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Assessment of long-term implementation of sea lice prevention technologies: efficiency in reducing infestations and impact on fish welfare

– part of CAC2016G Vindsvik: Integrated lice management - test of various combinations of preventive measures against lice. FHF project 901243

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Project Report

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Assessment of long-term implementation of sea lice prevention technologies: efficiency in reducing infestations and impact on fish welfare

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Summary (Norwegian):

I de senere år har det vært økt fokus på økt bruk av er en rekke forebyggende strategier mot lakselus i oppdrettsmerder. Dette studie dokumenterer hvordan ulike tiltak gjennom en produksjonssyklus egner er seg for bruk, med fokus på den lusereduserende effekt og laksens velferd. Forsøket ble gjennomført i kommersiell skala ved «Centre for Aquaculture Competence» (CAC, finansiert av CAC og FHF 901243), ved lokaliteten Vindsvik i Rogaland og varte i 13 måneder. Den adderende/ økende effekten av flere samtidige tiltak ble undersøkt i 12 merder. Forebyggende tiltak besto av enten bare rensefisk (gruppe A), rensefisk + funksjonelt fôr (gruppe B), rensefisk + funksjonelt fôr + dype lys og fôring (gruppe C) eller rensefisk + funksjonelt fôr + dype lys/ fôring + skjørt (gruppe D). Hver 3.-5. uke ble det tatt ut fisk for lusetelling av alle stadier og skåring av fisk for å vurdere laksens velferd. Miljøprofiler ble tatt daglig og ekkolodd ble benyttet i lange perioder. Påslag av lakselus varierte betydelig med sesong. Fiskegruppen som ble gitt samtlige forebyggende tiltak (D) hadde konsekvent lavere påslag enn gruppene som kun hadde rensefisk og funksjonelt fôr (A og B). Selv om det var store forskjeller i lusepåslag og svømmedyp var nivået av bevegelige lus likt mellom gruppene, og dermed var behovet for avlusing det samme. Potensielle årsaker kan være sammensatt og mangesidig, eksempelvis at rensefiskens effektivitet er redusert eller at representativiteten i uttakene ikke har vært lik mellom gruppene som svømmer på ulike dyp. Overordnet velferd hos laksen var lik mellom gruppene. De ulike forebyggende tiltakene hadde kommersielt potensial for å bli benyttet i forhold til velferd og produksjon. Men, det er behov for å etablere og vise mer stabil og bedre effekt mot lusepåslag. Muligens kan dette oppnås ved å kombinere bruken av tiltakene i takt med de varierende miljøforhold, med fokus på brakkvannslag, og laksens valg av svømmedyp.

Summary (English):

Salmon lice prevention strategies are steering towards passive implementation, and this study aimed to monitor these strategies in commercial cages over time, to determine the efficiency of these approaches and their effect on welfare. Four strategies were tested at a commercial scale at the Centre for Aquaculture Competence (CAC; funded by CAC and FHF), over a 13-month period. The additive effect of multiple treatments was established in 12 cages, which were assigned to a prevention strategy of either cleaner fish only (A group), cleaner fish and functional feed (B group), the previous two factors plus deep attractant lights and submerged feeding (C group), or the previous three factors plus a lice skirt (D group). Environmental profiles and school swimming depth were monitored throughout the study period, and sampling events occurred every 3 – 5 weeks to assess the infestation and welfare status of salmon. The rate of infestation fluctuated with season, however the group with all prevention strategies (D) maintained a lower rate of new infestations compared to the groups with in-cage prevention (A and B groups). However, even with strong differences in new infestations and swimming behaviour, the level of mobile lice was similar among all groups, thus incurring a similar frequency of delousing events. This is potentially due to unrepresentative sampling of salmon or reduced cleaner fish efficiency in treatment cages. There was no overall effect of these prevention strategies on welfare status of salmon during the study period. Thus, the tested prevention strategies have promising potential for commercial implementation; however, improved and consistent efficiency is likely to be achieved with flexible operation that changes with specific environmental conditions.

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1 Background

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. However, one of the challenges that has not yet been managed to a sustainable level 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, thereby causing high infestation pressures on wild salmonid populations that share the fjord environment (Serra-Llinares et al. 2014, Thorstad et al. 2015, Shephard and Gargan 2017). The impact of lice on wild populations has prompted action by the Norwegian government to strongly regulate production limits, whereby farmers will be allowed to increase their producible biomass depending on the infection 'status' of their regions (see Myksvoll et al. 2018). Therefore, there is strong pressure on farming companies to control and minimise the salmon lice infestation levels on their sites.

There is an increasing focus on prevention of sea lice infestation rather than new treatment methods, and investment of resources into research and development has expanded the possibilities for prevention innovations. However, there are a limited number of prevention measures available on a commercial scale, and even fewer with documented effects on infestation prevention and fish welfare. Realistically, farmers will utilise multiple prevention methods throughout a production cycle, and possibly even use methods simultaneously to maximise their integrated pest management (IPM) strategy. In this study, we focus on three prevention measures used in combination: submerged lights and feeding, a lice skirt, and functional feed.

The first two methods aim to mismatch the vertical positioning of the host from the expected shallow distribution of infective copepodid larval stage of the salmon lice. Salmon can be attracted to swim deeper in the sea cage by use of lights (Juell et al. 2003, Juell and Fosseidengen 2004, Oppedal et al 2007, Frenzl et al. 2014, Stien et al. 2014, Wright et al 2015) and by moving the feeding zone to match the lights' depth (Frenzl et al. 2014, Nilsson et al. 2017), thereby avoiding the shallow waters except for infrequent forays to the surface to refill their swim bladders (Korsøen et al. 2012). Positioning salmon deeper in the water column, whether with cage structures or otherwise, has been proven as an efficient way to prevent infestation (Stien et al. 2016, Oppedal et al. 2017) however is highly dependent on seasonal variation in salinity stratification and water temperature. If a brackish layer is present at the surface, sea lice larvae will actively avoid low-salinity waters and aggregate below the halocline (T. Crosbie, unpublished data, Heuch 1995) and the infective zone can be brought deeper in the water column. Alternatively, hydrodynamic mixing can also transport larvae to meet the deeper-swimming salmon. In contrast, warmer temperatures can also override phototactic preferences in the fish, resulting in shallow swimming if optimal temperatures are towards the surface (Oppedal et al. 2007, Oppedal et al. 2011, Stien et al. 2016).

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

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

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

The aim of this study was to document lice reduction ability and welfare effects of prevention strategies at a commercial salmon farming site, over a 13-month period covering most of a standard production cycle. The additive effect of multiple treatments was investigated: cleaner fish only (A group), cleaner fish and functional feed (B group), the previous two factors plus deep attractant lights and submerged feeding (C group), or the previous three factors plus a lice skirt (D group).

2 Materials and Methods

2.1 Cage setup

Experimental testing of the preventive measures was conducted at site operating at a fjord site near Vindsvik, western Norway. The project was hosted by the Centre for Aquaculture Competence (CAC), a company that undertakes large scale R&D, contributing to solve the greatest challenges in salmon farming, generating new knowledge and facilitating dissemination of new findings to the aquaculture industry as a whole. The Centre is owned and funded by Marine Harvest Norway, Skretting and AKVA Group. This specific project was funded by CAC and it's owners, the Norwegian Seafood Industry Research Fund (fhf.no) and NRC through the 'Skattefunn' programme. The experimental site had 12 circular sea cages (circumference = 120 m, 25 – 35 m deep) in two parallel rows (Fig. 1). Net depth became deeper when smolt nets were changed in late winter/spring of 2017. 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*), rock cook wrasse (*Centrolabrus exoletus*) and goldsinny wrasse (*Ctenolabrus rupestris*). Lump fish and some ballan wrasse were of farmed origin, while all others were from wild catches. Intended stocking density of clean fish was to be 5% of salmon number per cage, with restocking multiple times during the experimental period (Table 1). Although the operational manager of the site strived to keep stocking densities equal among the cages, only the quantities of cleaner fish added were known; mortality of cleaner fish was only recorded when individuals were found in the salmon mortality collection system. All cages had three hides (6 m long) installed, with the upper part of the hide fixed at the surface. Cleaner fish were provided with feed close to their hide daily.

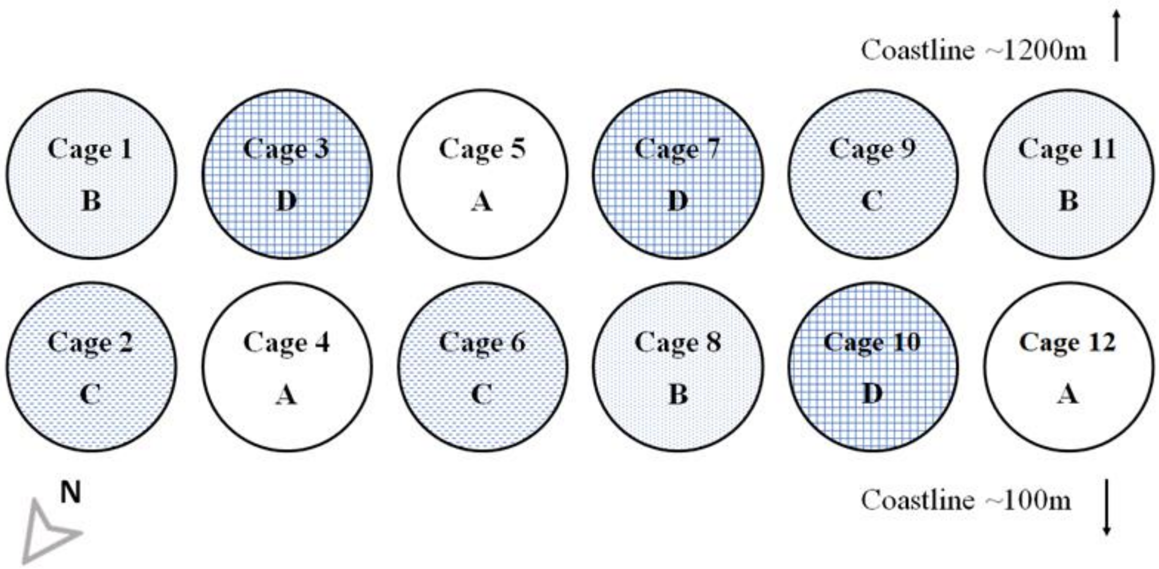

Fig 1. *Sea cage arrangement at the study site. Groups were additive lice management strategies categorised as: cleaner fish only (A), cleaner fish and functional feed (B), cleaner fish, functional feed and deep lights/feed (C), or cleaner fish, functional feed, deep lights/feed and lice skirts (D).*

Table 1. *Number of cleaner fish (all species) at the beginning of the study period, and the total number that were stocked at multiple time points throughout the study.*

Group	Cage	N at trial start	N stocked
A	4	4859	12107
	5	5878	8839
	12	4789	10656
B	1	4981	7451
	8	4593	10745
	11	4724	13435
C	2	4615	10049
	6	3591	11868
	9	4867	9577
D	3	4747	14949
	7	4580	16445
	10	4660	10533

Functional feed (Skretting Shield, Skretting, Norway) was provided to treatment cages (Group B, C, and D; see below) from trial initiation until trial completion. For all cages, fish were fed to satiation daily through visual monitoring during daylight hours. In December 2016, cages in C and D groups (see below) 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 D treatment cages (see below) that extended from the surface down to 6 m.

2.2 Experimental setup

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

To monitor group swimming behaviour, swimming depth distributions were continuously recorded using a PC-based echo integration system (CageEye, Oslo, Norway; Bjordal *et al.* 1993). The system includes a transducer submerged at 25-30 m deep inside every cage (moved deeper when nets were changed from the smolt net), positioned to face upwards with a 42° acoustic beam. The strength of the returned echo signal indicates the presence of fish, with higher signal strengths indicating more dense groups of individuals.

Environmental conditions of the water column were profiled daily using a CTD sensor (SD204, SAIV AS, Bergen, Norway), at a reference point outside of the experimental cages. Temperature and salinity were recorded from the surface to 40 m depth (Fig. 2).

The industry partner managing the facility retains an internal threshold of 0.2 adult females per fish at which a cage must be deloused (legislation requires treatment at 0.5 females per fish). Most delousing treatments were mechanical and non-chemical methods, applied on a cage-basis rather than whole-farm treatments. There was an exception to the non-chemical control, whereby a three-week period of delousing occurred using oral treatment (a chitin synthesis inhibitor in Dec 2016; not represented in Fig. 6), and another of hydrogen peroxide bath.

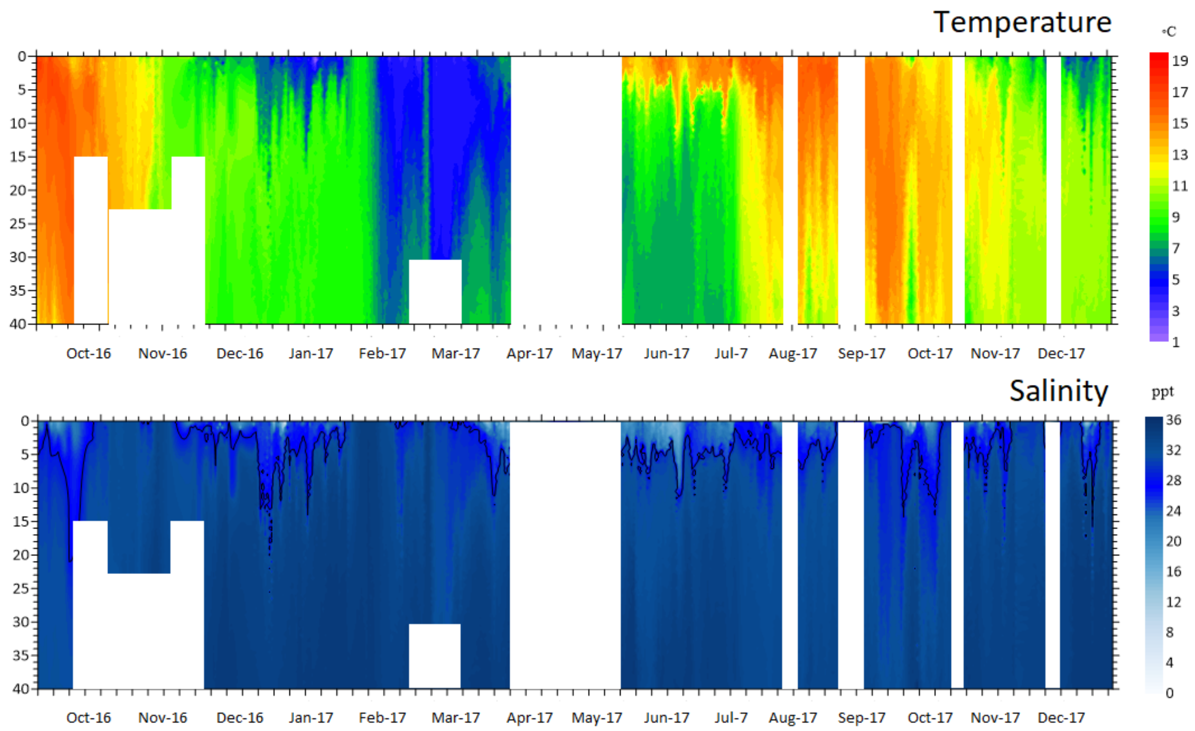

Fig 2. Temperature and salinity profiles over the study period, with colour contours indicated on the scale to the right of panels. The infective stage of salmon lice are assumed to aggregate below the brackish layer, so the salinity panel includes a black line highlights the contour where 28 ppt occurred. White blocks represent periods when data are not available.

