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Salmon lice loads on Atlantic salmon smolts associated with reduced welfare and increased population mortalities

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ABSTRACT: Modelling potential impacts of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon *Salmo salar* requires estimation of the levels that cause serious sub-lethal physiological impacts and direct mortality. Here we analysed results from existing laboratory experiments to identify 2 thresholds; the lower threshold (T1) estimates the level at which lice loads cause systemic sub-lethal effects on smolts likely to impact performance, and the upper threshold (T2) identifies the lice load causing direct mortality. T1 is an empirical value based on a catalogue of lice impacts according to impact type (superficial or systemic) and concentration, converted from a range of experiments. The onset of these indicators of welfare and behavioural impacts indicates T1 ≈ 0.08 lice g^{-1} of host. T2 represents 50% probability of onset of mortality under laboratory conditions quantified using a dose-response curve based on data collated from studies where mortality has occurred or not at specified lice concentrations. T2 ≈ 0.24 lice g^{-1} with a bootstrapped 95% confidence interval between 0.1 and 0.67 lice g^{-1}. These thresholds offer both an amending of the current approach to the management of lice concentrations on fish in the form of a sub-lethal welfare threshold and further evidence to support mortality-based lice thresholds already in use.

KEY WORDS: Parasite-induced mortality · Sea lice · Wild fish · *Lepeophtheirus salmonis* · *Salmo salar*

1. INTRODUCTION

Sea lice, a family of parasitic copepods, cause major economic and biological problems for farmed and wild salmonid fish (Torrissen et al. 2013), costing about 9% of total farm revenues (Abolofia et al. 2017). The key sea lice species causing these impacts in the Northern Hemisphere are the salmon louse *Lepeophtheirus salmonis* (Pike & Wadsworth 1999) and to a lesser extent some *Caligus* species (Hemmingsen et al. 2020). Feeding by lice on Atlantic salmon *Salmo salar* (hereafter referred to as 'salmon' unless specified) causes injuries including local tissue erosion (Nolan et al. 1999), reduced osmoregulatory capacity (Wells et al. 2005), stress (Finstad et al. 2000), impediments to swimming ability (Wagner et al. 2003) and reduced general immune response (Fast et al. 2002) and can compromise physiology and performance. If infestation levels are sufficiently high, then mortality can ensue (Wootten et al. 1982, Jónsdóttir et al. 1992, Torrissen et al. 2013).

Salmon lice not only damage farmed salmon but can affect the health of wild salmonid fishes, as determined, for example, by reduced mortality of groups of hatchery-reared salmon treated with an anti-sea-lice agent (Vollset et al. 2016). Female lice

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on salmonids produce strings of eggs which, after hatching, develop through 2 nauplii stages into planktonic larval copepodids. Copepodids are transported by water currents and may infect wild salmon in the marine environment (Taranger et al. 2015, Murray & Moriarty 2021). Modelling of larval lice transport has been progressively refined and resultant infection pressure validated (Salama et al. 2018, Sandvik et al. 2020), allowing increasingly accurate predictions of exposure and subsequent level of infestation of wild salmon. However, to assess the significance of this infestation, quantification of the impact of salmon lice on salmon is required.

A number of studies have assessed the impacts of lice on salmon, which can range from localised, superficial injuries (Nolan et al. 1999, Long et al. 2019) through more severe welfare impacts (Wagner et al. 2003, 2004, Wells et al. 2005), to mortality (Grimnes & Jakobsen 1996, Fjelldal et al. 2020). Currently, specific levels of mortality within salmon populations are used in management systems in Norway (e.g. Taranger et al. 2011, 2015), where 20% mortality is expected at 0.1–0.2 lice g^{-1}, 50% mortality at 0.2–0.3 lice g^{-1} and 100% at >0.3 lice g^{-1}. Damage from salmon lice at levels that are sub-lethal in controlled laboratory conditions may increase the risk of secondary viral and bacterial infection (Fast 2014) and, in the wild, may increase vulnerability to predation due to compromised performance (e.g. reduced swimming speed; Wagner et al. 2003, Bui et al. 2016). Furthermore, physiological stress may reduce growth rates, with additional consequences for survival and size at spawning (Fjelldal et al. 2020, 2022). Hence, a lower sub-lethal threshold that accounts for the increased risks of indirect mortality and reduced fitness was conceived (Hvas & Bui 2022). This threshold (T1) is defined as the lowest level at which substantial systemic sub-lethal impacts are observed. A direct impact threshold (T2) is the level of lice that can cause mortality due to the damage from sea lice alone under benign laboratory conditions.

