Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon

Luke T Barrett [1]*, Samantha Bui [2], Frode Oppedal [2], Tora Bardal [3], Rolf Erik Olsen [3], Tim Dempster [1]

[1] Sustainable Aquaculture Laboratory – Temperate and Tropical (SALTT), School of BioSciences, University of Melbourne, Victoria 3010, Australia

[2] Institute of Marine Research, Matredal 5984, Norway

[3] Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim 7010, Norway

* Corresponding author: luke.barrett@unimelb.edu.au

Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

ABSTRACT

Sea-cage salmon farming creates ideal conditions for ectoparasites such as the salmon louse *Lepeophtheirus salmonis*, with high lice densities leading to welfare challenges for stock and increasing lice burdens on wild salmonids. New treatments with low environmental impacts and minimal handling are needed to complement existing strategies. Irradiation by ultraviolet-C light (254 nm, UVC) at specific doses renders fertilised lice eggs inviable. We tested if this treatment can be applied directly to female lice with eggstrings while attached to salmon. We treated fish with attached adult lice with UVC light while they swam freely in tanks, to achieve a cumulative dose of ~0.1 J cm^{-2} on each side of the fish within a 6-day period. To compare to fish in tanks with no UVC (control), we collected and incubated eggstrings to measure survival of resulting larvae at the infectious copepodid stage. The UVC treatment resulted in up to a 99 % reduction in copepodid production relative to the control. However, UVC negatively impacted fish welfare, and was associated with early-stage cataract-like pathologies, poorer skin condition and behaviours indicative of discomfort. While UVC is highly effective at suppressing lice reproduction, the exposure regime tested here led to unacceptable animal welfare outcomes. More conservative exposure regimes may be acceptable with careful testing and calibration, but applications that do not expose host fish are preferable.

Keywords: parasite control; treatment; sea lice; salmon lice; animal welfare

INTRODUCTION

Parasitism of farmed Atlantic salmon (*Salmo salar*) by sea lice has severe impacts on production and welfare of both farmed and wild salmonids. The salmon louse *Lepeophtheirus salmonis,* in particular, is perhaps the most intractable issue facing salmon aquaculture in the northern hemisphere. Lice abundance is amplified by the high densities of salmon hosts in coastal sea-cages, causing spillover onto wild salmon and ongoing reinfestation of farmed salmon. High lice densities at salmon farms can reduce the welfare of farmed salmon (Wagner et al., 2008; Øverli et al., 2014) and contribute to declines in wild salmon populations (Krkošek et al., 2013). Accordingly, regular lice control is necessary to suppress lice populations, but many existing delousing treatments have significant drawbacks. Delousing is generally costly (Iversen et al., 2015), while some methods are also risky for the host (thermal, mechanical, H_2O_2: Overton et al., 2019), raise environmental concerns (chemotherapeutants: Langford et al., 2014), or are becoming less effective over time (chemotherapeutants: Aaen et al., 2015; Ljungfeldt et al., 2017). Stocking sea-cages with cleaner fish can be effective under some conditions but brings sustainability and welfare concerns for the ~60 million cleaner fish used each year (Mo and Poppe, 2018; Skiftesvik et al., 2013). New lice control methods are required for ongoing suppression of lice populations at salmon farms.

Ultraviolet wavelength radiation (UVA: 320–400 nm; UVB: 280–320 nm; UVC: 200–280 nm) has a long history of antimicrobial use for disinfection of substrates such as air, water and food preparation surfaces (Bintsis et al., 2000; Severin, 1980). UV radiation damages nucleic acids by causing strand breaks, and in the case of UVB and UVC, this produces pyrimidine dimers and pyrimidine and pyrimidone photoproducts (Friedberg et al., 2005). These mutations can block DNA transcription and lead to aberrant cell behaviour and/or loss of fidelity during replication. Cellular mechanisms exist to mitigate DNA damage, either by directly reversing modifications or by excising damaged elements (Friedberg et al., 2005), but sufficient exposure can exceed repair mechanisms and lead to cell death. UVC is the most widely used wavelength, as it is relatively inexpensive to produce using low pressure mercury vapor lamps with a peak 254 nm wavelength that is close to the maximal absorption spectrum of DNA, allowing lethal DNA damage to be induced with high efficiency (Cleaver, 2006; Friedberg et al., 2005).

A recent study introduced UVC radiation as a salmon lice control method (Barrett et al., 2019). Cumulative short UVC exposures were effective at reducing hatching and moulting success from salmon louse eggstrings at a range of up to 150 cm. It remains unknown if this treatment can be effectively applied to eggstrings on host fish in a sea-cage environment, but doses that reduce hatching success of salmon lice zygotes by 50-95 % (0.01-0.09 J cm^{-2}: Barrett et al., 2019) may be achievable over the developmental period of an eggstring in a sea-cage (1-3 weeks depending on

water temperature). If applied whenever lice loads are high, this control method could dramatically reduce the number of viable larvae released from a salmon farm that may infect the same farm or surrounding farms. Effects of UVC exposure on fish are not well known, as UVC radiation does not occur naturally in aquatic environments. However, skin and eye damage has been reported in fish after excessive UVA/UVB exposure (McArdle and Bullock, 1987; Cullen and Monteith-McMaster, 1993; Sweet et al., 2012), and given that UVC affects DNA via similar mechanisms to UVB (Friedberg et al., 2005; Rochette et al., 2006), it is likely that UVC will have similar effects at therapeutic doses.

Here, we tested whether an effective dose of UVC can be applied to salmon louse eggstrings *in situ* on salmon hosts, by exposing lice-infested salmon to daily UVC doses delivered by underwater UVC lamps in a tank environment and comparing (i) production of infective lice stages and (ii) fish welfare indicators from treated and untreated tanks.

MATERIALS AND METHODS

Experiment 1: efficacy

Study animals and husbandry

The UVC exposure trial took place at the Matre Research Station, Norwegian Institute of Marine Research, from March to May 2018. Six cylindrical tanks (3 m diameter, 70 cm depth, ~5 m^3) were stocked with 160 post-smolt Atlantic salmon (mean ± SD: 240 ± 10 g) per tank. Tanks were supplied with 100 ± 2 l min^{-1} filtered seawater (40 µm), pumped from 90 m depth in Matrefjorden. The temperature was 15 ± 0.2 °C and salinity 34.1 ± 0.2 ‰, with oxygen saturation maintained above 80 %. Automatic feeders delivered 4.5 mm standard feed pellets (Skretting, Norway) to satiation throughout daylight hours (12/12 daily light/dark cycle).

Wild adult female lice were collected from a salmon farm in Masfjorden and transferred onto host salmon (not those used for the UVC trial) in tanks to create a stock lice population (Hamre et al., 2009). Eggstrings were later harvested from the stock population by sedating and netting host salmon and removing mature eggstrings from gravid lice with forceps. Eggstrings were placed in small flow-through incubators (following methods outlined by Hamre et al., 2009). Once hatched and moulted, infective copepodid larvae were used to infest salmon in 3 of the 6 tanks designated for the UVC experiment. During infestation, tank flow was shut off and 3200 copepodids per tank (20 copepodids per fish) were added, with 45 min allowed for attachment before reinstating water flow. Supplemental oxygen was used to ensure that oxygen saturation did not drop below 60 % during the infestation procedure.

99

100 ***UVC treatment***

101 The UVC dose was delivered via low pressure mercury vapor lamps producing peak intensity at 254
102 nm (Planet Lighting Pty Ltd, Australia) for 10 days. Three of 6 tanks were randomly selected to
103 receive the UVC treatment (hereafter 'UVC' group), with three 40 W lamps hung vertically in a
104 triangular array around the centre of each tank (Fig. 1). Intensity was attenuated exponentially
105 through seawater, with measured mean intensities of 670 µW cm^{-2} at 10 cm, 191 µW cm^{-2} at 30 cm
106 and 9.5 µW cm^{-2} at 100 cm. During the first week of the trial, each lamp was enclosed within a 30 cm
107 diameter cylindrical mesh cage to prevent fish being exposed to irradiation >490 µW cm^{-2}. The
108 remaining three tanks did not receive UVC treatment (hereafter 'control' group) and instead
109 contained empty mesh cages in an identical array to function as procedural controls. Fish were
110 monitored daily throughout the experiment and any individuals with visible skin injuries or
111 behaviour indicative of distress or illness (e.g. excessive rubbing/jumping, loss of equilibrium) were
112 netted, sedated (1 g 100 L^{-1} metomidate hydrochloride; Aquacalm) and euthanised with a blow to
113 the head. After 6 days, superficial skin injuries from contact with the cages became apparent, so the
114 cages were removed from both control and UVC tanks for the remainder of the trial, with
115 unpowered lamps acting as procedural controls in the control group tanks. Throughout the study,
116 provisions were made to ensure that personnel were not exposed to UVC, including signage, barriers
117 and personal protective equipment. Given the risk of adverse effects on fish, the study plan was
118 assessed and found to meet ethical requirements (Mattilsynet FOTS number: 14862). Experiment 1
119 only commenced after a separate pilot study revealed no clear effects of a lower daily dose (~0.1 J
120 cm^{-2} over 10 days) on the welfare of 10 fish (these fish were not reused for Experiment 1).

