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Environment, lice levels, welfare and salmon swim depth at Kobbavika site with surface or deep feeding combined with artificial light.

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Project Report

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Title (Norwegian and English):
Miljø, lusenivå, velferd og laksens svømmedyp ved lokalitet Kobbavika ved bruk av utfôring i overflate eller under vann kombinert med kunstig lys

Environment, lice levels, welfare and salmon swim depth at Kobbavika site with surface or deep feeding combined with artificial light.

Authors:
Jonatan Nilsson, Lars Stien and Frode Oppedal

Summary (Norwegian):
For å motivere laksen til å svømme dypere i merdene og dermed romlig separere fisk og smittsomme lus, ble laks i to merder fôret på 7 m dybde og merdene opplyst med svake, fiolette LED-lys på 10 m dybde når det vanlige antikjønnsmodningslyset ikke ble benyttet. To kontrollmerder ble fôret på overflaten og hadde ikke noe ekstra lys unntatt fra januar til juni da antimodningsslysene var på. I løpet av denne perioden var det bare fôringsdybde som var forskjellig mellom behandling og kontrollgruppe. Ekkolodd overvåket kontinuerlig fiskens vertikale posisjon, miljøet ble profilert daglig og ved månedlig prøvetaking ble lusetall og fiskevelferd vurdert.

Kombinasjonen av undervannsfôring og dype LED-lys, og også undervannsfôring alene, gjorde at laksen svømte dypere i noen perioder, men dette ble ofte overstyrt av temperaturpreferanser. Det ble ikke funnet noen klare behandlingseffekter i påslag av lus, og ved de fleste prøvetakinger var det ingen forskjeller. De infesterende luselarvene (kopepoditten) unngår også redusert saltholdighet (<32/30) som ofte finnes i overflaten. Slikt brakvannslag var tilstede i lange perioder av studien, unntatt januar 2015 og oktober-november 2016. Dette kan ha begrenset effekten av det ønskede reduserte påslag av lus ved at laksen svømte dypere. En feilkilde kan også være at prøvetaking nær overflaten (<10 m dybde) fører til ikke-representative uttak av gruntsvømmende fisk. Sammehneger mellom svømmedyp og lusenivå på laksen bør undersøkes nærmere.

Velferd estimert som SWIM-score (Salmon Welfare Index model) var lik for de to behandlingene, noe som tyder på at dypfôring og svakt LED-lys ikke hadde noen negativ (eller positiv) effekt på velferden.

Summary (English):
In order to motivate salmon to swim deeper in the cage and thereby spatially separate fish and infective lice, salmon in two cages were fed at 7 m depth and cages were illuminated with weak violet LED lights at 10 m depth when the ordinary anti-maturation light were not present. Two control cages were fed at the surface and had no extra light except from January-June when the anti-maturation light were present. During this period only feeding depth differed between treatment groups. Echo sounders continuously monitored vertical position of the fish, environment was profiled daily and at monthly samples lice counts and fish welfare was assessed.

The combination of underwater feeding and deep LED lights, and also underwater feeding alone, did make salmon swim deeper in some periods, but in other environmental conditions temperature preference overruled this effect. No clear treatment effects on lice abundance were found, and on most sampling occasions there were no differences. Infective lice copepodites avoid lowered salinity (<32 / 30), and such brackish water layer was present in the upper meters for long periods of the study, except January 2015 and October-November 2016. This may have restricted the effect of deeper swimming on lice abundance. Also, sampling near the surface (<10 m depth) leading to non-representative samples may be a potential factor masking for treatment effects and needs to be further studied.

Welfare as estimated by SWIM scores (Salmon Welfare Index model) were similar for the two treatments, suggesting that deep feeding and weak LED light had no negative (or positive) effect on welfare.

Content

1 Background

The negative correlation between swimming depth of Atlantic salmon (Salmo salar) and their experienced lice infection pressure or lice levels in general is well described (Huse & Holm 1993; Osland et al., 2001; Hevrøy et al., 2003; Wright et al., 2017; Oppedal et al., 2017). 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., 2015) where infective stages of L. salmonis are most abundant (Heuch et al., 1995). A potential problem encountered when using skirts around the cages can however be poor oxygen conditions (Stien et al., 2012) or strong surface water-currents / rough weather in general, both of which make this strategy unsuitable for certain environments. Alternative strategies to combat lice in such environment and in areas with historically high lice infection rates and high treatment frequencies are therefore needed. 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. The notion of somehow separating salmon from lice or the depth where they are most abundant thus maintains its relevance.

It is common to use lights in salmon production to reduce the prevalence of early maturation (Taranger et al., 1999). These lights have typically been from 400 to 1000W full spectral (white) metal-halide lights, but the implementation of LED-lights of the blue spectrum in aquaculture is rapidly increasing. However, lights can only be used in certain periods of the year if they are to prevent rather than promote maturation (Oppedal et al., 2006). This period for S0-smolts will typically be in the range January to June the year after ponding, and use of lights outside this window, especially the continued use into the second autumn, will promote maturation after one winter in the sea, or grilsing. In order to use lights outside this window, narrow-spectered violet UV-LED lights (peak at 400 nm) have been developed. 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/cm2/sec (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).

Anti-maturation lights may also have a different function in that they attract fish to the depth at which they are placed, simultaneously stimulating salmon to continue schooling also during night-time (Juell et al., 2003; Juell & Fosseidengen, 2004; Oppedal et al., 2007; Frentzl et al., 2014), and can thus be used to draw fish towards a certain depth-range, e.g. outside the preferred depth of infective stages of salmon louse. Further, even lights of low intensity have been shown to attract fish towards the depth at which they are placed (Stien et al., 2014). Thus, if we deploy and keep lights at 10 m depth during a complete production cycle; violet UV-LED from first autumn to midwinter, anti-maturation lights at the same depth from mid-winter to mid-summer, and then revert back to violet UV-LED from mid-summer and until harvest, we may be able to continuously keep the fish deeper in the pen during night-time. By implementing lights over an entire production cycle, we will also disclose 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).

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; Oppedal et al., 2011), 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 are indeed 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.

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.

2 Material and methods

2.1 Experimental site

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 controls, 2 deep-cages). The placement and layout of the cages is illustrated in Fig. 1. Cages are 200 m (cone-nets) in circumference, with a depth of 35m (and later 45m), initially with a pen-wise volume of 47745 m^3 stocked with 196-198 000 smolts from transfer.

2.2 Experimental setup

In the two treatment cages (cages 1 and 3) system for deep feeding (feed entrance at 7 m depth) was installed for the entire production cycle, with the exception of shorter period when they were removed for technical reasons (Fig. 2). The control cages (2 and 4) 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 1 and 3 from trial start to 25th January, and from 15th July to end of trial, with the exception of shorter period when they were removed for technical reasons (Fig. 2). From 25th January to June (Fig. 2), standard anti-maturation lights with higher intensities was used in all 4 cages, placed at the same depth as LED-UV lights (10 m).

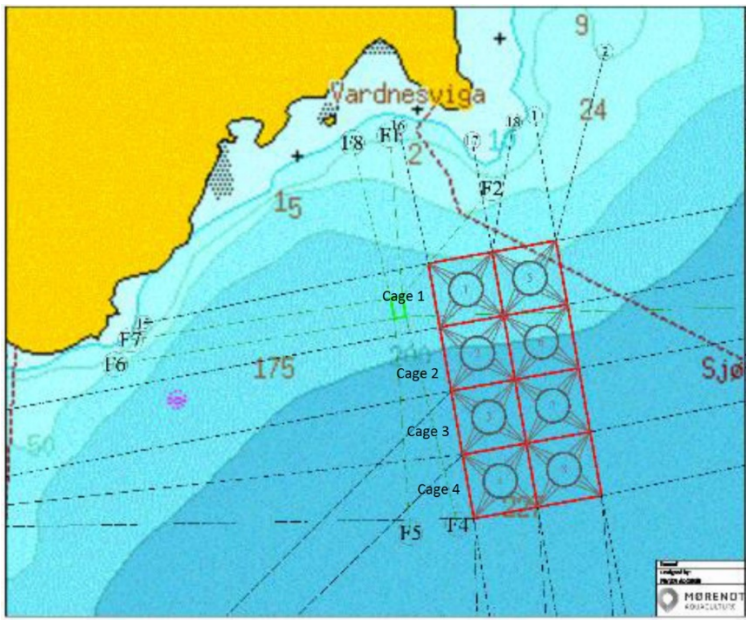


Fig. 1. Overview of the Kobbavika site layout. Cage 1 and 3 were treated (continuously submerged feeding and periodic weak violet light) and cage 2 and 4 acted as controls with surface feeding.

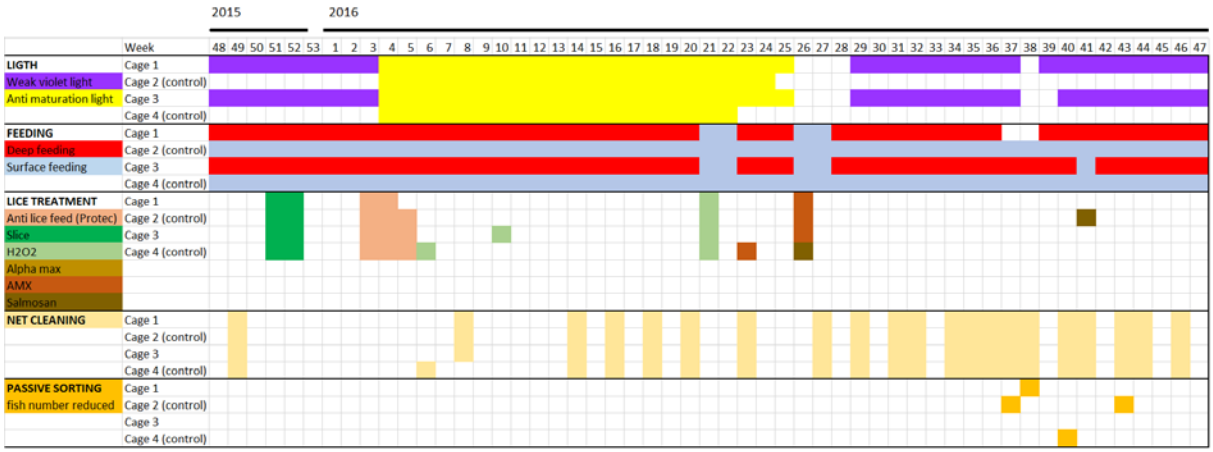


Fig. 2. Overview of events and treatments during the trial.

2.3 Cleanerfish

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 (Fig. 3).

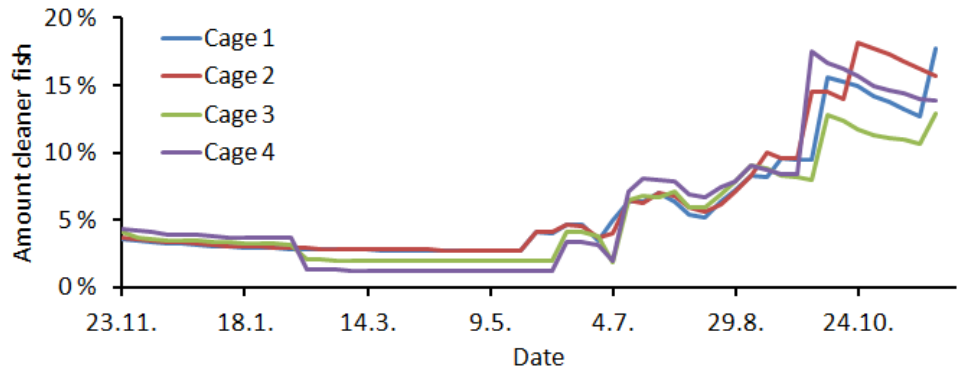


Fig. 3. Amount of cleaner fish in the experimental cages during the trial.

2.4 Environment

The Kobbavika site is positioned within the archipelago of islands and fjords east of Stavanger. Water movements are mainly driven by tidal currents, with periods of brackish water from precipitation. The environmental variables temperature and salinity at the site was measured on a single position at all depths within the depth range of the sea cages.

Environmental profiles were manually taken at daily intervals on the south-east corner of the barge. A multi probe instrument, CTD (SD204, SAIV AS, www.saivas.com) was set at 1 second measuring interval and slowly lowered to 45 m depth. The main parameters observed were temperature and salinity given at specific depths according to pressure reading. Oxygen data from the instrument are biased by a relatively high response time and are not included.

