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Efficiency and welfare impact of long-term simultaneous in situ management strategies for salmon louse reduction in commercial sea cages

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ABSTRACT

The Atlantic salmon (*Salmo salar*) aquaculture industry is faced with an obstacle in sustainability with increasing production, which is the control and prevention of the ectoparasitic salmon louse *Lepeophtheirus salmonis*. Lice prevention management is steering towards passive applications, and this study aimed to monitor multiple strategies in commercial cages over time, to determine the efficiency of these approaches and their effect on welfare. Four strategies were tested at a commercial scale over a 13-month period, covering a large proportion of a standard production cycle. The additive effect of multiple treatments was established in 12 cages, which were assigned to a prevention strategy of either: cleaner fish only, cleaner fish and functional feed, the previous two factors plus deep attractant lights and submerged feeding, or the previous three factors plus a lice skirt. Environmental profiles and school swimming depth were monitored throughout the study period, and sampling events occurred every 2–6 weeks to assess the infestation and welfare status of salmon. The rate of infestation fluctuated with season; however, the group with all prevention strategies maintained a lower rate of new infestations compared to the groups with cleaner fish or functional feed only. Cages with deep lights and feeding influenced the school swimming depth, with these groups generally swimming deeper; this meant that these cages also swam ~6 m deeper than the halocline when pooled over time. However, even with strong differences in new infestations and vertical distribution, the level of mobile lice was similar among all groups, thus incurring a similar frequency of delousing events. There was no effect of these prevention strategies on overall welfare status of salmon. This study shows the promise of utilising multiple lice prevention approaches and highlights the interaction between environment and infestation pressure.

1. Introduction

The national growth of the Atlantic salmon (*Salmo salar*) aquaculture industry has cemented Norway as the leading producer in the world, boasting 52% of global production in 2017 (FAO, 2019). Expansion has been facilitated by the industry's ability to refine and optimise production methods whilst responding rapidly to issues that arise with innovative solutions that stem from both research and commercial resourcefulness. However, despite enormous efforts, one of the challenges that has not yet been managed to a sustainable level is the issue of salmon lice (*Lepeophtheirus salmonis*) infestations. Salmon lice are an ectoparasite that have dramatically proliferated in parallel to the increasing abundance of farmed Atlantic salmon, thereby causing high infestation pressures on wild salmonid populations that share the fjord environment (Serra-Llinares et al., 2014; Shephard and Gargan, 2017; Thorstad et al., 2015). The impact of lice on wild populations has prompted action by the Norwegian government to strongly regulate production limits, whereby farmers will be allowed to increase their producible biomass depending on the infection ‘status’ of their regions (see Myksvoll et al., 2018). Therefore, there is strong pressure on farming companies to control and minimise the salmon lice infestation levels on their sites to enable further expansion of the industry (Olaussen, 2018).

As sea lice are increasingly resistance to the traditional oral and chemical treatments (Aaen et al., 2015; Denholm et al., 2002; Ljungfeldt et al., 2014; McNair, 2015) farmers are forced to use mechanical or thermal delousing methods (Overton et al., 2018). These methods require handling that stresses and in severe cases also physically harm the fish (Overton et al., 2018). There is therefore an urgent need for methods that prevent lice infestation. However, there is currently only a limited number of prevention measures available on a commercial scale, and even fewer with documented effects on

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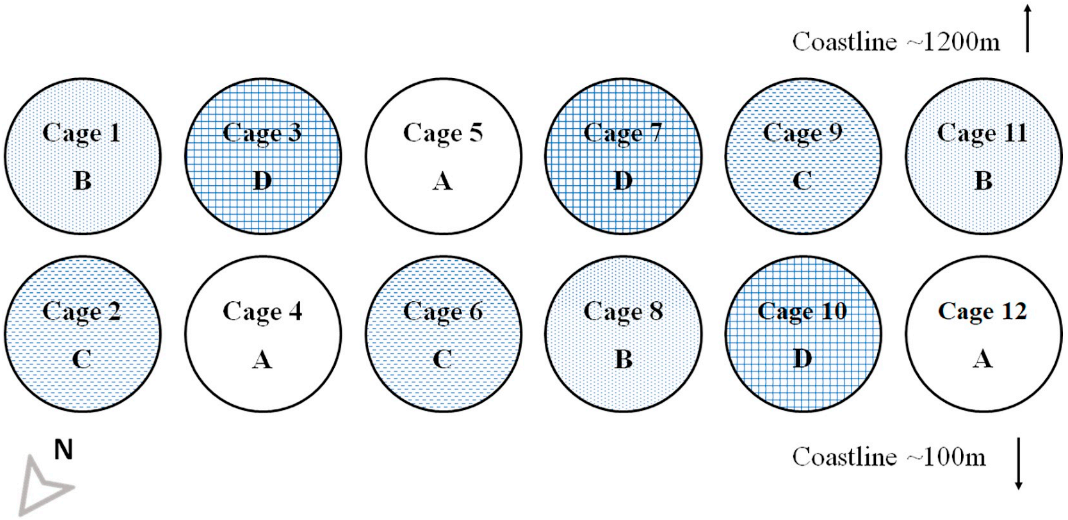

Fig. 1. Sea cage arrangement at the study site. Treatment groups were additive lice management strategies categorised as: cleaner fish only (A), cleaner fish and functional feed (B), cleaner fish, functional feed and deep lights/feed (C), or cleaner fish, functional feed, deep lights/feed and lice skirts (D).

infestation prevention and fish welfare (Noble et al., 2018). In practice, farmers will utilise multiple prevention methods throughout a production cycle, and possibly even use methods simultaneously to maximise their integrated pest management strategy. In this study, we focus on four control and prevention measures used in combination: attractors to make salmon swim deeper (submerged lights and feeding), a lice skirt, cleaner fish, and functional feed.

