Faglig sluttrapport for prosjektet:

AcuLice; Effekt av sammensatt akustisk lydbilde i sjø på lakselus, ett storskala 'proof of consept' studie

Utarbeidet av

Sigurd Handeland, UIB
Albert Imsland, Akvaplan-NIVA AS

Bergen 05.10.2021

Innholdsfortegnelse

Prosjekt finansiering

FoU delen av dette prosjektet (Prosjektnr: 901567, TilsagnsID: 49058) er i sin helhet finansiert av FHF. I tillegg har konsortie partnerne bidratt med en betydelig egeninnsats i form av uttesting av utstyr og innsamling av data.

Deler av dette prosjektet er blitt gjennomført som en mastergradsstudie ved Universitetet i Bergen. Mastergraden er skrevet på engelsk. Rapporten er, derfor, delvis på engelsk.

1. Sammendrag

Abstrakt

AcuLice™ er et nyutviklet system som bruker et sammensatt akustisk lydbilde med lavfrekvent lyd for å fjerne lakselus fra Atlantisk laks (heretter referert: *AcuLice*. Dette prosjektet undersøker og dokumenterer mulige stresseffekter ved bruk av systemet på Atlantisk laks samt effekten på lakselusdynamikken i storskala av *AcuLice* systemet. Effekten av *AcuLice* behandling på lakselusdynamikk ble målt ved ukentlig lakselustelling på anleggene fra uke 30, 2019 til uke 20, 2020. Antall lakselusbehandlinger i samme periode ble også registrert og sammenlignet mellom *AcuLice* - og referansegruppen. I tillegg ble antall uker fra utsett i sjø til første lakselusbehandlingen (mekanisk behandling) sammenlignet mellom de to gruppene. Funnene fra stressanalysene viste ingen signifikant forskjell i kortisol responsmålingene mellom *AcuLice* og kontrollgruppen. I tillegg, bortsett fra liten økning i plasma glukose, ble det ikke observert noen signifikant forskjell i den sekundære eller tertiære stressresponsen. For de modne hunn-lusene ble det vist et betydelig lavere antall for *AcuLice* gruppen. I tillegg ble det observert et lavere antall lakselusbehandlinger og en lengre produksjonsperiode før den første lakselusbehandlingen ved *AcuLice* anleggene. Dagens data tyder på at *AcuLice* behandling ikke har en negativ effekt på Atlantisk laks når det gjelder akutt stress og at behandlingen har en positiv effekt på reduksjon av lakselustrykket på produksjonsstedet.

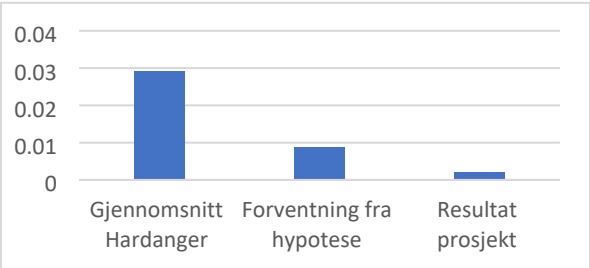
Abstract

AcuLice™ is a newly developed system which uses a composite acoustic sound image with low-frequency sound to remove salmon lice from Atlantic salmon (ref: *AcuLice)*. This project examines and documents the stress effects on Atlantic salmon as well as the effect on salmon lice dynamics in large-scale of the *AcuLice* system. The effect of *AcuLice* treatment on salmon lice dynamics was measured by weekly salmon lice counting's at the facilities from week 30, 2019 to week 20, 2020. The number of salmon lice treatments in the same period, was also recorded and compared between the *AcuLice* - and reference group. In addition, numbers of weeks from transfer to sea until the first salmon lice treatment (mechanical treatment) was compared between the two groups. The findings from the stress analyses showed no significant difference in the corticol response measurements between the *AcuLice* and control group. In addition, apart from slight increase in plasma glucose, no significant difference was observed in in the secondary or tertiary stress response measured. For the mature female salmon lice, a significant lower number was shown for the *AcuLice* group. In addition, a lower number of salmon lice treatments and a longer production period before the first salmon lice treatment occurred was observed at the *AcuLice* facilities. Present data suggest that *AcuLice* treatment does not have a negative effect on Atlantic salmon when it comes to acute stress and that the treatment has a positive effect on reduction of salmon lice pressure at the production site.

2. Bakgrunn

Bekjempelse av lakselus er en av de største utfordringene i norsk lakseoppdrett og kostet oppdretts- næringen mer enn 5 mrd. NOK i 2014 (Iversen et al., 2016). I sum tilsvarer dette ca 9 % av inntektene til det enkelte anlegg (Abolofia et al. 2017). Kampen mot lakselus foregår derfor på mange fronter og for næringen er det avgjørende at alle muligheter undersøkes. Herunder er det viktig å utvikle metoder som innbefatter høy effektivitet, lave kostnader og minimalt med negative effekter på fisk, samtidig som en unngår svinn, arbeidskrevende operasjoner og negative effekter på miljø.

Bruk av et sammensatt akustisk lydbilde (lavfrekvent lyd), er lansert som en ny metode for å hindre påslag av lakselus. Metoden er billig sammenliknet med andre metoder, krever ingen håndtering av fisken og har sannsynligvis små negative effekter på fisk og miljø. Siden lyd i liten grad forplanter seg fra vann til luft har metoden heller ingen negative HMS effekter. *AcuLice* tilfredsstiller dermed mange av kriteriene til en ideell bekjempelsesmetode mot lus. Spørsmålet er bare om metoden er effektiv nok i storskala. Det har siden 2011 blitt gjennomført en rekke undersøkelser og samarbeid mellom ulike forskningsinstitusjoner, Innovasjon Norge og FHF som har ledet frem til ny og innovativ teknologi for oppbygging av et akustisk lydbilde til bekjempelse av lakselus. Bremnes Seashore AS var det første oppdrettsselskapet som testet ut systemet i pilot fullskala. Forsøket ble gjennomført på lokaliteten, Nye Hessevik (2018), i samarbeid med NORCE og NIVA. Resultatene viste at bruk av et akustisk lydbilde resulterte i signifikant mindre lus på fisken sammenliknet med referansefisk uten slik behandling.



Figur 1. Resultat fra pilotstudien på 'Nye Hessevik' lokaliteten til Bremnes Seashore AS og som viser en klar forskjell andel modne lus i grupper med og uten *AcuLice* behandling.

Motivasjonen bak dette prosjektet har vært å utfordre de positive resultatene fra pilotundersøkelsen, herunder kartlegge om ett sammensatt akustisk lydbilde vil redusere nivået av lus i oppdrett i en større regional sammenheng (Delprosjekt 1).

Ved bruk av et sammensatt akustisk lydbilde mot lakselus så vil en operere i det samme frekvensområdet som sjøpattedyr og fisk kan oppfatte. Fisk med gassfylte indre strukturer er følsom for lydtrykk, og lavfrekvent lyd er derfor blitt brukt for å 'kalle inn' vill fisk i fjordsystem til fôringsstasjoner (Midling et al. 1987; Björnsson et al. 2010). I hvilken grad lyd fra *AcuLice* vil være forstyrrende og/eller stressende for fisk er usikkert. Denne problemstillingen vil bli utfordret i Delprosjekt 2 hvor vi ønsket å kartlegge effekten av et sammensatt akustisk lydbilde på velferd, stress og helse hos laks i oppdrett.

Skal havbruksnæringen ha mulighet for videre vekst er en avhengig av å redusere/eliminere lus som en utfordring. I 2011 ble det innført et nytt brakkleggingsregime for Hardangerfjorden, i tillegg til nye lusekoordineringsområder. Havbruksaktørene deltok aktivt, og koordinerte sin innsats gjennom de lokale/regionale fiskehelsenettverkene. En så at felles innsats hadde effekt, både internt og vis à vis omgivelsene. Utfordringen med lakselus, og følgeskadene av dette, må likefult løses i sin helhet. Det betyr at alle aktører må ta i bruk en metode og/eller teknologi i et felles forsøk på å få kontroll på lusa, og på en måte som ikke skader omkringliggende miljø. Disse problemstillingene er adressert i *AcuLice* prosjektet. Hovedmålsettingen har vært å undersøke om bruk av sammensatt akustisk lydbilde kan utvikles til å til å bli en regional behandlingsmetode mot lus i havbruksanlegg. Prosjektet er gjennomført i Produksjonsområde 3 (PO3) og har bestått av to delprosjekt med klart definerte delmål/resultatmål:

Delmål DP1; Å dokumentere effekten av *AcuLice* på infeksjonsrisiko mot lus hos oppdrettslaks i ett regionalt perspektiv.
Delmål DP2; Å kartlegge effekt av *AcuLice* på velferd, stress og helsesituasjon hos laks i oppdrett.

Organisering

Dette prosjektet er organisert gjennom PO3 Kunnskapsinkubator. Partnerne i *AcuLice* prosjektet er valgt ut fra et ønske om å bringe inn et tverrfaglig miljø med erfaring og ekspertise innen de aktuelle forskningsfelt. Prosjektet er administrativt koordinert av Even Søfteland. En kortfattet beskrivelse av partnerskapet er gitt i underliggende tekst.

Oppdrettspartnere PO3: Bremnes Seashore AS, Erco Seafood AS, Lerøy sjøtroll, Alsaker Fjordbruk AS.

Nasjonale FoU partnere: Universitetet i Bergen, Akvaplan-niva og NIVA Forskningspartnerne som inngår i konsortiet har lang erfaring fra nasjonale og internasjonale prosjekter. Fra UiB deltar Prof. Sigurd Handeland (vekst, miljøkontroll, smoltutvikling) og dr. Pablo Balseiro (molekylærbiologi, NORCE). I tillegg fikk siv. Ing kandidat Bibbi Hjelle en tilknytning til prosjektet via gjennomføring av master prosjekt. Akvaplan-niva sin aktivitet er ledet av Prof. Albert Imsland. Han har forfattet og med-forfattet mer enn 185 vitenskapelige publikasjoner i internasjonale tidsskrifter med refereeordning og har omfattende prosjektledererfaring fra FHF prosjekter. I tillegg har Dr. Sara Calabrese, NIVA deltatt i fiskevelferd evalueringen i DP1.

Kvalitetssikring

Prosjektbeskrivelse og plan er kvalitetssikret i henhold til interne rutiner hos UiB og gjennom FHFs rutiner for gjennomgang av faglig og næringsmessige relevans. Bremnes Seashore AS, har sammen med NIVA, UiB m.fl. gjennomført et forprosjekt knyttet til en lokalitet i PO3 (Vedlegg 1). Innhold, metoder og resultat ble kvalitetssikret og dokumentert gjennom fremleggelse til Mattilsynet i forkant av oppstart. Det er dette som skaper grunnlaget for en regional utprøving og dokumentasjon. Prosjektets innhold er gjennomgått av involverte samarbeidspartnere.

3. Problemstilling og formål

Havbruksnæringen har få verktøy i sin tilnærming til å eliminere lus som en utfordring på oppdrettsanleggene. Skal lakselus elimineres må en være villige til å samhandle i større regioner enten det gjelder antallet/størrelse på lokaliteter, avstander, soner, teknologi eller metodebruk. Bruk av *AcuLice* teknologi har vist seg effektivt ved lokaliteten, Nye Hessevik. Om bruken også viser seg effektivt på et regionalt nivå, innehar dette en næringsnytte av stor verdi. Men næringsnytten er avhengig av at en ikke påvirker omkringliggende miljø på en måte som er uheldig over tid, og/eller at en eventuell påvirkning kan styrkes gjennom avbøtende tiltak. Ett av de mest effektive forholdene med bruk av teknologien bak *AcuLice*, ligger i at denne er; 1. Enkel å bruke, 2. Krever lite innsats fra de ansatte på anleggene, 3. Forstyrrer ikke produksjonen, og 4. Bedrer risikobildet jfr. dagens operasjoner og reduserer antall avlusninger

2011 og 2012 var de to siste, gode, biologiske årene for havbruksaktørene på Vestlandet. Endring i regelverket i 2013 satt sammen med mangel på nye verktøy for avlusning førte næringen ut i en situasjon som har gitt et forverret biologisk bilde. Behandling for lus, og følgeskader av dette, koster i dag næringsaktørene i Sør mellom 10 og 15 kr/kg. I dette ligger selve avlusningen, utgang, sulting, nedsatt appetitt, forverret sykdomsbilde, økt bruk av helsefôr mm. Slik kan det ikke kan fortsette.
Norge har et sentralt fortrinn innen havbruk – dvs bruk av åpen merd i sjø. Skal Norge videreutvikle seg som en havbruksnasjon må alle involverte parter sette fokus på å utvikle nettopp dette fortrinnet.

Leveranser

Hendelser de siste årene knyttet til bruk av nye metoder, medikamenter mm. har vist et stort behov for nødvendig dokumentasjon i tilknytning til uttesting og normal bruk av nye produkt, teknologi og metoder. *AcuLice* prosjektet har således et behov for å få fram alle sider ved teknologien/metoden og dette er knyttet til behovet for å skape varighet over tid, uten forstyrrende/ødeleggende moment. All forskning som utføres i prosjektet skal kunne publiseres, og iht. FHF sin norm for bl.a. sluttrapportering. En oversikt over leveranser fra prosjektet er gitt under kapittel 7. Leveranser.

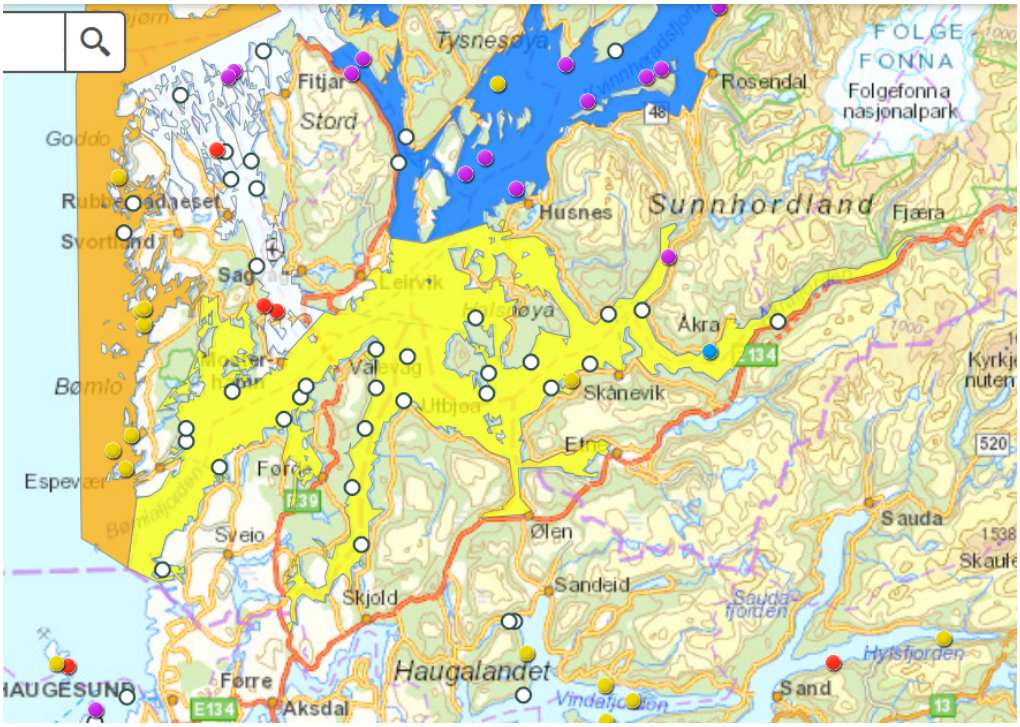
4. Prosjektgjennomføring

Delprosjekt 1. Effekt av *AcuLice* på infeksjonsrisiko for lakselus hos oppdrettet laks

Hensikt: Bekrefte/avkrefte om bruk av *AcuLice* er en effektiv metode for å redusere volumet lus i havbruksanleggene i PO3.

FoU-aktiviteter; gjennomføring og metode

I første fase av prosjektet ble teknologien bak *AcuLice* testet på 9 havbruksanlegg i Sunnhordland (navn på lokaliteter er gitt i tabell 1). I tillegg ble tre anlegg benyttet som kontroll, dvs. uten *AcuLice* behandling (navn på lokaliteter er gitt i tabell 1). Forsøket ble gjennomført i perioden mai 2019 til mai 2020. Oppstart på den enkelte lokalitet varierte pga ulik tid for utsett av forsøksfisk og øvrige bedriftsinterne forhold. I forkant av feltforsøket ble det i samarbeid med utstyrsleverandør gjennomført en innledende fase med fokus på oppsett av teknisk utstyr, herunder lokalisering av den elektromekaniske innretningen i sjø, plassering av elektronisk kabinett på flåte samt forbindelse mellom enhetene. Den elektromekaniske innretningen ble plassert i senter av anlegget. Lydbildet ble tilpasset hver enkelt lokalitet bl.a. for å minimalisere risiko for interferens fra omgivelsene. Denne fasen ble det også utarbeidet en komplett kravspesifikasjon til vedlikehold og drift. Installeringen tok fra 4 til 6 uker, dvs. fra en startet til alle anlegg er ferdig installert. En oversikt over forsøksområdet er gitt i bilde 1.



