Optimising handling in salmon aquaculture (2): The effect of mesh type on cryptic lesions and hygiene

Report:
UFAW grant application 25-20/21

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Summary

Atlantic salmon, *Salmo salar,* interact with humans during recreational and commercial activities, in both freshwater and marine environments. This involves routine hand netting, with a modest body of literature proving that handling techniques which reduce abrasion also minimises scale loss and likely improves fish welfare. In a recent study, the use of a rubber mesh, compared to a knotless equivalent, was shown to reduce scale loss in two size cohorts of the study species during routine movement between tanks.

The current study aimed to investigate this further via a Fluroscein dye technique, now commonly used to visualise cryptic damage to the mucus membranes of fish (eyes and more recently, skin). Although the technique was shown to work using easily available and cost-effective consumables, negligible damage was observed in any of the individually handled salmon regardless of mesh type. Previous studies have shown that the extent of observable skin damage is influenced by species, behaviour and anaesthetic technique. Although encouraging for this particular stock, it is unknown if higher net capture densities (i.e. more than one fish per net) or stock undergoing smoltification would be so robust.

Scale loss data collected simultaneously proved a link between fish size and the extent of scale loss. A positive correlation was of individual smolts netted with knotless mesh. This was not significant for rubber mesh which showed low scale loss regardless of fish size, suggesting a further benefit of using the latter mesh type and supporting the findings of previous studies.

Finally, *in vitro* microbiological study exposed sterile mesh fragments to effluent tank water allowing absorbance and adherence of bacteria to the mesh matrix. Subsequent incubation in sterile saline then permitted estimation of bacterial transfer between the exposed mesh to a further medium. Knotless mesh transferred more *Vibrio spp.* than rubber mesh, and this was significantly greater for total heterotrophic bacteria. This is likely due to the greater surface area and absorbance of infected water found in the filaments of knotless mesh. Encouragingly, following exposure to a standard aquaculture disinfectant, both mesh types were found to be sterile.

These findings further support the likelihood that rubber mesh reduces scale loss in Atlantic salmon, and also suggests reduced microbial transfer when rubber mesh is used for routine handling.

Keywords: animal welfare, salmonid, microbiology, mesh, skin damage, aquaculture

Introduction

Atlantic salmon (*Salmo salar*) interact with humans across several sectors, such as an expanding aquaculture industry, recreational and commercial fishing, and environmental sampling (Olaussen 2016, Malcolm *et al* 2019, Cook *et al* 2019, FAO, 2020). These activities involve capture and handling, often requiring the use of nets which remove protective mucus and scales. Subsequently, this may lead to pathogen invasion and osmoregulatory stress (Brydges *et al* 2009; Cook *et al* 2019). Minimising scale loss during routine handling operations would be beneficial to fish health and welfare, and for allied research sectors is an example of experimental refinement which can improve the outcome of experiments by reducing stress, disease and injury, and hence variation (Brydges *et al* 2009).

One approach is to use improved mesh and net designs during handling, with at least one study comparing angling handling practices using a wild salmonid population (brook trout *Salvelinus fontinalis;* Lizeé *et al* 2017). A more recent study (Powell, 2021) exposed two graded size classes of farmed Atlantic salmon to hand net mesh, finding that rubber coated mesh significantly reduced the number of lost scales, compared to a conventional knotless equivalent. This was proven discretely for two graded size classes, although since the provenance between stock was different, any statistical comparison between scale loss between size classes was not investigated. There also remains a need to examine if mesh types can differentially damage scale-less areas (eyes, fins) or mucus and epithelium adjacent to scales.

Fluorescein, a dye used to visualise damage in mucus membranes such as the surface of the eye, was proven as a clinical diagnostic tool for a variety of freshwater fish species *ca.* 20 years ago (Noga and Udomkusonsri 2002). Recently, Fluorescein has been used to visualise the effects of standard knotless mesh types (different plastic manufacture) on the integument in yellowtail tetra *Astyanax altiparanae* (Alvarez-Rubio, 2020). Compared to polypropylene or polyethylene, nylon mesh caused greater skin damage when visualised using Fluorescein. Following challenge (bacterial bath exposure), fish handled in nylon mesh recorded higher mortality rates. This interesting finding could be developed further, to

investigate the bacterial loading of mesh types. Simply, if knotless mesh has a greater surface area and is more absorbent than rubber mesh, over time this could harbour or transfer pathogens between fish or fish populations and deliver bacteria directly to damaged areas of the integument.

This project aims to investigate the extent that rubber mesh can further improve fish welfare by a) reducing mucus and epithelium loss, including scale-less areas such the fins and eyes; b) improving hygiene during fish handling, via a non-evasive approach (simulated net use in a fish tank and subsequent microbiological comparison between mesh types).

