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Cleaner Fish Do Not Impact the Pigmentation of Salmon Lice (*Lepeophtheirus salmonis*) in Commercial Aquaculture Cages

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Abstract: Salmon lice are one of the biggest challenges to sustainable salmonid aquaculture. The species display high evolutionary potential, which is evident by its development of resistance to numerous chemical compounds used for delousing. In response to this, salmon farms now use non-chemical delousing methods to minimize the damage done by salmon lice, including heavy reliance on cleaner fish. Anecdotal reports from farmers and fish health personnel in areas where cleaner fish are used have suggested that salmon lice are becoming less pigmented, potentially making them harder for cleaner fish to visually detect. This experiment investigated changes in the pigmentation of salmon lice in relation to the use of cleaner fish, louse stage and sex, temperature, preferred salmon swimming depth, daylength, and salinity. Salmon lice were sampled from snorkel cages on a commercial salmon farm where three cages were stocked with farmed lumpfish and ballan wrasse, and three cages were without cleaner fish. Water temperature, salinity, and depth were recorded using a conductivity, temperature, and depth recorder. Pigmentation was measured via photographic analysis of individual lice. Although louse pigmentation varied considerably throughout the experiment, using cleaner fish throughout a single production cycle did not reduce average louse pigmentation compared to control cages. On average, male lice were significantly darker pigmented than females, but otherwise there were no patterns in louse pigmentation in relation to life stage, salinity, temperature, or daylength. Salmon lice exhibit a high degree of evolvability and have become resistant to every chemical removal treatment developed thus far. The present data suggest that, with the densities and species of cleaner fish commonly used in commercial salmon production, there is not strong directional selection on louse pigmentation. Lice, at least with regard to visual appearance, are not likely to adapt in a way which reduces cleaner fish efficacy anytime soon.

Keywords: aquaculture; sea lice; pigmentation; cleaner fish; fish welfare

Key Contribution: The presence of cleaner fish did not lead to more transparent salmon lice. The present data suggest that, with the densities and species of cleaner fish commonly used in commercial salmon production; there is not strong directional selection on louse pigmentation.

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1. Introduction

One of the biggest challenges to the Atlantic salmon (*Salmo salar*) farming industry is ectoparasitic salmon lice (*Lepeophtheirus salmonis*) [1,2]. Salmon lice are a multi-faceted problem. Directly, be it wild or farmed salmon, lice injure their host by feeding on its skin, blood, and mucus [3]. Indirectly, regulations that aim to curb the growth of the louse population force farmers to pursue potentially risky and expensive management strategies. Salmon lice have an outstanding capacity to evolve, which is one of the primary reasons they are so difficult to combat. Numerous factors influence the rate of resistance evolution,

including intensity and frequency of selection, the population genetics and life history, and genetic mechanisms of resistance [4]. Salmon lice have a short generation time, especially in high water temperatures [5], which increases the possibility of new traits appearing rapidly [6]. Furthermore, the species is highly abundant and displays genetic variation in several key traits, including salinity and thermal tolerance [6]. With the high number of fish farms, farmed hosts vastly outnumber wild hosts [7], and as a result natural refugia are insufficient to reduce the selective pressure on salmon lice [4]. Thus, while gene flow may counteract local selective forces, when multiple farms apply the same treatments and therefore selection, this leads to strong population-wide selection [8]. Salmon farming also selects for a shorter generation time as the parasite fitness is maximized with early maturation and high fecundity, even if it damages the host [7]. Consequently, farming conditions favor rapid reproductive cycles as there is an abundance of mates, high host availability, and a need to reproduce before the farmer delouses or harvests the salmon [7].

Usage of chemical delousing was the leading delousing method from the 1980s to 2015 [9]. As a result, salmon lice evolved resistance and/or reduced sensitivity to four out of five chemical therapeutants [10]. The case of emamectin benzoate is a clear example demonstrating the evolutionary capacity of salmon lice; resistance appeared in a single farming region and then, due to strong selection and extensive use of chemicals, dispersed throughout the North Atlantic within just 8 years [11,12]. Furthermore, despite a decline in the use of chemical treatments in recent years [10], resistant strains still persist in regions wherein no chemotherapeutants are used [10–12]. Following the shift from chemical treatment to non-chemical delousing methods, there is the possibility that salmon lice may adapt similarly to these new methods as well.

The use of cleaner fish as a continuous louse control technique was developed in the late 1980s [2], and their use rapidly increased in Norway with the phase-out of chemical treatments [13–18] in favor of non-chemical delousing methods including mechanical and thermal treatments [17]. The cleaner fish used in Norwegian aquaculture are opportunistic feeders, meaning they feed on what is available [19], unlike obligate cleaner fish who primarily feed by cleaning other fish species [20]. Cleaner fish are less expensive and less stressful for the salmon than other delousing methods and are generally more acceptable to the public than chemotherapeutants [21]. With the widespread resistance to chemotherapeutants and the fact that non-chemical delousing strategies are stressful and elevate salmon mortality rates post-treatment [17,22], cleaner fish became a keystone control method in the fight against salmon lice. The lack of antagonistic behavior between Atlantic salmon and cleaner fish also helped spur investment [16]. The cleaner fish species mainly used in Norwegian aquaculture are ballan wrasse (*Labrus bergylta*), corkwing wrasse (*Symphodus melops*), rock cook (*Centrolabus exoladus*), goldsinny wrasse (*Ctenolabrus rupestris*), cuckoo wrasse (*Labrus mixtus*), and lumpfish (*Cyclopterus lumpus*) [23]. Lumpfish and ballan wrasse are mostly farmed for their use in aquaculture while the other species are only caught in the wild. Due to water temperature, there are limitations to the usage of cleaner fish with each species tolerating different temperature ranges. Lumpfish tolerate lower temperatures better [24] than wrasse, and typically, wrasse are best deployed in spring/summer while lumpfish are best deployed in autumn/winter [25]. Lumpfish and Atlantic salmon share feeding grounds in the wild [26], which may explain the non-antagonistic behavior of this species when reared together in salmon sea pens [27].