Bilde 1. Kartutsnittet viser gult området som er en del av forsøket.

Straks *AcuLice* var etablert og lokaliteten var klargjort ble utstyret aktivert og forsøket var igangsatt. Hver forsøksgruppe ble fulgt fra oppstart og frem til først påkrevde avlusning. Krav til lusebehandling ble besluttet av anlegget sin veterinær basert på ukentlige lusetellinger. Som en integrert del av forsøket ble det i hele forsøksperioden hentet inn produksjonsdata fra hver

lokalitet (*AcuLice* og kontroll) med fokus på ukentlig lusepåslag, vekt og overlevelse i tillegg til miljø data (salinitet, temperatur og oksygen). Lusepåslag ble registrert som små bevegelige lus og som kjønnsmoden hunnlus. Som en ekstra referanse til kontrollanleggene ble det besluttet å innhente informasjon fra 'andre grupper av fisk', dvs. vi benyttet en kombinasjon av tidligere statistikk for lusepåslag i området (data fra de fire siste årene), i tillegg til å hente data fra Barentswatch. Denne kombinasjonen av empiriske data fra prosjektet, erfaringer fra tidligere forsøk og data fra Barentswatch ble deretter benyttet til statistisk analyse av lusebelastning og antall uker frem til påkrevd lusebehandling under forhold med og uten bruk av *AcuLice*. Analysearbeidet innbefattet ett betydelig datamateriale (n=18 000), og hvor det forelå variasjon mellom de ulike anlegg mhp lusepåslag som følge av ulik lokalisering, utsettingstid, lusebelastning, annen lusebehandling (rensefisk) etc. Denne variasjonen har komplisert arbeidet med å analysere og tolke dataene. Effekten av variasjon er imidlertid kompensert for gjennom å øke størrelsen på datamaterialet hvilket har bidratt til å sikre en god styrke i de statistiske analysene. Det presenterte datagrunnlaget tilfredsstiller alle krav som er satt til bruk av parametriske statistiske metoder.

Følgende forutsetninger er lagt til grunn for analysen:

1. For alle fiskegrupper er forsøksperioden definert fra oppstart *AcuLice* og frem til første behandling (besluttet av veterinær).
2. Luseregistrering er gjennomført hver uke (4-8 merder pr lokalitet) og registreringen har innbefattet telling av små og store bevegelige samt modne hunnlus.
3. Tall fra Barentswatch er benyttet som kontroll for antall uker til behandling.
4. Effekt av behandling er undersøkt med Student's t-test og enveis ANOVA og signifikant effekt er påvist ved en p-verdi > 0.05.

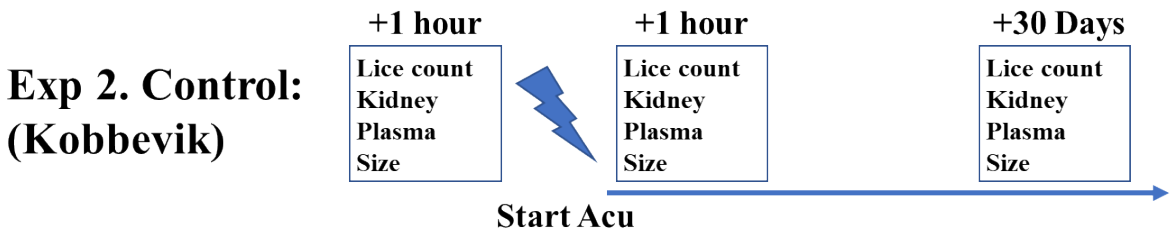
Delprosjekt 2. Effekt av *AcuLice* på velferd, stress og helsesituasjon hos laks i intensiv oppdrett

Hensikt: Bekrefte/avkrefte om bruk av *Aculice* teknologi har innvirkning på velferd, stress og fiskehelse hos laks.

FoU-aktiviteter; gjennomføring og metode

Denne delen av prosjektet har tatt utgangspunkt i en tilfeldig valgt lokalitet (Kobbevik og Furuholmen, Langenuen) og hvor fisken ble spesielt fulgt opp mhp. registrering av velferd, stress og fiskehelse, før og etter behandling med *AcuLice*.. Gjennomføring av forsøket fulgte samme protokoll som tidligere omtalte pilotprosjekt (Vedlegg 1).

Etter overføring til sjø, og klargjøring for *AcuLice* behandling, ble fiskene fulgt opp med tre prøveuttak; en time før oppstart behandling (kontroll), en time etter oppstart og 30 dager etter behandling. Første uttak er således uten *AcuLice* påvirkning og fremstår som en intern kontroll (Fig 2).



Figur 2. Skjematisk oppsett over Delprosjekt 2, Velferdstest av *AcuLice* på laks i sjø

Ved hvert uttak ble det tatt prøver av fisken mhp dokumentasjon av størrelse, kondisjon og kvalitet. Som en del av prøveprogrammet ble 20 fisk avlivet med bedøvelse og gjellebue #2 på venstre side ble tatt ut og overført til eppendorf rør med SEI (lagret på frys). Fra disse prøvene vil det bli analysert gjelle Na^+,K^+-ATPase (NKA) aktivitet ihht standardiserte metoder. I tillegg er det hentet inn prøver fra blod og vev. Fiskene er også undersøkt m.h.p. ytre morfologi; finneslitasje, gjellelokk og sår. For hver av de observerte velferdsindikatorer er det gitt en heltallsscore på mellom 0 og 5 (fra 0 til 2 for gjellelokk, katarakt og sår). Karakteren 0 tilsier ingen skade mens karakteren 5 (eller 2) betyr ett klart velferdsproblem. Blod er analysert for plasmakortisol. Videre vil blodplasma bli fulgt opp med nye analyser av glukose, ionenivå (Na^+, Cl^-). Fra de samme fiskene er det også tatt vev fra hjerte og hodenyre og som er overført til RNA later. Vevsprøvene er lagret og utvalgte grupper kan bli analysert ved tegn på sykdom, økt dødelighet eller redusert velferd (kun etter avtale med oppdretter). Slike prøver vil da bli analysert ved hjelp av real time PCR teknologi, med tilhørende primere og prober utviklet for deteksjon av ulike sykdoms agens hos laksefisk.
I tillegg er tegn på luseangrep, gjellestatus, sår og AGD score sjekket som en del av driften av anlegget. Lokalitetsansvarlig har vært ansvar for å sikre at prosedyre for telling og registrering av lakselus er gjennomført etter protokoll. Dødfisk, både laks og rensefisk, er registrert daglig (Fishtalk) med dato, antall fisk og eventuelle merknader med hensyn på mulig dødsårsak. Under testperioden ble det gjennomført daglige registreringer av utfôret mengde for å kunne beregne fôrutnyttelse. (Resultatene som ikke inngår i rapporten vil publiseres i masteroppgave).

Delprosjekt 5. Formidling og kommunikasjon; Helhetlig resultat evaluering, og offentliggjøring av funn til oppdrettere, myndigheter og allmennhet

Hensikt: Å sammenfatte resultatene fra DP1-2, kommunisere disse til oppdrettere, offentlige myndigheter og øvrige interessegrupper.

Aktiviteter; Gjennomføring og metode
Kommunikasjon fra prosjektet vil være svært viktig for å sikre en stor nytteverdi. All publisering i prosjektet vil bli klarert med Styringsgruppen, og rutiner for dette vil bli nedfelt i konsortieavtalen. Prosjektet vil formidle resultatene i 1) internasjonal journal med referee-ordning 2) fagtidsskrifter, f.eks. Norsk Fiskeoppdrett, 3) FHF-rapporter, og 4) deltakelse i industrifora som AquaNor, FHL-møter, AqKva konferansen i Bergen. Avklaring av eventuell påvirkning av miljø vil bli kommunisert direkte til Mattilsynet, Fylkesmann og Fiskeridirektoratet.

5. Oppnådde resultater, diskusjon og konklusjon

Delprosjekt 1. Effekt av *AcuLice* på infeksjonsrisiko for lakselus hos oppdrettet laks

Specific growth rate (SGR)

The *AcuLice* treated groups had a minimum value of SGR in weight at 0.32 % day^{-1} (Svollandsneset) and a maximum growth rate at 0.52 % day^{-1} (Grimsholmen, Hattasteinen) in the period from week 30, 2019 to week 20, 2020 (Fig. 5). For the reference group the minimum growth rate was 0.37 % day^{-1} (Maradalen) and maximum were 0.48 % day^{-1} (Mælen) in the same period. Overall, there were no significant differences in the mean SGR between the reference group and the *AcuLice* treated group (Student's t-test, $P > 0.05$, Fig. 3), in the experimental period (week 30, 2019 to week 20, 2020). The mean SGR for the *AcuLice* treated groups was 0.45 % day^{-1} and for the reference group 0.43 % day^{-1}.

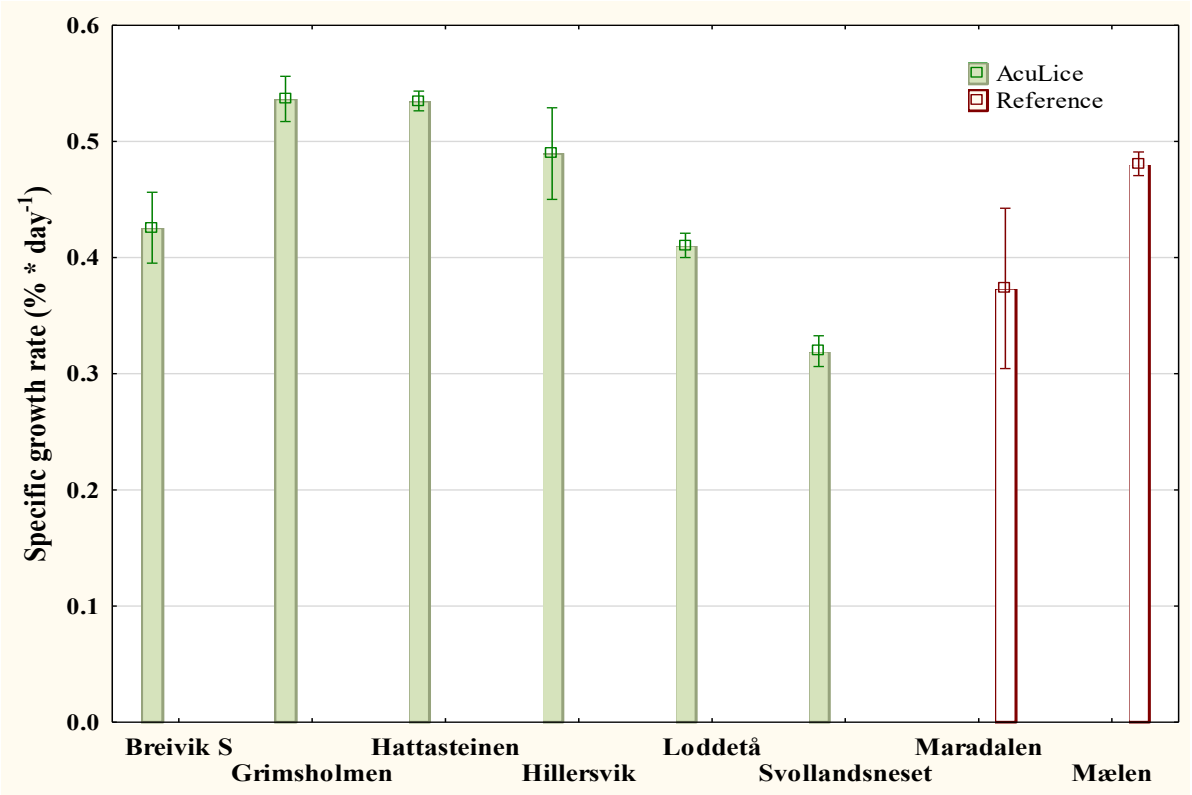


Fig. 3. Mean specific growth rate (SGR (% day^{-1})) calculated for the Atlantic salmon in each experimental facility in Experiment 2A in the period from week 30, 2019 to week 20, 2020. The *AcuLice* treated facilities are marked in green, and the reference group are marked in red. Data from each production facility is presented as mean ± SEM.

Effect on salmon lice dynamics – sessile and mobile salmon lice

The *AcuLice* treated groups showed a mean number of small (sessile and mobile) salmon lice from 0.39 (Loddetå) to 1.22 (Hillersvik) in the period week 30 in 2019 to week 20 in 2020 (Fig. 4). The reference group had, in the same period, a mean number of small salmon lice from 0.07 (Mælen) to 0.24 (Maradalen).

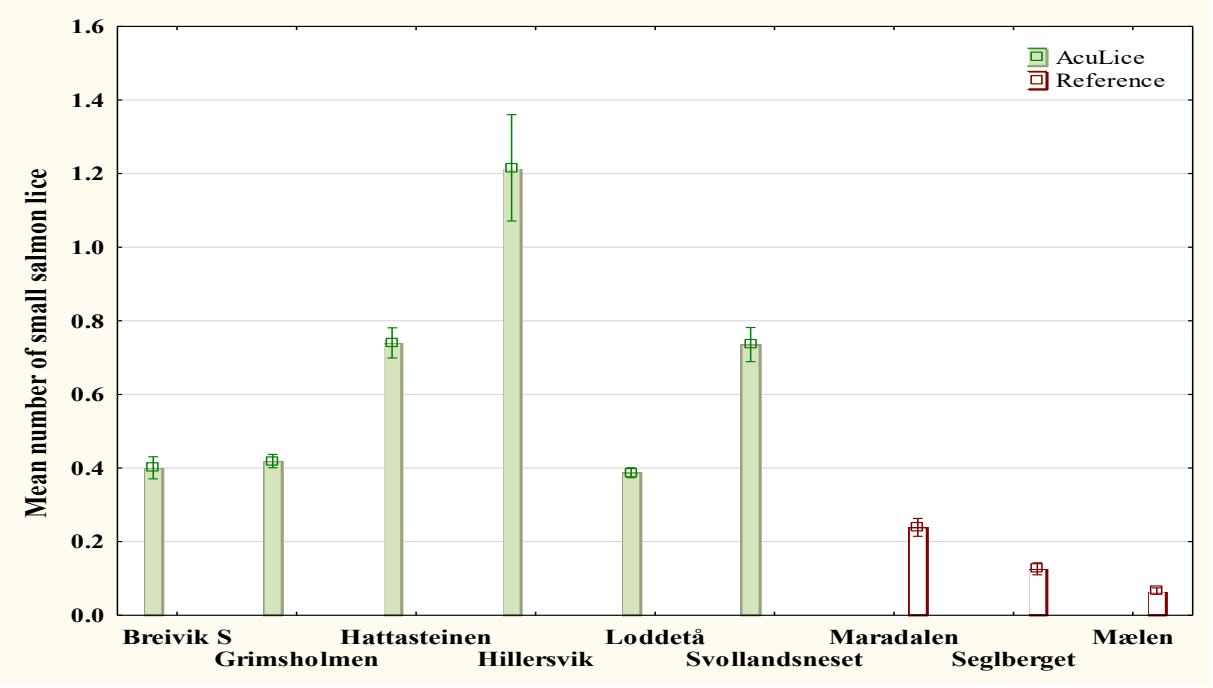


Fig. 4. Mean number of small (sessile and mobile) salmon lice measured per Atlantic salmon in the period from week 30, 2019 to week 20, 2020 at each facility. Groups of Atlantic salmon exposed to *AcuLice* treatment (*AcuLice*) compared to the reference group (Reference). Green marked columns are facilities with *AcuLice* treatment and red columns are the reference facilities. Data from each facility is presented as mean ± SEM.

Effect on salmon lice dynamics – mature female lice

The *AcuLice* treated groups had a mean number of mature female salmon lice from 0.12 (Breivik S) to 0.31 (Hillersvik) in the period week 30 in 2019 to week 20 in 2020 (Fig. 5). The reference group had in the same period a mean number of mature female salmon lice from 0.39 (Maradalen) to 0.49 (Mælen).

