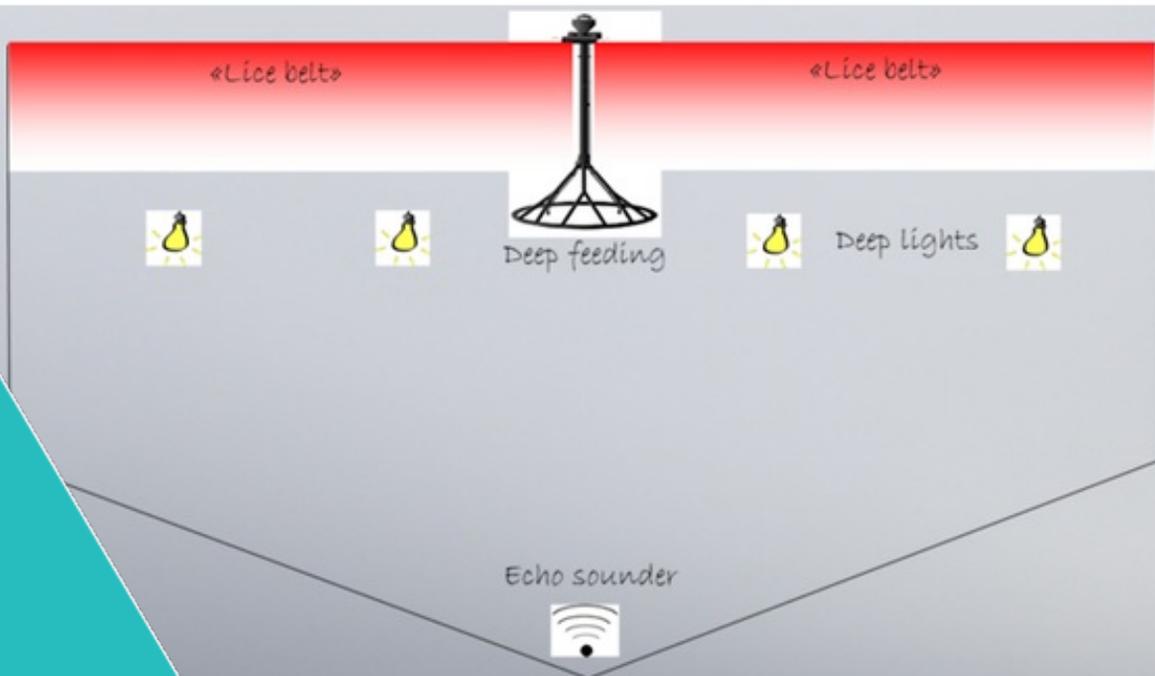# SUMMARY AND STATUS OF DEEP LIGHTS AND DEEP FEED USE IN COMMERCIAL SETTINGS: WELFARE, BEHAVIOUR AND INFESTATION AT THREE CASE STUDY SITES

End report from the FHF projects 901154 "Dypelysogfôring"

Samantha Bui, Frode Oppedal, Jonatan Nilsson, Tina Marie Weier Oldham og Lars Helge Stien (Havforskningsinstituttet)

«Lice belt» «Lice belt»

Deep feeding Deep lights

Echo sounder

Tittel (norsk og engelsk):

Summary and status of deep lights and deep feed use in commercial settings: welfare, behaviour and infestation at three case study sites

Bruk av dype lys og dyp fôring i kommersiell skala; effekter på velferd, atferd og lusepåslag ved 3 oppdrettsanlegg

Undertittel (norsk og engelsk):

End report from the FHF projects 901154 "Dypelysogfôring"

sluttrapport FHF 901154

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Sammendrag (norsk):

Infective salmon lice are attracted to light and are therefore more abundant near the surface than deep in the cage. Manipulating salmon swimming depth by the use of deep lights and deep feeding could therefore spatially separate fish and lice and reduce infestation rate. This was tested at three different commercial farms: during around a year at the fjordsites Kobbavika and Vindsvik in Rogaland, and during 2 months at the more coastal and exposed Skrubbholmen in Nord-Trøndelag. Control cages were fed at the surface and had deep light only as an anti-maturation management during the winter-spring season, while treatment cages were fed at 7 m depth and had deep lights throughout the period. At Vindsvik, a third group with a lice skirt in addition to deep light and feeding was included. Vertical position of the fish was monitored by echo sounders, environment was profiled daily and lice infestation and fish welfare was assessed at monthly samples.

The deep attractor made salmon swim on average 5.5 m deeper at Vindsvik, while the effect was generally small at Kobbavika. When there was a clear temperature stratification temperature preferences overruled the effect of the

attractors on swimming depth. Deep light and feeding had no significant effect on lice infestation status or on welfare score. Neither was there an effect of swimming depth on lice infestation rate. However, a halocline with brackish water above was present during much of the study period at both Kobbavika and Vindsvik, and as the infective lice avoid low salinities this may have pushed the lice deeper. Swimming distance from the halocline explained infestation rates better, irrespective of light and feeding regime, and high infestations usually occurred when fish swam near the halocline. It is therefore suggested that spatial separation between salmon and lice should take into account both the environment, salmon preference trade-offs and assumed lice vertical distribution, rather than only swimming depth *per se*.

Sammendrag (engelsk):

Lakseluslarvene som fester seg på laksen tiltrekkes lys og finnes derfor i større konsentrasjoner nær overflaten enn dypt i merden. Manipulering av laksens svømmedyp ved bruk av tiltrekkende dype lys og dyp fôring kan derfor skape et romlig skille mellom fisk og lus, og dermed redusere påslagsraten. Dette ble testet ved tre forskjellige kommersielle oppdrettsanlegg: i ca et år på fjordlokalitetene Kobbavika og Vindsvik i Rogaland, og i 2 måneder på den mer eksponerte kystlokaliteten Skrubbholmen i Nord-Trøndelag. Kontrollmerdene ble fôret på overflaten og hadde kun dype lys i vinter-/vårsesongen for å unngå kjønnsmodning, mens behandlingsmerdene ble fôret på 7 m dyp og hadde dype lys gjennom hele perioden. Ved Vindsvik ble en tredje gruppe med luseskjørt i tillegg til dypt lys og -fôring inkludert. Fiskens vertikale fordeling ble overvåket med ekkolodd, miljøet profilert daglig og lusenivå og fiskevelferd vurdert ved månedlige uttak.

Ved Vindsvik førte dype lys og dyp fôring til at laksen svømte i gjennomsnitt 5,5 m dypere enn kontrollfisk, mens effekten på atferd var generelt liten ved Kobbavika. Når det var en klar temperaturstratifisering overskygget laksens temperaturpreferanse effekten av attraktorene på svømmedybden. Dype lys og dyp fôring hadde ingen signifikant effekt på lusenivå eller på velferdsscore. Det var heller ikke noen effekt av svømmedyp i påslagsrate av lus. Store deler av forsøksperioden var det imidlertid en haloklin og brakkvann i overflaten både ved Kobbavika og Vindsvik. Da luselarvene unngår lave saltholdigheter kan dette ha presset også lusen dypere. Avstand til haloklinen forklarte påslagsrate bedre enn svømmedyp, uavhengig av lys- og fôringsregime, og høye påslag oppstod vanligvis når fisken svømte nær haloklinen. Det foreslås derfor at når man ønsker romlig adskillelse mellom laks og infesterende luselarver skal det tas hensyn til både miljøet, laksens avveiing mellom temperatur, lys og fôring, og lusens antatte vertikale fordeling, heller enn å bare fokusere på å øke laksens svømmedyp.

Innhold

Status of knowledge

The national growth of the Atlantic salmon (*Salmo salar*) aquaculture industry has cemented Norway as the leading producer in the world, boasting 53% of global production in 2015. 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. One of the major challenges that salmon aquaculture industries face is the issue of salmon lice (*Lepeophtheirus salmonis*) infestations, which is restricting the potential expansion of the Norwegian industry (Olaussen 2018). Salmon lice are an ectoparasite that have dramatically proliferated in parallel to the increasing abundance of farmed Atlantic salmon, prompting action by the Norwegian government to strongly regulate production limits. 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.

Strategies to combat lice are critical for disease management, with preventative measures being of high priority. This becomes increasingly important given the fact that the majority chemical compounds typically used to treat against lice have lost their effect completely or lice have become increasingly less sensitive to the active substance (Aaen *et al.* 2015). One strategy to mediate salmon lice infestations is depth-related prevention methods, where the distribution of the salmon in the water column is manipulated to mismatch the distribution of infective lice. Passive guidance of salmon swimming depth has been achieved using attractant lights and the provision of feed away from the surface (*e.g.* Oppedal et al 2011).