Pigmentation in salmon lice is what gives them their coloration. In copepods, pigment cells synthesize carotenoids and mycosporine-like amino acids (MAAs) to either function as a sunscreen or as scavengers of photo-produced radicals [28]. MAA is a water-soluble molecule found in many cyanobacteria and eukaryotic microorganisms, as well as aquatic life forms [29]. These molecules absorb UV radiation between 310 and 365 nm and act as sunscreen to protect against harmful levels of UV radiation [29]. The pigments from the pigment cells may distribute widely over the body surface, giving the lice a dark appearance, or the pigments can be concentrated in the pigment cells, leaving large areas of the louse transparent [8].

Broadly, in free-living crustaceans, pigmentation is often highly plastic and changes in response to UV exposure and predator cues [30]. In salmon lice specifically, there is evidence for both genetic and environmental determination of pigmentation [8]. The degree of pigmentation consistently differed between strains regardless of the environment, but also within strains the lice were consistently lighter when reared indoors compared to individuals reared outdoors [8]. Furthermore, louse placement on the fish is also a factor when it comes to pigmentation, where lice found on the dorsal side of the fish, which is most exposed to sunlight, were significantly darker than lice found on the ventral side of the fish [8]. As cleaner fish are thought to be dependent on eyesight to locate prey [31], their widespread and intense usage may be exerting selective pressure on the pigmentation of lice. No previous studies have examined the effect of cleaner fish predation on salmon louse pigmentation, but previous work has demonstrated that louse pigmentation is both genetically and environmentally influenced [8]. Given the high evolutionary capacity of salmon lice, there is some fear that lice may adapt to become less vulnerable to predation. One potential adaptive direction is altered appearance such that the lice become more difficult for the cleaner fish to visually detect.

The primary scientific purpose of this study was to determine whether the presence of cleaner fish in marine net cages alters louse pigmentation, with a secondary aim to examine the influence of other factors including season, environmental conditions, life stage, and sex on sea lice pigmentation.

2. Materials and Methods

2.1. Experimental Fish, Location and Sampling

All lice were collected from a commercial salmon farm located at Fosså (59.269 N, 6.143 E) in Boknafjorden, Hjelmeland municipality, Norway. Throughout this study, the farm consisted of six 200 m circumference polar circle cages equipped with 20 m deep snorkels (90 m circumference), two aeration devices positioned at 22 m depth (Midt-Norsk ringen, NorseAqua, Terråk, Norway), submerged feed distribution beginning at 18 m (SubFeeder, AKVA group, Klepp, Norway) and two submerged lights at 18 m (150 W/1200 W Aurora SubLED Combi light, AKVA group). In addition, three cages were stocked with a combination of farmed ballan wrasse and lumpfish throughout production, added when seasonally appropriate (Table 1), while three remained control cages with no cleaner fish. To maximize cleaner fish welfare and performance, cages with cleaner fish were also supplied with plastic kelp style hides [32–34] and species-specific cleaner fish feed.

Table 1. Species of cleaner fish used with stocking date and number of cleaner fish stocked in each cage.

		Lumpfish	
	Cage 1	**Cage 4**	**Cage 5**
Week 46 2020	10697	10521	10470
Week 8 2021	9792	9805	9817
		Ballan wrasse	
Week 21 2021	10,654	9876	9935

Salmon were collected from cages using a jumpnet. The jumpnet is a 5 × 5 × 5 m rectangular net with small buoys around the upper perimeter to hold the top of the net flush with the water's surface. Due to the fact that each individual salmon jumps, on average, at least once per day, jumpnets allow for the passive capture of salmon without the need for crowding or feed restriction. After having the net out for around 60 min, the fish were collected and placed in individual buckets where they were given an overdose of Finquel MS-222 (Tricaine Methanesulfonate).

At each site visit, 20–30 fish were sampled from each cage. Samplings were performed every 2–3 weeks between August and December 2021 for a total of seven samplings. For each fish, every louse was collected, and life stage recorded. The three early life stages, copepodite, chalimus I, and chalimus II, were not included in this trial because they are physically attached to the fish and are too small for consumption by cleaner fish [13]. The stages which are included in this study are pre-adult I, pre-adult II, and adults. After counting, all mobile lice were collected and placed in a seawater-filled Petri dish for photographic examination.

Temperature and salinity were recorded at a central reference location at the barge down to a depth of 30 m using a conductivity, temperature, and depth (CTD) recorder (SD204, www.saiv.no, (accessed on 10 August 2022)). Daylength was calculated as the time between sunrise and sunset, excluding twilight, and was determined using online data (www.timeanddate.com, (accessed on 10 August 2022)).

2.2. Photography and Measurement of Pigmentation

After all lice were counted and collected from an entire cage, the lice were prepared for photographing. To do this, lice were removed from the Petri dish and placed on tissue paper to remove excess water which could distort the image. After a quick drying, each louse was placed on a 240 lumen LED lightbox (Wafer 1, www.daylightcompany.com, (accessed on 5 August 2022)) to ensure even lighting from below. Several lice were then arranged according to stage, close together but not overlapping, next to a scale. An Olympus Tough TG-6 camera (Hamburg, Germany) atop an opaque, black polyvinyl chloride box was then placed over the scale and lice, such that all light was from the LED lightbox. In this way, the lighting conditions and camera position of all photographs were standardized and consistent between samplings, regardless of ambient conditions, with an exposure setting of +1.3.

Photographic analyses were performed using ImageJ (https://imagej.nih.gov/ij/download.html, (accessed on 5 August 2022)). To calibrate the size of the image, a 1 cm scale was included in each photo. Due to the fact that all photos were stored as jpg files which compress brightness information to complement human vision, each image required linearization before analysis. Images were linearized by photographing six grey standards ranging from 1 to 99% reflectance (https://www.xrite.com/, (accessed on 5 August 2022)) and modelling the linearization curve using the mica toolbox plugin (https://www.empiricalimaging.com/download/micatoolbox/, (accessed on 6 August 2022)). The resultant linear model was then used to generate a linear normalized version of each photograph.