2.3 Sampling regime (infestation and welfare status)

The first baseline sampling was conducted in November 2016, before the installation of the SubFeeder, lights, and skirt (Sample 0), but after functional feed had begun to be provided. Thereafter, every 3 to 5 weeks from January until December 2017 (a total of 15 sample events: see Supplementary table 1), fish were sampled and assessed for lice infestation levels and welfare status. Fish were captured using a hand net at the surface, a seine net at the surface, or small ring-net pulled from 5 or 10 m depth to the surface by a boat crane; cages were sampled using the same method and crew at each separate sample event. Sampling was conducted a minimum of 3 weeks after any delousing treatment, so that new infestations were certain to be unaffected by previous treatments; Sample 14 was an exception, where delousing occurred the week prior, and therefore is excluded from lice analyses. Delousing events were triggered by levels of 0.2 adult female lice fish^{-1}, which was assessed through weekly farmer counts rather than by this study's scientific lice counts. At each sampling point, 20 salmon from each cage were euthanised and any lice attached were quantified and staged. 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/normal to severely damaged/abnormal (Table 2). Welfare indicators are weighted in the model and used to calculate an overall welfare index, a value bounded from 0 (worst) to 1 (best) that represents an individual's welfare status. Occasionally more than 20 fish were captured from the cage, whereby only the first randomly-chosen 20 fish were assessed for welfare, and all lice were counted and divided by the total number of fish sampled. Gill diseases were also recorded, specially presence of PGI and AGD, however the prevalence of these diseases was negligible throughout the study.

Table 2. *Welfare indicators that comprise the Salmon Welfare Index Model (SWIM), each with weighting used to calculate an Overall Welfare Index for an individual (from Stien et al. 2013).*

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

2.4 Data handling and statistical analyses

Lice stages were categorised by whether they were new infestations since the previous sampling (copepodid, chalimus 1 and 2 stages), or could possibly have been present at the previous sampling (pre-adult 1 and 2, and adult stages). All lice considered a new infestation were summed and averaged by the number of individuals sampled per cage. This value was used to compare the efficiency of treatments on prevention of new infestation, using a generalised linear model (GLM) with poisson distribution. The model included treatment group and sample number as predictor variables, and cage number as random effect (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, FHF project 901283) based on the average sea temperature the 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. However, the efficiency of cleaner fish was qualitatively estimated by assessing the relationship between the estimated stocking density of cleaner fish the day prior to a sample event, with the mean abundance of mobile lice recorded at that sample time.

Echosounder data was frequently unavailable due to equipment damage or incorrect placement, however the data that was recorded provided information on the vertical dispersion of the salmon; 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 depths whereby more than 4% of fish were present. For qualitative 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 lice copepodids gradually avoid brackish waters of 28 ppt or fresher (T. Crosbie, unpublished data), the depth of 28 ppt was termed the halocline for this study. The estimated depth of the halocline was determined by the deepest depth with a salinity of 28 ppt or lower, calculated daily. The relationship between halocline depth (and therefore assumed depth of most infective copepodids), median salmon swimming depth, and actual average date for lice infestation (back-calculated from stage and temperature) was qualitatively assessed for the study period, whereby only days when all three parameters were available for a replicate cage were used.

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

3 Results

3.1 Lice infestation status, salmon swimming depth, and environment

Based on new lice infestation rates (i.e. sessile stages), the inclusion of treatment group was significant when compared to the null model. The full model showed that Group D was significantly different from A ($z = -2.8$, $p = 0.006$), whereas other groups and sample date as a factor were not different. The reduction in new infestations was most prominent in Group D (which had lice skirts, deep lights/feed, and function feed) with 51.3% and 63.3% fewer attached lice stages across the whole year compared to Group A and B, respectively (Fig. 3). However, this effect size varied with sample dates, ranging from 7.3% *more* lice (March 2017) to 92.6% *fewer* lice (July 2017) than the A group. A large proportion of the period showed promising efficiency, with 8 out of 13 samples where Group D had a reduction of > 40% compared to A, and only two samples where a reduction was not observed. This pattern was more evident in summer and autumn periods, and somewhat in winter; levels were low across all cages in spring. When compared to Group B, Group D had a 17% or more decrease in attached lice at all sample points except one (May 2017). In contrast, C cages had new infestation levels similar to A, with an overall average of 0.7% more lice than A groups. Peak levels of new infestations occurred during different seasons among groups, with the experimental period's maximum abundance observed in July 2017 for Groups A and B (2.48 and 2.85 lice $fish^{-1}$, respectively), but in December and March 2017 for Groups C (1.33 lice $fish^{-1}$) and D (0.72 lice $fish^{-1}$), respectively (Fig. 3). When lice attachment date was back-calculated, there were relatively large differences in infection success between days, within a short period of days to weeks (Fig. 4, middle panel). This suggests a significant role of day-to-day variation in infection pressure, and its interaction with halocline depth and salmon vertical distribution.

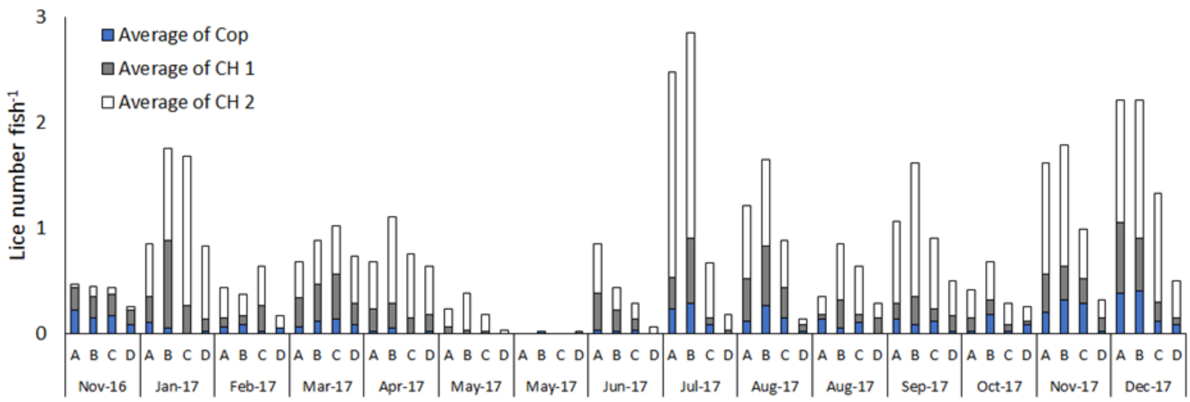

Fig. 3. *Average new lice infestation levels for each treatment group over the study period. Attached stages of lice are considered new infestations, with average abundance of copepodids, chalimus 1 and chalimus 2 represented.*

As the experimental site was in a narrow, inner fjord location, a stratification of temperature and salinity often occurred (Fig. 2). Brackish layers were present almost throughout the study period, sometimes extending below the protective depth of the lice skirt at 6 m (Fig. 4, upper panel). Salmon in all cages swam relatively shallow in the first months of the study, when there was little temperature difference in the water colomn. Thereafter, the preferred swimming depth of salmon exposed to deep lights and feed was often different to the cages without (Supplementary figure 1). Although the salmon responded to multiple environmental variables that fluctuated with time, there was a consistent difference in swimming depth when time-pooled means were estimated. The average median swimming depth of the shallowest school, over the entire study period, was 5.18 and 5.55 m for A and B cages without deep lights and feed, compared to 11.05 and 11.49 m for C and D cages. This translated to an average of ~0.03 and 0.16 m *above* the halocline for A and B cages over the period, whereas C and D cages were ~5.76 and 6.79 m *below* the halocline, but with larger variation between days (Fig. 4, lower panel).

Although many assumptions are made when calculating the daily infection success (i.e. date of lice attachment) and swimming depth of the school, an interesting pattern arises when plotting this data against halocline depth (Fig. 5). The distance of the school swimming depth from the halocline showed a weak relationship with infection success, as lice infestation rates were low for the available data points. However, the only peaks of infestation success above 2.0 lice per fish occurred when the school was within 5 m above or below the halocline (Fig. 5). The lack of infestation success peaks in the D cages suggests that the presence of lice skirts provides the additional preventive capacity compared to only deep lights and feed.

3.2 Mobile lice, treatment frequency, and cleaner fish effect

Mobile lice levels were relatively similar across groups throughout the experimental period, with only one sample date (November 2017) where C and D groups were more than 2 lice per fish lower than the A group (Fig. 6). Conversely, there were a few occasions where sampled fish in C and/or D cages had more mobile stages than A groups, with levels over 1 mobile per fish in comparison (Fig. 6). Even with delousing events, there was a peak in lice abundance in winter and late spring, and a gradual increase from autumn onwards (Fig. 6). The maximum mean number of mobile per fish was recorded as 7.6 in Group B followed by 5.7 in Group C, both of which occurred in December 2017. In contrast, the maximum infestation level in Group D was 3.5 mobile fish per fish, which was recorded in October 2017. The levels of mobile lice therefore triggered multiple delousing events, with no difference in the frequency of delousing among groups (Fig. 6). Group A and B underwent a total of 14 and 16 treatments across the replicate cages (mean 4.7 and 5.3 treatments per cage, respectively), while both C and D groups underwent 14 (mean 4.7 per cage).

Cleaner fish abundance in cages generally decreased, with stocking densities maintained by replenishment 5 to 9 times throughout the study period (Fig 7). The number of cleaner fish across treatment groups was similar from Oct 2016 until April 2017, however thereafter, there were periods were the B and D groups had fewer cleaner fish stocked (Fig 7). When assessing the relationship between stocking density and presence of mobile lice, it appeared that A cages had a positive linear correlation (R^2 = 0.19, 0.24, and 0.44 for replicate cages; Fig 8) whereby there were more mobile lice per fish when stocking densities increased. This was less apparent in B cages (maximum R^2 = 0.24), and negligible in C and D cages (maximum R^2 = 0.17 and 0.03, respectively; Fig 8).

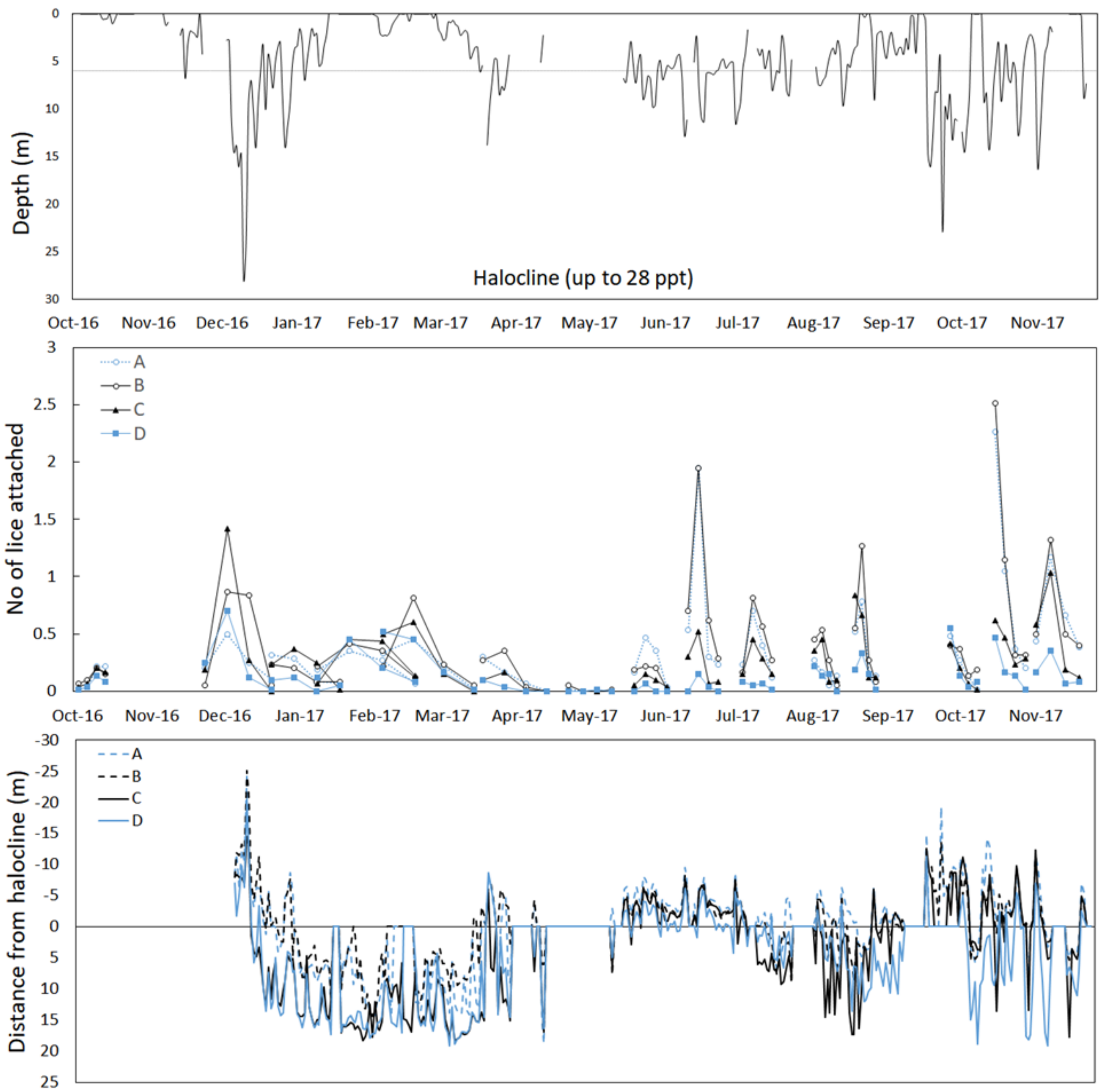

Fig. 4. Salinity profile, lice attachment date and salmon swimming depth over the study period. Upper panel: depth of halocline (determined as up to 28 ppt); middle panel: mean number of lice attached per fish, within treatment groups; lower panel: daily median swimming depth of schools per cage, compared to the halocline (28 ppt or lower) for that day. Negative values of the distance from halocline indicate salmon swimming above the halocline, whereas positive values indicate salmon swimming deeper in the cage. Times when either echosounder or environmental data were not available are not plotted. Dotted line at 6 m depth in upper panel represents the depth of the lice skirt in D groups.

