Research Article

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Sterilization of sea lice eggs with ultraviolet C light: towards a new preventative technique for aquaculture

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Abstract

BACKGROUND: Sea lice infestations on Atlantic salmon (*Salmo salar*) farms are a considerable burden on the industry and put wild salmonid populations at risk. Frequent delousing treatments are necessary to keep lice densities below allowable limits, but currently viable treatments have drawbacks in terms of financial cost, animal welfare, or environmental impacts. We tested if 254 nm ultraviolet C light (UVC) could function as a new preventative method to suppress reproduction of salmon lice (*Lepeophtheirus salmonis*) by sterilizing fertilized eggs. We exposed salmon lice eggstrings to a range of UVC intensities and durations to identify effective doses.

RESULTS: A cumulative dose of 0.008 J cm^{-2} induced 5% egg mortality, while 95% egg mortality occurred at 0.09 J cm^{-2}, indicating that UVC can be effective as a preventative treatment. The total cumulative dose appeared to be more important than the duration or number of individual exposures by which the total dose was achieved.

CONCLUSION: UVC treatment has immediate applications for the salmon aquaculture industry, including for the treatment of wastewater from delousing or other operations. Future work will assess the feasibility of UVC dose delivery on host salmon in sea cage environments that involves little or no fish handling and creates negligible environmental impacts.

Keywords: ectoparasites; parasite control; fish farming; salmon farming; UV irradiation

1 INTRODUCTION

Ectoparasitic sea lice infestations are one of the most serious problems facing salmon aquaculture across Europe and North and South America. Lice abundance is amplified by the high densities of salmon hosts held in sea cages, causing spill-back onto wild salmon and reinfestation of farmed salmon.[1,2] High lice densities present a survivorship challenge for wild salmon smolts on their migration to the open ocean in salmon farming regions, and can lead to significant reductions in recruitment,[3] while severe infestations on farmed fish create animal welfare concerns and reduced productivity.[4]

Promising new approaches can reduce the likelihood of new infections by minimizing encounters between parasites and hosts,[5–7] but regular delousing treatment is still necessary to reduce lice densities on farmed salmon. Delousing is costly[8] and most methods are stressful for the salmon, including mechanical, thermal and chemical treatments.[9] Moreover, some common delousing methods raise concerns for animal welfare, environmental impacts and growing treatment-resistance.[10–13] New chemical-free approaches with minimal handling of salmon and low environmental impacts are needed for ongoing suppression of lice populations at salmon farms and prevention of new infestations.

Ultraviolet (UV) light treatments may have the potential to reduce lice population growth, either by causing direct mortality of attached lice stages, or by rendering lice eggs or larvae inviable. At specific doses, UV light kills or inactivates viruses and cells by damaging DNA strands.[14,15] The UV wavelength components, UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm), have distinct properties, including their ability to penetrate substrates. UVA and UVB wavelengths pass through the Earth's atmosphere, so organisms exposed to direct sunlight have evolved physical and physiological defenses against these wavelengths.[14] The shorter UVC wavelength is filtered by the ozone layer and does not reach the Earth's surface. Cellular repair mechanisms are versatile and those that evolved in response to UVA and UVB radiation may also prepare cells for UVC radiation,[16] but UVC is relatively inexpensive to produce using low pressure mercury vapor lamps, and the peak 254 nm wavelength is readily absorbed by DNA but not proteins, allowing lethal DNA damage to be induced relatively efficiently.[15] Accordingly, 254 nm UVC has been the wavelength of choice for antimicrobial applications for over 50 years, including purification

