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Effect of water temperature and exposure duration on detachment rate of salmon lice (*Lepeophtheirus salmonis*); testing the relevant thermal spectrum used for delousing

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A B S T R A C T

Thermal delousing has become the most applied method for treatment against salmon lice. However, the temperature range used is strongly aversive for salmonids, and the method is associated with increased mortality. Treatment temperature × duration combinations should be tailored to maximise delousing efficiency and minimize welfare impacts on the host fish. We tested the detachment rate of sessile, pre-adult and adult male and female salmon lice (*Lepeophtheirus salmonis*) as a function of ambient temperature (11–16 °C), exposure temperature (28–36 °C), and exposure duration (0–120 s). Dead Atlantic salmon (*Salmo salar*) hosts were used to avoid negative fish welfare and detachment of lice due to fish behaviour. Within the range tested, higher exposure temperatures were associated with higher detachment rates among pre-adult and adult lice, while no sessile lice detached at any temperature. Moreover, no treatment combination detached 100% of lice of any stage, and at 28 °C, detachment of adult females was negligible. Most detachments occurred within the first 30 s of exposure. We conclude that for a given delousing efficiency, lower temperatures must be compensated for by considerably longer exposure durations. This may be a higher risk for the welfare of the host fish than higher temperatures and shorter exposure durations.

1. Introduction

The ectoparasitic copepod salmon louse (*Lepeophtheirus salmonis*), one of the biggest challenges for salmonid aquaculture, has become increasingly resistant against chemotherapeutants (Denholm et al., 2002; Coates et al., 2021). In response, the industry has shifted to non-medicinal delousing over the last decade, with thermal treatments now the most applied method in Norwegian farms. This method is also the most likely to induce mortality (Overton et al., 2019). Thermal treatments are carried out by bathing the fish in water with temperatures of 28–34 °C for 20–30 s, with the intention of exceeding the thermal tolerance of the mobile life-stages of lice so that they detach from the host (Brunsvik, 1997; Grøntvedt et al., 2015; Roth, 2016). Based on the vigorous behavioural escape responses exhibited by Atlantic salmon (*Salmo salar*) during treatment, which increase with treatment temperature (Nilsson et al., 2019), the Norwegian Food Authorities has recommended a maximum temperature of 34 °C (Sjømat Norge, 2020). However, the interaction between temperature and duration has not been well described, making it difficult to optimize the trade-off between delousing efficiency and fish welfare. Use of temperatures that do not trigger detachment in sea lice will be highly counterproductive, as the treatment procedure itself will still be stressful for fish irrespective of treatment temperature or delousing success, and exposure of sea lice to sub-lethal temperatures may effectively drive thermal resistance in sea lice (Ljungfeldt et al., 2017). Alternatively, using overly high temperatures or overshooting the required treatment time would compromise fish welfare more than necessary (Nilsson et al., 2019).

Results from commercial thermal treatment of Atlantic salmon suggests that the treatment temperature itself is more significant for delousing success than the duration of exposure, and that the temperature difference from that of ambient water (Δ°T) is crucial (Roth, 2016). In the Norwegian industry guidelines for controlling salmon lice (Sjømat

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Norge, 2020) a Δ°T in the range 20–22.5 °C is recommended, but to the best of our knowledge, this range has not been supported by any published controlled experiments. A recent study by Andrews and Horsberg (2021) describes an in vitro bioassay for testing the sensitivity of sea lice to thermal treatments. The authors compared the thermal sensitivity of different lice strains (pre adult II life stage, acclimated to 12 °C) by measuring detachment rates from a glass flask after 2 min exposure to different temperatures; 33 °C was required for full detachment of all strains (Andrews and Horsberg, 2021). Similarly, experimental treatments have found that 33.7–34 °C was required for successful delousing of the mobile life-stages after 30 s exposure for lice acclimated to 10 °C (Folkedal et al., 2021). These findings imply that for efficient thermal treatment, both fish and sea lice acclimated to normal sea temperatures must either be exposed to a treatment temperature close to, or above, the allowed maximum in Norway, or the exposure duration must be extended if lower temperatures are used.