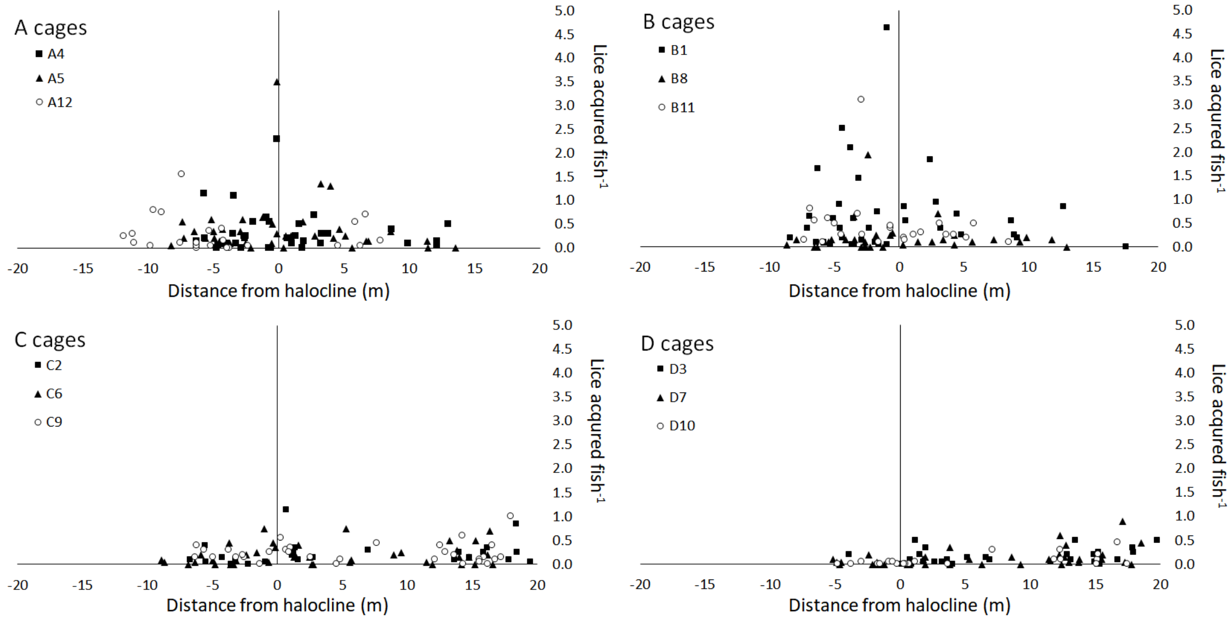

Fig. 5. *Lice infestation rate in relation to the salmon swimming depth, separated into panels for each treatment group (A, B, C, and D); negative values of the distance from halocline indicate salmon swimming above the halocline, whereas positive values indicate salmon swimming deeper in the cage. Data points are only represented if a day has values for lice attachment, salmon swimming depth, and salinity. Data are pooled over the experimental period.*

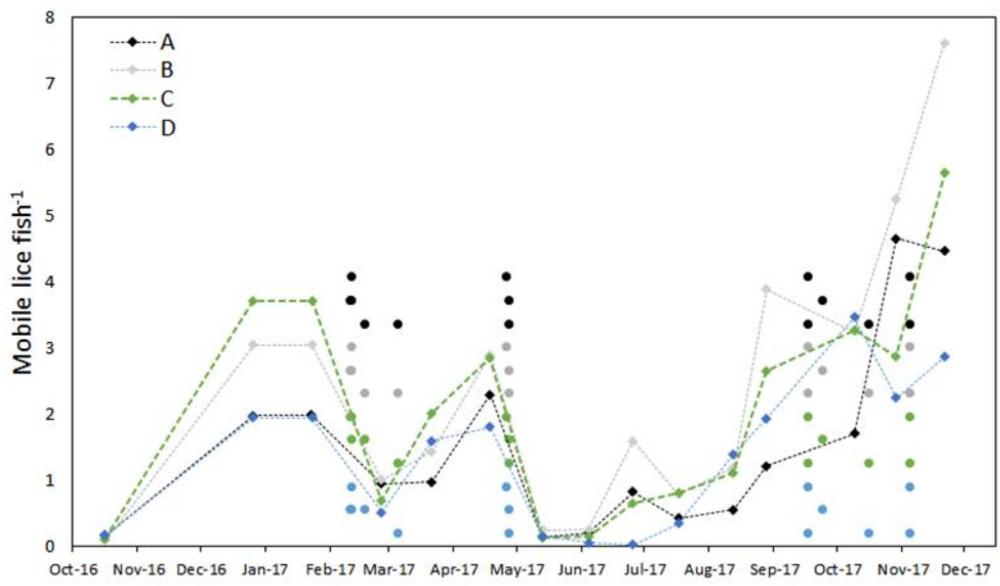

Fig 6. *Mean mobile lice abundance per treatment group, over the study period. Delousing events are shown (vertically staggered per cage on the actual treatment date) as circular markers, with the colour corresponding to the treatment group represented in the legend.*

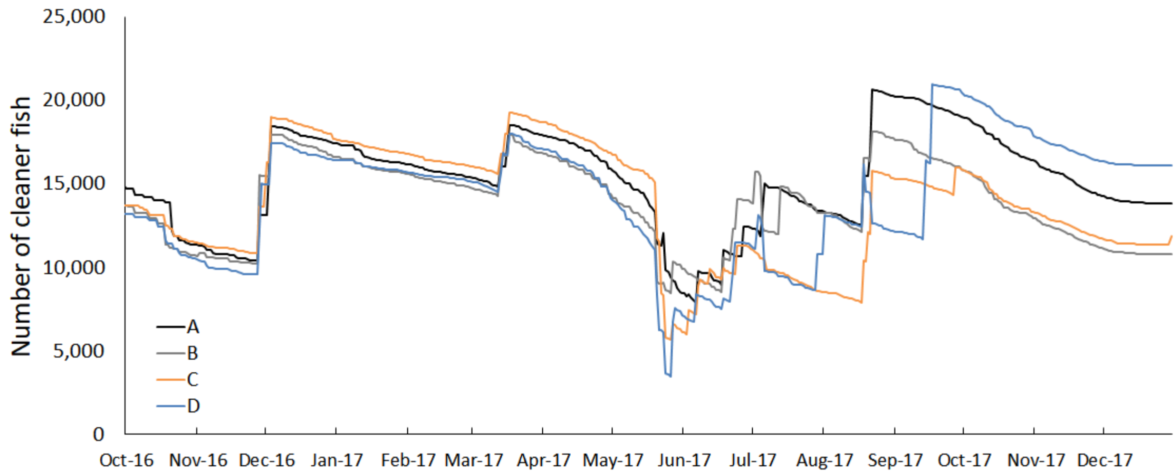

Fig 7*. Total abundance of cleaner fish in treatment groups over time, with number among replicate cages combined.*

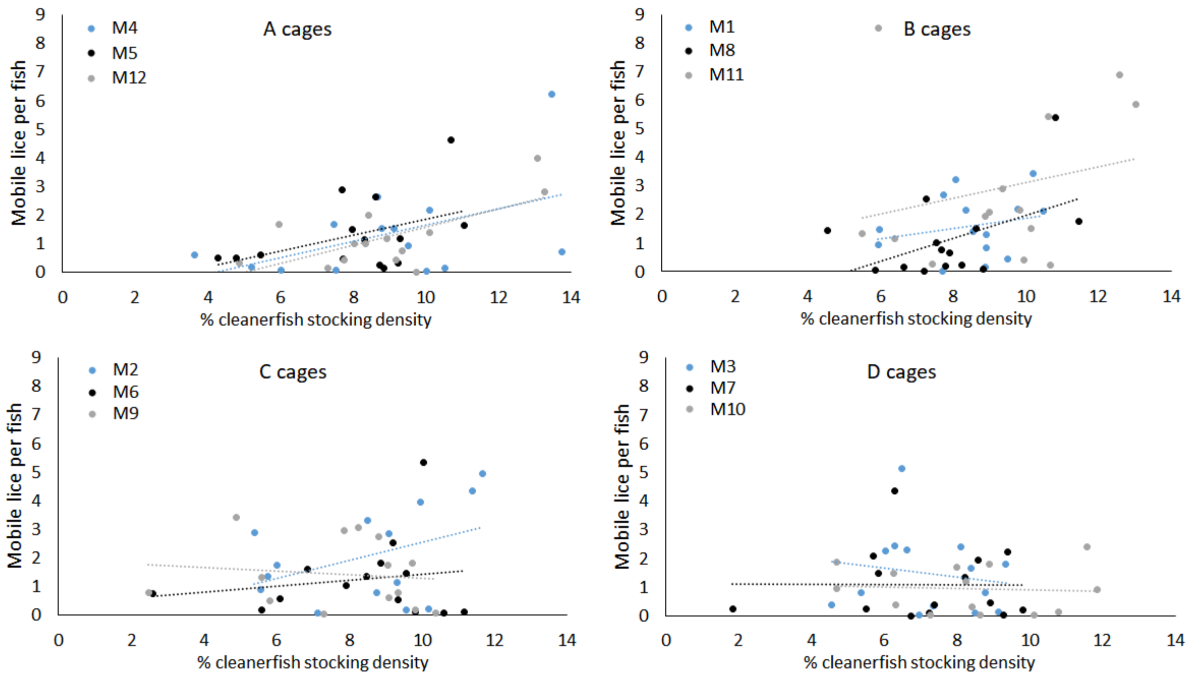

Fig 8. *Relationship between cleaner fish stocking density (pooled across fish species) and mobile lice levels per cage, over the study period. Each data point represents a sample event, and the estimated stocking density the day before sampling. Panels represent treatment groups (A, B, C, D). Abundance of mobile lice does not take into account recent cage-specific delousing events (minimum 3 weeks prior to sample date). Linear regressions are represented by dotted lines in colours matching data points.*

3.3 Welfare status

The overall welfare score of groups slightly deteriorated over time, but occasionally did reach initial levels (Fig. 9). The inclusion of submerged lights and feed (Group C) had a significantly improved welfare status ($z = 2.36$, $p = 0.018$) whereas the other groups were found to be similar. OWIs fluctuated with sample date, with samples from August 2017 onwards (excluding one date in September) showing large differences ($z < -3.31$, $p < 0.001$ for these samples; Fig 9). In particular, Group A and B had severely reduced OWIs of below 0.7 in July and August 2017; during this period, these groups had more severe eye conditions (Supplementary figure 2) and higher levels of new lice infestations (Fig. 3, Supplementary figure 3). Results of welfare scores for all samples and all cages are represented in Supplementary figure 3. Generally, severity of AGD was never high (maximum mean score observed was 0.9). PGI was prevalent in 9 out of 15 sample events, but scores were low and the lack of severity (and prevalence across individuals and cages) did not elicit veterinary action.

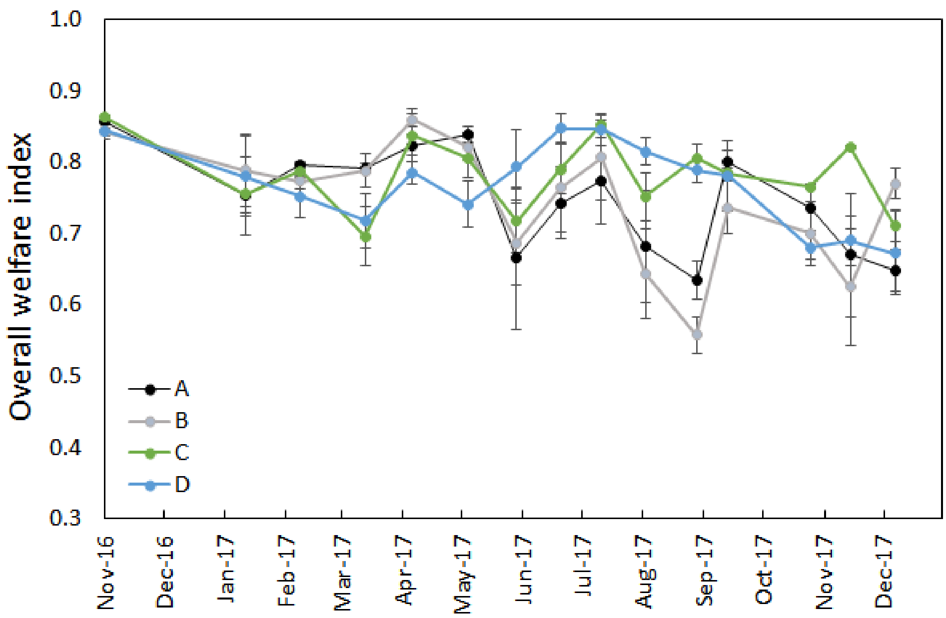

Fig 9. *Mean overall welfare score of fish in treatment groups, over the study period. The overall welfare index is calculated using the SWIM 1.1 model (Stien et al. 2013), which has a possible score range from 0 to the most positive score of 1.*

Growth of salmon was similar among treatment cages, with the increase in weight of sampled fish over time loosely following, to a lesser magnitude, the weight gain predicted by the farmer's management program (Fig. 10). Mean farmer-predicted end weights on the last sample date were very similar among groups, ranging only from 3624 g to 3857 g.