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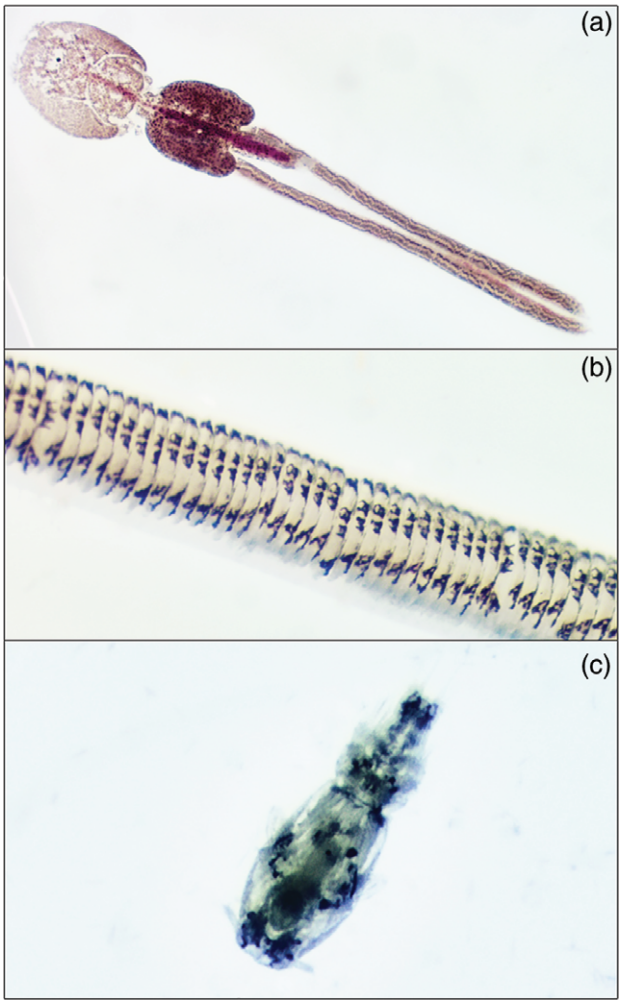

Figure 1. Adult female salmon louse *Lepeophtheirus salmonis* with eggstrings (a), closeup of developing embryos within an eggstring (b), and the infective copepodid stage (c). Image credit: Francisca Samsing.

of air, water and food preparation surfaces,[17,18] but has not yet been employed in the context of ectoparasite treatment.

Most current information of required doses relates to microbial organisms, but metazoans are also vulnerable; for example, complete mortality of newly laid insect eggs was induced by exposure to 56 J cm^{-2} of UVC irradiation,[19] while the same dose applied to larvae and pupae greatly reduced survival to adulthood. Lower doses may be effective against sea lice eggs, which are small and once fertilized, develop within a pair of external eggstrings trailing behind the female louse (Fig. 1). However, delivering an optimal dose *in situ* would require some control of the distance between the light source and the target organism, as the UVC wavelength is rapidly attenuated in water. Viable applications of UVC for salmon aquaculture may include treatment of water entering or exiting the cage (particularly during delousing operations where wastewater containing viable eggstrings is pumped out of the cage *via* pipes), or direct close-range exposure of lice and attached eggstrings on salmon within the sea cage. Salmon in sea cages typically swim in a circular path around the perimeter of the cage and could be exposed to numerous short UVC doses as they swim past stationary UVC lamps throughout the day.

In regions with a low density of wild hosts and a high density of salmon farms (most notably western Europe), the vast majority of new lice infestations on farmed salmon are caused by lice larvae that either originated from the same farm or were dispersed downstream from neighboring farms.[20,21] Effective suppression of lice reproduction within sea cages may therefore benefit the industry by reducing future infestation pressure. Here, we tested a range of doses of 254 nm UVC light to determine if they suppressed production of salmon louse (*Lepeophtheirus salmonis*) larvae. We conducted a multi-factorial experiment in which we varied light intensity, exposure duration, and number of exposures, to investigate the threshold at which the cumulative duration of multiple short exposures affected egg viability.

2 METHODS

2.1 Location and experimental setup

The experiment took place at the Institute of Marine Research (IMR) in Matre, Norway during November to December 2017. The UVC exposure trial was conducted in a rectangular raceway (260 × 41 × 16 cm, 170 L). Three 11 W mercury UVC lamps (254 nm) were located at one end of the raceway, housed within an open box lined with reflective material to direct light toward the raceway. To mimic the exposure regime that might occur as host salmon swim past stationary UVC lamps in a sea cage, eggstrings were exposed to timed bursts of UVC light using an opaque plastic divider that could be manually raised and lowered. No UVC light was detected in the raceway when the divider was lowered.