The first two methods aim to mismatch the vertical positioning of the host from the expected shallow distribution of infective sea lice larvae (Genna et al., 2005; Heuch, 1995). Salmon can be attracted to swim deeper in the sea cage by use of lights (Juell and Fosseidengen, 2004; Juell et al., 2003; Stien et al., 2014; Wright et al., 2015) and by moving the feeding zone to match the lights' depth (Frenzl et al., 2014; Nilsson et al., 2017), thereby avoiding the shallow waters except for infrequent forays to the surface to refill their swim bladders (Korsøen et al., 2012). It has been shown that positioning salmon deeper in the water column, whether with cage structures or otherwise, can be an efficient way to prevent infestation (Oppedal et al., 2017; Stien et al., 2016); however, it is highly dependent on seasonal variation in salinity stratification and water temperature. If a brackish layer is present at the surface, sea lice larvae will actively avoid low-salinity waters and aggregate below the halocline (Crosbie et al., 2019; Heuch, 1995) and the infective zone can be brought deeper in the water column. Alternatively, hydrodynamic mixing can also transport larvae to meet the deeper-swimming salmon. In contrast, warmer temperatures can also override phototactic preferences in the fish, resulting in shallow swimming if optimal temperatures are towards the surface (Oppedal et al., 2007; Oppedal et al., 2011; Stien et al., 2016).

As salmon swimming depth cannot be influenced with temporal certainty, the addition of a lice skirt to a cage with submerged feeding and lights offers an additional degree of prevention. The installation of a material sheeting in the upper depths around a sea cage acts to prevent larval sea lice from flowing into the cage and therefore, theoretically, causing infective lice to be mostly transported around the cage but occasionally also to depths below the skirt (Frank et al., 2015). The lice skirt proports that salmon can swim in the upper depths of the cage and be shielded from the infection zone, which has shown to be true at varying levels depending on both locality and season (Grøntvedt and Kristoffersen, 2015; Grøntvedt et al., 2018; Stien et al., 2018).

The use of functional feed could act as another prevention measure to complement the cage technologies, through improving the internal physiological defence of salmon. Functional diets aim to augment nutritional provision with additives (such as active plant or bacterial extracts) that benefit fish physiologically, and have formed a branch of preventative health strategies in commercial farming. In salmon aquaculture, various feed products are available that claim to mitigate adverse impacts of infection (e.g. by pancreatic disease or amoebic gill disease) or strengthen robustness through the provision of pre- and probiotics, vitamins, and immunostimulants (Tacchi et al., 2011). Laboratory trials have demonstrated the potential for functional feed to prevent and reduce lice infestation (Jensen et al., 2015; Jodaa Holm et al., 2016), however whether this translates to commercial-scale reality remains to be seen.

Submerged lights and feeding, lice skirts, and functional feed all act as a pre-emptive and passive approach to reducing potential lice infestations. Therefore, the lice that do surpass these prevention measures and indeed attach have no obstacles for their development to the reproductive stage. In some countries, there is a legislative threshold of lice levels that dictate when delousing treatments must occur; in Norway, this level is 0.2 adult female lice per fish during the spring salmon migration period and 0.5 the rest of the year (Norwegian Ministry of Fisheries and Coastal Affairs, 2012). Therefore, farmers are likely to use cleaner fish as an additional measure to graze on larger mobile lice stages, and keep lice levels low to avoid triggering mandatory delousing (Imsland et al., 2014; Imsland et al., 2018; Treasurer, 1996). In this study, we aim to determine the actual efficiency of industry use of these strategies (functional feed, submerged lights and feeding, lice skirts, and cleaner fish) on reducing infestation levels, and thus treatment frequency, over most of a grow-out period in a commercial farm. The effect of these measures on fish welfare is also a critical factor to describe before large-scale implementation, and thus welfare was also assessed throughout the experimental period.

2. Methods

2.1. *Experimental set-up and design*

Experimental testing of the preventive measures was conducted at a fjord site near Vindsvik, western Norway. The fish farm had 12 circular sea cages (cage circumference = 120 m, cage depth = 35 m) in two parallel rows (Fig. 1). In September 2016, approximately 65,000 smolt (~100 g) were transferred into each cage and raised with standard production procedures throughout the study. As this was a R & D concession site granted by the Norwegian Ministry of Trade, Industry and Fisheries, a specific ethics approval for this commercial study was not required.

Table 1
Welfare indicators that comprise the Salmon Welfare Index Model (SWIM), each with weighting used to calculate an Overall Welfare Index for an individual (from Stien et al., 2013).

Model	Welfare indicator	Value or score range
SWIM 1 (base model for welfare assessment)	Length	Value
	Weight	Value
	Condition factor	Score 1–3 based on value calculated from length and weight
	Emaciation	1–3
	Vertebral deform	1–3
	Sexual mature	1–4
	Smoltification state	1–6
	Fin condition	1–4
	Skin condition	1–7
	Number of sea lice	Score 1–5 based on lice cm^{-2}
SWIM 1.1 (extension model for fish health)	Eye status	1–5
	Gill status	1–3
	Opercula	1–5
	Mouth/jaw wound	1–3

Cages were assigned to one of four treatment groups (3 replicate cages per treatment; Fig. 1) that built on a control group with cumulative technologies added. That is, control cages (A group) were a standard production setup with only cleaner fish, where the B group had cleaner fish and were provided with functional feed. C group cages had cleaner fish, functional feed, and a submerged feeding system with attractant LED lights. D group cages had all of these preventive measures, and an additional lice skirt.

All cages were stocked with cleaner fish throughout the production cycle, using an industry-practice deployment and management strategy. Cleaner fish species used included the ballan wrasse (*Labrus bergylta*), lumpfish (*Cyclopterus lumpus*), corkwing wrasse (*Symphodus melops*), rock cook wrasse (*Centrolabrus exoletus*) and goldsinny wrasse (*Ctenolabrus rupestris*). Intended stocking density of clean fish was to be 5% of salmon number per cage, with restocking multiple times during the experimental period (supplementary Table 1). Although the operational manager of the site strived to keep stocking densities equal among the cages, only the quantities of cleaner fish added were known, whereas actual numbers of fish over time in the cage after mortality were unknown. All cages had three hides (6 m long) installed, with the upper part of the hide fixed at the surface. Cleaner fish were provided with feed close to their hide daily (industry practice guidelines, in Norwegian: http://lusedata.no/for-naeringen/veiledere-leppefisk/).