121 We aimed to expose lice eggstrings to the 95 % effective dose of 0.1 J cm^{-2} estimated by Barrett et al.
122 (2019). To calibrate the exposure regime to achieve the desired dose for the average individual, the
123 UVC intensity delivered by the 40 W lamps was measured according to: (a) increasing warm-up time;
124 and (b) distance through the water column, using a Solar Light PMA2100 radiometer and PMA2122-
125 WP sensor. A 20x20 cell grid was overlaid over a diagram of the tank and the maximum irradiance at
126 each cell estimated based on the distance from the nearest lamp. Then, prior to commencing the
127 exposure regime, the distribution of fish within each treatment tank was recorded using a top-down
128 photograph by a GoPro Hero3+ held 2.5 m above the centre of the tank. The 20x20 cell grid was
129 digitally overlaid over each photograph and fish were manually tallied within each cell. This allowed
130 estimation of the irradiance being received by each fish at the time of observation (based on the
131 estimated irradiance at the cell the fish was within at the time), and therefore estimation of the
132 mean intensity received by fish in the tank. This process was repeated for all 3 UVC tanks on each of

the first 4 days of the trial, with fish position was noted immediately before the daily UVC exposure began, and again 15 min after UVC lamps were switched on. Using this information, we designed an exposure regime that would provide an effective dose to both sides of the fish over the developmental period of an extruded eggstring. A heatmap representation of fish distribution (Fig. 1) was generated using the *filled.contour* function in base R (R Core Team, 2019).

The exposure regime consisted of a 15 min exposure once per day over a period of 10 days. Exposures were given in the late afternoon, during the light portion of the 12/12 diurnal cycle. The average eggstring was estimated to take 6 days from extrusion to hatching based on the water temperature in the tanks, so eggstrings sampled at the end of the trial had likely been extruded at least 4 days into the 10-day trial. The regime was calibrated to deliver the target dose (0.1 J cm^{-2}) within a 6-day window, with adult lice and the host fish cumulatively receiving 1.6x the target dose over the full 10-day period. The direction of tank flow was reversed each day to encourage redistribution of fish and ensure exposure of both sides of the body. We did not account for shading of individuals by those closer to the lamp, so doses may be marginally overestimated.

Eggstring sampling

Collection of eggstrings took place six weeks after infestation, coinciding approximately with the third or fourth eggstring pair produced by the female lice and following 10 daily UVC exposures received by female lice in the UVC tanks. Eggstring maturity was not assessed at the time of collection, and some will not have been exposed for the full 10 days. This is addressed in *Statistical analyses*. Before sampling, the tank water level was lowered to 20 cm and a light dose of sedative added (0.3 g 100 L^{-1} metomidate hydrochloride; Aquacalm) before netting randomly-selected fish into 500 L bins containing a full dose of the same sedative (1 g 100 L^{-1}). Tanks were sampled one at a time in random order.

To minimise lice loss and potential effects on eggstring viability in the sedation bin, sedated fish were removed as soon as they lost equilibrium and opercular movement slowed. Adult female lice with eggstrings were removed and transferred into incubator wells, and the fish immediately euthanised with a blow to head. Up to 24 eggstring pairs per tank (range 5-24, mean 19.7 pairs) were incubated.

Welfare assessments

Following euthanasia, remaining lice were counted and welfare assessments performed on the first 20 fish sampled from each tank (including fin, skin and eye condition) according to the Standardised Welfare Index Model v1.1 (SWIM; full methods in: Stien et al., 2013). This sample size was sufficient to detect the obvious adverse effects of UVC exposure; we prioritised bringing the trial to the

earliest reasonable endpoint rather than sampling additional fish. Earlier, SWIM assessments had also been done on a subset of fish after six daily exposures to check for any early welfare effects (these fish were also euthanised).

Fish welfare assessments also included behavioural monitoring. Counts of rubbing and jumping behaviours occurring in each tank were made using a live top-down video feed during a 10 min monitoring period per tank on 2 consecutive days (total 20 min per tank). All tanks were monitored in randomised order during a single block of time on each of the 2 days. The 10 min monitoring period was divided into two segments—5 min with the UVC lamps turned off and 5 min with the UVC lamps turned on—to assess whether behaviour is influenced by the immediate presence of UVC light or by cumulative symptoms of exposure. Whether lamps were turned on in the first or second half of the 10 min monitoring period was also randomised. In the control tanks, UVC lamps remained off for both 5 min segments.

Eggstring incubation and copepodid counts

Following collection, eggstring pairs were incubated with one eggstring pair per incubator well (Hamre et al., 2009) supplied with flowing filtered seawater at the same salinity and temperature as the experimental tanks (15 °C and salinity 34.1 ‰).

Larval salmon lice must undergo two moults (nauplius I and II) before reaching the infective copepodid stage. Accordingly, we used the number of copepodids produced from an eggstring as an indicator of reproductive success. Incubated eggstrings were checked for hatching daily throughout incubation, and copepodid larvae were counted 4 days after the first hatching was observed in an incubator well. This was sufficient time for all viable eggs to hatch and for nauplii larvae to moult into the copepodid stage. Eggstrings that remained unhatched 9 days after collection were considered non-viable. If live nauplii were observed during a copepodid count, the well was returned to the incubator for an additional day to allow more time for moulting before copepodid counts were recorded.

Experiment 2: host welfare threshold

Study animals and husbandry

This experiment also took place at the Matre Research Station, during March-April 2019. The UVC treatment was given in a single cylindrical tank (5 m diameter, 20 m^3), stocked with 40 post-smolt Atlantic salmon (1063 ± 146 g). As each predetermined dose was reached, a random subsample of 4 fish was netted and transferred to one of 4 smaller monitoring tanks (1.5 m^3), where they could be monitored for symptoms arising from UVC exposure (methods outlined in next section). All tanks

were supplied with filtered seawater at 9 °C and salinity at 34.1 ‰, with oxygen saturation maintained above 90 %. In the treatment tank, fish were fed twice daily to satiation (4.5 mm standard feed pellets: Skretting, Norway). Their behaviour was monitored twice daily for 10 min for evidence of distress, before and during feeding, both in the UVC treatment tank and monitoring tanks. In the monitoring tanks, fish were fed to satiation via automatic feeders throughout daylight hours and their welfare monitored daily.

UVC treatment

Exposures were delivered via a single 40 W low pressure mercury vapor lamp hung vertically in the centre of the treatment tank, spanning the full depth of the water column. Fish were kept between 200-250 cm from the light source by means of a plastic-coated steel mesh barrier encircling the centre of the tank. At this range, and through the mesh, fish received a mean measured intensity of 0.5 µW cm^{-2} at 254 nm. We assumed exposure to be uniform regardless of the fish's vertical position in the water column. Fish swam anti-clockwise throughout the trial so received the dose on the left side.

The UVC dose was given cumulatively via numerous 15-30 min exposures at 0.5 µW cm^{-2}, with subsamples of 4 random fish netted and transferred to monitoring tanks as each of 10 targeted cumulative doses were reached (Table 1). The 10 target doses were selected based on the copepodid production dose-response curve estimated in Barrett et al. (2019). This curve was also used to calibrate the exposure regime in Experiment 1. We set target doses according to their effect on copepodid production (effective dose, % reduction), with fish transferred at 10 % effective dose intervals from 0 % (0 J cm^{-2}) to 90 % (0.5 J cm^{-2}) reduction (Table 1).

During transfer to monitoring tanks, fish were fully anaesthetised with tricaine methanesulfonate (Finquel: 10 g 100 L^{-1}) and checked for obvious injury or illness. As fish from different dose level groups were mixed within the monitoring tanks (10 dose levels into 4 monitoring tanks), all fish were tagged dorsally with colour-coded Floy T-bar tags to allow individual welfare assessments to be matched with UVC dose levels. Dose level groups that were transferred on the same day were placed in the same monitoring tank to avoid repeatedly disturbing other groups (small tanks can lead to impact injuries when fish are startled). The 0% (and later the 90%) group was placed in Tank 1, the 10, 20, 30 and 40% groups in Tank 2, the 50 and 60% groups in Tank 3, and the 70 and 80% groups in Tank 4. One fish was euthanised during transfer due to a bacterial eye infection.

