

Article

Follow-Up Study on Acoustic De-Licing of Atlantic Salmon (*Salmo salar*): *Lepeophtheirus salmonis* and *Caligus elongatus* Dynamics over Four Consecutive Production Cycles

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Abstract: Acoustic lice treatment (AcuLice) is a newly developed system which uses a composite acoustic sound image with low-frequency sound to remove salmon lice (*Lepeophtheirus salmonis*) from Atlantic salmon (*Salmo salar*). The effect of AcuLice treatment on salmon lice dynamics was measured by weekly salmon lice counting at a full-scale production facility from mid-summer 2019 to late-spring 2024. We monitored four production cycles, with AcuLice applied for two of the production cycles and with no AcuLice treatment applied during the other two production cycles as control. This is a follow-up study to our previous work. The numbers of salmon lice treatments and of weeks until the first salmon lice treatment were also compared in the two experimental groups. For the small (sessile and mobile stages) salmon lice, a significantly lower number (mean $\pm$ SEM) was shown for the AcuLice group (0.73 ± 0.03) compared with the control group (1.18 ± 0.05). For the mature female salmon lice, a significantly lower number (mean $\pm$ SEM) was found for the AcuLice group (0.12 ± 0.01) compared with the control group (0.22 ± 0.03). In addition, the mean ($\pm$ SEM) number of *C. elongatus* varied between the two experimental groups and was higher in the control group (0.12 ± 0.01) compared with the AcuLice group (0.03 ± 0.01). In addition, a lower number (mean $\pm$ SEM) of salmon lice treatments (1.4 ± 0.17 vs. 4.22 ± 0.20) and a longer production period before the first salmon lice treatment occurred was observed for the AcuLice group (11.2 ± 0.1 weeks) compared with the control group (24.1 ± 2.3 weeks). These data suggest that the use of the AcuLice system significantly reduces the number of salmon lice (by 40–60%) and *C. elongatus* (by 70%) on farmed Atlantic salmon and reduces the need for traditional salmon lice treatments (by 65%).

Keywords: Atlantic salmon; acoustic de-licensing; salmon lice; sustainable aquaculture



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1. Introduction

Combating salmon lice is one of the biggest challenges in Atlantic salmon farming [1,2] and has been estimated to cost the salmon farming industry in Norway around 9% of their income [3]. In recent years, there has been a strong focus on the increasing salmon lice problem in the Norwegian aquaculture industry [4], and the large economic losses it causes in the form of lost growth, treatment mortality, reduced feed utilization, secondary infections, downgrading and treatment costs [5–8]. The aquaculture industry focuses on the prevention and control of the salmon lice species *Lepeophtheirus salmonis* and, in recent

years, *Caligus elongatus*. The fight against salmon lice is taking place on many fronts [4], and for the industry it is crucial that all options are explored. It is important to develop methods that are highly efficient, low cost and have minimal negative effects on fish, while avoiding waste, labor-intensive operations and negative effects on the environment. In sum, this indicates that the requirements for a new method will be that it reduces the pressure on fish, employees, authorities and technology.

A composite acoustic soundscape (low-frequency sound, AcuLice) has been launched as a new method by which to prevent the infestation of salmon lice [9]. The method is cheap compared with other methods and requires no handling of the salmon. As sound does not propagate from water to air to a large extent, it also has no negative health, safety and environment (HSE) effects. AcuLice thus satisfies many of the criteria of an ideal control method against lice. The only question is whether the method is effective enough on a large scale. The present study is a follow up study to our previous study [9] and emphasizes efficacy, long-term results, and possible reductions in both lice and treatment needs over four full-scale production cycles.

