

Salmon lice survive the straight shooter: A commercial scale sea cage trial of laser delousing



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ABSTRACT

Ectoparasitic salmon louse (*Lepeophtheirus salmonis*) infestations are costly for Atlantic salmon (*Salmo salar*) farmers in Norway. As a result, there is a strong desire for solutions to prevent and control infestations, and new technologies are typically developed and commercialised rapidly, without rigorous validation. Here, we tested the efficacy of a new commercially available control measure—delousing by underwater lasers—using a replicated design at full commercial scale. Laser delousing was used in combination with a preventive method (snorkel cages), with laser nodes deployed in 3 of the 6 sea cages at the site. The trial ran for 54 days, after which time there was no difference in infestation density of mobile salmon louse stages (pre-adult, adult male or adult female) in cages with or without laser nodes installed. By the end of the trial, adult female lice numbers in all cages were close to the legislated trigger for mandatory delousing (0.5 adult female lice per fish). The laser nodes delivered a large number of pulses relative to the number of lice in the cages, indicating that a lack of lethality rather than a lack of target detection was the limiting factor. If all pulses had been effective, they should have removed between 4–38 % of mobile lice each day. There was no effect on salmon welfare indicators such as skin condition or eye status. Our results highlight the importance of rigorous validation of new technologies across a range of conditions before widespread implementation by industry.

1. Introduction

Aquaculture is a relatively young industry compared to terrestrial production systems (Nash, 2011), with systematic research and development only becoming a key focus since the 1970s (Kumar and Engle, 2016). However, commercial production of finfish has become a highly lucrative industrial process analogous to modern agriculture (Asche et al., 2018; Ashche, 2008), with rapid technological advances facilitating substantial productivity growth in recent decades (Asche et al., 2018).

The farming of Atlantic salmon (*Salmo salar*) is a shining example of rapid industry growth, with commercial production initiated only ~50 years ago. Although the production volume of Atlantic salmon is less than 5% of global finfish production, it is the most valuable fish product (FAO, 2018). Norway is the principal producer of salmon, and its success can be partly attributed to technological innovation, productivity advancements, and efficiency at multiple levels of the production process. However, the industry faces significant obstacles in sustainability

(Klinger and Naylor, 2012; Olesen et al., 2010), with the most prominent risk factor being the proliferation of the ectoparasitic salmon lice, *Lepeophtheirus salmonis* (Murray et al., 2016). There has been substantial focus on prevention and control of lice in aquaculture, to reduce environmental and welfare impacts of infestations on both wild and farmed salmonids (Costello, 2009; Heuch et al., 2005; Krkosek et al., 2007; McVicar, 2004; Overton et al., 2018; Thorstad et al., 2015; Torrissen et al., 2013).

To manage and reduce the negative impacts of salmon lice, the Norwegian Ministry of Trade, Industry and Fisheries enforces a limit on infestation levels on all Norwegian salmonid farms, whereby companies must ensure less than 0.5 adult female lice on their fish (0.2 during the season of out-migration for wild salmonids) (Norwegian Ministry of Fisheries and Coastal Affairs, 2012). In the last decade, there has been an increase in technological innovation development around the management of lice in farms as traditional chemical or medicinal treatments have become less effective and unsustainable (Aaen et al., 2015; McNair, 2015). Most of these innovations can be considered short-term

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(i.e. minor adjustments to current farming practices), and classed as preventive, continuous, or immediate strategies (Brakstad et al., 2019). Preventive approaches aim to minimise exposure of the host to the infective planktonic stages of the lice, for example by shielding a section of the cage (Frank et al., 2015; Stien et al., 2018), changing the cage structure (Stien et al., 2016), or using stimuli to move the school's vertical distribution (Bui et al., 2019). Continuous approaches attempt to control levels of lice on the fish by use of invertivorous cleaner fish (Imsland et al., 2018; Treasurer, 2002) or functional feed (Jensen et al., 2015). Immediate delousing control methods are implemented when preventive or continuous strategies are unsuccessful and lice levels are nearing or exceeded the legislative threshold, requiring rapid reductions in infestation. Delousing treatments can include chemical therapeutants, freshwater bathing, mechanical removal, and thermal treatments (Overton et al., 2018).