This report aims to summarise the current status of knowledge for the use of submerged lights and feed zones as a means for lice prevention, and synergise recent studies that have tested this method at a commercial scale.

1.1 – Lice larvae distribution with depth

The salmon louse has 8 life-history stages, where the youngest stage, nauplius 1 are hatched from eggs carried in egg strings by the adult female; they are lecithotrophic and drift in this planktonic stage, undergoing two moults until they reach the infective copepodid stage (Hamre *et al.* 2013). It is this stage that starts exhibiting host-searching behaviours, whereby copepodids will actively disperse themselves near the surface of the water column. They are positively phototactic, but avoid brackish salinities that often are present in coastal or fjord waters (Heuch 1995, Genna *et al.* 2005). This strategy enables the largest possibility of encounter with salmonid hosts, who swim relatively shallow during their outmigration (Thorstad *et al.* 2012, Eldøy *et al.* 2017). Once a potential host is located, copepodids attach to the host and moult through two chalimus stages at that fixed position (termed sessile stages). When they reach the pre-adult 1 stage, they become mobile and begin to move around the host; after an additional pre-adult stage, lice moult into adults and females become reproductively active.

The concept of depth-related lice prevention technologies in aquaculture is to draw the salmon school away from the most parasite-risky surface waters, either through cage manipulations (*e.g.* snorkel structures, or submerged cages) or passive attraction using positive stimuli. Here, we focus on the latter method, with the use of submerged lights and provision of a deep feeding point as attractants.

1.2 – Use of light for influencing salmon behaviour and controlling sexual maturation

The observation that artificial lights, long used in salmon production to reduce early maturation, are highly attractive to salmon (Juell and Fosseidengen 2004, Oppedal *et al.* 2011, Wright *et al.* 2015) prompted researchers to explore the application of lights as a passive means of influencing salmon swimming depth. However, the use of artificial lights during a full production cycle has a potential negative side effect. Photoperiod, the alternation of light and dark in a 24-hour cycle, is a key environmental cue which modulates growth, reproduction and behaviour in fish (Falcón *et al.* 2007, Iigo *et al.* 2007). Because salmonids appear to lack any endogenous clock, production of the biological time keeping hormone, melatonin, is controlled entirely by photoreception in the retina and pineal gland (Falcón et al. 2007, Iigo *et al.* 2007). As a result, photoperiod modification with artificial lights has profound implications for the growth and development of farmed salmon. Currently, artificial lighting is widely used in salmon aquaculture to inhibit maturation in spring (Leclercq *et al.* 2011, Hansen *et al.*, 2017). By super-imposing artificial lights at night on the natural photoperiod from January to June, fish skip directly from the short daylengths of winter to the long daylengths of summer, and thus are signalled to have missed the spring window of opportunity for maturation (Migaud *et al.* 2010). However, while photoperiod advancement in spring inhibits maturation, extending summer light conditions into autumn can do the opposite and stimulate sexual development (Fjelldal *et al.* 2011, Fjelldal *et al.* 2018). Thus, behavioural modification using submerged lights is only a viable lice prevention measure if the photic signals which initiate sexual maturation in salmon are understood and accounted for.

Powerful, full spectrum (white) metal-halide lights from 400 to 1000W have traditionally been used to prevent maturation in the spring, but narrow-spectrum violet LED lights (peak at 400 nm) have been developed for use as an attractant the rest of the year. These lights are built to emit wavelengths with the least physiological effect on salmon, and light intensity from these 100W lights can be decreased as low as 0.1 µE cm^{-2} sec^{-1} (irradiance measured at 1 m) as compared to regular anti-maturation lights with an irradiance of 80-140 at the same distance (Migaud *et al.*, 2006, Leqlercq *et al.*, 2011; Hansen *et al.*, 2017). Studies on S1 smolts have shown that prolonged exposure to these lights did not induce increased maturation (Hansen *et al.*, in prep).

Thus, if lights are deployed and kept lights at 10 m depth during a complete production cycle, we may be able to continuously keep the fish deeper in the pen during night-time. Proposed regimes include using violet UV-LED from first autumn to midwinter, anti-maturation lights at the same depth from mid-winter to mid-summer, and then reverted back to violet UV-LED from mid-summer and until harvest. By implementing lights over an entire production cycle, we can also determine whether or not light attraction can override temperature (vertical gradients) which in the literature have been recognized as the main driver of vertical positioning in farmed salmon (Oppedal *et al.* 2011).

1.3 – The influence of feed on salmon swimming depth and behaviour

The provision of feed is a strong stimulus for salmon, whereby many of their behaviours can be anticipatory or related to feeding. As appetite is a strong driver of behaviour, salmon can be conditioned to new environments or negative stimuli (such as aversive flashing lights) through food reward (Bratland *et al.* 2010). Thus, although lights may keep the fish deep during the night, surface-feeding during day-time will still attract the fish towards the surface during feeding (Bjordal *et al.* 1993, Juell *et al.* 1994), and hungry fish will in addition be attracted to the surface at

times with no feeding (Juell *et al.* 1994, Frenzl *et al.* 2014). Contrary to trickle-feeding where fish will spend more time in the surface-layers where infective lice-stages are most abundant, shorter and more intensively distributed meals will leave the fish more time to position itself according to other preferences than feeding / hunger level (Bjordal *et al.* 1993, Juell *et al.* 1994), i.e. feeding motivation declines below some threshold level where other parameters are prioritized. Both empirical knowledge, also from several Marine Harvest sites, and a study done by Frenzl *et al.* (2014) show that salmon may be attracted towards greater depths when the feeding-area is re-situated from the surface and down to 5-8 m depth. The same study indicated that underfed fish are still attracted to the surface even if feed is distributed under water. This illustrates the importance of avoiding near continuous trickle-feeding and give priority to more intense feeding that may rapidly lower feeding motivation and therefore also tendencies to move towards the surface.

1.4 – Use of deep lights and feed as a lice prevention method

The negative correlation between swimming depth of Atlantic salmon and their experienced lice infection pressure or lice levels in general is well described (Huse and Holm 1993, Osland *et al.* 2001, Hevrøy *et al.* 2003, Stien *et al* 2016; Oppedal *et al.* 2017, Wright *et al.* 2017). The principle is shown in Figure 1. Similar results have been found when using skirts around farming cages to shield salmon from the upper water layers (Næs *et al.* 2012, Lien *et al.* 2014; Stien *et al* 2018) where infective stages of *L. salmonis* are most abundant (Heuch 1995, Heuch *et al.* 1995).
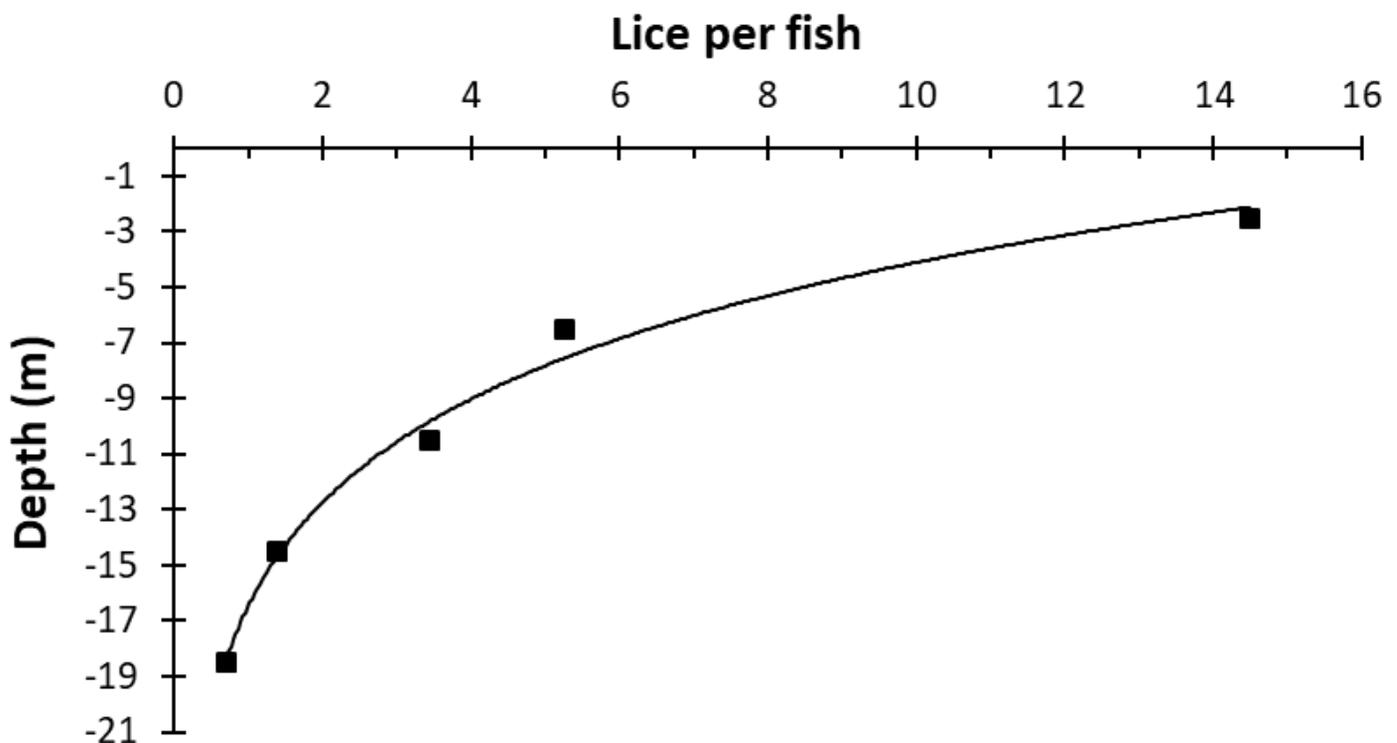

