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Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare

Daniel W. Wright[1,*,], Lena Geitung[2,**], Egil Karlsbakk[2], Lars H. Stien[1], Tim Dempster[3], Tina Oldham[4], Velimir Nola[1], Frode Oppedal[1]**

[1]Institute of Marine Research, Matre Research Station, 5984 Matredal, Norway
[2]Department of Biology, University of Bergen, 5005 Bergen, Norway
[3]Sustainable Aquaculture Laboratory – Temperate and Tropical, School of BioSciences, Parkville, VIC 3010, Australia
[4]Institute of Marine and Antarctic Studies, University of Tasmania, Launceston, TAS 7250, Australia

ABSTRACT: Surface environment modification is a potential parasite control strategy in Atlantic salmon sea-cage farming. For instance, a temporary low salinity surface layer in commercial-scale snorkel sea-cages has coincided with reduced amoebic gill disease (AGD) levels after an outbreak. We tested if a permanent freshwater (FW) surface layer in snorkel sea-cages would lower AGD and salmon lice levels of stock relative to snorkel cages with seawater (SW) only and standard production cages with no snorkels. Triplicate cages of each type with 2000 post-smolts were monitored in autumn to winter for 8 wk and sampled 4 times. Lower proportions of individuals with elevated AGD-related gill scores were registered in SW and FW snorkel cages compared to standard cages; however, these proportions did not differ between SW and FW snorkel cages. Individuals positive for AGD-causing *Paramoeba perurans* were reduced by 65% in FW snorkel relative to standard cages, but values were similar between SW snorkel cages and other types. While total lice burdens were reduced by 38% in SW snorkel compared to standard cages, they were unchanged between FW snorkel and other cage types. Fish welfare and growth were unaffected by cage type. Surface activity was detected in all cages; however, more surface jumps were recorded in standard than snorkel cages. Overall, fish in FW snorkel cages appeared to reside too little in freshwater to consistently reduce AGD levels and salmon lice compared to SW snorkel cages. Further work should test behavioural and environmental manipulations aimed at increasing freshwater or low salinity surface layer use.

KEY WORDS: Aquaculture · Cage environment · *Salmo salar* · *Lepeophtheirus salmonis* · *Paramoeba perurans* · Parasite control

INTRODUCTION

Sea-cage Atlantic salmon *Salmo salar* farming produces more than 2.3×10^6 t yr^{-1} (FAO 2017). This new and constant availability of large numbers of hosts has led to an increased scale of salmon parasite outbreaks in many marine ecosystems (Nowak 2007). Outbreaks of the salmon louse *Lepeophtheirus salmonis*, and of the amoeba *Paramoeba perurans* responsible for amoebic gill disease (AGD) (Young et al. 2007, 2008b, Crosbie et al. 2012) are of particular concern to the industry (Murray et al. 2016). Salmon lice outbreaks are thought to harm wild salmonids (Krkošek et al. 2011, 2013) and, as a result, strict regulations limit salmon lice loads on farmed fish. Many farmers must treat their fish repeatedly against sea lice during a production cycle, leading to increased costs and considerable risk to fish welfare (Overton

*Corresponding author: daniel.william.wright@imr.no
**Joint first authors

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et al. 2018). Norwegian authorities have also recently introduced the 'traffic light system', which limits allowable production volume in defined production zones according to the percentage of wild salmon in each production zone estimated to die due to salmon lice (Lovdata (2012)): <10% = increased production (green), 10–30% = no change in production (yellow), >30% = reduced production (red). In parallel, the expansion of AGD outbreaks to all major salmon farming regions has caused mass mortality events and a surge in AGD treatments (Shinn et al. 2015, Oldham et al. 2016). Innovating parasite controls to reduce both salmon lice and AGD could safeguard the ecological sustainability and future expansions of the salmon farming industry (Wright et al. 2017).

A range of chemotherapeutants can be used to treat salmon lice (organophosphates, emamectin benzoate, benzoyl ureas, hydrogen peroxide and pyrethroids) (Aaen et al. 2015), whilst AGD is currently treated with freshwater baths and hydrogen peroxide (Rodger 2014). Immersion in freshwater baths for 2 to 4 h removes freshwater-sensitive AGD-causing amoebae *P. perurans* from fish gills (Parsons et al. 2001, Clark et al. 2003, Rodger 2014). Unfortunately, short duration freshwater baths are unlikely to affect host-attached salmon lice which survive days to weeks in freshwater after developing past the first copepodid stage (Stone et al. 2002, Wright et al. 2016). In contrast, hydrogen peroxide use is rising rapidly (NIPH 2015, Murray 2016) due to its well known in-field efficacy against both salmon lice and AGD (Thomassen 1993, Adams et al. 2012). However, potential problematic effects on salmon welfare (Overton et al. 2018) and the evolution of chemical resistance against hydrogen peroxide (Helgesen et al. 2015, Helgesen et al. 2017) call into question the continued heavy reliance on this chemical. These factors are driving the development of chemical-free parasite controls. For salmon lice, these controls aim to prevent new lice from establishing themselves (fallowing, lice barrier skirt or snorkel cages, semi-enclosed cages, selective breeding of lice-resistant salmon) (Bron et al. 1993, Stien et al. 2012, Gharbi et al. 2015, Stien et al. 2016, Nilsen et al. 2017), or treat attached lice without chemicals (cleaner fish, laser, thermo-delousing, water jets) (Bjordal 1990, Aaen et al. 2015). The challenge for these substitute controls will be to simultaneously diminish both salmon lice and AGD.