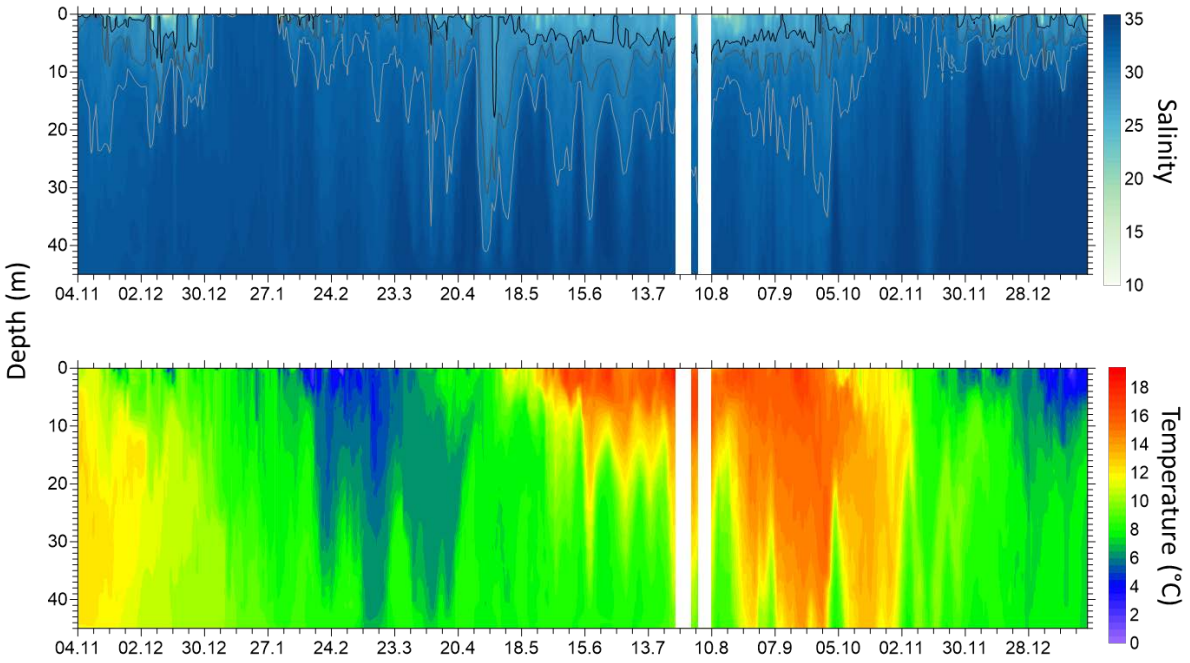


Fig. 4. Salinity and temperature throughout the study. A light grey, grey and black line are inserted at salinities 32, 30 and 28 respectively to illustrate brackish water were copepodids are less abundant. The infestative stage of the salmon lice normally start to avoid salinities of 32 with exponential increase of avoidance with reduced salinities (new results from ongoing FHF project TEMPLUS).

The temperature and salinity followed normal seasonal variation (Fig. 4). Lowest temperatures were observed in January to April with a clear gradient of warmer water with depth and the thermocline positioned between 5 and 10 m. After a short period of homogenous temperatures in May, the surface waters became warmest and the whole cage depth gradually warmed up by September with the depth of the thermocline increased over time. Late October to December the temperature was homogeneous around 8°C.

Salinities varied with freshwater run-offs and long periods of brackish surface waters was seen in November/ December of both years and in summer (Fig. 4).

2.5 Swim depth measured by echo sounders

Vertical distribution of the groups of salmon were observed using a PC-based echo-integration system (Lindem Data Acquisition, Oslo, Norway) connected to upward facing transducers with 42° acoustic beams (50 khz, 0.001 s pulse, 1 s echo listening, 4 s pulse interval). A wireless control box was placed on one cage and transducer cables connected from here to the three other cages. A transducer per cage was deployed between two ropes inside the cage and positioned approximately 1/3 in to the cage

diameter at maximum possible depth without touching the net wall. This placement was hypothesized to cover a substantial part of both the feeding and the lit area. A shadow area is found close to the surface along the net wall due to the position depth limitations of the transducers and coned shape of the net walls. A bias may have arised when fish have positioned themselves in the shadowed volume, typically seen at daytime when fish are not feeding and partly at night-time if the circular schooling structure of the fish are upheld.

2.6 Sampling from cages

Fish sample procedure was carried out by farm personnel. The population of caged fish was starved on the day of sample prior to the following procedure: A 10 m deep net was introduced next to the cage net wall and lowered a few metres. Hand-feeding commenced until a group of fish was observed above the net, followed by rapidly enclosure and crowding of fish at surface. From this aliquot random sampling of 20 individuals, maximum 5 per dip-netting, was performed. Fish were anaesthetised using Benzoak according to manufacturer´s prescription, and brought to the barge for lice counting, length and weight measurements and scoring of welfare (see below).

2.7 Lice counting

On Norwegian salmon farms, the most prevalent species of sea lice (*Copepoda*, *Caligidae*) is the salmon louse *Lepeoptheirus salmonis*. The salmon lice hatch from egg strings on adult females, then disperse in the plankton until they reach the infective stage, and attach to new salmonid hosts for their sedentary, chalimus life phase (Costello, 2006). A thorough description is given in Schram (1993), revised to 8 distinct stages by Hamre et al. (2013), see Fig. 5. Lice attaches externally to the fish during the 3rd stage (copepodid stage). The size of the lice at each stage display variation depending on the grow-out temperatures (Samsing et al., 2016).

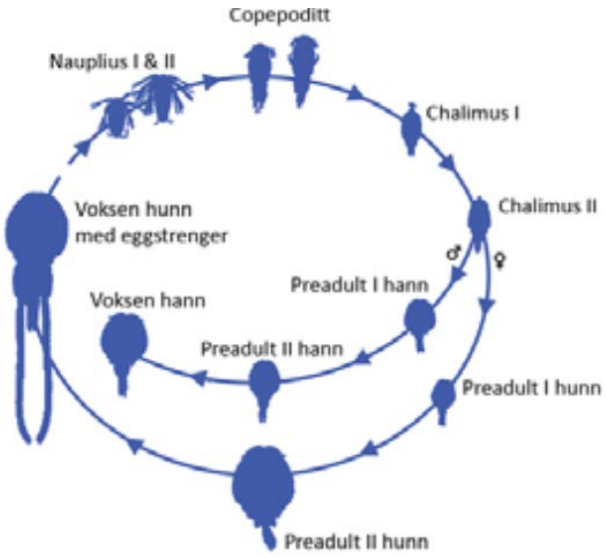


Fig. 5. The salmon lice life cycle. Modified from Schram (1993) adjusted by Hamre et al. (2013).

As part of management, the salmon farmer normally performs weekly or bi-weekly counts on sampled fish according to legislation whereby they distinguish between **sessile** (copepodite, chalimus I, II), **mobile** (preadult I, II and adult male), **female adult** lice while some also count with more precision to better follow development (e.g. Marine Harvest).

Here, all stages and their abundance on individual fish were documented. A trained technician or researcher recorded lice numbers using standard scientific procedures involving time available, good light conditions and water immersion if needed. Specifically, *L. salmonis* lice counts were categorized to each developmental stage (copepodid, chalimus I, chalimus II, preadult I, preadult II, adult and adult female with eggstring stages). Any lice remaining in the anesthetics, predominantly of mobile stages, were included in the recordings.

2.8 Welfare measurements

Production performance within sea cages may be measured as growth, mortality and welfare scoring, e.g. Salmon Welfare Index Model, SWIM (Stien et al., 2013). Here, weight and fork length was measured on the subsampled individual fish. Mortality are reported elsewhere. On the sampled fish, a SWIM score was given to each individual according to Stien et al. (2013) and Folkedal et al. (2016). Explanation of the SWIM scores are given in Table 1.

Table 1. Explanation of the SWIM scores.

Welfare indicator	Level 1	Level 2	Level 3	Level 4	Level 5	**Level 6**	**Level 7**
Sea lice (mobile lice/cm^2)	0	<0.05	0.05-0.08	>0.08			
Jaw deformation	Normal - Nothing to note	Potential - Suspected malformation, minor malformation	Clearly visible malformation	Strong/Extreme malformation			
Mouth jaw wound	No wound	Light wound	Clear bloddy wound				
Opercula	Normal opercula	Operculum only partly covering the gill on one side (unilateral	Opercula only partly covering the gills on both sides (bilateral)	Operculum unilaterally absent	Opercula bilaterally absent		
Gill status	Normal healthy gills	Mild signs of focal inflammation, necrosis (dead tissue), lesions or trauma	Severe signs of more generalized inflammation, necrosis, lesions or trauma				
Eye status	Functional, healthy eyes	Unilateral (one-sided), traumatic injury, moderate exophthalmia or haemorrhages inside the eye	Bilateral (two-sided), traumatic injury, moderate exophthalmia or haemorrhages inside the eyes	Bilateral (two-sided) cataract (more than 50% of lens coverage) or chronic condition with impaired vision	Severe exophthalmia or bilaterally blind individuals		
Skin condition	Normal healthy skin, nothing to comment	Scar tissue, healed	Scale loss (dislocated or missing scales)	Superficial wound or ulcer <1 cm^2	Superficial wound or ulcer >1 cm^2	Penetrating and/or multiple wounds or ulcers possibly infected	Large open wounds, life threatening
Fin condition	Normal healthy fins, nothing to comment	Scar tissue or slight necrosis	Moderate current skin damage and/or necrosis , including splitting and/or thickening	Severe skin damage and/or necrosis with bleeding, and/or inflammation and/or exposed fin rays and severe tissue loss			
Smoltification state	Fully smoltified	Parr, access to brackish water	Parr, incomplete smoltification, 10°C	Parr, incomplete smoltification, 14°C	Parr, incomplete smoltification, 7°C	Parr, incomplete smoltification, 20°C	
Sexual mature	Not mature	Precocious male	Mature male	Mature female			
Vertebral deformation	No external signs of vertebral deformities	'Short-tail' of normal weight	Short-tail' of low weight.				
Emaciation	Not emaciated	Potentialy emaciated	Distinctly emaciated				
Condition factor	>1.1	0.9-1.1	<0.9				

3 Results and discussions

3.1 Swim depth

Vertical distribution of salmon and detailed environmental profiles are shown in Fig. 6.

In November 2015, before trial start, fish in all cages showed a similar depth distribution with swimming near the surface during daytime and more spread during night. Night time density is relatively low, possibly derived from the salmon swimming outside of the echo transducers observation volume. When the violet LED lights were switched on and deep feeding started in cages 1 and 3 on 25th November, the fish in these two cages positioned themselves deeper in the cage at 5-10 m depth during daytime, while the control where more concentrated nearer the surface. Even so, some of the deep-fed fish went to the surface during feeding. At the time, some of these fish were thought to have high feeding motivation due to underfeeding, caused by lack of feeding depth under the pellet spread of the submerged feeder when small pellet size was used. The general pattern of deeper swimming at daytime continued in December and January with also a change to a slight avoidance of colder surface waters in all groups. Acoustic data from cage 4 are missing for December and most of January.

On the 25th of January anti-maturation light at 10 m depth were switched on in all cages, and fish in all cages distributed relatively similarly, deep in the cage during daytime and more spread during night, but clearly avoiding the cold surface water. Between 20 and 23 February the temperature was homogeneous throughout the depth interval, and fish swam higher in the water, around the depth of the lights, during these nights.

From mid-March when the surface temperature rose somewhat and fish in the control cages stayed closer to the surface during daytime and deep during night, while the deep fed fish avoided the surface water during both day and night. At the end of March and in April when the temperature was almost homogeneous fish in all cages stayed near the surface at daytime and more spread and generally less deep than during night in the earlier period.