Figure 1. *Lice infestation over a 12-week period with forced swimming at 0-5, 4-9, 8-13, 12-17 or 16-21 m depth in snorkel cages. Markers indicate average available swim depth below the snorkel with a logarithmic trendline and R2 value of 0.99. Data from Oppedal et al 2017.*

The approach of using lights and feed to attract salmon deeper in to the cage has a promising potential as a prevention strategy. Salmon can be attracted to swim deeper in the sea cage by use of lights (Juell *et al.* 2003, Juell and Fosseidengen 2004, Frenzl *et al.* 2014, Stien *et al.* 2014) and by moving the feeding zone to match the lights' depth (Frenzl *et al.* 2014, Nilsson *et al.* 2017). If successful, salmon will thereby be avoiding the shallow waters except for infrequent forays to the surface to refill their swim bladders (Korsøen *et al.* 2012). Positioning salmon deeper in the water column, whether with cage structures or otherwise, has been proven as an efficient way to prevent infestation (see above). However, the effectiveness of reducing infestation may be highly dependent on seasonal variation in salinity stratification and water temperature. If a brackish layer is present at the surface, sea lice larvae may actively avoid low-salinity waters and aggregate below the halocline (Heuch 1995, Wright *et al* 2018) and the infective zone can be brought deeper in the water column. Alternatively, constant or periodic 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 deep lights can attract fish to greater depth and stimulate schooling during night-time, and granted that deep feeding (removing the surface as a feeding area) stimulate fish to descend deeper in the cage during day-time, the combination of the two should in theory imply that the fish will stay deeper in the pen for significantly longer periods than will fish in control pens, and thus lice infection rates and levels in general should be reduced.

1.5 – Case studies

Three commercial sites provided case studies for the case of using submerged lights and feeding systems in a commercial production setting. Two sites were located in the Rogaland region of south-western Norway; Kobbavika was located at a relatively open site towards the entrance of Boknafjord (Nilsson *et al* 2017), whereas Vindsvik was situated in a narrow, more inland fjord that branched off Boknafjord (Bui *et al* 2018). Skrubbholmen is located further north, in Nord-Trøndelag region of Norway, at an open-water site with little salinity stratification (Nilsson *et al* 2018).

Methods

2.1 – Experimental setup

On all sites the principal setup was to use submerged lights positioned deep in the cages and deep feeding of which were to attract the salmon night and day to swim deeper, preferably under the so called "lice belt", and thereby be infested with less lice (Figure 2). Control cages were originally to have surface feeding and no lights.
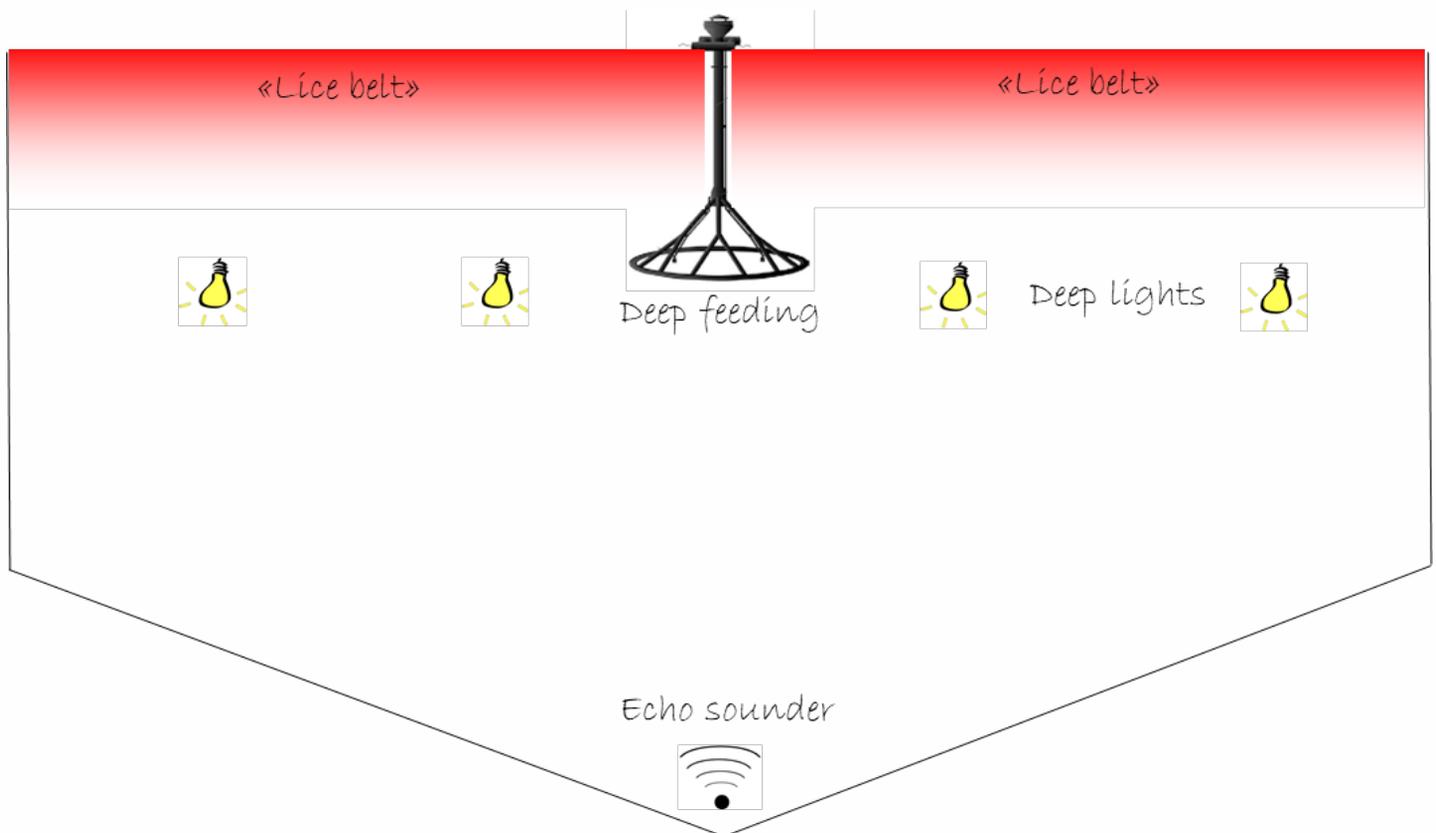

Figure 2. Principle of the experimental setup. Deep feeding and deep light are used to attract the salmon to swim below the hypothesized "lice belt" closer to the water surface. Echo sounders were to monitor swimming depth distribution of the fish.

Kobbavika: The site is positioned in Rogaland, Fognafjorden, on the south-east side of the island Fogn. The site consists of 8 circular cages, of which only 4 were used in the trial (2 surface fed control cages, 2 deep fed cages). Cages were 200 m (cone-nets) in circumference, with a depth of 35m (and later 45m), initially with a pen-wise volume of 47 745 m^3 stocked with 196-198 000 smolts from transfer. All cages had cleaner fish: around 4% at the start of the trial and between 13 and 18% at the end of the trial when the number of salmon had been reduced due to harvest. The study period was from December 2015 until December 2016.