Snorkel sea-cages incorporate a deep net roof opening into a central tarpaulin-lined narrow net-tube (snorkel) to the surface in an otherwise standard cage (Stien et al. 2016). This impedes contact between salmon hosts and free-swimming infective larval stages of salmon lice which are positively phototactic and pressure sensitive, causing them to typically aggregate near the surface (Heuch 1995, Heuch et al. 1995). The snorkel allows salmon to swim up and gulp air at the surface to replenish their open swim bladder for buoyancy regulation (Fahlén 1971, Dempster et al. 2011). Snorkel cages can reduce salmon lice infestations relative to standard cages at research- and commercial scales (Stien et al. 2016, Wright et al. 2017), with their effectiveness increasing with increased depth of the snorkel (Oppedal et al. 2017). AGD may also be treated using this technology by adding a freshwater surface layer inside a tarpaulined lined tube in the snorkel space (Wright et al. 2017) that would remove *P. perurans* from gills if the fish expose themselves sufficiently to freshwater (Parsons et al. 2001, Clark et al. 2003, Roberts & Powell 2003, Wright et al. 2016). Producing a temporary low salinity layer within the snorkels of commercial-scale cages has coincided with marked reductions in AGD levels after an outbreak, suggesting there is the potential for this technology to co-manage salmon lice and AGD (Wright et al. 2017). However, further testing is required to examine how variations of this surface environment modification, such as a permanent freshwater layer, affect AGD levels and to validate findings using standard production and seawater-filled snorkel cages for comparison.

In this study, we tested if snorkel sea-cages with a constant freshwater layer reduced AGD levels relative to standard cages and seawater-filled snorkel cages. Even though it is well established that snorkel cages reduce salmon lice levels (Stien et al. 2016, Oppedal et al. 2017), we also examined cage type effects on salmon lice infestations. Introducing freshwater into snorkel cages holding salmon might affect salmon lice infestations by influencing the behaviour and physiology of the host (McCormick et al. 1998, Oppedal et al. 2011) or parasite, particularly at the freshwater-sensitive copepodid stage (Bricknell et al. 2006, Wright et al. 2016). Additionally, we investigated if growth, mortality and other welfare indicators differed between cage types. Environmental conditions were closely monitored at the farm as well as within each snorkel cage to explain observed patterns.

MATERIALS AND METHODS

Study location and design

Nine steel frame sea-cages (12 × 12 m square, 12 m deep) were used at the Institute of Marine Research farm facility in Austevoll, southwest Norway (60° N).

These consisted of 3 unmodified standard cages and 6 snorkel cages (snorkel dimensions were 3 × 3 m square, 4 m deep), with 3 snorkels filled with seawater pumped (135 l min^{-1} pump, Xylem Water Solutions) from 4 m depth (hereafter 'SW snorkel' cages) and 3 snorkels filled with mains ozone-treated freshwater containing no chlorine or fluoride ('FW snorkel' cages). The 2 treatments (SW and FW snorkels) were interspersed in a block design at the facility. We stocked each cage with 2000 post-smolt Atlantic salmon, naïve to both AGD and salmon lice exposure, in a randomized block order from 26 to 28 October 2016. Fish (AquaGen strain) were produced at the Institute of Marine Research tank facility in Matre as 0+ out of season autumn smolts using standard protocols (e.g. Björnsson et al. 2000). Freshwater-filling of FW snorkels began after transfers were complete. Mean (± SD) fish weight was 76 ± 16 g, which led to stocking densities of 0.09 kg m^{-3} in standard and snorkel cages. Fish were continuously fed small portions throughout daylight hours to excess with a commercial diet (3 mm Spirit Supreme pellets, Skretting) via an automated system that operated screw pellet dispensers which released feed centrally in standard cages and into a pipe where it was transported by pumping seawater or freshwater to the top of snorkels. Because fish were fed to excess, no food conversion ratio (FCR) data was recorded in this trial. Inconsistencies in the management of one replicate FW snorkel cage compared to others led to its removal from all analyses.

Environmental depth profiles

Daily depth profiles of salinity and temperature were recorded by an automatic profiling CTD buoy (APB5, SAIV) programmed to measure between 0 to 12 m starting at 12:00 h daily at a reference location near the centre of the farm facility. We supplemented these measurements with weekly depth profiles between 0 and 12 m of salinity, temperature and dissolved oxygen (DO) using a CTD (SD204, SAIV) at the reference location and within each snorkel cage, to record differences between cage environments. Weekly profiles began the week following stocking and once freshwater layer creation was complete. Profiles involved lowering the CTD at a rate of 1 m min^{-1} to ensure the accuracy of oxygen recordings.