Ideally, parasite management should be based on knowledge of parasite tolerance thresholds before testing treatment effects on the host organism. For topical treatments such as thermal delousing in salmonids, the treatment strength (i.e. temperature level) and exposure duration are the key variables for establishing trade-offs between treatment success and impact on fish welfare. Testing of the isolated thermal treatment effect per se is not possible with the currently available devices used for commercial delousing, as the overall delousing effect is likely enhanced to an unknown degree by pumping, handling, flushing water and fish struggling behaviour (Grøntvedt et al., 2015; Roth, 2016). Even in controlled experiments with live fish, water temperatures relevant for delousing induce a strong behavioural response (Nilsson et al., 2019) that is likely to influence the detachment rate. To avoid confounding mechanical delousing effects, as well as welfare concerns when testing on live host fish, testing of lice sensitivity to temperature can be carried out in vivo by use of sacrificed salmon.

We tested the effect of water temperature and exposure duration on salmon lice detachment, using a custom-made apparatus for collection of lice detached from dead salmon at two relevant ambient sea temperatures.

2. Materials and methods

2.1. Host salmon and experimental site

Salmon of the Aquagen strain were used as host fish for the experiment. On 1 June 2020, 100 fish were randomly sampled from a group of ~4000 fish in a 12 × 12 m and 14 m deep sea cage and transferred to a 5 × 5 m and 5 m deep sea cage at the Institute of Marine Research, Austevoll. The fish were deloused on 15 June using a manual procedure which included anesthesia before thermal exposure (described in Folkedal et al., 2021). After this the fish were reared according to standard practice and were naturally infested with salmon lice. Thermal exposures were conducted on two occasions, 43 days apart (3 September 2020 and 16 October 2020) to compare lice naturally acclimated to different ambient temperatures (16 °C and 11 °C, respectively). The mean fish size was 45 ± 3 cm total length and 920 ± 236 g (± SD) on the first occasion and 50 ± 5 cm and 1359 ± 343 g on the second occasion.

2.2. Experimental setup

A flow tunnel was constructed for exposure of both host and lice to different temperatures (Fig. 1). The tunnel consisted of a plastic pipe with an inner diameter of 154 mm, bent 90° at each end of a 730 mm straight horizontal section. The end sections were left open to the air, allowing introduction of fish and water flow from the upstream end and outflow from the downstream end. The downstream end section could be swiveled downwards to fully empty the flow tunnel of water. The flow tunnel was placed on top of an 800 L container filled with approximately 400 L water. Water in the container was continuously pumped through a heater (T3C – DELTA from ELECRO engineering Ltd. and pump FLYGT SXM2SG/A) that maintained the desired temperature. A second pump (EHEIM Typ: 1262 21 0) moved water from the container into the flow tunnel at 45 L min^{-1}, resulting in a mean current speed of 4 cm s^{-1}. The water fell back into the container after exiting the tunnel.