2.2 Study animals and incubation system

Adult female lice were collected from salmon at research sea-cages in the area and transferred to hosts held in indoor tanks (Ø = 3 m, volume = 5 m^3) at 15 °C, 34‰ salinity. O_2 saturation was maintained above 85%. Over the course of this experiment, lice eggstrings were obtained from this population when required.

At this temperature, females extrude a set of eggstrings approximately every 5 days, with hatching time of eggs correlated with time of extrusion (S. Dalvin, unpublished data). Female lice produce two eggstrings concurrently (Fig. 1), so we used a matched pair design in which one eggstring served as the control, while the other underwent the treatment.

Prior to a treatment run, eggstrings were collected from adult females and placed into individual incubation wells.[22] The incubators were supplied with flowing seawater from the same header tank that supplied the stock holding tank. An eggstring pair was only collected if both eggstrings appeared to be the same length to the naked eye.

2.3 Experimental design

To test the effect of UVC on the hatching success of salmon lice eggstrings, eggstrings were exposed to UVC at different intensities, exposure frequencies (10, 100, and 250 exposures) and exposure durations (2 and 5 s). UVC intensity was adjusted by changing the distance between the light source and the eggstring (eight distances from 10 cm to 200 cm). Together, the design provided 48 treatment combinations (eight intensities × three exposure frequencies × two exposure durations), with five to six replicate eggstring pairs per treatment combination.

UVC wavelength light is rapidly attenuated by seawater according to a negative exponential function. To quantify UVC light attenuation, and thus estimate UVC intensity at a given distance from the light source, we used a germicidal UVC sensor (PMA2122-WP, Solar Light, PA; sensitivity: 254 nm, 10 nm bandwidth; range:

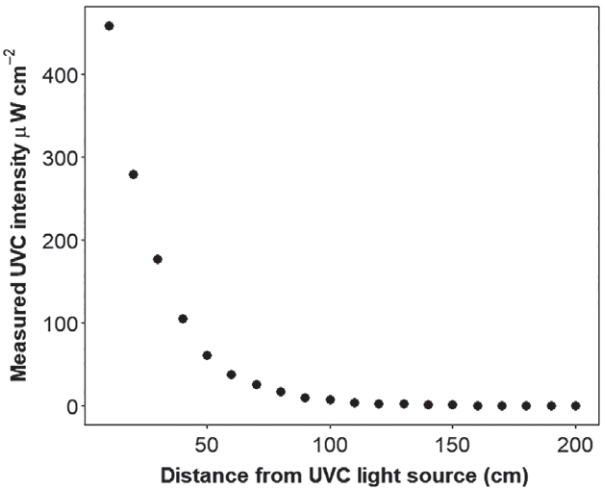

Figure 2. The maximum UVC irradiance (254 nm, 10 nm bandwidth) through seawater (salinity 34‰) at increasing distances, from three 11 W low pressure mercury lamps.

0–2000 μW cm^{-2}) and datalogging radiometer (PMA2100, Solar Light, PA, USA) to measure the maximum UVC intensity at 0.1 m intervals from the light source from 0 to 2 m in a raceway filled with seawater (15 °C temperature, 31.4‰ salinity, 8.7 mg/L oxygen). At each distance, the sensor was placed in the middle of the raceway and aligned to obtain the maximum intensity measurement. Measured intensity ranged from 459 μW cm^{-2} at 10 cm to 0.17 μW cm^{-2} at 200 cm (Fig. 2).

2.4 Exposure protocol

The raceway was filled with temperature-controlled water from the holding tank prior to each trial (15.1 °C ± 0.2, 8.9 mg/L O_2, 31.4‰ salinity). One eggstring from each pair was placed in the raceway at one of eight distances from the light source. 24 eggstrings could be treated simultaneously, with three eggstrings at each of the eight distances. At each distance, eggstrings were randomly allocated to the left, right or middle of the raceway. The location of each eggstring was noted so that the corresponding control eggstring in each pair could be placed in the same location for the subsequent trial.