After linearization, quantitative measurement of pigmentation was obtained by measuring the amount of light passing through each louse in a representative, fixed-size circular area on the cephalothorax (Figure 1). Due to the fact that size varies with louse life stage, specific diameters for the measurement area were chosen for each life stage: 50 pixels for adult females, 35 pixels for adult males and pre-adult II females, 25 pixels for pre-adult II males and pre-adult I females, and 20 pixels for pre-adult I males and *Caligus elongatus* (Figure 1). An example of pigmentation types is shown in Figure 2. A second circular area of the same size was measured next to each louse to provide a measurement of background lighting. To assess pigmentation, the average grey value of every pixel within the measurement area was calculated (mean grey value—MGV). To standardize for possible differences within and between each image, the MGV of each louse was subtracted from the MGV of the paired background area, giving a difference in MGV for each individual louse compared to the background (dMGV). Less pigmented lice are more transparent and have lower dMGV values, while more pigmented lice absorb light and have higher dMGV values. These measurements were taken on 3601 lice.

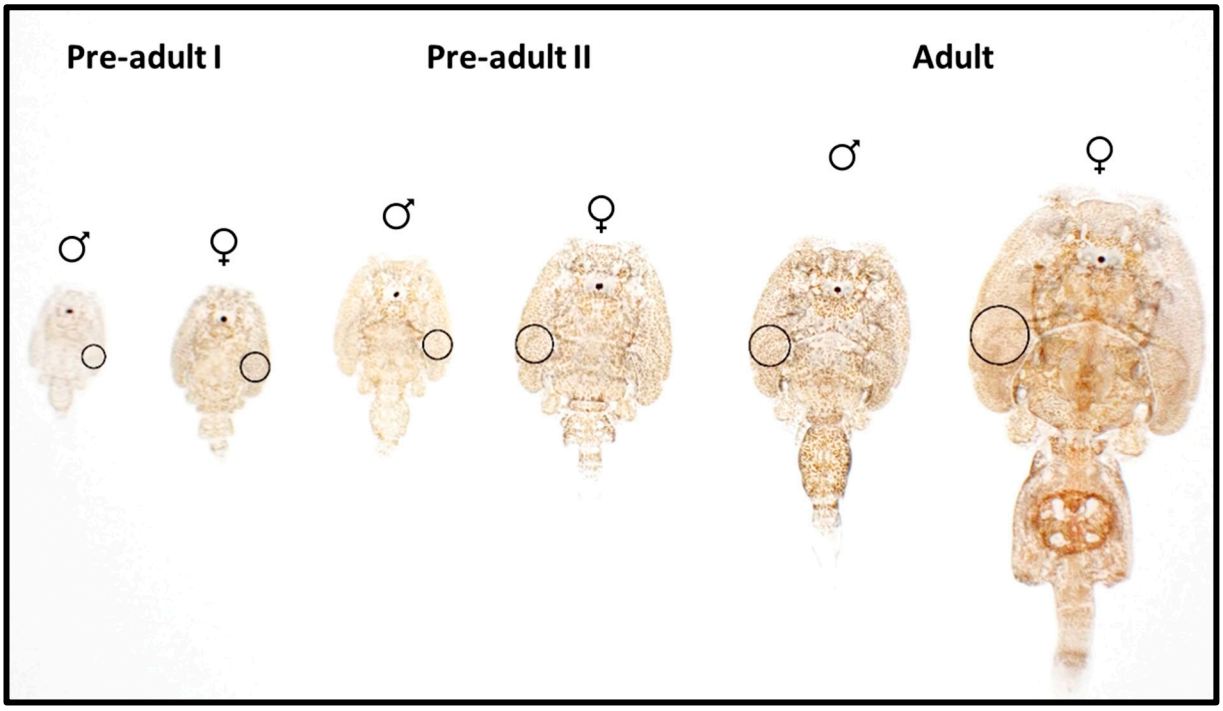

Figure 1. Typical examples of each gender and developmental stage of mobile *L. salmonis* sea lice included in the dataset. From left to right, male and female pairs of pre-adult I, pre-adult II, and adult salmon lice, respectively. The circles show the area where MGV was measured on the louse with the corresponding size of that area.

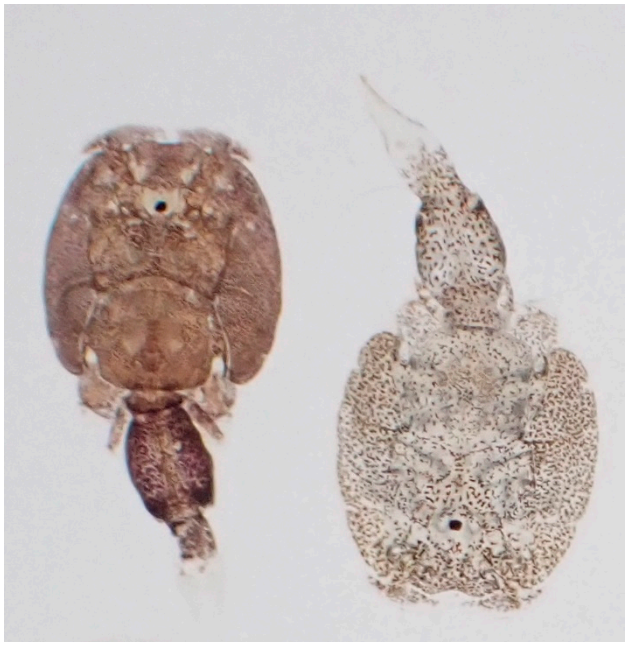

Figure 2. Two adult male *L. salmonis* with different degrees of pigmentation as measured by dMGV. The darker male on the left has a dMGV of 40.8 while the lighter louse on the right has a dMGV of 25.6.