Taranger et al. (2015) used combined results from experiments on 3 salmonid species, namely sea trout *Salmo trutta*, Arctic charr *Salvelinus alpinus* and Atlantic salmon (Nolan et al. 1999, Wagner et al. 2003, 2004, Tveiten et al. 2010), as part of a risk assessment framework, to create mortality probabilities of salmon based on lice loads. Wagner et al. (2008) reviewed the general physiological and immunological impacts of sea lice through a wide range of both field and laboratory studies on multiple salmonid species and identified general sub-clinical (beyond which physiological impacts are observed) and clinical (beyond which morbidity and epizootics occur) thresholds. Here, we focus on amalgamating available published data from laboratory experiments relating to wild-origin and farmed Atlantic salmon through a literature review, to identify the evidence to quantify T1 and T2. These thresholds can be used within modelling and management frameworks to understand and predict the impacts we could expect to see in wild populations. Here, we consider wider welfare impacts on wild salmon populations for use within such management frameworks, and we quantify evidence of direct lice-induced mortality through a dose-response analysis using data from the literature review.

2. METHODS

2.1. Literature review

A literature review was performed with the aim of collating studies assessing the physiological impacts of lice on salmon using PubMed/NCBI, Google scholar and Web of Science in January 2022 using the following search terms: (salmon* or smolt*) AND (sea lice* or *Lepeophtheirus salmonis*). The studies were manually screened, with those identified for inclusion focussed on lice impacts on salmon smolts and post-smolts, and allowed load to be calculated as lice g^{-1} wet fish weight either through direct inclusion of these values in the studies or calculation from fish weight and number of lice per fish. The studies used comparable methods for holding fish in controlled environments with lice added during the experiment. Those studies included in the dose-response analysis assigned the occurrence of salmon mortality (defined as death of salmon, occurring within a specific experimental replicate or sampling group) to specific treatment replicates, sampling events and time points. All studies incorporated groups of both lice-infested and control fish. Only 1 study (Fjelldal et al. 2020) reported mortality within control groups, which was attributed to a bacterial infection not found in the lice-infested tanks in that experiment. Seven studies reported measuring fish weight at the end of the experiment, whereas the others did not specify the point at which weight was measured (Nolan et al. 1999, Ross et al. 2000). Only 3 studies reported fish length. Therefore, lice loads were related to weight rather than length in our primary analysis, and a weight–length conversion was used to illustrate critical lice loads as a function of fish length, which is most readily measured in field studies. The conversion was per-

formed separately for farmed and wild smolts using regression equations derived from relevant populations (farmed: Pert et al. 2021; wild: Morris et al. 2019) (see Section 2.2). The relevant studies identified were: Finstad et al. (2000), Fjelldal et al. (2020), Grimnes & Jakobsen (1996), Long et al. (2019), Nolan et al. (1999), Ross et al. (2000), Wagner et al. (2003), Wagner et al. (2004) and Wells et al. (2005). Data from these studies are collated in Table 1. Of these studies, two stated explicitly that they used salmon sourced from wild populations (reared in hatcheries) (Finstad et al. 2000, Fjelldal et al. 2020; these studies are italicised in Table 1) with another study giving a stock number and river name, suggesting they were also reared from wild stock (Wagner et al. 2003). One other study (Wells et al. 2005) explicitly stated it used fish from farmed stock (bold in Table 1), the rest only included the use of Atlantic salmon without comment on specific source other than hatchery reared (Grimnes & Jakobsen 1996, Nolan et al. 1999, Ross et al. 2000, Wagner et al. 2004, Long et al. 2019). Five of the studies were performed in Norway, 2 in Canada, 1 in Ireland and 1 in Scotland, with the Canadian studies using Pacific strains of *Lepeophtheirus salmonis*.

Table 1. Data collated from Finstad et al. (2000) (F), Fjelldal et al. (2020) (FJ), Grimnes & Jakobsen (1996) (G), Long et al. (2019) (L), Nolan et al. (1999) (N), Ross et al. (2000) (R), Wagner et al. (2003) (W3), Wagner et al. (2004) (W4), and Wells et al. (2005) (W), with calculated lice g^{-1} values, average fish weights, the length of experiment before it ended or mortality was recorded (days post infection, DPI), and the recorded temperature if given (Fjelldal et al. 2020 included temperatures for rearing but not for the experiment itself and so these are not included). In the mortality column, N = no mortality occurred, Y = mortality occurred. Number of fish is the number of fish sampled for lice measurements if given or the number in the experimental replicate. Studies that explicitly stated using farmed fish stock are highlighted in **bold** (Wells et al. 2005), and those that stated using wild fish stock are *italicised* (Finstad et al. 2000, Fjelldal et al. 2020). Aim refers to the original intent of the study. Sub-lethal means the study looked at predominantly physiological effects such as tissue damage, whereas stress means the study studied stress responses through measurement of hormones such as cortisol. PA: pre-adult; A: adult; Ch: chalimus; NA: data not available