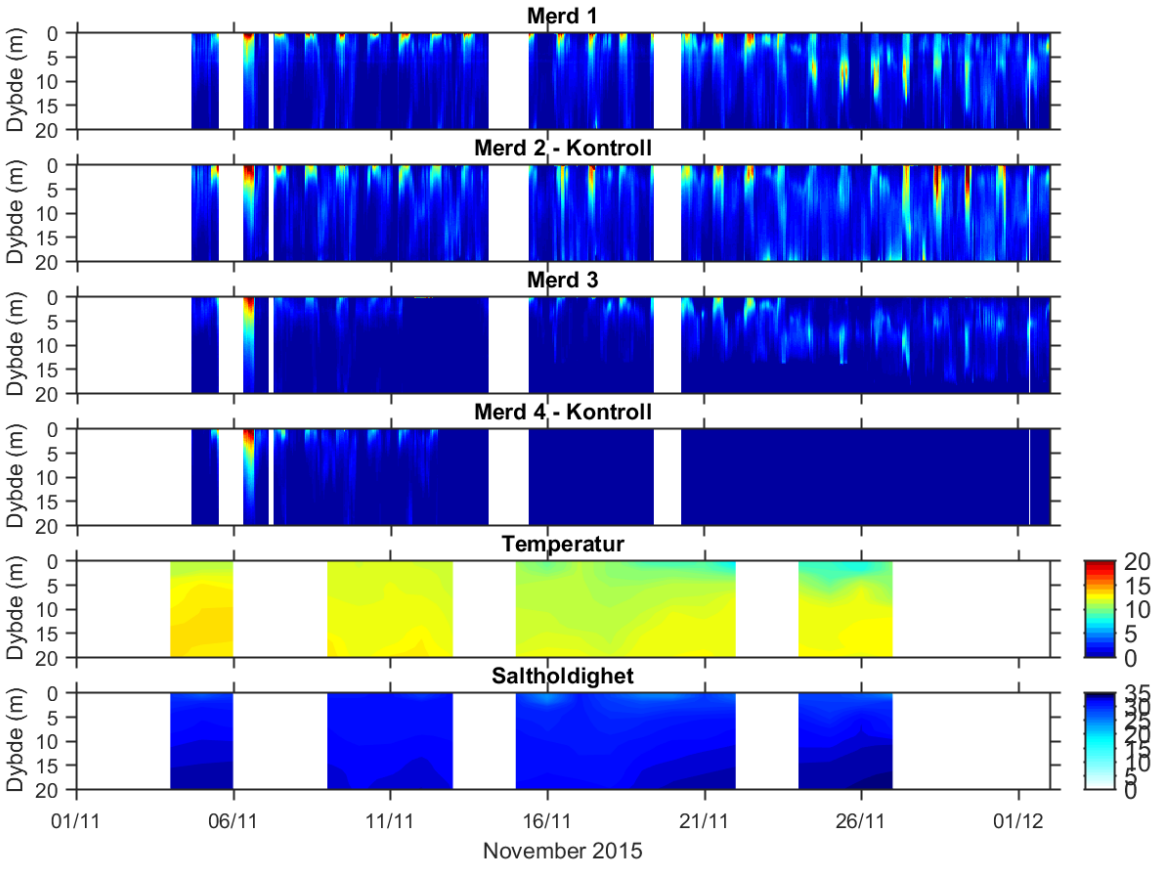
In May when the water near the surface had become warmer than deeper down salmon in all cages showed a fairly similar distribution with schooling near the surface during daytime and a more spread and deeper vertical distribution during night. In both March and April the clear reduction in total echo backscatter at night could indicate that a large proportion of the fish may swim outside the observation volume of the transducer, i.e. along the net wall, away from the central daytime feeding area or in the restricted volume below.

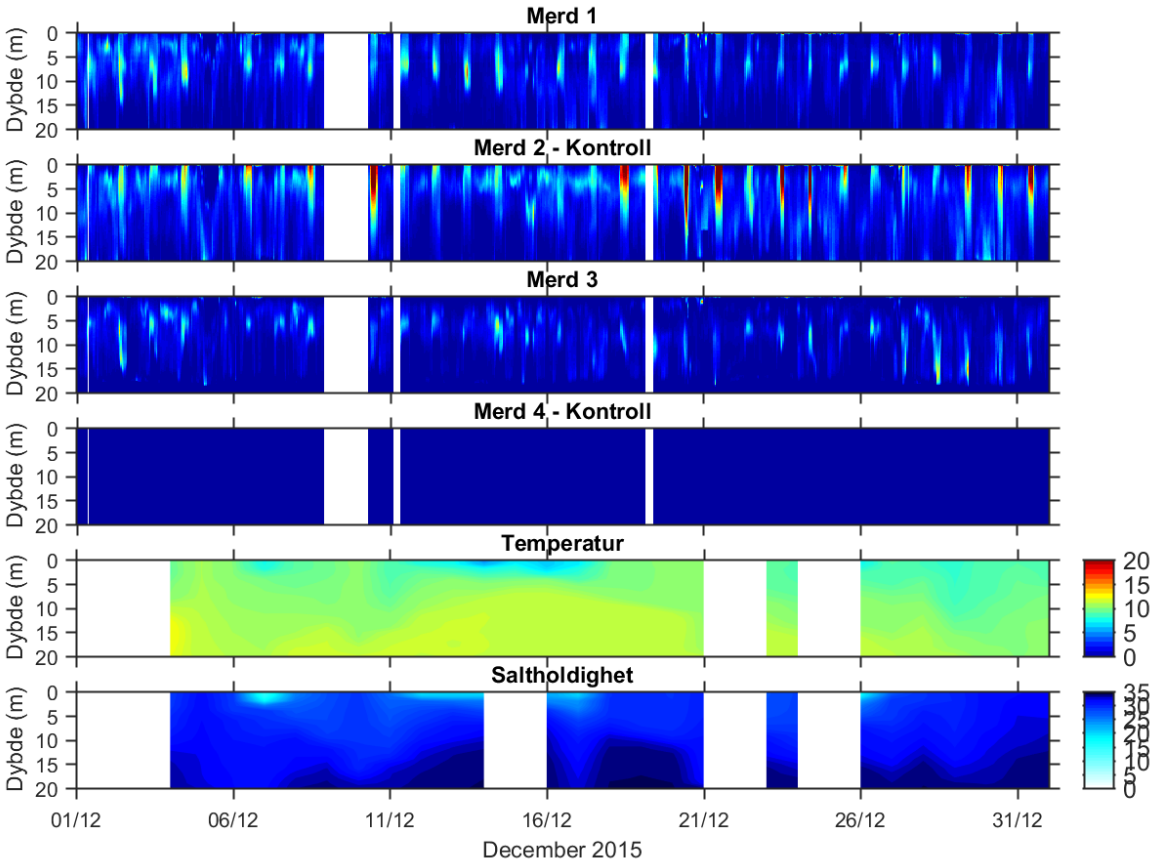
During the first weeks of June when the surface water had warmed up further (>15 °C) fish in all cages avoided the upper few meters and swam between 5 and 10 m during daytime (10-15 °C). At night, fish spread out more during the short hours of darkness. In essence fish seemed to avoid the “too” warm surface layer and the “cooler”, <10 °C, deep layer. In mid-June the temperature rose also deeper down in the cage making the temperature stratification less distinct, which affected the swimming depth so that the fish were more vertically spread both day and night, but still below the surface layer. No distinct differences over several days between treatment groups were observed.

The anti-maturation lights were removed the 7th (cage 4), 17th (cage 2) or 26th (cage 1 and 3) June, and the deep feeders in cages 1 and 3 were removed and fish fed at the surface from 26th June. All cages were attempted deloused with AMX between 27th and 29th June. Thus, in the beginning of July there were no treatment differences between any cages, and fish in all cages had a relatively wide vertical depth range both day and night with much fish near the surface during daytime. The deep

feeders in cages 1 and 3 were taken into use again from and 12th July and the weak violet LED lights in these cages switched on 15th July, and the fish in these cages showed a more pronounced diurnal distribution, swimming mainly between 5 and 10 m during daytime and deeper during night, while the control cages had a more spread distribution both day and night. As surface temperature rose further from about mid-July, fish again distinctly avoided the warm surface layer (>16 °C).

In August and September when the water temperature was relatively high throughout the depth range salmon in all cages were relatively spread both during day and night, but fish with deep feeding and deep light swam to a lesser extent near the surface than in the two control cages. Acoustic data from October/ November are missing or some way obscured by placement or equipment failure and comparisons or patterns are based on few observations and have been left out.