2.3. Statistical Analyses

All data analyses were performed using R version 4.1.2 [35]. A Levene's test was conducted to check for homogeneity of variance for the fixed effects chosen for this study by using the car package in R [36]. A two-way mixed nested analysis of variance (ANOVA, [37]) was applied to check for the interaction between factors and their relevance to the re-

sponse value dMGV. In this analysis, the cages (random) were nested within the predictor (fixed) variables. As most tests revealed highly significant Levene's test (indicating non-homogeneity of the variances between groups), it was decided to apply a non-parametric Kruskal–Wallis test [37] for all one-way combinations. In cases of significant Kruskal–Wallis test, a post hoc Dunn test was performed to test for possible differences between experimental groups. All data presented are mean ± SD unless otherwise specified.

3. Results

3.1. dMGV by Treatment

There were no significant differences in louse pigmentation between cleaner fish (dMGV = 17.4 ± 5.6 to 20.4 ± 5.4) and control treatments (dMGV = 15.9 ± 6.2 to 23.0 ± 8.7) at any time point (Figure 3). Furthermore, there was no clear trend with time as the treatment with highest dMGV changed between samplings. In three out of seven samplings, control cages had higher mean dMGV than cleaner fish cages. In three samplings, the dMGV's were identical in the two treatment groups, and for one sample cleaner fish cages had higher mean dMGV.

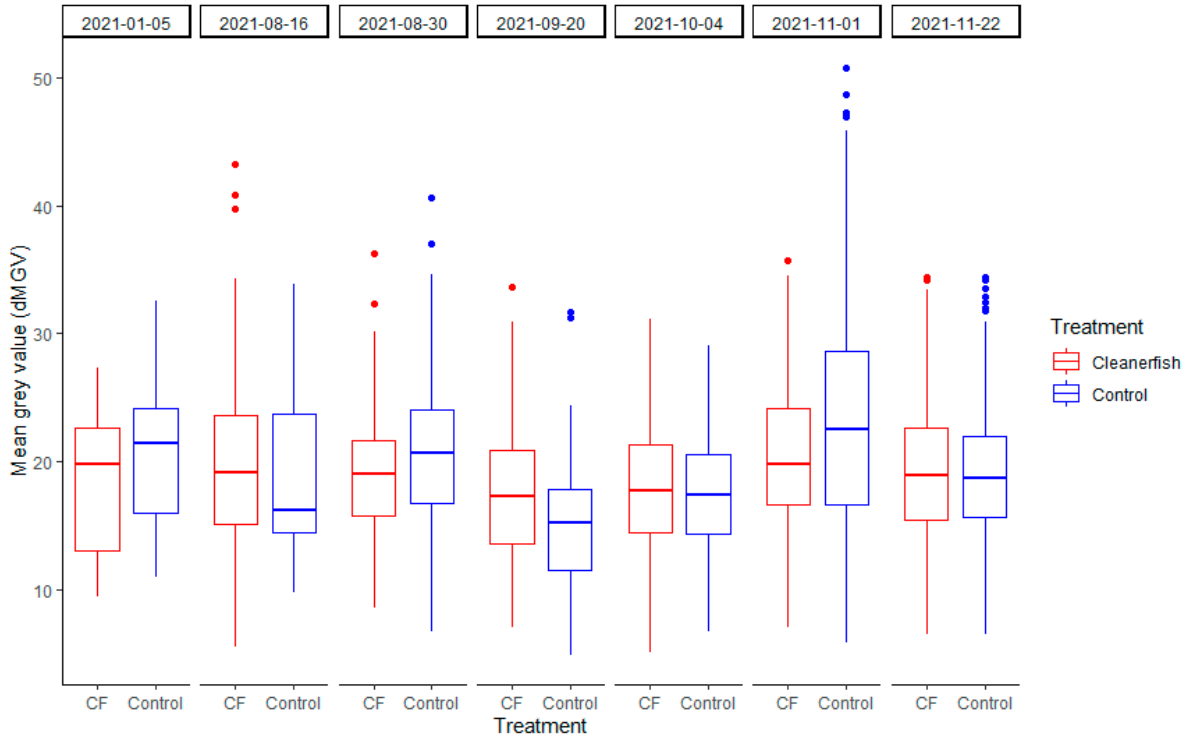

Figure 3. Boxplot showing the interaction between treatment and date. Y-axis shows mean grey value (dMGV) while the x-axis shows treatment. The treatment is compared at each date with cleaner fish cages in red and control cages in blue. Whiskers indicate minimum and maximum values, while boxes indicate Q1, median, and Q3 quartiles. Outside the whiskers, outliers are presented as individual data points.

3.2. Seasonal Changes of dMGV

3.2.1. Sea Temperature

Average temperatures during samplings ranged from 5.3 to 17.4 °C near the surface (0–15 m) and from 6.8 to 16.0 °C in the deeper waters (15–30 m). Although the two-way ANOVA revealed that there was a significant interaction between temperature and treatment ($F_{4, 66}$ = 2.59, $p < 0.05$), dMGV differed minimally between cleaner fish (dMGV = 17.8 ± 5.0 to 19.7 ± 6.5) and control treatments (dMGV = 17.3 ± 4.7 to 21.1 ± 6.4) with no clear pattern (Figure 4).