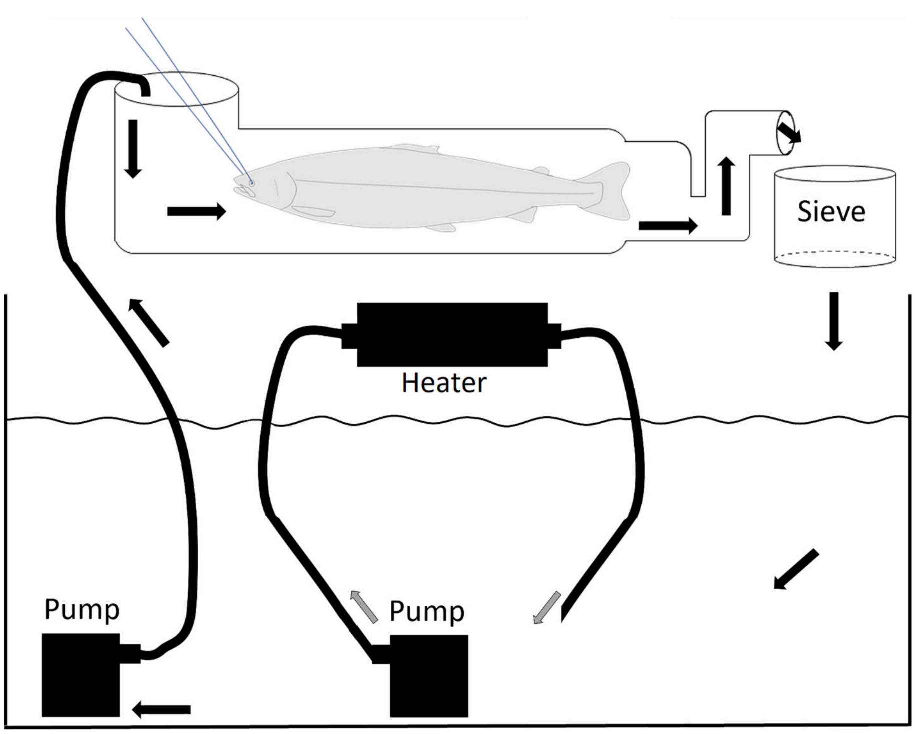

Fig. 1. Illustration of the setup. Arrows indicate the direction of the water flow. Not to scale.

2.3. Experimental procedure

From each ambient temperature, freshly-euthanised salmon with attached lice were exposed to one of six different treatment temperatures: 28, 30, 32, 34, 36 °C (n = 5–6 salmon per temperature, Table 1).

Salmon were carefully netted from their holding sea cage into a 1 m^3 container with oxygenated seawater at ambient temperature, before being moved in groups of 10–20 fish to the experimental room. Salmon were taken out one at the time and killed with a blow to the head. Immediately thereafter, a steel wire was attached to the head, from which the fish was suspended during the thermal test. The test started within 30 s after the fish was killed, by lowering it approximately 20 cm into the horizontal section of the tunnel (Fig. 1). A sieve (23 cm diameter, 180 μm mesh size) was held below the outflow to collect lice detached from the host salmon. The sieve was replaced every 15 s for 2 min to allow detachment rates to be scored over 15 s intervals. Thereafter, the fish was removed, and the end section of the tunnel was lowered to drain the tunnel and ensure complete replenishment. This water was also filtered through a sieve to collect any remaining lice. The number of individual lice of each stage was carefully counted in each sieve, identified as stages chalimus 1, chalimus 2, pre-adult, adult male or adult female. The smallest sessile stage, the copepodite, was not included as they were too small to be reliably counted under these conditions. Due to time constraints, no distinction was made between the pre-adult 1 and pre-adult 2 stages, between male and female pre-adults, or between adult females with or without egg strings. After being removed from the tunnel, the fish were examined for remaining lice, which together with the number of lice collected in the sieves, gave the total number of lice of each stage. Lice were not counted on the fish before the exposure, as it was considered more important that the lice be exposed quickly following the death of the host. In a pilot study of 8 hosts, we never found fewer lice in the sieves and post-exposure count than in the pre-exposure count, while the opposite was the case in 5 of the 8 hosts. This suggests that few lice are missed by the post-exposure counts, and/or that pre-exposure counts, given the need to perform them quickly, are likely to underestimate the number of attached lice.

To estimate the time delay between lice detaching and entering the sieve, 19 dead adult female lice were released manually 20 cm into the flow tunnel, approximately where the head of the host salmon were placed during exposure, and the exact time taken to reach the sieve was measured. The median and mean transit time was 19 and 20.7 s, respectively, ranging from 7 to 40 s. As lice detaching from the head will have the longest distance to travel before entering the sieve, we assume that the median delay from the entire host body is slightly shorter. Estimated time for detachment was therefore adjusted with 15 s, so that lice collected in the first sieve, held below the tunnel exit from 0 to 15 s of exposure, were considered to be those that detached immediately when the salmon was introduced to the tunnel (0 s), lice collected in the sieve between 15 and 30 s were considered to have detached between 1 and 15 s, and so forth.