Welfare assessments

At 6 days following their last UVC exposure, 2 of the 4 fish at each dose level were euthanised (Finquel: 20 g 100 L^{-1}) and given a formal welfare assessment for skin and eye health (Sample 1). The

remaining 2 fish per dose level were all sampled on the same day (Sample 2), 14 days after the highest dose group (90%) completed its exposure regime. Lower dose groups had completed their exposure regimes earlier, so had a longer recovery time to Sample 2 (ranging from 18 days for the 80% group to 22 days for the 10% group and 24 days for the 0% group). Because of this considerable difference in time spent in the monitoring tanks, Sample 2 data are not presented.

Metrics of fish welfare were modified from the SWIM assessment (Stien et al., 2013) to target UVC-related symptoms based on observations in Experiment 1. We assessed scale loss (1: no scale loss; 2: minor scale loss <10 cm^2; 3: extensive scale loss), skin damage consistent with sunburn or physical injury (1: no damage; 2: small old wounds or superficial injury; 3: small open wound (<1 cm^2); 4: multiple small open wounds or a single large wound; 5: severe condition, potentially life-threatening), and eye opacity indicative of cataract development (1: no cataracts; 2: early stage in a single eye; 3: early stage in both eyes or developed cataract in a single eye; 4: developed cataracts in both eyes; 5: likely blindness). We did not conduct histopathology on eye samples, so cataract assessments were made based on gross characteristics.

Skin histology

To detect damage from UVC irradiation not apparent to the naked eye, we also conducted histolopathology on skin samples from 2 fish from the 0, 30, 60 and 90% effective dose groups (total 8 fish). At Sample 1, we excised 1x2 cm samples incorporating the dermis and subcutaneous fat layer from consistent locations on the pigmented (dorsolateral) and non-pigmented (ventrolateral) skin areas between the dorsal and pelvic fins, on the exposed left and unexposed right side of each fish. Samples were preserved in 4 % phosphate-buffered formaldehyde at 4°C until dehydration and embedding using a tissue processor (TP1020, Leica Biosystems, Germany). Samples were sectioned at 5-7 µm thickness using a microtome (RM2255, Leica Biosystems, Germany) and stained with HE (dorsolateral samples) or Alcian Blue-Nuclear Fast Red (ventrolateral samples). Glass slides were scanned at 40x magnification with a digital slide scanner (NanoZoomer, Hamamatsu Photonics, Japan). Measurements were done using scanner software (NDP, Hamamatsu Photonics, Japan).

Statistical analyses

Experiment 1

Numbers of adult lice on UVC and control fish were compared using a generalised linear mixed model fitted using the glmmTMB package for R (Brooks et al., 2017; R Core Team, 2018). Individual fish were treated as replicates, with treatment (UVC, control) as a fixed effect and tank identity as a random effect (nested within treatment) to account for non-independence between tank-mates. As

there were more fish with few or no lice than would be expected in a Poisson distribution, we specified a negative binomial model family.

The probability of at least one egg in a given eggstring hatching was compared between UVC and control groups using a X^2 test of proportions, while mean copepodid production per eggstring was compared between groups using a generalised linear mixed effects model fitted using the glmmTMB package for R (Brooks et al., 2017; R Core Team, 2018). Replication was at the level of female lice (i.e. eggstring pairs), with treatment (UVC, control) specified as a fixed effect and tank identity as a random effect nested within treatment. We specified a negative binomial model family to account for the large number of eggstrings that produced few or no copepodids. As some female lice only had a single eggstring attached upon collection, the number of copepodids produced by a female was divided by the number of eggstrings to provide an estimate of copepodids produced per eggstring.

Some eggstring pairs were extruded late in the trial and as a result, did not receive the full target dose of 0.1 J cm^{-2} over a 6-day window. To account for this, we noted the date of first hatching for incubated eggstrings and estimated the likely date of extrusion based on temperature-dependent development times (Samsing et al., 2016). Dose response curves were then fitted to model copepodid counts per eggstring according to the estimated number of days of exposure using the drc package for R (Ritz et al., 2015). To avoid overfitting, model functions were ranked by Akaike's Information Criterion to identify the most parsimonious function. Data were best fitted by a three-parameter log-logistic (sigmoidal) function. The significance of function parameters was tested using the *coeftest* function in the *lmtest* package (Zeileis and Hothorn, 2002). To account for variance heterogeneity, we used a robust covariance matrix computed by the *sandwich* package (Ritz et al., 2015; Zeileis, 2004). Dose-response plots were produced in *ggplot2* (Wickham, 2009) using model predictions provided by the *drc* package. Eggstring pairs from two adult female lice were omitted from the analysis because they were the only replicates to receive <2 days of exposure. The dose-response model also omitted 10 UVC eggstring pairs (14 % of UVC eggstring pairs) that did not hatch, as it was not possible to estimate the date of extrusion without a hatching date. Accordingly, estimated effective dose levels provided by the dose-response model will be somewhat conservative.

Experiment 2

Metrics of fish welfare were not formally analysed due to the low sample size and issues with interpreting models fitted to qualitative score data. However, to assist in visual interpretation, the data are plotted with generalized additive model fits overlaid. Model fits were generated using the R

296 package *mgcv* (Woods 2011) and plotted using the *ggplotify* and *ggplot2* packages (Guangchuang
297 2019; Wickham 2019).

298

299 **RESULTS**

300 **Experiment 1: efficacy**

301 ***Infestation density***

302 Numbers of attached lice were highly variable across individual fish and tanks, despite careful
303 quantification of copepodids and all tanks being subject to identical infestation protocols. UVC
304 irradiation was not associated with a lower density of adult lice stages (mean lice per fish ± SE; UVC:
305 1.2 ± 0.2, Control: 1.0 ± 0.2; negative binomial GLMM: $z_{115} = 0.56$, $p = 0.57$).

306 ***Copepodid production***

307 Among UVC treated eggstrings, 86 % of eggstrings underwent at least partial hatching, whereas
308 100% of eggstrings from control tanks hatched (N wells = 72 (UVC) + 46 (control), $X^2_1 = 5.3$, $p = 0.02$).
309 The presence of large numbers of dead nauplii (stage I and II) indicated that UVC exposure affected
310 moulting success of nauplius larvae. 25/72 incubator wells in the UVC group underwent at least
311 partial hatching with low moulting success (≤10 copepodids), compared with 1/46 wells in the
312 control group. In many cases, hundreds of nauplii hatched but died before moulting into copepodids.

313 The UVC treatment drove a 66 % reduction overall in the number of copepodid larvae produced by
314 treated eggstrings (mean copepodids ± SE; UVC: 46 ± 8; Control: 136 ± 9; negative binomial GLMM:
315 $z_{101} = 2.4$, $p = 0.015$). The number of days of exposure to the UVC treatment and therefore
316 cumulative dose received (estimated from date of first hatching) strongly predicted the magnitude
317 of this effect in the UVC group (dose-response modelling: Fig. 2; Supplementary Table 1). There was
318 a sharp decline in copepodid production in eggstrings that had been exposed to the UVC regime for
319 4 days (60 mins, ~0.06 J cm^{-2} UVC), and copepodid production was reduced by >99 % after 6 days of
320 exposure (Table 2). The dose-response function estimated a 10 % effective dose for the UVC group
321 at 3.4 days (~0.050 J cm^{-2}), 50 % at 3.7 days (~0.055 J cm^{-2}), and 90 % at 4.0 days (~0.060 J cm^{-2}).

322 Copepodid production in the control group appeared to increase with more 'exposure days' (Fig. 2).
323 This is an experimental artefact arising from non-zero daily mortality for eggstrings in incubation -
324 eggstrings with more exposure days prior to collection spent less time in the incubator before
325 hatching and therefore had higher hatching rates. As UVC and control groups were subject to
326 identical incubation conditions, this effect is expected to be uniform across both UVC and control
327 groups but masked by the strong effect of the UVC treatment in the UVC group.

Fish welfare

Fish in the UVC group are estimated to have received a cumulative dose of ~0.15 J cm^{-2} per side by the end of the 10 day trial (0.015 J cm^{-2} day^{-1}), although this is a mean value and despite efforts to redistribute fish by changing flow direction, some individuals will likely have received a considerably higher or lower dose.