AcuLice treatment against salmon lice is based on the use of a complex acoustic soundscape (low-frequency sound). The use of acoustics to address sea lice infestation in salmonid farming is a promising innovative method [10,11]. Underwater sound is characterized by two connected components: sound pressure and particle motion. While sound travels through water in the form of a pressure wave, water particles move related to this wave but do not travel with it. While mammal hearing is mainly associated with the sensing of pressure, most fish and invertebrates instead detect particle motion [12]. The sound levels generated by an acoustic source will propagate in the body of water and attenuate with distance. This attenuation is primarily due to the geometric dispersion of the sound energy. In water, the velocity is five times higher than in air but varies throughout the water column depending on the water density, influenced by temperature, salinity and hydrostatic pressure. Sound propagating to deeper water will be deflected by barriers, such as the bottom or temperature/salinity transition layers, and will continue travelling in a sound channel that is limited by the surface and the submerged barrier. When sound propagates in a sound channel, sound energy loss will be less than predicted from geometrical energy loss. The attenuation of sound in water is frequently dependent on locations in which high frequencies are absorbed quicker than low-frequency waves. Sole et al. repeatedly exposed Atlantic salmon to 350 Hz and 500 Hz tones in three- to four-hour exposure sessions, reaching received sound pressure levels of 140 to 150 dB [10]. Gross pathology and histopathological analysis performed on exposed salmon's organs did not reveal any lesions that could be associated with sound exposure. Further, Sole et al. [11] found that *L. salmonis* is sensitive to low frequency sounds. Specifically, the study found that the central nervous system in all stages and the A/B cells (responsible for the secretion of the precursor of frontal filament) in the copepodit and chalimus stages of *L. salmonis* were affected by sound exposure, leading to reduction of the capacity of the sea lice to infest its host [11]. Earlier studies have shown that sea lice react with 'aggressive behavior' if exposed to low-frequency sound in the frequency range of 1–5 Hz [13].

In the study of Hjelle et al. (2022) [9] the effect of AcuLice treatment on salmon lice dynamics was measured by weekly salmon lice counting at the facilities from mid-summer 2019 to late-spring 2020. For the mature female salmon lice, a significantly lower number remained for the AcuLice exposed group compared with the reference group. In addition, fewer salmon lice treatments and a longer production period before the first salmon lice treatment occurred was observed at the AcuLice facilities, when compared with the reference facilities. Aside from a slight increase in plasma glucose, no significant difference was observed in the primary, secondary or tertiary stress responses of the Atlantic

salmon. Overall, the data of Hjelle et al. (2022) [9] suggest that the use of the AcuLice system reduces the need for traditional salmon lice treatments with no added stress to the fish. However, it remains to be seen if the positive effect of the AcuLice system can be observed in several consecutive production cycles.

Accordingly, the overarching goal of this study is to present analyses that clarifies the potential impact of AcuLice as a non-destructive method of the de-licing of salmon in cages. More specifically, data on salmon lice and *C. elongatus* from individual growing seasons, as observed in full-scale commercial production facilities with, and without, AcuLice in operation will be compared. We aim to answer the following questions:

- Is the number of weeks prior to the first conventional de-licing different?
- Is the total number of conventional de-licing actions in an individual growing season different?
- Is the number of mobile, sessile and mature salmon lice females different?
- Is the number of *C. elongatus* different?

2. Materials and Methods

2.1. Field Trials—Effect of AcuLice Treatment on Salmon Lice and *Caligus elongatus* Under Commercial Conditions

2.1.1. Fish Material and Farming Conditions

The Atlantic salmon used in the field trial originates from the Salmobreed strain and was farmed from hatching to smolt at a recycling facility operated by Hardingsmolt AS in Tørvikbygd, Kvam, Norway. The hatching and juvenile protocols followed the same steps as given in our previous study [9], including the use of constant light and feeding according to temperature and fish size [14]. A traditional photoperiod regimen was performed to stimulate the transformation of parr smolts [15]. After completing the parr-smolt transformation, the fish were reared for seven weeks in a semi-closed facility at Koløy, Fitjar (GreenBag). When the fish reached approx. 500 g, the group was transferred to open cages (160 m and volume of 58,900 m³) at Hattasteinen (59.628° N, 5.252° E) production facility, Hardangerfjord, Norway.

2.1.2. AcuLice Installation Process

The installation process for AcuLice processing was carried out in collaboration with the equipment supplier and followed the same steps as in our previous study [9]. This involves plugging in the speaker, usually in the center of the venue (depth of 10–20 m), positioning the processor, and connecting the component to the internet (Figure 1). The exact source location (geographic and depth) is based on the outcome of sound propagation modelling. Using local bathymetry and oceanographic conditions as input, the model predicted the position with minimum impact of acoustic interference between the transmitted pulse and deflected/reflected signals. For further information on the installation process, see Hjelle et al. [9].