Materials and Methods

Ethical statement. The current study counted lost scales at opportune moments during infrequent stock management culls (i.e. the primary purpose of fish handling and culling was never to collect data), or employed microbiology of culture system water (and did not require the use of animals). Ethical approval was approved by University of Stirling AWERB (application (19/20) 207) as a "non-ASPA" study permitting the use of up to 60 animals.

Salmon stock, aquarium system and husbandry. The approach was similar as described in Powell (2021) with minor alterations. Incoming smolts (*ca.* 100g) used in this study (sourced from Niall Bromage Freshwater Research Unit, Institute of Aquaculture, Stirling University) arrived at MERL in late October 2020 and were initially stocked in 6000L stocking tanks at 15 kgm^{-3} stocking density. In early December, 70 salmon were relocated to 2m diameter stock tank (2000L, stocking density *ca.* 6 kgm^{-3}) and were not handled for 90 d until a management cull was required for a proportion of the stock. Salinity and temperature varied between entry to the facility and management cull (29-33 ‰, 5-10°C).

Salmon data collection. An overview of salmon manipulation and Fluorescein exposure is provided in Figure 1. Smolts (total n = 37; weight 292 ± 12.0 g) were removed individually from tanks in a rapid, fluid motion, remaining emersed in the net for *ca.* 2 s. Individual salmon were placed into meticulously

cleaned cylindrical containers, containing 50 L seawater to minimise collision, with the mesh type alteration and scale counting procedure as described in Powell (2021). Upon reaching deep anaesthesia (Tricaine methanesulfonate, 100 mgL^{-1} seawater, Pharmaq Ltd, Fordingbridge, UK), death was confirmed by both pithing and destruction of cervical vertebrae using a scalpel. All manipulation was via the oral cavity and gill arch (S-shaped meat hook) to reduce any artificial mucus loss and epithelial damage from manual handling.

A third (true control) treatment was granted via the Ethical Application. This was not stated in the grant application, since it was not possible to guarantee commissioning during a management cull. Terminal anaesthesia and confirmation of death via rigor mortis *in situ* (i.e. in the tank) is a valid method to cull large numbers of fish at MERL, and employing this method would not require net manipulation of any kind. However, the decision was made to not proceed (see Results and Observation section).

To reduce quenching of Fluroscein dye (Davis et al., 2008), individual salmon were suspended in 50L seawater for 3 min to remove any adhering anaesthetic, prior to exposure to excess dye. Salmon were then exposed to Fluorescein disodium salt (trade name Acid Yellow 73; $0.5gL^{1}$, 5 min; Drain Tracing Dye, Monument Tools, Wallington, UK). To reduce Fluorescein carryover (Davis et al., 2008), individual salmon were finally rinsed in in 50L seawater for a further 1 min. Salmon were then individually photographed using a mobile phone (Samsung A40, Samsung C&T U.K. Ltd, London, UK) on both sides, under either white light or in darkness using a handheld UV torch (51 LED, 395 nm; Youthink brand) with both torch and camera maintained in a tripod at *ca.* 40 cm height. The salmon were then weighed (Mettler Toledo Spider 2S; Mettler Toledo Ltd, UK). Images of individual salmon flanks were prepared for analysis after Colotelo and Cooke (2011), with the intention to use ImageJ software and pixilation to calculate the proportion of fluorescent skin areas over the entire outline. In addition, scales were collected as described in Powell (2021).