Amoebic gill disease and salmon lice

At fortnightly intervals, on 8–9 November (Time 1), 22–24 November (Time 2), 5–7 December (Time 3) and 20–21 December (Time 4), 20 fish from each cage were sampled. Fish were caught by ceasing feeding at least 24 h prior, lowering a hoop net and hand feeding to motivate surfacing of fish, followed by swift lifting of the hoop net. We subjected sampled fish to a lethal dose of anaesthetic (Finquel), then transferred them to seawater-filled trays for counts of all salmon lice stages (copepodid, chalimus I, chalimus II, preadult I, preadult II male, preadult II female, adult male, adult female and adult female with eggstrings). Counts of mobile stages in buckets holding the sampled fish were also included in the total counts. New lice at each sampling time were considered to be attached copepodid, chalimus I and chalimus II lice stages, which developed in ≤2 wk at mean observed temperatures of 9°C in the trial (Stien et al. 2005). Next, AGD-related gill scoring (0–5, with 0 for no gill pathology and 1–5 for increasing severity of gill pathology, using lesion-covered gill surface area categories) was carried out on each of the 8 gill arches (Taylor et al. 2009). The AGD-related gill score given to an individual fish was based on the maximum score of its arches. At Time 3, when gill scores remained elevated, swabbing of the third right gill arch (a half turn on the front and a half turn on the back) was performed on 10 fish in each cage type. The swab was inserted into 1 ml vials of RNAlater and stored at 4°C for 24 h and thereafter at −18°C until PCR analysis for *P. perurans* detection (Pharmaq, Bergen, Norway). Analysed samples returned a cycle threshold (CT) value indicating *P. perurans* presence when below a cut-off of 30.0, with co-analysed control samples recording CT values above it. We created a *P. perurans* load index, where a CT value of 30.0 or greater had a *P. perurans* load of 0, and lower CT values were transformed by subtracting 30 and reversing the sign of the resulting value (e.g. CT value of 28 = *P. perurans* load index of 2). AGD-related gill scores and *P. perurans* load were positively correlated based on individuals swabbed at Time 3 (Pearson's correlation, $t = 2.8$, $p < 0.05$) providing support that gill scores resulted from AGD-causing *P. perurans*, as reported by others (e.g. Young et al. 2008a, Bridle et al. 2010).

Growth, mortality and other welfare indicators

At Time 4, sampled fish were measured for fork length (cm) and weight (g), condition factor (K) calculated as (weight × $length^{-3}$)/100 (Bolger & Connolly 1989), and scores of individual welfare indicators (emaciation, vertebral deformity, sexual maturation,

smoltification state, fin condition, skin condition, eye status, opercula, mouth jaw wound, upper jaw deformity, lower jaw deformity) contributing to the Semantic Welfare Index Model (SWIM) version 2.0. Lice and gill welfare indicators were not incorporated into overall SWIM scores. Numbers of mortalities in each cage were recorded from checks performed 3 times per week.

Surface activity

Beginning from the first sampling time, jumps and rolls were counted in a 5 min period within each cage on the same day at weekly intervals (Dempster et al. 2008). These numbers were recalculated to jumps per fish per day.

Statistical analyses

Proportions of AGD-related 'light plus' gill scores (≥2, with higher scores indicating increased gill pathology) used as a measure of AGD levels in salmon cages within industrial and research settings (Maynard et al. 2016) were compared. Generalised linear models with binomial error distributions, including treatment (standard, SW snorkel and FW snorkel) and cage (1–8) as factors, compared light plus gill scores at each time (using the glm function in R; Crawley 2012). For each comparison, models incorporating treatment × cage, treatment + cage and treatment only were built and the simplest model was selected if no significant difference was identified between them via ANOVA tests (anova function in R). Arcsine-transformed proportions of fish with gills found to be *P. perurans*-positive in each cage were compared between treatments using *t*-tests (t.test function in R).

We assessed differences in new lice per fish (count data with overdispersion) between treatments at each time using generalised linear models with quasi-Poisson error distributions, which included treatment and cage as factors. As before, a simpler model was chosen from more complex ones if no difference was found from ANOVA tests between models. For an overall assessment of lice infestation levels that fish incurred during the study, we examined total lice numbers (including sessile and mobile stages) on sampled fish and in their bucket for each cage at the final sampling (Time 4). These total counts per cage were compared between treatments via *t*-tests.

At Time 4, when fish had experienced the different cage type treatments the longest, growth (based on weight), condition and square-root-transformed SWIM scores of sampled fish were compared using linear mixed-effect models, with treatment as a fixed effect and cage as a random effect (lme function in R). At Time 4, arcsine-transformed proportions of fish with fin (scores ≥3), skin (scores ≥3), eye (scores ≥2) and cumulative mortalities in each cage were also compared between treatments via *t*-tests, which were also used to compare square-root-transformed jumps per fish per day in each cage, pooled from all weekly assessments, between treatments. Error distributions were checked for variance and normality (plot function in R). Results are presented as means (±SE) and 95% confidence intervals (CIs).