Lice stage	Lice g^{-1}	Mortality	Number of fish	Fish weight (g)	Temperature (°C)	Source	DPI	Aim
Ch, PA	*0.683*	*N*	*15*	*60*	*7–10*	*F*	*21*	*Stress*
Ch, PA	*1.95*	*Y*	*15*	*60*	*7–10*	*F*	*18*	*Stress*
PA	*0.583*	*N*	*15*	*60*	*7–10*	*F*	*25*	*Stress*
PA	*1.567*	*Y*	*15*	*60*	*7–10*	*F*	*25*	*Stress*
PA	*0.383*	*Y*	*15*	*60*	*7–10*	*F*	*29*	*Stress*
PA	*0.9*	*Y*	*15*	*60*	*7–10*	*F*	*28*	*Stress*
PA, A	*0.383*	*N*	*15*	*60*	*7–10*	*F*	*32*	*Stress*
PA, A	*0.6*	*Y*	*15*	*60*	*7–10*	*F*	*30*	*Stress*
PA, A	*0.116*	*N*	*15*	*60*	*7–10*	*F*	*40*	*Stress*
PA, A	*0.367*	*Y*	*15*	*60*	*7–10*	*F*	*40*	*Stress*
PA	*0.46*	*Y*	*55*	*40*	*NA*	*FJ*	*28*	*Stress*
PA	*0.33*	*Y*	*55*	*40*	*NA*	*FJ*	*28*	*Stress*
PA	*0.36*	*Y*	*55*	*40*	*NA*	*FJ*	*28*	*Stress*
PA	1.525	N	28	40	10–11	G	27	Sub-lethal
PA	1.95	Y	19	40	10–11	G	27	Sub-lethal
PA	1.2	N	7	40	10–11	G	31	Sub-lethal
PA	1.425	Y	17	40	10–11	G	29	Sub-lethal
PA, A	1.225	N	5	40	10–11	G	34	Sub-lethal
PA, A	1.2	Y	7	40	10–11	G	31	Sub-lethal
A	0.002	N	10	90.1	10	L	7	Sub-lethal
A	0.002	N	10	90.1	10	L	7	Sub-lethal
A	0.044	N	10	90.1	10	L	7	Sub-lethal
PA, A	0.0133	N	7	225	15	N	10	Stress
PA, A	0.0267	N	7	225	15	N	10	Stress
PA, A	0.0444	N	7	225	15	N	10	Stress
PA	2.97	Y	40	60	9–14	R	12	Sub-lethal
PA	2.97	Y	40	60	9–14	R	17	Sub-lethal
A	0.02	N	12	609.3	9.5	W3	31	Swimming
A	0.13	N	7	609.3	9.5	W3	31	Swimming
A	0.08	N	9	656.7	9.5	W4	38	Swimming
PA	**0.224**	**Y**	**10**	**97.4**	**13.6**	**W**	**14**	**Sub-lethal**
PA	**0.504**	**Y**	**10**	**97.4**	**13.6**	**W**	**14**	**Sub-lethal**
PA/A	**0.341**	**Y**	**10**	**97.4**	**13.6**	**W**	**14**	**Sub-lethal**
PA/A	**0.252**	**Y**	**10**	**97.4**	**13.6**	**W**	**21**	**Sub-lethal**

These experiments were designed primarily to observe physiological effects of salmon lice and hence were terminated when mortalities started to occur. Therefore, we used a binary classification of mortality or no mortality (Table 1) and cannot evaluate the levels of mortality that could have occurred within experiments. It seems probable that had experiments been allowed to continue, mortality rates would have been higher than those observed and perhaps may have occurred at lower lice loads. However, an experiment with the explicit aim of killing multiple animal subjects would be ethically objectionable. In light of this constraint, we believe our current approach to be an appropriate and ethically sound alternative. Treatments <7 d were excluded from our analysis, as even under severe physiological compromise, fish may be morbid for several days prior to mortality actually occurring. This cut-off excluded a number of replicates from Long et al. (2019) of 1–5 d duration despite lice being adults when infestation took place. Details of each experiment included in the analysis are provided in Table 1.

2.2. Calculating lice per gram in reviewed experiments

For sub-lethal impacts, some studies supplied lice g^{-1} values directly, whereas in others, the average fish weight was used with defined lice numbers to calculate a comparable figure. Similarly, most of the data employed to derive the dose-response curve used reported lice abundance divided by average fish weight to calculate lice g^{-1} of host fish (Table 1).

Fish weights (W, g) were converted to lengths (L, mm) using the equation $L = 0.63 \times W + 144.77$ for farmed smolts and $L = 2.13 \times W + 83.76$ for wild smolts derived from 2 datasets where n = 3770 (Pert et al. 2021; farmed) and n = 4278 (Morris et al. 2019; wild). Weight/length ranges from these 2 datasets had almost no overlap; therefore, conversions were split into discrete ranges based on separate regression equations.

Lice numbers used here were those measured on the fish when mortality occurred or the experiments ended (mean experiment length 18 d; range 7–40 d) (Table 1), apart from 1 study which did not state when lice were counted, although analyses from that study showed no significant change in infestation levels over time (Nolan et al. 1999). There are differences in reported lice development stage during infestation between these studies, but all lice were recorded as having reached pre-adult/adult stages by the end of each experiment.