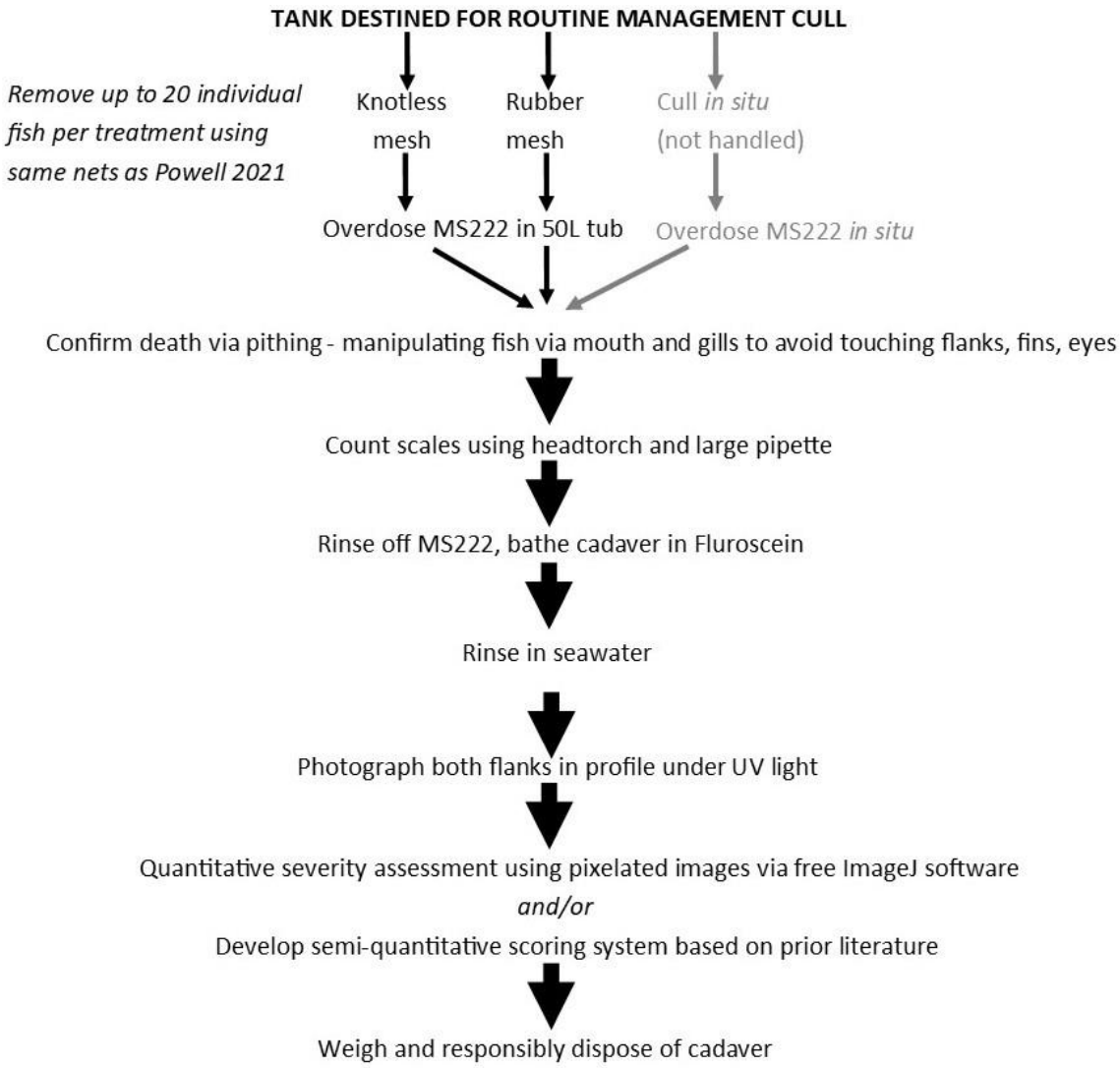

Figure 1. *Overview of net handling procedure and data collection*

Mesh and water samples. Both rubber and knotless nets were thoroughly rinsed and allowed to air dry for 10 d. Irregular mesh sections were cut from both nets (n = 12) and weighed (Mettler Toledo AB104, Mettler Toledo Ltd, UK; rubber mesh, 2.254 ± 0.103 g; knotless mesh, 2.608± 0.150 g) and then autoclaved in individual foil parcels (121 °C, 3 h). A 1 L quantity of MERL tank system effluent water from a 2m diameter tank containing 30 salmon, *S. salar,* (*ca.* 15 kg biomass) was taken as a common source for further microbiology and placed in a clean stoppered vessel on ice.

Microbiology. Under sterile technique, mesh fragments were exposed to system water and a standard aquaculture disinfectant as described below (Figure 2). To ascertain system water bacterial load,

aliquots (*n* = 4, 1 ml) were taken from system water stock as a positive control and placed on ice. Further samples (24 x 50 ml) were removed sequentially into sterile 60 ml containers. Individual mesh samples added aseptically, briefly agitated and incubated at an average annual water temperature (60 min; 11 °C). Half (*n* = 6) mesh samples were removed aseptically, added to 1 ml sterile seawater and agitated constantly (60 min; 11°C) using a rock tumbler (Manchester Minerals Ltd, UK). Aliquots were removed and placed on ice.

In addition to the work plan stated in the grant application, the efficacy of disinfectant was compared between mesh types. After the initial 60 min agitation in system water, the remaining mesh samples (*n* = 6) were individually added to 50 ml Halamid (Chloramine T) at the recommended concentration to disinfect nets (1%, 30 min; Axcentive SARL, 2020). To remove all traces of disinfectant before cell culture, mesh samples were individually rinsed sequentially 3 times (50ml excess sterile 3% NaCl, 5 min). Finally, mesh samples were agitated for a further 60 min at 11° C with water samples aliquoted as above. Control system water and all mesh water aliquots were serially diluted (sterile 3% NaCl) and plated in sextuplicate (20 µL drops) on Tryptic Soy Agar (TSA) + 1.5% NaCl and Thiosulphate Citrate Bile Salts-sucrose (TCBS) agar either neat (without dilution) or up to x 1000 dilution. Plates were incubated at 11 °C for up to 7 days, with CFU calculated as CFU g^{-1} mesh or CFU ml^{-1}.