Optical or laser delousing is a unique innovation that has overcome multiple technical challenges to produce an instrument that combines machine learning louse detection with targeted louse removal. The method aims to control salmon lice infestation levels using laser pulses (concentrated photons) targeted at machine-identified lice to injure the parasite but not the host. 'Nodes' submerged under buoys within the sea cage contain an automated camera system that scans passing fish, identifies potential lice on the fish, and instigates a pulse of light directed at the suspected lice. The images of lice on salmon collected from nodes are transmitted back to the database and are used to continually train the machine learning system. The pulses apparently do not harm the skin of the salmon, while the system can identify and avoid the fish's eye (Brown, 2016; Frenzl, 2017). Generally, multiple nodes are deployed in each sea cage, and are monitored externally by a technical support team. The system has been commercially available since 2014 and at present, is reportedly in use in around 150 cages in Norway.

Here, we present a case study for a technology that has been adopted by industry, but has not yet been validated scientifically. This study aimed to test the efficacy of laser delousing for the control of salmon lice at a commercial salmon farm, as well as its short-term effects on salmon welfare. The farm had applied an integrated pest management strategy – prevention (snorkel cages; Geitung et al., 2019) in combination with continuous control (lasers). The laser delousing strategy was implemented over 54 days, to a point where the lice level was nearing the threshold that triggers delousing action.

2. Methods

The trial was conducted at a commercial fish farm (Låva) in Jelsafjorden, western Norway (59.1°N, 5.6°E). The farm had 6 circular sea cages ($\varnothing = 50.9$ m, C = 160 m, 36 m deep) arranged in a single row from the feed barge (from south-west to north-east). All cages had a 16 m deep snorkel ($\varnothing = 28.4$ m, C = 90 m) installed before the fish arrived. The cages were stocked with Atlantic salmon (*Salmo salar*, autumn-transferred smolts, SalmoBreed strain) one month before trial start. Throughout the experimental period the farm was managed according to standard rearing and feeding procedures for commercial salmon aquaculture.

The trial ran for 54 days between 6 December 2018 to 28 January 2019. At the start of the experiment, each cage held between

157000–165000 salmon with an average weight of 250–450 g. Every second cage (i.e. the 2nd, 4th and 6th cages heading north-east along the single-row site) was equipped with 2 lasers (Optical Delousing™, Stingray Marine Solutions AS, Norway), as recommended for cages of this size and this number of fish, to undergo the continuous laser delousing treatment. The remaining 3 cages had no additional delousing strategies and therefore acted as controls. The lasers were installed at the location by Stingray's service team in cooperation with the fish farmers on 5 December 2018. As is the standard protocol, daily operation and monitoring of the laser nodes were performed by a technical support team at Stingray's main offices in Oslo, not onsite. Each day had a variable operational time, the period during which the lasers were active; this varied depending on the decision of Stingray's operations team and is directly correlated with the number of pulses emitted. Thus, pulses per day was the best measure of effective operation and was used as such in the analyses. Daily updates on salmon positioning from fish farmers were used to place the laser nodes at the most optimal depth in the cage (the largest proportion of fish visible to the cameras). Nodes could be rotated and moved vertically from the surface to a maximum depth of 25 m. The nodes were cleaned once a month during the experimental period, according to manufacturer recommendations. On day 34 of the experimental period (9 January 2019) one laser was taken out of Cage 6 and sent for repair. Cage 6 continued with a single laser for the remainder of the trial.

Daily salinity and temperature profiles between 0–40 m depth were collected from a reference point (the feed barge) using a Conductivity, Temperature and Depth (CTD) recorder (SD208, SAIV-AS, Bergen, Norway). Thermal stratification with cooler upper layers occurred throughout most of the study period, with temperatures ranging from 9.2 to 4.9 °C in the surface and from 10.8 to 7.4 °C in the deeper waters. The salinity profile was relatively stable throughout, with high salinity (> 28.9 ppt) and only minor stratification.