Cumulative mortality among the treatment cages fluctuated from the standard cage, with A group cages having the lowest mortalities for most of the study period, apart from ~January to May 2017 when D cages were in fact lower (Fig. 11). Through May to August 2017, mortality rate was similar among B, C, and D groups, and around the end of September, the A group increased to converge to similar levels of the other groups. By the end of the study period, C cages had the lowest cumulative mortality rates, approximately 8600 individuals fewer than worst group, B.

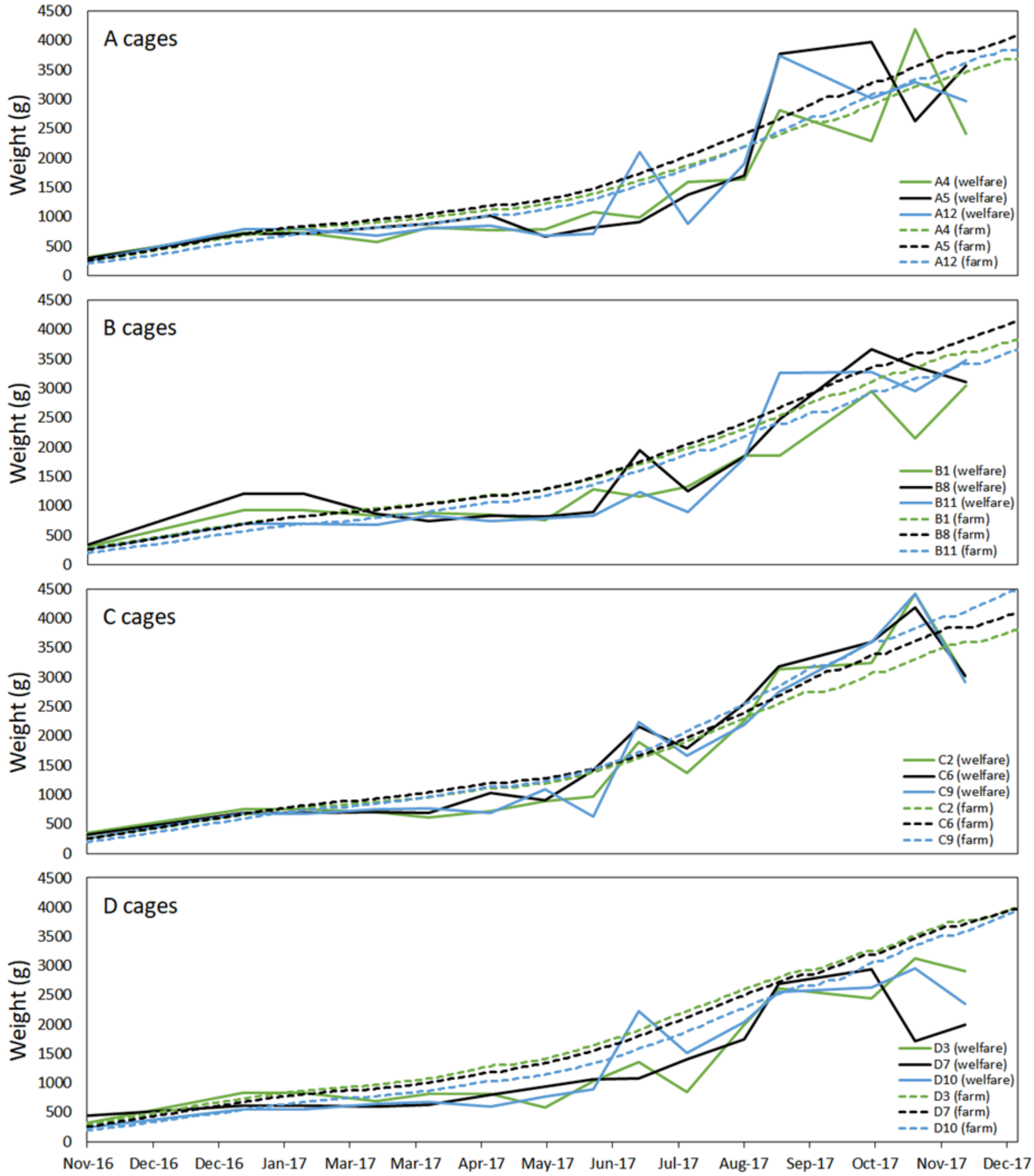

***Fig 10**. Weight of salmon over the study period, as recorded by fish sampled for the welfare assesments ('welfare' data) and as estimated by the farm management software ('farm' data). Panels represent individual replicate cages, categorised into treatment groups.*

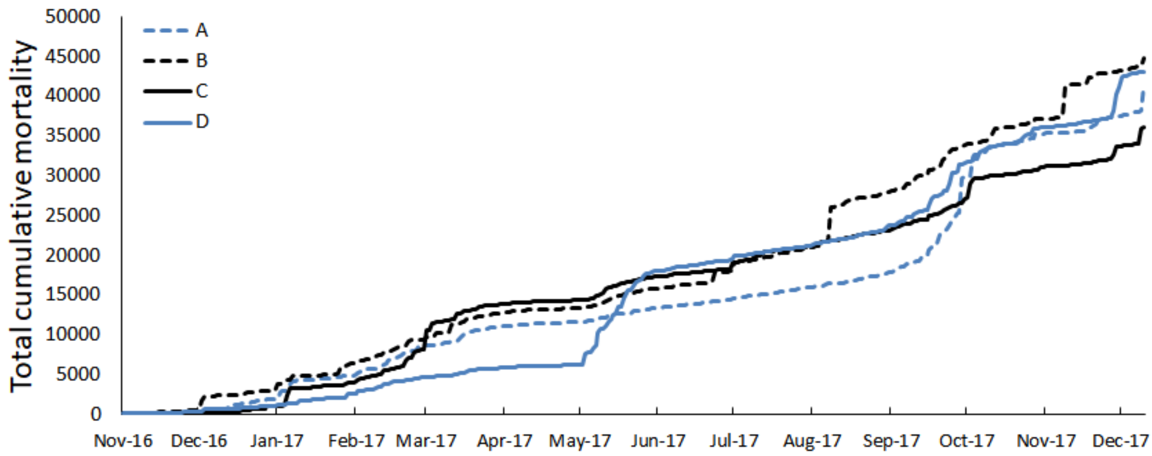

Fig 11. *Cumulative mortality over the study period, with total value represented for replicate cages within treatment groups (A, B, C, and D).*

4 Discussion

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

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

As salmon lice are positively phototactic, but assumed to avoid brackish waters and therefore distribute just below a halocline (Heuch 1995), the depth of the halocline in relation to where salmon are dispersed is a significant relationship. The use of deep lights and feed aims to draw the salmon deeper in the cage away from this parasite-risky area, however this strategy is unlikely to be efficient during periods when the halocline extends below the depth of the lights/feeders. For example, during October 2017, the halocline was deeper than the skirt and feeding zone depth, and the predicted date of attachment showed little difference among groups as all schools swam at similar depths (Fig. 4).

At separate commercial site that used analogous deep lights and feeders, no difference in infestation levels or salmon swimming depth were found between control and treatment cages (Nilsson et al. 2017). In this study, salmon swimming depth was distinctly different between cages with submerged lights and feed compared to those without, whereby the attractant lights and deeper feed zone encouraged the school to distribute deeper in the cage during most periods. The average depth difference between A/B cages versus C/D cages was approximately 6 m over the study period, with continuous variations observed in winter and autumn. There is a suggested link between peaks of infection pressure resulting in increased infection success (i.e. higher rate of lice attachment) when fish were swimming close to the halocline, which was more prevalent in cages without deep lights and feed. It must be noted that the data here was processed using daily average values, which would not reflect diurnal differences in salmon swimming depth preferences (Oppedal et al. 2011). If differences between daytime and night time swimming depths were large, the median value calculated per day would mask realistic school interactions with the halocline.

4.2 Mobile lice and delousing frequency

Although new infestations were reduced in D cages overall, this did not translate to a reduction mobile lice levels and therefore delousing events. There are three possible reasons for the discrepancy between infestation levels and subsequent mobile lice abundances that triggered delousing: reduced efficiency of cleaner fish in cages with sub-lights/feeders or skirts, unrepresentative sampling of fish for assessment, or the farm company's lower threshold that triggers delousing (0.2 adult females fish-1).

To our knowledge, no studies have assessed the impact of cage technologies on cleaner fish behaviour or efficiency. The presence of a lice skirt can reduce oxygen levels near the surface by 5 – 35 percentage points saturation (Stien et al. 2012, Stien et al. 2018), which may influence cleaner fish distribution or cleaning behaviour. As their hides were situated within the skirt, chronic exposure to slightly reduced oxygen levels could reduce their welfare status and subsequently, their delousing potential. However, the lack of positive relationship between mobile lice levels and cleaner fish stocking density (Fig. 8) suggests that the presence of the skirt in this study did not influence their efficiency. The use of deep lights/feed aims to draw salmon deeper in the cages, and could also create a mismatch compared to the vertical distributions of cleaner fish. The maximum distance from a client at which a cleaner fish will exhibit inspection or cleaning behaviours has not been documented, and there is likely to be differences in searching behaviours among cleaner fish species used. As such, if there are large differences in depth distributions of salmon and cleaner fish, any spontaneous opportunity for cleaning is reduced. Interestingly, there was lack of effect of stocking density on mobile lice abundance (Fig. 8), if density estimates are accurate. For some individual cages in A, B, and C groups, there appeared to be a positive relationship between mobile lice levels and cleaner fish stocking density, which suggests that further comprehensive studies are required to understand their delousing efficiency.

True estimates of lice loads are vulnerable to sampling error, through either capture methods or treatment for examination (Heuch et al. 2011). Euthanising fish in a water bath and conducting lice counting inspections directly afterwards is a more accurate method for recording lice abundances, compared to a blow to the head and individual bagging (Copley et al. 2005). Therefore, in this study, capture methods are the likely source of unrepresentative sampling, if indeed this was the case. In a sea cage, differences in spatial location of individual salmon within the school is driven by size (Folkedal et al. 2012), hunger, physiological state (i.e. emaciation status, Vindas et al. 2016), and infection status (Bui et al. 2016), all of which are likely to interact with the presence of lice skirts or submerged lights/feed; if individuals caught for sampling are only from the upper 5 m depth, it is possible that the sampled individuals are unrepresentative due to these factors. In fact, over the study period, the median swimming depth of the shallowest school for C and D groups was ~6 m below the halocline (Fig. 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.

The discrepancy between the reduction of new infestations and the similar frequency for delousing events could be due to the internal limits set by the partner company (CAC, operated by Marine Harvest), which is lower than the national legislation (0.2 compared to 0.5 adult female lice fish-1, respectively). It could be possible that the reduction of infestations can be translated to reduced adult female lice abundances, but the difference occurred above the 0.2 lice threshold. However, when comparing the prevalence of 0.2 versus 0.5 adult female lice fish-1 across samples, a similar pattern exists, whereby cages would have needed to be deloused at the same inter-group rate (i.e. the number of times a cage exceeded 0.5 adult females per fish was similar across groups; see Supplementary table 2). If the experimental facility followed the national legislation level of 0.5 female lice fish^{-1} instead, treatment frequency (as predicted by this study’s scientific lice counts, conducted only every 2 – 3 weeks) would have been reduced from 63 occasions to only 28 (Supplementary table 2). In fact, some cages barely reached the limit of 0.5 adult female lice fish^{-1}; one A cage never surpassed 0.5 adult females, and two D cages only passed this limit once.

An alternative theory for the converging abundance of mobile lice among groups could be the availability of brackish waters in the cages without lice skirts; salmon in these cages would have access to low-salinity waters and their forays to brackish salinities could affect the development success of attached lice. There were periods in this study whereby the halocline was around or above the depth of the skirt, thus potentially providing a brackish environment for A, B, and C groups (Fig. 4); however, there needs to be improved understanding of the effect of brackish water on lice attached to hosts to fully explore this possibility.

4.3 Welfare status

The prevention technologies tested here were not expected to cause negative specific welfare effects, although progression of growth and condition factor was particularly interesting with the submerged feeder and functional feed. The mean Overall Welfare Index (OWI) score over time was between 0.74 and 0.78 for all groups, which is comparable to standard commercial sites using only SWIM 1.0 assessments (Folkedal et al. 2016). The lowest average OWI in this study was 0.56 in Group B (Sample 10). At this particular sample event, the extreme low scores were driven by a higher rate of worst eye status scores, resulting in individual OWI's of 0.00. The range maximum of OWIs during the experimental period was similar among groups (0.86 for Group A, B, and C, and 0.85 for Group D), however Group A and B had lower minimums (0.63 and 0.56, respectively) compared to Group C and D (0.70 and 0.67, respectively).

When investigating the drivers of low OWIs, no consistent pattern in severity of welfare indicators was observed among treatment groups (Supplementary figure 2), suggesting that spikes and differences among cages are likely to be due to lice infestation levels, which are incorporated into the welfare model. Another possible driver of low welfare scoring could be procedures such as handling during delousing. During the experimental period, this farm site avoided chemotherapeutants and mainly utilised mechanical delousing methods for control of mobile sea lice, which could result in more frequent spikes of severe welfare scores due to treatment. For instance, there was a spike in prevalence of severe mouth/jaw wounds in C and D groups (Sample 3) and D and B groups (Sample 14), however overall the level remained below 15% of sampled fish exhibiting severe scores. However, these peaks did not align with recent delousing events closely (Fig. 6), so could be a delayed effect or cause by other husbandry procedures. Eye status was the indicator that was most often scored highly. The most severe eye score will be recorded if the fish have high eye-area coverage cataracts in both eyes, or severe exophthalmia that renders them blind (see Pettersen et al. 2014). This can be caused by a number of biological and abiological factors (Noble et al. 2012), however the most likely influencers pertinent to this study's cage environment are the rapid increase in temperature and growth during spring months (Bjerkås et al. 2001), or common aquaculture practices such as pumping, or secondary infections. Although the cages that had all prevention technologies had more peaks of eye severity, there was not a consistent difference among groups that could be attributed to the presence of lice skirt or submerged lights (Supplementary figure 2).