Functional feed (Skretting Shield, Skretting, Norway) was provided to salmon in treatment cages (Group B, C, and D) from trial initiation until trial completion. For all cages, salmon were fed to satiation daily through visual monitoring during daylight hours. In December 2016, cages in C and D groups had a structure installed in the centre that supplied feed at 7 m depth (AKVA SubFeeder; AKVA Group, Norway). In addition, five UV LED lights (Aurora SubLED Combi, AKVA Group, Norway) were suspended between 7 and 10 m depth (the depth recommended by the provider). The lights emitted a deep violet (120 W) colour from end of Dec 2016 – mid-Jan 2017, a green-blue anti-maturation (600 W) colour from mid-Jan until mid-June 2017, then returned to deep violet colour thereafter. At the same time, a semi-permeable canvas lice skirt (Norwegian Weather Protection, Norway) was installed outside of D treatment cages that extended from 1 m above the surface to 6 m below. Treatments were assigned to cages in a randomised block design (Fig. 1).

To monitor group vertical distribution, the salmon were continuously recorded using a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway; Bjordal et al., 1993). The system includes a transducer submerged at ~30 m deep inside every cage, positioned to face upwards with a 42° acoustic beam. The strength of the returned echo signal indicates the presence of fish, with higher signal strengths indicating more dense groups of individuals. Environmental conditions of the water column were profiled daily using a CTD sensor (SD204, SAIV AS, Bergen, Norway), at a reference point outside of the experimental cages. Temperature and salinity were recorded from the surface to 40 m depth.

The industry partner managing the facility retains an internal threshold of 0.2 adult females per fish at which a cage must be deloused (legislation requires treatment at a farm-average level of 0.5 females per fish during non-migration seasons). Most delousing treatments were mechanical and non-chemical methods (with the exception of one group of delousing that were oral treatment, and another of hydrogen peroxide bath), applied on a cage-basis rather than whole-farm treatments.

2.2. Sampling regime for welfare and infestation status

The first baseline sampling was conducted in November 2016, before the installation of the SubFeeder, lights, and skirt (Sample 0), but after functional feed had begun to be provided. Thereafter, every 2 to 6 weeks from January until December 2017 (a total of 15 sample events: see Supplementary table 2), fish were sampled and assessed for lice infestation levels and welfare status. Fish were captured using a hand net at the surface, a seine net at the surface, or small ring-net pulled from 10 m depth to the surface by a boat crane; cages were sampled using the same method at each separate sample event. Sampling was conducted a minimum of 3 weeks after any delousing treatment, so that new infestations were certain to be unaffected by previous treatments; Sample 14 was an exception, where delousing occurred the week prior, and therefore is excluded from lice analyses. Delousing events were triggered by levels of 0.2 adult female lice fish per fish, which was assessed through weekly farmer counts rather than by this study's scientific lice counts. At each sampling point, 20 salmon from each cage hand-netted out and immediately euthanised in a sedative bath (overdose of benzocaine; Benzoak vet.), and lice on the host or in the sedation vessel were quantified and staged. Each fish was also evaluated using the standardised SWIM 1 and 1.1 models (Stien et al., 2013), which involves the scoring of 14 indicators of welfare ranging from undamaged/normal to severely damaged/abnormal (Table 1). Welfare indicators are weighted in the model and used to calculate an overall welfare index (OWI), a value bounded from 0 (worst) to 1 (best) that reflects an individual's welfare status. Occasionally > 20 fish were captured from the cage, whereby only the first randomly chosen 20 fish were assessed for welfare, and all lice were counted and divided by the total number of fish sampled. Gill diseases were also recorded, specially presence of proliferative gill inflammation and amoebic gill disease, however the prevalence of these diseases was negligible throughout the study, whereby scores were low and the lack of severity (and prevalence across individuals and cages) did not elicit veterinary action.

2.3. Data handling and statistical analyses

Lice stages were categorised by whether they were new infestations since the previous sampling (copepodid, chalimus 1 and 2 stages), or could possibly have been present at the previous sampling (pre-adult 1 and 2, and adult stages). All lice considered a new infestation were summed and averaged by the number of individuals sampled per cage. Date of lice attachment was back-calculated for new infestations stages using their estimated temperature-dependent development rate (Hamre et al., 2019) based on the average sea temperature 2–3 weeks prior to the sample event. For example, if the sea temperature was approximately 10 °C, a pre-adult 2 male louse would have taken 25.6 days to develop to that stage, and therefore was estimated to have attached 25.6 days prior to the sample date.

New infestation levels and welfare scores were compared among groups with a generalised linear mixed model, with treatment group and sample date as predictor variables, and cage number as a random effect (package 'glmmTMB' in R; Brooks et al., 2017). The model of lice data used a Poisson distribution, whereas the welfare model used a binomial distribution. Null models were created and compared to the full model using a Chi-Squared test to determine the effect of the factor Treatment.

Existing infestations (mobile lice stages) were not analysed as many confounding factors could affect the value (such as actual cleaner fish stocking density or recent delousing treatments), which could vary between sample points.

Echosounder data was frequently unavailable due to equipment damage or incorrect placement, however the data that was recorded provided information on the vertical dispersion of the salmon; in all cages, occasionally the school exhibited a bimodal distribution where there were 2 (and rarely, 3) main groups within the school. The echosounder data gave echo strength values along the vertical water column that indicated the relative signal strength (i.e. density or presence of fish) at 7 cm depth bands. Data were recorded continuously and so the relative strength was used to determine the vertical distribution of the school over time. Using the mean upper and lower depth limits of the shallowest school (i.e. the middle depth of the shallowest school, to represent the main group of fish), the average median depth of this range was used to estimate the daily value of swimming depth for that cage, across all hours within the day. This approach of describing the shallowest school allows investigation into the potential interaction between the school and the halocline.

As lice copepodids gradually avoid brackish waters of 28 ppt or fresher (Crosbie et al., 2019), the depth of 28 ppt was termed the halocline for this study. The estimated depth of the halocline was determined by the deepest depth with a salinity of 28 ppt or lower, calculated daily. To investigate the relationship between halocline depth (and therefore assumed depth of infective copepodids), salmon vertical distribution, and actual lice infestation (back-calculated from stages), daily values of school swimming distance from halocline was calculated for each cage. The potential of swimming depth alone, or swimming depth in relation to halocline depth to influence lice infestation rates were both tested using a generalised linear model. As there were few days with data for all three parameters available, data points were pooled over the experimental period and used in the model with cage number as a random effect.