2.3. Data analysis

For T1, a catalogue of critical lice levels at which a range of physiological responses become evident was derived from published studies to highlight where impacts shift from localised minor injuries to compromised general physical condition and welfare. Below T1, impacts are typically limited to the area around the louse feeding site and superficial in nature (e.g. minor lesions and cellular damage). Above T1, the impacts are detectable in whole-body responses that impact the welfare and physical condition of the host (e.g. decreases in red blood cell count and cardiac output). Reported results in each study were examined to identify the lowest recorded lice concentration at which there was a significant change in a physiological response from a control level. Specifically, T1 was chosen as the lowest lice load where there is evidence of systemic physiological impacts and compromised physical condition. T2 was derived from a meta-analysis of studies to quantify the threshold level at which onset of mortality is evident in a benign environment.

The T2 threshold was assessed as 50% probability of onset of mortality within a group of salmon and was derived using a dose-response curve for mortality which was fitted using the 'drc' package version 3.0-1 (Ritz et al. 2015) within the R statistical environment version 4.1.2 (R Core Team 2021). We applied a constrained, 2-parameter log–logistic curve where e represents the lice per gram at which 50% probability of mortality occurs, and b is the exponent, described by the function:

$$Y = \frac{1}{1 + \left(\frac{X}{e}\right)^{b}} \quad (1)$$

where Y = probability of mortality for a given value of X (lice per gram of fish), $b = -0.976559$ and $e = 0.242949$.

In the statistical analysis package R, this equation can be derived using the syntax:

$$\text{Mortality} \sim \text{lice load, weight} = \log(\text{fish numbers}), \text{fct} = \text{LL.2}(), \text{type} = \text{Binomial} \quad (2)$$

Mortality/survival (recorded as a binary response) indicates whether mortality occurred within the experiment/replicate. The lice load represents the lice g^{-1} for that data point (i.e. the lice concentration at which mortality was/was not recorded). LL.2 specifies the type of model used, in this case 2-term log–logistic. Some replicates were terminated for fish welfare reasons and therefore mortality is available

only as a population level binary response. Number of fish is the number sampled for lice measurements if given or the number in the experimental replicate (Table 1). The model was bootstrapped with 1000 iterations to test both the confidence interval bounds and estimate due to high variance in the upper bounds of the data.

3. RESULTS

The literature review found 9 studies that fit the criteria outlined above; the sub-lethal thresholds used in the determination of T1 can be found in Table 2. The collated data extracted from the studies and used in the calculation of T2 are given in Table 1.

3.1. Sub-lethal T1 threshold

Studies have measured a range of parameters related to lice impacts on individual salmon (Table 2). Skin disruption was measured at lice loads of >0.008 lice g^{-1} after 5 d post infection (DPI), with the more severe impacts, such as reductions in swimming ability and cardiac output, beginning at lice loads of >0.08 lice g^{-1} after 35 DPI. Breakdown of osmoregulation was first identified at lice loads of >0.21 lice g^{-1} after 21 DPI. In all cases where parameters were repeatedly measured over time, lice-impact thresholds decreased with increasing time. For example, osmolality increased at lice loads of 0.44 lice g^{-1} after 14 DPI, but similar effects were seen at 0.21 lice g^{-1} after 21 DPI (Table 2).

Our selection of T1 hinged on the occurrence of key physiological effects with potential for indirect lethal impacts in natural conditions. These effects occur at ~0.08 lice g^{-1} with measurable impacts on cardiac output, swimming capabilities and reductions in haematocrit. Further systemic physiological impacts have been shown at similar values between 0.12 and 0.14 lice g^{-1}, giving further evidence that for management purposes, a threshold at T1 = 0.08 lice g^{-1} could be used to identify a critical lice loading.

3.2. Mortality dose-response analysis and the T2 threshold

Experiments under benign laboratory conditions confirm a direct association between load per gram of *L. salmonis* and mortality of salmon. For instance,

Table 2. Threshold values of lice g^{-1} for change in sub-lethal physiological measurements derived from specified studies. Direction indicates whether the parameter increased or decreased from control level. Days post infection (DPI) are recorded at the point at which significant differences from control groups were recorded. In cases where 2 values exist for the same physiological measurement, the order matches the order of lice g^{-1} values. Rows in *italics* are those where lice g^{-1} values were supplied directly from the source article