2.1.3. Experimental Facility

Field trials with AcuLice took place in Sunnhordaland between 2017 and 2023 at the Hattasteinen (Bømlo, Vestland, Norway, location number 11511) full-scale production facility. During this period, Hattasteinen had 2 production periods (2019 and 2023) with AcuLice treatment and 2 periods without AcuLice treatment (2017 and 2021), with a fallowing period of 2–4 months after each production cycle was ended, in accordance with regulations [16]. The number of sea cages in each production cycle was as follows: 2017—4 sea cages; 2019—4 sea cages; 2021—9 sea cages; 2023—5 sea cages. The Hattasteinen facility followed an ordinary production protocol for salmon farming for commercial

consumption, see [9]. Hattasteinen is a fjord facility with a salinity of 30–32‰. The mean (SEM) temperatures during the experimental period were as follows: 2017—10.6 °C (0.7); 2019—11.2 °C (0.6); 2021—10.8 °C (0.6); 2023—10.6 °C (0.7). In addition, Hattasteinen had a density of 10–12% of cleaner fish present in the cages during each production cycle.

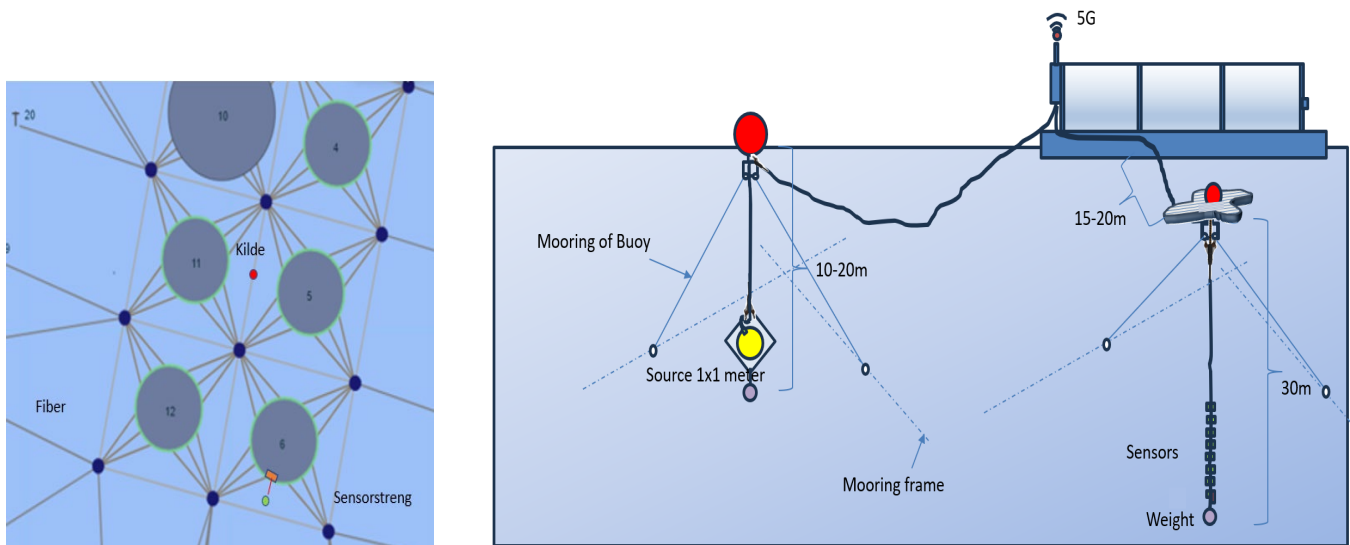


Figure 1. Horizontal (**left** panel) and vertical (**right** panel) views of the set up of the AcuLice system in a commercial salmon farm. The AcuLice system consists of a central control room with monitoring, an electronic processor located on the facility (not shown) and a component in the sea as shown here. An anchored source rig that transmit low frequency sound waves is located centrally among the pens (marked with red dot in left panel). Environmental information was received from sensors hanging in a wire at the pen periphery (marked “sensorstreng” in left panel). The whole system is connected to the internet.