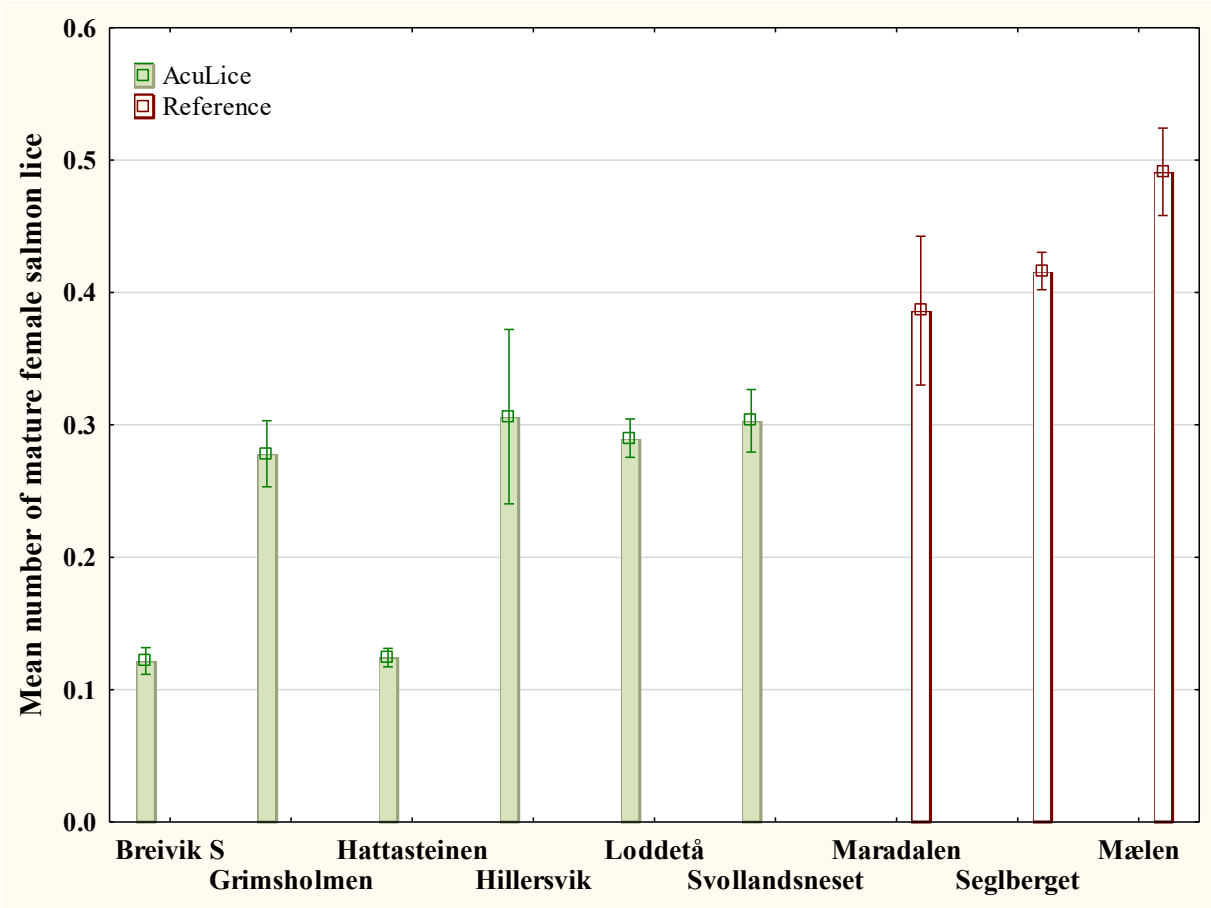


Fig. 5. Mean number of mature female salmon lice measured per Atlantic salmon in the period from week 30, 2019 to week 20, 2020 at each facility. Groups of Atlantic salmon exposed to *AcuLice* treatment (*AcuLice*) compared to the reference group (Reference). Green marked columns are facilities with *AcuLice* treatment and red columns are the reference facilities. Data from each facility is presented as mean ± SEM.

Effect of AcuLice treatment in salmon lice population composition

Higher number of small salmon lice was observed for the *AcuLice* treated groups compared to the reference groups during the experimental period (one-way ANOVA, $P < 0.001$, Fig. 6A). In contrast, a lower number of mature female salmon lice was observed for the *AcuLice* treated groups compared to the reference in the same period (one-way ANOVA, $P < 0.001$, Fig.)6.

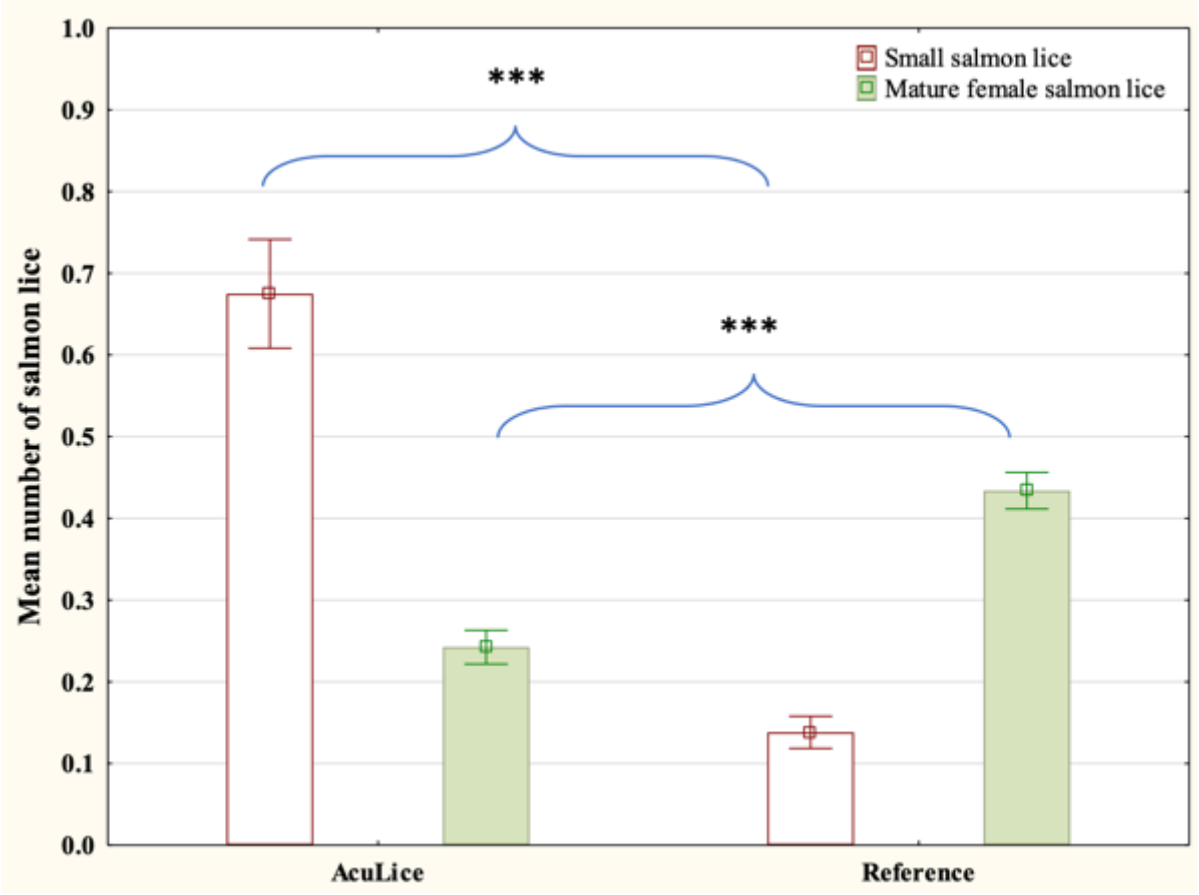


Fig. 6A. Mean number of small salmon lice (red columns) and mature female lice (green columns) measured as per Atlantic Salmon between group exposed to *AcuLice* treatment compared to the reference group, during the period (week 30, 2019 to week 20, 2020). Asterisks indicates the level of significance between the two groups: *** $P < 0.001$. Data from each treatment group is present as a mean ± SEM.

Numbers of salmon lice treatments in the experimental period

The *AcuLice* treated group had significant lower number (3.1) of salmon lice treatments (Student's t-test, $P < 0.05$) during the 43 weeks period (week 30, 2019 to week 20, 2020) compared to the reference group (6.3, Fig 6B).

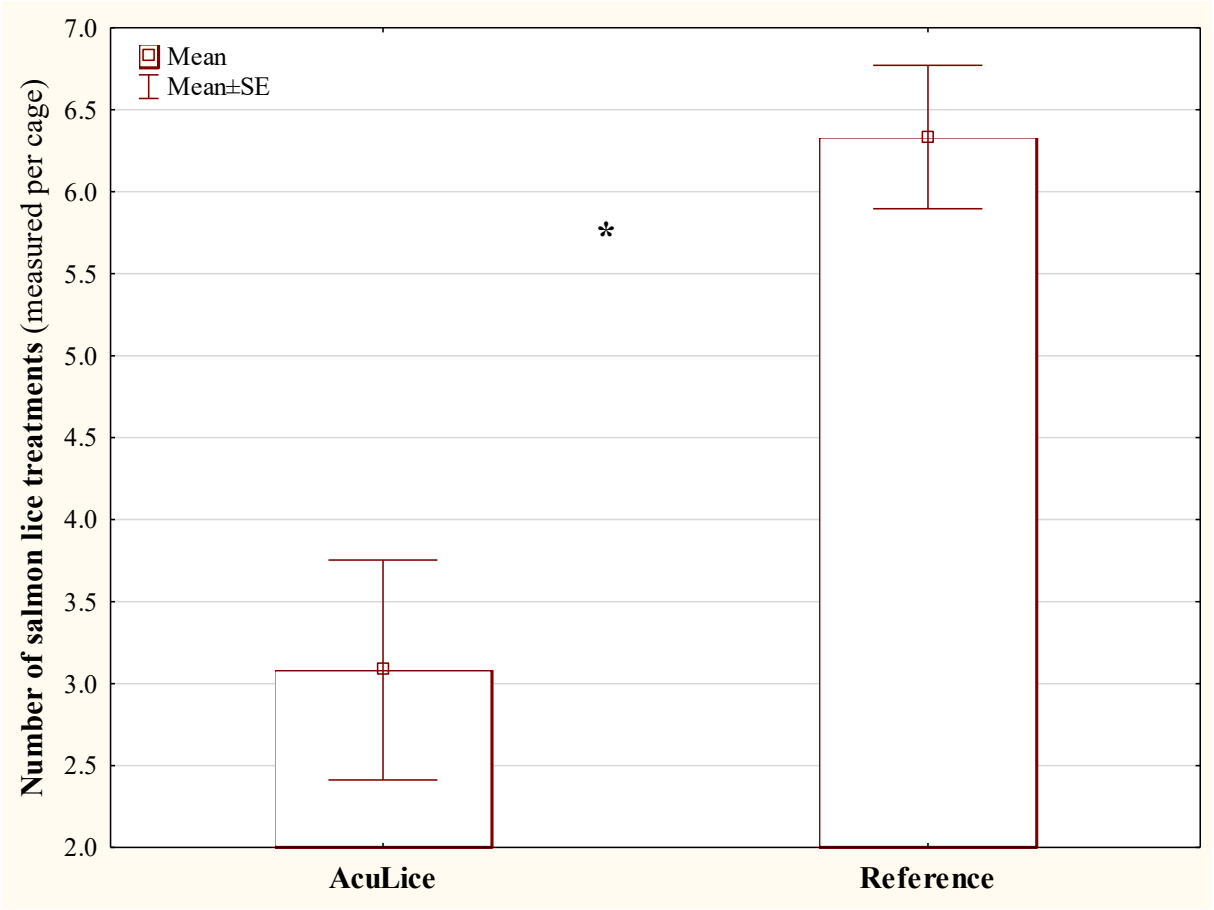


Figure 6B. Mean number of salmon lice treatments during the period (week 30, 2019 to week 20, 2020) measured as treatment per cage between group exposed to AcuLice treatment compared to the reference group. Asterisks indicates the level of significance between the two groups: * $p < 0.05$. Data from each facility is presented as mean ± SEM.

Numbers of weeks to first salmon lice treatment

For the *AcuLice* treated facilities the minimum number of weeks was 22 (Grimsholmen, Fig. 7) and the maximum number was 40 weeks (Loddetå). The reference group had a period of 16 to 25 weeks (Seglberget, Maradalen) before the first treatment was necessary. Overall, the mean number of weeks until the first salmon lice treatment increased significantly (Student's t-test, $P < 0.05$) from 20.3 weeks in the reference groups, to 33.2 weeks in the *AcuLice* treated groups.

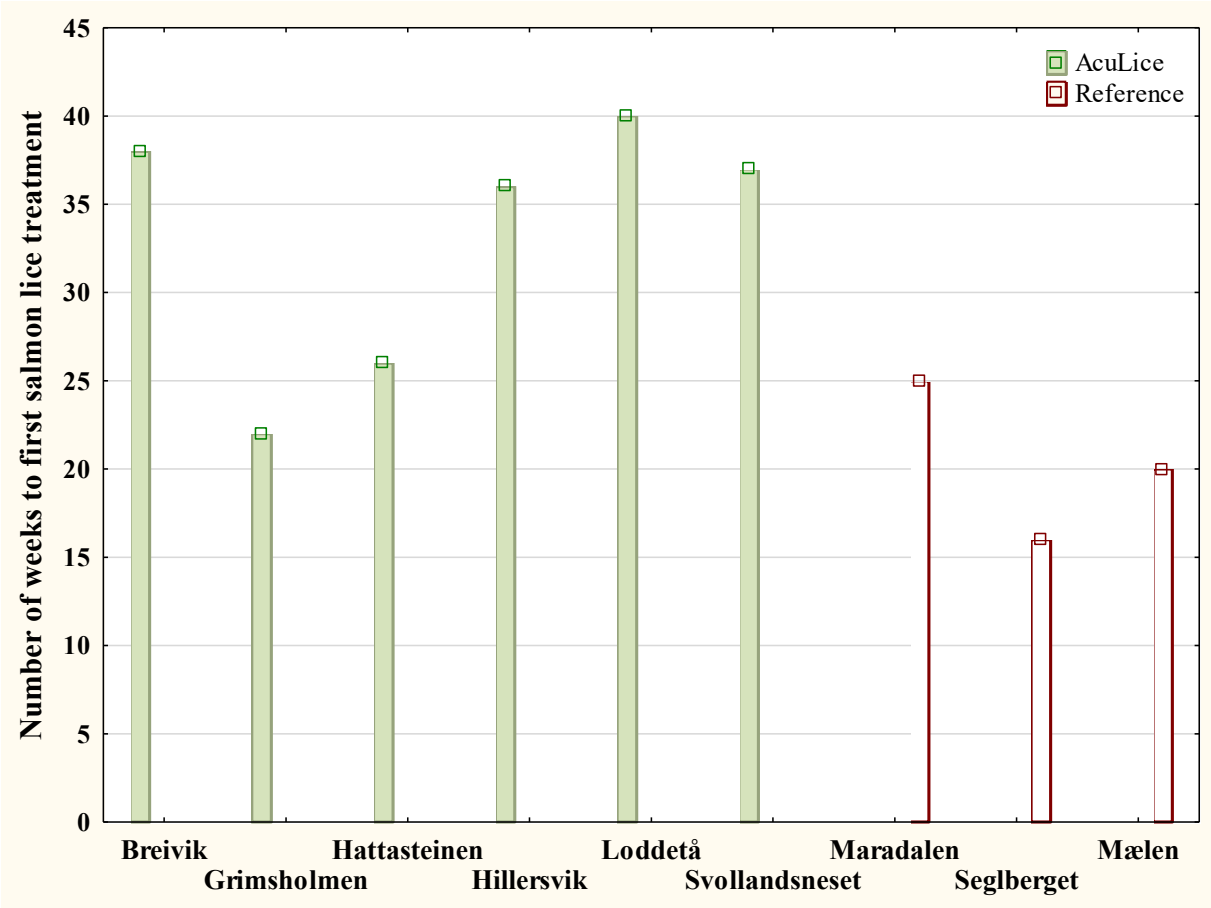


Fig. 7. Mean number of weeks to the first salmon lice treatment at the experimental facilities in Experiment 2. Green marked columns are facilities with *AcuLice* treatment and red columns are the reference facilities.

Delprosjekt 2. Effekt av *AcuLice* på velferd, stress og helsesituasjon hos laks i intensivt oppdrett

Plasma cortisol concentration

The mean value of plasmatic cortisol concentration was 29.72 mmol L^{-1} at the first sampling (Control), and 35.50 mmol L^{-1} at the second sampling (1 hour with *AcuLice* treatment) and did not vary (two-way nested ANOVA, $P > 0.05$, Fig. 8) between the two sampling points.