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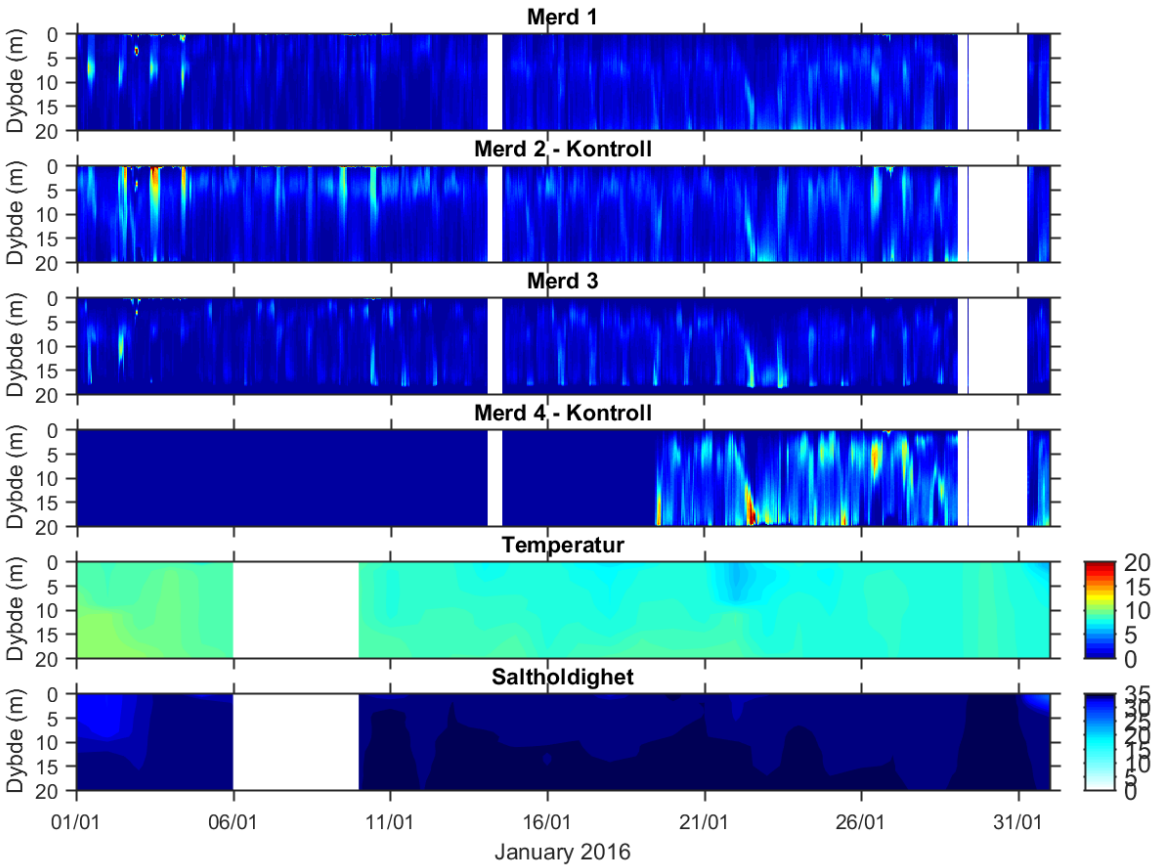
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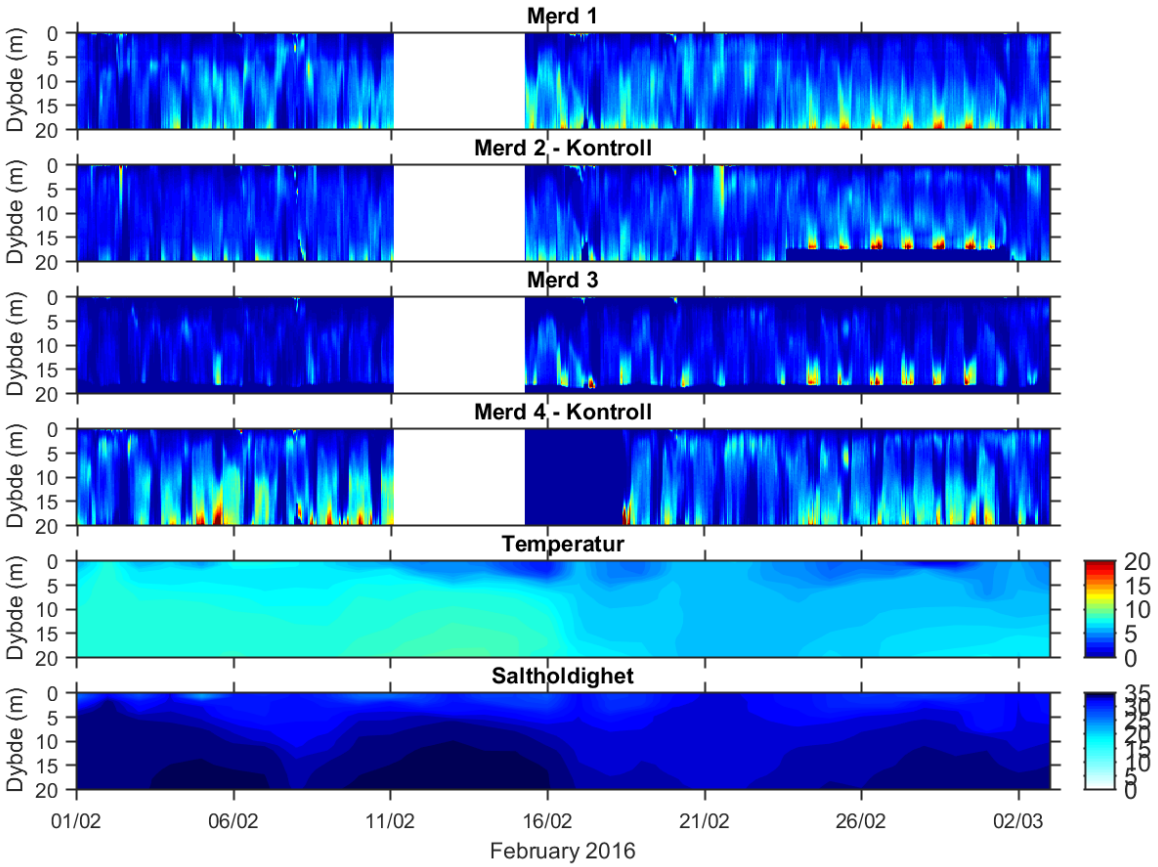
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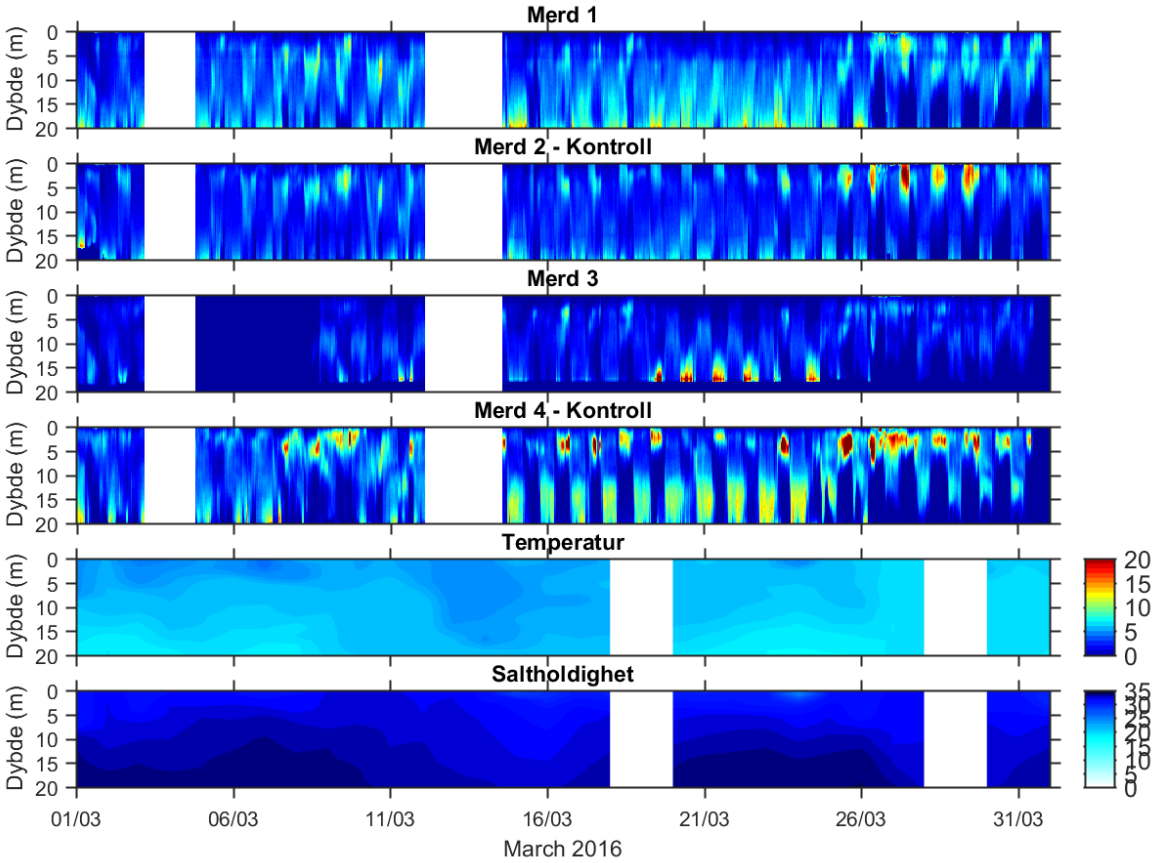
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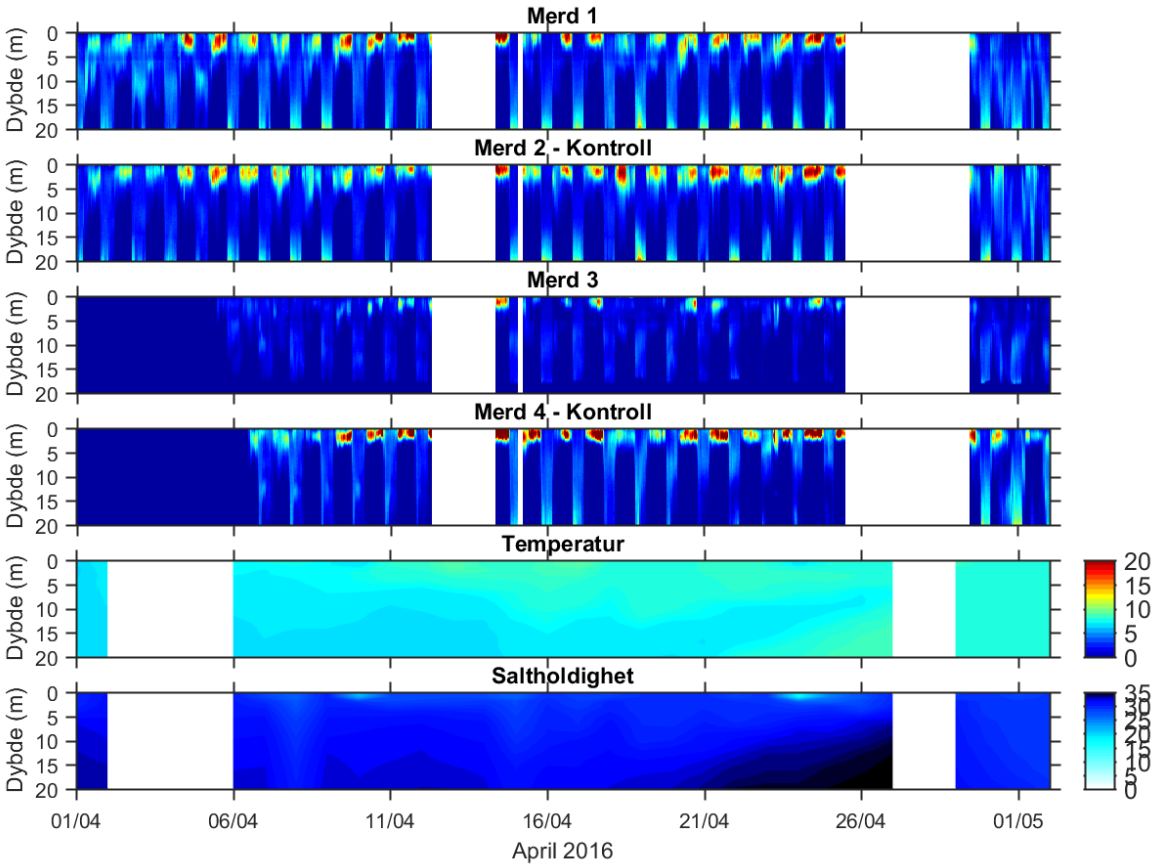
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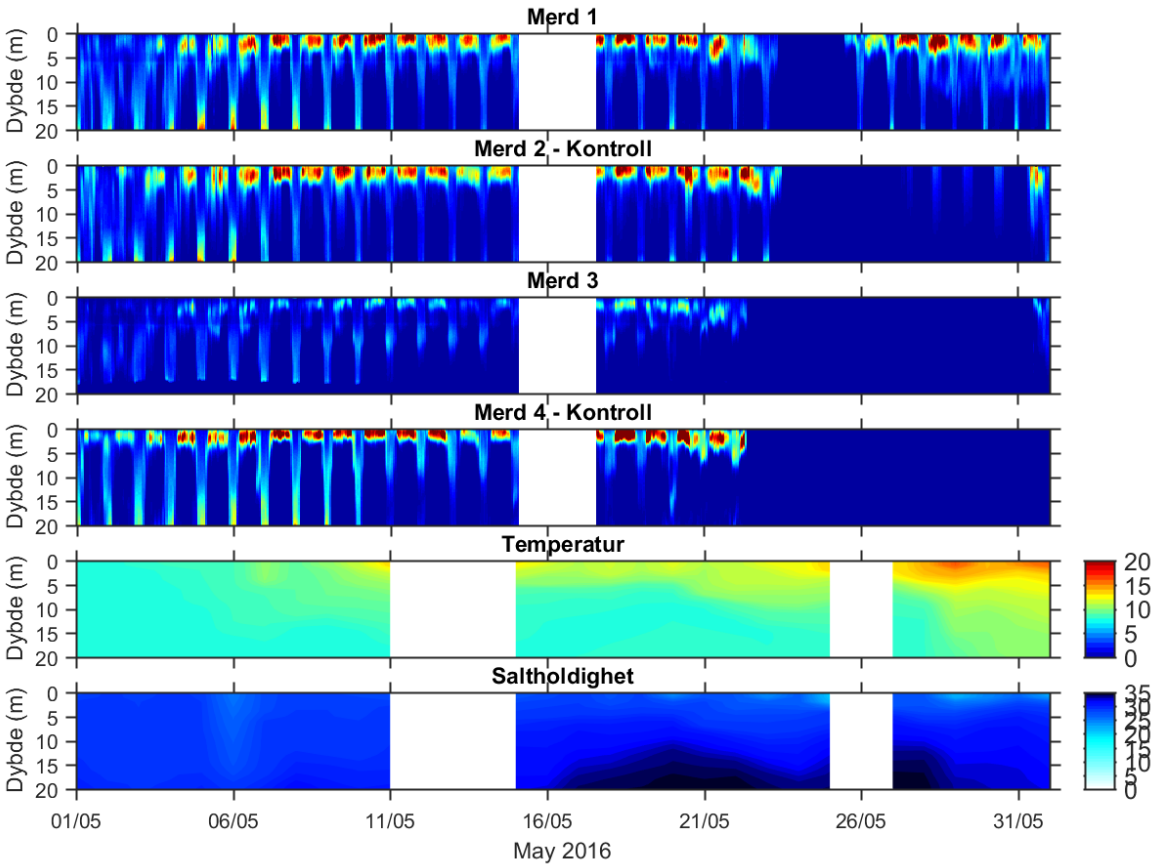
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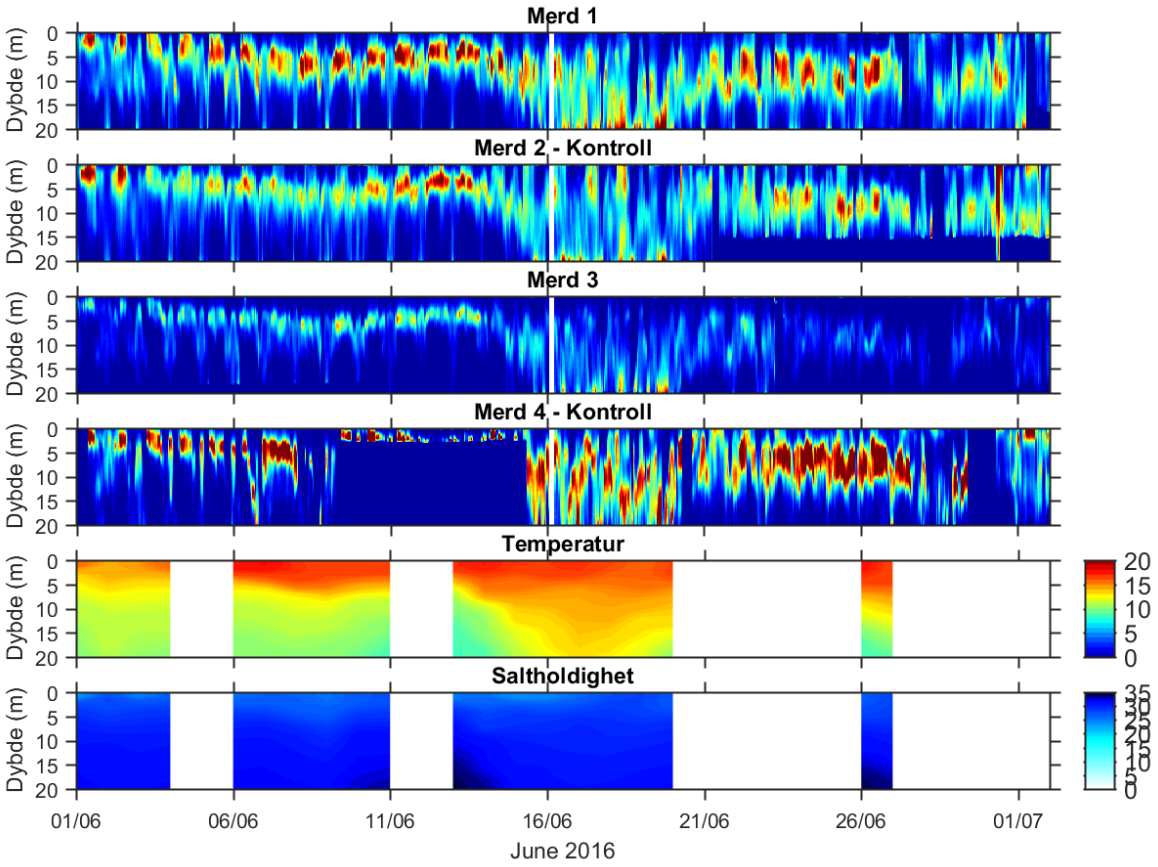
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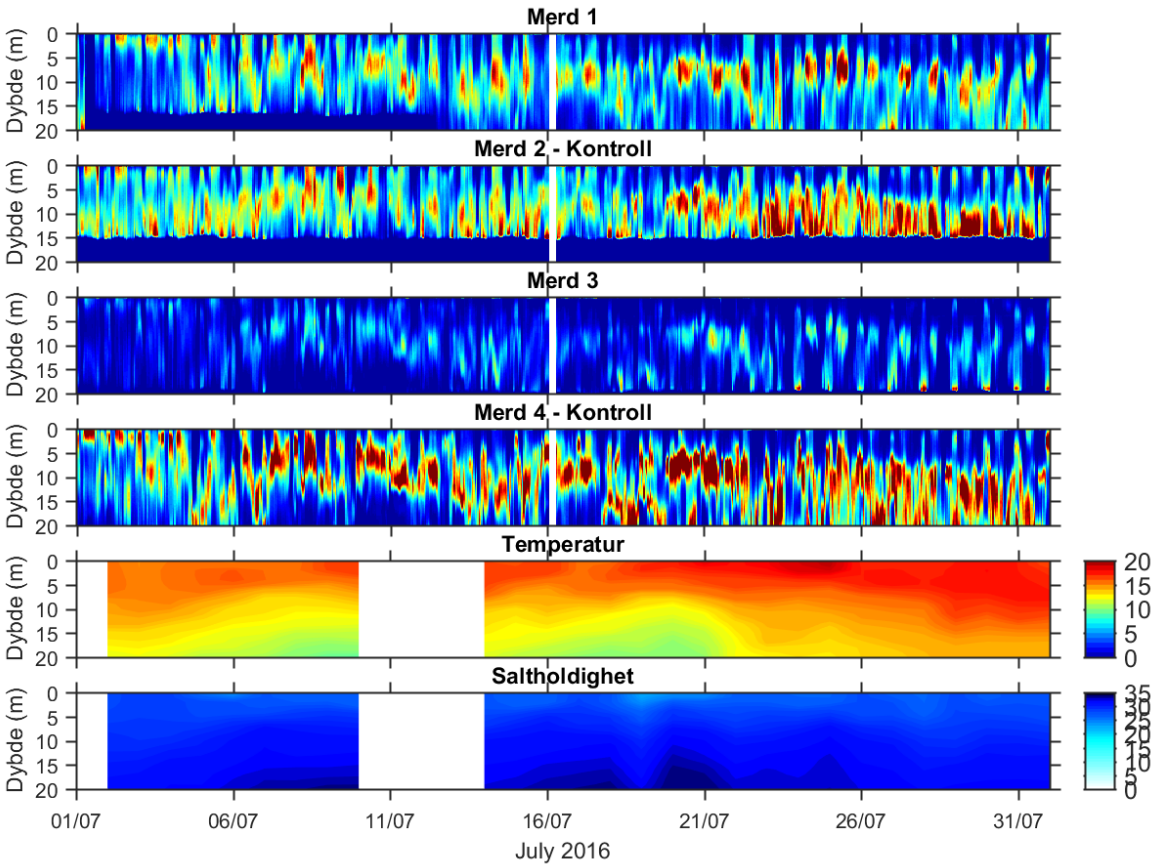
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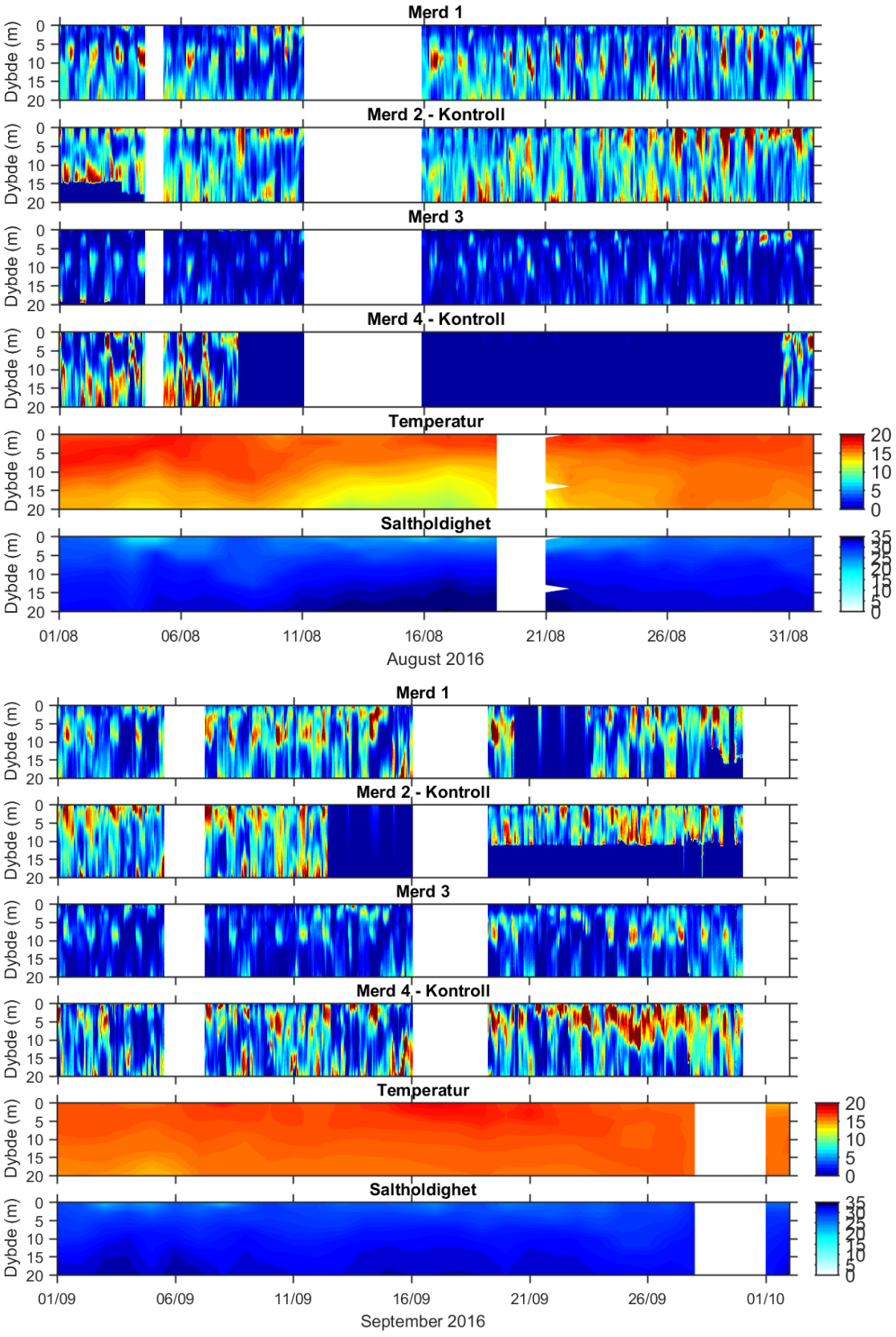