RESULTS

Environment

Salinity remained high (>28.3) and non-stratified at the reference location (reflecting conditions in standard cages) and in the SW snorkel cages (Fig. 1, Table S1 in the Supplement at www.int-res.com/articles/suppl/q010p255_supp.pdf). Thermal stratification with cooler upper layers occurred sporadically in both standard and SW snorkel cages, though was less severe in SW snorkels because snorkel water was constantly replenished with pumped warmer seawater from 4 m depth (Fig. 1, Table S1). In FW snorkel cages, a stable freshwater layer was continuously maintained (salinity ≤1 in top 2 m), of predominantly lower temperature than underlying water (Fig. 1, Table S1). As a result, temperatures between 0 and 1 m depth in FW snorkels were 2.6, 1.4, 0.7 and 1.4°C cooler than SW snorkels and 1.5, 1.2, 0.0 and 0.7°C cooler than in standard cages in the sampling interval periods before Times 1, 2, 3 and 4, respectively (Table S1). DO saturation remained stable between 77 and 85% for standard and SW snorkel cages, but levels were much higher (up to 148%) in the freshwater surface layer of FW snorkel cages, particularly preceding Times 1 and 4, due to the ozone treatment of freshwater (Table S1).

Amoebic gill disease

Soon after stocking at Time 1, AGD-related gill scores remained low and the proportion of fish with light plus scores (≥2) were similar between cage

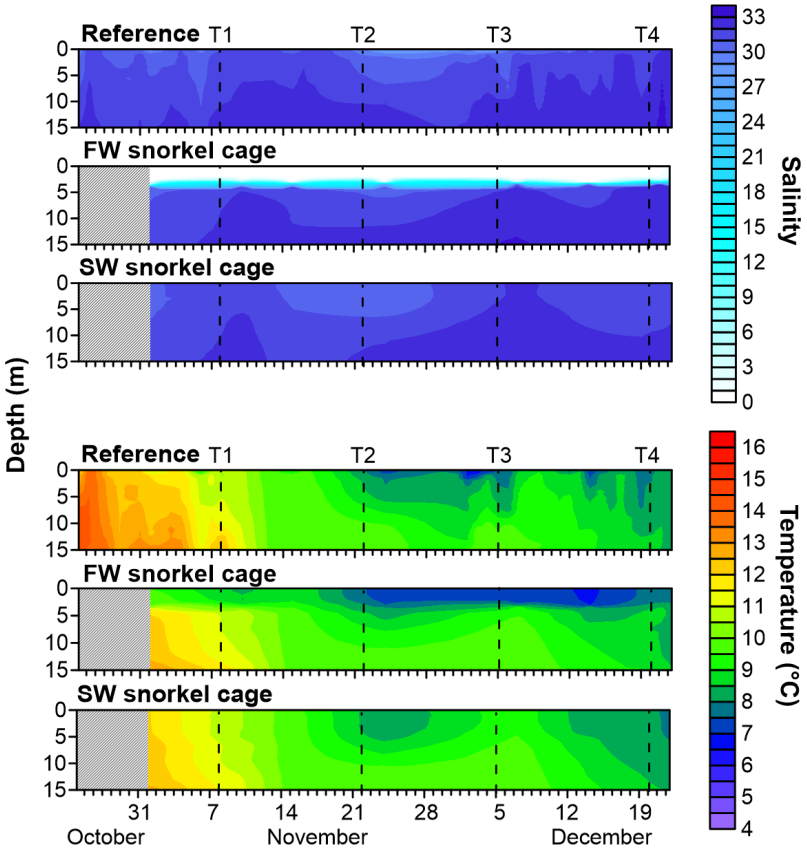

Fig. 1. Depth profiles over time of salinity (top) and temperature (bottom) in a study of the effects of surface environment modification on parasites in farmed Atlantic salmon *Salmo salar* in southwest Norway. Profiles were measured daily by an automatic profiling CTD buoy at a reference location, indicative of standard cage conditions and weekly using a CTD in snorkel cages filled with seawater (SW snorkel) or freshwater (FW snorkel). Measurements at the reference location were taken from 24 October 2016. Measurements in snorkel cages started on 1 November once freshwater layers were established, and the preceding period is shown as grey shading. Values are from a single FW and a single SW snorkel cage, with similar conditions observed in replicate cages. The 4 sampling times (T1 to T4) are shown by dashed vertical lines

types ($z \geq -1.7$, $p \geq 0.1$; Fig. 2, Table S2). Gill scores increased thereafter and became highest in standard cages compared to SW snorkel cages at Times 2 and 3 ($z \geq 3.1$, $p < 0.05$), but not at Time 4 ($z = 1.7$, $p = 0.1$; Fig. 2, Table S2). These scores also remained lower in FW snorkel relative to standard cages at Times 2–4 ($z \leq -2.4$, $p < 0.001$; Fig. 2, Table S2). No differences were observed in gill scores between SW and FW snorkel fish at Times 2–4 ($z = 0.01$ to 1.9, $p > 0.06$; Fig. 2, Table S2). Cage and treatment × cage interactions were present for most comparisons between cage types at Times 2–4 (Fig. 2, Table S2). At Time 3, there was a 65% reduction in the proportion of fish with gills testing positive for *Paramoeba perurans* in FW snorkel (15% of fish) compared to standard cages (43% of fish) ($t = -4.7$, $p < 0.05$), but not between SW snorkel cages (20%) and other types ($t \geq -2.6$, $p \geq 0.1$; Fig. 2).