Lice g^{-1}	Physiological measurement	Direction	Fish weight g^{-1}	DPI	Source
0.008–0.054	Skin disruption	Increase	90.1	5–7	Long et al. (2019)
0.0133–0.044	Skin disruption	Increase	200–250	5–10	Nolan et al. (1999)
0.0133–0.044	Gill epithelial disruption	Increase	200–250	5–10	Nolan et al. (1999)
0.044	Plasma calcium	Decrease	90.1	7	Long et al. (2019)
0.08	*Cardiac output*	*Decrease*	*656.8*	*35*	*Wagner et al. (2004)*
0.08	*Haematocrit*	*Decrease*	*656.8*	*35*	*Wagner et al. (2004)*
0.08–0.13	*Swimming performance*	*Decrease*	*609.3–656.8*	*35, 32*	*Wagner et al. (2003, 2004)*
0.12	Plasma chloride	Increase	60	40	Finstad et al. (2000)
0.13	*Plasma chloride*	*Increase*	*609.3*	*32*	*Wagner et al. (2003)*
0.13–0.14	Haematocrit	Decrease	97.4	21, 14	Wells et al. (2005)
0.18	*Plasma chloride*	*Increase*	*40*	*30*	*Fjelldal et al. (2020)*
0.2–0.4	Cortisol	Increase	97.4	21, 14	Wells et al. (2005)
0.21–0.44	Osmolality	Increase	97.4	21, 14	Wells et al. (2005)
0.22	*Plasma sodium*	*Increase*	*40*	*30*	*Fjelldal et al. (2020)*
0.38–0.6	Plasma chloride	Increase	60	29, 21	Finstad et al. (2000)
0.6	Cortisol	Increase	60	7	Finstad et al. (2000)
1.525–1.875	Condition factor	Decrease	40	27, 25	Grimnes & Jakobsen (1996)
1.525–1.875	Plasma chloride	Increase	40	27, 25	Grimnes & Jakobsen (1996)
1.525–1.875	Haematocrit	Decrease	40	27, 25	Grimnes & Jakobsen (1996)
1.525–1.875	Serum albumin	Decrease	40	27, 25	Grimnes & Jakobsen (1996)
1.525–1.875	Serum protein	Decrease	40	27, 25	Grimnes & Jakobsen (1996)

Grimnes & Jakobsen (1996) found that ~95% mortality of the population occurred at high lice densities once lice moulted to the pre-adult life cycle stage. Fjelldal et al. (2020) found that mortalities increased as lice densities on fish increased, rising from 3% at 0.2–0.3 lice g^{-1} to 29% at >0.6 lice g^{-1} after 28 DPI.

Data included in the mortality dose response analysis contain 34 experimental groups, 16 with no mortality and 18 with some mortality (Table 1). These data were collected as end points in experiments designed to assess physiological consequences of lice infection. Therefore, it is not possible to assess the proportion of each population that may have died due to the lice infection. However, it is possible to model with a binomial distribution to assess whether there was any mortality in treatments with lice relative to controls.

We found a significant relationship between lice loads and probability of mortality (SE = 0.071, t = 3.428, p = 0.0006) (Fig. 1). The 50% probability of any mortality (±13%) occurs at lice loads of T2 = 0.24 lice g^{-1}. The bootstrap process found that the 95% confidence interval (CI) calculated for the dose (lice load) T2 lies between 0.11 and 0.67 lice g^{-1}, with the bootstrapped median confirming the T2 estimate at 0.24 lice g^{-1}. As expected, the lower bound and the estimate both show strong agreement with those calculated from the original model, but the upper bound is higher than the original estimate.

Threshold loadings of lice in absolute numbers vary with fish weight. The implication for critical numbers of lice to reach thresholds across the range of smolt lengths typically seen in wild and culture scenarios is illustrated in Fig. 2. For example, for small wild smolts of 11 cm, the calculated T1 is 0.9 lice $fish^{-1}$, with T2 at 2.6 lice $fish^{-1}$, whereas equivalent levels for large farmed smolts (20 cm) are ~8 and >20 lice $fish^{-1}$, respectively.

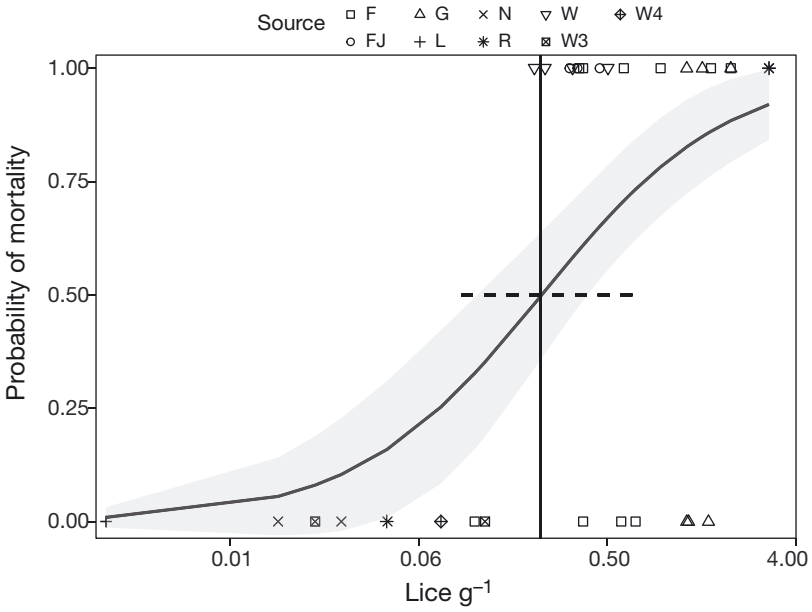

Fig. 1. Observed abundance of preadult/adult lice (lice g^{-1}) infection associated with no mortality = 0 or some mortality = 1 and predicted dose-response curve for lice load versus proportion of populations in which mortality occurs. Shaded band shows 95% confidence interval for probability of mortality at a given lice load. Vertical line shows lice load at which there is predicted 50% probability of mortality (T2), with dashed line showing bootstrapped 95% CI around this threshold as lice g^{-1}. Symbols represent experimental replicates with (1) or without (0) mortality, N = 34 (Table 1). Source refers to study data from Finstad et al. (2000) (F), Fjelldal et al. (2020) (FJ), Grimnes & Jakobsen (1996) (G), Long et al. (2019) (L), Nolan et al. (1999) (N), Ross et al. (2000) (R), Wagner et al. (2003) (W3), Wagner et al. (2004) (W4), and Wells et al. (2005) (W)