2.4. Data analysis

Detachment rates were analysed using hierarchical logistic regression models, with the probability of detachment predicted by exposure temperature (integer), exposure duration (integer) and lice stage (factor), including full interaction terms. To limit model complexity, separate models were fitted for the two ambient temperatures (Model 1: 11 °C, Model 2: 16 °C). The response variable was specified in binomial form, i.e. cumulative counts of detached and non-detached lice at the end of each 15 s interval. The identity of the host fish was specified as a random intercept term to account for non-independence of lice from the same fish or flow tunnel run. Exposures at the ambient temperature were omitted to improve the fit of the model to data from treatment temperature exposures (28–36 °C). The analysis was conducted using the R programming language (R Core Team, 2022), with models fitted using the *glmmTMB* function in the *glmmTMB* package (Brooks et al., 2017) and assessed using the *simulateResiduals* function in the *DHARMa* package (Hartig, 2019) and the *Anova* function in the *car* package (Fox and Weisberg, 2019). Interaction effects were interpreted using adjusted predictions generated by the *ggpredict* function in the *ggeffects* package (Lüdecke, 2018).

An additional model (Model 3) was fitted to detachment data for pre-adult lice only, simplifying the model enough that it was possible to test the effect of ambient temperature or Δ°T. The same could not be achieved by omitting pre-adult lice, as there were too few adult lice present on fish taken from 16 °C ambient temperature (Table 1). To identify whether exposure temperature or Δ°T was the best predictor of detachment rates, we fitted two competing models (Model 3A and 3B). Detachment rates and fish identity were specified as for Models 1 and 2,

Table 1
Number of host salmon, total number of lice for all hosts combined for each stage per exposure temperature (bold font), median (regular font) and range (in brackets) number of lice of each stage per host and exposure temperature. Data are presented for occasions in September, when the ambient sea temperature was 16 °C, and October when the ambient sea temperature was 11 °C.

		Ambient	28 °C	30 °C	32 °C	34 °C	36 °C
September (16 °C ambient)	# host salmon	6	5	5	5	5	5
	Chalimus 1	**40** 6.5 (0–15)	**21** 5 (2–5)	**18** 4 (0–6)	**11** 2 (1–4)	**12** 1 (1–5)	**9** 2 (0–4)
	Chalimus 2	**13** 1 (0–9)	**7** 1 (0–4)	**11** 0 (0–11)	**6** 1 (0–2)	**1** 0 (0–1)	**10** 2 (1–3)
	Pre-adult	**42** 7 (5–10)	**50** 7 (5–21)	**65** 12 (9–19)	**74** 17 (10–18)	**75** 15 (13–16)	**121** 14 (10–57)
	Adult male	**3** 0.5 (0–1)	**5** 0 (0–3)	**16** 2 (0–11)	**7** 2 (0–2)	**16** 4 (1–5)	**22** 3 (0–12)
	Adult female	**1** 0 (0–1)	**14** 1 (0–12)	**22** 2 (0–17)	**2** 0 (0–1)	**14** 0 (0–11)	**9** 2 (0–4)
October (11 °C ambient)	# host salmon	5	5	5	5	5	5
	Chalimus 1	**0** 0 (0–0)	**0** 0 (0–0)	**0** 0 (0–0)	**0** 0 (0–0)	**0** 0 (0–0)	**0** 0 (0–0)
	Chalimus 2	**3** 0 (0–2)	**1** 0 (0–1)	**0** 0 (0–0)	**2** 0 (0–1)	**3** 0 (0–2)	**1** 0 (0–1)
	Pre-adult	**38** 9 (5–9)	**52** 12 (7–13)	**101** 21 (16–23)	**77** 15 (13–18)	**58** 14 (0–17)	**107** 20 (10–33)
	Adult male	**20** 4 (3–5)	**41** 8 (6–10)	**40** 7 (4–12)	**43** 8 (7–11)	**27** 6 (0–8)	**35** 7 (4–10)
	Adult female	**43** 9 (6–10)	**48** 8 (5–15)	**59** 11 (9–16)	**45** 12 (2–14)	**44** 9 (0–15)	**59** 12 (10–13)

while the fixed effects varied: Model 3A included exposure temperature and exposure duration with an interaction effect, while Model 3B included Δ°T and exposure duration with an interaction effect.