The SWIM assessments revealed negative effects among fish exposed to the UVC treatment. UVC treated fish generally scored more poorly (higher) than control fish in skin (UVC: median = 4, range = 3-6 vs. Control: median = 3, range = 1-3) and eye condition (UVC: median = 3.5, range = 2-5 vs. Control: median = 2 , range = 1-3). Median fin scores did not differ (UVC: median = 2, range = 1-3 vs. Control: median = 2, range = 1-3). Poorer eye condition scores were usually due to a higher incidence of suspected early stage cataracts in UVC fish (typically a diffuse 1-3 mm cloudy area in the centre of the lens or cornea). The skin of UVC-exposed fish typically had superficial haemorrhaging at the base of the scales, consistent with physical trauma (Fig. 3). The median skin score of 4 reflected the presence of superficial haemorrhaging at the base of the scales, consistent with physical trauma, as well as a small lesion or scratch (<10 mm). Fish with multiple or larger lesions were given scores >4. Fins were generally in good condition, with the median fin score of 2 reflecting partial fin membrane splits that likely occurred during netting.

Fish in the UVC group also exhibited behaviours consistent with skin irritation, including more frequent jumping (2.7 x) and rubbing against the tank material (32 x) than the control group (Fig. 4). This difference was observed whether the UVC lamps were switched on at the time of observation or not. Bruising from jumping and abrasion from rubbing likely exacerbated the decline in skin condition. Final sampling and euthanasia were conducted as soon as possible after discovering these adverse fish welfare outcomes. 67/480 fish from the UVC group died or were euthanised before the trial endpoint, compared to 4/480 fish in the control group. 37/67 mortalities in the UVC group occurred during the last 72 hrs of the trial.

Experiment 2: host welfare threshold

Fish welfare

No signs of irritation, such as rubbing or jumping, were observed during twice-daily behaviour monitoring sessions either in the treatment tank or monitoring tanks. However, skin and eye health assessments indicated that condition decreased with increasing dose (Fig. 5). Worsening of welfare over time was most apparent in skin condition, with all fish exposed to higher dose levels (equivalent to >60 % copepodid reduction) developing minor skin injuries. No fish received a skin condition score of 4 or higher (i.e. multiple small open wounds or worse). Scale loss did not appear to be related to

UVC exposure and remained approximately constant over time regardless of dose level. Cataract prevalence was highly variable but appeared to increase monotonically with dose level. Of the 26/40 fish that were scored as having cataracts, 13 were in the left eye only, 11 were in both eyes, and 2 were in the right eye only.

Skin histology

The UVC treatment led to general loss of cellular organisation of the epidermis, especially the basal layer epithelial cells. There was clear evidence of oedema increasing with exposure from the 0% to 90% exposure groups. Epidermal thickness increased with exposure, especially on skin samples from the ventral surface (Fig. 6). In dorsal samples, the increase in thickness and oedema was obvious in the 90% group only. The epidermis underlying and protected by the scales had fewer changes than the layer overlying the scales. Under the scales, the epidermis was only thickened in the ventral samples from the 90% group, with no major changes observed in the dorsal samples of any exposure group. Goblet cell abundance decreased in exposed epidermis according to dosage, with a notable change in position from more scattered throughout the epidermis in low exposure groups, to a more apical concentration at high exposure groups (Fig. 6). This was most noticeable in ventral samples. In the epidermis protected by the scales, the decrease in goblet cell density was only observed at highest (90%) dose on the ventral side, while no clear effects were seen in dorsal samples. In the worst-affected samples, severe sloughing of the epidermis had exposed the underlying tissue, accompanied by bleeding and malformation of tissue.

DISCUSSION

UVC irradiation of lice-infested Atlantic salmon was highly effective at reducing production of the infective salmon lice stages, with >99 % fewer copepodids per eggstring produced by the UVC group relative to the control group after 6 days of UVC exposure (Experiment 1). However, the exposure regime had negative effects on the welfare of exposed fish, with mild symptoms arising by Day 6 and worsening until the termination of the trial on Day 10. The results of the host welfare threshold experiment (Experiment 2) indicate that there may be no completely safe dose for salmon, but short-term welfare effects were relatively mild in fish that received doses corresponding to a 0-60 % copepodid reduction.

Copepodid production

Reductions in copepodid production (Experiment 1) occurred at broadly similar estimated doses to those in a recent study that exposed eggstrings already detached from lice (Barrett et al., 2019).

Intensity and duration of UVC exposure was precisely controlled in that study, so it is likely that small discrepancies here reflect either: (a) inaccuracy in dose estimates from the present study due to practical limitations in delivering precise doses to eggstrings while attached to free swimming host fish; and/or (b) effects of differing exposure regimes (i.e. daily 15 min exposures over several days vs. numerous 2-5 sec exposures in a single day: Barrett et al., 2019).

Delayed effects of UVC exposure were common, with many UVC-treated eggstrings undergoing at least partial hatching but with subsequent mass mortality of nauplii larvae. This may be consistent with UVC exposure during the zygote or embryonic stage creating DNA photoproducts that block transcription and lead to errors during later cell replication (Friedberg et al., 2005). DNA damage from UV exposure also has carcinogenic effects in many taxa (Cleaver and Crowley, 2002; Setlow et al., 1989), but it is not known whether this occurs in salmon lice.

Fish welfare

UV-induced skin damage occurs primarily via DNA strand breaks in epidermal cells. Some amount of DNA damage can be repaired by various mechanisms, but cells with a heavy mutational burden or genomic instability may instead undergo programmed cell death (apoptosis) in the hours or days following exposure and be replaced (Friedberg et al., 2005). Symptoms of UV damage typically arise in the days following exposure. Histological studies of salmonid skin exposed to UVB irradiation have found loss of mucosal (goblet) cells, intercellular oedema, necrosis and sloughing (Bullock and Roberts, 1992; Blazer et al., 1997; Noceda et al., 1997). We found similar symptoms following UVC exposure in the present study. The loss of goblet cells here did not appear to be a consequence of sloughing, but rather a generalized reduction throughout the epidermis. Protected areas underlying scales were clearly less affected compared to unprotected areas of epidermis, and the dorsal side appeared less sensitive than the ventral side. The reason for the latter is unclear, as the pigmented dorsal layer is beneath the epidermis.

Exposure to UVC in Experiment 1 also prompted excessive rubbing and jumping behaviours in affected fish, which likely led to additional injuries resulting from the physical contact with the tank walls or lamp apparatus. These injuries generally consisted of minor haemorrhaging at the base of the scales, and in the most severe cases, loss of scales and visible scratches or lesions from contact with abrasive surfaces. In addition to higher rates of skin injury, chronic UV exposure suppresses wound healing in fish (Bullock and Roberts, 1992). This may have worsened the accumulation of skin damage from small abrasions over the course of the trial. Mortality rates were higher in the UVC group, perhaps driven by a combination of immunosuppression from UVC exposure and osmotic stress from skin wounds. Fish did not appear to avoid UVC lamps whether they were switched on or

off (Fig. 1), and 2-3 days after the first UVC exposure, rubbing and jumping occurred regardless of whether the lamps were switched on at the time of observation. This indicates that fish do not suffer immediate discomfort from exposure to UVC and accordingly do not attempt to avoid potentially harmful exposure. Although we did not observe behavioural indications of irritation in Experiment 2, the presence of minor scratches on most fish in the 70-90 % dose level groups is consistent with findings from Experiment 1.

Cataracts and other eye pathologies are common in farmed salmon. Cataract formation has been attributed to a variety of intrinsic and environmental causes, including triploidy, dietary deficiencies, temperature and UV exposure (Ersdal et al., 2001; Sambraus et al., 2017), while other pathologies may arise though physical trauma, osmotic stress or infection (Pettersen et al., 2014). In this study, there was a higher frequency of eye pathology in the UVC group, primarily suspected early stage cataracts, indicating rapid cataract formation following UVC exposure. Studies in mammals indicate that UV-induced cataract formation stems from DNA breaks in the epithelium (Hightower, 1995; Li and Spector, 1996), with epithelial cell death occurring within 24-48 hours following exposure to UVB, accompanied by changes to gene regulation, ion imbalances and development of tissue opacity deeper within the eye (Söderberg, 1990; Hightower, 1995; Li and Spector, 1996). In lake trout, small cataracts became apparent within 48 hours of exposure to UVB doses >0.5 J cm^{-2}, with some recovery in the form of increased clarity observed after 5 days (Cullen and Monteith-McMaster, 1993). At higher doses (>1 J cm^{-2}) there was no evidence of recovery after 7 days (Cullen and Monteith-McMaster, 1993). Cataracts may occur at even lower doses of UVC relative to UVB, as the shorter wavelength of UVC is more specifically absorbed by the corneal epithelium. In the present study, early cataract formation occurred after 6 daily UVC exposures in Experiment 1 (cumulative ~0.1 J cm^{-2}), with some increase in prevalence and severity observed after an additional 4 days exposure (the time of final sampling). To avoid further animal welfare issues, we did not hold fish to test for recovery of the lens or cornea. In Experiment 2, cataract prevalence in Sample 1 increased gradually with UVC exposure (Fig. 5). There was no evidence of recovery at Sample 2.