2.1.4. Experimental Design

The installation of the AcuLice equipment at Hattasteinen in week 13 followed the general description as previously described (see Section 2.1.1 and [9]). Each trial period for salmon lice counting was set from start-up at week 16 in spring to week 20 the following spring (a period of 57 weeks) so both the first and second year of each production cycle was monitored in each case. The number of salmon lice treatments was also counted for this period (see [9]). Due to the ordinary operation at Hattasteinen the facility had to comply with the authorities’ delousing regulations if sexually mature female salmon lice exceeded the limit of 0.5 sexually mature female lice per salmon, or, in the spring, 0.2 sexually mature female lice per salmon. Throughout the different trial periods in the four-year period, delouse treatments occurred when necessary.

2.1.5. Protocol for Sampling

As an integral part of the field experiment, production data were collected with a focus on weight, weekly salmon lice infestation and number of salmon lice treatments. Fish were randomly collected from each sea cage ($N = 20$ from each cage) using hand nets. The fish were then stunned as directed on the given agent used (Benzocaine (Benzoak)). Registration of *C. elongatus* was registered in the same measurements. The salmon lice and *C. elongatus* count was undertaken by qualified salmon lice counters by carefully examining each individual fish. Salmon lice were classified into the following stages: sessile sea lice, mobile sea lice and adult female lice. The number of *Caligus elongatus* was also counted. Total mean values for each category were calculated for the data collected over the 57-week period at the Hattasteinen production facility. Apart from a mean of 0.05 sessile sea lice in the 2023 production cycle, no lice were detected at the beginning of each production cycle.

2.1.6. Data Collection

To analyze the number of weeks until the first sea lice treatment was required, data for all production groups were collected in the period from the sea transfer of Atlantic salmon to the first salmon lice treatment undertaken in each production cycle, in line with the description in our previous study [9].

2.2. Statistical Analysis

The distribution of all response variables was checked for normality and variance homogeneity using the Shapiro–Wilk test and the Levene test. No deviations from normality or homogeneity of variances were found. A general linear model (bidirectional random effects nested ANOVA) analysis was adapted between each of the response variables and predictor variables, “AcuLice” and “control” (i.e., production without AcuLice installed), and with replicate sub-sampling (random effect) as a nested factor within the predictor variables. A Student’s *t*-test was used to analyze the AcuLice and control groups in terms of the number of salmon lice treatments and the number of weeks from the time the Atlantic salmon were transferred to sea cages until the first salmon lice treatment occurred. A significance level of $\alpha = 0.05$ was used for all statistical models.

3. Results

3.1. Effect on Salmon Lice Dynamics—Sessile and Mobile Salmon Lice

A significantly lower number of small salmon lice was observed for the AcuLice-treated groups compared with the reference groups during the experimental period (one-way ANOVA, $p < 0.001$, Figure 2). The AcuLice-treated groups showed a mean ($\pm$ SEM) number of small (sessile and mobile) salmon lice from 0.69 ± 0.03 (in 2019) to 0.79 ± 0.02 (in 2023). The control group had a mean number of small salmon lice from 1.01 ± 0.04 (in 2021) to 1.34 ± 0.07 (in 2017).