3. Results

There was considerable variation in the density of lice between host individuals, louse life stages, and months (ambient temperatures) (Table 1). Chalimus 1 and 2 stages were more prevalent in September (16 °C ambient temperature) than in October (11 °C ambient temperature), while adult stages exhibited the opposite pattern (Table 1).

No sessile lice (chalimus 1 or 2) were detached by exposure to any of the treatment temperatures tested, up to the 120 s maximum duration. Mobile stages were more susceptible to detachment when exposed to high temperatures, with all exposures to treatment temperatures leading to higher detachment rates than ambient temperatures, regardless of the mobile stage (pre-adult, adult male or adult female) or ambient temperature (11 or 16 °C) (Fig. 2). However, none of the temperatures tested consistently removed 100% of pre-adult, adult male or adult female lice, even after 120 s (Fig. 2).

The statistical models revealed that detachment rate was predicted by significant interactions between exposure temperature, exposure duration and louse stage. For lice from 11 °C ambient temperatures (Model 1), there were significant two-way interactions between all three model terms, while the three-way interaction was not statistically significant (Table 2). For lice from 16 °C ambient temperatures (Model 2), only the temperature × duration and duration × stage interactions were significant (Table 2). All interaction effects modulated the size but not direction of the main effects, such that higher temperatures and/or longer durations reliably led to higher detachment rates for each life stage (Fig. 2). Higher temperatures reduced the duration required to achieve a given detachment rate, yet longer durations did not necessarily reduce the temperature required. Instead, there was some evidence that cumulative detachment rates plateau well below 100% when lice are exposed to treatment temperatures in the range of 28–30 °C for realistic durations (Fig. 2).

Among pre-adult lice, both exposure temperature and Δ°T significantly predicted detachment rates, via interactions with exposure duration. However, the comparison of Models 3A and 3B indicated that exposure temperature was a better predictor than Δ°T (Akaike Information Criterion: 1687 cf. 1753, respectively). We therefore proceeded with a model containing exposure temperature and exposure duration, but also included ambient temperature and full interaction effects (Model 3, Table 2). Detachment rates were strongly predicted by the combined effects of exposure temperature and duration, but not by the ambient temperature main effect, nor any interaction effects involving ambient temperature (Table 2). However, the nonsignificant effect of ambient temperature was in the expected direction (higher detachment rates among pre-adult lice acclimated to 11 °C than 16 °C: Fig. 3).

4. Discussion

The present study shows that mobile salmon lice detach faster at higher temperatures, but with incomplete delousing achieved for any temperature × duration combination that is likely to fall below the lethal threshold for salmon (Nilsson et al., 2019). The delousing effect was modest at 28 and 30 °C, while exposures >30 °C were considerably more effective. However, within a typical exposure duration of 30 s, as is normal for thermal treatment of farmed salmonids, the detachment rate was generally <80% for mobile lice, even at 34–36 °C.