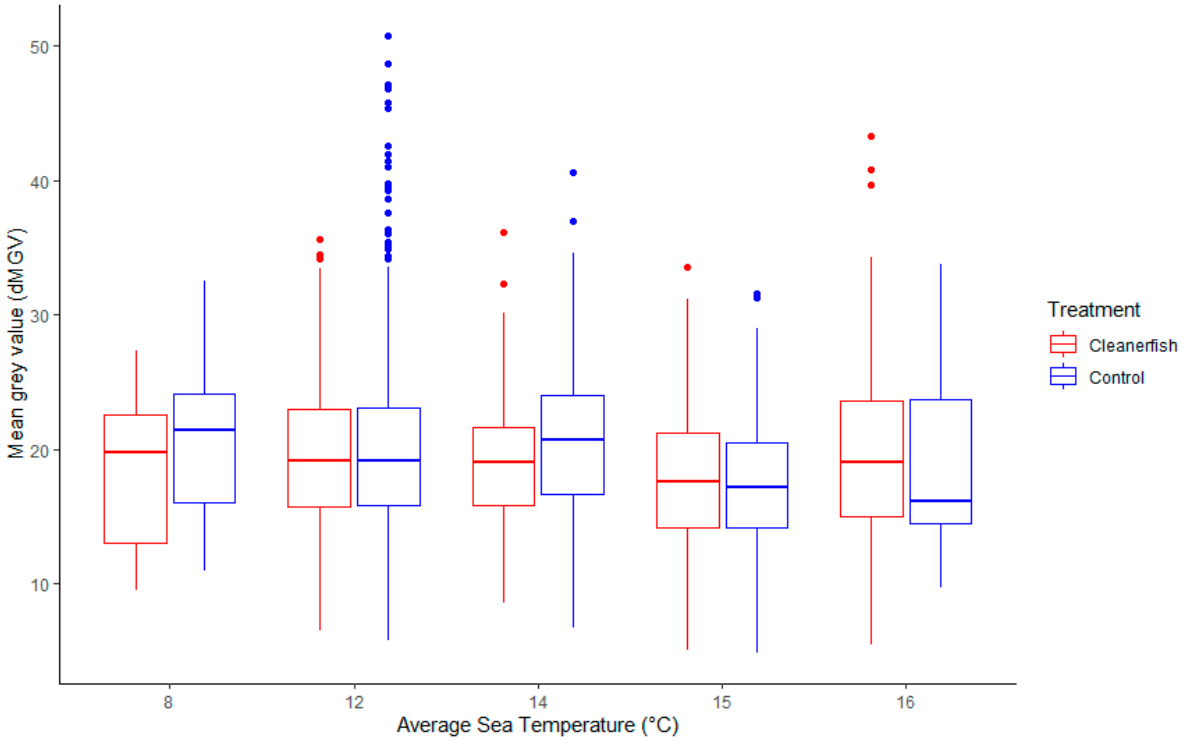

Figure 4. Boxplot showing the interaction between average sea temperature and treatment. Y-axis shows mean grey value (dMGV) while the x-axis shows the average sea temperature. The treatment is compared at each temperature with cleaner fish cages in red and control cages in blue. Symbols as in Figure 3.

3.2.2. Hours of Daylight

The longest day of those sampled lasted 15.5 h while the shortest day lasted just 6.2 h. Like temperature, although there was a significant interaction between daylight hours and treatment on dMGV (two-way ANOVA, $F_{6, 65} = 6.29$, $p < 0.001$), observed differences were minor and followed no clear trends. Average dMGV of lice in cleaner fish cages ranged from 17.4 ± 5.6 to 20.4 ± 5.4, while in control cages dMGV ranged from 15.9 ± 6.2 to 23.0 ± 8.7 (Figure 5). Furthermore, there was no clear correlation between dMGV and daylength. The highest measured dMGV values occurred when daylength was 8.7 h for both cleaner fish (dMGV = 20.4 ± 5.4) and control (dMGV = 23.0 ± 8.7) treatments. The lowest measured dMGV occurred when daylength was 12.4 h for both control (dMGV = 15.9 ± 6.2) and cleaner fish treatment groups (dMGV = 17.4 ± 5.6) and fluctuated on either side of those measures (Figure 5).

3.2.3. Salinity

Surface salinity ranged between 20.6 and 31.9 ppt. Pigmentation varied minimally with salinity in both treatments (Figure 6), cleaner fish (dMGV = 19.1 ± 5.1 to 19.7 ± 6.5), and control (dMGV = 19.0 ± 6.6 to 20.2 ± 7.4). Furthermore, there was no significant interaction between salinity and treatment on dMGV (two-way ANOVA, $F_{2, 3499} = 1.72$, $p > 0.25$).