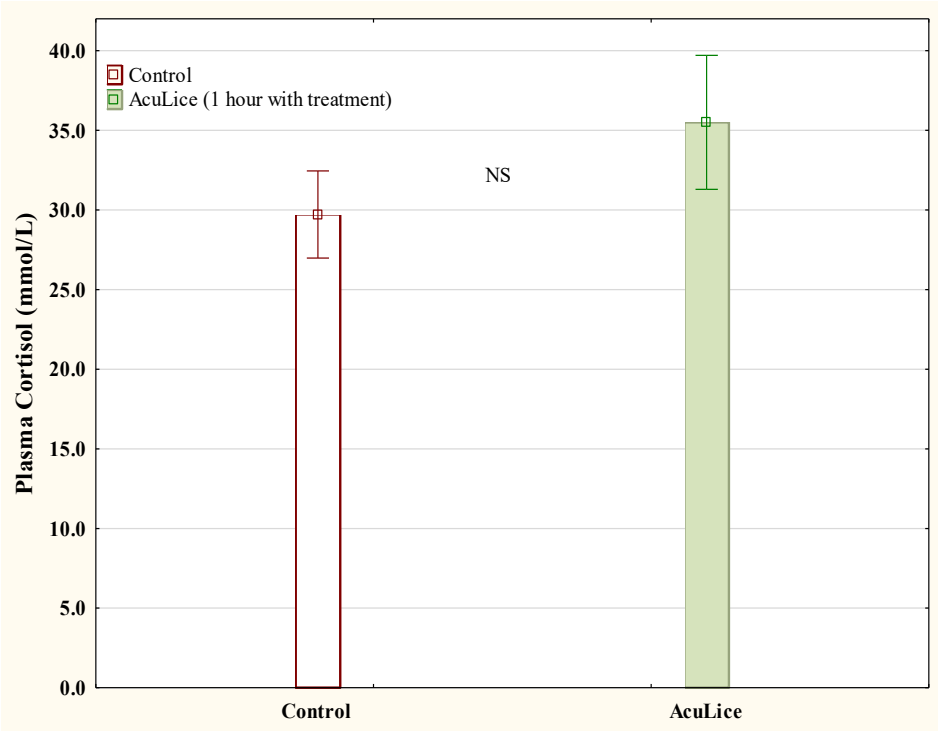


Fig. 8. Average plasma cortisol (mmol L^{-1}) consentration for Atlantic salmon before starting the *AcuLice* treatment (control) and 1 hour after starting the *AcuLice* device (*AcuLice*). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, N=30.

Plasma glucose concentration

Plasma glucose concentration increased (two-way nested ANOVA, $P < 0.05$, Fig. 9) from initial (Control) mean value of 5.75 mmol L^{-1}, to 6.13 mmol L^{-1} at the second sampling.

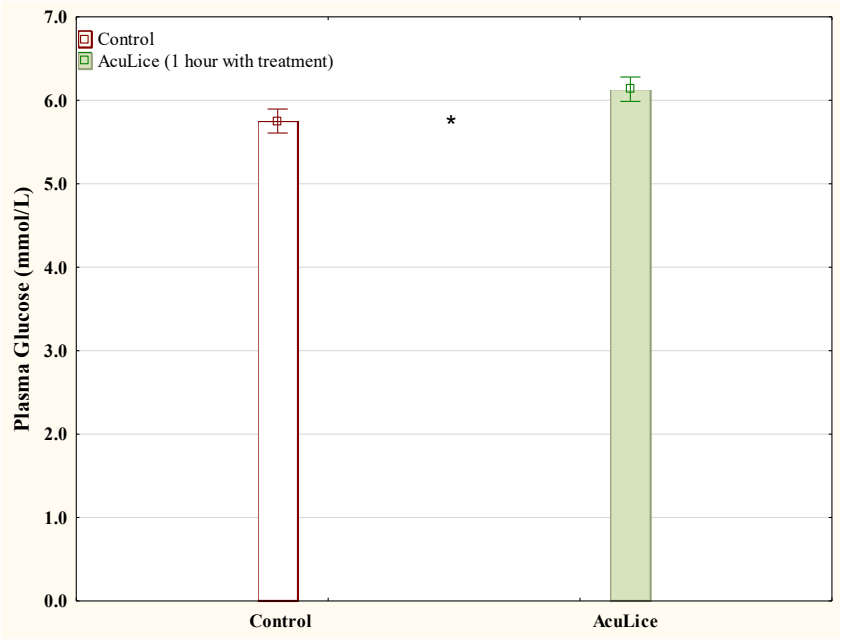


Fig. 9. Average plasma glucose (mmol L^{-1}) concentration for Atlantic salmon before starting the *AcuLice* treatment (control) and 1 hour after starting the *AcuLice* device (*AcuLice*). Asterisks indicates the level of significance between the two groups: * $P < 0.05$. Each data sampling is present as a mean ± SEM, N=30.

Plasma lactic acid concentration

The mean value of plasma lactic acid concentration was 2.70 mmol L^{-1} at the first sampling (Control), and 2.68 mmol L^{-1} at the second sampling (1 hour with *AcuLice* treatment) and did not vary (two-way nested ANOVA, $P > 0.05$) between the two sampling points.

Plasma chloride concentration

The mean value of plasmatic chloride concentration was 127.40 mmol L^{-1} at the first sampling (Control), and 126.28 mmol L^{-1} at the second sampling (1 hour with *AcuLice* treatment) and did not vary (two-way nested ANOVA, $P > 0.05$) between the two sampling points.

Plasma calcium concentration

The mean value of plasmatic calcium concentration was 2.67 mmol L^{-1} at the first sampling (Control), and 2.68 mmol L^{-1} at the second sampling (1 hour with *AcuLice* treatment) and did not vary (two-way nested ANOVA, $P > 0.05$) between the two sampling points.

Plasma magnesium concentration

The mean value of plasmatic calcium concentration was 0.87 mmol L^{-1} at the first sampling (Control), and 0.85 mmol L^{-1} at the second sampling (1 hour with *AcuLice* treatment).

Discussion

Possible stress effects of AcuLice treatment

The cortisol results in Experiment 1 did not show any difference in concentration between the control sampling and after one hour with treatment. Cortisol has a central role in the stress response and homeostasis related to stress, in addition to impact other processes, such as growth, behavior, reproduction and osmoregulation (Wendelaar Bonga, 1997; Mommsen et al., 1999). The cortisol concentration will increase rapidly after the fish has been exposed to a stressor (Wendelaar Bonga, 1997) and go towards normal levels within one or more hours. In present experiment, samples were taken 1 h after *AcuLice* treatment was started. No behavior alteration was observed via camera in the moment the *AcuLice* treatment was started, which can support the findings that the fish was not stressed. If the fish were affected in this moment, it would have been a mild stress and there will be a possibility that the cortisol levels had already dropped to the normal levels when the sampling took place. However, present results show no significant difference in the concentration levels in blood between the two samplings. The observation with no behavior alteration can substantiate that the Atlantic salmon in Experiment 1 did not have a primary stress response.

In Experiment 1, a significantly higher concentration of glucose was observed when the group had been exposed to *AcuLice* treatment for one hour. The plasma glucose concentration is affected by an increase in cortisol levels but can also be influenced by other factors such as diet and nutrient type (Mommsen et al., 1999). The elevations in plasma cortisol stimulate glycogenolysis (conversion of glycogen stored in the tissue to glucose that is released into the blood) and an increase will be a slow response to a stressor (Fast et al., 2008). According to

Olsen et al. (2002), a maximum concentration of glucose in the blood will be achieved after approximately 3 – 6 hours after the salmon has been exposed to a stressor. Since the sampling took place one hour after the start of the treatment, it could indicate that the elevated concentration had either not reached the maximum concentration or that the glucose levels could be influenced by other factors, as feeding. Studies have shown that Atlantic salmon has a normal concentration of glucose in blood around 3.3 mmol L^{-1} (Fast et al., 2008) and values under 6 mmol L^{-1} is observed to be in the normal range (Skjervold et al., 2001). The mean values measured in the present study were 5.75 and 6.13 mmol L^{-1} for the control and *AcuLice* groups, respectively, so both can be considered to fall within the normal range for Atlantic salmon. Glucose levels in fish blood is also known to have a great variability and has been considered a poor indicator of secondary stress (Mommsen et al., 1999). In addition, the low values of lactic acid concentration in the plasma support the indication that the increase in glucose that has occurred is due to factors other than stress, such as diet. Based on this the increase in plasma glucose levels found in the present study does not have to be directly correlated with the *AcuLice* treatment.

No significant difference in the plasma lactic acid concentration between the control and treated group in the Experiment 1 was observed. Lactic acid is a result of a limited amount of oxygen accessible for aerobe cell metabolism and can be achieved by hard physical activity or low oxygen levels in the water (Milligan and Girard, 1993). In relation to a stressor, lactic acid indicates that there has been a high muscle activity which can be correlated with a fish being exposed to a stressor (Iversen et al., 2003). As a result of a stressor the lactic acid concentrations is observed to be over 6 mmol L^{-1} in blood plasma (Iversen et al., 2003). This can indicate that present results, with concentration levels around 2.7 mmol L^{-1}, is in the normal range of lactate concentration. It does also correspond to the schooling behaviour observed trough camera, showing no changes in swimming behaviour during the treatment period. There were also not found a significant difference in plasma chloride concentration between the control sampling and one hour after the *AcuLice* was started in the present study. In SW the plasma chloride concentration will increase when an acute stressor occurs due to leakage through the tight junctions of the epithelium (McDonald and Milligan, 1997). For a non-stressed Atlantic salmon in SW the plasma chloride concentration has been reported to be around 135 ± 2.5 mmol L^{-1} (Fivelstad et al., 1998). Present observations are thus lower and indicates no elevated values associated with a stressor.

There were not found any differences in the magnesium concentration between the two experimental groups. Previous studies have shown that there is a high connection between increased plasma magnesium and mortality after a fish is undergoing a stressor (Liebert and Schreck, 2006; Iversen and Eliassen, 2009). Changes in the magnesium concentration are a good indicator of acute stress (Stewart et al., 2016). Normal plasma magnesium concentration is typically less than 1 mmol L^{-1} for salmonids (Liebert & Schreck, 2006; Iversen and Eliassen, 2009), which is consistent with the current values in this experiment.

Overall, the findings of current trial indicates that the secondary stress response was not activated during the one-hour treatment with *AcuLice*. The glucose levels did increase during the experiment, but in relation to the other parameters as well as results from previous studies, this is potentially based on factors other than the treatment.

Effect of AcuLice treatment in field

No differences in specific growth rate was found between the *AcuLice* and reference production facilities during the 42 week trial period. This indicates no tertiary stress response in the treatment group. A chronic stress factor will negatively affect the growth, reproductive ability and immune system (Schreck, 2010) and present data indicate that this was not the case in the current study.

To find out if *AcuLice* treatment has a salmon lice removal effect, the number of salmon lice was counted weekly and categorized. In the salmon farming context, the two categories small salmon lice and mature female salmon lice, are the most relevant in connection with accumulation and the delicing limit (Torrissen et al., 2013; Mattilsynet, 2021). Therefore, these main categories were analyzed. The results showed that there was a difference in the number of salmon lice between the two experimental groups in the 42-week study period. The *AcuLice* sites had a significantly larger proportion of small salmon lice in their facilities. This may indicate that the salmon lice pressure at the sites with *AcuLice* treatment was higher and thus has a significantly greater salmon lice impact on these facilities compared to the reference group. Based on the results that the *AcuLice* sites had a significantly higher number of small salmon lice, this would lead to that the other salmon lice stages would be accumulated in a larger number than at the reference sites (Stien et al., 2005). However, the results showed that the *AcuLice* sites had a significantly lower number of mature female salmon lice than the reference sites. This is contrary to the expected development where a larger number of small salmon lice should lead to more mature female salmon lice (Kristoffersen et al., 2014). The lower proportion of mature female salmon lice thus indicates that salmon lice are removed or disappeared during the salmon lice life cycle at the localities using *AcuLice*.

A previous study by Heuch and Karlsen (1997) has observed the anterolateral flow field from a swimming salmonids is one of the most important factors for successful infestation with a host for a salmon lice. The flow field is derived from water being moved when the salmonid is swimming and is in a low frequency range of 1 – 5 Hz (Kalmijn 1988, 1989; Heuch and Karlsen, 1997). Therefore, low frequencies in this range can be used to mask the water pressure signature from a potential host. 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. It is conceivable that salmon lice that have infected the salmon become unsure whether it is in the right species and therefore choose to jump off while waiting for the apparently correct host where the sound frequency comes from. Another possible reason is that the salmon lice were disturbed by the constant frequency which causes them to stop eating on the salmon skin and thus end up dying.

The results indicated that salmon lice disappeared in the period from when they are defined as small salmon lice to the stage of mature female salmon lice. Due to the fact that the study includes localities that produce fish during ordinary operation, these must follow national regulations with delice at the limit of 0.5 mature female salmon lice. An average of 3.1 delice operations per cage has been carried out in the *AcuLice* facilities, which is a significant lower number of treatments compared to the reference group with an average of 6.3 delice during the period from week 30, 2019 to week 20, 2020. This suggests that delicing is not the cause of lower number of mature female salmon lice in *AcuLice* facilities. Furthermore, it supports

previous findings that *AcuLice* has a lower number of mature female lice which leads to fewer salmon lice treatments.

Overall, the results indicate that the *AcuLice* sites have had a greater salmon lice pressure with a significantly larger number of small lice during the period. In addition, the results suggest that salmon lice are removed from the fish during the salmon lice life cycle at the *AcuLice* sites and that the number of delice treatments compared to the reference sites is significantly lower. Based on these results, it appears that *AcuLice* influences the removal of salmon lice and has a significant effect on reduction of the salmon lice burden in Atlantic salmon commercial production.

Conclusion

The Atlantic salmon group reared with low frequent sound treatment (*AcuLice*) for one hour in commercial open sea cages showed minor or no acute stress response compared to the control. Long term field study showed changes in salmon lice composition, number of salmon lice treatments and in the number of weeks until the first needed treatment, indicating that *AcuLice* treatment had a significant effect on reduction of the salmon lice burden in Atlantic salmon commercial production.

Referanser

Abolofia, J., Asche, F., Wilen, J.E. 2017. The Cost of Lice: Quantifying the Impacts of Parasitic Sea Lice on Farmed Salmon. Marine Resource Enocomics 32, 329-349.

Björnsson, B., Karlsson, H., Guðbjörnsson, S., 2010. The presence of experienced cod (Gadus morhua) facilitates the acoustic training of naïve conspecifics. ICES CM 2010/I:09.

Fast, M.D., Hosoya, S., Johnson, S.C., Afonso, L.O.B., 2008. Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short- and long-term stress. Fish and Shellfish Immunology 24, 194–204. doi:10.1016/j.fsi.2007.10.009

Fivelstad, S., Haavik, H., Løvik, G., Olsen, A.B., 1998. Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): Ion regulation and growth. Aquaculture 160, 305–316. doi:10.1016/S0044-8486(97)00166-X

Heuch, P.A, Karlsen, H.E., 1997. Detection of infrasonic water oscillations by copepodids of *Lepeophtheirus salmonis* (Copepoda: Caligida). Journal of Plankton Research 19, 735–747. https://academic.oup.com/plankt/article/19/6/735/1521406

Iversen, A. et al., 2016. The cost impact of lice in Norwegian salmon farming - Presentation at "Zero LICE", Day zero of the NASF 2016, Bergen March 1. http://www.seafoodinnovation.no/page/65/Zero_Lice_01_03_2016.

Iversen, M., Finstad, B., McKinley, R.S., Eliassen, R.A., 2003. The efficacy of metomidate, clove oil, Aqui-STM and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture 221, 549–566. doi:10.1016/S0044-8486(03)00111-X

Iversen, M.H., Eliassen, R.A., 2009. The effect of allostatic load on hypothalamic-pituitary-interrenal (HPI) axis before and after secondary vaccination in Atlantic salmon postsmolts (*Salmo salar* L.). Fish Physiology and Biochemistry 40, 527–538. doi:10.1007/s10695-013-9863-x

Kalmijn, A.J., 1988. Hydrodynamic and Acoustic Field Detection. In: Atema J., Fay R.R., Popper A.N., Tavolga W.N. (Eds) Sensory Biology of Aquatic Animals. Springer, New York, pp. 83–130. doi:10.1007/978-1-4612-3714-3_4

Kalmijn, A.J., 1989. Functional Evolution of Lateral Line and Inner Ear Sensory Systems. In: Coombs, S., Görner, P., Münz, H. (Eds) The Mechanosensory Lateral Line. Springer, New York, pp. 187-215. doi:10.1007/978-1-4612-3560-6_9

Kristoffersen, A.B., Jimenez, D., Viljugrein, H., Grøntvedt, R., Stien, A., Jansen, P.A., 2014. Large scale modelling of salmon lice (*Lepeophtheirus salmonis*) infection pressure based on lice monitoring data from Norwegian salmonid farms. Epidemics 9, 31–39. doi:10.1016/j.epidem.2014.09.007

Liebert, A.M., Schreck, C.B., 2006. Effects of acute stress on osmoregulation, feed intake, IGF-1, and cortisol in yearling steelhead trout (*Oncorhynchus mykiss*) during seawater adaptation. General and Comparative Endocrinology 148, 195–202. doi:10.1016/j.ygcen.2006.03.002

Mattilsynet., 2021. Lakselus. Mattilsynet (29 January 2018). Retrieved 10.05.21 from https://www.mattilsynet.no/fisk_og_akvakultur/fiskehelse/fiske_og_skjellsykdommer/lakselus/ (in Norwegian).

McDonald, G., Milligan, L., 1997. Ionic, osmotic and acid-base regulation in stress. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.) Stress and Health in Aquaculture. Cambridge University Press, Cambridge, pp. 119-145.

Midling, K.Ø., Kristiansen, T.S., Ona, E., Øiestad, V., 1987. Fjordranching with conditioned cod. ICES CM 1987/F:29.