3. Results

3.1. Salmon vertical distribution and interaction with environment

Although the salmon responded to multiple environmental variables that fluctuated with time, there was a consistent difference in mean depth distribution of the shallowest school between treatment groups, when time-pooled means were estimated. Cages with deep lights and feed (C and D groups) swam deeper than those without (A and B groups), whereby C and D cages where significant factors in the model (C group estimate ± SE: 5.85 ± 1.18, $z = 4.95$, $p < .001$; D group estimate SE: 6.30 ± 1.18, $z = 5.33$, $p < .001$). This translated to a pooled mean swimming depth of 11.05 m (SE: ± 0.2 m) for fish in C cages and 11.49 m (± 1 m) for D cages. In contrast, the average median swimming depth of the shallowest school, over the entire study period, was 5.18 (± 1.7) and 5.55 (± 0.4) m for A and B cages without deep lights and feed or skirt. The presence of more than one school (i.e. bimodality) occurred for 24–25% of the study period in A and B groups (range: 15–32%), and 31% for C and D groups (range: 21–40%).

As the experimental site was in a narrow, inner fjord location, a stratification of temperature and salinity often occurred (Fig. 2 upper panel). Brackish layers were present almost throughout the study period, sometimes extending below the protective depth of the lice skirt at 6 m (Fig. 2 middle panel). The pooled mean swimming depth translated to an average of ~0.03 and 0.16 m *above* the halocline for A and B cages over the period, whereas C and D cages were ~5.76 and 6.79 m *below* the halocline, but with larger variation between days (Fig. 3, lower panel).

3.2. Preventative efficiency (new infestations)

Peak levels of new infestations occurred during different seasons among groups, with the experimental period's maximum abundance observed in July 2017 for Groups A and B (2.48 and 2.85 lice fish^{-1}, respectively), but in December and March 2017 for Groups C (1.33 lice fish^{-1}) and D (0.72 lice fish^{-1}), respectively (Fig. 4). When lice attachment date was back-calculated, there were relatively large differences in infection success between days, within a short period of days to weeks (Fig. 3, upper panel).

Treatment as a factor contributed significantly to the observed variation in lice infestation levels ($\chi^2 = 12.1$, $p = .007$). Compared to the control group, B and C groups did not have reduced lice loads over the study period ($p > .33$ for both groups). However, cages with all prevention strategies (D group) significantly reduced the rate of parasite acquisition (intercept estimate: −0.43, estimate ± SE: −1.03 ± 0.34, $z = -3.04$, $p = .002$), with 51.3% and 63.3% fewer attached lice stages across the whole year compared to Group A and B, respectively (Fig. 4). The GLMM also showed that sample date was a strong influencer of lice levels ($z = 2.03$, $p = .042$). Thus, this effect size varied with sample dates, ranging from 7.3% *more* lice (March 2017) to 92.6% *fewer* lice (July 2017) than the A group. A large proportion of the period showed promising efficiency, with 8 out of 13 samples where Group D had a reduction of > 40% compared to A, and only two samples where a reduction was not observed. In contrast, C cages had new infestation levels similar to A, with an overall average of 0.7% more lice than A groups.

When investigating the relationship between distance of salmon swimming from halocline depth and subsequent lice acquired, analyses showed that neither swimming depth alone or distance from halocline explained infestation levels, however a trend was found whereby distance from halocline could be contributing more to the lice variation ($z = -1.86$, $p = .062$) compared to swimming depth alone ($z = -1.64$, $p = .101$; Fig. 5).

3.3. Mobile lice and treatment frequency

When pooled across the study period, mobile lice levels were highest on fish in B and C cages (mean ± SE: 2.4 ± 0.5 and 2.0 ± 0.4 mobile lice fish^{-1}), followed by A (1.5 ± 0.4 mobile lice fish^{-1}) and D (1.4 ± 0.3 mobile lice fish^{-1}). Temporally, mobile lice intensities were relatively similar across groups throughout the experimental period, with only one sample date (November 2017) where C and D groups were > 2 lice per fish lower than the A group (Fig. 6). Even with delousing events, there was a peak in lice abundance in winter and late spring, and a gradual increase from autumn onwards (Fig. 6). The levels of mobile lice triggered multiple delousing events, with no difference in the frequency of delousing among groups. Group A and B underwent a total of 14 and 16 treatments across the replicate cages (mean 4.7 and 5.3 treatments per cage, respectively), while both C and D groups underwent 14 (mean 4.7 per cage).

3.4. Welfare status

There was no significant contribution of treatment towards OWI compared to a null model ($\chi^2 = 0.25$, $p = .969$), indicating that the various prevention strategies did not affect welfare status over the experimental period. However, OWIs irrelevant of treatment fluctuated temporally, with differences in scores between sample dates and a trend of welfare decline over the experimental period (Fig. 7). The range maximum of OWI's during the experimental period was similar among groups (0.86 for Group A, B, and C, and 0.85 for Group D), however