In the two treatment cages, systems for deep feeding at 7 m depth (AKVA SubFeeder; AKVA Group, Norway) was installed for the entire production cycle, with the exception of shorter periods when they were removed for technical

reasons. The control cages were fed at the surface. In addition to deep feeding, nine low-intensity violet LED-UV lights were installed at 10 m depth in treatment cages from trial start to 25th January, and from 15th July to end of trial, with the exception of shorter periods when they were removed for technical reasons. From 25th January to June, standard anti-maturation lights with higher intensities were used in all 4 cages, placed at the same depth as LED-UV lights (10 m).

Vindsvik: The site was located in the same region as Kobbavika, but further inland in the long, narrow fjord of Jøsenfjorden. The experimental site had 12 circular sea cages (circumference = 120 m, 35 m deep) in two parallel rows. In September 2016, approximately 65 000 smolt (~100 g) were transferred into each cage and raised with standard production procedure throughout the study. All cages were stocked with cleaner fish throughout the production cycle, using whichever species were available at the time. Cleaner fish species used included the ballan wrasse (*Labrus bergylta*), lumpfish (*Cyclopterus lumpus*), corkwing wrasse (*Symphodus melops*) and goldsinny wrasse (*Ctenolabrus rupestris*). Intended stocking density of cleaner fish was to be 5% of salmon number per cage, with restocking multiple times during the experimental period. The study period was from October 2016 until December 2017.

Cages were assigned to one of four treatment groups (3 replicate cages per treatment), however in this report, only 3 groups will be represented: surface-fed cages (cleaner fish and functional feed provided), deep lights + feed cages (cleaner fish, functional feed, and deep lights + feed), and deep lights + feed + skirt cages (cleaner fish, functional feed, deep lights + feed, and a lice skirt).

Functional feed (Skretting Shield, Skretting, Norway) was provided from trial initiation until trial completion. In December 2016, relevant treatment cages 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 to 10 m depth. 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 – 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 skirt treatment cages that extended from the surface down to 6 m.

Skrubbholmen: The site was located in a relatively exposed area south of Nærøysund in Nord-Trøndelag. Four cages (circumference = 157 m, 15 m deep + cone) were used in the study. The cages were stocked with 162 000 salmon in each, transferred on 22 May 2017. The fish were around 2.7 kg on average at trial start and 3.3 kg at trial termination. All cages had 5 m deep lice skirts and 6 LED lights at 9 m depth. The lights were of the same type as used at Vindsvik (AKVA Aurora SubLED Combi), with the green-blue anti-maturation colour switch on continuously throughout the study period. Two of the cages were fed at 7 m depth by the AKVA SubFeeder (AKVA Group, Norway). The remaining two control cages were fed at the surface. Prior to the trial all fish had been surface fed. The study period was from 24 January until 22 March 2018.

2.2 – Welfare and infestation assessments

Lice have a temperature-dependent growth rate, whereby the time taken to reach the mobile pre-adult stage is between 6 days (at 21°C) to 69 days (at 3°C) post-infestation for males (S. Dalvin, unpublished data). For evaluating the effectiveness of deep lights and feeding on preventing infestation, we only use data on abundances of sessile lice stages (i.e. copepodid, chalimus 1 and chalimus 2 stages) found on salmon. This is due to the

uncertainty of the grazing effect cleaner fish had on mobile stages, and the occurrence of delousing treatments that often only target mobile stages.

Procedures for assessing welfare and recording infestation status was the same for all sites. Every 3 to 4 weeks during the study period (except during summer at Kobbavika), 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 from the surface and 10 m deep, or small ring-net pulled from 5 or 10 m depth to the surface by a boat crane; cages were sampled using the same method at each separate sample event. At Vindsvik, sampling was conducted a minimum of 3 weeks after any delousing treatment, so that new infestations were certain to be unaffected by previous treatments. At Kobbavika, there were delousing events approximately two weeks before Sample 3 (Cage 4 only) and Sample 4 (Cage 3 only); these has not been excluded from analysis. At each sampling point, 20 salmon (occasionally more) from each cage were euthanised and any lice attached were quantified and staged. Also lice in the anesthetization tub was counted, and all lice were counted and divided by the total number of fish sampled. Each fish was also evaluated using the standardised SWIM 1 and 1.1 model (Stien *et al.* 2013), which involves the scoring of 14 indicators of welfare ranging from undamaged ormal to severely damaged/abnormal (Table 2). Welfare indicators are weighted in the model and are used to calculate an overall welfare index, a value bounded from 0 (worst) to 1 (best) that represents an individual's welfare status. Gill diseases were also recorded, specially presence of PGI and AGD, however the prevalence of these diseases were negligible throughout the study.

2.3 - Monitoring of swimming depth and environment

To monitor group swimming behaviour, swimming depth distributions were continuously recorded using a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway; Bjordal *et al.* 1993, now CageEye AS, cageeye.no). 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). Profiles of temperature and salinity were taken from the surface down to 40 m depth, dependent on depth at the site, at a reference point outside of the experimental cages.

2.4 - Data handling

Skrubbholmen lacks comprehensive data for swimming depth and therefore we only represent and discuss lice data for this short experimental period.

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. This value was used to compare the efficiency of treatments on prevention of new infestation, using a generalised linear model (GLM). The model included treatment, site, and sample date as predictor variables, with cage number as random effect and used a Poisson distribution (package 'glmmTMB', function *glmmTMB* in R). Date of lice attachment was back-calculated for new infestations stages using their temperature-dependent development rate (S. Dalvin, unpublished data) based on the average sea temperature 2-3 weeks prior

to the sample event.

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, particularly at the Skrubbholmen site. Thus, analyses of salmon swimming depth were only conducted on the Kobbavik and Vindsvik sites. The data provided information on the vertical dispersion of the salmon; occasionally the school exhibited a bimodal distribution where there were 2 (and rarely, 3) main groups within the school. The swimming depth of salmon was estimated using the relative vertical distribution seen from the echosounder data, at 1-m depth intervals whereby more than 4% of fish were present. For analysis of school vertical distribution in relation to halocline depth, values for daily swimming depth were calculated as the median depth of the upper (shallower) school from the mean of their upper and lower depth limits, pooled across all hours within the day.

As copepodids gradually avoid brackish waters, particularly of 28 ppt or fresher (Wright et al 2018), a stratification with salinity value of 28 ppt was considered 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. The relationship between the distance of median salmon swimming depth from the halocline depth (and therefore assumed depth of infective copepodids), and actual lice infestation (back-calculated from stages) was assessed for the study period, whereby only days when all three parameters (environment, salmon swim depth, and lice attachment rate) were available for a replicate cage were used. Intensity of lice acquired per fish was analysed with a GLM using distance from halocline (as an absolute value) and site as predictor variables, with cage as a random factor. Data were pooled across treatments, as distance from halocline was the most interesting factor and is intrinsically linked with the influence of deep lights and feed on school swimming depth.