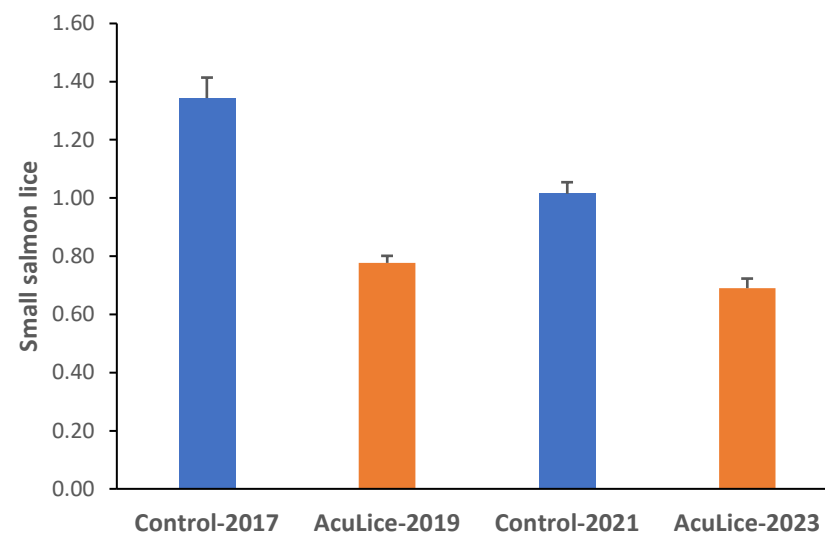


Figure 2. Mean number of small (sessile and mobile) salmon lice measured per Atlantic salmon in the four production cycles at the Hattasteinen production facility. Data are presented as mean $\pm$ SEM (N = 228–513 i.e., 4–9 sea cages for 57 weeks).

3.2. Effect on Salmon Lice Dynamics—Mature Female Lice

A significantly lower number of mature female salmon lice was observed for the AcuLice-treated groups compared with the reference groups during the experimental period (one-way ANOVA, $p < 0.001$, Figure 3). The AcuLice-treated groups had a mean ($\pm$ SEM) number of mature female salmon lice from 0.09 ± 0.01 (in 2019) to 0.15 ± 0.01 (in

2023), whereas the mean number of mature female salmon lice in the control group varied from 0.17 ± 0.03 (in 2017) to 0.27 ± 0.03 (in 2021).

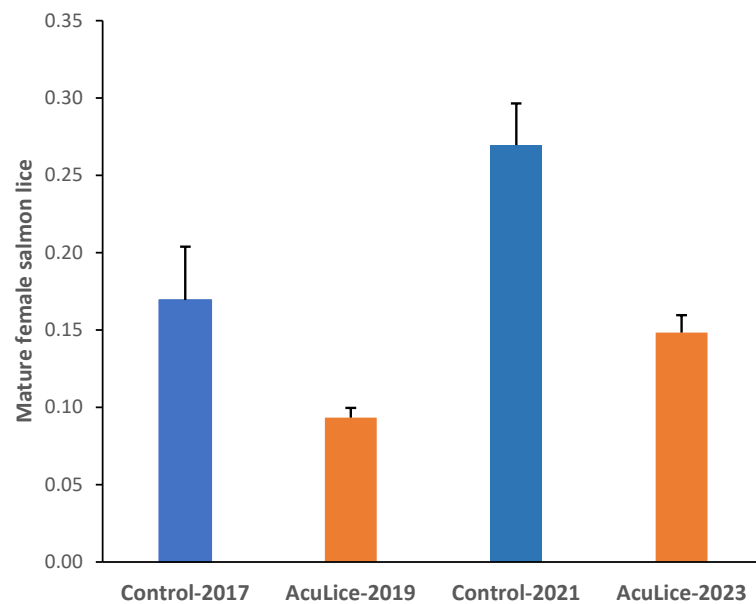


Figure 3. The mean number of mature female salmon lice measured per Atlantic salmon in the four production cycles at the Hattasteinen production facility. Data are presented as mean $\pm$ SEM (N = 228–513 i.e., 4–9 sea cages for 57 weeks).

3.3. Effect on Salmon Lice Dynamics—*Caligus elongatus*

A significantly lower number of *C. elongatus* was observed for the AcuLice-treated groups compared with the reference groups during the experimental period (one-way ANOVA, $p < 0.001$, Figure 4). The AcuLice-treated groups had a mean ($\pm$ SEM) number of *C. elongatus* from 0.02 ± 0.01 (in 2019) to 0.05 ± 0.01 (in 2023) whereas the mean number of *C. elongatus* in the control was 0.12 ± 0.02 (in 2021). There were no established routines at Hattasteinen for the registration of *C. elongatus* in 2017, so no data on *C. elongatus* were available for the control group in that year.