At fortnightly intervals, 20–50 fish from each cage were sampled (Table 1). Fish were randomly netted and subjected to a lethal dose of anaesthetics (Benzoak vet., benzocaine, 200 mg ml⁻¹, VESO Vikan, Namsos, Norway) before salmon lice were assessed on each fish while submerged in seawater-filled trays. The number of lice were counted and classified according to life stages: copepodid, chalimus I, chalimus II, preadult I, preadult II (male and female) and adult (male, and female with and without egg-strings). Counts of mobile stages that had detached from the host and were found in sedation vessels were also included in the total counts, and divided among the individuals sampled in that vessel. In addition to lice counts, fish welfare was scored according to the Salmon Welfare Index Model 1.0 (SWIM 1.0) (Stien et al., 2013). Ten welfare indicators described the condition of the individual, including assessments of emaciation status, presence of deformities (vertebral, opercula, upper jaw, lower jaw), fin and skin condition, eye and gill status, and presence of mouth/jaw wounds. Individual indicators were scored from 1 (good condition) to a maximum of 3–7, with higher scores representing increasing severity (Stien et al., 2013).

2.1. Data handling and statistical analyses

To test for an effect of the laser treatment on mobile lice abundance, pre-adult, adult male and adult female lice densities were tracked over

Table 1

Dates and corresponding day number since experiment start for sampling events. The number of fish sampled across 3 replicate cages are shown for each sample point.

Experimental Day	Date	N fish sampled in laser-free cages	N fish sampled in laser cages
1	6 Dec 2018	62	61
11	17 Dec 2018	62	61
28	3 Jan 2019	61	59
40	15 Jan 2019	153	158
53	28 Jan 2019	93	93

time in cages with and without lasers deployed. Rates of increase in lice density are not expected to be linear, so lice density data were modelled using generalized additive models (GAMs) fitted using the mgcv package for R (Wood, 2011; R Core Team, 2019). Treatment group (with or without laser) and cage ID were fitted as factors, with trial day as the smoothing term ($k = 3$). A treatment \times cage ID interaction term was also tested and removed from the model if not significant. Model fit was checked using the gam.check function in mgcv. Cage-level mean louse densities at each sampling date were treated as replicates. We avoided using individual fish as replicates due to the non-independence of individuals within the same cage.

To determine the effect of laser treatments on individual welfare indicators, a multivariate ANOVA model was run with all welfare measures included as response variables, and treatment and sample day as predictor variables, with cage ID as a random factor.

3. Results

After the 54 days of laser operation, mean abundance of all mobile lice stages was similar between cages with laser delousing (1.26 ± 0.08 lice fish⁻¹) and those without (1.25 ± 0.20 lice fish⁻¹). This lack of effect remained when focusing on specific lice stages (Table 2); mean abundance of adult females was similar between cages with lasers present (0.17 ± 0.03 females fish⁻¹) and those without lasers (0.15 ± 0.004 females fish⁻¹), and abundance of adult male (laser: 0.32 ± 0.02 ; no laser: 0.31 ± 0.03 males fish⁻¹) and pre-adult lice stages (laser: 0.29 ± 0.10 ; no laser: 0.31 ± 0.12 pre-adults fish⁻¹) also did not differ between treatment groups (Supplementary Table 1).

Tracking relative lice abundance over time (GAM analysis) revealed a considerable increase in pre-adult, adult male and adult female lice abundance over the 54-day trial (Sample day smoothing term: Table 2). All cages started with very few lice, then acquired up to $20\times$ more lice during the study period (Fig. 1). This trend was not significantly affected by the presence of lasers, regardless of lice stage (Treatment term: Table 2). A power analysis based on the R^2 of the fitted models indicated that there was adequate power to detect an effect with

Table 2

Results of generalised additive models fitting temporal changes in pre-adult (Model 1), adult male (Model 2) and adult female (Model 3) lice abundance in 6 sea cages (Cage ID term). Three cages contained an operational laser lice removal system, while 3 did not (Treatment term). Sample day was the smoothing term, fitted within treatment groups ($k = 3$).