Overall Welfare Indexes (OWI) were averaged across individuals in a cage and compared among groups using a GLM. Similar to the lice model, treatment, site and sample date were included as predictor variables in the model.
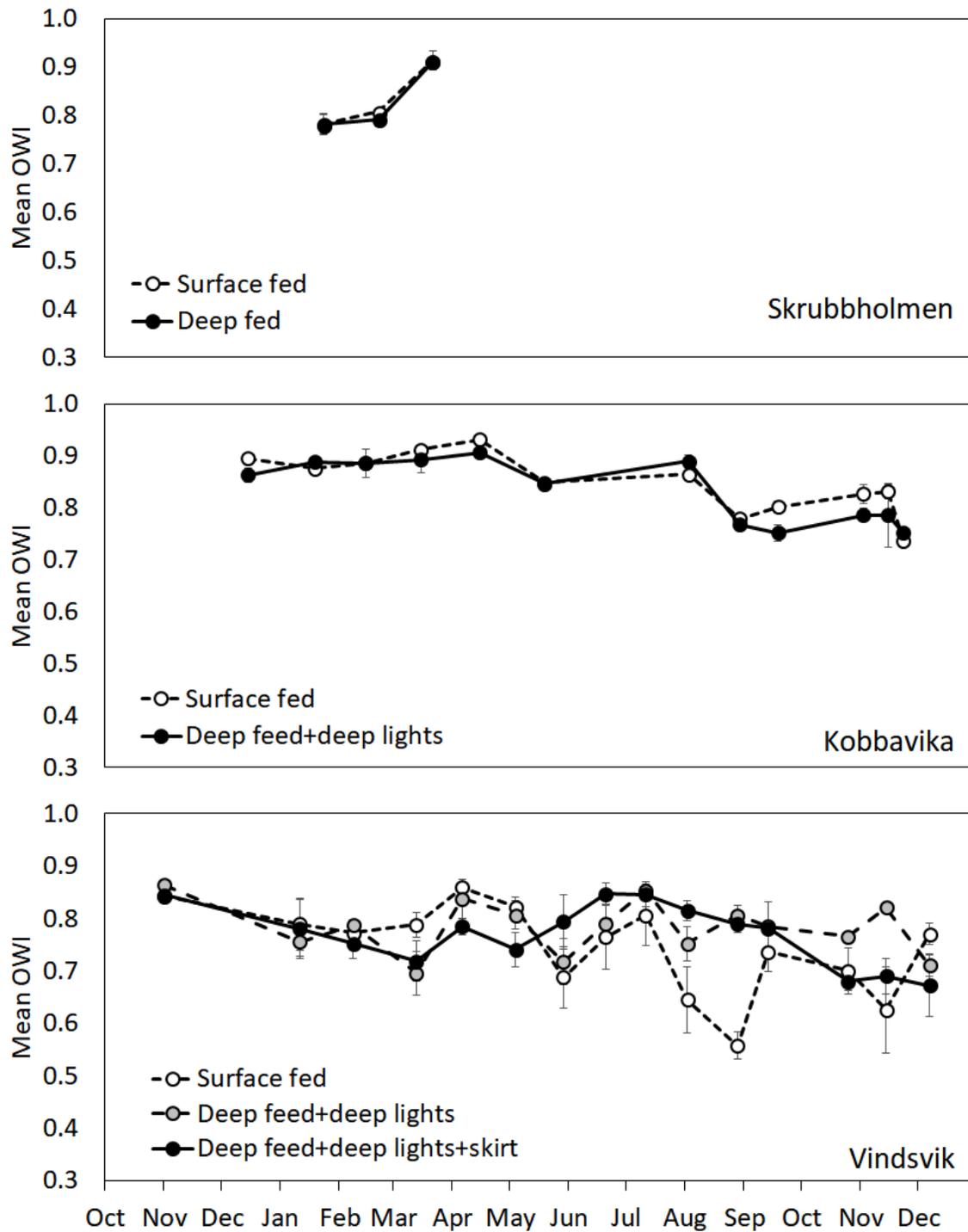
Results

3.1 - Salmon welfare

Figure 3. Mean overall welfare score of fish in treatment groups, for each study site, over the study period. Periods differ in year between sites: Kobbavika shows data from 2015-2016, Vindsvik from 2016-2017, and Skrubbholmen within 2018. The overall welfare index is calculated using the SWIM model (Stien et al. 2013), which has a possible score range from 0 to the most positive score of 1.

Overall welfare, as calculated using the SWIM model, was similar between standard cages and those with deep lights and feed; the null model was not different from the full model that included treatment (χ^2 = 0.277). The full model therefore did not show a significant effect of deep lights and feed on welfare compared to control cages, but OWI was different between sites (Vindsvik from the other two; z = -7.61, p < 0.001) and among sample points (z = -4.70, p < 0.001). For Kobbavika, OWI was similar among all cages for the 12-month study period, with a small non-significant drift of treatment cages in autumn months (Figure 3). At Skrubbholmen, the shorter study period showed almost identical welfare statuses among cages. Vindsvik was more variable, with the largest discrepancy between groups also occurring in autumn; however, it was the surface-fed cages that suffered lower welfare status in August to October, reaching the lowest OWI score of 0.56 (Figure 3). The addition of a lice skirt did not affect welfare, however scores were lower than deep feed and lights cages in October to December.

3.2 – Environment and swimming depth

The exposed site Skrubbholmen had relatively homogenous water with temperature and salinity changing little with depth, and no halocline was present at this site. As this site was studied during winter-spring, the temperatures were low (< 4 °C) and falling throughout the study period (Figure 4). Kobbavika was studied over almost a year and temperature changed both with time and depth. Kobbavika is located in a fjord and therefore affected by freshwater, and a halocline (salinity < 28 ppt) was present for long periods, usually between 5 and 10 m depth (Figure 4). Vindsvik was also studied over a year, with temperature changing with time and depth. Vindsvik is located in a more inland fjord site, and is more affected by freshwater than Kobbavika, with a more pronounced and partly deeper halocline (Figure 4).
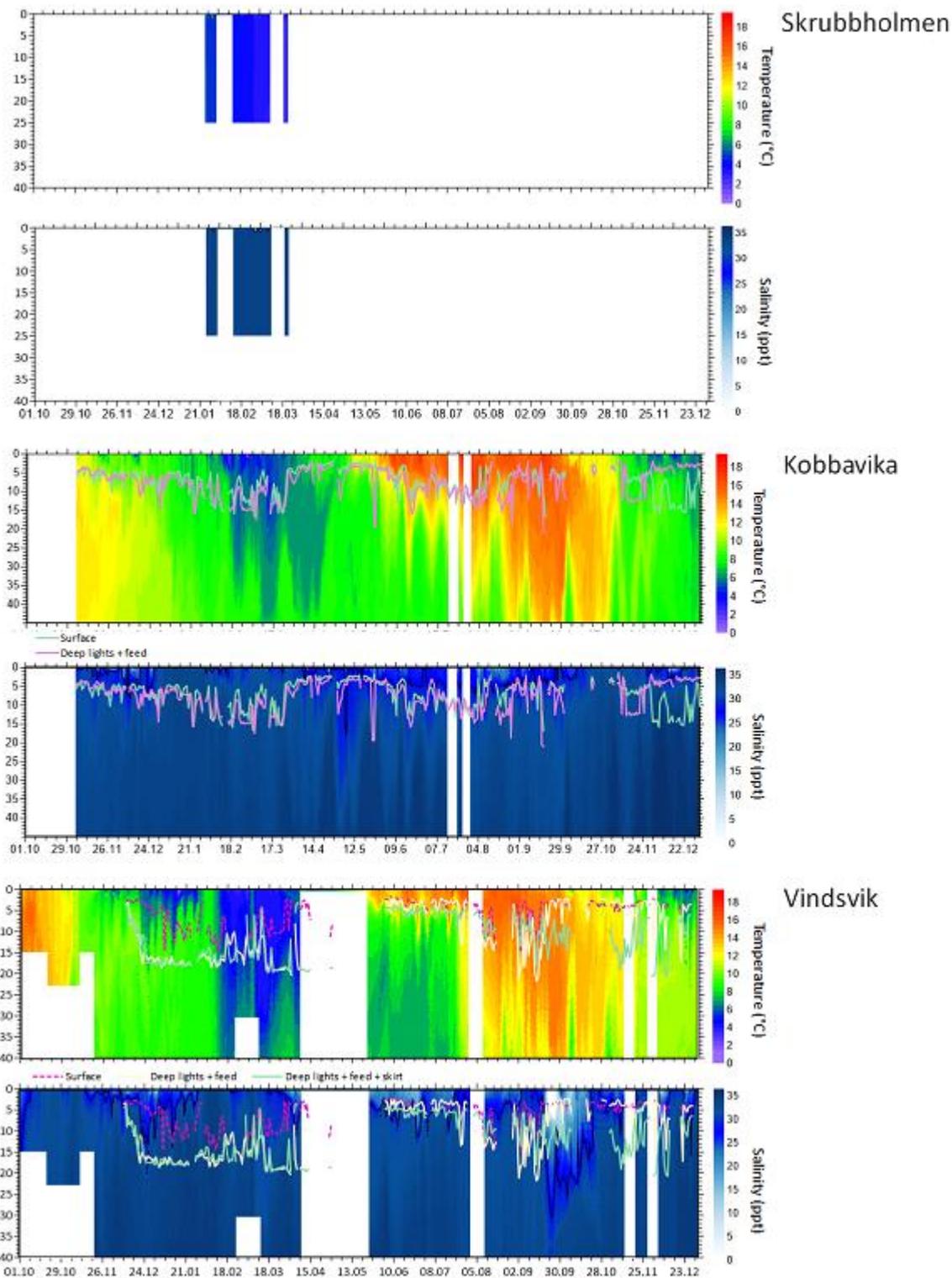
Figure 4. *Environmental profiles of temperature and salinity for the case study sites largely differ in their halocline characteristics: Skrubbholmen (top panels) is unstratified, whereas Kobbavika (middle panels) has a shallow halocline and Vindsvik (bottom panels) has a deeper halocline. The halocline limit of 28 ppt represent the black contour line. White blocks indicate periods where environment data is not available. Overlaid onto the environment profiles is the median daily swimming depth of experimental fish at Kobbavik and Vindsvik, calculated from the shallowest-swimming school. Treatments represented are standard commercial-fed cages (Surface), deep lights and feed (Deep lights + feed), and deep lights and feed, and a lice skirt (Deep lights + feed + skirt, at Vindsvik only). Periods differ in year between sites: Kobbavika: 2015-2016; Vindsvik: 2016-2017; Skrubbholmen: within 2018.*

Brackish layers were present almost throughout the study period, sometimes extending below the depth of the deep lights and feeders (Figure 4).