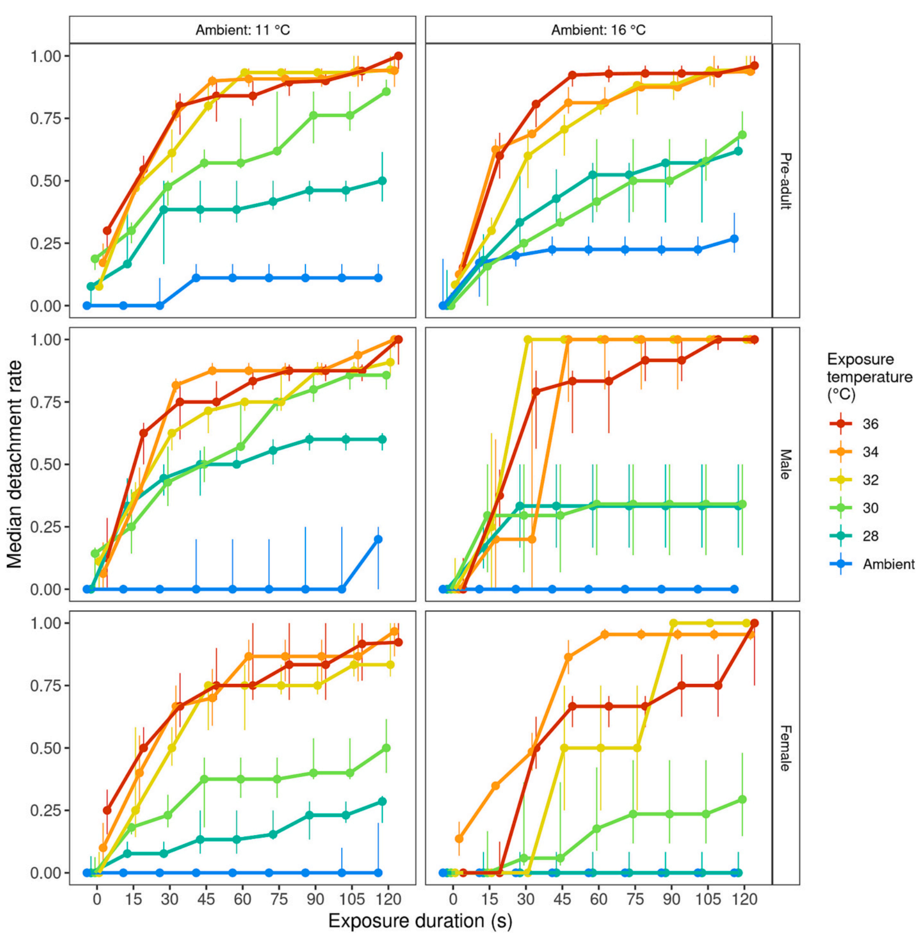

Fig. 2. Cumulative proportion of salmon lice detaching from the host at 15 s time intervals during a thermal treatment applied within a flow tunnel. Data are plotted by stage (pre-adult, adult male or adult female lice) and ambient temperature (11 or 16 °C). Points show median values and error bars show 25th and 75th percentile values. The exposure duration variable is adjusted for the estimated time delay between lice detaching and being collected by the sieve at the pipe outlet, see section 2.3. for details.

Table 2
Analysis of variance tables for Models 1, 2 and 3. Models were fitted using the glmmTMB package for R, with a binomial family and logistic link function. ANOVA tables were generated using the car package for R, with type II sums of squares. Model terms are denoted as follows: treatment temperature, $T_{Treatment}$; duration, $D_{Treatment}$; louse stage, *Stage*; ambient temperature, $T_{Ambient}$. Each model tests a subset of the complete data set: Model 1: $T_{Ambient} = 11$ °C; Model 2: $T_{Ambient} = 16$ °C; Model 3: *Stage* = Pre-adult.

Model 1	X^2	df	P
$T_{Treatment}$	81	1	<0.0001
$D_{Treatment}$	995	1	<0.0001
Stage	163	2	<0.0001
$T_{Treatment} \times D_{Treatment}$	62	1	<0.0001
$T_{Treatment} \times Stage$	41	2	<0.0001
$D_{Treatment} \times Stage$	19	2	<0.0001
$T_{Treatment} \times D_{Treatment} \times Stage$	2	2	0.36

Model 2	X^2	df	P
$T_{Treatment}$	54	1	<0.0001
$D_{Treatment}$	671	1	<0.0001
Stage	59	2	<0.0001
$T_{Treatment} \times D_{Treatment}$	59	1	<0.0001
$T_{Treatment} \times Stage$	0.1	2	0.95
$D_{Treatment} \times Stage$	10	2	0.006
$T_{Treatment} \times D_{Treatment} \times Stage$	4	2	0.13