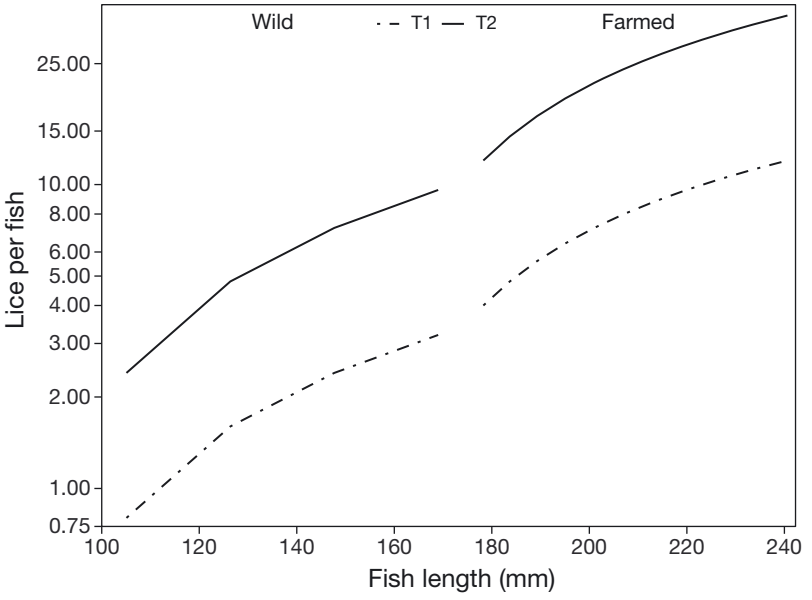

Fig. 2. Number of pre-adult/adult lice per fish needed for severe sub-lethal impacts to occur (T1) and 50% probability of mortality commencing in benign laboratory populations (T2) in relation to fish length. Data were converted from weight using equations L = xW + y, where x and y are derived from measurements of farmed (Pert et al. 2021) and wild (Morris et al. 2019) smolts in separate equations

4. DISCUSSION

Here we have combined results from the literature regarding sub-lethal physiological impacts of salmon lice on Atlantic salmon smolts. We have catalogued a range of lice loads at which specific physiological measurements show significant differences from uninfected control groups. Broadly, these impacts are expressed as stress responses and superficial injuries at ~0.008 lice g^{-1} (Long et al. 2019) and become more severe (i.e. reduced osmoregulatory ability, reduced cardiac output and reduced swimming performance) at ~0.08 lice g^{-1} (Wagner et al. 2004). The critical threshold T1 may therefore be considered to be 0.08 lice g^{-1}, as these lice-induced changes all impact the ability of a salmon smolt to migrate safely to its oceanic feeding grounds through direct reductions in physical endurance and performance, which can lead to reduced productivity and indirect mortality. The level of lice infestation at which the probability of mortality reaches 50% in most populations even under benign laboratory conditions was calculated as T2 = 0.24 lice g^{-1}. No experimental studies have explicitly measured mortality as the primary response to infestation levels with sea lice; rather, mortality was observed as an unwanted outcome of efforts to measure physiological responses to lice infestations. Therefore, mortality could not be expressed as a continuous variable in our analysis. If experiments had continued for longer periods, then higher levels of mortality may have occurred and mortality may have ensued at lower lice loadings, but that was not explicitly tested in the experiments reviewed. In some cases, experiments or replicates within experiments were ended early due to welfare concerns; for instance, Wells et al. (2005) ended the high-level infestation replicate due to high levels of mortality after 21 DPI. For these reasons, estimates of T2 values could be overestimated. Data on impacts of salmon lice on salmon health continue to be published (Fjelldal et al. 2022, Hvas & Bui 2022), and therefore these estimates may continue to be refined. In practice, it is ethically questionable to use mortality as a deliberate experimental end point and therefore the analysis presented here is a best approximation of the maximum lice loading at which mortality ensues under controlled laboratory conditions.

4.1. Sub-lethal lice threshold (T1)

Catalogued responses to lice infestation that are sub-lethal in a controlled laboratory environment vary widely in both severity and type (see Table 2). At lice loads below T1 (0.008–0.08 lice g^{-1}), the impacts are limited to local, relatively superficial surface-layer and cellular damage (Nolan et al. 1999, Long et al. 2019). As lice loads increase in excess of T1, the impacts are more severe and generalised, with larger-scale physiological changes becoming apparent. For instance, decreases in haematocrit were seen in lice loadings of 0.14 lice g^{-1}, with osmolality and a large range of other parameters varying significantly from control levels at ~0.2 lice g^{-1} (Wells et al. 2005). These impacts reflect generalised stress and difficulties in osmoregulation. Other studies have found broadly similar results, such as increased plasma chloride in lice-infested fish following induced exercise at 0.13 lice g^{-1} (Wagner et al. 2003), at routine levels of activity at 0.12 (Finstad et al. 2000) and 0.18 lice g^{-1} (Fjelldal et al. 2020), indicating impaired physical condition and dehydration. This is further evidenced by a 19% reduction in swimming performance at 0.13 lice g^{-1} (Wagner et al. 2003).