At Kobbavika, salmon in all cages swam relatively shallow in the first months of the study, when there was little temperature difference in the water column, and generally swam between 5 to 15 m over the study period. For Kobbavika, there was little difference in the swimming depths of treatment and control cages over time. At Vindsvik, fish exposed to deep lights and feed began to swim deeper than control fish almost straight away, but then fluctuated between matching swimming depths and swimming deeper between February to April. During this period, fish were more likely to swim at the same depth if there was no thermal stratification. From June to August, swimming depths were very similar between groups, as the preference for the thermocline was higher than any attraction to lights. Larger differences in swimming depths were then observed for both sites in the late autumn to early winter months, but with contrasting patterns: treatment cages swam shallower than control fish during this period at Kobbavika, but deeper at the Vindsvik site (Figure 4).

3.3 - Lice infestation status

Across the study period and case study sites, the provision of deep lights and feed did not influence the number of lice salmon acquired. The full model was significantly different from the null model which excluded treatment (χ^2 = 0.001), however the variation that treatment contributed to lice was largely driven by the lice skirts in the full model (deep lights and feed and skirt cages compared to deep lights and feed: $z = -2.89$, $p = 0.004$; control cages compared to deep lights and feed only treatment: $z = 1.71$, $p = 0.087$). Control cages Site and sample date were significant factors in the model (Skrubbholmen compared to Kobbavika: $z = -3.30$, $p < 0.001$, and Sample: $z = 4.60$, $p < 0.001$, respectively).

The rate of lice acquisition over the study period varied, with relative peaks occurring in July onwards (Figure 5) at both Kobbavika and Vindsvik sites. The period of study for Skrubbholmen was in winter months, and therefore overall lice levels were low throughout. The exposed and halocline conditions of Kobbavika resulted in higher lice levels of almost 8 lice per fish, compared to the highest level of nearly 3 lice per fish in Vindsvik (Figure 5).
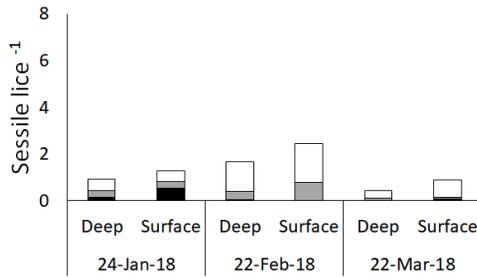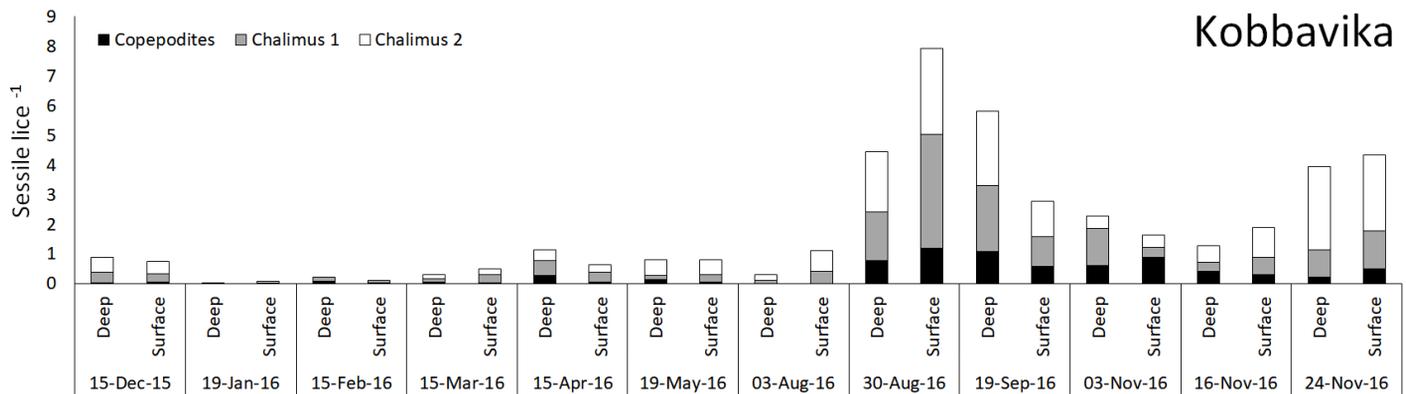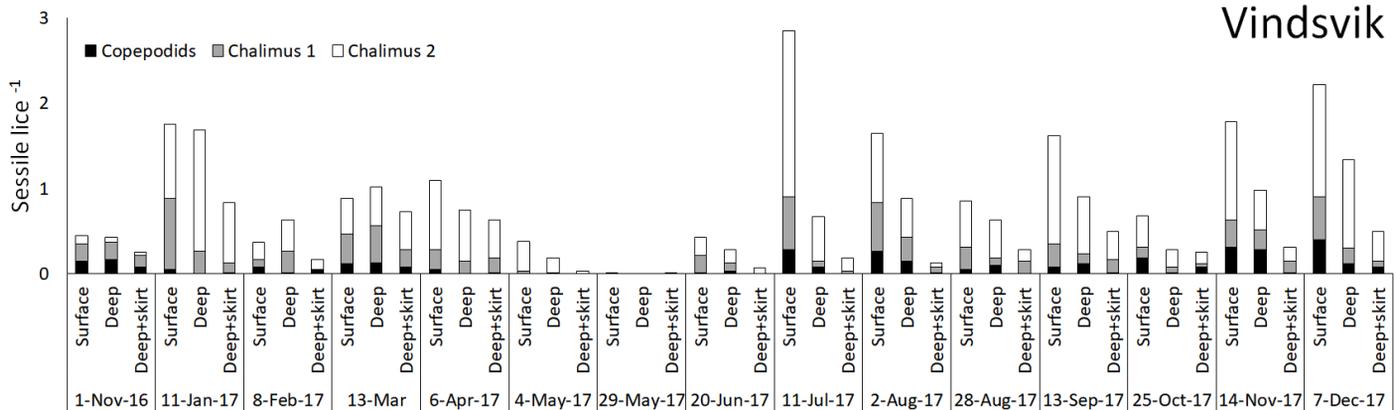
Figure 5. *Average sessile infestation levels per fish for treatment groups over the study period, for the three case study sites: an unstratified site (Skrubbholmen), a shallow-halocline site (Kobbavika), and a deep-halocline site (Vindsvik). Sessile stages of lice are copepodids, chalimus 1 and chalimus 2 which are represented within the columns. The first sample point at Vindsvik and Skrubbholmen were conducted prior to the treatments initiation.*

The largest spikes in infestation rates occurred in the latter part of the year for both Kobbavika and Vindsvik, but did not necessarily correlate to periods with particular halocline characteristics (Figure 6). Although the salmon responded to multiple environmental variables that fluctuated with time, there was a consistent difference in swimming depth at Vindsvik when time-pooled means were estimated. The average median swimming depth of the shallowest school, over the entire study period, was 5.55 m for cages without deep lights and feed, compared to 11.05 m for deep lights/feed and 11.49 m for deep lights/feed + skirt cages. This translated to an average of ~ 0.16 m *above* the halocline for control cages over the period, whereas both deep lights and feed groups were ~5.76 and 6.79 m *below* the halocline, but with larger variation between days (Figure 6). However, at Kobbavika, average median swimming depth was very similar between control and treatment cages (5 and 6 m, respectively), which

Summary and status of deep lights and deep feed use in commercial settings: welfare, behaviour and infestation at three case study sites

