and salmon epidemiology. Philos Trans R Soc B 371:20150203

Hamre LA, Glover KA, Nilsen F (2009) Establishment and characterisation of salmon louse (*Lepeophtheirus salmonis* (Kroyer 1837)) laboratory strains. Parasitol Int 58:451–460

Hamre LA, Eichner C, Caipang CM, Dalvin ST and others (2013) The salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. PLOS ONE 8:e73539

Hansen TJ, Fjelldal PG, Folkedal O, Vågseth T, Oppedal F (2017) Effects of light source and intensity on sexual maturation, growth and swimming behaviour of Atlantic salmon in sea cages. Aquacult Environ Interact 9:193–204

Heuch PA, Nordhagen JR, Schram TA (2000) Egg production in the salmon louse [*Lepeophtheirus salmonis* (Kroyer)] in relation to origin and water temperature. Aquacult Res 31:805–814

Horreo JL, Machado-Schiaffino G, Griffiths AM, Bright D, Stevens JR, Garcia-Vazquez E (2011) Atlantic salmon at risk: apparent rapid declines in effective population size in southern European populations. Trans Am Fish Soc 140:605–610

Hvas M, Folkedal O, Imsland A, Oppedal F (2017) The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar.* J Exp Biol 220:2757–2764

Jensen AJ, Karlsson S, Fiske P, Hansen LP, Ostborg GM,

Hindar K (2014) Origin and life history of Atlantic salmon (*Salmo salar*) near their northernmost oceanic limit. Can J Fish Aquat Sci 71:1740–1746

Johannessen A (1977) Early stages of *Lepeophtheirus salmonis* (Copepoda, Caligidae). Sarsia 63:169–176

Johansson D, Juell JE, Oppedal F, Stiansen JE, Ruohonen K (2007) The influence of the pycnocline and cage resistance on current flow, oxygen flux and swimming behaviour of Atlantic salmon (*Salmo salar* L.) in production cages. Aquaculture 265:271–287

Johnson SC (1993) A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naive Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. In: Boxshall GA, Defaye D (eds) Pathogens of wild and farmed fish: sea lice. Ellis Harwood, Chichester, p 68–80

Johnson SC, Albright LJ (1991a) Development, growth, and survival of *Lepeophtheirus Salmonis* (Copepoda, Caligidae) under laboratory conditions. J Mar Biol Assoc UK 71: 425–436

Johnson SC, Albright LJ (1991b) The developmental stages of *Lepeophtheirus Salmonis* (Kroyer, 1837) (Copepoda, Caligidae). Can J Zool 69:929–950

Jones MW, Sommerville C, Bron J (1990) The histopathology associated with the juvenile stages of *Lepeophtheirus Salmonis* on the Atlantic salmon, *Salmo salar* L. J Fish Dis 13:303–310

Jonsdottir H, Bron JE, Wootten R, Turnbull JF (1992) The histopathology associated with the preadult and adult stages of *Lepeophtheirus Salmonis* on the Atlantic salmon, *Salmo Salar* L. J Fish Dis 15:521–527

Montory JA, Cumillaf JP, Cubillos VM, Paschke K, Urbina MA, Gebauer P (2018) Early development of the ectoparasite *Caligus rogercresseyi* under combined salinity and temperature gradients. Aquaculture 486:68–74

Morales-Serna FN, Rivas-Salas AI, Gómez S, Fajer-Ávila EJ (2015) Developmental stages and fecundity of *Lepeophtheirus simplex* (Copepoda: Caligidae) parasitic on bullseye puffer fish (*Sphoeroides annulatus*). Folia Parasitol 62:004

Novales Flamarique I, Browman HI, Belanger M, Boxaspen K (2000) Ontogenetic changes in visual sensitivity of the parasitic salmon louse *Lepeophtheirus salmonis.* J Exp Biol 203:1649–1657

Oppedal F, Dempster T, Stien LH (2011) Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. Aquaculture 311:1–18