The low growth in the early months of the study are not uncommon for newly-transferred smolts, as they prefer to swim close to the surface and as a result, experience colder temperatures (Oppedal et al. 2011). However, a notable discrepancy in predicted and actual weight is observed in the A and D group, where A cages fluctuate above and below the predicted, but fish sampled in D cages are often smaller than predicted (Fig. 10). Unrepresentative sampling could have also affected the welfare status results of the D group in later sample points. Average weight at the sample point in November was recorded as 2599 g, compared to the estimated weight by the farmers of 3442 g (Fig. 10). Similarly, in December the recorded versus estimated was 2418 g and 3694 g (respectively; Fig. 10). This indicates that the fish collected for welfare assessments were likely to be smaller than the school average, and therefore those individuals could have experienced different conditions that impact welfare, such as environment or infestation pressure. This is particularly the case if those smaller fish that were sampled were growth-stunted individuals (loosely termed 'loser fish'), which have a different welfare status and physiological state than 'normal' fish (Vindas et al. 2016). Growth-stunted fish are often severely emaciated and moribund, and therefore farmers attempt to remove them from the cages as soon as possible; as the prevalence of growth-stunted fish at this site was reasonably high, this could account for the jump in mortalities recorded in March (C group), May (D group), and September (A group) 2017 (Fig. 11).

4.4 Adaptive prevention

In the cages with both skirts and the deep light/deep feeding 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. For instance, higher lice acquisition is likely to occur if a fraction of the school chooses to swim at the depth of the lights and feed (i.e. 7 – 9 m), but at the same time, a deep brackish layer distributes copepodids deeper than the skirt's protective depths to overlap with the distribution of potential hosts.

This study 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, lice skirts could be lowered deeper if there is a brackish layer at the surface and salmon are swimming deep, or completely removed if salmon prefer the shallow brackish depths. Similarly, submerged lights and feed could encourage the school's depth preferences towards areas with lower infective risk, as predicted by the salinity profile.

4.5 Practical implementation

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

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6 Supplementary figures and tables.

Supplementary table 1. *Summary of sample dates over the study period.*

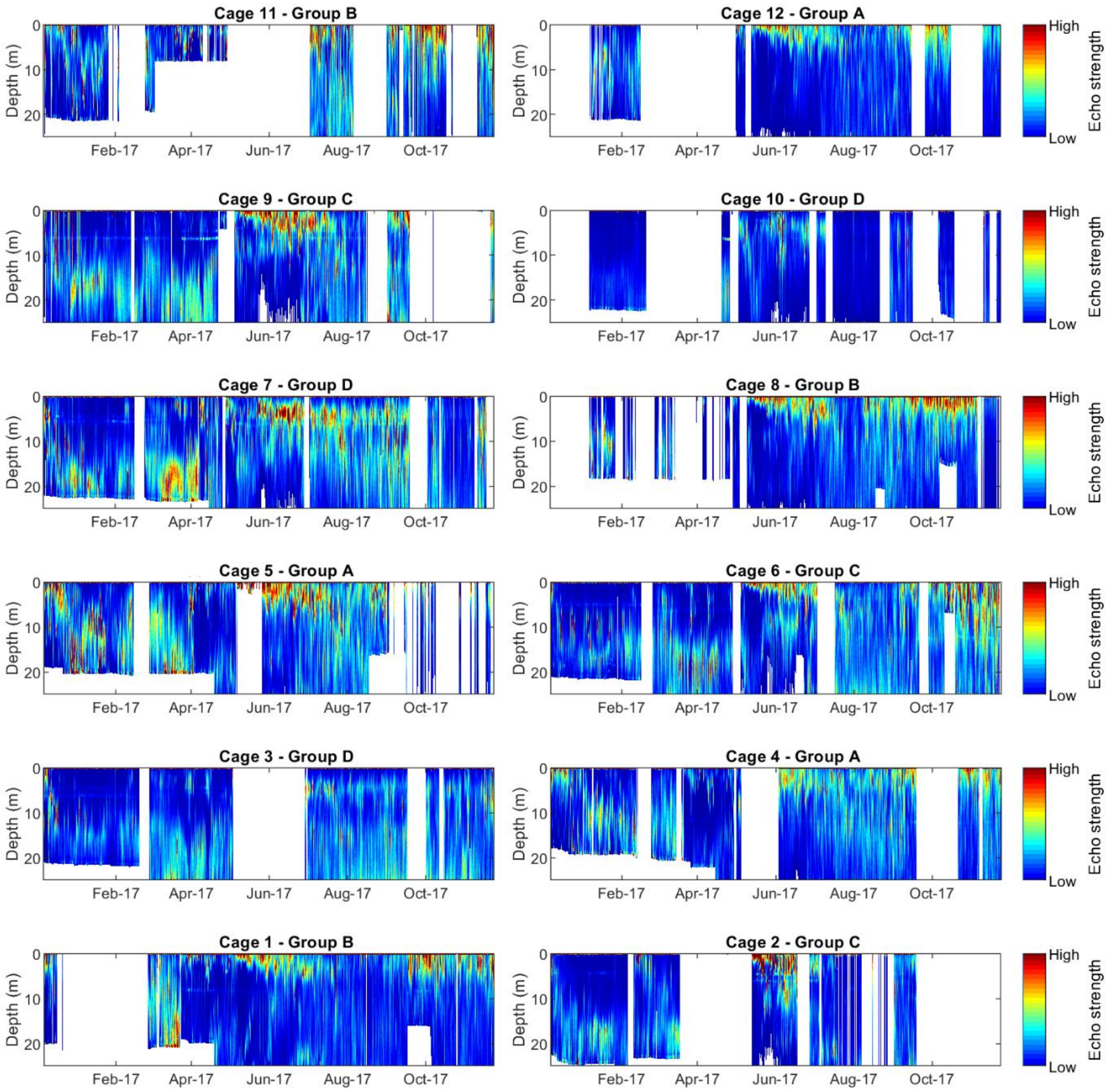
Sample number	Date
0	1 November 2016
1	11 January 2017
2	8 February 2017
3	13 March 2017
4	6 April 2017
5	4 May 2017
6	29 May 2017
7	20 June 2017
8	11 July 2017
9	2 August 2017
10	28 August 2017
11	13 September 2017
12	25 October 2017
13	14 November 2017
14	7 December 2017

Supplementary table 2. *Summary of mean adult female lice per fish at each sample point for each cage (labelled with treatment group letter and actual cage number), and the subsequent frequency of occurrences whereby adult female lice exceeded either 0.2 or 0.5 females per fish.*

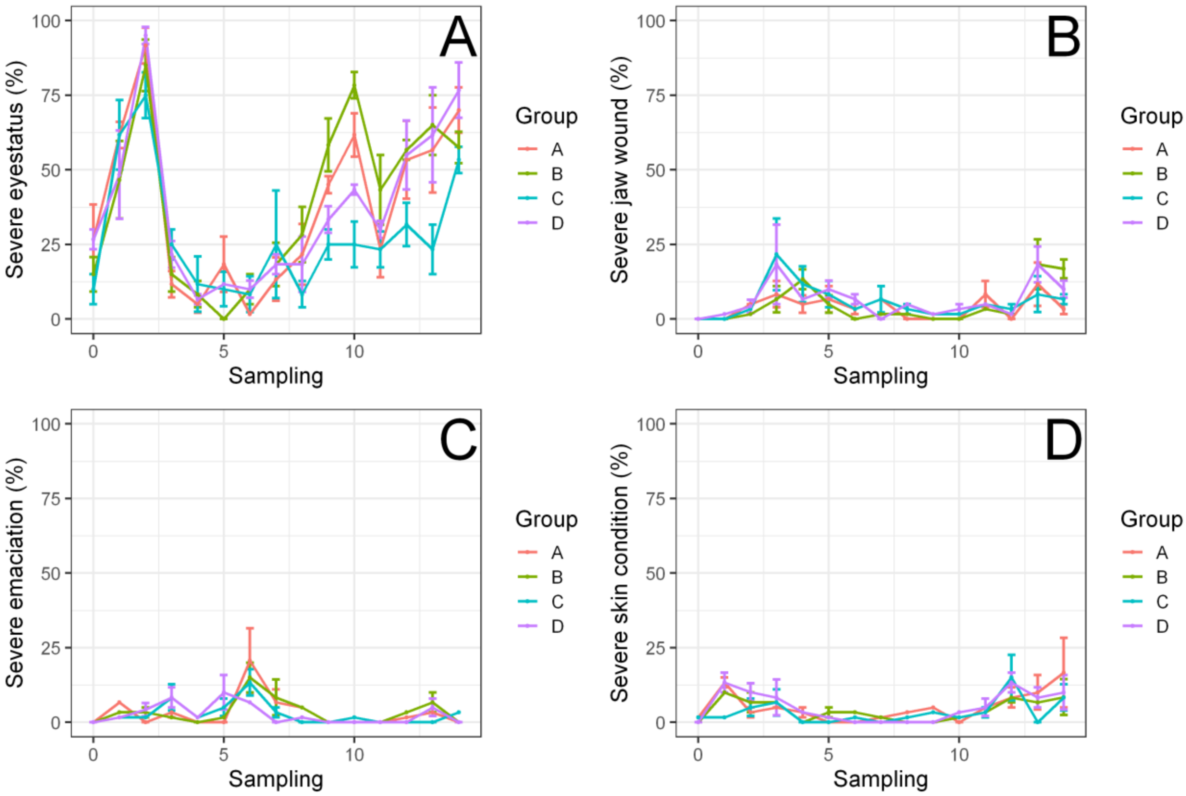
Date	A4	A5	A12	B1	B8	B11	C2	C6	C9	D3	D7	D10
Nov-16	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00
Jan-17	0.33	0.15	0.24	0.00	0.00	0.35	0.83	0.00	0.48	0.35	0.46	0.41
Feb-17	0.33	0.15	0.24	0.00	0.00	0.35	0.83	0.00	0.48	0.35	0.46	0.41
Mar-17	0.15	0.10	0.35	0.15	0.00	0.14	0.10	0.05	0.10	0.10	0.15	0.00
Apr-17	0.20	0.10	0.00	0.10	0.05	0.15	0.30	0.10	0.25	0.00	0.15	0.20
May-17	0.70	0.95	0.50	0.67	0.85	0.14	1.60	0.71	0.76	0.65	0.43	0.45
May-17	0.09	0.05	0.00	0.10	0.00	0.05	0.05	0.05	0.00	0.00	0.00	0.10
Jun-17	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00
Jul-17	0.00	0.04	0.05	0.04	0.00	0.19	0.00	0.10	0.14	0.05	0.00	0.00
Aug-17	0.10	0.10	0.00	0.10	0.00	0.25	0.05	0.14	0.10	0.05	0.05	0.05
Aug-17	0.00	0.00	0.10	0.10	0.05	0.15	0.05	0.05	0.00	0.30	0.10	0.40
Sep-17	0.14	0.00	0.14	0.24	0.09	2.29	0.43	0.29	0.45	0.65	0.10	0.59
Oct-17	0.05	0.45	0.38	0.14	0.86	2.10	1.00	0.62	0.40	1.80	0.79	0.25
Nov-17	0.62	0.09	0.30	0.09	0.60	0.57	0.29	0.09	0.20	0.20	0.19	0.20
Dec-17	0.19	2.40	0.24	3.90	0.17	0.53	1.78	1.62	0.38	2.00	0.05	0.00

	A	**B**	**C**	**D**
Samples above 0.2 lice fish^{-1}	14	13	19	17
Samples above 0.5 lice fish^{-1}	4	9	9	6

Supplementary figure 1. *Raw data from the echosounder transducers, indicating the relative echo strength over time. Warmer colours denote higher echo signal strengths, which translates to the depth at which highest fish density observed at that time point. Periods or depths with missing data are represented by the white blocks.*

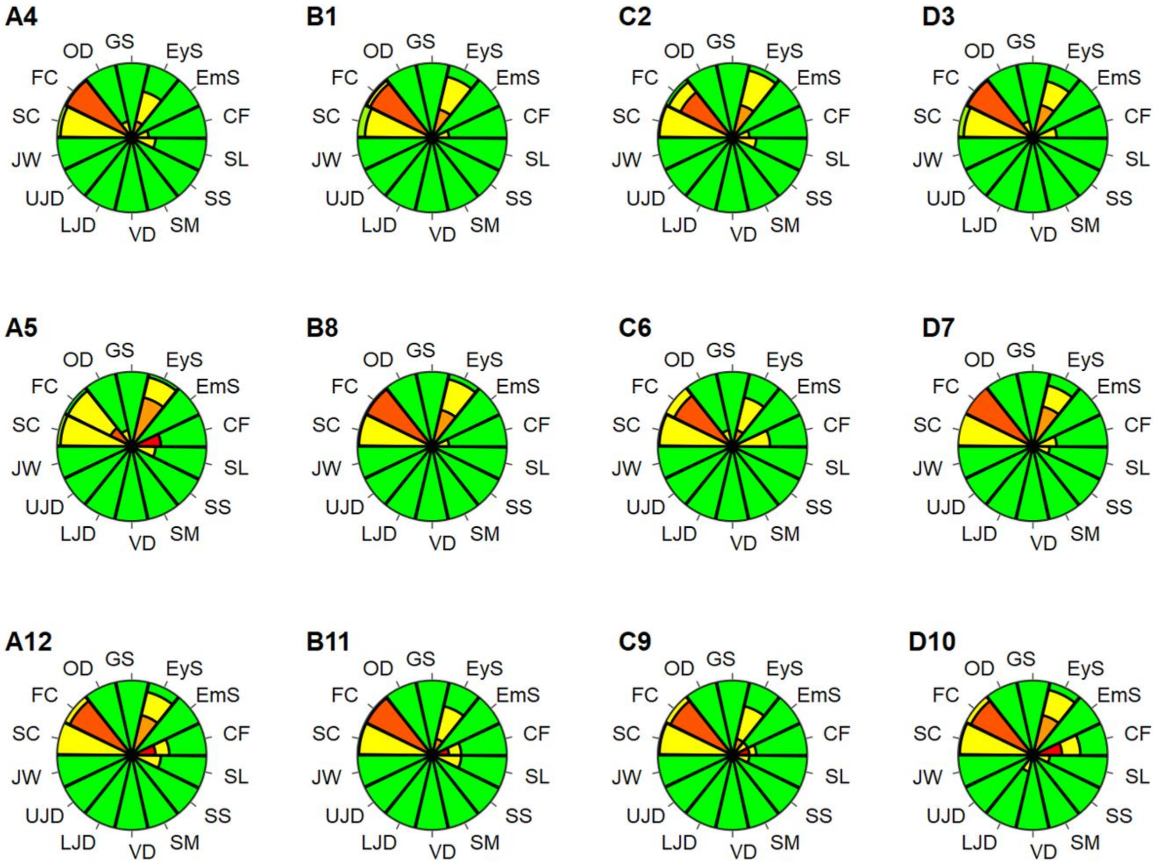


***Supplementary figure 2**. Frequency of the most severe scores for eye status (panel A), jaw wounds (panel B), emaciation (panel C), and skin condition (panel D), over all sample events. Sampling number correlates to the sample dates in Supplementary table 1.*