Fig. 6. Monthly vertical distribution of biomass estimated with acoustics, and vertical profiles of temperature (°C) and salinity (ppt) for the same periods measured with CTD.

An example of short term variation in swim depth distribution is given in Figure 7. Here we see a clear difference in daytime distribution whereby deeply fed fish are around the feeding depth, with surface fed fish occupying surface waters during feeding. Attraction to the LED light sources at night is not clearly visible, but a difference between control and treated cages are present.

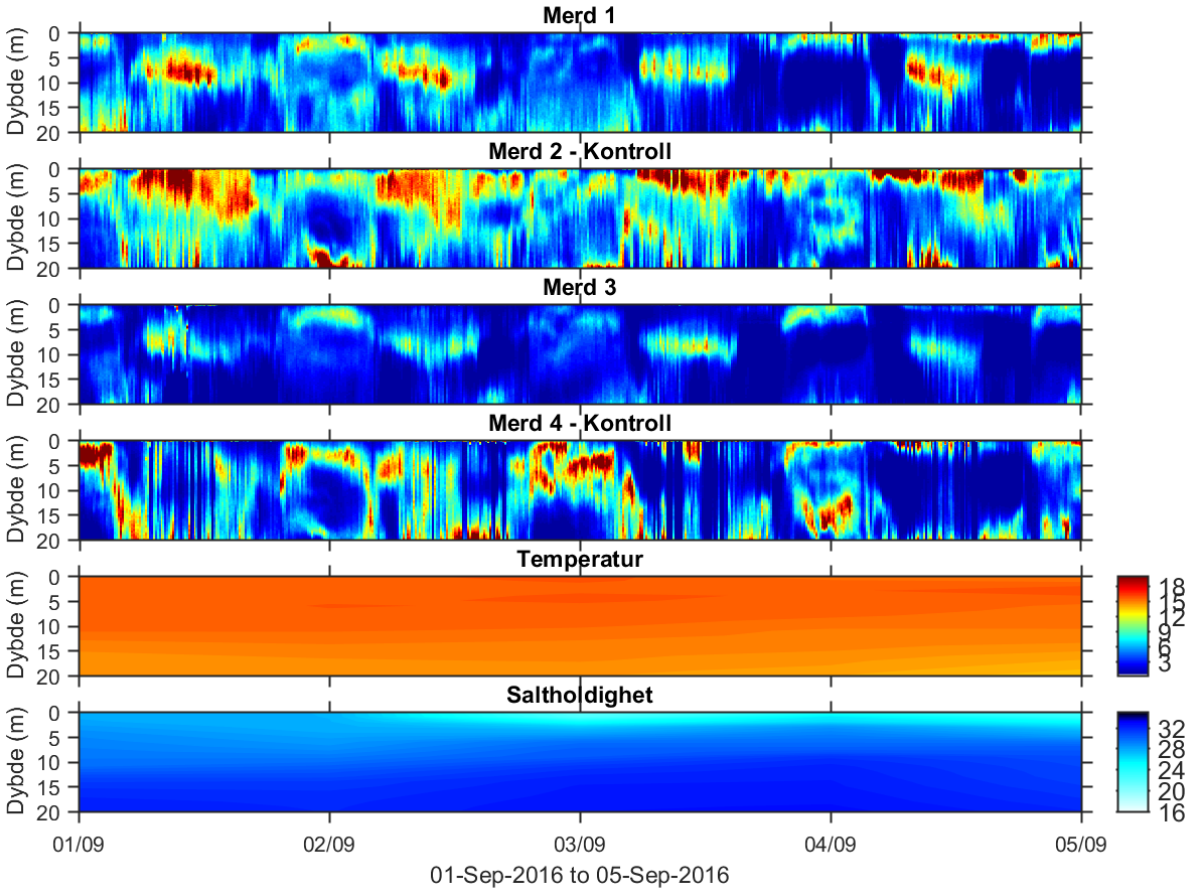


Fig. 7. Example where underwater feeding attract the fish away from the surface layer at daytime. Ticks indicate midnight. Five days (1-5 September 2016) of vertical distribution of biomass estimated with acoustics, and vertical profiles of temperature (°C) and salinity (ppt) for the same periods measured with CTD (scaling differs from fig 6).

3.2 Lice, total numbers

The total amount of lice found was relatively low during winter but increased during spring and was high throughout the autumn (Fig. 8). As the mobile stages of lice are highly affected by the variable effects of cleaner fish and other treatments we here focus on the sessile stages for comparisons of treatments.