Industry applications

Potential applications involving direct exposure of infested fish would have to overcome two major challenges: (1) achieving a sufficient, cost-effective dose given the rapid attenuation of UVC wavelength light in seawater, and (2) suppressing lice reproduction without reducing the welfare of salmon over the duration of a grow-out cycle. The first challenge may be best tackled by installing numerous small light sources, for example in the form of a ‘curtain’ of hanging UVC lamps that individuals must pass through while swimming around the cage, by using lamps in combination with a ‘snorkel’ salmon lice barrier to expose individuals at close range for short durations (Stien et al.,

2016), or by exposing fish near the surface during feeding times. Lower seawater temperatures may favour such an approach, as eggstrings develop more slowly and so may be exposed over a longer period (Samsing et al., 2016). The second challenge—side effects on fish—is more difficult to solve, and may be prohibitive. Careful control of doses would be required to achieve smaller reductions in copepodid production while maintaining acceptable fish welfare. Before any potential deployment in the industry, research is needed to identify how exposure regimes influence side-effects, including long-term symptoms over the course of a grow-out cycle (for example, development of cancer or chronic skin lesions). Further, exposure to UVA and UVB light can interact with other conditions such as skin parasites (*Ichthyobodo*: Bullock, 1985) and pre-existing wounds (Bullock and Roberts, 1992); these interactions may also be relevant for UVC. If cost-effective doses can be given with minimal short-term side effects, there may be cases where suppression of salmon lice reproduction by UVC exposure is a useful method. Specifically, we envision a situation where production of infective stages on farms with high lice levels can be suppressed while awaiting delousing or harvesting. Conversely, if it is not possible to suppress lice reproduction within sea-cages without harming salmon, then applications of UVC that target lice life stages prior to infestation or after delousing may remain possible, such as treatment of cage inflow or sterilisation of delousing wastewater. A proportion of fertilised eggs remain viable following bathing treatments (e.g. Bravo et al., 2015; Toovey and Lyndon, 2000), while mechanical delousing systems employ physical filtering to avoid releasing viable eggstrings.

Conclusions

Long term suppression of salmon lice populations in the salmon farming industry will likely depend on multiple complementary strategies, spanning pre-infection prevention to post-infection control. The application of UVC light to lice-infested salmon via underwater lamps can prevent fertilised salmon lice eggstrings from developing into infective stages, but the exposure regime tested here led to unacceptable welfare outcomes for host salmon and should be avoided. The feasibility of UVC deployment in full-scale industrial settings would depend on identifying an exposure regime that offers more modest but still cost-effective reductions in lice reproduction with minimal side effects for host fish. Delivery methods that do not expose host fish may be preferable.

ACKNOWLEDGEMENTS

This study was funded by the Norwegian Seafood Research Fund (FHF) (project number: 901460, 2017-2019). Approval for ethical use of animals was obtained from the Norwegian government (Mattilsynet FOTS number: 14862). We thank Sinclair Park (Planet Lighting; planetlighting.com) for fruitful discussions and for providing the UVC lamps. Tone Vågseth (IMR), Georgia Macaulay (UoM), Michael Sievers (IMR) and Thomas Crosbie (UoM) provided technical assistance during the trial. Kjell Maroni (FHF), Geir Magne Knutsen (Bremnes Seashore) and Solveig Willis (Salmon Group) provided useful comments on industrial relevance. The authors declare no conflicts of interest.

REFERENCES

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol. 31, 72–81. https://doi.org/10.1016/j.pt.2014.12.006

Barrett, L.T., Pert, C.G., Bui, S., Oppedal, F., Dempster, T., 2019. Sterilization of sea lice eggs with ultraviolet C light: towards a new preventative technique for aquaculture. Pest Manag. Sci. https://doi.org/10.1002/ps.5595

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potential applications of ultraviolet light in the food industry – a critical review. J. Sci. Food Agric. 80, 637–645. https://doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1

Blazer, V.S., Fabacher, D.L., Little, E.E., Ewing, M.S., Kocan, K.M., 1997. Effects of ultraviolet-B radiation on fish: histologic comparison of a UVB-sensitive and a UVB-tolerant species. J. Aquat. Anim. Health 9, 132-143. https://doi.org/ 10.1577/1548-8667(1997)009<0132:EOUBRO>2.3.CO;2

Bravo, S., Silva, M.T., Agusti, C., Sambra, K., Horsberg T.E., 2015. The effect of chemotherapeutic drugs used to control sea lice on the hatching viability of egg strings from *Caligus rogercresseyi*. Aquaculture 443, 77–83. https://doi.org/10.1016/j.aquaculture.2015.03.011

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. Modeling zero-inflated count data with glmmTMB. bioRxiv 132753. https://doi.org/https://doi.org/10.1101/132753

Bullock, A.M., 1985. The effect of ultraviolet-B radiation upon the skin of the plaice, *Pleuronectes platessa* L., infested with the bodonid ectoparasite *Ichthyobodo necator* (Henneguy, 1883). J. Fish Dis. 8, 547–550. https://doi.org/10.1111/j.1365-2761.1985.tb00970.x

522 Bullock, A.M., Roberts, R.J., 1992. The influence of ultraviolet-B radiation on the mechanism of
523 wound repair in the skin of the Atlantic salmon, *Salmo salar* L. J. Fish Dis. 15, 143–152.
524 https://doi.org/10.1111/j.1365-2761.1992.tb00648.x

525 Cleaver, J.E., 2006. Cells have long experience of dealing with UVC light. Nature 442, 244.
526 https://doi.org/10.1038/442244b

527 Cleaver, J.E., Crowley, E., 2002. UV damage, DNA repair and skin carcinogenesis. Front. Biosci. 7,
528 1024–1043. https://doi.org/10.2741/A829

529 Cullen, A.P., Monteith-McMaster, C.A., 1993. Damage to the rainbow trout (*Oncorhyncus mykiss*)
530 lens following an acute dose of UVB. Curr. Eye Res. 12, 97–106.
531 https://doi.org/10.3109/02713689308999477

532 Ersdal, C., Midtlyng, P.J., Jarp, J., 2001. An epidemiological study of cataracts in seawater farmed
533 Atlantic salmon *Salmo salar*. Dis. Aquat. Organ. 45, 229–236. https://doi:10.3354/dao045229

534 Friedberg, E.C., Walker, G.C., Siede, W., Wood, R.D., 2005. DNA repair and mutagenesis, 2nd ed.
535 American Society for Microbiology Press, Washington, DC, DC.

536 Guangchuang, Y., 2019. ggplotify: Convert plot to 'grob' or 'ggplot' object. R package version 0.0.4.
537 https://CRAN.R-project.org/package=ggplotify

538 Hamre, L.A., Glover, K.A., Nilsen, F., 2009. Establishment and characterisation of salmon louse
539 (*Lepeophtheirus salmonis* (Krøyer 1837)) laboratory strains. Parasitol. Int. 58, 451–460.
540 https://doi.org/https://doi.org/10.1016/j.parint.2009.08.009

541 Hightower, K.R., 1995. The role of the lens epithelium in development of UV cataract. Curr. Eye Res.
542 14, 71–78. https://doi.org/10.3109/02713689508999916

543 Iversen, A., Hermansen, Ø., Andreassen, O., Brandvik, R.K., Marthinussen, A., Nystøyl, R., 2015. Cost
544 drivers in salmon farming (In Norwegian). Report 41/2015 from Nofima, Trondheim. Available
545 from: https://nofimaas.sharepoint.com/sites/public/Cristin/Rapport%2037-2018.pdf

546 Krkošek, M., Revie, C.W., Gargan, P.G., Skilbrei, O.T., Finstad, B., Todd, C.D., 2013. Impact of
547 parasites on salmon recruitment in the Northeast Atlantic Ocean. Proc. R. Soc. B. 280.
548 https://doi.org/10.1098/rspb.2012.2359

549 Langford, K.H., Øxnevad, S., Schøyen, M., Thomas, K. V., 2014. Do antiparasitic medicines used in
550 aquaculture pose a risk to the Norwegian aquatic environment? Environ. Sci. Technol. 48,
551 7774–7780. https://doi.org/10.1021/es5005329

552 Li, W.-C., Spector, A., 1996. Lens epithelial cell apoptosis is an early event in the development of

553 UVB-induced cataract. Free Radic. Biol. Med. 20, 301–311.
554 https://doi.org/https://doi.org/10.1016/0891-5849(96)02050-3

555 Ljungfeldt, L.E.R., Quintela, M., Besnier, F., Nilsen, F., Glover, K.A., 2017. A pedigree-based
556 experiment reveals variation in salinity and thermal tolerance in the salmon louse,
557 *Lepeophtheirus salmonis*. Evol. Appl. 10, 1007–1019. https://doi.org/10.1111/eva.12505