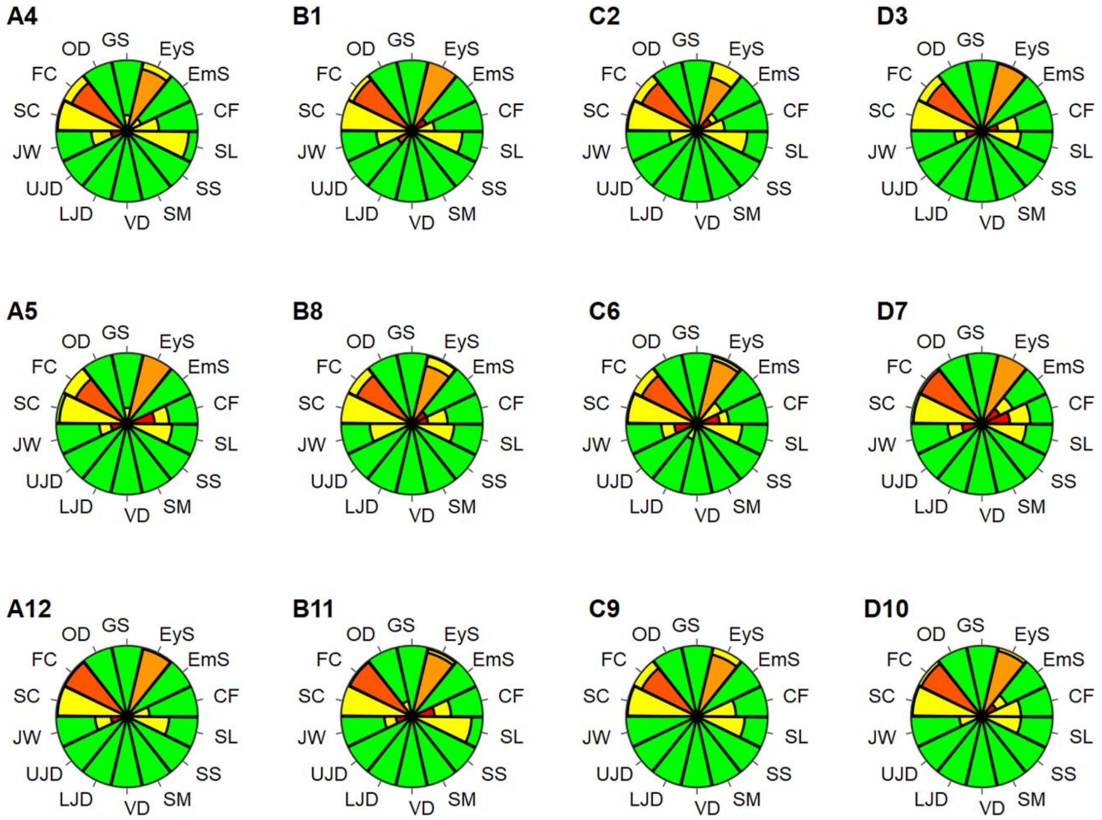


Supplementary figure 3. Welfare scores of individual cages (label: treatment group and cage number) for each sample. Severity of indicators is indicated by warmer colours representing higher scores (see Table 1). Welfare indicators shown are GS = Gill status, EyS = Eye status, EmS = Emaciation status, CF = Condition factor, SL = Sea lice, SS = Smoltification state, SM = Sexual mature, VD = Vertebral deformity, LJD = Lower jaw deformity, UJD = Upper jaw deformity, JW = Jaw wound, SC = Skin condition, FC = Fin condition, OD = Opercula deformity.

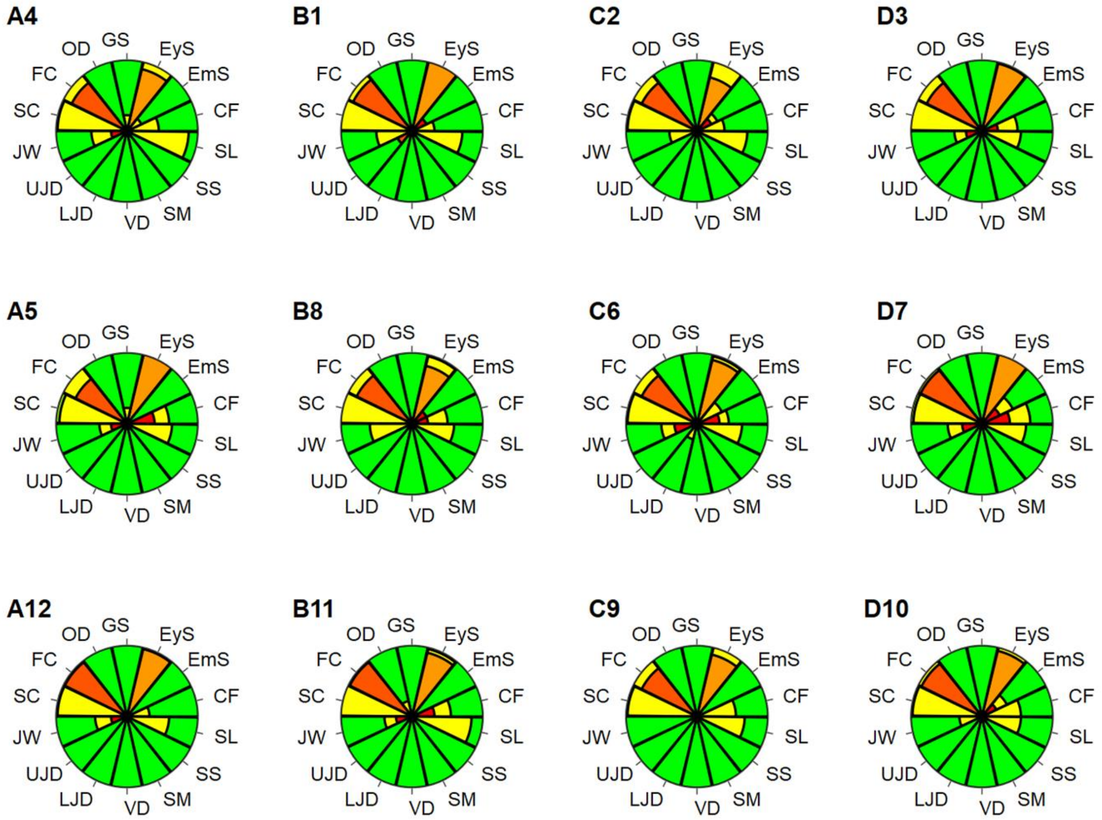
Sample 0 (1 Nov 2016)



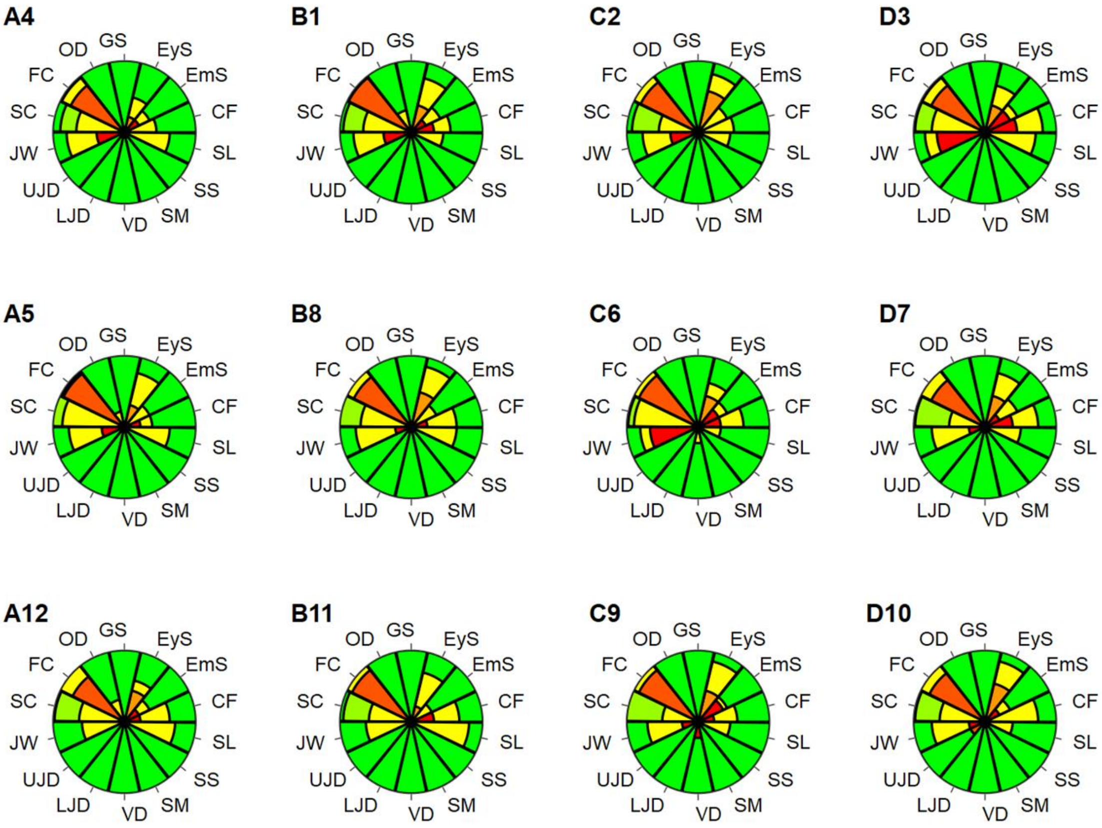
Sample 1 (11 Jan 2017)



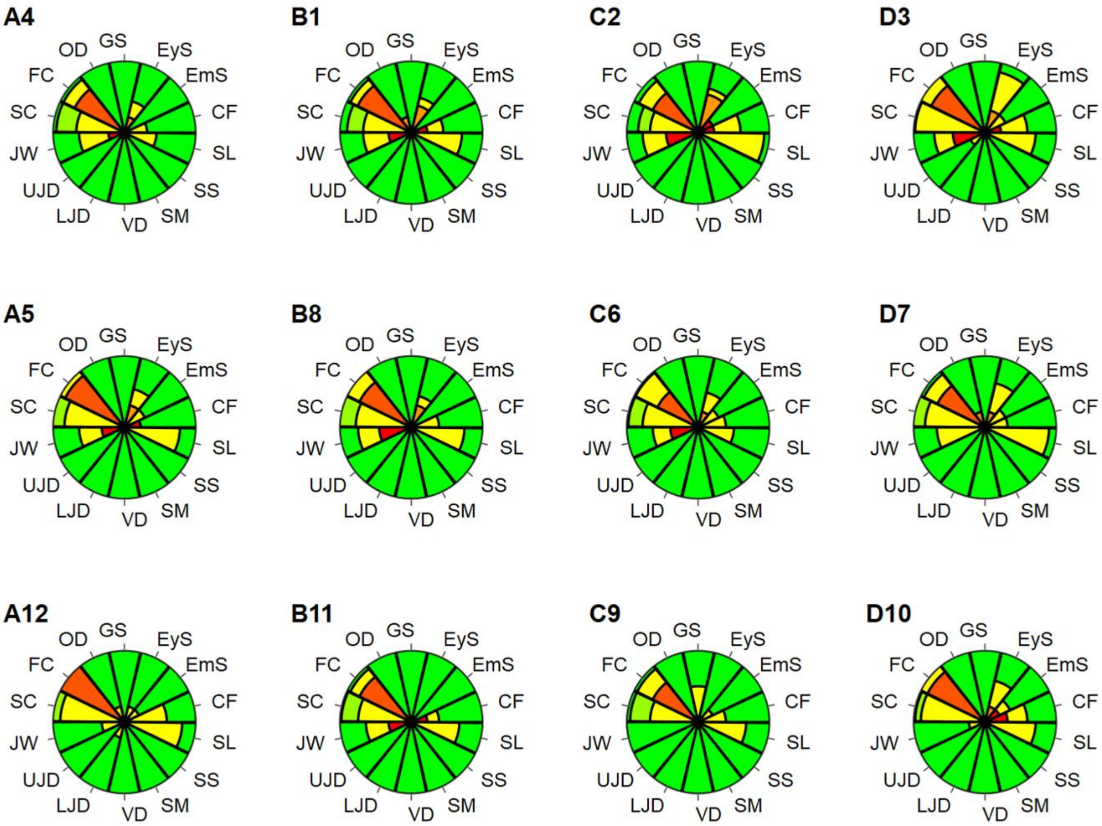
Sample 2 (8 Feb 2017)