Salmon lice

New lice (copepodid and chalimus stages) per fish were lower in SW snorkel relative to standard cages at Times 1 (means of 1.6 vs. 2.8) ($t = -4.3$, $p < 0.001$), 2 (means of 1.6 vs. 3.0) ($t = -5.3$, $p < 0.001$) and 4 (means of 3.3 vs. 5.1) ($t = -2.4$, $p < 0.05$), but not at Time 3 (means of 1.6 vs. 2.7) ($t = -1.2$, $p = 0.9$). At Time 3, an interaction between treatment and cage occurred ($t = -2.0$, $p < 0.05$; Fig. 3). FW

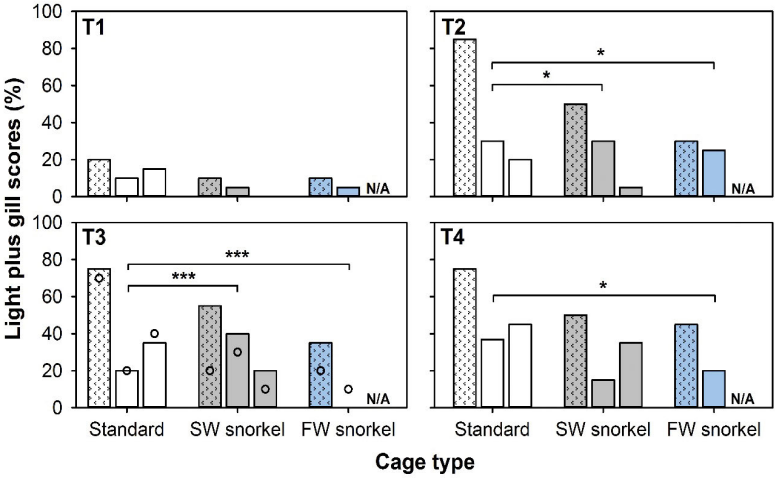

Fig. 2. Proportions of 'light plus amoebic gill disease (AGD)-related gill scores' (scores of ≥2; see 'Materials and methods' for further details) for farmed Atlantic salmon in different cage treatments. Results are shown for each replicate (n = 3 replicates) standard (white bars), SW snorkel (grey bars) and FW snorkel cage (blue bars) at Times 1–4. See Fig. 1 legend for details of cage treatments and sampling times. Stippled bars indicate cages positioned closest to other AGD-affected cages at the farm and expected to be under increased infection pressure. Open circles at Time 3 denote proportions of *Paramoeba perurans*-positive fish from gill swab PCR analysis of 10 fish in each replicate cage. N/A indicates 1 FW snorkel cage discarded from analyses. *$p < 0.05$, ***$p < 0.001$

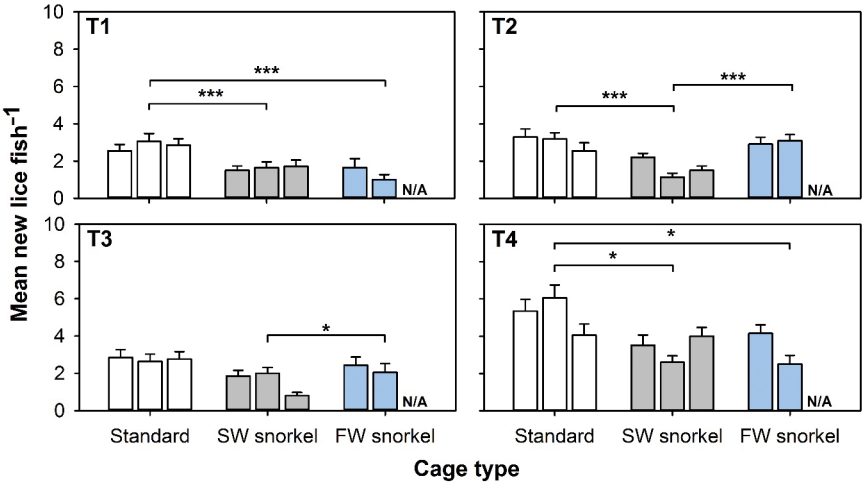

Fig. 3. Mean counts (±SE) of new lice per fish (attached lice or copepodid, chalimus I and chalimus II lice stages) in farmed Atlantic salmon for each replicate standard (white bars), SW snorkel (grey bars) and FW snorkel cage (blue bars) at Times 1–4. See Fig. 1 legend for details of cage treatments and sampling times. N/A indicates 1 FW snorkel cage discarded from analyses. *p < 0.05, ***p < 0.001