558 McArdle, J., Bullock, A.M., 1987. Solar ultraviolet radiation as a causal factor of "summer syndrome"
559 in cage-reared Atlantic salmon, *Salmo salar* L.: a clinical and histopathological study. J. Fish Dis.
560 10, 255–264. https://doi.org/10.1111/j.1365-2761.1987.tb01070.x

561 Mo, T.A., Poppe, T.T., 2018. Risk of using cleaner fish in fish farming (In Norwegian), Norsk
562 Veterinærtidsskrift nr. 2/2018. Oslo. Available from: https://www.vetnett.no/norsk-
563 veterinartidsskrift-nr-2-2018

564 Noceda, C., Sierra, S.G., Martínez, J.L., 1997. Histopathology of UV-B irradiated brown trout *Salmo*
565 *trutta* skin. Dis. Aquat. Organ. 31, 103–108. https://doi.org/10.3354/dao031103

566 Oppedal, F., Juell, J.-E., Johansson, D., 2007. Thermo- and photoregulatory swimming behaviour of
567 caged Atlantic salmon: implications for photoperiod management and fish welfare.
568 Aquaculture 265, 70–81. https://doi.org/https://doi.org/10.1016/j.aquaculture.2007.01.050

569 Oppedal, F., Taranger, G.L., Juell, J.-E., Hansen, T., 2001. Growth, osmoregulation and sexual
570 maturation of underyearling Atlantic salmon smolt *Salmo salar* L. exposed to different
571 intensities of continuous light in sea cages. Aquac. Res. 30, 491–499.
572 https://doi.org/10.1046/j.1365-2109.1999.00362.x

573 Øverli, Ø., Nordgreen, J., Mejdell, C.M., Janczak, A.M., Kittilsen, S., Johansen, I.B., Horsberg, T.E.,
574 2014. Ectoparasitic sea lice (*Lepeophtheirus salmonis*) affect behavior and brain serotonergic
575 activity in Atlantic salmon (*Salmo salar* L.): perspectives on animal welfare. Physiol. Behav. 132,
576 44–50. https://doi.org/10.1016/j.physbeh.2014.04.031

577 Overton, K., Dempster, T., Oppedal, F., Kristiansen, T.S., Gismervik, K., Stien, L.H., 2019. Salmon lice
578 treatments and salmon mortality in Norwegian aquaculture: a review. Rev. Aquac. 11, 1398–
579 1417. https://doi.org/10.1111/raq.12299

580 Pettersen, J. M., Bracke, M. B., Midtlyng, P. J., Folkedal, O., Stien, L. H., Steffenak, H., Kristiansen, T.
581 S., 2014. Salmon welfare index model 2.0: an extended model for overall welfare assessment of
582 caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish
583 health professionals. Rev. Aquacult. 6, 162–179. https://doi.org/10.1111/raq.12039

584 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for

585 Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

586 Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10, 1–13.
587 https://doi.org/10.1371/journal.pone.0146021

588 Rochette, P.J., Bastien, N., Todo, T., Drouin, R., 2006. Pyrimidine (6-4) pyrimidone photoproduct
589 mapping after sublethal UVC doses: nucleotide resolution using terminal transferase-
590 dependent PCR. Photochem. Photobiol. 82, 1370. https://doi.org/10.1562/2004-12-01-RA-390

591 Sambraus, F., Fjelldal, P.G., Remø, S.C., Hevrøy, E.M., Nilsen, T.O., Thorsen, A., Hansen, T.J., Waagbø,
592 R., 2017. Water temperature and dietary histidine affect cataract formation in Atlantic salmon
593 (*Salmo salar* L.) diploid and triploid yearling smolt. J. Fish Dis. 40, 1195–1212.
594 https://doi.org/10.1111/jfd.12594

595 Samsing, F., Oppedal, F., Dalvin, S., Johnsen, I., Vågseth, T., Dempster, T., 2016. Salmon lice
596 (*Lepeophtheirus salmonis*) development times, body size, and reproductive outputs follow
597 universal models of temperature dependence. Can. J. Fish. Aquat. Sci. 73, 1841–1851.
598 https://doi.org/10.1139/cjfas-2016-0050

599 Setlow, R.B., Woodhead, A.D., Grist, E., 1989. Animal model for ultraviolet radiation-induced
600 melanoma: platyfish-swordtail hybrid. Proc. Natl. Acad. Sci. USA 86, 8922–8926.
601 https://doi.org/10.1073/pnas.86.22.8922

602 Severin, B.F., 1980. Disinfection of municipal wastewater effluents with ultraviolet light. J. Water
603 Pollut. Control Fed. 52, 2007-2018.

604 Skiftesvik, A.B., Bjelland, R.M., Durif, C.M.F., Johansen, I.S., Browman, H.I., 2013. Delousing of
605 Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture
606 402–403, 113–118. https://doi.org/10.1016/j.aquaculture.2013.03.032

607 Söderberg, P.G., 1990. Experimental cataract induced by ultraviolet radiation. Acta ophthalmologica
608 - Supplement 196, 1–75.

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

614 Stien, L.H., Dempster, T., Bui, S., Glaropoulos, A., Fosseidengen, J.E., Wright, D.W., Oppedal, F., 2016.
615 "Snorkel" sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon
616 with minimal welfare impacts. Aquaculture 458, 29–37.

https://doi.org/10.1016/j.aquaculture.2016.02.014

Sweet, M., Kirkham, N., Bendall, M., Currey, L., Bythell, J., Heupel, M., 2012. Evidence of melanoma in wild marine fish populations. PLoS ONE 7. https://doi.org/10.1371/journal.pone.0041989

Toovey, J.P.G. and Lyndon, A.R., 2000. Effects of hydrogen peroxide, dichlorvos and cypermethrin on subsequent fecundity of sea lice, *Lepeophtheirus salmonis*, under fish farm conditions. Bull. Eur. Ass. Fish Pathol. 20, 224-228.

Wagner, G.N., Fast, M.D. and Johnson, S.C., 2008. Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids. Trends Parasitol. 24, 176–183.

Wickham, H., 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag New York.

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. B 73, 3–36

Zeileis, A., 2004. Econometric computing with HC and HAC covariance matrix estimators. J. Stat. Softw. 11, 1–17. https://doi.org/10.18637/jss.v011.i10

Zeileis, A., Hothorn, T., 2002. Diagnostic checking in regression relationships. R News 2, 7–10.

Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

632 **TABLES**

633

634 **Table 1.** UVC dose levels given during Experiment 2.

Copepodid reduction (%)	Cumulative dose (J cm^{-2})	Day transferred	Exposure time (×15 min)
0	0 (control)	1	0
10	0.009	3	20
20	0.010	3	22
30	0.011	3	25
40	0.012	3	27
50	0.014	4	31
60	0.017	4	38
70	0.021	5	47
80	0.029	7	65
90	0.051	11	113

635 Dose levels are expressed as (left to right) percent efficacy against lice eggstrings (in terms of
636 copepodid reduction), cumulative dose, day of the trial at which the fish were transferred from the
637 treatment tank to the monitoring tank, and the number of 15 min UVC exposures received before
638 being transferred.

639

640 **Table 2.** Copepodid production per eggstring according to estimate dose received by eggstring (UVC-
641 treated tanks only)

Est. days exposed	Cumulative dose (J cm^{-2})	N (adult female lice)	Copepodids per eggstring (mean ± SD)
2	0.030	11	101 ± 60
3	0.045	15	103 ± 62
4	0.060	7	16 ± 28
5	0.075	9	9 ± 21
6	0.090	19	0.2 ± 0.7

642

643

644 **FIGURE CAPTIONS**

645

646 **Figure 1**. Layout of UVC lamps ('+') within 3 m diameter treatment tanks. Heatmaps depict the
647 relative distribution of fish in tanks with UVC lamps powered off (upper panel) and on (lower panel).
648 Data on fish position is the sum of fish in all three UVC tanks over the first 4 days of the trial. Units
649 are relative density.

650

651 **Figure 2.** Copepodid production (copepodids eggstring^{-1}) according to treatment group (UVC,
652 Control) and estimated cumulative exposure to UVC (corresponding to the estimated number of
653 days between extrusion and collection of the eggstring). The estimated date of eggstring extrusion
654 was based on the number of days between collection and first hatching and unpublished data on
655 temperature-dependent developmental rates. The dose-response relationship is fitted by a 3-
656 parameter log-logistic function.