Once the eggstrings were in place, UVC lamps were switched on with the divider lowered. After waiting 15 min to ensure that the lamps had reached their full output, the divider was repeatedly raised to expose the eggstrings for the chosen duration (2 or 5 s) with a period of 2 s between each exposure. After 10, 100 or 250 exposures, the eggstrings were removed and placed in individual incubation wells (one eggstring per well). The strongest treatment combination – 250 × 5 s exposures at 10 cm distance from the lamp array – provided a cumulative exposure of 0.57 J cm^{-2}.

The process was then repeated with the lamps switched off to apply the control treatment (using the second eggstring from each female). To avoid confounding temporal effects, the trial order (treatment-control or control-treatment) was randomly assigned throughout the experiment, and both eggstrings from each female were treated on the same day.

L. salmonis has three planktonic, free-swimming larval stages: eggs hatch as the planktonic nauplius I, then shortly after molt into the nauplius II and then the infective copepodid stage. Therefore survival after two molts (i.e. from hatching to copepodid) indicates potentially viable, infective individual. We monitored the incubation wells every day after treatment and recorded the date of first hatching for each eggstring. The number of living copepodids was measured 3–8 days after the first hatching date, as all lice should have reached the copepodid stage by day 3 and would still be viable up to 8 days at 15 °C (R. Skern-Mauritzen, unpublished data).

2.5 Statistical analysis

Two variables were recorded to test the effect of UVC treatment: hatching success, taken as a binary response (hatched or not hatched, with any nauplii production being sufficient for a score of 'hatched'), and developmental success of larvae, taken as the number of larvae per eggstring that successfully hatch and molt into the copepodid stage.

Hatching rates were compared across control and treated eggstrings using a χ^2 test of proportions. Developmental success across UVC doses was compared by fitting non-linear dose–response regression models in the *drc* package for R.[23] To avoid overfitting, model functions were ranked by Akaike's Information Criterion to identify the most parsimonious function. The significance of function parameters was tested using the coeftest() function in the *lmtest* package.[24] To account for variance heterogeneity, we used a robust covariance matrix computed by the *sandwich* package.[23,25] Dose–response plots were produced in *ggplot2*[26] using model predictions provided by the *drc* package. As there is considerable variation in the quality of eggstring pairs across females and batches, and it is difficult to detect treatment effects on eggstrings that would have had low hatching success regardless of treatment, we omitted data from 23/240 eggstring pairs in which the control eggstring produced <10 copepodids.

3 RESULTS

The UVC treatment had a strong effect on larval survival, but did not typically cause total hatching failure for salmon louse eggstrings; treated eggstrings had a 93% probability of producing at least one nauplius larva (224/240), compared to 95% (229/240) for control eggstrings (test of proportions: $\chi^2 = 0.6$, df = 1, $P = 0.4$). However, eggstrings exposed to UVC irradiation produced fewer copepodids. We observed large numbers of dead nauplii in incubator wells containing treated eggstrings; some died during or shortly after hatching, while others lived for several days as nauplii but did not molt into the copepodid stage. The reduction in copepodid production with UVC dose exhibited a sigmoidal dose–response relationship that was best fitted by a five-parameter log-logistic function (Fig. 3). Model parameters for the slope and inflection point of the response curve were highly significant (Table 1), indicating a significant dose-dependent effect of UVC exposure on copepodid production. Unexposed eggstrings produced 112 ± 4 copepodids per eggstring, while the strongest treatment resulted in only 9 ± 6 copepodids per eggstring. We observed 5% mortality at 0.008 J cm^{-2} and 95% mortality by 0.09 J cm^{-2}, although modelled estimates for effective doses came with considerable uncertainty at the upper end (Table 2). The 95% effective dose of 0.09 J cm^{-2} is equivalent to approximately 20 min at a distance of 50 cm (through seawater) from a 40 W low pressure mercury vapor lamp.