snorkel fish also had fewer new lice than those in standard cages at Times 1 (means of 1.3 vs. 2.8 new lice per fish) (t = –3.9, p < 0.001) and 4 (means of 3.3 vs. 5.1) (t = –3.3, p < 0.05), although similar counts were observed at Times 2 (means of 3.0 vs. 3.0) (t = –0.1, p > 0.05) and 3 (means of 2.2 vs. 2.7) (t = –1.3, p < 0.05) (Fig. 3). Fish had fewer lice in cages with SW snorkels than with FW snorkels at Times 2 (t = –5.3, p < 0.001) and 3 (t = –2.1, p < 0.05; Fig. 3). By Time 4, when all lice stages were present in the 3 cage types, total lice per fish differed between standard and SW snorkel cages (means of 15.7 vs. 9.8; i.e. a 38% reduction) (t = 7.5, p < 0.05), but not between standard and FW snorkel cages (means of 15.7 vs. 12.6) (t = 0.9, p = 0.5) or SW and FW snorkel cages (means of 9.8 vs. 12.6) (t = 0.9, p = 0.5; Fig. 4).

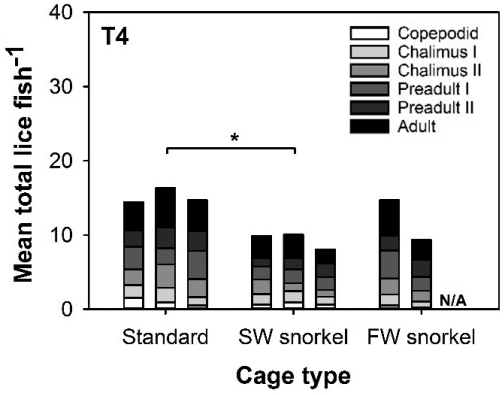

Fig. 4. Mean numbers of copepodids, chalimus I, chalimus II, preadult I, preadult II and adult lice per fish (later stages in increasingly darker shades from white to black) in farmed Atlantic salmon for each replicate standard, SW snorkel and FW snorkel cage at Time 4. See Fig. 1 legend for details of cage treatments and sampling times. N/A represents 1 FW snorkel cage discarded from analyses. *p < 0.05

Growth, welfare and mortality

At the last sampling point, there were no differences in the weight ($\chi^2 \leq 2.2$, p ≥ 0.1) or condition factor ($\chi^2 \leq 2.7$, p ≥ 0.1) of sampled fish between cage types (Table 1). Adequate and comparable welfare scores of salmon were upheld in all cage types ($\chi^2 \leq 3.5$, p ≥ 0.1; Table 1). When individual welfare indicators were analysed separately, no differences in observed skin ($t \leq 3.2$, p ≥ 0.1), fin ($t \geq -0.4$, p ≥ 0.8) or eye damage ($t \leq 2.5$, p ≥ 0.1) existed between treatments. Mouth damage was only detected in standard cages (3.4% of stock), and no fish were atypical for other welfare indicators (Table 1). Cumulative mortalities were similar between cage types ($t \geq -0.6$, p ≥ 0.6).

Table 1. Mean (±SE) values for condition of farmed Atlantic salmon *Salmo salar* in southwest Norway held in standard cages, and in snorkel cages filled with seawater (SW snorkel) or freshwater (FW snorkel). Higher values for condition factor and overall Semantic Welfare Index Model (SWIM) score indicate better condition. Individual welfare indicator scores show proportions of individuals with high scores indicating deviance from the normal condition

Parameter	Standard	SW snorkel	FW snorkel
Mean weight (g)	197.1 ± 13.1	177.3 ± 4.8	179.7 ± 7.6
Mean condition factor	1.15 ± 0.02	1.14 ± 0.02	1.19 ± 0.01
Mean overall SWIM score	0.92 ± 0.01	0.93 ± 0.00	0.93 ± 0.00
Fin damage (scores ≥3)	64.4%	65.0%	62.5%
Skin damage (scores ≥3)	74.6%	63.3%	85.0%
Eye damage (scores ≥2)	84.7%	58.3%	40.0%
Mouth damage (scores ≥2)	3.4%	0.0%	0.0%
Emaciation (scores ≥2)	0.0%	0.0%	0.0%
Smoltification (scores ≥2)	0.0%	0.0%	0.0%
Sexual maturation (scores ≥2)	0.0%	0.0%	0.0%
Vertebral deformity (scores ≥2)	0.0%	0.0%	0.0%
Upper jaw deformity (scores ≥2)	0.0%	0.0%	0.0%
Lower jaw deformity (scores ≥2)	0.0%	0.0%	0.0%

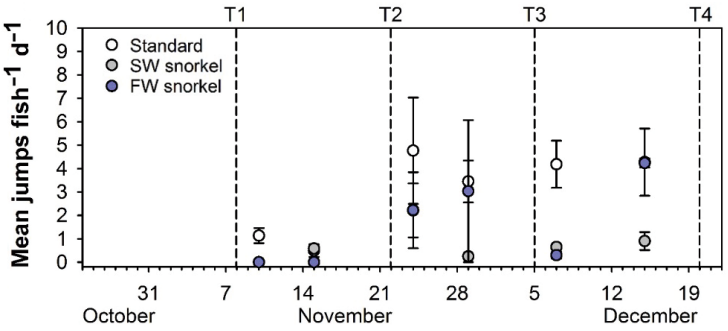

Fig. 5. Mean number (±SE) of jumps per fish per day by farmed Atlantic salmon in standard (white circles), SW snorkel (grey circles) and FW snorkel cages (blue circles) at weekly assessments. Values for each cage type are aggregates from replicate cages (n = 3 replicates). See Fig. 1 legend for details of cage treatments. The 4 sampling times (T1 to T4) are indicated by dashed lines