Model 1: pre-adult lice				
Term	df	F	p	
Treatment	1	2.1	0.16	
Cage ID	5	1.5	0.24	
Smoothing term				
Sample day (with laser)	edf	Ref.df	F	p
Sample day (with laser)	1.81	1.96	5.8	0.02
Sample day (without laser)	1.85	1.98	9.6	0.002
Model 2: adult male lice				
Term	df	F	p	
Treatment	1	0.05	0.82	
Cage ID	5	4.1	0.01	
Smoothing term				
Sample day (with laser)	edf	Ref.df	F	p
Sample day (with laser)	1.88	1.99	22.7	< 0.0001
Sample day (without laser)	1.79	1.96	24.2	< 0.0001
Model 3: adult female lice				
Term	df	F	p	
Treatment	1	0.4	0.54	
Cage ID	5	2.8	0.047	
Smoothing term				
Sample day (with laser)	edf	Ref.df	F	p
Sample day (with laser)	1.60	1.84	7.9	0.002
Sample day (without laser)	1.78	1.95	7.7	0.002

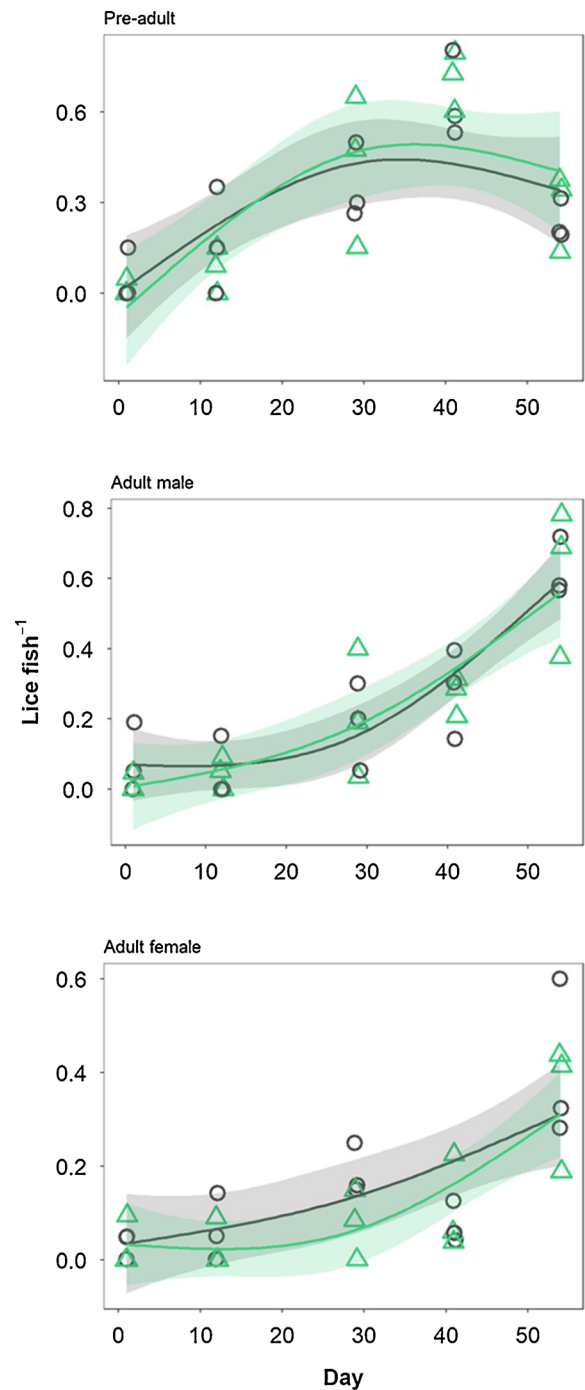


Fig. 1. Mean infestation density of fish in cages with (green triangles) and without (grey circles) lasers present, with the three replicate cages represented. Panels show abundances of three categories of mobile lice stage (top to bottom: pre-adult 1 and 2 with sexes pooled; adult males; adult females). Temporal patterns in infection levels are represented by generalised additive model (GAM) fits, with $k = 3$. Shaded areas indicate the 95 % confidence interval around the GAM fit (green: lasers; grey: no lasers) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

sampling days within cages treated as replicates (for power = 0.80, required sample size for pre-adult lice: $n = 15.1$; adult male lice: $n = 6.8$; adult female lice: $n = 11.6$).