Piasecki W, Mackinnon BM (1995) Life-cycle of a sea louse, *Caligus Elongatus* von Nordmann, 1832 (Copepoda, Siphonostomatoida, Caligidae). Can J Zool 73:74–82

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ritchie G (1993) Studies on the reproductive biology of *Lepeophtheirus salmonis* (Kroyer, 1838) on Atlantic salmon (*Salmo salar*, L.). PhD thesis, University of Aberdeen

Rittenhouse MA, Revie CW, Hurford A (2016) A model for sea lice (*Lepeophtheirus salmonis*) dynamics in a seasonally changing environment. Epidemics 16:8–16

Samsing F, Oppedal F, Dalvin S, Johnsen I, Vagseth T, Dempster T (2016) Salmon lice (*Lepeophtheirus salmonis*) development times, body size, and reproductive outputs follow universal models of temperature dependence. Can J Fish Aquat Sci 73:1841–1851

Sandvik AD, Bjørn PA, Ådlandsvik B, Asplin L and others (2016) Toward a model-based prediction system for salmon lice infestation pressure. Aquacult Environ Interact 8:527–542

Schram TA (1993) Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In: Boxshall GA, Defaye D (eds) Pathogens of wild and farmed fish: sea lice. Ellis Harwood, Chichester, p 30–47

Singh S, Mishra G, Omkar (2018) Plasticity in reproductive output and development in response to thermal variation in ladybird beetle, *Menochilus sexmaculatus.* J Therm Biol 71:180–188

Skern-Mauritzen R, Torrissen O, Glover KA (2014) Pacific and Atlantic *Lepeophtheirus salmonis* (Krøyer, 1838) are allopatric subspecies: *Lepeophtheirus salmonis salmonis* and *L. salmonis oncorhynchi* subspecies novo. BMC Genet 15:32

Stehfest KM, Carter CG, McAllister JD, Ross JD, Semmens JM (2017) Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors. Sci Rep 7:4545

Stien A, Bjørn PA, Heuch PA, Elston DA (2005) Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. Mar Ecol Prog Ser 290: 263–275

Stien LH, Lind MB, Oppedal F, Wright DW, Seternes T (2018) Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. Aquaculture 490:281–287

Taranger GL, Carrillo M, Schulz RW, Fontaine P and others (2010) Control of puberty in farmed fish. Gen Comp Endocrinol 165:483–515

Taranger GL, Karlsen Ø, Bannister RJ, Glover KA and others (2015) Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES J Mar Sci 72: 997–1021

Torrissen O, Jones S, Asche F, Guttormsen A and others (2013) Salmon lice — impact on wild salmonids and salmon aquaculture. J Fish Dis 36:171–194

Tucker CS, Sommerville C, Wootten R (2000a) The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon, *Salmo salar* L. J Fish Dis 23:309–320

Tucker CS, Sommerville C, Wootten R (2000b) An investigation into the larval energetics and settlement of the sea louse, *Lepeophtheirus salmonis*, an ectoparasitic copepod of Atlantic salmon, *Salmo salar.* Fish Pathol 35:137–143

Tucker CS, Norman R, Shinn AP, Bron JE, Sommerville C, Wootten R (2002) A single cohort time delay model of the life-cycle of the salmon louse *Lepeophtheirus salmonis* on Atlantic salmon *Salmo salar.* Fish Pathol 37:107–118

Wagner GN, Fast MD, Johnson SC (2008) Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids. Trends Parasitol 24:176–183

Warton DI, Hui FKC (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10

Wootten R, Smith JW, Needham EA (1982) Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. Proc R Soc Edinb (Biol) 81:185–197

Wright DW, Stien LH, Dempster T, Vagseth T, Nola V, Fosseidengen JE, Oppedal F (2017) 'Snorkel' lice barrier technology reduced two co-occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. Prev Vet Med 140: 97–105

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