657

658 **Figure 3.** Comparative images of eyes and skin condition of healthy (A-B) and UVC-exposed (C-D)
659 Atlantic salmon post-smolts. Image C shows suspected early stage cataract development (indicated
660 by an area of opacity at the centre of the eye) and a small haemorrhage on the iris. This was a
661 common eye pathology in UVC-exposed fish. Descriptions are based on externally visible symptoms.
662 Image D shows haemorrhaging at the base of the scales.

663

664 **Figure 4.** Frequency of rubbing and jumping behaviours in host salmon within Control and UVC
665 tanks. Values of stacked bars represent the sum of behaviour counts for all three tanks within each
666 treatment group. Observations were made during a 5 min period per tank, on two consecutive days
667 (total 10 min per tank), while lamps in the UVC tanks were turned on (left panel) and off (right
668 panel).

669

670 **Figure 5.** Salmon welfare scores from Experiment 2 (upper panel: skin condition; lower panel:
671 cataract severity) in relation to UVC exposure. Exposure levels are relative to effective doses (%
672 reduction in copepodid production) estimated during Experiment 1. Only data from Sample 1 are
673 shown (welfare assessments done at 6 days post exposure). The relationship between welfare score
674 and exposure level is illustrated using fits from a generalized additive model (skin condition) and
675 linear model (cataract).

676

677 **Figure 6.** Ventrolateral (left panels) and dorsolateral (right panels) epidermal sections from fish
678 exposed to varying UVC doses, showing thickening of the epidermis with a loss of cellular
679 organisation and reduced density of goblet (mucosal) cells. Panels A and B: no exposure; C and D:
680 30% effective dose; E and F: 60% effective dose; G and H: 90% effective dose. Scale bar is 50 µm.
681 Samples were sectioned at 5-7 µm thickness and stained with Alcian Blue-Nuclear Fast Red
682 (ventrolateral) or HE (dorsolateral).