The length of time elapsed since lice infestation of the fish was an important determinant of severity of impact. The sub-lethal T1 thresholds for plasma chloride decreased with time after infection. Wagner et al. (2003) found a significant change in plasma chloride at 0.13 lice g^{-1} but only after exercise after 32 DPI, and Fjelldal et al. (2020) saw a similar effect at 0.18 lice g^{-1} after 30 DPI. With increased time, 40 DPI, a similar effect was then seen at 0.12 lice g^{-1} (Finstad et al. 2000), showing that the longer lice are attached, the more severe the physiological effects can be and so the estimate of T1 is lower.

Another major factor affecting determination of lice thresholds is the life cycle stage of lice on the fish, particularly as a cause of mortality. All of the studies used in our analysis reported that almost all mortality occurred once lice entered pre-adult/adult stages. Lice of these stages move between feeding sites, leading to increased wounds, and their gut contents contain a greater proportion of fish blood (Braden et al. 2020). Grimnes & Jakobsen (1996) used very high lice densities and yet saw very little mortality until after the lice moulted into the pre-adult stage at ~23 DPI. At this stage, survival of the fish was ~95% and yet 3 d later, survival had dropped to ~65% and by the end of the experiment after 32 DPI, only ~5% of infected fish were still alive. Even for sub-lethal effects, life cycle stage plays a critical role in severity of impact. A recent study found no differences between measures of respiratory condition between low and high lice densities when all lice were at the chalimus life cycle stage. However, once lice had reached pre-adult stages, significant differences were found across all measures between fish

with pre-adult lice and those with chalimus-stage lice and controls (Hvas & Bui 2022).

As the host fish grows, the infestation level expressed as the number of lice per gram reduces. For example, smolt length on day D may be modelled as $L_D = L_0 e^{0.0059D}$ (Mork et al. 2012), where L_0 is initial length. From this relationship, over a 16 d period between infection and lice becoming mobile, a fish with a starting length of 125 mm grows to 137 mm, corresponding to a weight change of 19.5 to 25.2 g (see Section 2.2) and to a 30% increase in lice numbers per fish that would be needed to reach T1 or T2. For extrapolations of lice thresholds to wild smolts, typical weights and sizes are sometimes lower than the weight/length ranges of the fish used in the experiments included in our analyses (Fig. 2). Hence, there is an assumption that the threshold in terms of lice per gram of fish is constant across sizes of smolts.

4.2. Lethal lice threshold (T2)

Our analysis of mortality (Fig. 1) quantifies a level at which the probability of any mortality in a group exceeds 50% (T2 = 0.24 lice g^{-1}). Unfortunately, the reporting of mortality did not allow accurate numbers or proportions within groups to be established; however, published evidence suggests that the proportion of individuals that die increases with lice infestation level. For instance, the lowest lice loads where mortality occurred were found by Wells et al. (2005), where at 0.224 lice g^{-1}, 2 fish died from a group of ~30. At 0.341 lice g^{-1}, 8 fish were reported to have died, whereas at 0.504 lice g^{-1}, 26 individuals died, with the group being discontinued due to welfare concerns. In experiments with higher lice loads of 1.2–2.97 lice g^{-1}, mortality and morbidity occurred for almost all fish in experimental groups, with some replicates having been ended early due to extremely high levels of mortality (Grimnes & Jakobsen 1996, Ross et al. 2000). Grimnes & Jakobsen (1996) observed mortality at 30 lice per 40 g fish, which equates to 0.75 lice g^{-1}. Across all studies included in our analysis, no mortalities were recorded in any of the experimental populations at loads <0.2 lice g^{-1} (Fig. 1). This value of T2 = 0.24 lice g^{-1} may be termed a critical load under controlled conditions.

4.3. Transfer of thresholds to wild conditions

In the studies reviewed herein, fish were held under laboratory conditions. In the wild, impacts at lice infestation levels above T1 that would be sub-lethal in laboratory tanks may be compounded by factors such as co-infection with viruses, bacteria or parasites, pollution, thermal stress, and osmoregulatory stress. For instance, increased infestations of *Lepeophtheirus salmonis* lead to more skin lesions and higher levels of mortality when co-infected with the bacterium *Moritella viscosa* (Carvalho et al. 2020), one of the main causes of winter ulcer disease. Co-infection in a farm or wild setting can have a substantial impact on fish health and mortality rates. Environmental factors such as temperature have been shown to affect impacts of salmon lice, as well as their growth and maturation (Pike & Wadsworth 1999). Impacts of lice on infested fish increase with rising temperatures (Godwin et al. 2020). Temperature was recorded in the studies analysed here, but the range was small (9–15°C) and was not reported consistently. Therefore, temperature was not included in the dose-response model. Water quality can also interact with lice infection; for example, smolts originating from acidified rivers may be more vulnerable to lice when they enter the sea (Finstad et al. 2007).