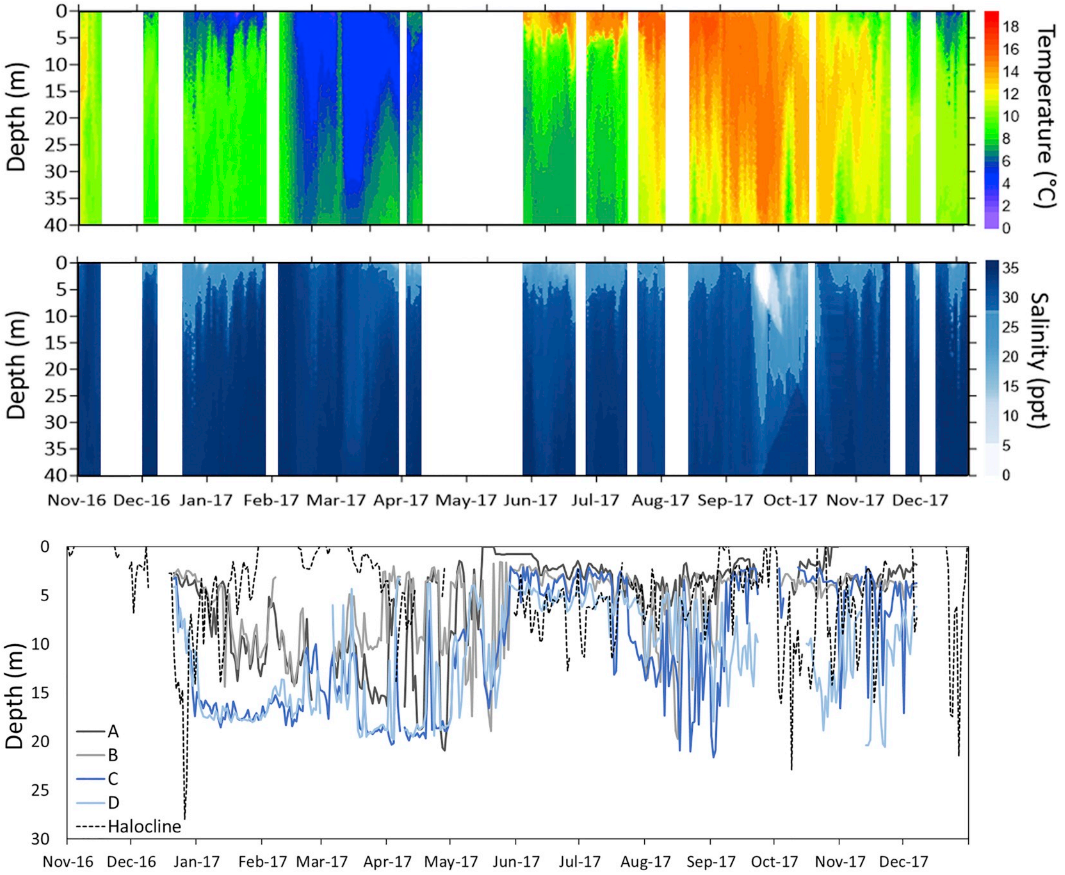

Fig. 2. Temperature (upper panel) and salinity profiles (middle panel), and estimated salmon swimming depth (lower panel) over the study period. White blocks in the environmental profiles represent periods when data are not available. Swimming depth of the school was estimated as the mean depth of the shallowest school (when there was bimodal distributions) per day, averaged across the three replicate cages in the treatment groups. The infective stage of salmon lice is assumed to aggregate below the brackish layer, so the swimming depth panel includes a black dotted line which highlights where 28 ppt occurred. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Group A and B had lower minimums (0.63 and 0.56, respectively) compared to Group C and D (0.70 and 0.67, respectively). Samples from August 2017 onwards (except for one date in September) showed large differences in OWI (Fig. 7). In particular, Group A and B had moderately reduced OWIs of below 0.7 in August and September 2017; during this period, these groups had more severe eye conditions (Supplementary Fig. 1) and higher levels of new lice infestations (Fig. 4).

4. Discussion

4.1. Preventing new infestations, and interaction with environment and salmon depth preference

The largest effect of prevention was found in cages that had all tested strategies, indicating that the cage addition of deep lights and feed, and a lice skirt, was the most effective in deterring new infestations. Previous commercial tests conducted with only deep lights and feed had found no overall reduction using only this strategy for 11 months (Nilsson et al., 2017). Hence, the addition of the lice skirt promotes prevention efficiency, with a reduction in new infestations recorded almost throughout the study period, without affecting the welfare status of salmon. This aligns with results from Stien et al. (2018) who used 10 m skirts at a commercial site, from May – September; however, the prevention efficiency they found was variable, and most effective in August. Cages with deep lights and feed, but no lice skirt, demonstrated a trend towards reduced new infestation levels but this was not consistent enough over time. The provision of functional feed did not influence rate of infestation compared to control cages, but may have interacted with the cage strategies to improve efficiencies that is undetectable otherwise.

The presence of an impermeable material surrounding the upper metres outside a sea cage is expected to prevent most of the surface waters from entering the cage, however under some conditions the water can be redirected vertically and potentially enter cage underneath the skirt (Frank et al., 2015). It is unknown whether this is likely to occur with a 10 m lice skirt, and so the variability in preventative efficacy in Stien et al. (2018) and this study could be due to halocline depth or reduced protection by the skirt. The ratio of water entering the cage versus being forced around the cage can vary with factors such as flow conditions and skirt deformation (Lien et al., 2014), cage position relative to other structures, and fish schooling behaviour.

As salmon lice are positively phototactic, but assumed to avoid brackish waters and therefore distribute just below a halocline (Crosbie et al., 2019; Heuch, 1995), the depth of the halocline in relation to where salmon are dispersed is an important relationship. The use of deep lights and feed aims to draw the salmon deeper in the cage away from the surface depths where infective copepodids are assumed to distribute, however this strategy is unlikely to be efficient during periods when the halocline extends below the depth of the lights/feeders. For example, during October 2017, the halocline was deeper than the skirt and feeding zone depth, and the predicted date of attachment showed little difference among groups as all schools swam at similar depths (Fig. 3). There is a suggested link between peaks of infection pressure resulting in increased infection success (i.e. higher rate of lice attachment) when fish were swimming close to the halocline, which was more prevalent in cages without deep lights and feed or skirts