Results

meant a similar distance from halocline over time (~1.8 and 2.8 m, respectively; Figure 6).
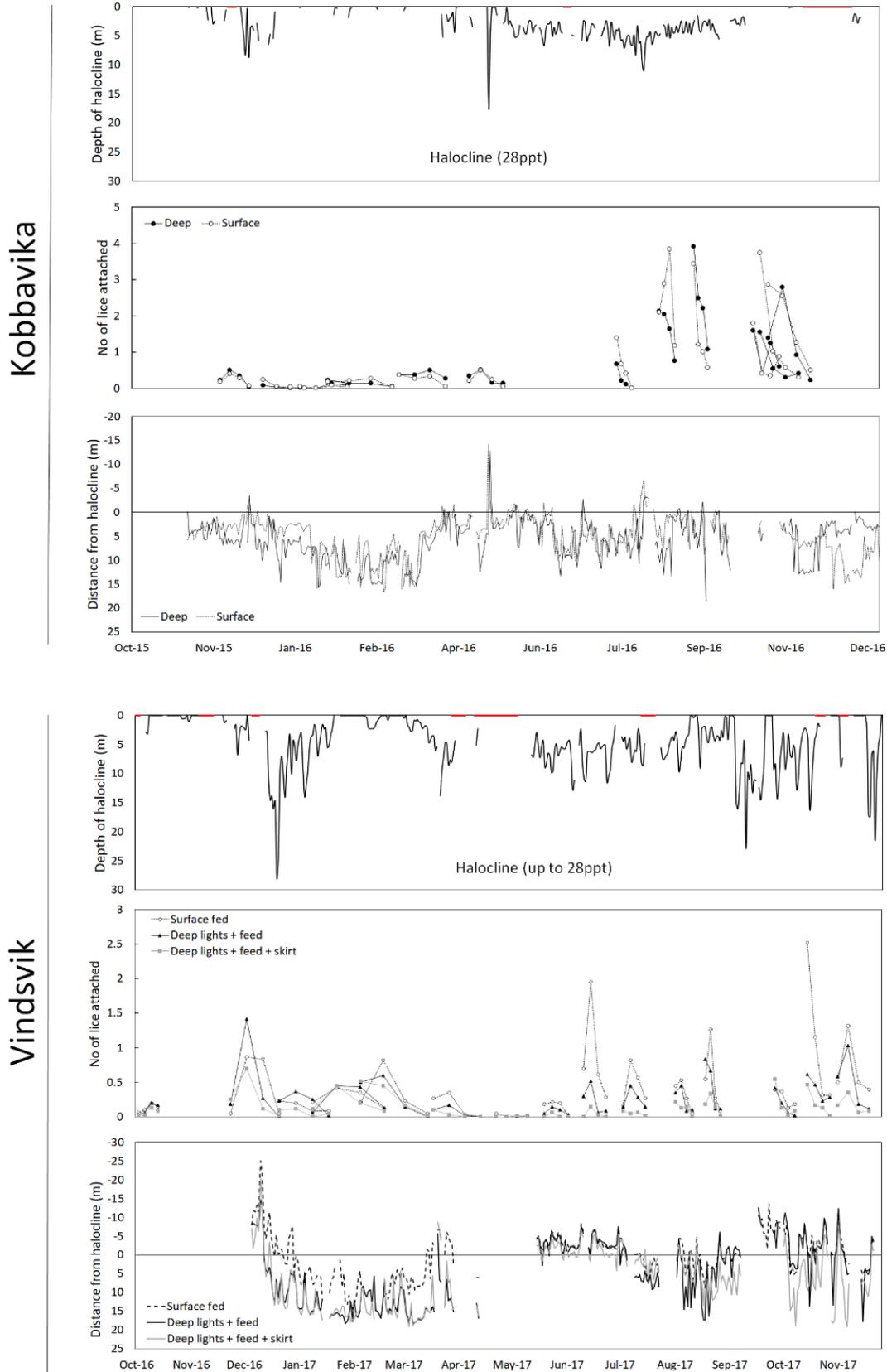
Figure 6. *Halocline depth (defined as up to 28 ppt), lice attachment date and salmon swimming depth over the study period for Kobbavika (upper 3 panels) and Vindsvik (lower 3 panels). Infestation rate per day is represented by mean number of lice (using data from copepodids, chalimus 1, chalimus 2 and pre-adult 1) attached per fish, within treatment groups. Daily median swimming depth of schools relative to halocline is shown, with negative values of the distance from halocline indicating salmon swimming above the*

halocline, whereas positive values indicate salmon swimming deeper in the cage. Times when environmental data were not available are marked out with red horizontal bars.

Numerous assumptions were made when calculating the daily infection success (i.e. date of lice attachment) and swimming depth of the school, however an interesting pattern arises when looking at the relationship between the schools' relative distribution around the halocline and the number of lice acquired (Figure 7). The correlation between distance from halocline and lice acquired was weak but non-significant for the model that included all data ($z = -1.83$, $p = 0.067$). However, salmon in cages that also had a lice skirt are theoretically not interacting with the halocline in the same way if it were within the skirt depth, so these data were removed for a subsequent analysis. When pooling data only from control cages and those with deep lights and feed, the model thereby demonstrates a significant relationship between larger distances away from the halocline and lice acquired ($z = -2.40$, $p = 0.017$), with site also as a significant factor ($z = -3.80$, $p < 0.001$). To investigate whether swimming depth, irrespective of halocline, influenced the rate of infestation, a GLM was used with the same variables included as the distance from halocline model but inserting swim depth data instead. This model did not reveal a difference in lice acquired due to swimming depth but suggested that a trend was present with ($z = -1.66$, $p = 0.096$) and without the skirt cages included ($z = -1.85$, $p = 0.064$).
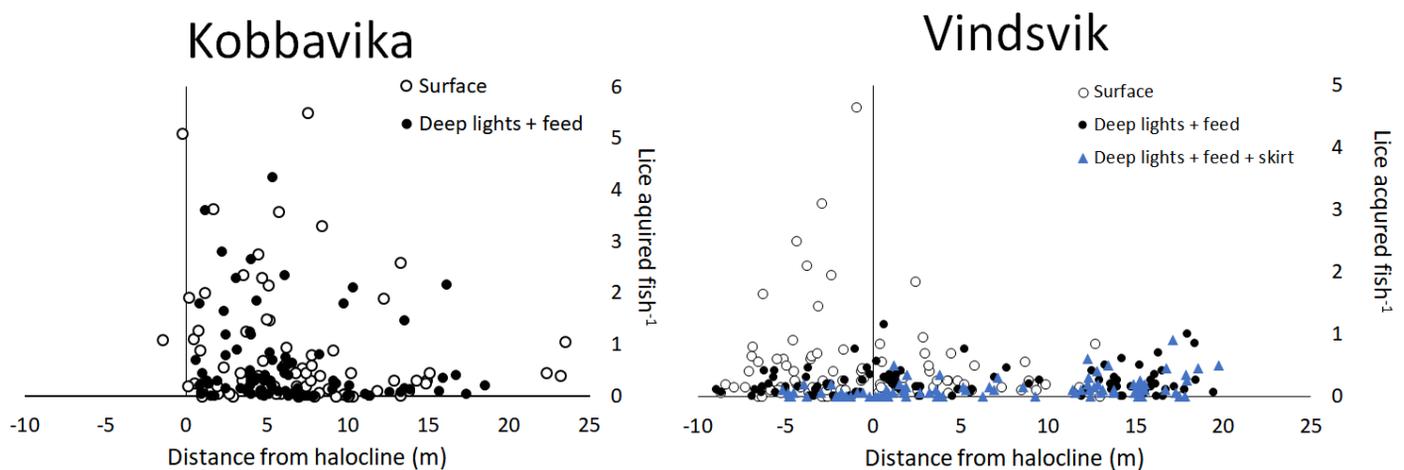
Figure. 7. *Lice infestation rate in relation to the salmon swimming depth, for sites Kobbavika and Vindsvik, separated into panels for each treatment group. Negative values of the distance from halocline indicate salmon swimming above the halocline, whereas positive values indicate salmon swimming deeper than the halocline. Data points are only represented if a day has values for lice attachment, salmon swimming depth, and salinity. Data are pooled over the experimental period.*
Caption

Discussion

4.1 – Impact of deep lights and feeders on salmon welfare

The implementation of deep lights and a deep feeding zone was not anticipated to affect the physical condition of salmon, but welfare indicators related to growth could possibly be influenced by a deeper feeding zone. The results from these case studies using deep lights and feed for 14 months showed that there was no long-term effect of this technology on salmon welfare, and in the case of Vindsvik, could actually provide an advantage over standard cages. Short-term declines in welfare status at Vindsvik could be driven by higher lice infestation levels in standard cages, or indicators such as eye status (Bui et al 2018). At Kobbavika, the largest difference in Overall Welfare Index (OWI) was 0.05, which is a negligible variation. Welfare status at Skrubbholmen was exceedingly similar over the 2 sample points during the experimental period, demonstrating no short-term effect on applying deep lights and feed at later stages of the production cycle.