Milligan, C.L., Girard, S.S., 1993. Lactate metabolism in rainbow trout. Journal of Experimental Biology 180, 175–193. doi:10.1242/jeb.180.1.175

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9, 211–268. doi:10.1023/A:1008924418720

Olsen, R.E., Sundell, K., Hansen, T., Hemre, G.I., Myklebust, R., Mayhew, T.M., Ringø, E., 2002. Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study. Fish Physiology and Biochemistry 26, 211–221. doi:10.1023/A:1026217719534

Schreck, C.B., 2010. Stress and fish reproduction: The roles of allostasis and hormesis. General and Comparative Endocrinology 165, 549–556. doi:10.1016/j.ygcen.2009.07.004

Skjervold, P.O., Fjæra, S.O., Østby, P.B., Einen, O., 2001. Live-chilling and crowding stress before slaughter of

Atlantic salmon (*Salmo salar*). Aquaculture 192, 265–280. doi:10.1016/S0044-8486(00)00447-6

Stien, A., Bjørn, P.A., Heuch, P.A., Elston, D.A., 2005. Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. Marine Ecology Progress Series, 290, 263-275. doi:10.3354/meps290263

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., Horsberg, T.E., Jackson, D., 2013). Salmon lice - impact on wild salmonids and salmon aquaculture. Journal of Fish Diseases 36, 171–194. doi:10.1111/jfd.12061

Wendelaar Bonga, S.E., 1997. The Stress Response in Fish. Physiological Reviews 77, 591–625. doi:10.1152/physrev.1997.77.3.591

6. Hovedfunn

- Resultatene fra feltstudien viser at *AcuLice* behandling mot lakselus bidrar til en signifikant økning i effektiv produksjonstid fra oppstart og frem til første påkrevde behandling mot lus. Itillegg ble det observert et signifikant lavere antall lakselusbehandlinger i *AcuLice* anleggene.

- Videre viste forsøket en signifikant nedgang i forholdet mellom liten og moden lus i perioden fra oppstart og frem til første påkrevde lusebehandling ved bruk av *AcuLice*.

- For de modne hunnlusene ble det vist et signifikant lavere antall for *AcuLice* gruppen.

- Blodplasma analysene viste ingen signifikante akutte eller langtidseffekter på stressrespons hos laks ved bruk av *AcuLice*. Resultatene er i samsvar med tidligere pilotforsøk. Samlet indikerer disse studiene ingen utfordringer knyttet til velferd hos fisk som er behandlet med *AcuLice* mot lakselus.

7. Leveranser

Hendelser de siste årene knyttet til bruk av nye metoder, medikamenter mm. har vist et stort behov for nødvendig dokumentasjon i tilknytning til uttesting og normal bruk av nye produkt, teknologi og metoder. *AcuLice* prosjektet har således et behov for å få fram alle sider ved teknologien/metoden og dette er knyttet til behovet for å skape varighet over tid, uten forstyrrende/ødeleggende moment. All forskning som utføres i prosjektet skal kunne publiseres, og iht. FHF sin norm for bl.a. sluttrapportering. Følgende konkrete leveranser og milepæler (MP) følger av prosjektet.

Lev. og Milepæl	Beskrivelse	AP	Type	Gradering	Leveranse- mnd.
1	Oppstartsmøte prosjektgruppe og styringsgruppe	1-5	Referat	Internt	Juni 2019
2	Prosjektgruppe møter	1-5	Referat	Internt	Månedlig
3	Styringsgruppe møter	1-5	Referat	Internt	Vår og høst
4 (MP1)	DP1; Nytt teknisk utstyr er montert og testet på lokalitetene	1	Notat	Internt	Juni 2019
5 (MP2)	DP1; Resultat frå effekt av *Aculice* på infeksjonsrisiko for lakselus er klar	1	Rapport	Offentlig	Medio des 2020
6	DP5; Kommunikasjon av resultat	5	Presentasjon	Offentlig	Fortløpende
7	Analyse av resultat, faglig rapport	1-5	Rapport	Internt	Høst 2021
8	Faglig og administrativ sluttrapport	1-5	Rapport	Internt	Høst 2021

Vedlegg 1. Dokumentasjon av tilvekst og velferd hos postsmolt i forbindelse med bruk av lavfrekvent lyd.

Utarbeidet av

Sigurd Handeland, UNI Researsch AS
Lena Geitung, Bremnes Seashore AS
Sara Calabrese, NIVA

Bakgrunn

AcuLice er en metode som er blitt utviklet for å bekjempe lakselus i felt som er basert på bruk av lavfrekvent lyd. Metoden tar utgangspunkt i at lakselus har interne reseptorer for lydbølger hvilket vil fremkalle flukt adferd gitt eksterne forstyrrelser i miljøet. Innledende undersøkelser av lus, testet i laboratoriet, har vist tydelige atferdsreaksjoner ved påføring av forskjellige lydbilder. Resultat fra småskala forsøk har videre vist klare indikasjoner på at bruk av denne metoden vil kunne redusere infeksjonsrisiko hos laksefisk med opptil 70 %. Metoden antas derfor å kunne bidra positivt til å redusere lusebelastningen rundt oppdrettsanlegg. Resultatene fra innledende forsøk viser ingen negative effekter på velferd hos laks. Bakgrunnen for dette pilot prosjektet har vært å teste effekten av lavfrekvent lyd mot lakselus under kommersiell drift (feltforsøk) med fokus på dokumentasjon av tilvekst, helsetilstand, velferd og påslagsrisiko for lus hos Atlantisk laks.

Gjennomføring

Dette forsøket innbefattet en ferskvannsfase og en etterfølgende matfiskfase. Ferskvannsdelen ble gjennomført på Bremnes Seashore sine settefiskanlegg, Gjeravåg og Skålevik (Bømlo), og tok utgangspunkt i to smoltgrupper (1+) med en snittstørrelse på ca. 140 gr. All fisk hadde samme biologiske, genetiske og produksjonstekniske opphav. Ved siste del av ferskvannsfasen ble hver av gruppene overført til enten forsøkslokaliteten Hesvik (n=100 000, snitt vekt: 155 gr.) eller referanselokaliteten Apalviknes (n=100 000, snitt vekt: 130 gr.). Referanselokaliteten ligger 2 km fra forsøkslokaliteten. Etter overføring til sjø ble forsøksgruppen (Hesvik) eksponert for en kontinuerlig lavfrekvent lyd (Hydrofon ble plassert oppi merden). Det ble etablert et justert lydbilde for å hindre potensielle forstyrrende virkninger for omgivelser. Under forsøket ble det benyttet en installasjon med 2 hydrofoner plassert på ca. 50-100 cm dyp i senter av merden og rettet på skrå nedover. Lyden ble sendt vertikalt fra overflaten og ned mot sjøbunnen. Det ble satt opp en internett-tilkoblet PC som var koblet til hydrofonene og som kontinuerlig kunne styre lydbildet i merden. På referanselokaliteten ble det benyttet ett standard produksjonsanlegg uten lydpåvirkning. Tidligere statistikk fra begge lokalitetene viste at disse typisk fikk påslag av lus på fisken.

Innsamling av prøver

I ferskvann

I løpet av de siste ukene i ferskvann ble det tatt prøver av fisken mhp. dokumentasjon av størrelse, kondisjon og smoltkvalitet. I tillegg ble det hentet inn prøver fra blod og vev.
Prøver av innsamlet blod er under analyse for plasmaionenivå (Na^+, Cl^-, Pentra-400) og plasmakortisol (Elisa). Resultat fra disse prøvene vil bli fremlagt når de er klargjort og kvalitetssikret.

I sjøvann

Etter overføring til sjø, ble hver fiskegruppe (forsøksfisk og referanse) fulgt opp gjennom fire prøveuttak (09.05.2018, 07.06.2018, 05.07.2018 og 29.08.2018). I forbindelse med hvert prøveuttak ble det registrert lengde og vekt på 60 laks fra hver av lokalitetene. Målingene ble benyttet til å beregne kondisjon og spesifikk vekstrate (SGR).Kondisjonsfaktor (KF) ble beregnet ved hjelp av følgende formel: $KF = vekt/(lengde)^3 * 100$, mensSpesifikk vekstrate (SGR) ble beregnet etter følgende formel: $SGR = (\ln(W2)-\ln(w1))/(T2-T1) * 100$

Ved prøveuttak 4 ble det tatt en serie gjelleprøver fra tilfeldig utvalgte fisk (n=20, Apalviknes og Hesvik). Gjelleprøvene ble lagret på henholdsvis SEI-buffer og RNA later. Fra de samme fiskene ble det også hentet inn vev fra hjerte og hodenyre (lagret på RNA later) med tanke på fremtidige analyser hvis tegn på sykdom eller økt dødelighet skulle oppstå i gruppen. Ved økende dødelighet vil slike prøver bli analysert ved hjelp av real time PCR teknologi med tilhørende primere og prober utviklet for deteksjon av sykdoms agens hos laksefisk. Følgende agens varianter er aktuelle: Salmonid pox virus (SGPV), ILA virus (ISAV), PD virus (SAV), Piscisine retrovirus (PRV), IPN virus (IPNV), Piscisine myocarditis virus (PMCV), Branciomonas cycticola (Ca.Bc, epiteliocyctis), Paranucleospora theridon (PT), Paramoeba perurans (P.sp.), Ichtyobodo salmonis (I.sal.) og Tetramitus sp. (TSSU).

Ved hvert av prøveuttakene ble tegn på luseangrep, gjellestatus, sår og AGD-score samt standard SWIM-registrering (Stien et al., 2013) sjekket på 60 fisk fra hver lokalitet. Lusekontrollen ble koordinert mellom de to lokalitetene. På hver lokalitet ble det også gjennomført daglige registreringer av utfôret mengde (kg/dag). Fôrtype og endringer i kvalitet ble registrert (produsent, type) og lagret på anlegget sitt Aquafarmer system. Dødfisk ble registrert i Aquafarmer med dato, antall fisk og eventuelle merknader med hensyn på mulig dødsårsak.

Vannanalyser

Parallelt med de biologiske analysene ble tatt vannprøver fra produksjonsenhetene. Vannprøver i sjø ble hentet inn med vannhentere (5m dyp) på begge lokaliteter (Hesvik og Apalviknes). Vannprøvene ble analysert for metall (Al, Fe, Cu, Mg), CO_2, HCO_3^- and $CO_3^{\ 2}$ TAN, TOC, alkalitet og Ph av NIVA, Bergen. På hver lokalitet ble det gjennomført registreringer av O_2 metning, temperatur og salinitet (3m dyp). Temperatur og pH ble målt med et Multi 3410 meter med SenTix980 pH probe (WTW, Oberbayern, Tyskland), salinitet med en TetraCon probe (WTW, Oberbayern, Tyskland) og oksygen med en Handy Polaris (Oxyguard, Farum, Danmark). Turbiditeten ble målt på ferske prøver med Turbiquant 1500 IR (Merck, Darmstadt,

Tyskland). Total karbonat konsentrasjonen, som er summen av CO_2, HCO_3^- and CO_3^{2-}, ble bestemt ved hjelp av karbondioksid elektrode ifra Orion (Model 9502). Karbondioksid konsentrasjonen ble beregnet ifra prosentdel karbondioksid av total karbonat og målt karbonat konsentrasjon (Gebauer et al., 1992). Prøveflaskene til TAN analysene ble syrevasket på forhånd. Prøvene forsures til pH < 2 ved bruk av konsentrert svovelsyre og lagres ved ca. 4 °C i kjøleskap. TAN bestemmelsen ble gjennomført etter Norsk Standard 2005, NS-EN ISO 11732 ved hjelp av auto-analysator.

Statistisk behandling av data

Verdier som er gitt i ulike tabeller er gitt som gjennomsnittsverdier. All statistisk analyse er gjennomført ved bruk av programmet Statistica 64. I forkant av hver analyse ble alle data testet for normalitet samt homogen varians mellom de ulike grupper ved hjelp av en Kolmogornov-Smirnov test og en Hartley F-max test. De ulike snittverdiene er testet mot hverandre ved hjelp av to-veis nested ANOVA (GLM) etterfulgt av Student Newman Keul test hvis resultatene fra GLM analysen viste signifikant effekt. For alle testene ble signifikansnivået satt til 0.05.

Resultat

Innsamlede resultat fra forsøket er gitt i tabell 1 til 6.

Tabell 1. Registrerte vannkvalitetsparametre på Apalviknes og Hesvik i forsøksperioden.

Lokalitet	Oksygen (%)	Salinitet (ppt)	Temperatur (°C)
Apalviknes	94,3	25,5	13,9
Hesvik	97.0	25,1	14,5

Tabell 2. Resultat fra vannanalyser gjennomført ved Apalviknes og Hesvik ved ulike prøveuttak (09.05.18, 07.05.18, 05.07.18 og 29.08.18).

	09.05.2018		07.06.2018		05.07.2018		29.08.2018	
Lokalitet	Hesvik	Apal.	Hesvik	Apal.	Hesvik	Apal.	Hesvik	Apal.
Merd	3	3	3	3	3	3	3	3
Temperatur (°C)	9,1	10,1	18,3	18,5	17,8	17,5	16,3	16,5
Salinitet (ppt)	24	24,3	23,1	23,2	20,4	20,2	19,2	25,1
pH (21 °C Eurofins)	8	8,1	8,1	8,1	7,9	8	8	8
pH (temp. korrigert)	8,2	8,3	8,1	8,1	7,9	8,1	8,1	8,1
Total ammonium nitrogen (TAN; µg/L)	10	5,8	<3	<3	17	17	17	10
Ammoniakk (µg/L)*	0,11	0,08	<0,08	<0,08	0,24	0,30	0,27	0,17
Totalt organisk karbon (TOC; mg C/L)	3,2	2,1	2	2,1	1,9	2	2,8	2,7
Turbiditet (FNU)	0,51	0,47	0,55	0,7	0,35	0,37	2,3	1,5
Alkalitet (mmol/L)	1,66	1,66	1,6	1,62	1,38	1,38	1,3	1,67
CO_2 (mg/L)	0,47	0,36	0,39	0,39	0,60	0,46	0,44	0,50

Tabell 3. Gjennomsnittlig vekt, lengde og kondisjonsfaktor (KF) hos forsøksfisken ved Apalviknes og Hesvik ved ulike prøveuttak

Lokalitet	Dato	L (cm)	V (g)	KF
Apalviknes	09.05.2018	22,2	131,9	1,20
Hesvik		23,2	149,6	1,19
Apalviknes	07.06.2018	25,6	180,4	1,08
Hesvik		26,8	190,9	0,99
Apalviknes	05.07.2018	30,9	335,4	1,14
Hesvik		29,9	274,5	1,03
Apalviknes	29.08.2018	39,7	785,4	1,25
Hesvik		39,3	722,0	1,19

Tabell 4. Periodisert spesifikk vekstrate hos forsøksfisken ved Apalviknes og Hesvik mellom ulike prøveuttak (09.05.18, 07.05.18, 05.07.18 og 29.08.18)

Lokalitet	SGR S1-S2	SGR S2-S3	SGR S3-S4	SGR S1-S4
Apalviknes	1,20	2,30	1,55	1,65
Hesvik	0,94	1,34	1,76	1,46

Tabell 5. Resultat fra SWIM analysen hos forsøksfisken ved Apalviknes og Hesvik ved ulike prøveuttak (09.05.18, 07.05.18, 05.07.18 og 29.08.18)

Lokalitet	Emaciation	Rygg-deform.	Smoltifisering	Finne	Skinn	Øye
Apalviknes	1,0	1,0	1,0	3,4	3,0	2,3
Hesvik	1,0	1,0	1,0	3,2	3,0	1,6
Apalviknes	1,0	1,0	1,0	3,3	3,1	1,3
Hesvik	1,0	1,0	1,0	3,3	3,1	1,2
Apalviknes	1,0	1,0	1,0	3,6	3,0	1,0
Hesvik	1,0	1,0	1,0	3,6	3,1	1,0
Apalviknes	1,0	1,0	1,0	3,7	3,1	1,0
Hessvik	1,0	1,0	1,0	3,6	3,1	1,2

Tabell 5 forts. Resultat fra SWIM analysen hos forsøksfisken ved Apalviknes og Hesvik ved ulike prøveuttak (09.05.18, 07.05.18, 05.07.18 og 29.08.18)

Lokalitet	Gjelle status	Gjellelokk	Sår munn/kjeve	Øvre kjeve deform.	Nedre kjeve deform.
Apalviknes	1,0	1,0	1,1	1,0	1,0
Hesvik	1,0	1,0	1,2	1,0	1,0
Apalviknes	1,1	1,1	1,0	1,0	1,0
Hesvik	1,0	1,0	1,0	1,0	1,0
Apalviknes	1,0	1,1	1,0	1,0	1,0
Hesvik	1,0	1,0	1,0	1,0	1,0
Apalviknes	1,1	1,0	1,0	1,0	1,0

Hessvik	1,0	1,0	1,0	1,0	1,0

Tabell 6. Gjennomsnittlig antall nye lus (copepoditt, chalimus I og chalimus II) og totalt antall lus hos forsøksfisken ved Apalviknes og Hesvik på ulike prøveuttak

Snitt av lus pr fisk	Dato	Nye lus	Totalt lus
Apalviknes	09.05.2018	0,17	0,17
Hesvik		0,15	0,15
Apalviknes	07.06.2018	0,02	0,17
Hesvik		0,03	0,37
Apalviknes	05.07.2018	0,02	0,23
Hesvik		0,00	0,52
Apalviknes	29.08.2018	0,13	0,36
Hesvik		0,03	0,84

Kortfattet diskusjon og konklusjon

Ved samtlige prøveuttak viste resultatene en god vannkvalitet med lave nivåer av fritt CO_2, total ammonium nitrogen og ammoniakk på begge lokalitetene (Tabell 1 og 2). Det er en liten økning i turbiditet og organisk karbon ved det siste uttaket (29. August), men økningen er lik på begge lokalitetene. Oppsummert viser resultatene god vannkvalitet og liten variasjon mellom lokalitetene. Sannsynligheten for at ulik vannkvalitet har påvirket lusepåslag og fiskevelferd på lokalitetene ansees som minimal.