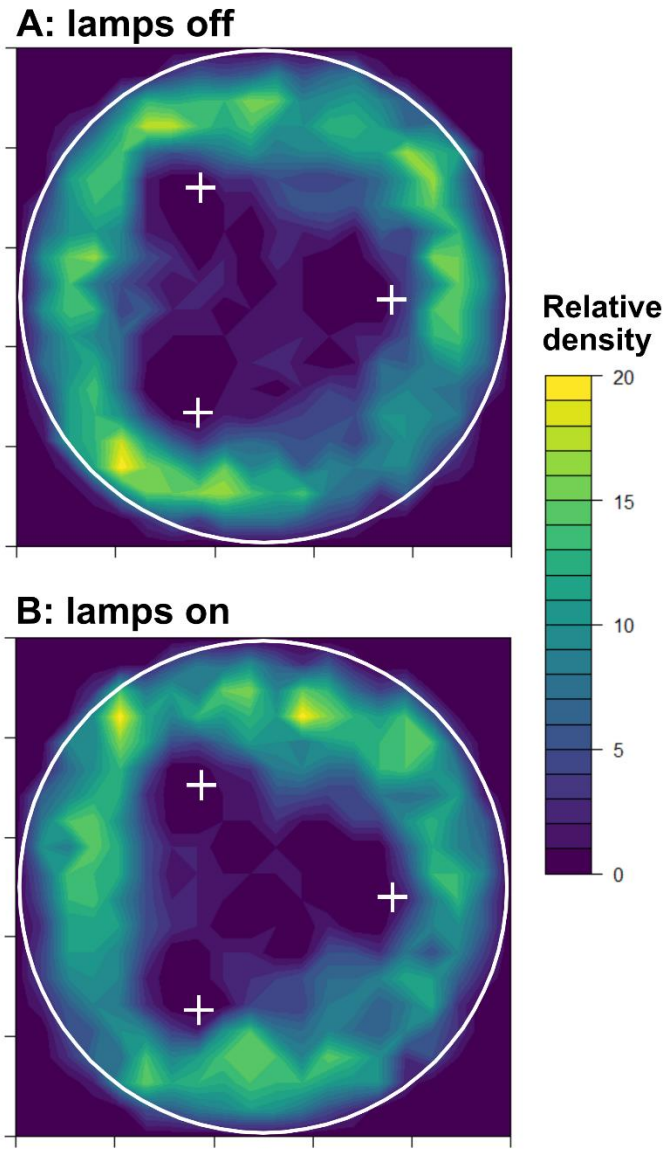
683

Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

684 **FIGURES**

685 **Figure 1**

686

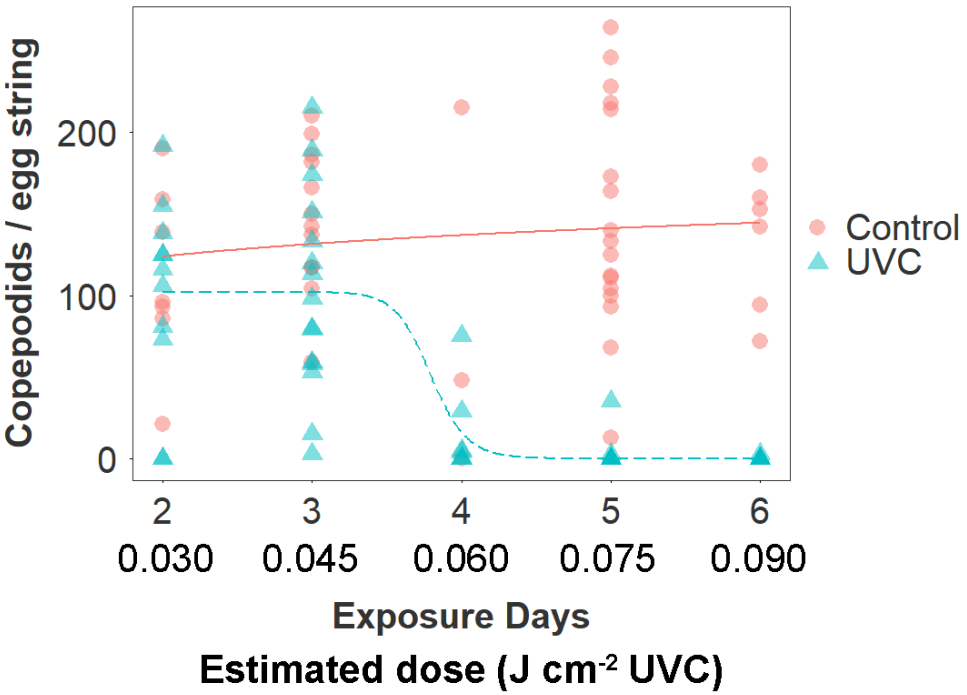


687

688

689 **Figure 2**

690



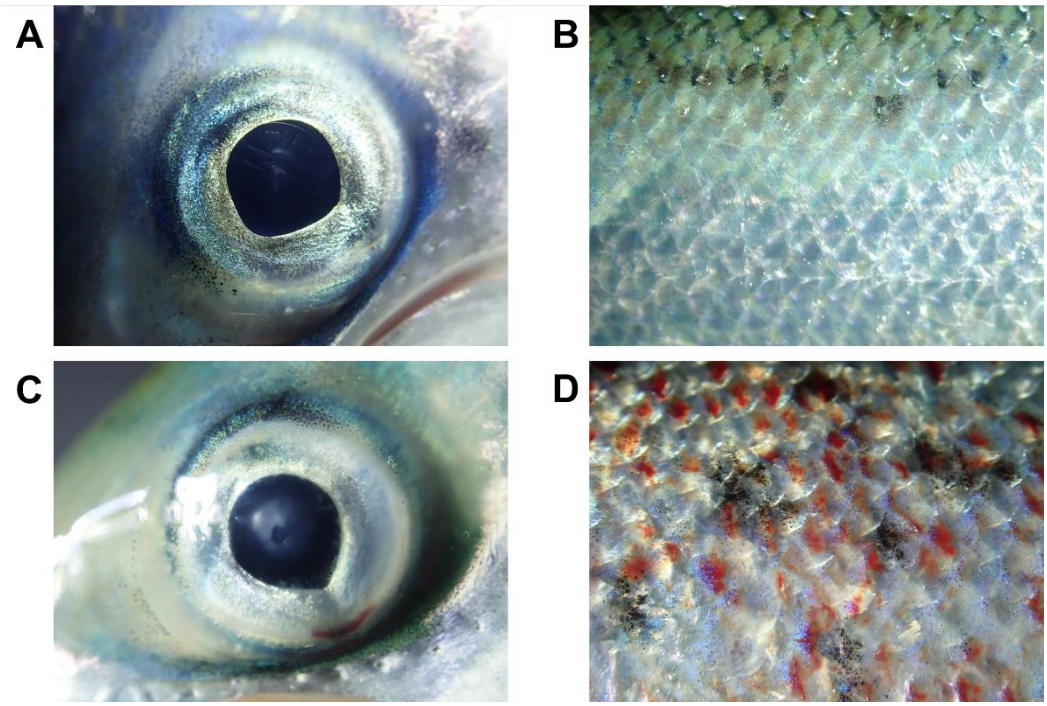
691

692

Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

693 **Figure 3**

694



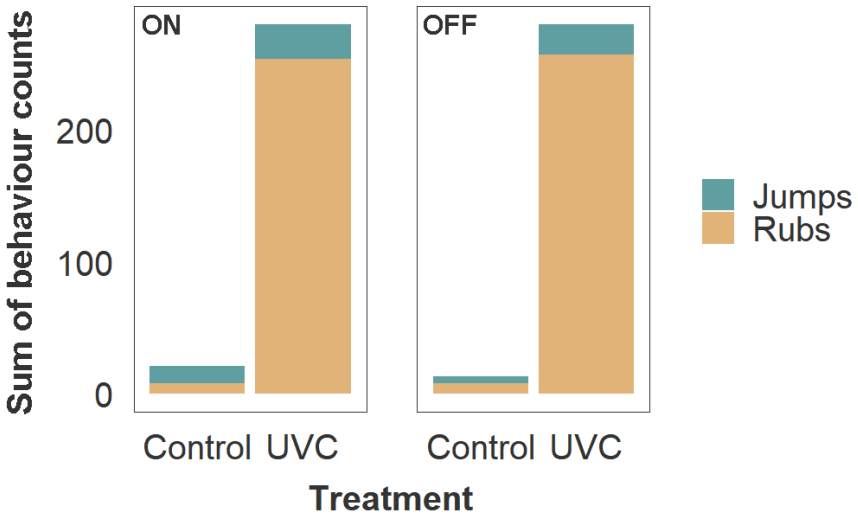
695

696

Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

697 **Figure 4**

698



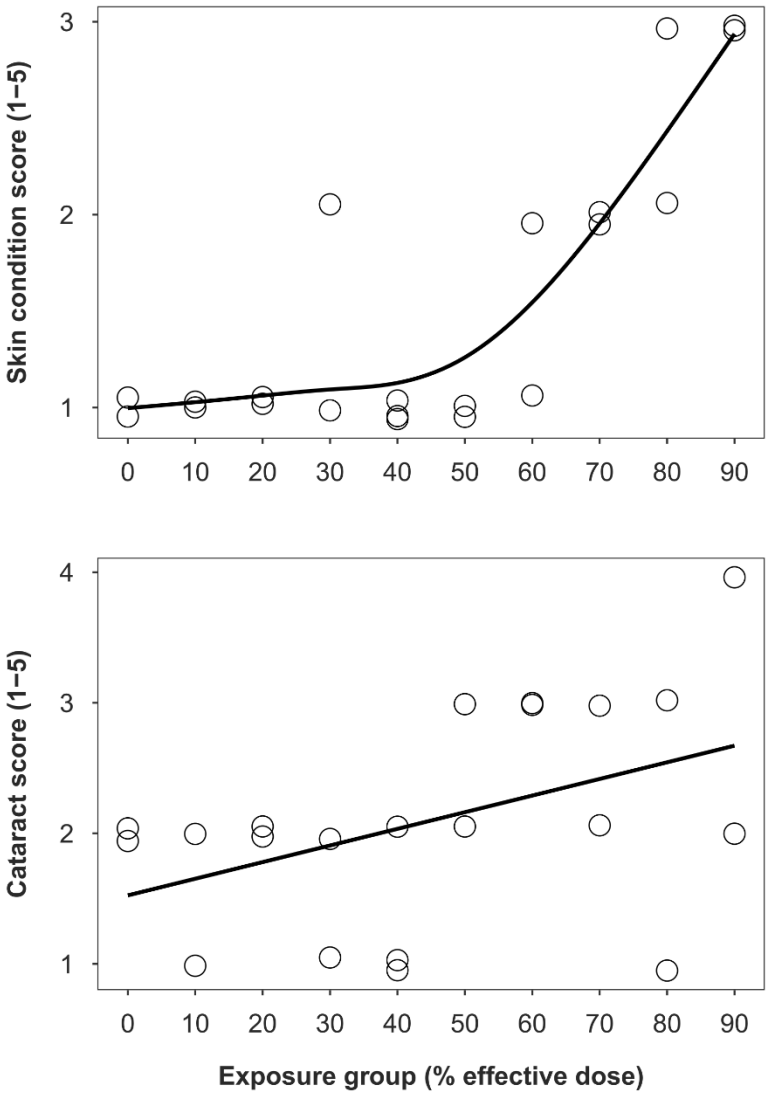
699

700

Cite as: Barrett LT, Bui S, Oppedal F, Bardal T, Olsen RE, Dempster T (2020) Ultraviolet-C light suppresses reproduction of sea lice but has adverse effects on host salmon. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2020.734954

701 **Figure 5**

702

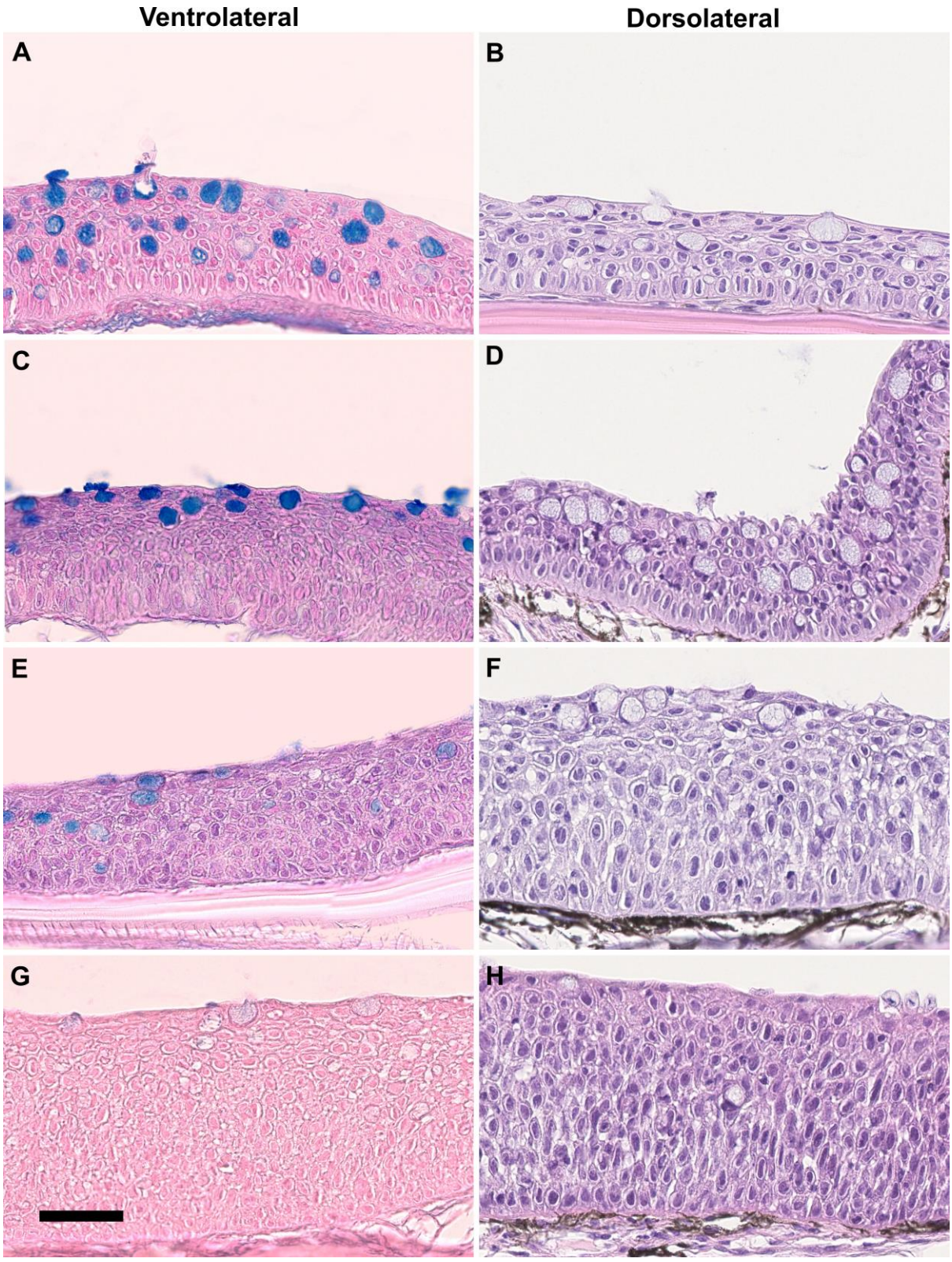


703

704

705 **Figure 6**

706



707

708