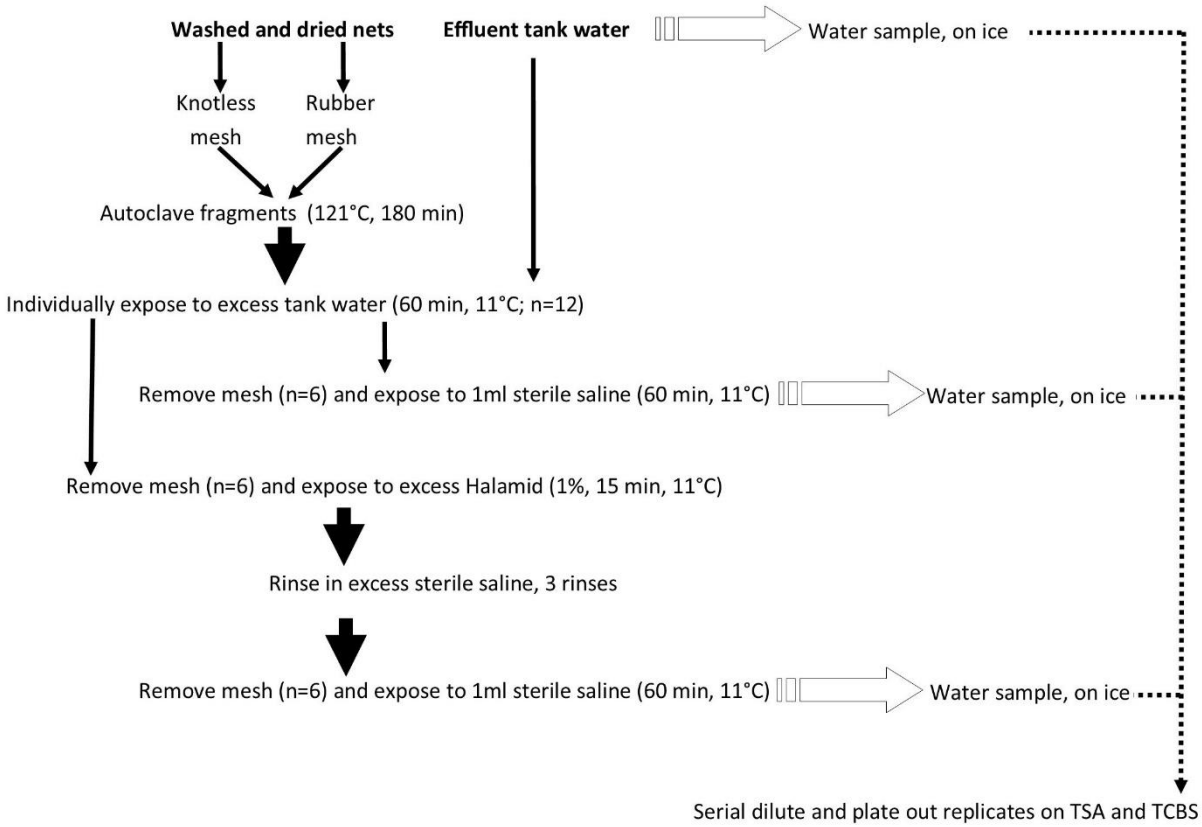

Figure 2. *Overview of mesh sampling and microbiology*

Data analysis. All data was analysed using GraphPad-Prism (GraphPad Software Inc San Diego, USA). All data shown are mean ± 1 SEM (other than regression where a 5 % confidence limit is shown surrounding regression lines) and were confirmed for normality and homogeneity of variances (Kolmogorov-Smirnov test; Bartlett's test respectively) prior to further analysis. Salmon weight and scale loss data were analysed using regression (scale loss *vs* wet weight) to ascertain the equation of a straight line, 95 % confidence limit and goodness of fit (r^2). An F statistic was employed to ascertain slopes were significantly different between mesh types. For microbiology, unpaired Student's *t*-test (with Welch's correction, since variance were different between data sets) was used to compare bacterial loading between mesh types and treatments, discretely for all culturable total heterotrophic bacteria (TSA data) or *Vibrio spp.* (TCBS data).

Results and observations

Salmon weight, scale loss and cryptic damage. During initial data collection, it became apparent that despite obvious and recordable scale loss, negligible or zero cryptic damage could be observed by Fluorescein treatment after netting with either mesh type. Fluorescence was only obvious during an initial trial with salmon skin fillet, the eye of one individual experimental fish showing mild unilateral exopthalmia, and another individual with experimentally damaged skin performed after culling (see appendix). Additionally, the nostril openings of all experimental fish were coloured by Fluorescein, providing a positive control that the process had worked for every photographed individual (see appendix). This proved that the technique was working using the product, concentration time and photographic method used.