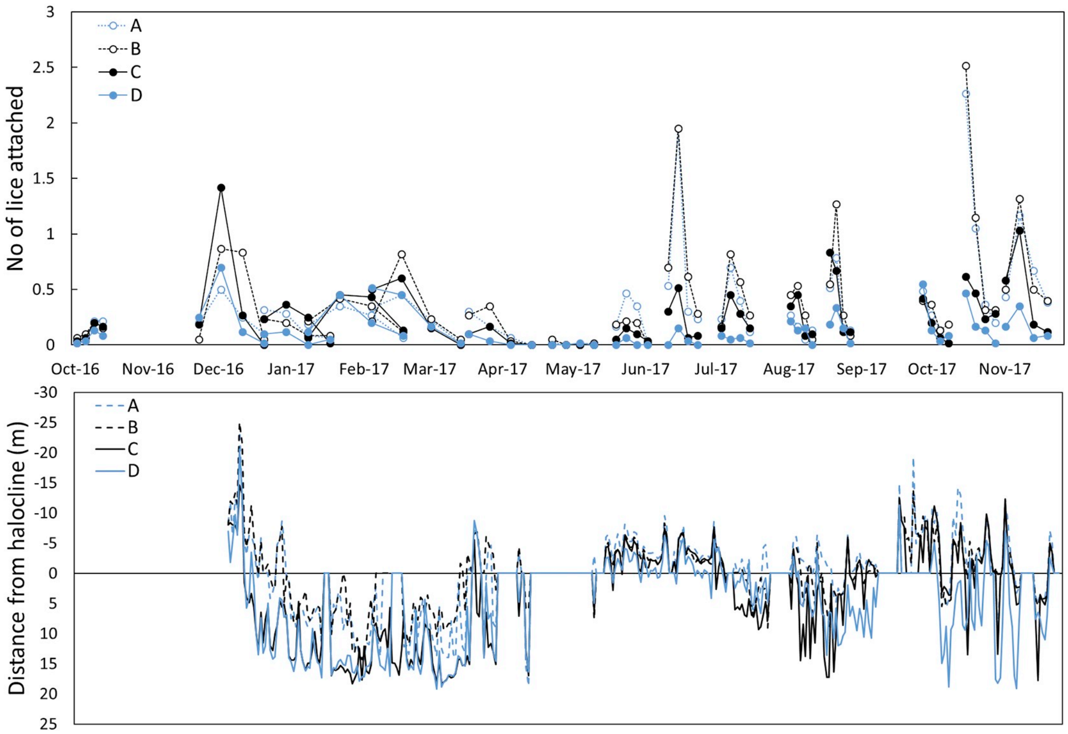

Fig. 3. Lice attachment date and salmon swimming depth over the study period. Upper panel: mean number of lice attached per fish (as calculated from sessile stages and pre-adult 1 counts), within treatment groups; lower panel: daily median swimming depth of schools per cage, compared to the halocline (28 ppt or lower) for that day. Negative values of the distance from halocline indicate salmon swimming above the halocline, whereas positive values indicate salmon swimming deeper in the cage. Times when either echosounder or environmental data were not available are not plotted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

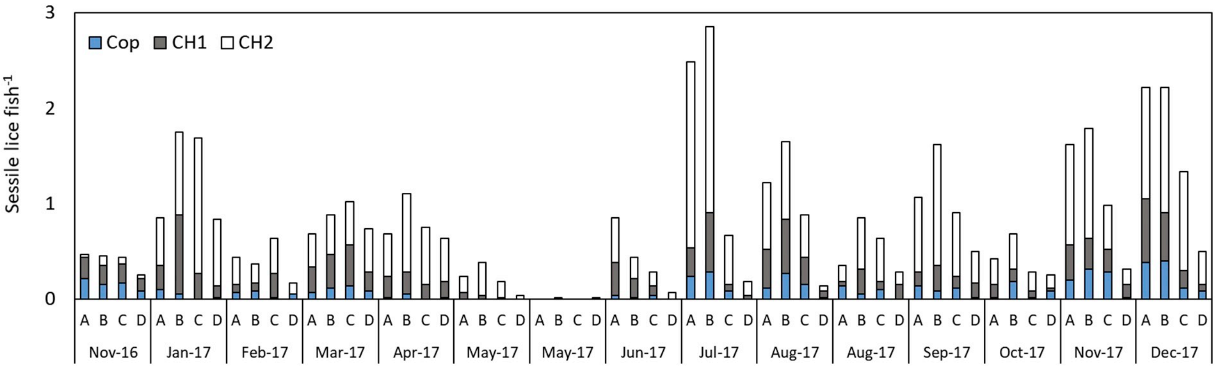

Fig. 4. Average new lice infestation levels for each treatment group (A-D) over the study period. Sessile stages of lice are considered new infestations, with average abundance of copepodids (Cop), chalimus 1 (CH1) and chalimus 2 (CH2) represented. The November 2016 sample was conducted prior to the implementation of prevention treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 5). These results suggest considerable day-to-day variation in infection pressure that interacts with halocline depth and salmon vertical distribution, which should be investigated further with finer-scale monitoring of these parameters.

At a separate commercial site that used analogous deep lights and feeders, no difference in infestation levels or salmon swimming depth were found between control and treatment cages (Nilsson et al., 2017). In the present study, depth distribution of the shallowest school was distinctly different between cages with submerged lights and feed compared to those without, whereby the attractant lights and deeper feed zone encouraged the school to distribute deeper in the cage during most periods. The average depth difference between A/B cages versus C/D cages was approximately 6 m over the study period, however there were periods when swimming depths were similar across groups. The presence of the deeper attractants did not appear to influence vertical bimodality of the school. Salmon exhibit a trade-off between temperature and attraction to artificial lighting; if temperature is slightly lower at the illuminated depth than at other depths, salmon prefer the higher temperature during the day and illumination during night, while at larger differences in temperature the higher temperature is preferred also at night (Oppedal et al., 2007; Oppedal et al., 2011). Here, all groups swam in the warmer water above the illuminated zone during the summer while salmon with deep light swam deeper when there was a clear temperature stratification with warmer water near the surface (e.g. June – July; Fig. 2).

4.2. Mobile lice and delousing frequency

The results from this study highlights the complexity of large-scale studies conducted at commercial sites with so many interacting factors,