Model 3	X^2	df	P
$T_{Treatment}$	141	1	<0.0001
$D_{Treatment}$	1425	1	<0.0001
$T_{Ambient}$	2	1	0.18
$T_{Treatment} \times D_{treatment}$	52	1	<0.0001
$T_{Treatment} \times T_{Ambient}$	0.7	1	0.40
$D_{treatment} \times T_{Ambient}$	1.7	1	0.19
$T_{Treatment} \times D_{treatment} \times T_{Ambient}$	0.5	1	0.50

Lice levels may vary greatly in time and between individuals (Bui et al., 2020a, 2020b), which was also the case in the present study. Due to low densities of adult lice in September (16 °C ambient temperature), it was not possible to test whether detachment of these stages was influenced by Δ°T. However, among pre-adult lice, the absolute exposure temperature was a better predictor of detachment rates than Δ°T, at least for the range of ambient and exposure temperatures included in the present study. This finding is not in accordance with the recommendation of a Δ°T of 20–22.5 °C (Sjømat Norge, 2020), although we note that the present study was not designed to test effects of Δ°T, and it is possible that a wider range of ambient temperatures would have revealed an effect of Δ°T on detachment rates at a given exposure temperature.

The detachment rate of sea lice was highest during the first 30 s of thermal exposure, especially at 34–36 °C, which is in line with Roth (2016) who concluded that the exposure temperature is more significant than the exposure duration. Most fish, irrespective of treatment temperature, still had mobile lice attached after 120 s of exposure, indicating that complete delousing will be difficult to achieve with this method, and certainly not with temperature and duration combinations that support fish welfare. Although not documented in the present study, lice that remain attached might very well survive. This is supported by a recent finding that for most strains, >50% of copepodites and pre-adult lice remained alive 24 h after an exposure to 35 °C for 120 s (Andrews and Horsberg, 2020).

Pre-adult lice generally detached faster than the adult lice, which corresponds with the higher survival rates of adult lice than pre-adult lice when exposed to freshwater (Wright et al., 2016). Using abundance of pre-adults as a threshold for when to treat would therefore allow for shorter treatment durations and reduce the welfare load per treatment. However, thermal treatment appears to have little effect on the sessile chalimus 1 and 2 stages, as shown by Grøntvedt et al. (2015) and Roth (2016) in addition to the present study, as the sessile stages are attached to the host with a frontal filament. Chalimus 2 can develop to