Operational time of the lasers was 83 % per day on average (range: 22–99 %), i.e. 20 h per day. Rate of laser activity was likely influenced by lice levels in cages but was also controlled externally by the service

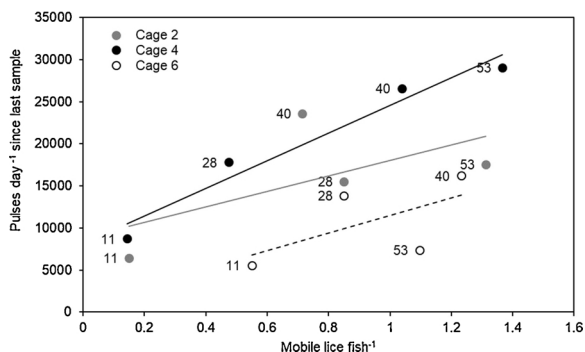


Fig. 2. Correlation between mean pulses per day during the period between sample points (11 – 17 days) in treatment cages with recorded mobile lice per fish, over the experimental period. Regression lines indicate linear correlation between infection intensity and laser delousing function; R^2 values ranged from 0.38 (both Cage 6, black dotted line, and Cage 2, grey solid line) to 0.96 (Cage 4, black solid line). Each data point is labelled with the experimental day of the sample point. Mobile lice includes the pre-adult and adult life-history stages. Note that cage 6 only had one laser node operating from Day 34 onwards, while the remaining two cages continued with 2 operating nodes.

provider and adjusted according to their management strategy. Even so, for each period between sampling days where lice numbers were manually assessed, there was a weak positive relationship between the number of lice per fish recorded on sample days, and the number of pulses per day (since last sample day; i.e. 11–17 days between). More pulses were emitted when higher lice numbers were present on the fish in Cage 2 ($R^2 = 0.38$) and Cage 6 ($R^2 = 0.38$), whereas Cage 4 exhibited a stronger correlation ($R^2 = 0.96$; Fig. 2). After Day 7, 2 cages with 2 lasers installed emitted an average of 17,500–21,664 pulses per day. One cage (Cage 6) only had one laser operational from Day 34 onwards.

To compare the activity of the laser to the number of lice ‘available’ to detect, the total number of mobile lice in each cage was estimated by multiplying the number of fish by the mean density of lice observed during manual counts. Thus, when considering the possible population of lice available to be targeted in a cage, there were 2–16 × more lice available than the number of pulses per day (Table 3), excluding the last sample where Cage 6 (with one operational laser) had 24 times more lice present than pulses shot. This translated to 0.04–0.38 pulses per louse per day (Table 3).

Differences in welfare scores between treatment groups were

Table 3

Estimated number of lice available in a cage to be targeted by the laser delousing technology, and the ratio of laser pulses to available lice. Note that cage 6 only had one laser node operating from Day 34 onwards, while the remaining two cages continued with 2 operating nodes.

Day	Cage	N fish	Mobile lice per fish	Total lice in cage [§]	Pulse per day*	Daily pulse per louse
11	2	156,317	0.15	23,448	6455	0.28
	4	160,378	0.14	22,911	8718	0.38
	6	161,495	0.55	88,822	5562	0.06
28	2	155,996	0.85	132,597	15,523	0.12
	4	160,145	0.47	75,858	17,814	0.23
	6	161,304	0.85	137,108	13,872	0.10
40	2	155,583	0.71	111,131	23,585	0.21
	4	159,725	1.04	165,752	26,586	0.16
	6	160,797	1.23	198,125	16,222	0.08
53	2	154,977	1.31	203,407	17,562	0.09
	4	159,276	1.37	217,677	29,058	0.13
	6	160,237	1.10	175,744	7400	0.04

[§] Total number of fish in the cage multiplied by the mean lice density recorded during manual sampling events.

* Mean pulse over the days between sample points.

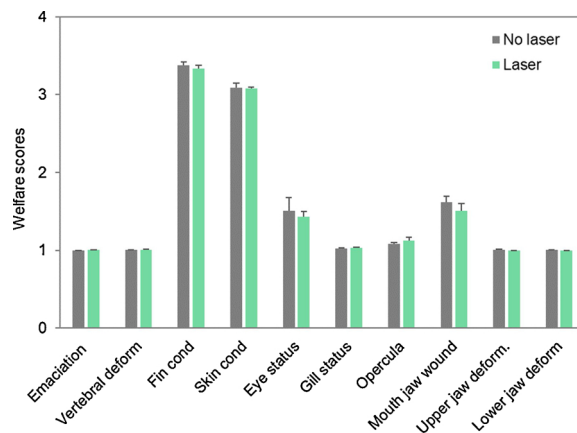


Fig. 3. Mean scores of individual welfare indicators for salmon in cages with laser delousing and cages without, over the 54-day study period.