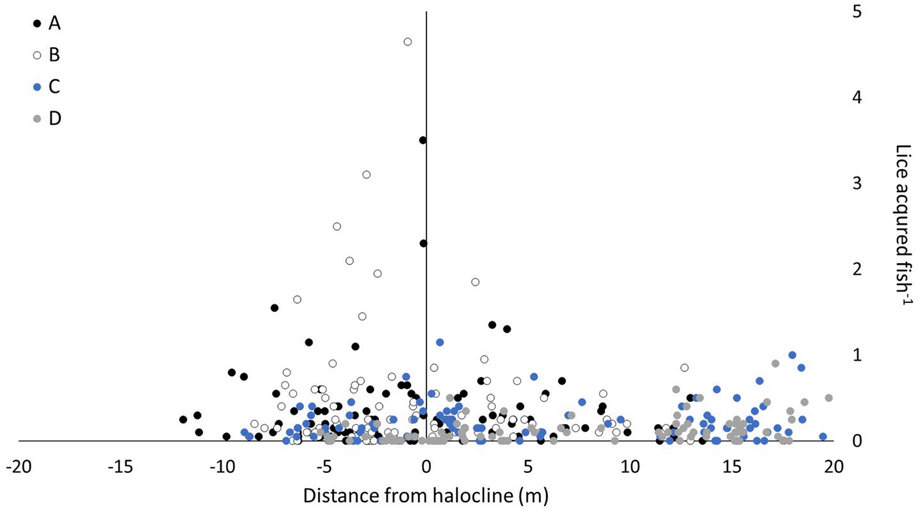

Fig. 5. Lice infestation rate in relation to the salmon swimming distance from the halocline depth, for each treatment group (A, B, C, and D); negative values of the distance from halocline indicate salmon swimming above the halocline, whereas positive values indicate salmon swimming deeper in the cage. Data points are only represented for days that have values for lice attachment, salmon swimming depth, and salinity. Data are pooled over the experimental period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the subsequent convolution of interpreting results. There are numerous factors that could have influenced the infestation status throughout the study, some of which would be uncontrollable elements (e.g. individual cleaner fish efficacy, impact of delousing events through the different teams conducting the process, or even differences in the work quality of staff). However, this study does provide a broader conclusion about the efficacy of these strategies in reducing lice infestations under realistic commercial conditions; although new infestations were reduced in D cages overall, this did not translate to a reduction in mobile lice levels and therefore delousing events. There are three possible reasons for the discrepancy between infestation levels and subsequent mobile lice abundances that triggered delousing: reduced efficiency of cleaner fish in cages with deep lights and feeders or skirts, unrepresentative sampling of fish for assessment, or the farm company's lower threshold that triggers delousing (0.2 adult females per fish).

In this study, the use of cleaner fish was largely tested as the concept of aquaculture's use and management strategy of cleaner fish, rather than the specific effectiveness of cleaner fish with controlled stocking densities. Deployment was managed by the farmers and mortality estimated from using the salmon collection system, as is standard practice. Thus, this study demonstrates the lice control effectivity of cleaner fish as an industry-accepted strategy when combined with other prevention strategies. To our knowledge, no published studies have assessed the impact of cage technologies on cleaner fish behaviour or efficiency. Evidence from this site suggested that corkwing wrasse (*Symphodus melops*) were the poorest cleaners in cages with lice skirts for 3 months during late summer, with a mean of only 0.2 lice in their stomach contents compared to 1.8 in wrasse from C cages (Gentry et al., 2019). The in-cage technologies could influence the efficacy of cleaning behaviours, through mismatching vertical distributions with the deeper salmon attractants. Alternatively, the presence of a lice skirt can reduce oxygen levels near the surface by 5–35 percentage points saturation (Stien et al., 2018; Stien et al., 2012), which may influence cleaner fish distribution or cleaning behaviour. As their hides were situated within the skirt, chronic exposure to slightly reduced oxygen levels could reduce their welfare status and subsequently, their delousing potential.

True estimates of lice loads are vulnerable to sampling error, through either capture methods or treatment for examination (Heuch et al., 2011). Euthanising fish in a water bath and conducting lice counting inspections directly afterwards is a more accurate method for recording lice abundances, compared to a blow to the head and individual bagging (Copley et al., 2005). Therefore, in this study, capture methods are the likely source of unrepresentative sampling, if indeed this was the case. In a sea cage, differences in spatial location of individual salmon within the school is driven by size (Folkedal et al., 2012), hunger (Juell et al., 1994), physiological state (i.e. emaciation status, Vindas et al., 2016), and infection status (Bui et al., 2016), all of which are likely to interact with the presence of lice skirts or submerged lights/feed; if individuals caught for sampling are only from the upper 5 m depth, it is possible that the sampled individuals are unrepresentative due to these factors. Nilsson and Folkedal (2019) recently found that sampling of caged salmon is size-biased, even when the entire population is crowded before fish are netted out and a large number of fish are sampled. In fact, over this study period, the median swimming depth of the shallowest school for C and D groups was ~11

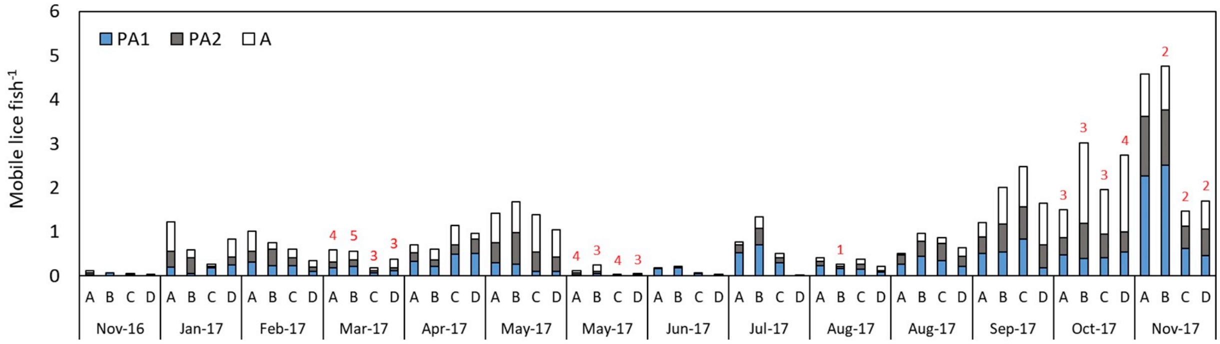

Fig. 6. Average mobile lice infestation levels for each treatment group (A-D) over the study period. Mobile stages include pre-adult 1 (PA1), pre-adult 2 (PA2), and adult (A) lice, with females and males pooled for each stage. Red data labels above bars indicate if delousing occurred before this sample point, with numbers indicating the total number of delousing events within the treatment group during that time (over 3 cages). The November 2016 sample was conducted prior to the implementation of prevention treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