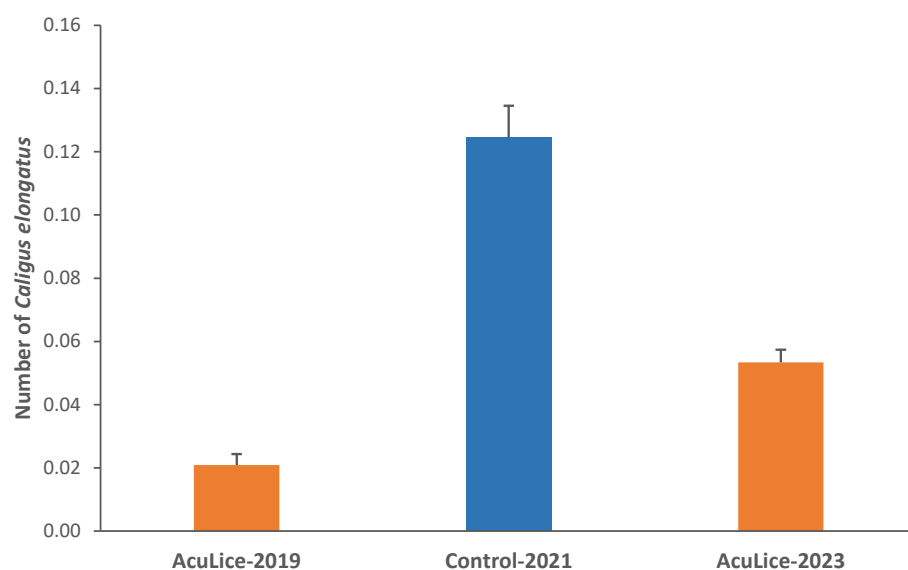


Figure 4. Mean number of *Caligus elongatus* measured per Atlantic salmon in the four production cycles at the Hattasteinen production facility. Data are presented as mean $\pm$ SEM (N = 228–513 i.e., 4–9 sea cages for 57 weeks). No data on *C. elongatus* were available for the first production cycle (control 2017) due to lack of registration routines.

3.4. Numbers of Salmon Lice Treatments in the Experimental Period

A significantly lower number of salmon lice treatments was observed for the AcuLice-treated groups compared with the reference groups during the experimental period (one-way ANOVA, $p < 0.001$, Figure 5). The AcuLice-treated groups had a mean number ($\pm$ SEM) of salmon lice treatments from 1.00 ± 0.13 (in 2019) to 1.80 ± 0.22 (in 2023), whereas the mean number of salmon lice treatments in the control varied from 4.00 ± 0.10 (in 2017) to 4.44 ± 0.19 (in 2021).

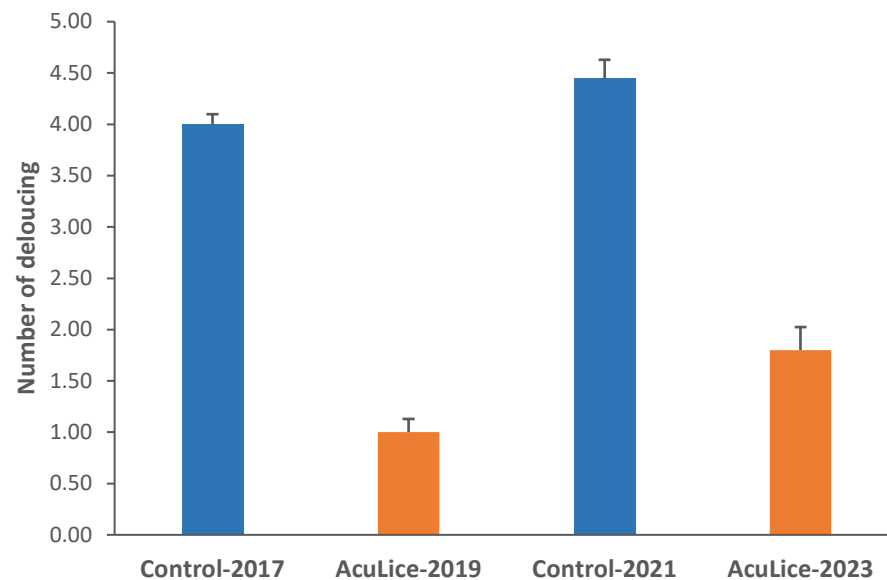


Figure 5. The mean number of mechanical sea lice treatments (number of delousing) performed over the four production cycles at the Hattasteinen production facility. Data are presented as mean $\pm$ SEM (N = 4–9 sea cages throughout the production cycle).