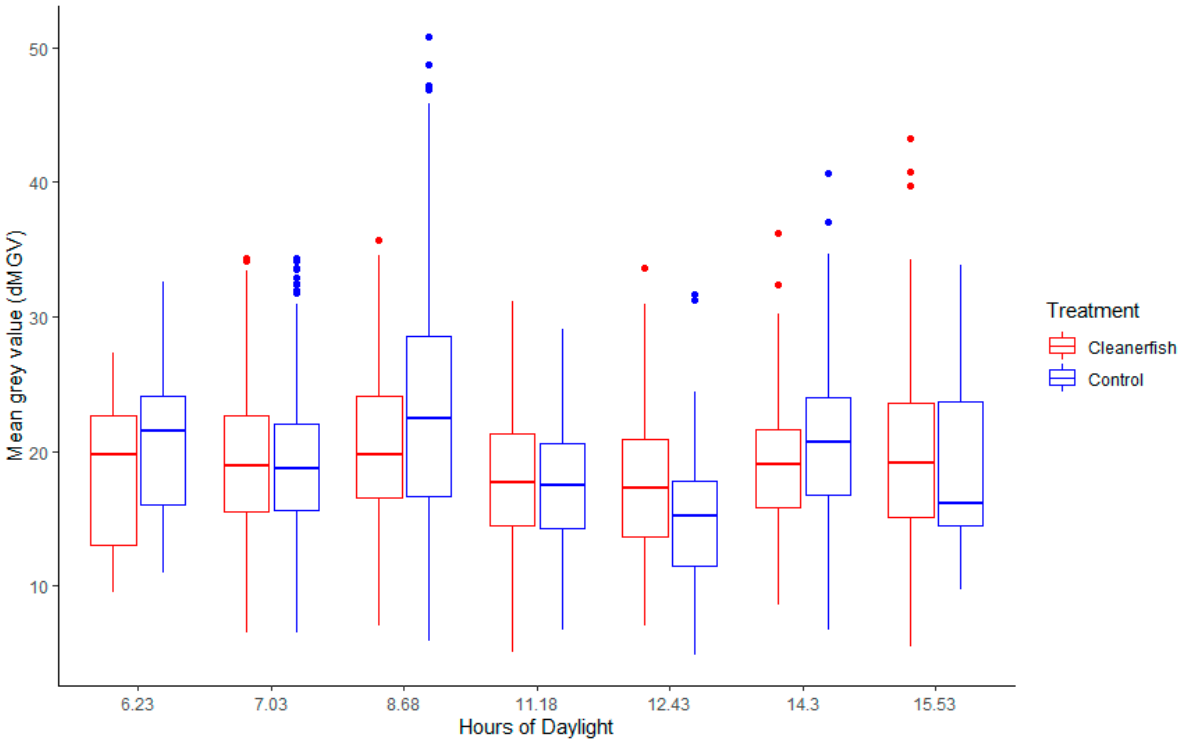

Figure 5. Boxplot showing the interaction between hours of daylight and treatment. Y-axis shows mean grey value (dMGV) while the x-axis shows the length of days. The treatment is compared at each daylength with cleaner fish cages in red and control cages in blue. Symbols as in Figure 3.

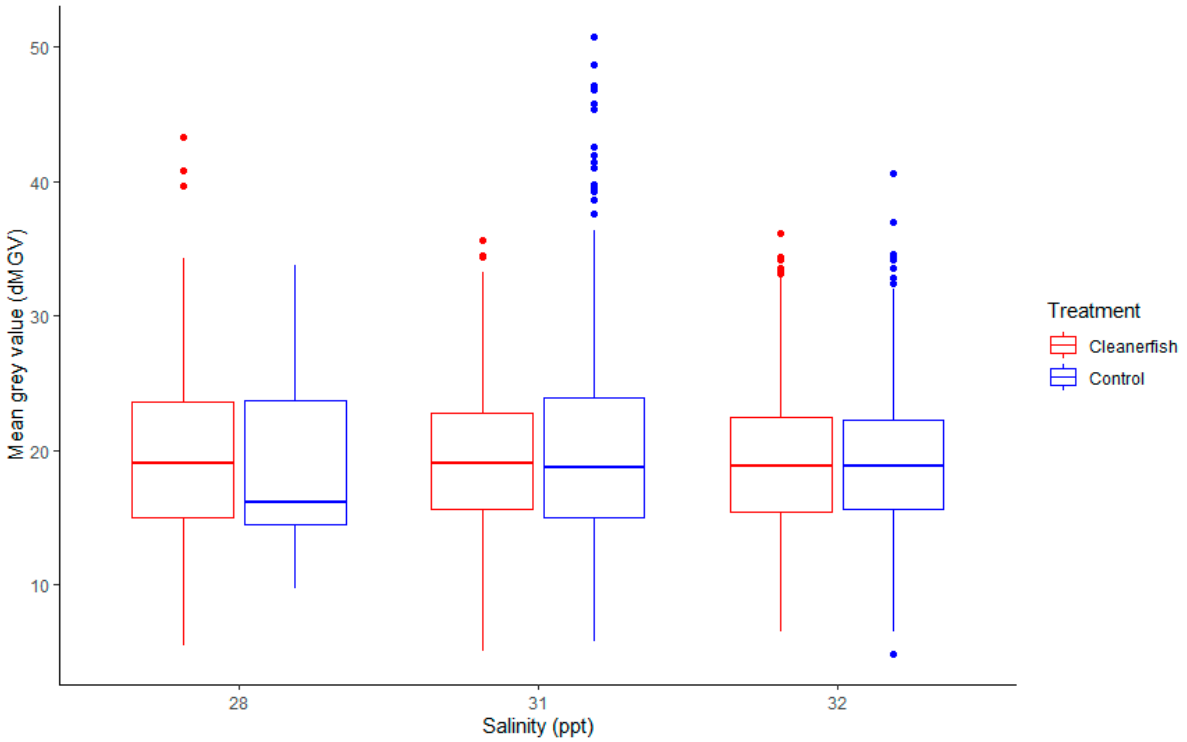

Figure 6. Boxplot showing the interaction between salinity and treatment. Y-axis shows mean grey value (dMGV) while the x-axis shows the salinity (ppt). The treatment is compared at each point of salinity with cleaner fish cages in red and control cages in blue. Symbols as in Figure 3.

3.3. dMGV by Stage and Sex

3.3.1. dMGV by Stage

A total of 1627 adult, 598 pre-adult I, and 1376 pre-adult II were all recorded individually, and each stage included both males and females. There was a significant interaction between louse stage and date on dMGV (two-way ANOVA, $F_{12, 3578} = 6.41$, $p < 0.001$); however, there were only minor differences between the adult (dMGV = 17.3 ± 6.0 to 22.4 ± 7.1), pre-adult II (dMGV = 16.6 ± 6.2 to 19.3 ± 4.8), and pre-adult I stages (dMGV = 16.6 ± 4.3 to 20.8 ± 5.6) (Figure 7). Furthermore, there was no clear trend as the stage with highest dMGV oscillated between samplings.