Due to these circumstances, the additional third control treatment (culling *in situ* without any netting) was not attempted, as it was unlikely that this process would yield any fluorescence data and certainly no scale loss data. However, initial investigation of fish weight and scale loss data showed that the tank, which had not been graded recently, displayed a relatively wide weight range. To complete the management cull, remaining fish were netted to increase the *n* number for rubber and knotless mesh treatments, to investigate any relationship between mesh type, scale loss and fish weight for individually handled fish from the same tank and cohort. This was deemed the best course of action to take and would also provide novel data to support the findings by Powell (2021).

Significantly fewer scales were lost for smolts handled using rubber, compared to knotless mesh (Figure 3; difference between slopes extremely significant, $P<0.01$). Scale loss increased with weight for fish handled in knotless mesh ($r^2 = 0.046$; regression significantly non-zero, $P < 0.001$) but was not proven for fish handled in rubber mesh ($r^2 = 0.61$; regression not significantly non-zero).

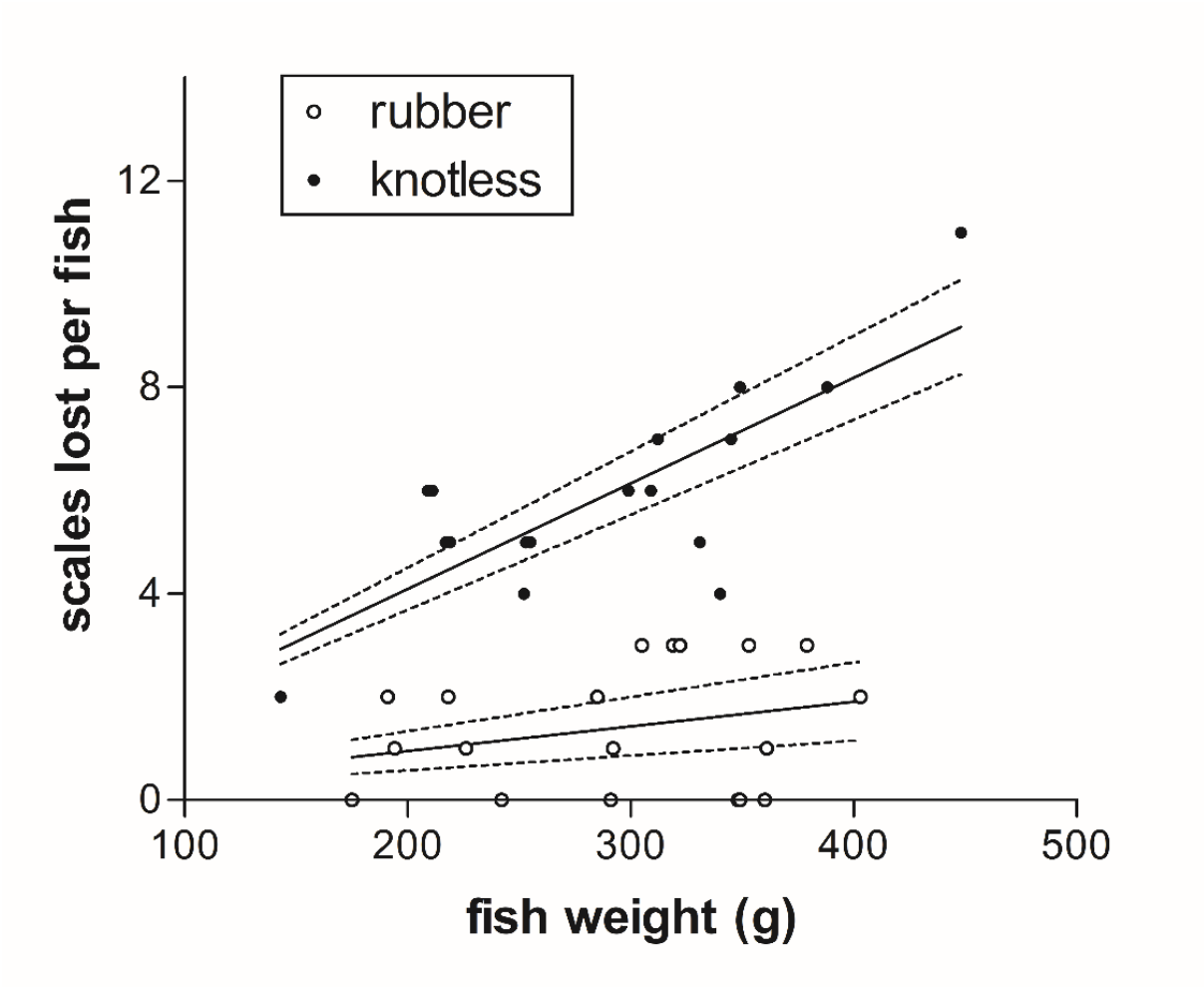

Figure 3. Atlantic salmon smolts *S. salar,* individually exposed to rubber mesh (294.5 ± 15.93 g, n=19) or knotless mesh (287.7 ± 17.62 g, n=18) during simulated hand netting. Dashed lines show 95 % confidence limit. Equation for rubber mesh: y = 0.003739 * x + 0.3166. Goodness of fit r^2 = 0.046. Slope is not significantly non-zero (F = 0.81, P > 0.05, DFn = 1, DFd = 17). Equation for knotless mesh: y = 0.02017 * x +0.08691. Goodness of fit r^2 = 0.61. Slope is significantly non-zero (F = 24.57, P < 0.001, DFn = 1, DFd = 16). Rubber and knotless mesh slopes are significantly different (F = 7.99, P < 0.01, DFn = 1, DFd = 33). For clarity, graph is shown with line forced through origin, however statistics performed on non-corrected data.

Net microbiology. The mean bacterial load of the system water used in the experiment was 4542 CFU/ml (TSA plates; total heterotrophic bacteria) and 404 CFU/ml (TCBS plates; *Vibrio spp.*). Following incubation in system water, both mesh types transferred significant numbers of bacteria to sterile water, allowing them to be recovered.

For total heterotrophs, the bacterial loading of knotless mesh was 831.4 ± 219.4 CFU/g, significantly higher than rubber mesh (185.2 ± 19.7 CFU/g; unpaired Student's *t*-test with Welch's correction, P = 0.0325). This corresponded to *ca.* 18% and 4% of the bacterial loading of the system water for knotless and rubber mesh, respectively (Figure 4). For TCBS, *Vibrio spp.* loading for knotless mesh (21.1 ± 7.1 CFU/g, *ca.* 5% system water) was higher than rubber mesh (6.1 ± 1.2 CFU/g, *ca.* 1.5% system water), but not statistically significant (unpaired Student's *t*-test with Welch's correction, P = 0.0907).

For the other net samples additionally incubated in Halamid disinfectant and sequential multiple rinsing in sterile saline, no bacteria were cultured for either mesh type, replicate or on any agar (data not shown; Figure 4).