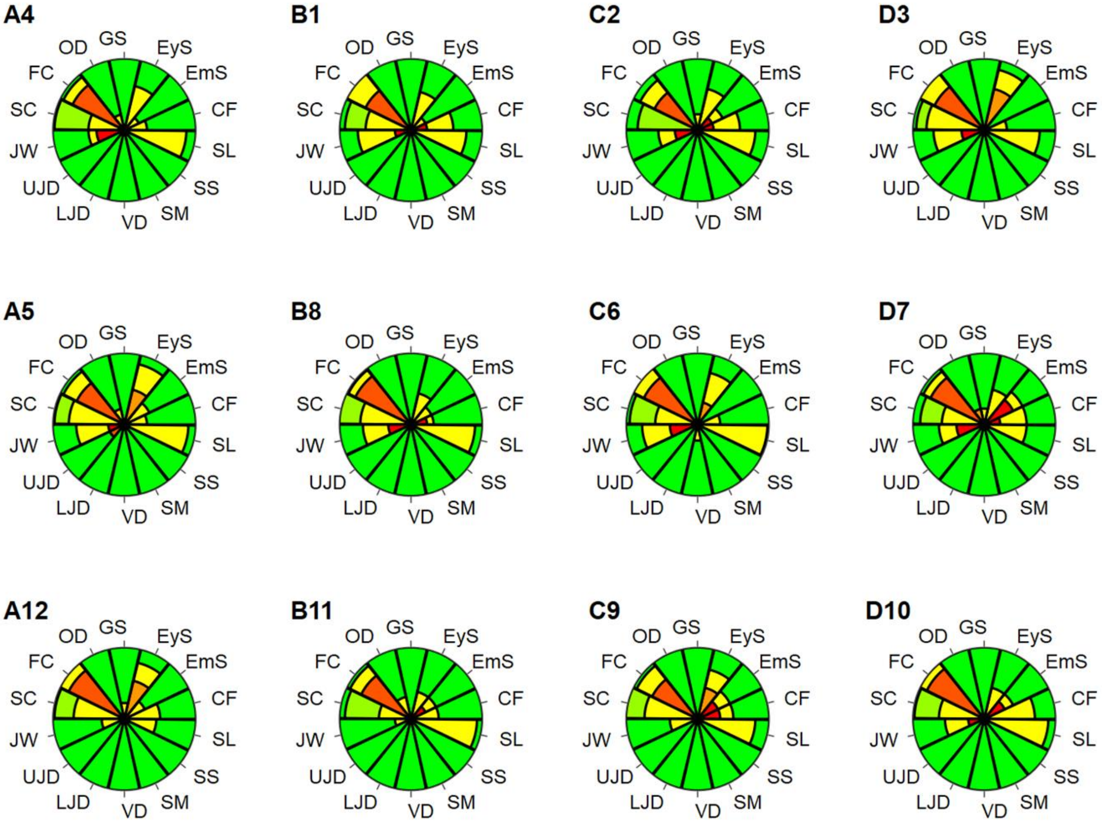
Sample 3 (13 Mar 2017)



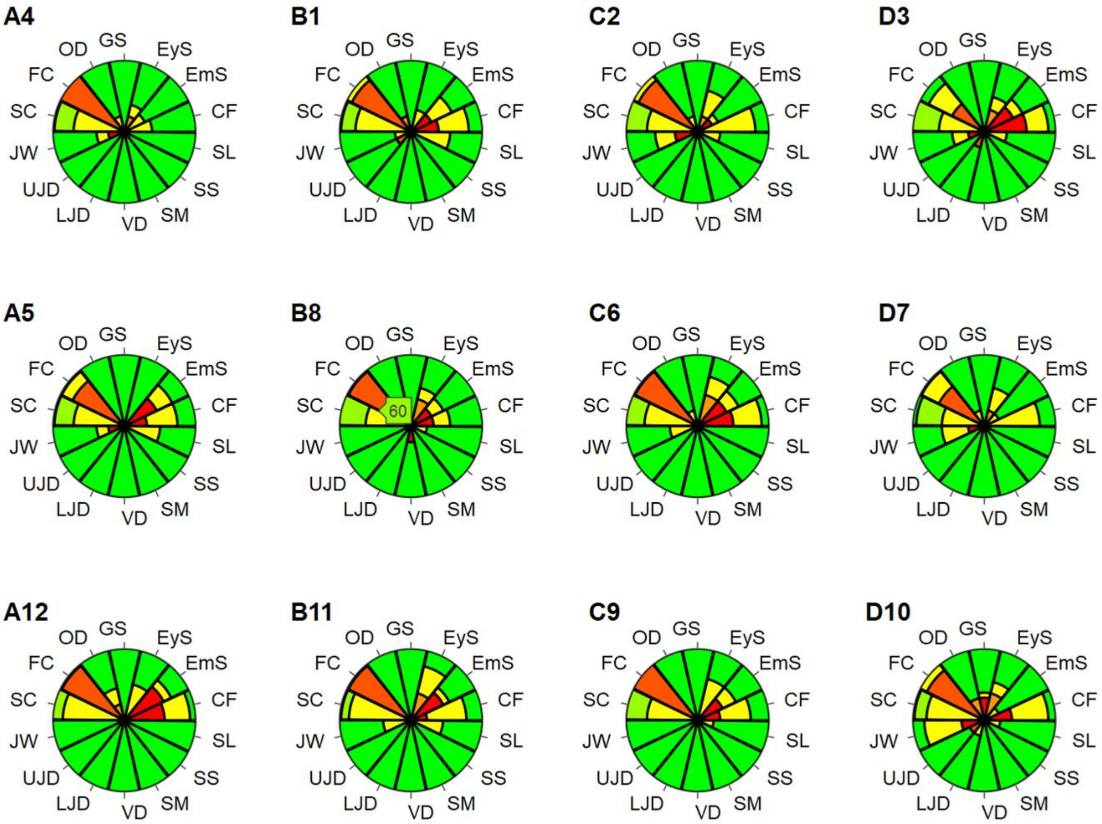
Sample 4 (6 Apr 2017)



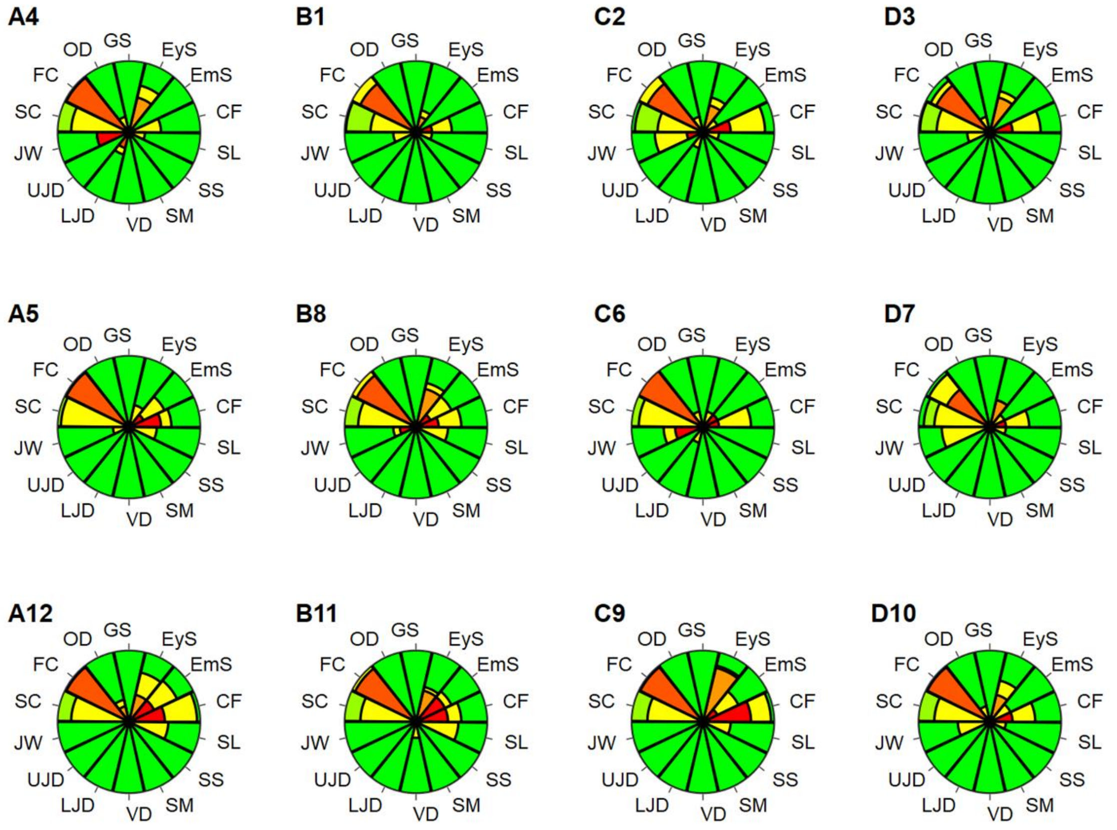
Sample 5 (4 May 2017)



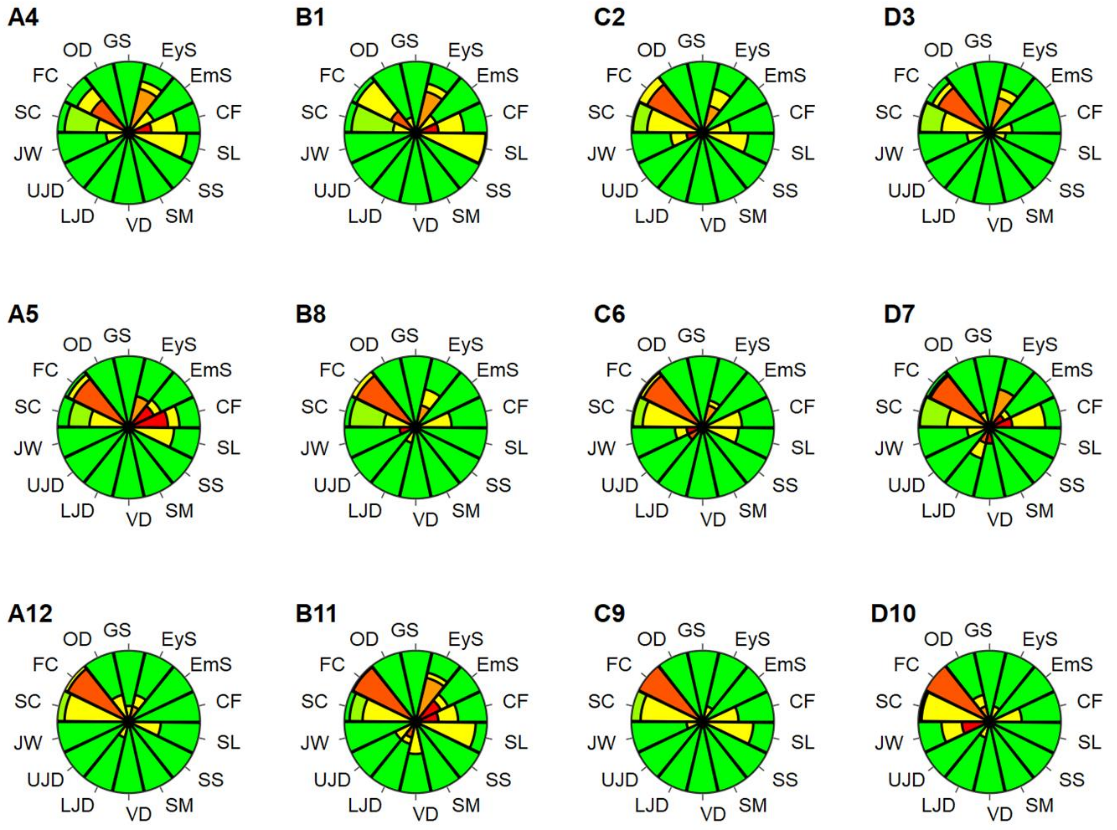
Sample 6 (29 May 2017)



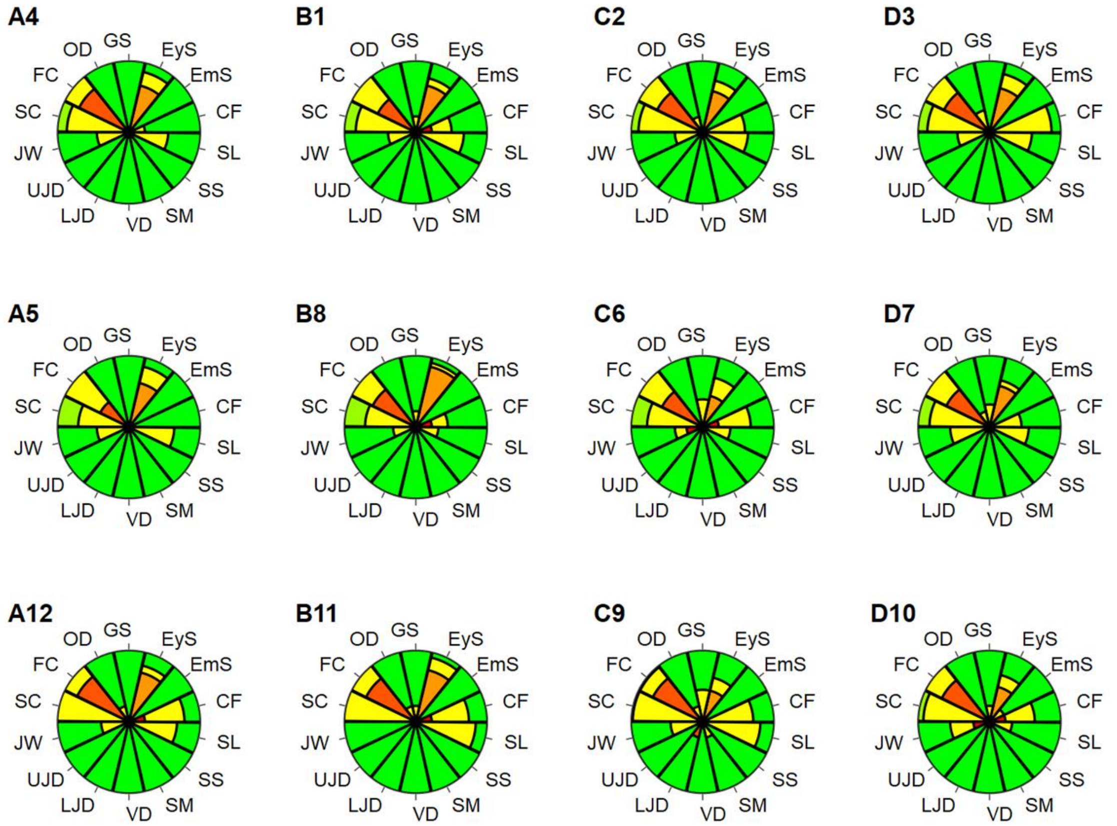
Sample 7 (20 Jun 2017)