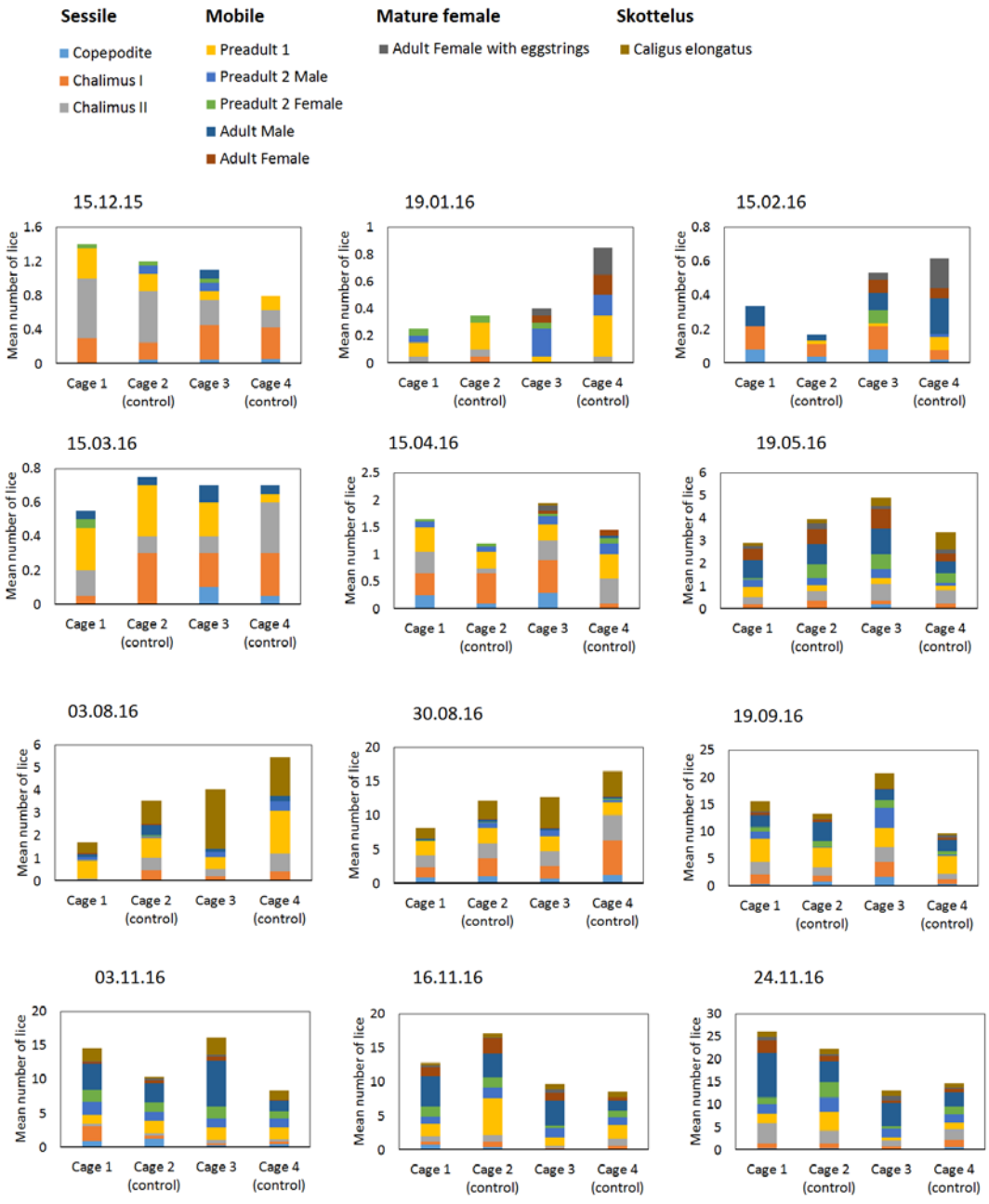


Fig. 8. Mean number of lice on all sampling dates. Different colours indicate different stages, see label. Vertical axis changes between samples to enhance readability of stage compositions.

3.3 Lice, numbers of lice at sessile stages

New infestations that may have been altered by treatments or environmental conditions is best considered through evaluation of the number of lice within the sessile stages of copepodids, chalimus I and Chalimus II. **No systematic differences in amount of sessile lice were found between cages with deep feeding and weak deep light and control cages** (Fig. 9). While number of sessile lice was somewhat lower on the deep fed salmon on samplings in early (low overall numbers) and late August (high overall numbers), the opposite pattern was found on the September sampling. On most sampling occasions the lice amount overlapped between treatment groups. No clear explanations for the indicated potential differences among treatments in August and September can be seen from the plots of vertical distribution. The brackish water layer was relatively constant, and deep (5- 10 m with salinities of 28-32), during the infective period from July through September and lack of variation cannot explain the trend.

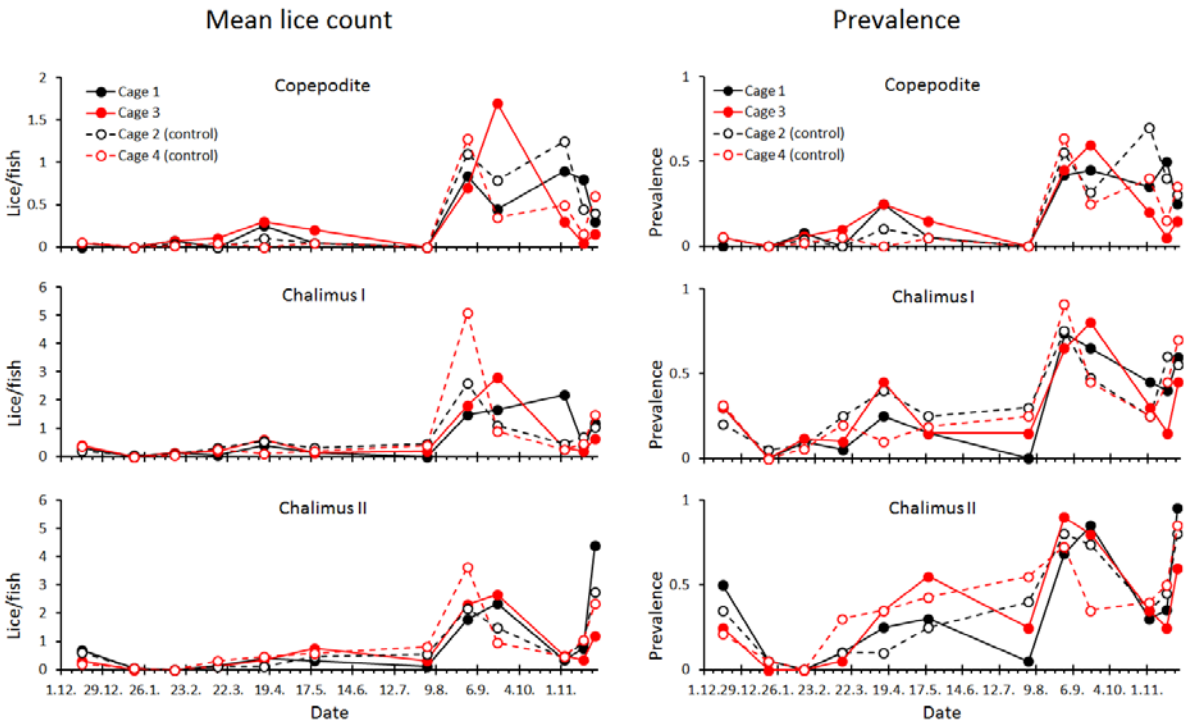


Fig. 9. Mean number of sessile lice (left) and prevalence (the proportion of individuals with lice) on all sampling dates. Filled circles: Cages with deep feeding; Open circles: Control cages. Major ticks (x axis): 28 days; minor ticks: 7 days.

3.4 Welfare

The OWI was generally good during the winter and spring but started to fall in May and had decreased further in August and during the autumn (Fig. 10). **No systematic differences in SWIM Overall Welfare Index (OWI) were found between treatment groups**. Looking at the scores of the individual welfare indicators, on which OWI is calculated, the majority of the individuals had the best possible score (score 1) on most indicators during winter and spring (Fig. 11). In August a larger proportion of fish had reduced scores on indicators gill, skin and fin condition, eye status and sea lice. This pattern remained and was partly strengthened throughout the autumn samplings (Fig. 11).

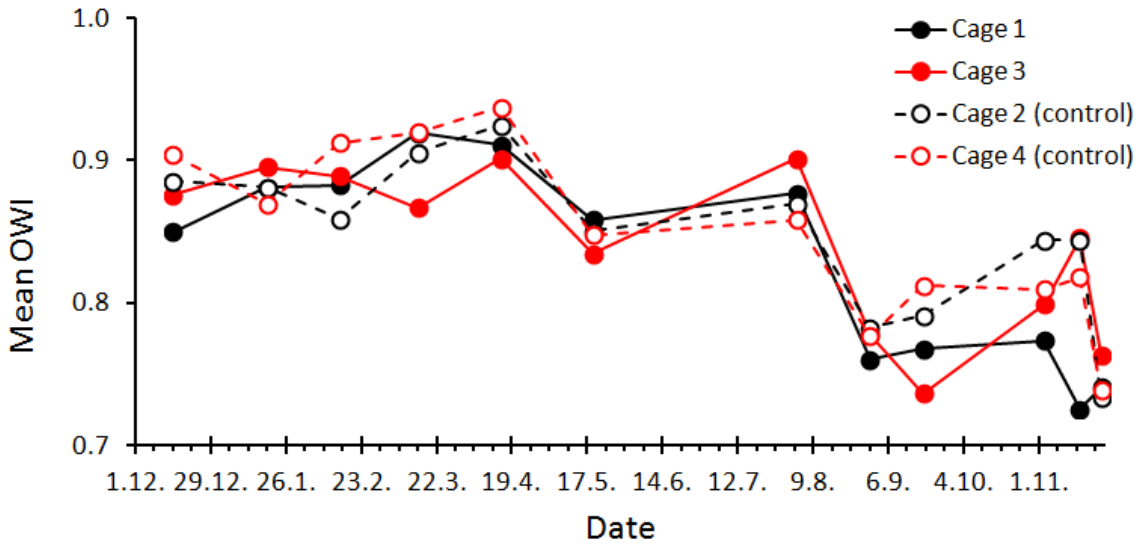
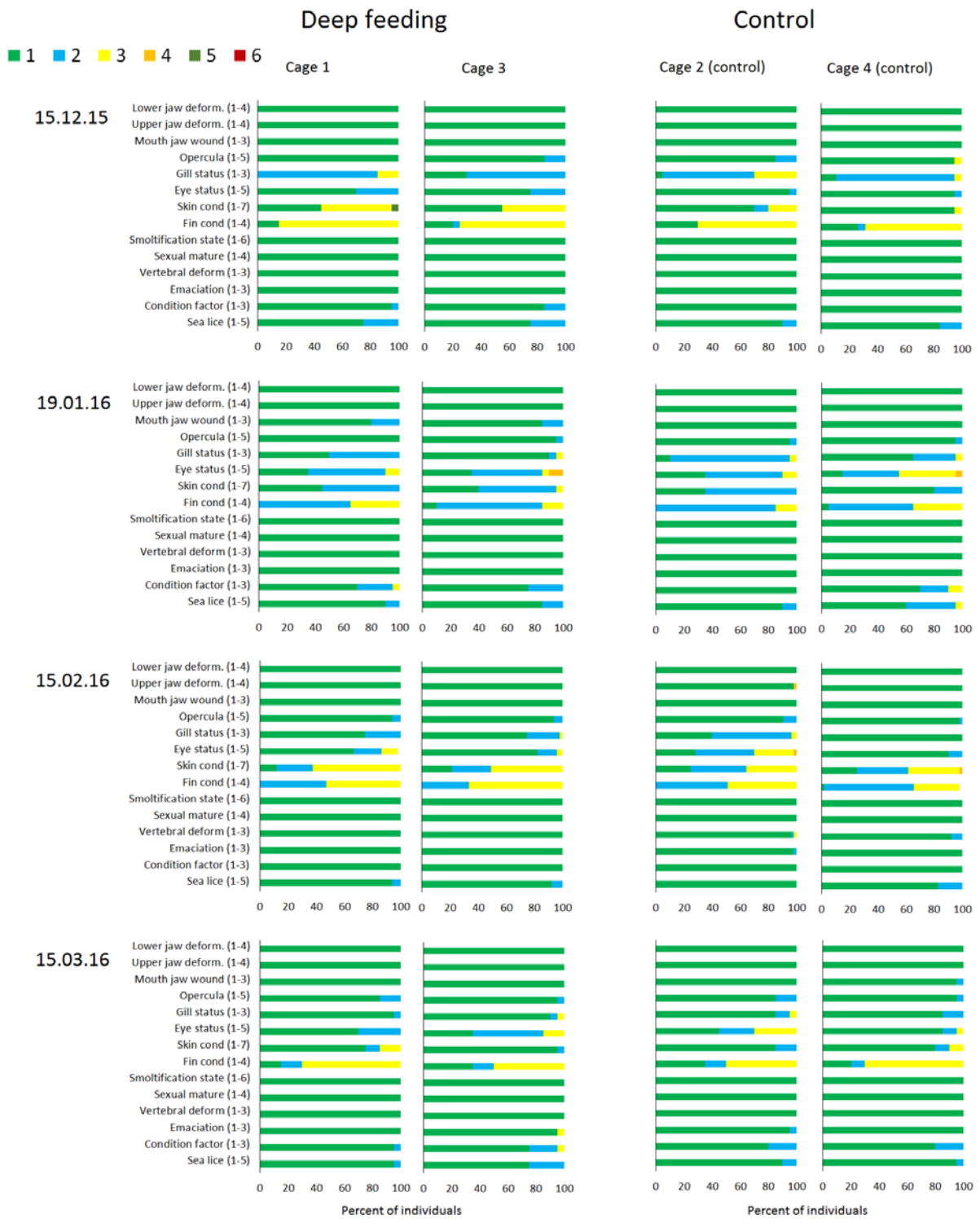
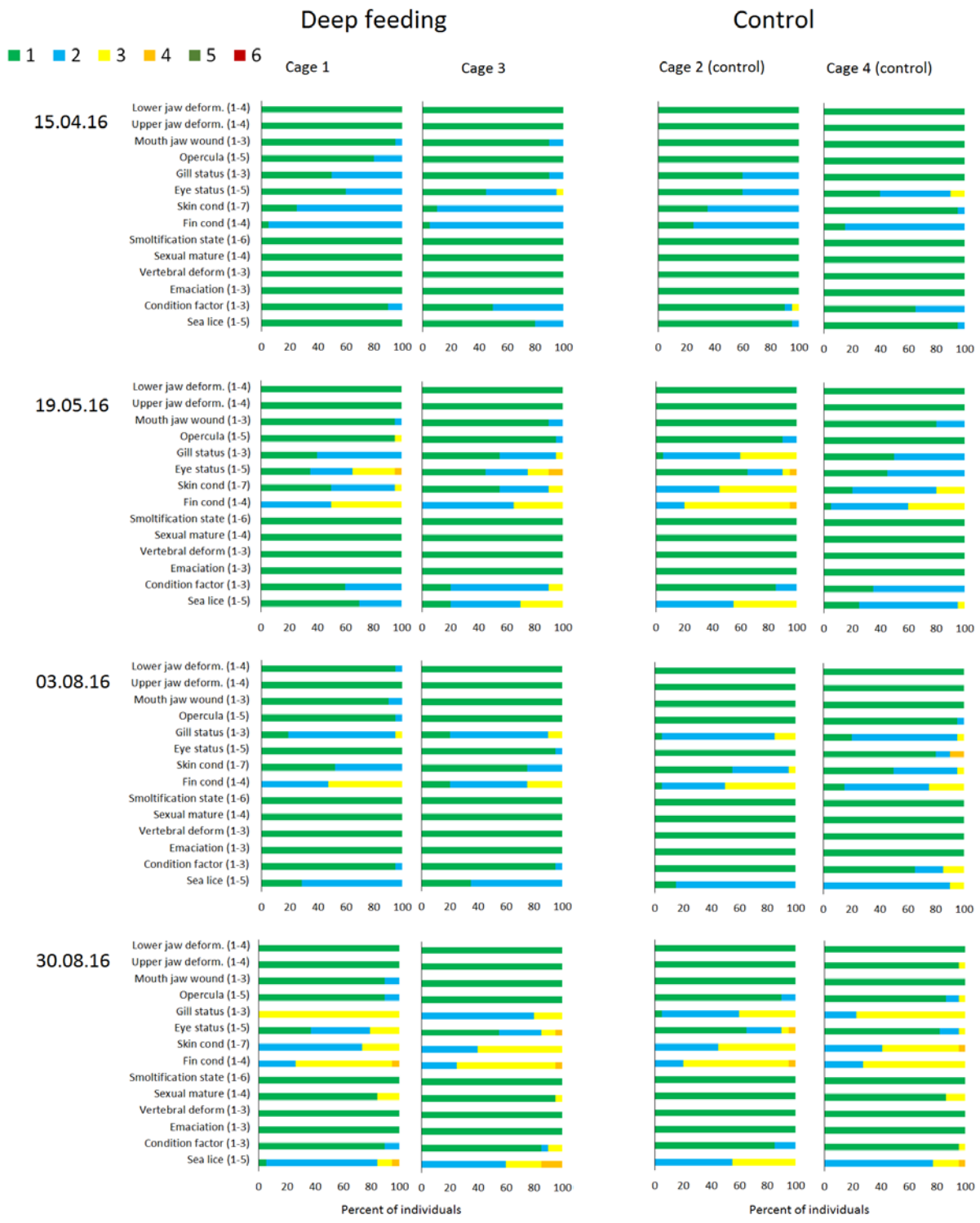