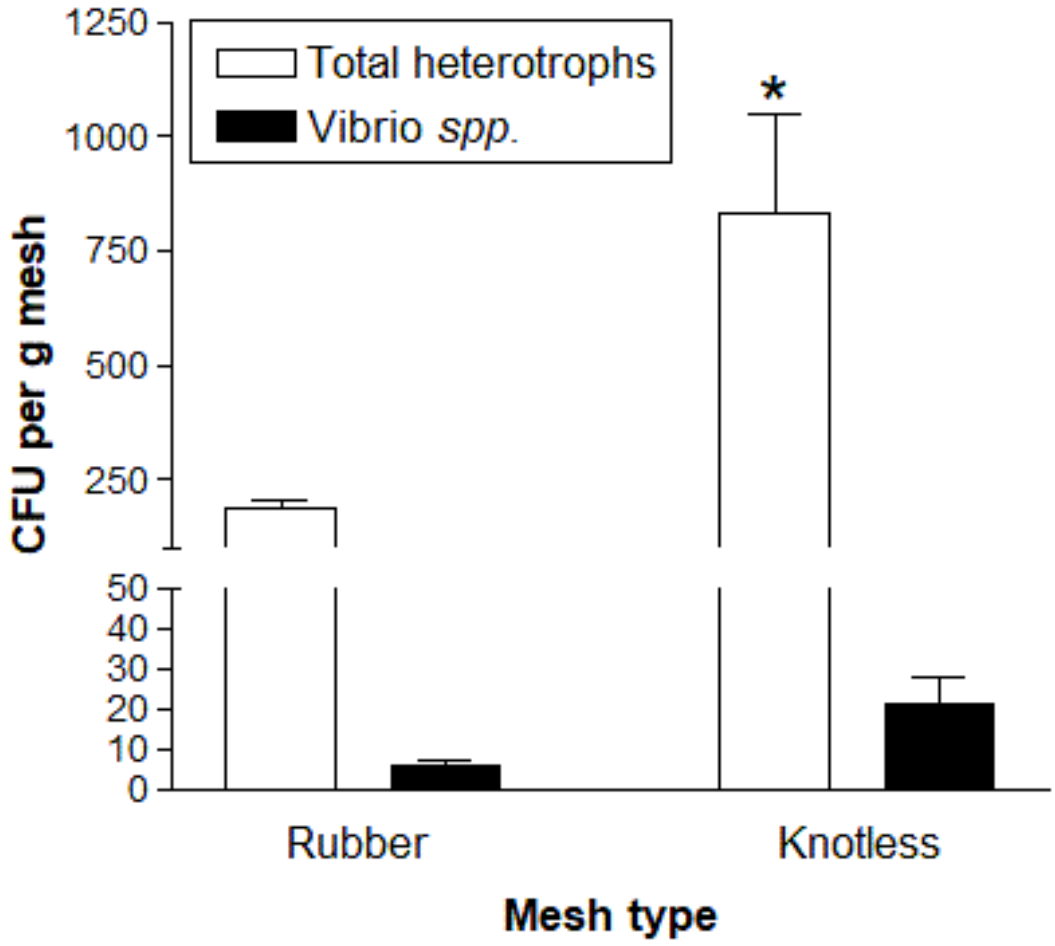

Figure 4. Microbiology of rubber and knotless mesh fragments (*n*=6), exposed to system water outflow from *S. salar* stock tank. Culturable bacteria converted to CFU/g mesh, either total heterotrophs (TSA) or *Vibrio spp.* (TCBS). Data for mesh exposed to Halamid not shown, since no bacteria were recovered in any sample. Asterisk denotes significantly higher total heterotroph load for knotless compared to rubber mesh (Unpaired Student's *t*-test with Welch's correction, $P<0.05$).

Discussion

From our observations, Fluorescein treatment did not highlight any cryptic damage to salmon smolts following a few seconds handling, for either standard knotless mesh or rubber mesh. Whilst fluorescein treatment has highlighted cryptic epithelial damage in several fish species, the severity and location appears to be influenced by species (Noga and Udomkusonsri, 2002), by behaviour during capture (Colotelo and Cooke, 2011) and in freshwater species may be more severe due to a lack of buffering when using MS222 anaesthetic (Davis et al., 2008). Furthermore, in some instances a significant physical insult was required to elicit damage, for example experimental injury on culled fish using a scalpel (e.g. Noga and Udomkusonsri, 2002), or prolonged handling (Alvarez-Rubio 2020; Davis an Ottmar, 2006).

In contrast, the salmon stock in the current study were maintained at a low density, had not been handled for many weeks, had undergone smoltification, benefitted from natural buffering of seawater and were exposed to minimal handling, suggesting several reasons for the negligible wounding observed. Since the technique appeared to be functioning satisfactorily (see appendix), it must be concluded that the brief netting protocol in our stock was apparently benign, in terms of maintaining the integrity of mucus and epithelial tissue (if not minor scale loss). Whilst this is encouraging in terms of day-to-day fish handling and welfare in our facility, only individual fish were handled (rather than multiple fish, at higher net capture densities), and minor scale loss was still observed in most handling replicates. Although not studied, it is possible that recently arrived fish from freshwater, entering smoltification, would have been more sensitive.