Det ble ikke funnet noen signifikante forskjeller i størrelse (lengde og vekt), kondisjon og tilvekst mellom de to lokalitetene (Tabell 3 og 4). Tilsvarende ble det heller ikke funnet noen signifikante forskjeller i resultatene fra SWIM analysen (Emaciation, rygg deform., smoltifisering, finne, skinn, øye gjeller, munn og kjeve) mellom lokalitetene (Tabell 5) (Stien et al., 2013). I sum indikerer dette ingen forskjell i helsetilstand og velferd mellom fiskene på de to forsøkslokalitetene (Apalviknes og Hesvik).

Som en del av selskapets lusebekjempelsesstrategi ble det satt ut rognkjeks på begge lokaliteter i begynnelsen av april. I tillegg ble det på Apalviknes satt ut leppefisk mellom 15. juli til 15. august, samt kjørt en Slice-kur i tidsrommet 6. til 12. juni 2018. Resultatene fra lusetellingene er gitt i tabell 6. Ved de to siste prøveuttakene viste lusetellingene mindre nye lus på Hesvik (lydpåvirket) sammenliknet med Apalviknes (0.00 mot 0.02 og 0.03 mot 0.13). Den totale lusebelastning ved siste prøveuttak var imidlertid høyest på Hesvik (0.84) sammenliknet med Apalviknes (0.36). Ingen av disse trendene kunne imidlertid påvises ved bruk av statistiske metoder. På Hesvik hadde imidlertid selskapet problemer med at strømaggregatet ble ustabilt (fom. 20. august), hvilket medførte at den lavfrekvente lydkilden ble ustabil og etterhvert havarerte (3. september). Ny lydkilde ble montert og satt i drift 8.september.

Oppsummert: Basert på våre innhentede lusetall fra felt, og ovenfor nevnte tekniske utfordringer, er det vanskelig å kunne konkludere om metoden har en reell effekt på lus i felt, eller ei. Resultatene fra SWIM analysen viste ingen forskjell i helse og velferd. Dette indikerer at eksponering til lavfrekvent lyd har ingen effekter på helse og velferd hos laks i felt.

Anbefalt litteratur:

Stien, L.H., Bracke, M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P.J., Vindas, M.A., Øverli, Ø., 2013. Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Reviews in Aquaculture 5, 33-57.

Vedlegg 2. Manuskript til peer-review vitenskapelig artikkel

Det understrekes at manuskriptet ikke kan kopieres, refereres, eller på annen måte brukes før etter at dette er publisert som forfatterevaluert (per-reviewed). Manuskriptet sendes til den vitenskapelige journalen Aquaculture høsten 2021.

Acoustic delicing of Atlantic salmon (*Salmo salar*): Fish welfare and salmon lice (*Lepeophtheirus salmonis*) dynamics

Bibbi Maria Kállay Hjelle[1§], Albert Kjartan Dagbjartarson Imsland[1,2*§], Pablo Balseiro Vigo[1], Sigurd Olav Handeland[1§]

[1]Department of Biological Sciences, University of Bergen, High Technology Centre, 5020 Bergen, Norway
[2]Akvaplan-niva Iceland Office, Akralind 4, 201 Kópavogur, Iceland

Running title: Acoustic delicing of Atlantic salmon

Keywords: Atlantic salmon; Acoustic delicing; Salmon lice; Fish welfare

∗ Corresponding author at: Akvaplan-niva Iceland Office, Akralind 4, 201 Kópavogur, Iceland. E-mail address: aki@akvaplan.niva.no (A.K.D. Imsland).
§Equal authorship between: Hjelle, Imsland and Handeland

Draft - do not copy or cite

Abstract
AcuLice™ is a newly developed system which uses a composite acoustic sound image with low-frequency sound to remove salmon lice from Atlantic salmon. This study examines and documents the stress effects on Atlantic salmon as well as the effect on salmon lice dynamics in large-scale of the AcuLice™ system. The effect of AcuLice™ treatment on salmon lice dynamics was measured by weekly salmon lice counting's at the facilities from week 30, 2019 to week 20, 2020. The number of salmon lice treatments in the same period, was also recorded and compared between the AcuLice™ - and reference group. In addition, numbers of weeks until the first salmon lice treatment (mechanical treatment) was compared between the two groups. The findings from the stress analyses showed no significant difference in the cortisol response measurements between the AcuLice™ and control group. In addition, apart from slight increase in plasma glucose, no significant difference was observed in in the secondary or tertiary stress response measured. For the mature female salmon lice, a significant lower number was shown for the AcuLice™ group. In addition, a significant lower number of salmon lice treatments and a longer production period before the first salmon lice treatment occurred was observed at the AcuLice™ facilities. Present data suggest that AcuLice™ treatment does not have a negative effect on Atlantic salmon when it comes to acute stress and that the treatment has a positive effect on reduction of salmon lice pressure at the production site.

1. Introduction

The biggest pathogenic threat to the Atlantic salmon aquaculture industry is considered to be related to salmon lice (*Lepeophtheirus salmonis*) (Mackinnon, 1998; Mustafa et al., 2000). Salmon lice feed on blood, skin and mucus from the salmonid, where the extent damage of the host varies from mild skin damage to more serious wounds on the individual salmon which can cause death (Dawson et al., 1999; Bowers et al., 2000). Furthermore, the infestation can lead to several negative factors as decreased growth rate, appetite and pre-convection efficiency (Dawson et al., 1999; Pike et al., 1999). Salmon lice will also have a negative impact on wild salmonids (Torrissen et al., 2013). In addition, the parasite constitutes a huge economic cost for the aquaculture industry due to treatment and preventive efforts and did cost the Norwegian salmon farming industry approximately NOK 5 billion in 2016 (Iversen et al., 2017), which corresponds to about 9% of the income of the individual production facility (Abolofia et al., 2017). The increasing salmon lice pressure has also led to the parasite becoming a decisive factor when it comes to establishment of new aquaculture concessions and have led to a reputation challenge for the aquaculture industry.

Based on the desire to develop new and effective methods that include high efficiency, low costs and minimal negative effects on fish, while avoiding wastage, labor-intensive operations and negative effects on the environment, a treatment called AcuLice™ has been developed. AcuLice™ is a method to prevent the spread of salmon lice with the use of a complex acoustic sound image which produces and sends out constant low frequency sound to the water masses. The system emits sound waves and sound occurs when water molecules

Draft - do not copy or cite

are set in motion and pressed closer together so that the pressure increases (Kvadsheim et al., 2017). The sound levels generated by an acoustic source will propagate in the water mass and be attenuated with distance. In water, the velocity is five times higher than in air, but varies through the water column depending on the water temperature, salinity and hydrostatic pressure. Sound will also be deflected towards depths where the sound speed is lowest and thus lead to the formation of sound channels. In water, high-frequency energy will be absorbed quickly, while low-frequency energy is almost not absorbed. This means that 99% of the energy in a sound pulse of 100 kHz will be absorbed after approximately 10m, while the corresponding distance for a sound pulse of 100 Hz is 10 km

A previous study has shown that salmon lice react with 'aggressive behavior' if they are exposed to low-frequency sound in the frequency range 1-5 Hz (Heuch and Karlsen, 1997). The study showed that the copepodites increased the swimming activity in this frequency range and at a frequency of 3 Hz the highest activity was observed. The frequency area of 1-5 Hz is the same that salmon produce when swimming. Hydrodynamic signals have been observed to be one of the factors salmon lice use to select the right host fish to infect. The AcuLice™ system is thought to disturb the salmon lice so that it enters a state of dormancy where it does not absorb food and dies or leave the host salmon to find a more suitable host. In a previous pilot study (Handeland et al., 2018), there was observed effects regarding removal of salmon lice over time and no welfare challenges were shown in measurements of stress, fin condition and growth when exposed to AcuLice™ treatment. Based on the positive results in the previous pilot study, it was decided to conduct a large-scale study to follow the effects in an ordinary production situation with focus on fish welfare and health, as well as the effect on salmon lice.

High density of salmon lice, including salmon lice treatments, diseases and noise are some factors that can cause increased levels of stress in teleost (Handeland et al., 1996; Smith et al., 2004; Torrissen et al., 2013; Gallardo et al., 2019). Chronic stress occurs if the stress response is activated repeatedly or is persistent. Due to the magnitude and duration of exposure to a stressor, the stress response can be divided into primary, secondary and tertiary stress responses (Tort, 2011; Wendelaar Bonga, 2011). To determine if a fish is exposed to a stressor the changes that occur during a possible impact can be measured. The primary response can be measured by analyzing the concentration of cortisol in blood. Since secretion of catecholamines occurs faster than for cortisol (Saligaut et al., 1999), and the biological half-life of adrenaline and noradrenaline being as short as 10 min in fish blood (Wendelaar Bonga, 2011), the catecholamines are not an useful indicator of primary stress response. To determine the secondary stress response, measurements of plasma concentration of calcium, chloride, glucose, lactic acid and magnesium are performed. The stress-induced homeostasis caused by primary and secondary stress response will usually fall back to almost normal values in a chronic state of stress (Schreck, 2010; Sterling, 2012). This makes measurements of these parameters challenging in the context of detecting a tertiary stress response. However, the tertiary stress response can be analyzed by measuring, for example, survival and the specific growth rate.

Draft - do not copy or cite

This study aims to map the effect of AcuLice™ on the welfare, stress and health situation of salmon in intensive farming and document the effect of AcuLice™ on the risk of infestation for salmon lice in farmed salmon in a commercial perspective.

2. Materials and Methods

2.1 Experiment 1 – Acute stress effects of AcuLice™ treatment

2.1.1 Fish Material and Rearing Conditions

The Atlantic salmon (N=60) used in Experiment 1 originated from the Salmobreed strain and was reared from hatching to smolt at a recycling facility drifted by Hardingsmolt AS in Tørvikbygd, Kvam. After hatching the juveniles were fed a standard dry diet (Ewos, Skretting, Norway), in circular fiberglass tanks (rearing volume 5-50 m3) at constant light and in heated water (approximately 12 – 14°C). Later (at size 6–8 g), they were transferred from start feeding tanks to grow out RAS tanks (8 – 12 m, circular, fiberglass, volume 90 – 150 m3). Following transfer, the fish are reared at constant light and further fed a standard dry diet according to temperature and fish size (Austreng et al., 1987). All groups are vaccinated at a size of 40 – 60 g and then transferred to new (grow larger out tanks) 12 – 15 m tanks (circular, fiberglass, volume 150 – 350 m3) where they are supplied with environment temperature freshwater and reared as described above. Oxygen content in outlet water was measured regularly and kept above 80%. During the experimental period, the fish experienced a freshwater temperature ranging from 12 to 14°C. A traditional photoperiod regime was conducted to stimulate parr smolt transformation (Handeland and Stefansson, 2001). After completed parr-smolt transformation the fish were reared for seven weeks in a semi-closed system at Koløy, Fitjar (GreenBag). When the fish did reach approximately 500 g the group was transferred to open sea cages (160 m and volume of 37.000 m3) at Brattavika 60.044°N, 5.303°E).

2.1.2 AcuLice™ Installation Process

The installation process for AcuLice™ treatment was performed in collaboration with the equipment supplier. Prior to the installation itself, extensive local measurements were made at the respective fish farms to be able to model and map the local fixed factors. After preliminary investigations and modelling of the site, the sound image was adapted to each individual site. The system was then installed at sea by the supplier. This involves connecting the speaker, usually in the centre of the site (depth of 10 – 20 m, Fig. 1), placing the processor and connecting the component to internet. During this phase, a complete requirements specification for maintenance and operation was also prepared. The system is continuously monitored electronically. Once the AcuLice™ system was installed, it could be turned on by the equipment supplier whenever desired.

Draft - do not copy or cite

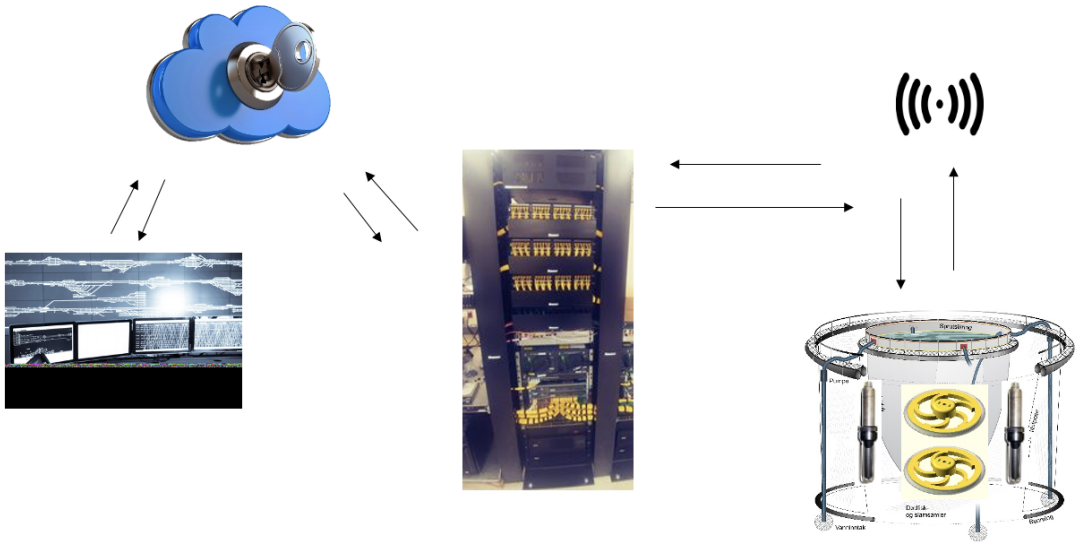
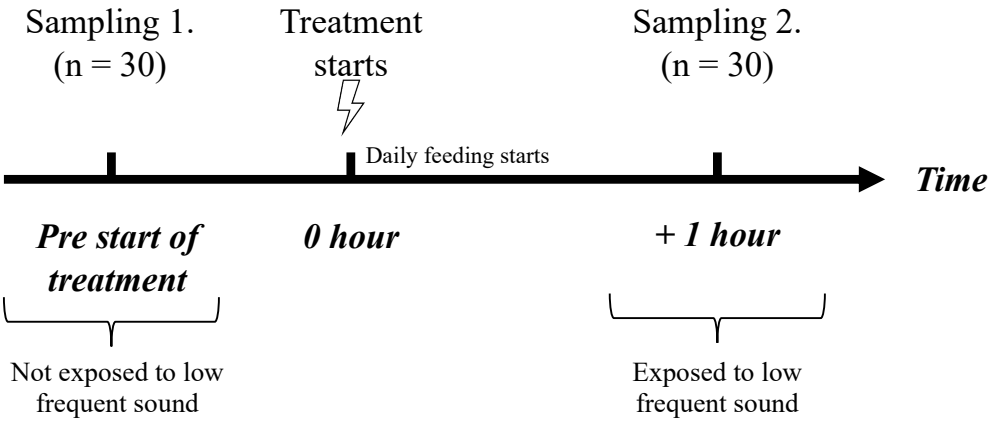


Fig. 1. The AcuLice™ system consists of a central control room with monitoring, an electronic processor located on the facility and a component in the sea (that sends out low frequency sound waves). The whole system is connected to internet.