In this study, the total dose was delivered cumulatively by numerous exposures at a given distance. The effect of the dose was not strongly dependent on the specific exposure regimen of the number and duration of individual exposures comprising the total

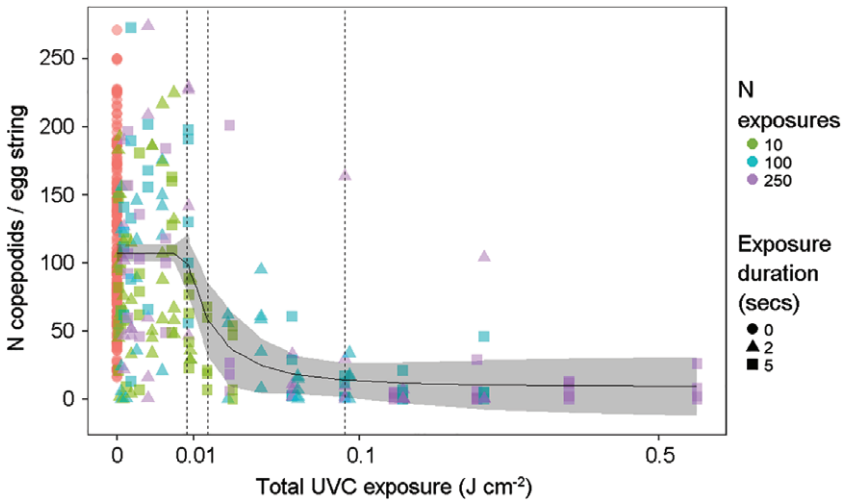

Figure 3. Number of copepodids produced per eggstring, according to total UVC exposure (combined light intensity and cumulative exposure duration). ($n = 434$ eggstrings). Symbols are colored and shaped according to the number and duration of short exposures comprising the cumulative total dose. Vertical dashed lines indicate modelled estimates for 5%, 50% and 95% effective doses (L-R).

Table 1. Tests of model parameters for five-parameter log-logistic function fitted to dose–response data. The model fits the relationship between UVC dose and copepodid production per eggstring. The standard error (SE), t-statistic (t) and *P*-value (*P*) are given for each model parameter

Parameter	Estimate	SE	t	*P*
b: Asymptote 1	29	33	0.9	0.38
c: Asymptote 2	9.4	8.7	1.1	0.28
d: Slope	107	3.3	33	**<0.0001**
e: Inflection	0.008	0.001	7.4	**<0.0001**
f: Asymmetry	0.04	0.03	1.2	0.21

Table 2. Predicted effective UVC doses (ED) for preventing hatching in 5%, 50% and 95% of lice eggs. The standard error (SE) and 95% confidence interval are given for each predicted effective dose

Parameter	Dose (J cm^{-2})	SE	95% CI
ED_5	0.008	0.001	0.005, 0.011
ED_{50}	0.01	0.004	0.006, 0.023
ED_{95}	0.09	0.13	−0.17, 0.35

dose (Fig. 4). However, at doses above ED_5, the most UVC-resistant eggstrings (inferred on the basis of high copepodid production) tended to be those for which the prescribed dose was spread over a larger number of exposures (Fig. 4). Dose–response functions fitted to data grouped by the number and duration of exposures were similar in inflection points and slopes, although the minimum asymptote differed between 2 and 5 s exposure groups (Table 3).

4 DISCUSSION

4.1 Effects of UVC light on salmon lice eggstrings

Exposure to UVC wavelength light caused substantial reduction in salmon louse copepodid production. The number of copepodids produced correlated negatively with the total dose received, with near total mortality induced at the highest doses.

Mortality occurred over several stages of egg and larval development. Although 93–95% of eggstrings underwent at least partial hatching regardless of the treatment, a smaller proportion of larvae that hatched from UVC-treated eggstrings successfully molted into the infective copepodid stage, leading to the observed reductions in copepodid production with UVC exposure.