The effect of fish size on scale loss is an interesting addition to the findings of Powell (2021). Briefly, this study investigated handling of two graded size classes of *S. salar*, showing that rubber mesh reduced scale loss compared to a knotless alternative. However, due to the different stock provenance and potential tank effect, any effect of fish size on scale loss was not analysed. In the current study, a range of fish sizes from *ca.* 150-450g from the same origin and tank were handled and analysed

without any potential confounding influences. The results detail a negligible effect of fish weight and scale loss when handled with rubber mesh, in comparison to a clear correlation of fish weight and scale loss when a standard knotless mesh is used. This adds further evidence that rubber mesh is less abrasive, and that for knotless mesh at least, scale loss corresponds to fish weight, muscular power and likely skin attrition against the mesh during increasingly aggressive net roll (Barthel *et al* 2003; Olsen *et al* 2012, Powell 2021). Whether a reduction in mesh size or shape could reduce skin attrition in larger fish would be a useful further avenue for commercial net development.

The microbiological comparison of net types proved that meshes adsorb bacteria, including *Vibrio spp.* from system water, and have the potential to transfer a significant proportion (up to 18%) back into adjacent water. Although this was a simulated *in vivo* study using effluent water, it is reasonable to assume that a more sophisticated study (e.g. molecular typing of species; use of fish mucus as a bacterial source, or perhaps swabbing of fish before and after contact with nets) would reach similar conclusions.

Bacterial biofilms have been suggested as reservoirs for infection in aquaculture operations, with netting filaments, in particular, detailed as substrates which are colonised by pathogenic bacteria (Cai and Arias, 2017). The higher bacterial loading of knotless mesh is likely to be due to the greater surface area of the material allowing more water to be absorbed, retained and bacterial cells to attach, (compared to rubberised surfaces which have reduced filaments), since the bacterial count was normalised in terms of CFU/g mesh. In a commercial setting where hand nets are unlikely to be rinsed in sterile water, and where knotless mesh is likely to remain damp for a long period of time, it is again reasonable to suggest that bacterial loading could be greater if a biofilm developed.