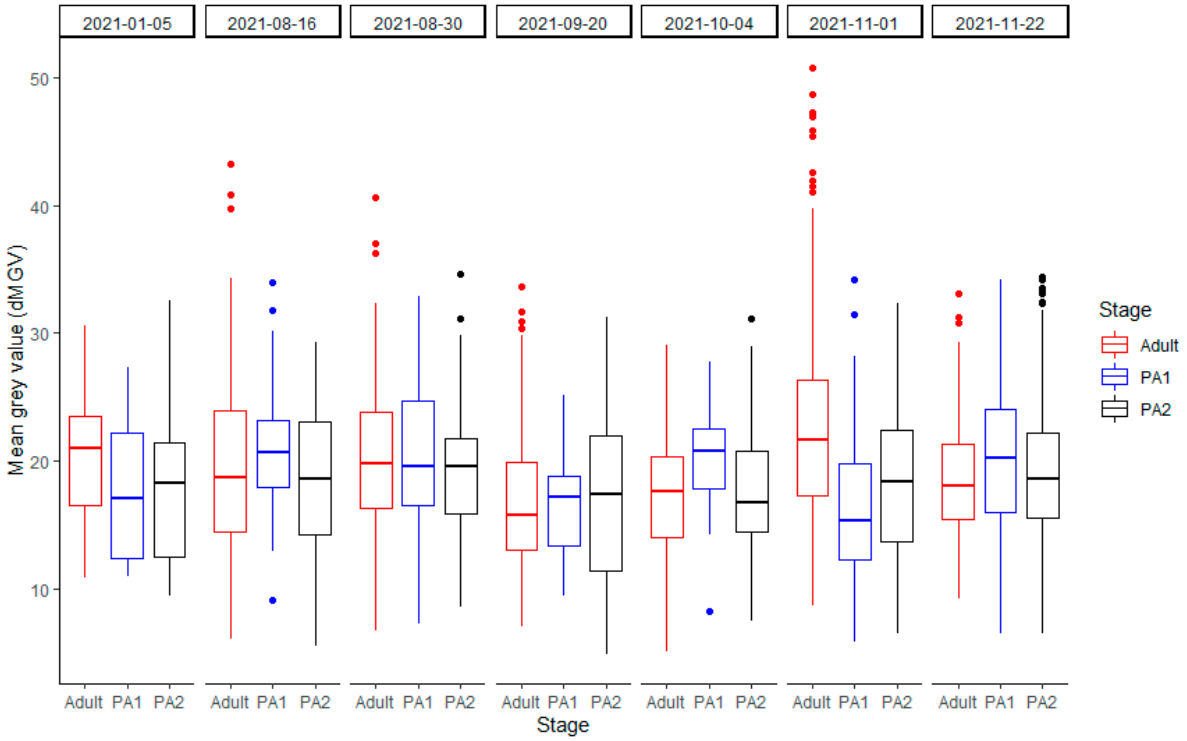

Figure 7. Boxplot showing the interaction between louse life-stage and date. Y-axis shows mean grey value (dMGV) while the x-axis shows the life-stage. The stages are compared at each date with adult in red, pre-adult I in blue, and pre-adult II in black. Symbols as in Figure 3.

3.3.2. dMGV by Sex

In total, 1827 males and 1774 females were evaluated. There was a significant interaction between sex and date on dMGV (two-way ANOVA, $F_{6, 3583} = 17.9$, $p < 0.001$, Figure 8), with a clear difference between the sexes. Males were darker than females in six out of the seven samplings, with a maximum dMGV of 24.1 ± 7.9 compared to 19.1 ± 5.5 for females. In contrast, the lowest mean dMGV occurred in females at 14.9 ± 4.3 compared to 18.1 ± 6.1.

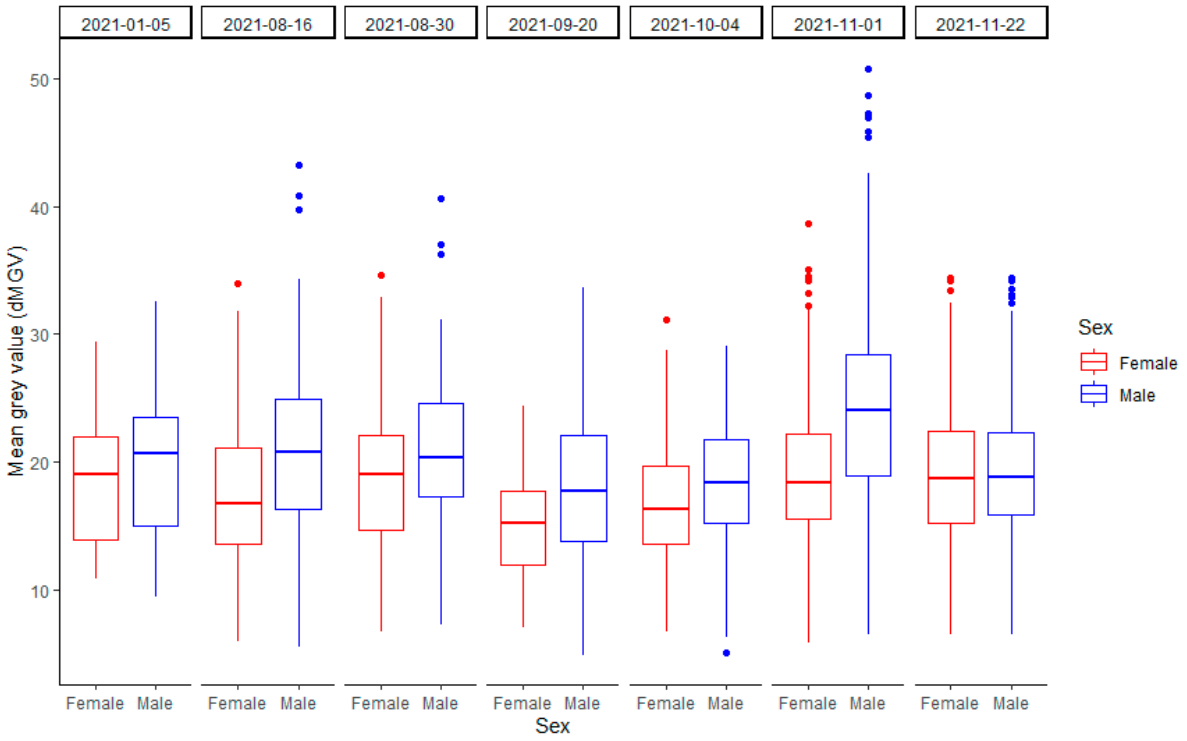

Figure 8. Boxplot showing the interaction between sex and date. Y-axis shows mean grey value (dMGV) while the x-axis shows the sex. The treatment is compared at each date with females in red and males in blue. Symbols as in Figure 3.