Surface activity

Surfacing by salmon was observed in all cage types and increased during the study, particularly after Time 2 (Fig. 5). Fish in standard cages performed more jumps per fish per day (mean of 3.0) than SW snorkel (mean of 0.8) and FW snorkel cages (mean of 1.3) ($t \geq 2.8$, $p < 0.05$; Fig. 5). No differences in jump frequency were detected between SW and FW snorkel fish ($t = 0.04$, $p = 0.97$; Fig. 5).

DISCUSSION

SW and FW snorkel cages outperformed standard cages in terms of lowered AGD-related gill scores and reduced numbers of new salmon lice at some time points. All cage types had similar fish welfare and growth outcomes. However, we did not consistently detect reduced AGD and lice levels in FW snorkels compared to SW snorkels as initially predicted, with increases in new lice in FW compared to SW snorkel cages at certain time points. Daytime surfacing behaviour by salmon appeared unaffected between SW and FW snorkel cages. This suggests that while salmon frequently accessed the freshwater surface layer, their exposure durations were likely inadequate to alter AGD or salmon lice levels significantly below those in SW snorkel cages. Our results contrast with the AGD suppression observed in a commercial trial where freshwater was added to a snorkel to combat an AGD outbreak (Wright et al. 2017). There are several possible reasons for this, including differences in snorkel sizes that may affect salmon behaviours, and the multiple ways the freshwater layer water in the FW snorkel differed from the SW snorkel other than salinity, including temperature and oxygen content.

Effects of standard, SW and FW snorkels cages on AGD

AGD-related gill scores, correlated with loads of AGD-causing *Paramoeba perurans* during the study, were often higher in standard cages, but similar between FW and SW snorkel cages. A higher proportion of *P. perurans*-positive fish were also found in standard compared to FW snorkel cages. Harvest-sized fish with high AGD-related gill scores were held in shallow cages within the research farm facility, measuring ~30 m width × 120 m length. As swimming in the same depth and locality as AGD-affected individuals may increase AGD risk (Young et al. 2014), shallow swimming by fish in standard cages could have partially explained their higher AGD-related gill scores than snorkel fish. The lack of difference in AGD-related gill scores between FW and SW snorkel fish suggested that salmon mostly failed to enter freshwater sufficiently to decrease *P. perurans* populations on their gills (2 to 4 h freshwater baths are effective; Parsons et al. 2001, Clark et al. 2003, Rodger 2014).

Effects of standard, SW and FW snorkel cages on salmon lice

The lack of salmon lice reductions in FW snorkel cages indicated that the development of salmon lice on Atlantic salmon was unhindered by regular freshwater exposures during surface jumps (mean of 2.3 jumps per fish per day) and other possible times of residence. Thus, these periods were likely too short to eliminate freshwater-sensitive attached copepodids which takes 1 to 3 h (Wright et al. 2016). High salmon lice infestations of wild sea trout *Salmo trutta* are associated with entry into shallower brackish water or rivers, possibly for self-treatment against lice (Gjelland et al. 2014). Once completing their seaward out-migration, wild post-smolt Atlantic salmon also use less saline environments and this may also be a reaction to new salmon lice recruits (Mitamura et al. 2017). Despite the potential for Atlantic salmon to self-treat against salmon lice by moving from seawater to freshwater or low salinity environments, this did not occur under the conditions created in FW snorkel cages within the current trial.

In some instances, FW snorkel cages increased new salmon lice infestations compared to SW cages. There are several possible reasons for this. Firstly, the freshwater exposures that salmon were subjected to may have removed mucus or induced stress, making them more susceptible to salmon lice infestations as has been documented for other external parasites such as *Neobenedenia girellae* skin flukes (Yamamoto et al. 2011). Reduced sheltering by fish inside snorkels filled with freshwater could also increase salmon lice infestations of FW compared to SW snorkel cages. While harvest-sized salmon have been found to position themselves almost exclusively below 4 m deep SW snorkels in identical cages in autumn (Stien et al. 2016), periodic post-smolt presence inside 4 to 16 m deep SW snorkels, inferred from low oxygen conditions, was detected by Oppedal et al. (2017). Therefore, greater fish residency inside SW snorkels may contribute to lice reduction effects typically seen in this cage type (see Oppedal et al. 2017). More work is needed to reveal differences in depth distribution of Atlantic salmon among standard, SW and FW snorkel cage types.

Effects of standard, SW and FW snorkels cages on fish welfare and growth

Fish welfare indicators and weights were similar between snorkel and standard cages, including where snorkels were filled with freshwater, confirming conclusions reached in previous snorkel cage investigations that use of this technology does not affect fish welfare (Oppedal et al. 2017, Wright et al. 2017). Snout damage, likely due to collisions with net roof and snorkel structures, has been observed in one research scale snorkel cage study (Stien et al. 2016) but we did not observe this negative effect here.