Whilst it is encouraging that the correct use of a net disinfectant apparently reduced bacterial numbers or viability to zero in the current study, in commercial recreational sport fisheries there is evidence suggesting that disinfectant net dips are not maintained adequately and this can reduce disinfection efficacy (Tidbury et al., 2018). The reduced knot filaments found in rubber meshes could

reduce bacterial infection further, in addition to improved management practices or antibacterial components within the mesh (Canada et al., 2020; Tidbury et al., 2018).

Conclusions

These findings support the modest but growing literature base that rubber mesh should be used in preference to knotless meshes, since they remove fewer scales during routine handling of Atlantic salmon, particularly for larger specimens. Rubber mesh also has a reduced bacterial load with respect to mesh weight, and presumably less ability to transfer bacteria to other fish, or to areas of scale loss during the physical process of fish handling. Whilst no cryptic damage was observed in this trial using fluorescein treatment, the current study suggests that the technique can be performed with simple, cheap and widely available technology (i.e. mobile phone camera, simple UV torches, non-scientific grade Fluorescein).

Acknowledgements

The author wishes to thank all colleagues at MERL. The current study was kindly funded via a UFAW small grant award (grant application 25-20/21). This study does not promote or criticise any brand of net or disinfectant, which were chosen simply due to similarities in mesh aperture size and bag volume. The author declares no conflict of interest.

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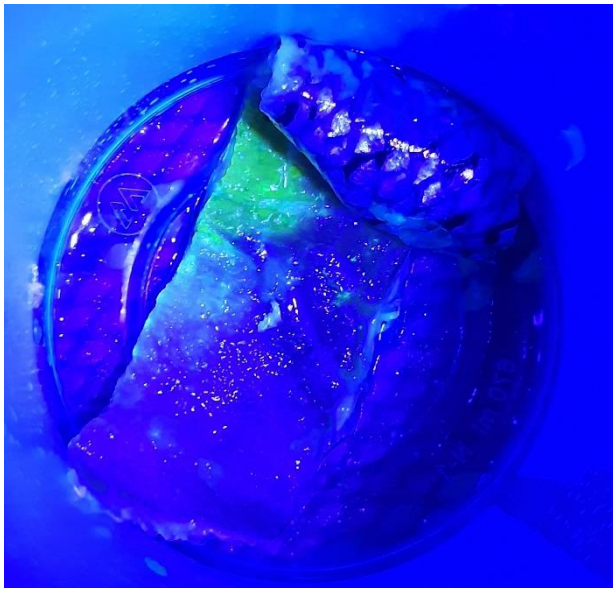
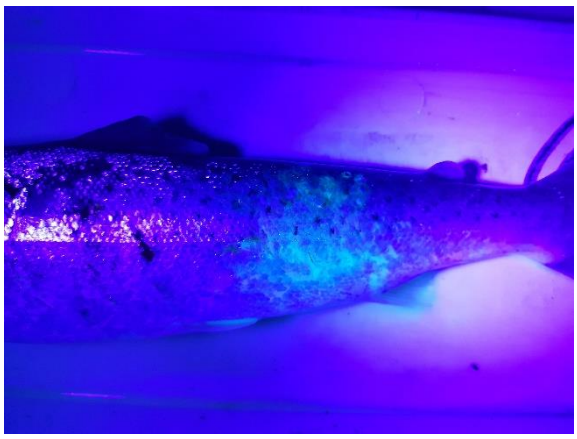
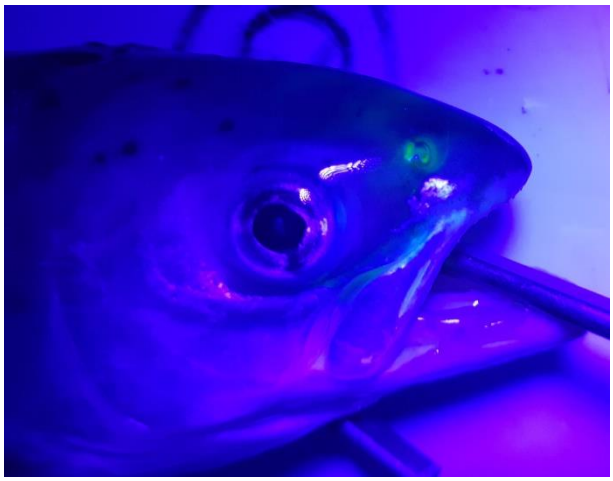
Appendix







Positive Fluorescein dye reactions with Atlantic salmon, *S. salar.* Clockwise from top left: Piece of skin fillet (test); slightly exophthalmic fish showing slight corneal ulcer; experimental post-mortem damage; typical netted fish showing no obvious fluorescence; internal nostril fluorescence seen on all fish and used as a positive control to check process had worked.