2.1.3 Experimental Design

Experiment 1 was carried out on 24 October 2019 and did include a control sampling and a treatment sampling (Fig. 2). The control sampling (Sampling 1) took place prior to start of the AcuLice™ treatment and the treated group (Sampling 2) were done one hour after start of the AcuLice™. During the start of AcuLice™ treatment, the farmed salmon were monitored using an underwater camera, and no changes in the fish's behavior were observed. All measurements and plasma collections were performed at the feed barge at the facility. To get to and from the edge of the sea cage a boat was used. Daily feeding started at the same time as the AcuLice™ treatment.



ct - do not copy

Fig. 2. Experimental design for Exp1 at Brattavika. Sampling 1 took place before treatment (Control) and when the sampling was conducted the AcuLice™ treatment (and daily feeding) were started. One hour after treatment start, Sampling 2 took place

Pellets were used to attract the fish, before the fish were captured using hand net. Then the fish was humanly euthanized with an anaesthetic overdose of Benzocaine (Benzoak vet.® 20%, ACD Pharma AS, Norway). The blood was collected within 1 – 3 min to limit the effects of stress. The blood (2 mL) was taken from the caudal vein using heparinized syringes with 21G needles. The plasma was separated from blood cells by centrifugation (4 min at 5000 rpm). The fish was then measured in size (weight (g) and length (cm) to the nearest 0.1 g and 0.1 cm. When the first subset with N = 10, was done, the same procedure was followed for the two next subsets, subset number 2 and 3. When all the three subsets in sampling 1 were done, the AcuLice was turned on, and sampling 2 was performed one hour after.

2.1.4 Analysis of plasma cortisol concentration

Cortisol quantification from plasma was carried out using competitive ELISA (DEMEDITEC Cortisol ELISA Kit) following the manufacturer protocol (Demeditec Diagnostics GmbH, DEH3388, Kiel, Germany). The plasma samples were analysed in triplicates (10 µL) in a 96-well microplate. Every plate included two internal control samples and standards of a known concentration. Sample (10 µL), control or calibrator were dispensed into each well. Enzyme conjugate (200 µL) was dispensed using a multi-channel pipette. Enzyme conjugate consist of cortisol linked to horseradish peroxidase. The plate was then incubated for 60 min on an orbital shaker at room temperature. After incubation the unbound conjugate is washed off. Substrate Solution, which included TBM (3,3',5,5'-Tetramethylbenzidine), was added. The plate was incubated for 30 min at room temperature and in dark to let the reaction happen. After the incubation, a stop solution (containing hydrochloric acid to decrease the pH) was added to each well. The intensity of colour developed is measured at 450 nm in a multimode microplate reader (Tecan Spark®, Tecan, Männedorf, Switzerland). With the help of known concentration standards, cortisol concentrations were calculated using a 4 Parameters Marquardt logistic regression with an extrapolation factor of 1 in the SparkControl Magellan v2.2.10 software.

2.1.5 Analysis of plasma chloride, glucose, lactic acid, calcium and magnesium

The concentration of chloride in the plasma samples was measured by potentiometry using the Pentra c400 clinical chemistry analyser with Ion-Selective Electrode (ISE) module (HORIBA, Kyoto Prefecture, Japan). Calibration of the ISE module was done using the ABX Pentra Standard 1, ABX Pentra Standard 2, and ABX Pentra Reference. The samples (>180 µL) were measured using a specific electrode. Chloride in the sample induce a change in the potential difference across the electrode membrane which is then compared with the reference electrode.

Draft - do not copy or cite

The measurements of glucose, lactic acid, calcium and magnesium were analysed using the Pentra c400 by colorimetric spectrophotometry determination. Each required reagent was calibrated using the ABX Pentra Multical and a quality control was performed using ABX Pentra P and N controls, as stated in the manufacturer's protocol.

Glucose was measured using a quantitative in vitro diagnostic assay using ABX Pentra Glucose HK CP reagent (HORIBA). The method used is the hexokinase method where the production of the phosphorylated glucose-6-phosphate is coupled with the subsequent production of D-gluconate-6-phosphate and reduction of NAD^+. The concentration of glucose is proportional to the increase in the NADH concentration. This is measured by spectrophotometry at 340/380 nm (Burrin and Price, 1985).

Lactic acid is analysed by a quantitative in vitro diagnostic determination using ABX Pentra lactic acid reagent (HORIBA). Lactate oxidase triggers the release of hydrogen peroxide which further reacts with 4-aminoantipyrine and ESPAS (N-ethyl-N-sulfopropyl-m-anisidine) to form a coloured complex. The reaction is dependent on peroxidase access and is measured bichromatically at 550/700 nm. The concentration of lactic acid is proportional to the intensity of the colour.

To measure the calcium concentrations in the plasma samples, a method based on the metallochromogen Arsenazo III was used using ABX Pentra Calcium AS CP reagent (HORIBA). In the reaction, calcium ions (Ca^{2+}) will react with Arsenazo III (2.2 '- [1,8-Dihydroxy-3,6-disulfonapthyylene-2,7-bisarzo] -bisbenzenearsonic acid). The result of the reaction is a purple-colored chromophore with a pH of 6.75. The sample (5 µL) was mixed with reagent (300 µL) and water (10 µL). The absorbance of the Ca-arsenazo III complex was then measured bichromatically at 660/700 nm. The increase in absorbance of the reaction mixture is directly proportional to the calcium concentration.

Magnesium was determined from a quantitative in vitro diagnostic assay using ABX Pentra 1and reagent (250 µL) are mixed. The magnesium ions in an alkaline solution will form a purple-coloured complex. GEDTA (glycolethylenediamine tetraacetic acid) is included in the reagent and forms complexes with calcium ions, which ultimately renders the reaction specific. The magnesium concentration is proportional to the intensity of the coloured magnesium complex measured.

2.2 Experiment 2 – Effect of AcuLice™ treatment in field

2.2.1 Fish Material and Rearing Conditions

As a result of different companies being involved in Experiment 2, the fish at the various facilities came from Salmobreed stain but were farmed at different hatcheries in the Hardanger region (Hordaland, Norway). All the fish did follow a general procedure for hatchery-production as the previously described in section 2.1.1.

2.2.2 Experimental Facilities and Locations

Draft - do not copy or cite

Experiment 2 took place in Sunnhordaland at 9 full scale facilities (Table 1) within the fjords: Bømlafjorden, Klosterfjorden, Ålfjorden og Skåneviksfjorden, collectively, they are further referred to as Hardangerfjorden. Assignment of facilities into reference or AcuLice™ treatment groups were based on current regime of the area. This has been done in order to avoid that salmon lice released from an AcuLice™ treated facility infest a reference facility placed downstream.

Table 1. Experiment 2 facilities divided into the two treatment groups (AcuLice or reference) with site number, company that operates the facility and coordinates of the location of the sites.

	Site name	Site number	Company that operates the facility	Coordinates (°N/°E)	
AcuLice	Breivik S	11574	Bremnes Seashore AS	59.671	5.312
	Grimsholmen	11559	Sjøtroll Havbruk AS	59.657	5.404
	Hattasteinen	11511	Bremnes Seashore AS	59.628	5.252
	Hillersvik	10300	Erko Seafood AS	59.608	5.312
	Loddetå	28996	Bremnes Seashore AS	59.692	5.543
	Svollandsneset	22955	Bremnes Seashore AS	59.685	5.589
Reference	Maradalen	12134	Fjeldberg-. Nordsjø-. Sunnhordaland- & Tysnes Fjordbruk AS	59.762	5.687
	Seglberget	17015	Fjeldberg-. Nordsjø-. Sunnhordaland- & Tysnes Fjordbruk AS	59.730	5.788
	Mælen	12127	Fjeldberg-. Nordsjø-. Sunnhordaland- & Tysnes Fjordbruk AS	59.699	5.724

2.2.3 Experimental Design

Each experimental group was followed from onset into sea during the spring of 2019, until week 20 in 2020. The start-up at each locality varied due to different times for when production fish were transferred to sea cages and other company-internal conditions. The installation of the AcuLice™ equipment at the facilities in Experiment 2 follows the general description as previously described (see section 2.1.3). All the experimental facilities had started the AcuLice™ treatment no later than week 30, 2019. The experimental period for salmon lice counting was set from week 30 in 2019 to week 20 in 2020 (a period of 43 weeks). Number of salmon lice treatments was also counted for this period. In addition, numbers of weeks between fish were transferred to sea water (SW) cages to first salmon lice treatment (defined as mechanical delice in the present study) was required, was measured and calculated (see below). All equipment maintenance during the period was performed by the supplier. Daily follow-up was carried out by employees at the facility. Due to ordinary operation of the facilities included in Experiment 2, the facilities had to follow the regulations on delice if

Draft

mature female salmon lice exceed the limit. Throughout the production period delice treatments did occur when required for all the facilities.

2.2.4 Sampling Protocol

As an integral part of the experiment, production data was collected from each locality throughout the trial period (AcuLice and reference) with a focus on weight, weekly salmon lice infestation and numbers of salmon lice treatments. The facilities did follow a salmon lice counting protocol in accordance with regulations (The Veterinary Institute, 2009). Fish were randomly collected from three to six different, randomly chosen, cages (N = 20 from each pen) using hand nets. The fish was then anesthetized as directed on the given agent used.

The salmon lice counting was done by qualified salmon lice counters by carefully examining each individual fish. Salmon lice were classified into the stages: sessile salmon lice, mobile salmon lice and adult salmon lice (adult male and female salmon lice). As a result of anaesthetic treatment salmon lice that fall off in the tub were counted and categorized. Subsequently, the average for each category and cage was calculated and registered in the Barentswatch database (BarentsWatch).

2.2.5 Data Processing and Calculations

Data collected during the 43-week period (week 30, 2019 – week 20, 2020) were processed. First the classifications of the salmon lice life stages that were registered during salmon lice counting were split up and changed into the new categories: small salmon lice (including copepodite, *chalimus* 1 and 2 life stages) and mature female salmon lice. Total average values for each different category were calculated for the data collected in the 43-week period at each facility in Experiment 2.

Specific growth rate on weight (SGR) was calculated for the period in the reference and AcuLice groups, with the exception of Seglberget due to missing weight measurements. The SGR was calculated according to the formula:

$$\text{SGR} = \frac{lnW_2 - lnW_1}{\Delta T},$$

where W_1 is weight at the first measuring point, T_1, and W_2 is weight at second measuring point T_2. ΔT is numbers of days between T_2 and T_1.

2.2.6 Data Collection

For analyzing the numbers of weeks until the first salmon lice treatment was required, data for all the production groups in Experiment 2 was collected from the database BarentsWatch (https://www.barentswatch.no/fiskehelse/), in the period from SW transfer of Atlantic salmon until the first salon lice treatment did occur. Number of mature female salmon lice the week before first salmon lice treatment occurred was also retrieved from the database. A salmon lice treatment in this study is defined as a mechanical salmon lice treatment solely conducted due to an excessive number of female mature salmon lice (regulated limit of 0.5 mature female

Draft - do not copy or cite

salmon lice per salmon (0.2 in summer)). The use of biological treatment (cleaner fish) and chemical treatment (bath or added to feed) is omitted from the definition in this thesis. Requirements for salmon lice treatment were decided by the company veterinarian based on weekly salmon lice counts.

2.3 Statistical analysis

All statistical analysis and figures were performed using the Statistica™, v.13 (TIBCO Software Inc, Palo Alto, CA, US) software. Data in all graphical illustrations are presented by the means of each group and standard error of means (SEM). Statistical outliers with values greater than 1.5 times the interquartile range were excluded from the datasets using the Tukey fence method in Microsoft® Excel v. 16.41 (Microsoft, Redmond, Washington, US). The distributions of all response variables were checked for normality and homogeneity of variance using the Shapiro-Wilk test and the Levene test. A General Linear Models (two-way random effects nested ANOVA) analysis was fitted between each of the response variables and the predictor variables, "AcuLice sites" and "control regime site"/"reference regime sites", and with replicate sub-samplings (random effect) as a nested factor within the predictor variables. A student t-test was used to analyse the specific growth rate, numbers of salmon lice treatments and numbers of weeks from Atlantic salmon was transferred to SW cages until first salmon lice treatment did occur between the AcuLice and reference group. A significance level of α=0.05 was used for all statistical models while asterisks was used to indicate significant differences between groups, NS = no significant difference, ($p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***)).

3. Results

3.1 Experiment 1 – Acute stress effects of AcuLice treatment

3.1.1. Plasma cortisol concentration

The mean value of plasmatic cortisol concentration was 29.72 mmol L^{-1} at the first sampling (Control), and 35.50 mmol L^{-1} at the second sampling (1 hour with AcuLice treatment) and did not vary (two-way nested ANOVA, $P > 0.05$, Fig. 3) between the two sampling points.

Draft - do not copy or cite

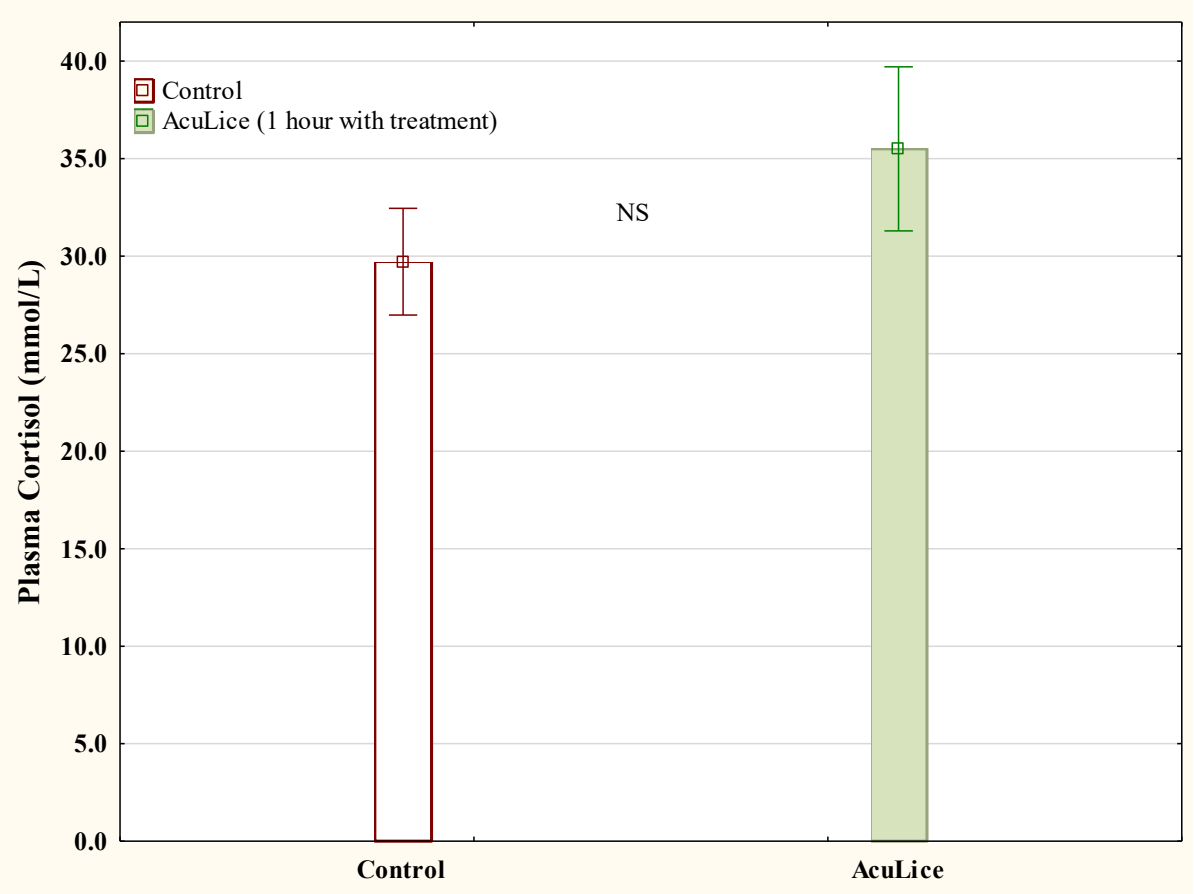


Fig. 3. Average plasma cortisol (mmol L^{-1}) consentration for Atlantic salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). NS indicates no significance between the two groups. Each data sampling is present as a mean ± SEM, N=30.

3.1.2. Plasma glucose concentration

Plasma glucose concentration increased (two-way nested ANOVA, $P < 0.05$, Fig. 4) from initial (Control) mean value of 5.75 mmol L^{-1}, to 6.13 mmol L^{-1} at the second sampling.