4. Discussion

There was no significant difference in mean degree of pigmentation between lice in cages with and without cleaner fish. Using cleaner fish did not therefore result in less pigmented lice during this study. These results are in contrast to previous work, where *Daphnia* were observed to become less pigmented when under selective pressure from predators, even in high UV environments [30]. This is despite evidence previously found that there is both genetic and environmental control of pigmentation in *L. salmonis*, demonstrating the potential for both plastic and adaptive responses to selection [8]. One possible explanation for the lack of change in average lice pigmentation in the present study is that there may have been insufficient selection pressure exerted by the cleaner fish. Firstly, eyesight may not be the only sense used by cleaner fish to detect prey. Lumpfish use olfaction to detect potential predators [38] and may also be used for foraging. If olfaction is used by cleaner fish for foraging, this would reduce the possibility for selection on pigmentation by cleaner fish. Secondly, both species of cleaner fish used in this experiment are opportunistic feeders [39]. Although previous research has shown that at 8% density cleaner fish can reduce the number of salmon lice found within a cage to equal or lower than previously recorded counts [16], lumpfish also eat crustaceans, salmon feed, and hydrozoans when used in salmon cages [19]. According to Imsland et al. [19], only 33–38% of lumpfish had ingested sea lice after 77 days in salmon cages. Therefore, even if cleaner fish are entirely reliant on eyesight to locate prey, selective pressure on louse pigmentation could still be weak if cleaner fish are primarily feeding on alternative food sources. For example, although both ballan wrasse and lumpfish have been observed to eat salmon lice, the swimming speed of Atlantic salmon is higher than that of both cleaner fish species and may be a reason why they do not eat enough lice to change pigmentation [40]. In addition, the cages used in this study were snorkel cages, which may also affect cleaner fish performance. Snorkel cages work by uncoupling salmon from salmon louse larvae while providing access to surface air [41], pushing salmon to stay deeper in snorkel cages than in standard cages. This could affect the interaction between species as their depth

distribution may be shifted compared to standard cages. Ballan wrasse are found in deeper, warmer, and more saline water than lumpfish, which is found at shallower, cooler, and more brackish water [42]. Ballan wrasse spends most of their day at 15 m or deeper [43], while lumpfish spend most of their day at 10 m or above and used hides extensively [43]. Different depth distribution for salmon and cleaner fish leads to less interaction between them, and likely reduces lice feeding [44].

Lice pigmentation varied with temperature, but with no apparent pattern. Temperature may have both direct and indirect effects on lice pigmentation. The water temperature's primary function for salmon lice is to dictate the growth rate of the lice with lower temperatures, making their metabolism slow down while higher temperature speeds it up [45]. As lice body size is correlated with pigmentation, with larger individuals being darker [8], and higher temperatures making them grow faster [46], co-selection may occur for size and color [47]. Temperature is also a key environmental factor influencing salmon swimming depth and density. The optimal temperature for growth of post-smolt Atlantic salmon is 13–16 °C [48], and they adjust their vertical position depending on where the temperature most closely resembles their preference. Due to the fact that salmon avoid thermal extremes [49], it is possible that salmon were swimming closer to the surface during the experimental period, exposing lice to more UV radiation. Therefore, even if the temperature does not affect pigmentation directly, it may affect the lice by changing their position on the host or changing the preferred depth of the host.

As with temperature, although pigmentation varied with daylength, there was no consistent increase in dMGV with increasing hours of daylight in either treatment. This is despite an earlier study finding that louse pigmentation is strongly influenced by environmental conditions, likely light [8]. From comparison of genetically similar outdoor and indoor-reared lice, the outdoor reared lice were found to be significantly darker pigmented. Pigmentation, however, is costly and slows growth for other free-living crustacean species such as *Daphnia* [30]. This trade-off may also be the case for salmon lice. As this experiment was conducted on a commercial salmon farm where fish are held in marine net cages, subject to the highly variable conditions of nature, other factors may override the purported influence of light observed in controlled tank trials. Further work is required to understand the role light exposure may play in driving pigmentation of salmon lice in the natural environment.

Lastly, although neither salinity nor life stage meaningfully influenced pigmentation, males were consistently darker pigmented than females. One possibility is that, since male lice are smaller than females, approximately half the size [50], pigmentation cells may be dispersed differently in their body. This hypothesis also aligns with the previous observation that smaller lice are significantly darker than larger individuals, regardless of sex [8]. Another point worth considering is the placement of the different sexes on the host. Individuals positioned on the dorsal side of the fish would receive the most UV exposure, whereas those on the sides or ventral sections would receive considerably less. Adult males are mostly found on the dorsal section of the fish, while adult females are typically found on the head and may be positioned ventrally or on the fishes' side [1] Unfortunately, Bui et al. [1] did not separate the sexes at the pre-adult stages. Pigmentation may also help camouflage lice if it reflects the coloration of their position on the host. Previous work has shown that lice located on the ventral section of salmon were lighter than those positioned dorsally, a pattern consistent in both sexes [8].

5. Conclusions

Although louse pigmentation varied considerably throughout the trial, overall, there were no meaningful differences in pigmentation between lice in cages with cleaner fish and those without. The presence of cleaner fish did not lead to more transparent salmon lice. Additionally, and contrary to expectation, there were no clear trends in louse pigmentation with environmental variables including temperature, salinity, and daylength. The only clear trend observed was that males were, on average, significantly darker than females.

These results contrast with expectations based on previous work in *L. salmonis* and similar species and suggest that, while controlled tank trials are useful for understanding basic biology and adaptive capacity, the interactions between numerous variables occurring in the natural environment render outcomes of interventions such as cleaner fish introduction difficult to predict.

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