3.5. Numbers of Weeks to First Salmon Lice Treatment

Significantly higher (one-way ANOVA, $p < 0.001$) mean ($\pm$ SEM) number of weeks until first salmon lice treatment was observed in the AcuLice groups in 2019 (26.5 ± 0.3) and 2023 (21.6 ± 4.3) compared with the control groups in 2017 (5.0 ± 0.4) and 2021 (17.4 ± 0.2 SEM).

4. Discussion

In the salmon farming context, two categories—small salmon lice and mature female salmon lice—are most relevant in connection with accumulation and the de-licing limit [1,16,17]. Therefore, these main categories were analyzed. The results show that the AcuLice group had a significantly lower proportion of small salmon lice, mature female lice and *C. elongatus* lice compared with the control group during the entire experimental period. This (may) indicate that salmon lice and *C. elongatus* are removed or disappear during the salmon lice life cycle during the production cycles in which AcuLice was in use.

A previous study [13] has observed that the anterolateral flow field from a swimming salmonid is one of the most important factors for successful infestation of a host for the salmon louse. The flow field is derived from water being moved when the salmonid is swimming, producing infrasound at a low frequency range of 1–5 Hz [13,18,19]. As shown in the present study, some of the salmon lice had disappeared during the AcuLice treatment, and it is unclear exactly why this occurs. There is well-founded documentation that salmon lice behavior is stimulated by low frequency sound [10,11,13]. Further, there is growing evidence showing that other crustaceans also react to exposure to low frequency sound [12]; for example, and as shown by Hubert et al. (2018) [20], the way in which low frequency sound impacts food uptake in crabs. Further, Popper et al. (2018) [21] have underlined the

impact of particle motion on the sensory system of crustaceans. Our hypothesis is thus that sea lice are impacted by low frequency sound, and that they might leave their host because they feel uncomfortable under the influence of the AcuLice soundscape. Alternatively, their ability to feed under this uncomfortable regime might be reduced, causing them to leave due to starvation.

The results indicate that salmon lice disappeared in the period in which they are defined as small salmon lice and reach the stage of mature female salmon lice. As the present study includes a production locality that produces fish during its ordinary operation, it must follow national regulations, with delousing occurring at the limit of 0.5 mature female salmon lice. An average ($\pm$ SEM) of between 1.00 ± 0.13 and 1.80 ± 0.22 delousing operations per cage was carried out during the AcuLice periods, which was a significantly lower number of treatments compared with the reference group, with an average between 4.00 ± 0.10 and 4.44 ± 0.19 . This supports previous findings [9] showing AcuLice treatment to lead to a lower number of mature female lice, in turn leading to fewer salmon lice treatments.

An important aspect of the present study is the economic viability of the findings. Does the reduction in delousing treatment surpass the cost of the implementation and operation of AcuLice? In the present study there were three times more delousing treatments in the production cycles without AcuLice (an average of 4.2 delousing treatments per cage) compared with those production cycles using AcuLice (an average of 1.4 delousing treatments per cage). What does this mean in possible cost savings? At present delousing costs are around NOK 500,000 per cage. Facilities with six cages will then have a delousing cost of MNOK 3/delousing i.e., costs without AcuLice: MNOK $3 \times 4 =$ MNOK 12 vs. costs with AcuLice: MNOK $3 \times 1 =$ MNOK 3. *Thus, the saved costs amount to around 9 MNOK per production cycle due to reduced delousing costs in the AcuLice groups.* Further, when delousing, one has to starve the fish before treatment, typically for 4–5 days. It also often takes some time before appetite and pre-absorption are back to normal levels. It can take a week before you are back to normal levels. One can perhaps assume that one loses 7 feeding days/lice treatment. If one assumes a normal production of 1500 tons/permit/year and a production time of 365 days, one will lose $1500/365 = 4.11$ tons (round) per day. This will correspond to 3.58 tons gutted per day. If one assumes a sales price of NOK 80 and a production cost of NOK 60, this will mean NOK 71,600 per day: Loss without AcuLice (28 lost feeding days): NOK $71,600 \times 28$ lost feeding days $\times 3$ permits = MNOK 6 vs. Loss with AcuLice (7 lost feeding days): NOK $71,600 \times 7$ lost feeding days $\times 3$ permits = MNOK 1.5. *Thus, the saved costs amount to around 4.5 MNOK per production cycle due to reduced production loss in the AcuLice groups.* The third aspect is that of reduced mortality.