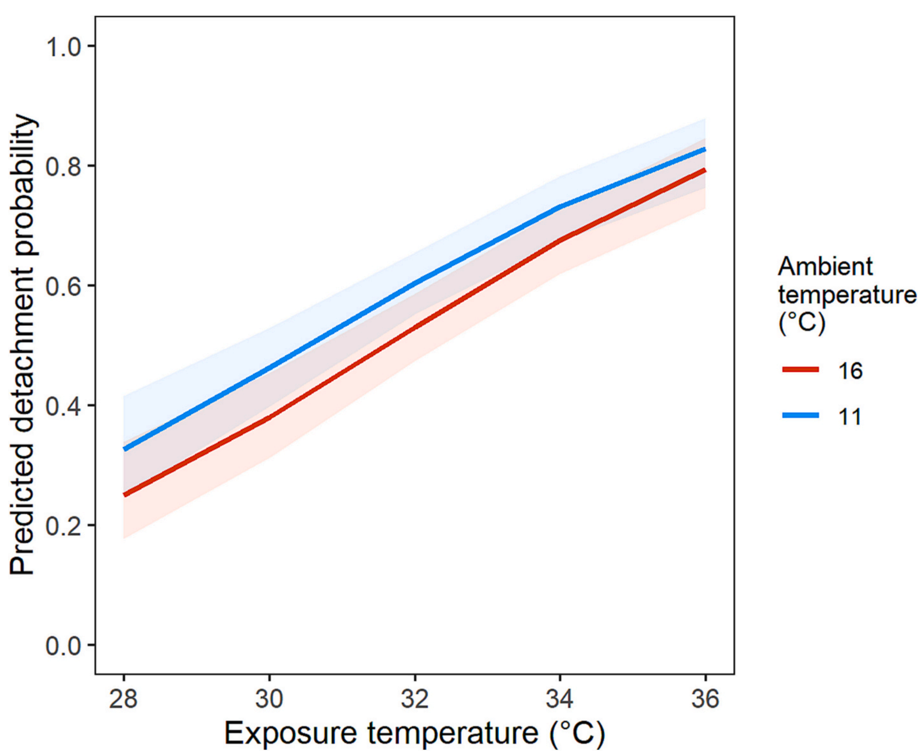

Fig. 3. Predicted proportion of pre-adult lice detached from the host after exposure to treatment temperatures between 28 and 36 °C, according to the ambient temperature at which the lice were acclimated (11 or 16 °C) (Model 3). Predicted detachment rates are conditional on 30 s of exposure to the treatment temperature, as this is a common duration for thermal treatments applied within the industry.

pre-adult 1 within a few days at summer temperatures (Hamre et al., 2019), and treatments based on abundance of pre-adults rather than of adult females would therefore have to occur more frequently and likely increase the total welfare load on the fish and the cost for the farmer.

The test apparatus used in the current study proved useful in obtaining estimates of lice detachment sensitivity with respect to temperature. However, the transit speed from detachment to collection is variable, probably due to turbulence in the tunnel, and salmon lice are spread over the entire body surface of the host salmon (Bui et al., 2020a), affecting the distance between the detachment point and the collection sieve. Together, these sources of error will have added some variance to the exposure duration effect, although they are not expected to have driven any systematic bias.

Bioassays have been developed for testing the sensitivity of lice to chemotherapeutants (Helgesen and Horsberg, 2013) and non-medicinal methods such as low salinity and temperature (Andrews and Horsberg, 2020, 2021). The findings from the present study support the validity of the bioassay by Andrews and Horsberg (2021), which scored detachment from a glass flask rather than from a host. Comparing pre-adult lice collected from the same geographical area, similar ambient temperature (12 °C) and using the same thermal exposure window as the present study, Andrews and Horsberg (2021) found detachment rates of 70 and 80% after 120 s at 30 and 31 °C respectively, and 100% at ≥32 °C. Here, using lice collected from 11 °C, we found detachment rates of 85% after 120 s at 30 °C and ~ 95% at 32–36 °C. Unlike Andrews and Horsberg (2021), we also observed lice detaching at ambient temperature (16% after 120 s). Fish handling prior to exposure in our study, including killing with a blow to the head, may explain that difference.

The detachment rate in the present study, with dead and thus motionless salmon in slow running water, is likely lower than would have occurred in an industry setting. In a commercial delousing operation, the host fish are subjected to mechanical impact (crowding, pumping and dewatering) before entering the treatment chamber. The strong behavioural response to the heated water, associated with pain and potential tissue damage (Nilsson et al., 2019; Gismervik et al., 2019; Moltumyr et al., 2021; Moltumyr et al., 2022), is likely to contribute further to the detachment of lice. However, in an ideal world, delousing efficiency should not depend on stress responses by the hosts, and treatments should aim to minimize stress (Folkedal et al., 2021). We therefore consider detachment rates independent of host behaviour to be more relevant for the improvement of thermal delousing techniques.

In conclusion, increasing treatment temperatures from 28 to 36 °C has a large effect on the lice detachment rate, with a weak delousing effect at 28–30 °C, especially for adult female lice. At temperatures between 32 and 36 °C, most detachment occurred within the first 30 s, and the additional delousing effect gained by extending exposure duration was modest. Salmon panic behaviour and loss of control also increases over time when exposed to these temperatures (Nilsson et al., 2019). A trade-off between treatment success and impact on fish welfare should therefore focus on the higher temperatures and shorter exposure durations, not vice versa. Combinations of temperature and duration that result in acceptable fish welfare and delousing effects should be identified via experiments with small numbers of fish, and bioassays should be employed on-site before delousing to reduce the need for repeated treatments of thermally-tolerant lice strains.

Author contribution

AMJ and TH designed and built the flow tunnel apparatus. JN, AMJ, VN, TH and OF contributed during the experiments. LTB did the statistical analysis of the data. JN and OF wrote the first draft of the manuscript, and all authors contributed to and approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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