Fig. 10. Mean Overall Welfare Index (OWI) for the individuals sampled on each sampling date. OWI ranges from 0 (worst) to 1 (best). See Stien et al. (2013) for explanation on how OWI is calculated. Filled circles: Cages with deep feeding; Open circles: Control cages. Major ticks (x axis): 28 days; minor ticks: 7 days.


Deep feeding
Control
1 2 3 4 5 6
Cage 1
Cage 3
Cage 2 (control)
Cage 4 (control)
15.12.15
19.01.16
15.02.16
15.03.16
Lower jaw deform. (1-4)
Upper jaw deform. (1-4)
Mouth jaw wound (1-3)
Opercula (1-5)
Gill status (1-3)
Eye status (1-5)
Skin cond (1-7)
Fin cond (1-4)
Smoltification state (1-6)
Sexual mature (1-4)
Vertebral deform (1-3)
Emaciation (1-3)
Condition factor (1-3)
Sea lice (1-5)
0 20 40 60 80 100
Percent of individuals


Deep feeding
Control
1 2 3 4 5 6
Cage 1
Cage 3
Cage 2 (control)
Cage 4 (control)
15.04.16
19.05.16
03.08.16
30.08.16
Lower jaw deform. (1-4)
Upper jaw deform. (1-4)
Mouth jaw wound (1-3)
Opercula (1-5)
Gill status (1-3)
Eye status (1-5)
Skin cond (1-7)
Fin cond (1-4)
Smoltification state (1-6)
Sexual mature (1-4)
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Condition factor (1-3)
Sea lice (1-5)
0 20 40 60 80 100
Percent of individuals

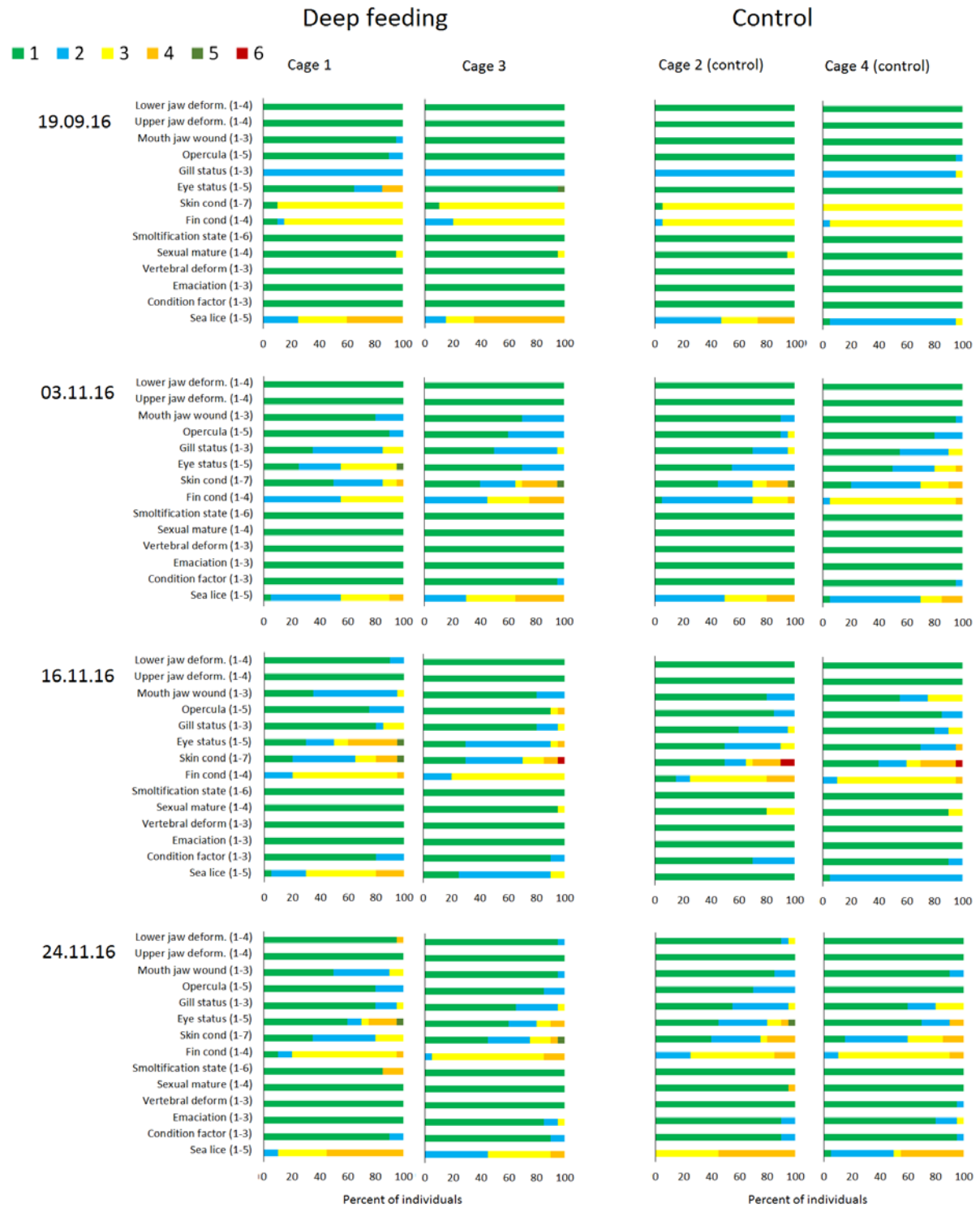

Fig. 10. Distribution (%) of SWIM scores on the individuals sampled on each sampling date. Different colours indicate different scores, see label.

4 General discussion

4.1 Swimming depth and lice infestation

Salmon lice in the planktonic infective copepodite stage are positively phototactic and are therefore most abundant near the surface where the light level is highest (Hevrøy et al., 2003; Heuch et al., 1995). The idea behind deep feeding and deep lights is to motivate salmon to swim deeper in the cage and thereby avoid infestation. Spatial separation of salmon and infective lice has proven efficient in other studies (e.g. Næs et al., 2012; Lien et al., 2015; Oppedal et al., 2017). Both underwater feeding alone (Fig. 6, March) and in combination with deep LED-UV lights (Fig. 6, December-January and August-September) did in some periods have an effect on swimming depth in that salmon stayed less near the surface than the control. In other times of the year (February, April) other environmental parameters, especially temperature, overruled this effect. The principle of motivating salmon to swim deeper by the use of deep feeding and light did thus prove to work under the right environmental conditions. However, copepodites avoid fresh and brackish water, and when there is a surface layer of brackish water the copepodites where thought to be mainly found in the most surface-near water with a salinity above 28 (anectodal evidence). The more scientifically evident say that copepodids are attracted to light and full salinity (Heuch 1995, Heuch et al. 1995), although the exact salinity avoidance thresholds remain unclear. Recent, and new knowledge from on-going trials at IMR (FHF project 901283 Templus) show that there is no clear cut-off at a salinity level of 28 for the brackish water that the copepodids start to avoid. There is a peak of lice copepodids already under a halocline of brackish water of salinity 32, a >50% reduced number of copepodids in brackish water of 30, 28 with low numbers at lower salinities and copedids more or less absent below a salinity of 20. As such, fish swimming just below the halocline are then most exposed for infestation of lice. In the present study a layer with brackish (<28) water from the surface down to around 5 m depth was present periodically during the winter and almost continuously from May to the beginning of October, while layers of 30 was seen 8-10 m deeper in the same and extended periods and layers of brackish water of salinity 32 extended below 10 m depth for most periods, except January 2015 and October-November 2016 (Fig. 4). This fact may clearly explain why the difference in swimming depth between treatment groups in several cases did not result in differences in lice abundance. In August salmon with deep feeding and lights were mainly found below the halocline and had less sessile lice than the control fish that distributed more widely. The differences in depth distribution were however relatively small, and with a similar depth distribution in September the deep fed fish had more sessile lice than the control fish. It is therefore possible that these differences represent a chance effect rather than a true effect of treatments.

4.2 Welfare score (SWIM)

Most individuals in all cages got the best possible score (score 1) on most welfare indicators during winter and spring, with the exception of gill, skin and fin condition, and thus the OWI was relatively high during this period. It is normal that skin and fin are reduced (Folkedal et al., 2016), as both may be inflicted by netting during sampling (e.g scale loss and fin splitting, both level 3). The OWI were reduced during the autumn 2016 as welfare indicators skin condition and sea lice got worse, with more individuals having high scores. It is likely that high loads of lice have negatively affected the skin on some individuals. Generally, a welfare score level below 0.8 is considered poor and below 0.7 very poor. The lack of clear differences in SWIM score between fish fed deep and control fish suggest that reduced welfare is not related deep feeding or deep LED-UV lights.

4.3 Possible sampling bias

By using underwater feeding at 7 m depth and LED-UV lights at 10 m depth we aimed at attract fish to stay as much as possible below 7 m. As fish were netted with a 10 m deep net it is likely that those individuals that were not attracted by deep lights and feeding, and thus did not gain possible effects of deeper swimming, were overrepresented in the samples. This may have masked treatment effects. Representative sampling from large groups of fish in large volumes is challenging. Fish with different characteristics may have different preferences in where in the cage they position themselves. For instance, larger fish spend more time deeper in the cage than smaller individuals (Nilsson et al., 2013) resulting in mean weight changing with depth (Folkedal et al., 2012). Fig. 12 shows mean weight of the sampled individuals on all sampling occasions and cage mean obtained from Akvafarmer. Poor sampling would result in deviations from the cage mean, and the data shows that sampling was biased in terms of weight on some occasions, but generally the mean weight of the sampled fish reflected that of the cage. If size bias also reflects bias in lice abundance is not known, but the possibility cannot be ruled out. Furthermore, hungry fish are more likely to be attracted by the hand-feeding in connection with sampling. Sampling populations with assumed differences in vertical distribution with the same surface-near sampling method is therefore a risk, but no known better alternatives exist today.