negligible (Fig. 3), indicating that there was no impact of laser delousing on the salmon welfare indicators monitored (Pillai’s trace = 0.33, $F_{(10,12)} = 0.59$, $p = 0.79$). There was a slight decrease in welfare scores over time for both treatment groups (Pillai’s trace = 0.77, $F_{(10,12)} = 4.1$, $p = 0.01$). For skin condition, severe scores (5–7) only occurred on 3 fish out of the 607 assessed across both treatment groups. All 3 were from control cages. For eye status, severe scores (4–5) were observed on 7 out of 607 fish, 5 of which were from cages with lasers. Mortality during the experimental period was on average 1.05 % (± 0.15 % SE) of total fish in control cages, and 0.98 % (± 0.10 % SE) for treatment cages.

4. Discussion

Automated laser delousing had no detectable benefit when deployed for 54 days within snorkel cages at a commercial salmon farm. Legislative thresholds of lice levels on Norwegian farms focus on limiting the adult female stage; abundances of adult female stages increased throughout the trial and were expected to reach the limit within a few weeks of the study’s conclusion (Fig. 1).

It is not clear why the laser system was ineffective, but one possibility is that the automated detection system was unable to detect or fire an effective pulse at a sufficient number of lice. Snorkel structures, as used in the present study, can have reduced water exchange and could conceivably concentrate particulate matter or plankton in areas that the nodes occupy. However, conditions in this trial were relatively conducive to laser delousing, with generally low turbidity and algal concentrations at the time of the trial (winter 2018/2019). Salmon behaviour may also be influential; while salmon are not expected to actively avoid nodes, their depth preferences and changes in swimming patterns may frequently draw them away from the nodes’ target areas and limited action distance. For example, salmon exhibit strong behavioural responses to feed stimuli and increased activity during feeding periods, particularly during the first feed deliveries of the day (Juell et al., 1994; Oppedal et al., 2011). Environmental conditions such as temperature or light will also drive depth preferences (Oppedal et al., 2007, 2011), with most schools displaying considerable changes in swimming depth (Føre et al., 2017; Johansson et al., 2009). As a result, the node depth must be changed frequently to match that of the school. Further, in a typical commercial cage (~50,000 m³), only a small proportion of salmon are likely to pass within effective range (~1.5 m) of a node over a given duration. Snorkel structures in the cages confine salmon to a smaller volume of water and could improve encounter rates between salmon and nodes (Geitung et al., 2019; Stien et al., 2016), assuming the school is not beyond the maximum operating depth of the nodes (25 m). However, while all of the above are considerations in deployment of laser delousing nodes, in the present study, the large

number of pulses per day (relative to the estimated number of available lice: Table 3) suggests that infrequent target detection was not the reason for the apparent lack of effect.

A more likely explanation is that most laser pulses did not result in the removal of a louse, either due to an imperfect detection system resulting in the targeting of non-lice objects (we do not have access to data on this), or because the laser pulse is not sufficient to remove a correctly targeted louse. Anecdotal reports from manufacturer testing indicate that each laser pulse is close to 100 % effective, yet results from the present study are consistent with lice staying alive and attached. Specifically, the number of laser pulses relative to the estimated number of mobile lice suggests that if all pulses were lethal, around 4–38 % of the lice population would have been removed each day (Table 3). Instead, we found no significant effect on lice density after 54 days. Occasionally, individual lice were found during manual sampling events with superficial damage that could be attributed to a laser pulse – these were still alive and attached to the host (pers. obs. L. Geitung). It is not known how lice survive the laser pulse (e.g. insufficient power after travelling some distance and attenuating through the water column, or imperfect accuracy resulting in shots to non-lethal areas of the body). Further testing in a range of environmental conditions and cage types may help to identify the potential drivers of poor detection and/or lethality. Adjustments in management tactics of the laser delousing system at a site could also be explored, such as increasing nodes per cage or more frequent changes in depth distribution. Close-range pulses may be more accurate and attenuate less through the water column.