FW in snorkels: contrasting results in commercial and experimental trials

Commercial snorkels (10 m circle diameter × 10 m deep; volume 6448 m^3; Wright et al. 2017) have a volume 179 times greater than our research snorkels. The greater volume within the snorkel may promote greater fish residence time. Greater numbers and densities of fish within larger snorkels may enable them to school in their standard circular swimming pattern (~500 individuals are required to initiate schooling behaviour in 500–2000 m^3 cages; Oppedal et al. 2011) and thus spend longer periods at a given depth. However, the smaller snorkels used in this study may have limited this behaviour and allowed only enough room for surfacing for swim-bladder refilling before returning to swimming in a school formation below the snorkel. Further, while feed entered the snorkel at the surface in both the commercial trial and this experiment, we observed that fish in the commercial trial entered the snorkel to take the feed, while in this trial they mostly waited until the feed had fallen below the snorkel depth. The restricted space in the smaller snorkel may have inhibited formation of the typical feeding aggregation at the surface and limited use by salmon of this layer.

In this trial, the freshwater layer was created by applying mains ozone-treated freshwater, whereas in the commercial trial, where far greater quantities were required, snorkels were filled with freshwater from a local river (Wright et al. 2017). These different methods of application and volumes of added freshwater created quite different outcomes in the surface layer's salinity, temperature and oxygen levels. Due to larger freshwater volumes and greater instability in a larger snorkel, salinity conditions achieved by filling snorkels with freshwater at a commercial scale (salinity of 4–5) were higher than the current study (always <1) (Wright et al. 2017). Salinity gradients also tended to be steeper in this research scale study (stable salinity between 0 and 2 m depth, then constantly increasing salinity between 2 and 4 m) compared to the commercial-scale study (constantly increasing salinity throughout the snorkel) (Young et al. 2014). The higher salinity and its more gradual gradient may have provided a more attractive self-treatment space for Atlantic salmon to enter than an abrupt change to an almost completely fresh layer.

Freshwater filling with cooler temperature water, less preferred by salmon, has been typical in commercial- (Wright et al. 2017) and research-scale snorkels. At the commercial scale, surface water temperatures in individual snorkels filled with freshwater to varying degrees were 0.6 to 1.9°C cooler at the surface than reference conditions at one time (Wright et al. 2017), whereas in this study FW snorkels ranged from 1.5, 1.2, 0 and 0.7°C cooler than reference conditions across 4 sampling points. Similarly lower surface temperatures in FW snorkels between these 2 studies point to these relatively small temperature differences being unimportant in freshwater layer use by salmon. However, a more attractive water temperature within the FW area should be tested to increase fish residence (Oppedal et al. 2011).

Oxygen supersaturation from ozone treatment occurred in the research scale FW snorkels used here,

reaching levels (maximum 148% DO saturation at Time 4) approaching those known to cause stress, gas bubble disease and behavioural and physiological changes in parr and pre-smolt Atlantic salmon (Brauner et al. 2000, Espmark & Baeverfjord 2009, Espmark et al. 2010). In contrast, the low salinity layer in commercial snorkel cages filled from a local river had DO saturations of <100% and was not ozone treated (Wright et al. 2017). Therefore, the oxygen supersaturation and, potentially, residual ozone in the surface freshwater layer in this study may have acted as a deterrent. However, limited information exists on the effects of oxygen supersaturation and residual ozone in post-smolt Atlantic salmon, so we are unable to gauge the extent of this possible effect in this trial.

Mean AGD-related gill scores in snorkel cages (mean gill scores in cages up to 1.9) in this study were lower than in the commercial trial, which experienced a major outbreak (mean gill scores in cages up to 2.8; Wright et al. 2017). Low stocking densities and holding caged fish at declining water temperatures in autumn to winter, rather than increasing temperatures in summer to autumn (elevated water temperature is associated with increased AGD incidence; Oldham et al. 2016), potentially contributed to the lower AGD-related gill scores and limited the detection of gill score differences between cage types. A follow-up investigation, where salmon in SW and FW snorkel cages experience a more severe AGD outbreak, would improve the detectability of AGD differences between these cage types.

CONCLUSIONS

In our autumn to winter study, a permanent freshwater surface layer maintained within snorkel lice barrier sea-cages holding Atlantic salmon did not affect their freshwater-sensitive ectoparasites, *Paramoeba perurans* and salmon lice. Salmon may have had limited contact time with the freshwater layer because of how they vertically positioned within snorkel cages or because they avoided the cool, super-oxygenated freshwater surface layer created to the extent that the parasites were not exposed sufficiently to the freshwater layer to produce an effect. Multiple changes to the freshwater surface layer to attract salmon to it are possible, including temporary night lighting strategies (Juell & Fosseidengen 2004, Wright et al. 2015) and making surface waters warmer, less hyperoxic and more saline (Oppedal et al. 2011). These may intensify freshwater or low salinity layer use by salmon to the point where *P. perurans* and salmon lice are reliably diminished.

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