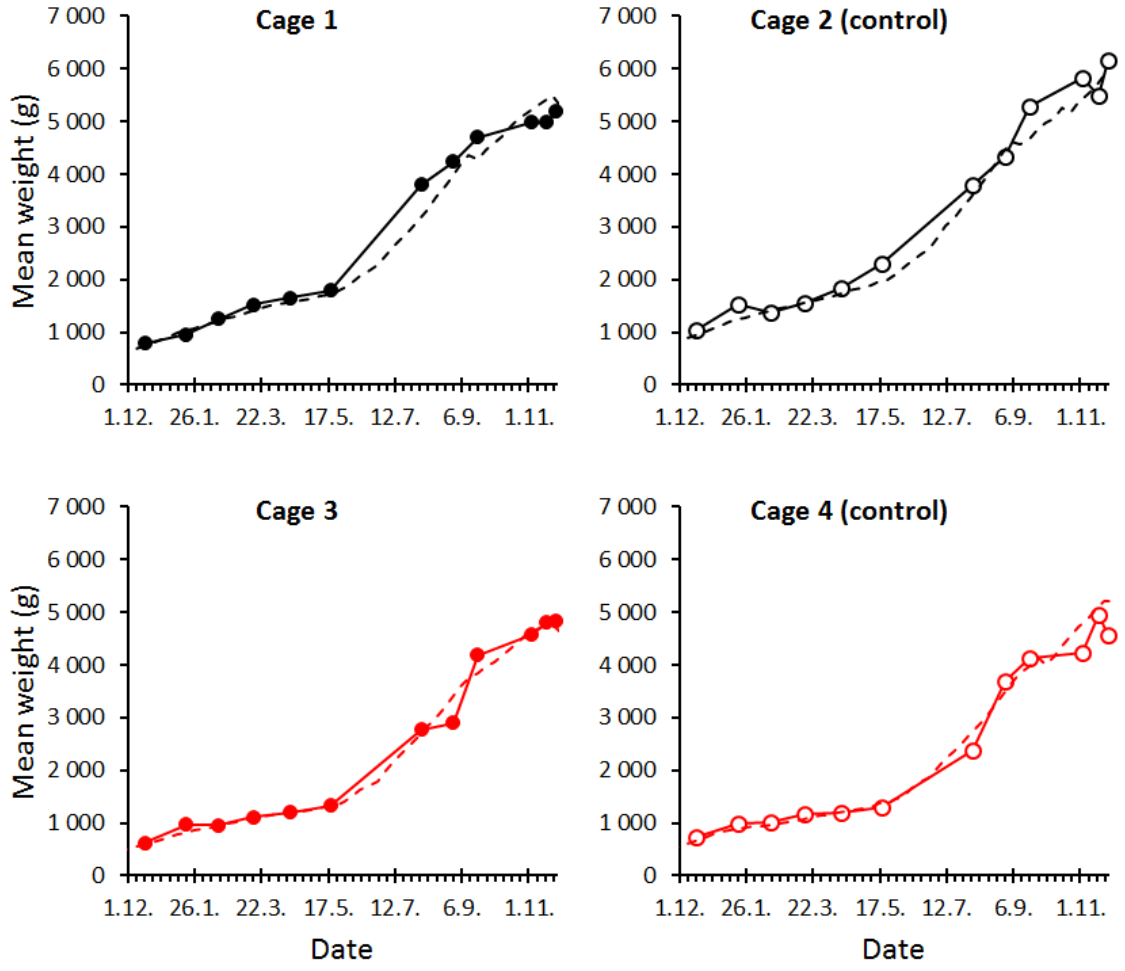

Fig. 12. Mean weight from sampled fish (circles and solid line) and mean weight estimated from Akvafarmer (dashed line). Filled circles: Cages with deep feeding; Open circles: Control cages. Major ticks (x axis): 56 days; minor ticks: 7 days.

4.4 Suggested improvements

In the deep fed cages feed entrance was at 7 m depth, and lights somewhat deeper at 10 m depth. During a significant proportion of the study period, especially during summer, a layer of brackish water was present from surface down to approximately 5-15 m depth dependent on what salinity to consider. Infective copepodites, avoiding brackish water, may therefore have had the highest concentration below the halocline, near the depth the fish were supposed to be attracted to. Placing the feed entrance and possibly the light deeper than what was the case here may be an option to increase the chance that fish and copepodites are spatially separated as much as possible. Oppedal et al. (2017) recently showed that in snorkel cages the infection rate decreased dramatically with the depth of the snorkel.

5 References

Bjordal, Å., Juell, J.E., Lindem, T., Fernö, A., 1993. Hydroacoustic monitoring and feeding control in cage rearing of Atlantic salmon (Salmo salar L.). In: Reinertsen, H., Dahle, L.A., Jørgensen, L., Tvinnereim, K. (Eds.), Fish Farming Technology. Balkema, Rotterdam, pp. 203–208.

Folkedal, O., Stien, L. H., Nilsson, J., Torgersen, T., Fosseidengen, J. E., Oppedal, F. 2012. Sea caged Atlantic salmon display size-dependent swimming depth. Aquatic Living Resources 25, 143–149

Folkedal, O., Pettersen, J. M., Bracke, M. B. M., Stien, L. H., Nilsson, J., Martins, C., Breck, O., Midtlyng, P. J., Kristiansen, T. S. 2016. On-farm evaluation of the **SALMON WELFARE INDEX MODEL (SWIM 1.0) - THEORETICAL AND PRACTICAL CONSIDERATIONS. ANIMAL WELFARE 25, 135–149**

Frenzl, B., Stien, L. H., Cockerill, D., Oppedal, F., Richards, R. H., Shinn, A. P., Bron, J. E. & Migaud, H., (2014) Manipulation of farmed Atlantic salmon swimming behaviour through the adjustment of lighting and feeding regimes as a tool for salmon lice control. *Aquaculture* **424**, 183-188.

Hansen, T., Stefansson, S.O., Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquac. Fish. Manage. 23, 275–280

Hansen, T.J., Fjelldal, P.G., Folkedal, O., Vågseth, T., Oppedal, F., 2017. Effects of light source and intensity on sexual maturation, growth and swimming behaviour of Atlantic salmon in sea cages. Aquacult. Environ. Interact. 9, 193-204

Hamre, L. A., C. Eichner, C. M. Caipang, S. T. Dalvin, J. E. Bron, F. Nilsen, G. Boxshall and R. Skern-Mauritzen (2013). "The Salmon Louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two Chalimus stages." PLoS One 8(9): e73539.

Heuch PA (1995) Experimental Evidence for Aggregation of Salmon Louse Copepodids (*Lepeophtheirus Salmonis*) in Step Salinity Gradients. Journal of the Marine Biological Association of the United Kingdom 75:927-939

Heuch, P.A., Parsons, A., Boxaspen, K., 1995. Diel vertical migration: a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids. Canadian Journal of Fisheries and Aquatic Sciences 52, 681–689.

Hevrøy, E.M., Boxaspen, K., Oppedal, F., Taranger, G.L., Hol, J.C., 2003. The effect of artificial light treatment and depth on the infestation of the sea louse *Lepeophtheirus salmonis* on Atlantic salmon (*Salmo salar* L.) culture. Aquaculture 220, 1–14.

Huse, I., Holm, J. C. 1993. Vertical distribution of Atlantic salmon (*Salmo salar*) as a function of illumination. J. Fish Biol. 43, 147-156.

Juell, J.E., Fernö, A., Furevik, D., Huse, I., 1994. Influence of hunger level and food availability on the spatial distribution of Atlantic salmon, Salmo salar L., in sea cages. Aquac. Fish. Manage. 25, 439–451.

Juell, J. -E. & Fosseidengen, J. E. (2004) Use of artificial light to control swimming depth and fish density of Atlantic salmon (*Salmo salar*) in production cages. *Aquaculture* **233(1)**, 269-282.

Juell, J. E., Oppedal, F., Boxaspen, K. & Taranger, G. L. (2003) Submerged light increases swimming depth and reduces fish density of Atlantic salmon *Salmo salar* L. in production cages. *Aquaculture Research* **34(6)**, 469-478.

Leclercq, E., Taylor, J. F., Fison, D., Fjelldal, P. G., Diez-Padrisa, M., Hansen, T. & Migaud, H. (2011) Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (*Salmo salar*) post-smolts. *Comparative Biochemistry and Physiology, Part A* **158**, 116-125.

Lien, A., Stien, L.H., Grøntvedt, R.N., Frank, K. (2015). Permanent skjørt for redusering av lusepåslag på laks. Trondheim: SINTEF Fiskeri og havbruk AS.

Migaud,H., Taylor, J.F., Taranger,G.L.,Davie, A., Cerdá-Reverter, J.M., Carrillo, M., Hansen, T., Bromage, N.R., 2006. Pineal gland sensitivity to light intensity in salmon (*Salmo salar*) and sea bass (*Dicentrarchus labrax*): an in vivo and ex vivo study. J. Pineal Res. 41, 42–52.

Nilsson, J., Folkedal, O., Fosseidengen, J. E, Stien, L. H., Oppedal, F. 2013. PIT tagged individual Atlantic salmon registered at static depth positions in a sea cage: Vertical size stratification and implications for fish sampling. Aquacultural Engineering 55, 32– 36

Næs, M., Heuch, P. A., & Mathisen, R. (2012). Bruk av «luseskjørt» for å redusere påslag av lakselus *Lepeophtheirus salmonis* (Krøyer) på oppdrettslaks. Vesterålen fiskehelsetjeneste.

Oppedal, F., Taranger, G. L., Juell, J. E., Fosseidengen, J. E. & Hansen, T. (1997) Light intensity affects growth and sexual maturation of Atlantic salmon (*Salmo salar*) postsmolts in sea cages. *Aquatic Living Resources* **10(6)**, 351-357.

Oppedal, F., Berg, A., Olsen, R.E., Taranger, G.L., and Hansen, T. 2006. Photoperiod in seawater influence seasonal growth and chemical composition in autumn sea-transferred Atlantic salmon (*Salmo salar* L.) given two vaccines. Aquaculture 254, 396-410.

Oppedal, F., Juell, J.-E., Johansson, D. 2007. Thermo- and photoregulatory swimming behaviour of caged Atlantic salmon: Implications for photoperiod management and fish welfare. Aquaculture 265, 70-81.

Oppedal, F., Dempster, T. & Stien, L. H. (2011) Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. *Aquaculture* **311(1)**, 1-18.

Oppedal, F., Samsing, F., Dempster, T., Wright, D. W., Bui, S., Stien, L. H. 2017. Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. Pest Management Science http://dx.doi.org/10.1002/ps.4560.

Osland, H., Sandvik, J.I., Holm, J., Heuch, P.-A., Bakke, S., 2001. Studie av lakseluspåslag og tilvekst hos Atlantisk laks (*Salmo salar*) i nedsenkede merder. HSF-report, R-NR 4/01 (22 pp. (In Norwegian)).

Stien, L. H., Nilsson, J., Oppedal, F., Kristiansen, T.S., Lien, A.M., Folkedal, O. 2012. Skirt around a salmon sea cage to reduce infestation of salmon lice resulted in low oxygen levels. Aquacultural Engineering 51, 21-25.

Stien, L. H., Bracke, M. B. M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P. J., Vindas, M. A., Øverli, Ø., Kristiansen, T. S. 2013, Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Reviews in Aquaculture, 5: 33–57. doi:10.1111/j.1753-5131.2012.01083.x

Stien, L., Fosseidengen, J.E., Malm, M., Sveier, H., Torgersen, T., Wright, D.W., Oppedal, F. 2014. Low intensity light of different colours modifies Atlantic salmon depth use. Aquacultural Engineering 62, 42-48.

Samsing, F., Oppedal, F., Dalvin, S., Johnsen, I., Vågseth, T., Dempster, T. 2016. Salmon lice (*Lepeophtheirus salmonis*) development times, body size and reproductive outputs follow universal models of temperature dependence. Canadian Journal of Fisheries and Aquatic Sciences. 73, 1841-1851

Schram TA (1993) Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In: GA BoxshallD Defaye. Pathogens of wild and farmed fish: sea lice. New York: Ellis Horwood. pp. 30-47

Wright, D.W., Glaropoulos, A., Solstorm, D., Stien, L.H., Oppedal, F. 2015. Atlantic salmon *Salmo salar* instantaneously follow vertical light movements in sea cages. Aquaculture Environment Interactions 7, 61-65.

Wright, D.W. Stien, L.H., Dempster, T., Vågseth, T., Nola, V., Oppedal, F., 2017a. 'Snorkel' lice barrier technology reduced two co-occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. Preventive Veterinary Medicine 140, 97-105

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