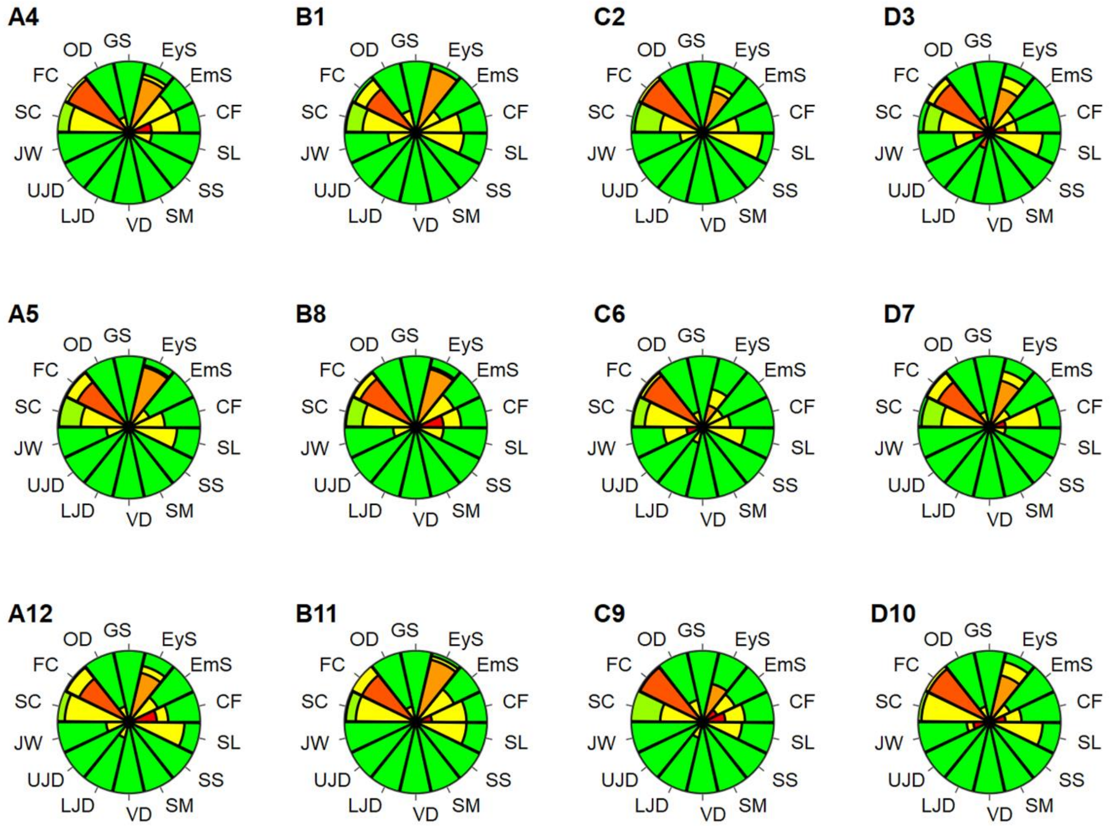
Sample 8 (11 Jul 2017)



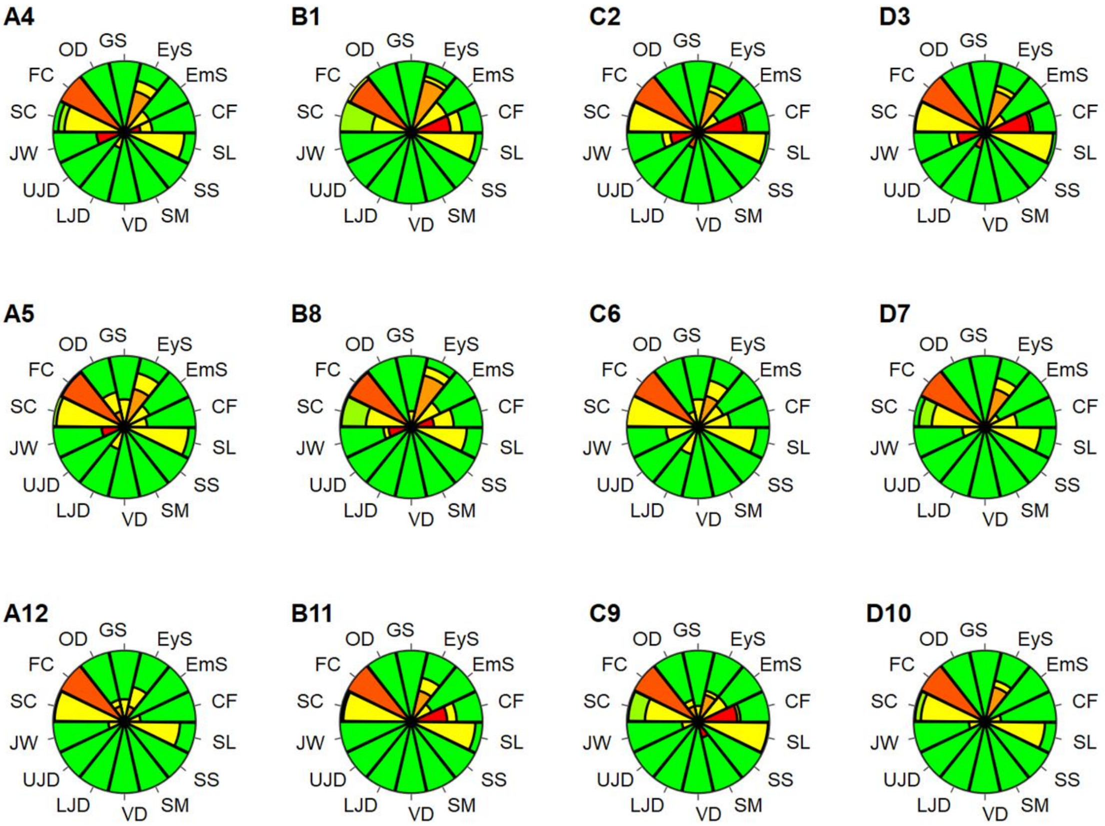
Sample 9 (2 Aug 2017)



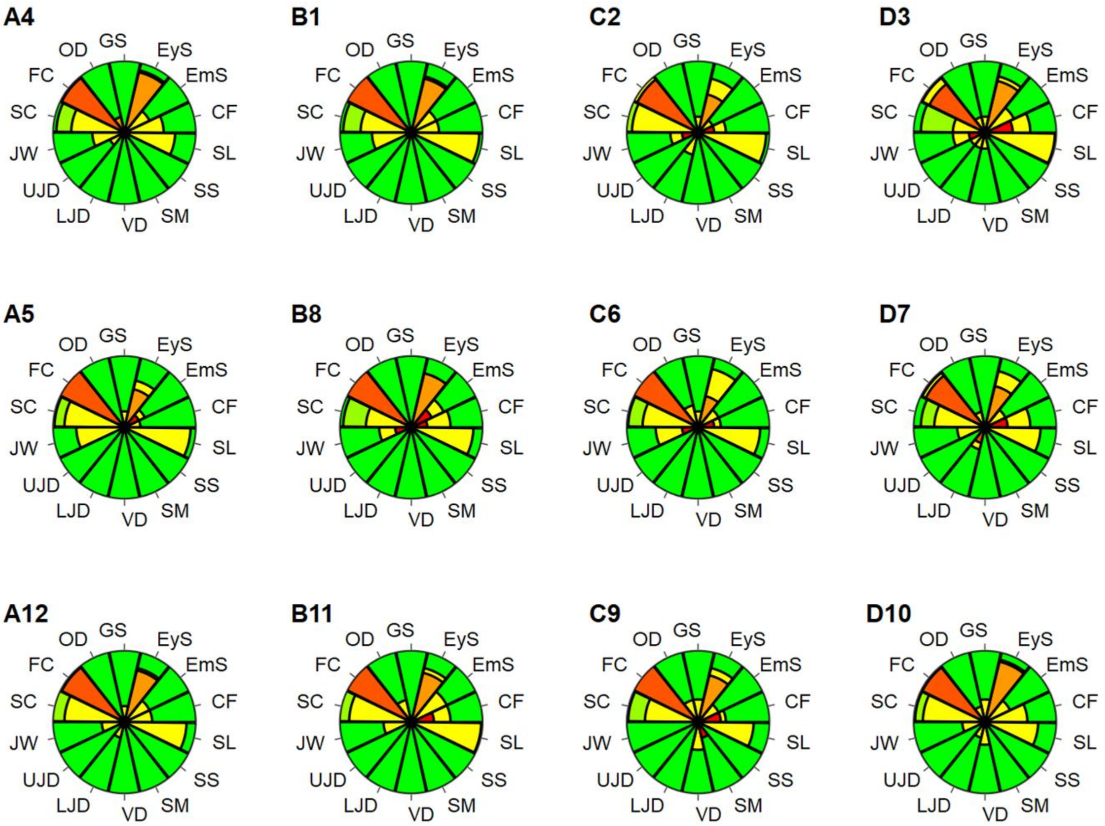
Sample 10 (28 Aug 2017)



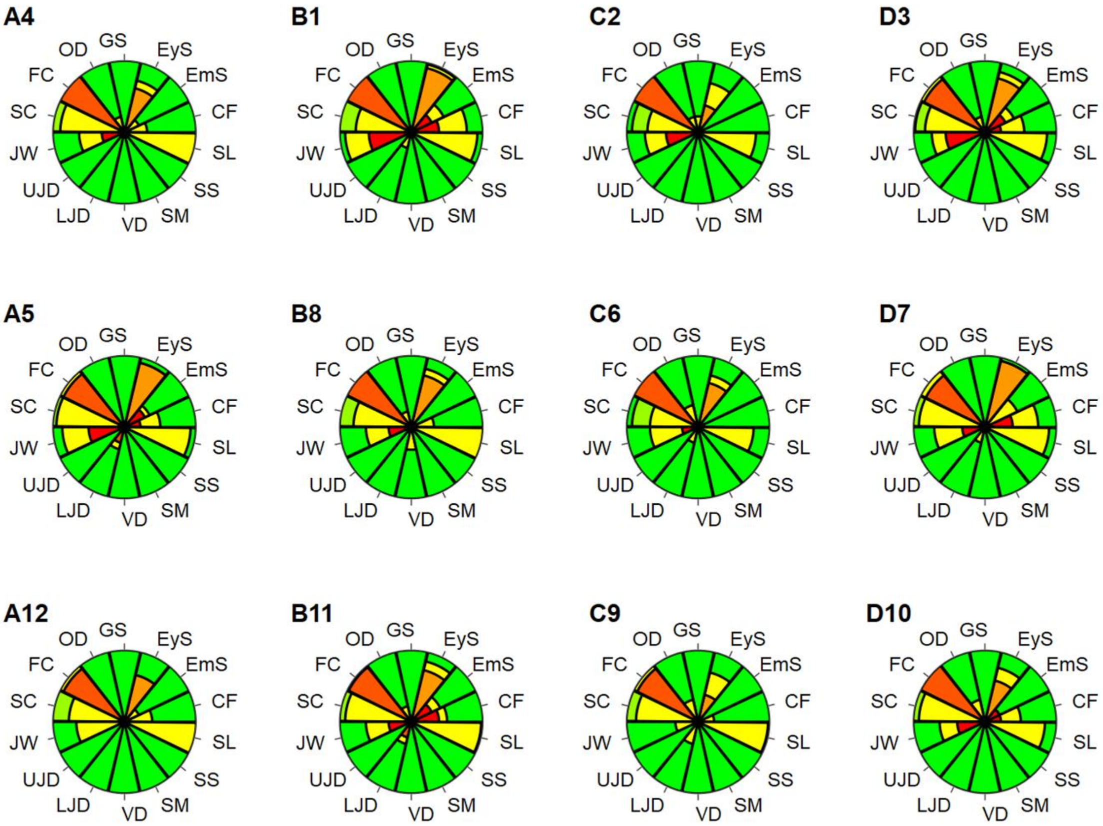
Sample 11 (13 Sep 2017)



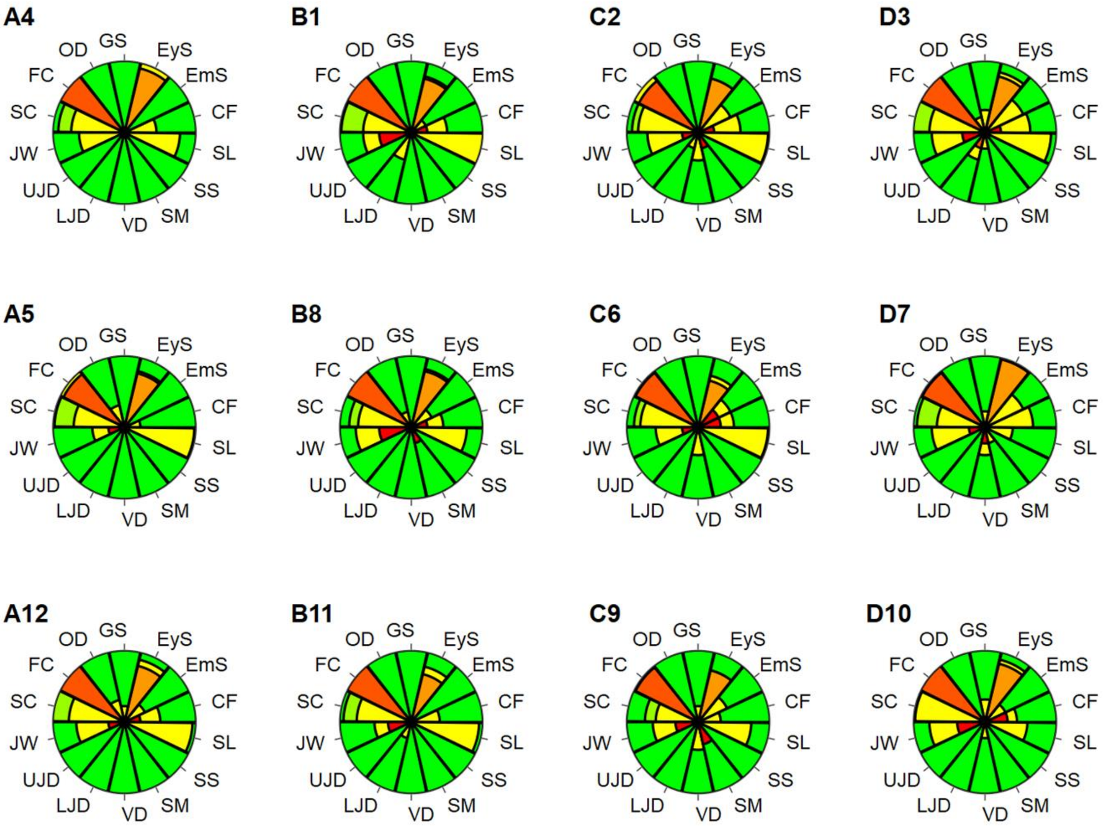
Sample 12 (25 Oct 2017)



Sample 13 (14 Nov 2017)



Sample 14 (7 Dec 2017)



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