On average, mortality in fish farms along the Norwegian coast is at present at about 16%. It is estimated that between half and two-thirds are either directly caused by delousing or by the consequential mortality of fish that are weakened by viral diseases and thus cannot tolerate delousing. Thus, the use of AcuLice can potentially reduce current operation mortality by 50%. The delousing treatment will take place in the last part of the production cycle and the fish will then usually weigh 3–5 kg. If one starts with a fish of 4 kg and a value of 60 NOK (production cost/gutted salmon (factor 0.87)), then, with a stocking of 325,000 salmon/permit, mortality rates of 16% (without AcuLice) and 8% (with AcuLice) and an average weight of the slaughtered fish of 5.5 kg (round weight), this amounts to the following: Loss without AcuLice (8% mortality): MNOK 5.43×3 permits = MNOK 16.3, vs. Loss with AcuLice (2% mortality): MNOK 1.36×3 permits = MNOK 4.1. *Thus, the saved costs amount to around 12 MNOK per production cycle due to reduced mortality in the AcuLice groups.* In total this amounts to around $(9 + 4.5 + 12) = 25.5$ MNOK in saved costs per production cycle when using AcuLice. The current operating costs of AcuLice for a

production site of six sea pens are around MNOK 2.7/year. The total cost without AcuLice (12 + 6 + 16.3) is MNOK 34.3, the saved cost with AcuLice (25.5–2.7) is MNOK 22.8 and the reduced costs in percentage is 66%. i.e., the reduction in delousing treatment greatly surpasses the cost of the implementation and operation of AcuLice.

Johnson et al. (2004) [22] have estimated that, in marine and brackish water fish cultures, 61% of copepod infestations are caused by members of the Caligidae family, 40% of which are caused by species of *Caligus* and 14% by species of *Lepeophtheirus*. A major difference between *L. salmonis* and *Caligus* spp. lies in their host specificities. *L. salmonis* is essentially a parasite of salmonid fish [23], whereas many *Caligus* spp. tend to be much less host specific [23,24] and have been found on >80 fish species [23]. In the central and northern parts of Norway, high *C. elongatus* abundance on farmed fish frequently occurs in autumn [24]. Mature stages of *C. elongatus* are smaller than mature *L. salmonis* [25], with both sexes of equal size (around 6 mm). *C. elongatus* is a much better swimmer than *L. salmonis* and can re-infect other fish species if removed from the original host [24–27]. In the present study, AcuLice was demonstrated to be an effective treatment to significantly lower the number of *C. elongatus*, which may be very valuable given the possibly high infection rate and notoriety of *C. elongatus*.

Overall, the present findings clearly suggest that salmon lice and *C. elongatus* are removed from the fish during the salmon lice life cycle during the use of AcuLice and that the number of delousing treatments compared with the control production cycles is significantly lower. Based on these results, it appears that AcuLice influences the removal of salmon lice and *C. elongatus* and has a significant effect in reducing the burden of salmon lice and *C. elongatus* on the commercial production of Atlantic salmon. The present findings add further strength to our previous study [9], as we here present a time-series on the effect of AcuLice at the same production site over four consecutive production cycles, each covering both the first and second year of salmon in the sea. It should be noted, however, that the present experimental design, comparing AcuLice-treated and control groups, involved samplings conducted in different years. However, both baseline lice data and environmental data were very similar in all production cycles, adding strength to our findings.

5. Conclusions

The long-term field studies in four consecutive production cycles showed changes in salmon lice and *C. elongatus* composition, in the number of salmon lice treatments and in the number of weeks until the first required treatment, indicating that AcuLice treatment had a significant effect on reducing the salmon lice burden in Atlantic salmon commercial production. Understanding the functional mechanism behind these results is a subject for future studies.

Author Contributions: A.K.D.I., P.B. and S.H. planned the research, designed the study and analyzed the data. A.K.D.I., S.H. and O.R.G. wrote the article and all authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The present field trials were approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by that authority.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be obtained by contacting the authors.

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Conflicts of Interest: Author Olav Rune Godø was employed by the company Husgod Holding AS. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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