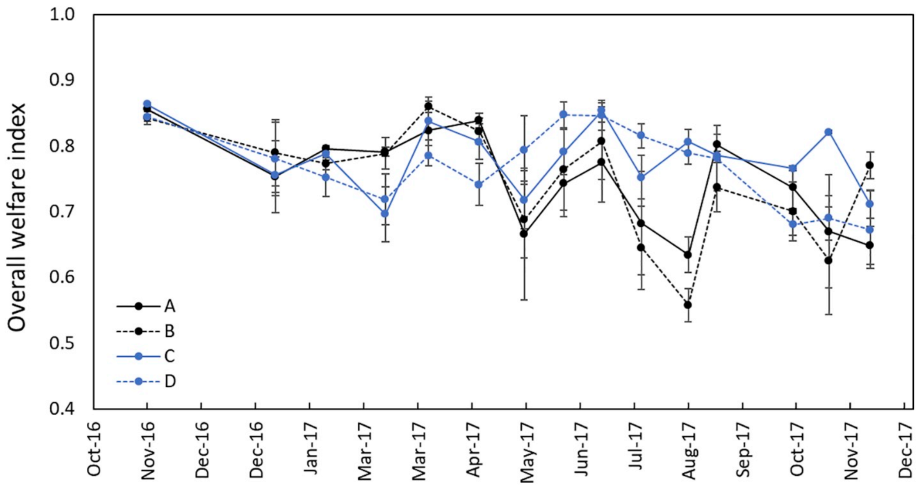

Fig. 7. Mean overall welfare score of fish in treatment groups, over the study period. The overall welfare index is calculated using the SWIM 1.1 model (Stien et al., 2013), which has a possible score range from 0 to the most positive score of 1. Note that the first sample point in November 2016 was before treatments were implemented in any of the cages. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to 12 m (Fig. 2), and therefore collections from shallower than 10 m is likely to capture individuals that are not in the larger school. This was attempted to be mitigated by the restriction of feed in the sample cage prior to netting fish: as these production fish are fed well throughout the day, withholding feed and then hand-throwing pellets before capturing should theoretically improve the chance for sampling representative fish.

The discrepancy between the reduction of new infestations and the similar frequency for delousing events could be due to the internal limits set by the farming company (CAC, operated by Mowi AS), which is lower than the national legislation (0.2 compared to 0.5 adult female lice per fish, respectively). It could be possible that the reduction of infestations can be translated to reduced adult female lice abundances, but the difference occurred above the 0.2 lice threshold. However, when comparing the prevalence of 0.2 versus 0.5 adult female lice fish per fish across samples, a similar pattern exists, whereby cages would have needed to be deloused at the same inter-group rate (i.e. the number of times a cage exceeded 0.5 adult females per fish was similar across groups; see Supplementary table 3). If the experimental facility followed the national legislation level of 0.5 female lice fish per fish instead, treatment frequency (as predicted by this study's scientific lice counts, conducted only every 2–6 weeks) would have been reduced from 63 occasions to only 28 (Supplementary table 3). In fact, some cages barely reached the limit of 0.5 adult female lice fish per fish; one A cage never surpassed 0.5 adult females, and two D cages only passed this limit once.

An alternative theory for the converging abundance of mobile lice among groups could be the availability of brackish waters in the cages without lice skirts; salmon in these cages would have access to low-salinity waters and their forays to brackish salinities could affect the development success of attached lice. There were periods in this study whereby the halocline was around or above the depth of the skirt, thus potentially providing a brackish environment for A, B, and C groups but not D groups (Fig. 3); however, evidence from laboratory trials suggest that lice development is affected when exposed to salinities < 20 ppt for > 9 consecutive hours (Sievers et al., 2019).Improved understanding of the effect of brackish water on lice attached to salmon in the field is required, to fully explore this possibility.

4.3. *Welfare status*

The prevention technologies tested here were not expected to cause negative specific welfare effects, although progression of growth and condition factor was particularly interesting with the submerged feeder and functional feed. The mean Overall Welfare Index (OWI) score over time was between 0.74 and 0.78 for all groups, which is comparable to standard commercial sites using only SWIM 1.0 assessments (Folkedal et al., 2016). The occurrence of extreme low scores (e.g. when cage mean was 0.56 in Group B) were driven by a higher rate of worst eye status scores, resulting in individual OWIs of 0.00.

When investigating the drivers of low OWIs, no consistent pattern in severity of welfare indicators was observed among treatment groups. A possible driver of low welfare scoring could be procedures such as handling during delousing. During the experimental period, this farm site avoided chemotherapeutants and mainly utilised mechanical delousing methods for control of mobile sea lice, which could result in more frequent spikes of severe welfare scores due to treatment. For instance, there was a spike in prevalence of severe mouth/jaw wounds in C and D groups (Sample 3) and D and B groups (Sample 14), however overall the level remained below 15% of sampled fish exhibiting severe scores (Supplementary Fig. 2). Although skin and fin condition were poor throughout the study period, eye status fluctuated and was often scored highly. The most severe eye score will be recorded if the fish have high eye-area coverage cataracts in both eyes, or severe exophthalmia that renders them blind (see Pettersen et al., 2014). This can be caused by a number of biological and abiological factors (Noble et al., 2012), however the most likely influencers pertinent to this study's cage environment are the rapid increase in temperature and growth during spring months (Bjerkås et al., 2001), or common aquaculture practices such as pumping and mechanical delousing, or secondary infections.

4.4. *Adaptive prevention*

In the cages with both skirts and the deep light and feed system, there is likely to be a complex interaction between the temperature profile inside the cage, the thermo-regulatory swimming depth preference of the school, and the distribution of infective lice in response to salinity gradients. Preliminary evidence from this study and another analogous case study using only deep lights and feed (Nilsson et al., 2017) showed that the distance of the school from the halocline could be a greater driver of infestation prevention over a long period than the swimming depth. This interaction is temporally dynamic, thus using a static approach with installations at fixed depths for the duration of a production cycle is unlikely to maximise the preventative efficiency of these integrated pest management strategies.

This study and other previous works with depth-related prevention approaches (Oppedal et al., 2017; Stien et al., 2018; Stien et al., 2016) demonstrate that the concept of host-parasite mismatching can be successful, but under certain conditions. With constant changes in environmental conditions that drive the behaviour of the salmon and their interaction with cage prevention technologies, an approach to maximise efficiency is to utilise these tools in response to specific environments. By understanding under what conditions a technology successfully functions (through both host and parasite behaviours), and having flexible responses to the current temperature and salinity profile, farmers could maximise lice prevention potential. For instance, lice skirts could be lowered deeper if there is a brackish layer at the surface and salmon are swimming deep, or completely removed if salmon prefer the shallow brackish depths. Similarly, submerged lights and feed could encourage the school's depth preferences towards areas with lower infective risk, as predicted by the salinity profile.

4.5. Practical implementation

The addition of lice skirts and the submerged lights and feed system increased the amount of equipment suspended in or around the cage, leading to higher workload in maintenance and during procedures compared to standard production cages. However, the anecdotal experience at this site demonstrates that the management of the cage technologies was easily achievable with good planning and skilled staff. The Norwegian salmon industry is currently exploring all viable options for preventing salmon lice infestations, with some farms applying multiple approaches, and therefore greater workload related to equipment and cage structures is likely to be inevitable.

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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.

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