Using the Salmon Welfare Index Model (SWIM) to assess overall welfare status is an invaluable tool to compare large groups of salmon that are exposed to different experimental conditions; the OWI over time for these sites were ~ 0.76 (Vindsvik), ~ 0.84 (Kobbavika), or ~ 0.83 (Skrubbholmen) which is comparable to standard commercial production cages (Folkedal et al 2016). Kobbavika and Vindsvik sites showed a temporal decline in OWI, with a maximum reduction of 0.16 or 0.17 in OWI, respectively; this is not uncommon for salmon held in production 14 months after sea-transfer.

In terms of the feed uptake of salmon with the deep feeding zone, the indirect measure of coping could be growth of these groups compared to those with standard surface feed. At Vindsvik, cages with deep feed grew to ~ 8.5 times their original sampled body weight (i.e. from 322 g to 2701 g), compared to ~ 10.5 in standard-fed cages. This discrepancy could be due to reduced feed intake either because of the feeding method, or because they experienced alternative temperatures, or a combination of both. Alternatively, the recorded weights could be confounded by unrepresentative sampling, whereby emaciated or smaller-class fish were captured instead of individuals from the deeper-swimming main school (see discussion below). In contrast, the deep fed fish at Kobbavika grew more (~ 7 times heavier at sampling 12 than at sampling 1, estimated on sampled fish) than the surface fed controls (~ 6 times heavier). At both sites, growth predicted by the managing program estimated an increase of 7.4 and 15.9 times for the deep fed fish (at Kobbavika and Vindsvik, respectively) and 7.1 and 16 times for the surface fed. This may indicate some sampling bias and suggests a smaller deviation in growth between the groups that were fed deep and those with standard feed regimes.

4.2 – Swimming depth of salmon in relation to light and feed system

The difference in swimming depth between deep light and feed attracted fish was small and inconsistent at Kobbavika, even if there were tendencies of deep attracted fish swimming deeper during periods with little temperature stratification. In contrast, the deep attracted fish at Vindsvik swam on average 5.5 m deeper than the control fish, but with small difference when there was a clear temperature stratification with warmer water near the surface (*e.g.* July). Manipulation of swimming depth with the use of lights has been demonstrated in several studies (Juell *et al.* 2003, Juell and Fosseidengen 2004, Oppedal *et al.* 2007, Frenzl *et al.* 2014), and also with weak violet

lights as used when the anti-maturation lights were switched off in the present study (Stien *et al.* 2014; Wright *et al.* 2015). Salmon do however trade-off between illumination and temperature. 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). This is in accordance with the observations at Vindsvik with all groups swimming in the warmer water above the illuminated zone during the summer while salmon with deep light swam deeper when the temperature stratification was smaller. Also at Kobbavika all fish were swimming above the illuminated depth when the temperature was highest near the surface during the spring, and there were tendencies of fish with deep lights swimming deeper during late summer when the warm water was less stratified. Generally, the differences in swimming depth between the Kobbavika groups was small with all groups swimming relatively near the illumination depth most of the study period, which may explain the minor effect of the light and feed attractants.

During the spring, also the surface fed control groups had lights at the same depth as the treatment groups, in all sites. Still, deep fed fish at Vindsvik swam deeper than control fish during much of this period, suggesting that deep feeding attracted salmon to swim deeper at this site. In the period with anti-maturation lights when the only difference between groups was feeding depth, all groups in Kobbavika swam at depths similar to the feeding depth most of the period, except when warmer surface water attracted them to swim higher, and thus no clear effect of feeding depth was found. In the study Frenzl *et al.* (2014) there was no clear additional effect on swimming depth of deep lights and deep feeding compared to deep light only, but the dubious results could be because some of the deep fed fish that did not find the food stayed close to the surface as they became hungry (Juell *et al.* 1994). Trials with groups without any additional light that are either fed at the surface or deep fed would be more suited to study the isolated effect of deep feeding on salmon swimming depth.

4.3 – Lice infestation rate in relation to swimming depth and distance from the halocline

Infective lice larvae are positively phototactic and are therefore more abundant towards the surface (Heuch 1995), and previous findings have found that infestation is negatively affected by salmon swimming depth (Osland *et al.* 2001, Hevrøy *et al.* 2003, Oppedal *et al.* 2017, Wright *et al.* 2017). In the present study lice infestation rates was not clearly correlated by swimming depth. However, as lice larvae avoid brackish water (Heuch 1995) the maximum density of larvae and thus probability of being infested is not at the surface if a halocline with brackish water above is present, which was the case during large parts of the study period in both Kobbavika and Vindsvik sites. Lice would then be most abundant as near the sun light as possible but within the preferred salinity, i.e. just below the halocline. In accordance with this, distance from halocline explained the lice infestation rates better than swimming depth *per se* in the present study. One must keep in mind that in our model several assumptions and simplifications were made. For instance, halocline depth was defined as the deepest point with a salinity of 28 ppt or less, and lice assumed to prefer salinities > 28 ppt and avoid lower salinities. In reality the avoidance breakpoint is more gradual (Wright *et al.* 2018). Furthermore, swimming depth was boiled down to one depth value per day (daily median value of the mean depth of the shallowest school), while in reality the depth distribution of the school usually several meters and varied during the day, and there were sometimes more than one school. In addition, the possibility of some degree of sampling bias when collecting fish from the surface in large cages should not be ignored. Still, the infestation rates declined with the estimated distance from the halocline, and most of the higher infestation rates

occurred when the distance to the halocline was low. We therefore consider distance from the halocline to be a better predictor of infestation rate than swimming depth *per se*. In order to avoid infestations salmon should therefore not necessarily be attracted to swim deeper, but further away from the halocline. In this study, salmon were attempted to be attracted to deep feeding at 7 m and to lights located at 9-10 m depth, and these depths were often similar to the halocline depth. In cases with a deep halocline it would possibly be more efficient to attract the fish towards the surface, while when the halocline is shallow or non-existing deep attraction is a better option. Dynamic control of the swimming depth attractors then requires monitoring of fish vertical distribution and the vertical profile of the environment, and operational depth adjustment of the attractors.

4.4 – Sampling method effect

True estimates of lice loads and welfare status are vulnerable to sampling error, through either capture methods or processing techniques (Heuch *et al.* 2011). For lice assessments, 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, capture methods are the likely source of any unrepresentative sampling for both welfare and lice assessments. 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 deep lights and feed; if individuals caught for sampling are only from the upper 5 or 10 m depth, it is possible that the sampled individuals are unrepresentative due to these factors. In fact, the median swimming depth of the shallowest school for deep lights and feed groups at Vindsvik was ~ 11 m (Figure 4), 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 improves the chance for sampling 'normal' fish. On the other hand, Kobbavika is less likely to have biased sampling as schools swam at similar depths in both treatment and control cages (Figure 4), on average ~6.5 m deep over the study period. Thus, the discrepancy in weight between groups at Vindsvik but not Kobbavik could be a by-product of unrepresentative sampling in relation to the main school's swimming depth.

Conclusion: flexible management strategies

In the cages with deep lights and deep 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 two case studies 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, and that attracting fish with lights and feed should take this into account. Although data points for the analysis of distance from halocline and subsequent lice acquisition was limited and carried many assumptions, this model also showed that cages with lice skirts masked the preventive effect of distance from halocline. This suggests that dealing with a dataset of this nature using the 'actual swim depth-halocline depth-lice acquired' approach could be a promising method for understanding the host-parasite interaction at a finer scale. These case studies showed no difference in lice infestation between control or treatment cages, however time-specific preventive successes were observed, suggesting that calculating distance of school swimming depth from halocline could be an explanatory variable during these periods.

These case studies and other previous works with depth-related prevention approaches (Stien *et al.* 2016, Oppedal *et al.* 2017, Stien *et al.* 2018) 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, submerged lights and feed could encourage the school's depth preferences towards areas with lower infective risk, as predicted by the salinity profile and halocline depth, if the expected temperature preference matches the intended depth. The use of lice skirts could facilitate prevention during periods when shallow temperatures are likely to override light or feed attraction, and skirts could also be lowered deeper if there is a brackish layer at the surface.

5.1 – Main findings

- Welfare is not affected by the use of deep light and deep feeding./ *Dype lys og dyp fôring påvirker ikke fiskens velferd.*

- Deep light and feeding encouraged the fish to swim deeper in periods./ *Laksen svømte periodevis dypere ved bruk av dype lys og fôring.*

- Reduction in new lice infestation was not consistent with deep light and deep feeding, but a pattern for prevention efficiency was present./ *Reduksjon av lusepåslag med dype lys og dyp fôring var ikke konstant, men et forebyggende mønster ble observert.*

- Increased distance from halocline (brackish surface waters) seemed to reduce susceptibility to lice infestation./ *Økende svømmedyp fra haloklinen (overgang brakkvann-sjøvann) reduserte lusepåslag.*

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