Although our analyses indicated no effect of laser delousing on lice abundance or salmon welfare status at this site, the power to detect such differences must be considered. A post-hoc power analysis indicated that our sample size was adequate for a difference to be detected, however we cannot be certain that the fish sampled were representative of the much larger group in the cage. For the vast majority of studies at commercial scale, the logistical challenge of sampling even 1% of the fish in a sea cage of 150 000 individuals, and repeating that for multiple cages, is impractical. Because estimation of sea lice levels in commercial sites is required for mandatory reporting, there has been a focus on modelling the representativeness of different sampling strategies, and these strategies can similarly be applied to sampling for welfare metrics. With the skewed distribution of abundance of lice infections and correlation with prevalence, current literature emphasises the approach of sampling “few fish from many pens” being more beneficial than “many fish from few pens” (Revie et al., 2007). Even with low sample numbers, prevalence can be indicative of abundance and thus cage-level differences can inform the status of the site (Jeong and Revie, 2020). Similarly, prevalence of poor welfare scores in a sampled group will indicate any rise in negative welfare status. Nevertheless, conclusions from sampling protocols of low sample numbers in such large groups should be interpreted with caution.

Overall welfare score declined over the experimental period for all cages, which is commonly observed in commercial settings (Bui et al., 2018; Folkedal et al., 2016). However, during the period of operation, the laser delousing strategy did not negatively impact salmon welfare in any of the welfare indicators recorded. Concerns have been raised around the potential for injuries to the eyes (which could be mistaken for a louse) and skin, however both of these metrics scored similarly after 50 days of laser operation (Fig. 3). The long-term effect of continuous exposure to light pulses is unknown past 50 days, but any related welfare issues on other commercial sites that have used laser delousing over a full production cycle have not been brought to light. As the exposure rate of individual salmon to laser pulses is unknown, further testing is needed to map impacts on welfare metrics such as cellular skin damage, mucosal layer and eye health, and to ensure that exposure does not cause behavioural distress in salmon. If physical and

behavioural welfare indicators are unaffected by laser delousing, then welfare concerns could be allayed for this technology. More powerful lasers may be required to increase lethality; if so, their effects on welfare should be rigorously tested.

In general, there is a disparity between the rapid rate of commercial product development in the aquaculture industry, and scientific validation of those technologies. The Norwegian salmon farming industry benefits from continuous innovation, leading to advances in productivity and efficiency (Asche et al., 2013). There are substantial opportunities for start-up businesses and corporations to develop products that can be available on the market relatively quickly, with anecdotal evidence or personal connections largely driving the acquisition of these new strategies by farming companies (Brakstad et al., 2019). A case study of one relatively small Norwegian salmon farming company estimated production losses from lice and related management expenses totalled approximately 6.89 million NOK per licence (site) in 2016 (Brakstad et al., 2019). This substantial financial and social pressure drives aquaculture companies to seek immediate solutions, and the nature of the Norwegian market is such that rapid commercialisation of technologies is possible. The industry is responsive to emerging innovations and are quick to adopt new strategies to combat salmon lice. This fast-paced acquisition and implementation of technologies can promote progress, but if the strategy is not fully developed or well-tested, investments and resources may be misdirected. Few strategies are robustly validated before implementation.

New technologies, especially those central to production and disease control, should be validated at a commercial scale across a range of conditions. This is particularly important when there are potential welfare implications beyond the scope of Norwegian Food Safety Authority assessments. An example of thorough validation is the snorkel cage for prevention of salmon lice infestations. Its efficacy and potential welfare impacts have been experimentally documented under a range of conditions (Oppedal et al., 2019), with different iterations of the snorkel structure (Oppedal et al., 2017), with a range of fish sizes (Oppedal et al., 2019; Stien et al., 2016), focusing on secondary infections (Wright et al., 2017), and finally at commercial scale over a full production cycle (Geitung et al., 2019). In contrast, there are several examples of lice control technologies that have been widely adopted by the industry without a full understanding of their efficacy or their consequences for animal welfare. These include cleaner fish (small number of studies, mostly poorly replicated and at less than commercial scale: Overton et al., 2019), and thermal and mechanical delousing (Gismervik et al., 2019; Overton et al., 2018). In both cases, the evidence base is now improving, but too late to (a) avoid unacceptable welfare outcomes for billions of vertebrate animals, or (b) guide prudent financial investment by the industry (Brakstad et al., 2019). Proper validation of new techniques before widespread uptake is crucial for the maintenance of high ethical, environmental and financial standards in the industry.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2020.105063>.

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