The 95% effective UVC dose in this study (0.09 J cm^{-2}) is higher than the 100% effective dose for antimicrobial applications (0.00005–0.002 J cm^{-2}[18]:) and similar to effective doses for some UV-adapted microbes,[25] but much lower than the dose used to kill 100% of khapra beetle eggs and 98% of early stage khapra beetle larvae (57 J cm^{-2}[19]:). Large variation in effective doses is consistent with the properties of UVC wavelength light and biological tolerance. All else being equal, smaller organisms may be more vulnerable due to reduced attenuation through tissues, but there is also significant variation in evolved radiation tolerance between taxa regardless of size. UVC radiation selectively damages DNA *via* two potentially mutagenic base modifications: cyclobutane pyrimidine dimers (CPDs), and less frequently, pyrimidine (6–4) pyrimidone photoproducts (6–4PP).[15,27] Left unchecked, this DNA damage can lead to loss of cell proliferation (germ cells) or aberrant cell behaviour (somatic cells). Cellular mechanisms exist to mitigate DNA damage, either by directly reversing modifications or by excising damaged elements,[15] but when repair mechanisms are insufficient, cell death will occur. Cell death is catastrophic for single celled organisms and gametes, but in metazoans, damaged epidermal cells can undergo cell suicide and be recycled and replaced, perhaps allowing the organism to recover from relatively higher doses. Accordingly, we expect that higher doses would be required to kill attached lice stages than early stage zygotes.

4.2 Opportunities and challenges for industry deployment

This study demonstrates that UVC light has the potential to be an effective preventative lice treatment for the salmon farming industry by reducing the abundance of infective copepodid stages exported from farms. Atlantic salmon farms with relatively high numbers of adult female lice can produce an enormous quantity of larvae (150–965 eggs per eggstring[28]:), and thus are likely to contribute the vast majority of copepodids in coastal waters around fish farms.[29] Reducing export of copepodids from farms

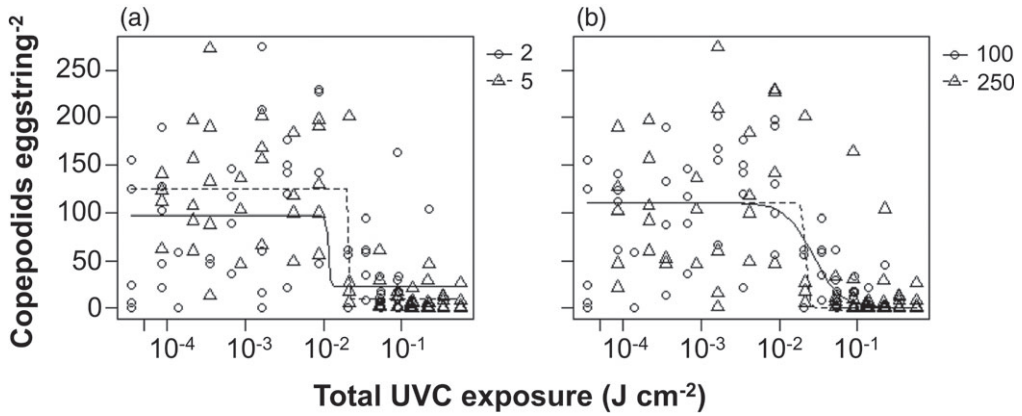

Figure 4. Number of copepodids produced per eggstring, according to total UVC exposure. Replicates are grouped by (a) the duration of individual exposures (2 or 5 s), and (b) the number of individual exposures (100 or 250 exposures). A three-parameter log-linear function best fitted the data.

Table 3. Comparison of dose–response function parameters according to (i) duration and (ii) number of individual UVC exposures comprising the total dose. The standard error (SE), t-statistic (t) and *P*-value are given for each model parameter

Parameter	Estimate	SE	t	*P*
(i) Exposure duration (2 *vs* 5 s)				
b: Slope	−150	11 810	−0.01	0.98
c: Max asymptote	14	12	1.1	0.26
d: Min asymptote	−28	13	−2.1	**0.04**
e: Inflection point	−0.01	0.03	−0.4	0.74
(ii) N exposures (100 *vs* 250 exposures)				
b: Slope	−18	1311	−0.01	0.99
d: Min asymptote	0.24	14	0.02	0.99
e: Inflection point	0.005	0.02	0.25	0.80

Data split by exposure duration (i) were best fitted by a four-parameter log-logistic function; data split by number of exposures and (ii) were best fitted by a three-parameter log-logistic function.

will result both in considerably lower infestation pressure for adjacent wild salmonid populations and lower farm re-infestation rates following delousing treatments.