Reductions in smolt swimming speed and cardiac output due to lice infestation that are sub-lethal in controlled laboratory settings are likely to both increase vulnerability to predation, and reduce feeding efficiency in the wild (Wagner et al. 2003). Low feeding efficiency would reduce growth, resulting in reduced size at maturity leading to reduced reproductive output (Vladic & Petersson 2015). For example, hatchery-reared wild salmon smolts infested with 0.38 lice g^{-1} grew at 0.4% d^{-1}, compared to 1% d^{-1} for uninfected fish (Fjelldal et al. 2020). Hence, smolts that survive lice infestation may suffer long-term effects that can lead to reductions in population reproductive output. Even following delousing with freshwater, lice-infested fish were shown to be shorter than uninfested fish following 38 d recovery time, although weight differences found during infestation had disappeared (Fjelldal et al. 2022). Furthermore, since mortality of salmon smolts in the lower river and at sea is inversely related to size and condition (Armstrong et al. 2018), it is likely that effects of sea lice on growth and energy reserves will similarly affect subsequent survival.

Smolts with infestation levels above T2 would not only be at high risk of mortality from the direct effects of lice grazing on body tissues, but also likely highly vulnerable to predation and open to attack from other infections and infestations.

4.4. Application of T1 and T2 in management models

In a management context, to assess risk from or impact of sea lice on wild smolts, T1 and T2 thresholds can be used as part of a modelling framework. Integration of best available data on salmon smolt dispersal together with sea lice dispersal, survival and infestation behaviour can be used to estimate numbers of lice on wild salmon as a function of lice on farmed salmon (Murray & Moriarty 2021, Murray et al. 2022). Such models currently use simple generalisations of lice and fish dispersal, but in future can be extended to an individual-based particle as further information becomes available regarding local hydrology and wild smolt behaviour. These models need threshold values to assess implications of modelled lice loadings. From our review, it is reasonable to take T1 as a threshold above which there is a seriously heightened risk of impacts of sea lice on growth and survival of wild salmon. We see T2 as representing a level where substantial mortality due to lice may be inevitable, even in cases where there are few predators and environmental stressors to add to direct impacts of lice damage seen in a laboratory environment.

Mortality profiles due to sea lice in wild smolts with lice levels ranging between T1 and T2 can be expected to vary substantially in time and space depending on the predator community, food availability, disease risk and environmental stressors (Myksvoll et al. 2018). Efforts have been made to arrive at a standardised estimate of possible mortality as part of the Norwegian Traffic Light System for managing open-pen aquaculture to protect wild salmon. In this case, equivalents to our T1 and T2 are put forward by Taranger et al. (2011, 2015) who, combining data for various salmonids together with expert opinion, arrived at assumptions that mortality is 20% at 0.1–0.2 lice g^{-1} and 50% at 0.2–0.3 lice g^{-1}. The lower threshold level (0.1–0.2 lice g^{-1}) seems reasonable in view of our estimate that risk from lice increases substantially above 0.08 lice g^{-1}. The upper level (0.2–0.3 lice g^{-1}) also seems reasonable as a management threshold in light of our T2 calculation of 0.24 lice g^{-1}. An earlier review (Wagner et al. 2008) also explored thresholds for sub-clinical physiological and clinical impacts. However, as with Taranger et al. (2015), these cover multiple salmonid species and also contain estimates from field studies. Despite this, the sub-clinical threshold (T1 equivalent) proposed by Wagner et al. (2008) for salmonids (0.1 lice g^{-1}) is approximately the same as in Taranger et al. (2015). The threshold (0.08 lice g^{-1}) proposed in our paper is specifically for Atlantic salmon. The clinical threshold (morbidity/mortality) derived by Wagner et al. (2008) is also derived directly from the literature but is higher (0.75 lice g^{-1}) than the equivalent T2 figure calculated using the dose-response model here (0.24 lice g^{-1}) and in Taranger et al. (2015) (0.2–0.3 lice g^{-1}). This figure of 0.75 lice g^{-1} is based on citations from 3 reviews (Pike & Wadsworth 1999, Tully & Nolan 2002, Fast & Johnson 2004) and is the same as the level derived from Grimnes & Jakobsen (1996) (30 lice for a 40 g salmon). Given our results and the results of papers published since then, we posit that this is an overestimate of the lice g^{-1} value at which salmon mortality is likely to occur.

In conclusion, we have compiled available information from laboratory trials to quantify 2 threshold impact levels for Atlantic salmon. T1 may be considered a threshold for elevated risk; T2 is a level at which, under laboratory conditions, mortality occurs due to direct damage from lice. These thresholds provide important context for interpreting consequences of lice recorded or predicted on wild salmon smolts.

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