UVC irradiation has immediate applications for aquaculture. Many current delousing methods for salmon farms (such as mechanical and thermal treatments) produce wastewater containing live lice, viable eggstrings and other pathogens that can enter the environment and increase reinfection risk. Filtering of this wastewater is increasingly being used, but concentrated high doses of UVC irradiation applied to wastewater may be a less expensive and more reliable method of preventing viable eggs from entering the environment.[17] UVC treatment may also be applied to other forms of wastewater, such as outflow from dead fish collection systems. Light intensities to achieve an effective dose will depend on flow rates and turbidity of water.[18]

Other potential applications require additional research before deployment. One possibility is to target salmon lice eggs while still attached to the host, by directly exposing infested salmon to specific doses of UVC irradiation in the sea cage environment. The doses leading to 50–95% egg mortality (0.01–0.09 J cm^{-2}) could be achieved over the developmental period of an eggstring in a sea cage (1–3 weeks depending on water temperature), and if applied whenever lice loads are high, may dramatically reduce densities of infective stages in and around salmon farms. Eggstrings in sea cages would likely be exposed to numerous small doses as hosts repeatedly swim past UVC light sources. Reflecting this, we used a dosing regime of numerous short exposures, with a varying number of exposures, exposure durations and distances from the light source. Importantly, there was no clear effect of the exposure regime on efficacy of the cumulative dose – a promising result for in-cage UVC treatment. It is worth noting that differential effects may occur with exposure regimes not tested here (*e.g.* one continuous exposure or long durations between multiple exposures). Research is needed to assess the effects of in-cage UVC irradiation on host fish, as well as any environmental impacts (*e.g.* plankton communities passing through sea cages).

This study primarily assessed effects of UVC on mortality of lice eggs and larvae, but UVC irradiation at sublethal levels may also have important suppressive effects on lice populations. Sterilized individuals, especially males, can disproportionately impact populations by deceiving conspecifics into investing in infertile matings.[30] This strategy has been successfully used to control other pest species, including the release of male mosquitos sterilized by radiation to function as 'ovitraps' for female mosquitos,[31] and may be particularly effective against sea lice as female lice are frequently mate-limited.[32]

Lethal or sterilizing doses may conceivably be applied to all lice life stages. Combined with existing lice control methods to minimize encounter rates or remove attached lice from stock, UVC has potential to assist in the ongoing suppression of sea lice populations. Benefits will be maximized by having continuous treatment at key sites to 'disconnect' lice populations, thus reducing reinfection risk and slowing the evolution of treatment-resistant sea lice.[33]

5 CONCLUSION

Sustainable growth of the Atlantic salmon farming industry requires new techniques to reduce the amplifying effect of farms on salmon lice abundance, for the benefit of both farmed and wild fish. We have demonstrated that sufficient doses of UVC irradiation can dramatically reduce production of infective stages from salmon lice eggstrings and recommend immediate deployment for treatment of delousing system outflow and other wastewater from salmon aquaculture. Future work will investigate the feasibility of treating eggstrings on lice attached to salmon in tank and sea cage environments.

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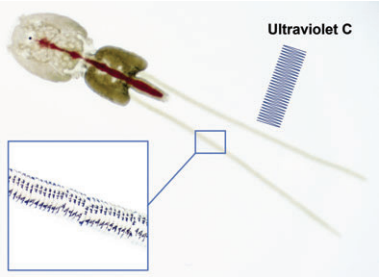
Research Article

New methods are needed to prevent and control sea lice infestations in salmon aquaculture. This article presents a test of ultraviolet C irradiation for reproductive suppression in sea lice.



Sterilization of sea lice eggs with ultraviolet C light: towards a new preventative technique for aquaculture

Luke T Barrett, Cassandra G Pert, Samantha Bui, Frode Oppedal and Tim Dempster