Draft - do not

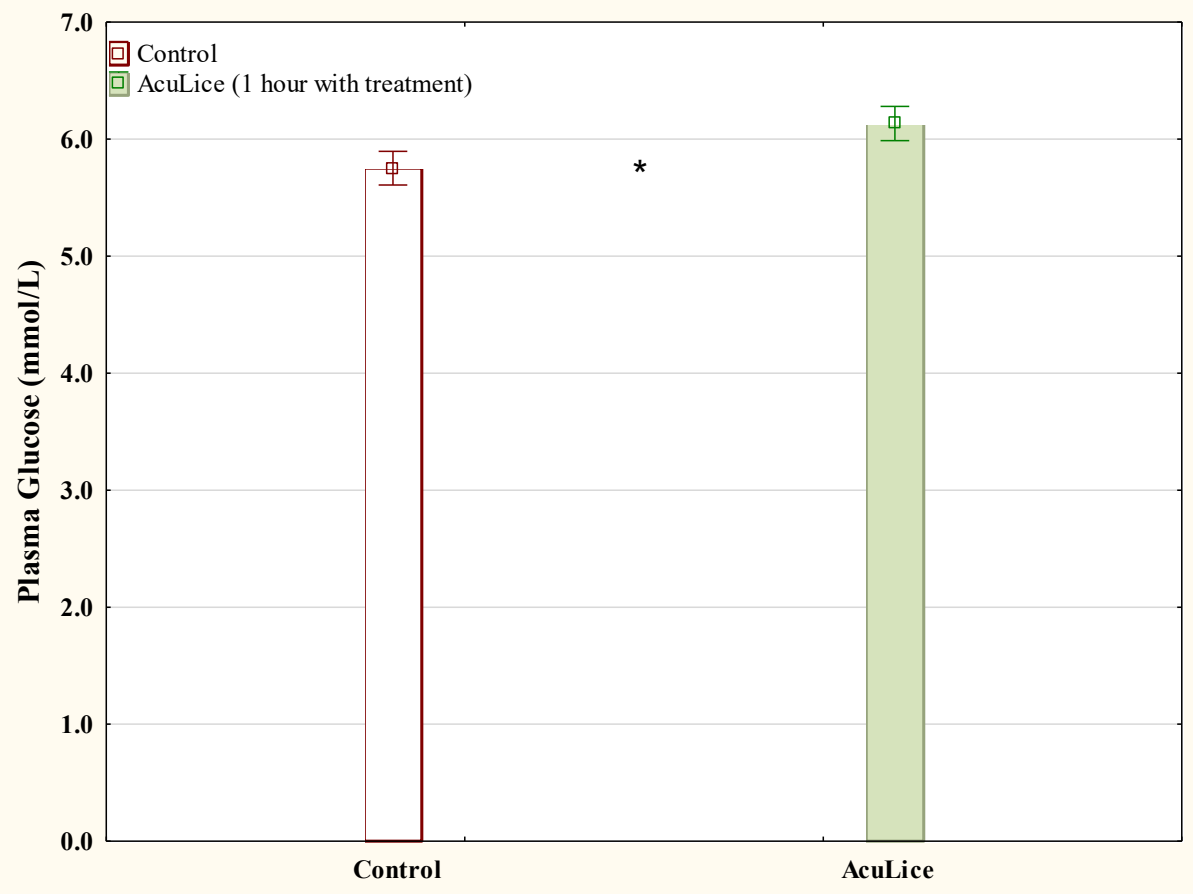


Fig. 4. Average plasma glucose (mmol L^{-1}) concentration for Atlantic salmon before starting the AcuLice treatment (control) and 1 hour after starting the AcuLice device (AcuLice). Asterisks indicates the level of significance between the two groups: * $P < 0.05$. Each data sampling is present as a mean ± SEM, N=30.

3.1.3 Plasma lactic acid concentration

The mean value of plasma lactic acid concentration was 2.70 mmol L^{-1} at the first sampling (Control), and 2.68 mmol L^{-1} at the second sampling (1 hour with AcuLice treatment) and did not vary (two-way nested ANOVA, $P > 0.05$) between the two sampling points.

3.1.4 Plasma chloride concentration

The mean value of plasmatic chloride concentration was 127.40 mmol L^{-1} at the first sampling (Control), and 126.28 mmol L^{-1} at the second sampling (1 hour with AcuLice treatment) and did not vary (two-way nested ANOVA, $P > 0.05$) between the two sampling points.

3.1.5 Plasma calcium concentration

The mean value of plasmatic calcium concentration was 2.67 mmol L^{-1} at the first sampling (Control), and 2.68 mmol L^{-1} at the second sampling (1 hour with AcuLice treatment) and did not vary (two-way nested ANOVA, $P > 0.05$) between the two sampling points.

Draft - do not

3.1.6 Plasma magnesium concentration

The mean value of plasmatic calcium concentration was 0.87 mmol L^{-1} at the first sampling (Control), and 0.85 mmol L^{-1} at the second sampling (1 hour with AcuLice treatment).

3.2 Experiment 2 – Effect of AcuLice™ treatment in field

3.2.1 Specific growth rate (SGR)

The AcuLice treated groups had a minimum value of SGR in weight at 0.32 % day^{-1} (Svollandsneset) and a maximum growth rate at 0.52 % day^{-1} (Grimsholmen, Hattasteinen) in the period from week 30, 2019 to week 20, 2020 (Fig. 5). For the reference group the minimum growth rate was 0.37 % day^{-1} (Maradalen) and maximum were 0.48 % day^{-1} (Mælen) in the same period. Overall, there were no significant differences in the mean SGR between the reference group and the AcuLice treated group (Student's t-test, $P > 0.05$, Fig. 5), in the experimental period (week 30, 2019 to week 20, 2020). The mean SGR for the AcuLice treated groups was 0.45 % day^{-1} and for the reference group 0.43 % day^{-1}.

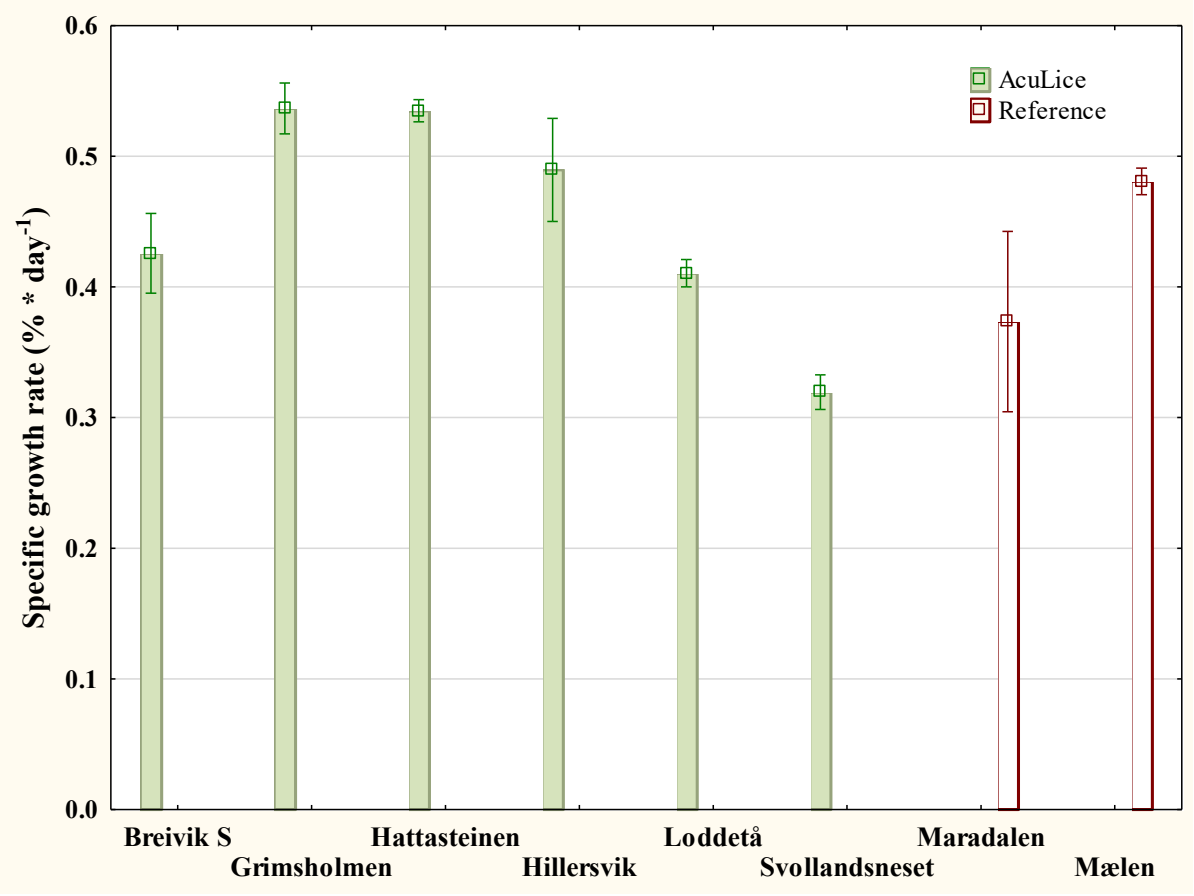


Fig. 5. Mean specific growth rate (SGR (% day^{-1})) calculated for the Atlantic salmon in each experimental facility in Experiment 2A in the period from week 30, 2019 to week 20, 2020. The AcuLice treated facilities

Copyright site

are marked in green, and the reference group are marked in red. Data from each production facility is presented as mean ± SEM.

3.2.2 Effect on salmon lice dynamics – sessile and mobile salmon lice

The AcuLice treated groups showed a mean number of small (sessile and mobile) salmon lice from 0.39 (Loddetå) to 1.22 (Hillersvik) in the period week 30 in 2019 to week 20 in 2020 (Fig. 6). The reference group had, in the same period, a mean number of small salmon lice from 0.07 (Mælen) to 0.24 (Maradalen).

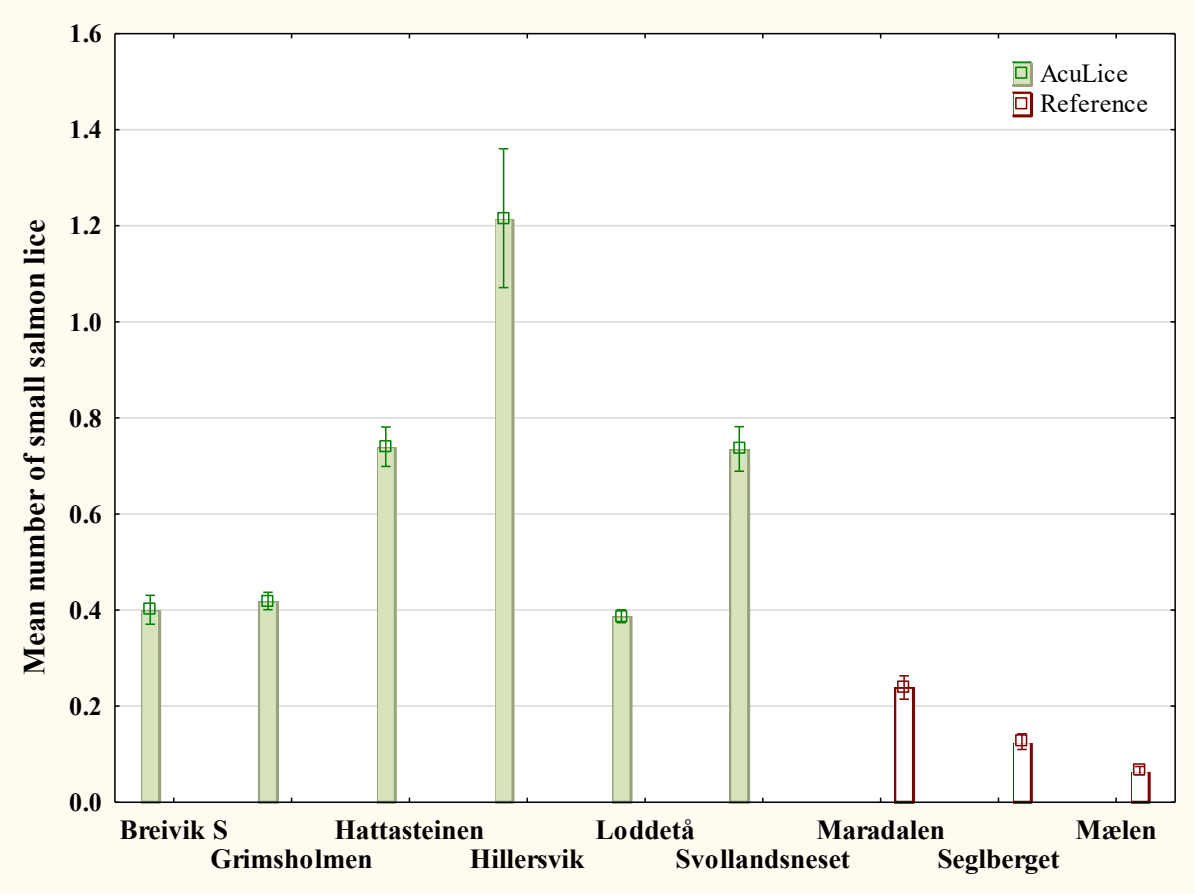


Fig. 6. Mean number of small (sessile and mobile) salmon lice measured per Atlantic salmon in the period from week 30, 2019 to week 20, 2020 at each facility. Groups of Atlantic salmon exposed to AcuLice treatment (AcuLice) compared to the reference group (Reference). Green marked columns are facilities with AcuLice treatment and red columns are the reference facilities. Data from each facility is presented as mean ± SEM.

3.2.3 Effect on salmon lice dynamics – mature female lice

The AcuLice treated groups had a mean number of mature female salmon lice from 0.12 (Breivik S) to 0.31 (Hillersvik) in the period week 30 in 2019 to week 20 in 2020 (Fig. 7). The reference group had in the same period a mean number of mature female salmon lice from 0.39 (Maradalen) to 0.49 (Mælen).

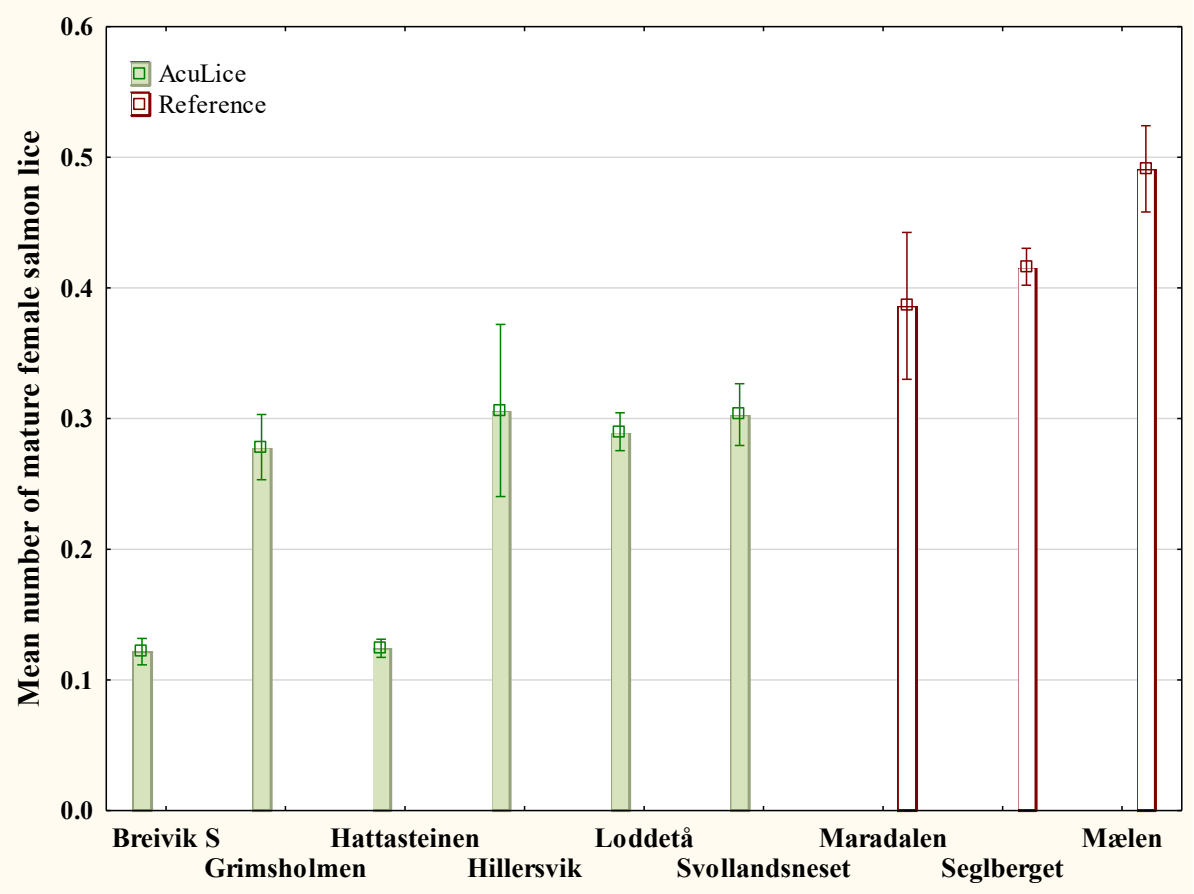


Fig. 7. Mean number of mature female salmon lice measured per Atlantic salmon in the period from week 30, 2019 to week 20, 2020 at each facility. Groups of Atlantic salmon exposed to AcuLice treatment (AcuLice) compared to the reference group (Reference). Green marked columns are facilities with AcuLice treatment and red columns are the reference facilities. Data from each facility is presented as mean ± SEM.

3.2.4 Effect of AcuLice treatment in salmon lice population composition

Higher number of small salmon lice was observed for the AcuLice treated groups compared to the reference groups during the experimental period (one-way ANOVA, $P < 0.001$, Fig. 8). In contrast, a lower number of mature female salmon lice was observed for the AcuLice treated groups compared to the reference in the same period (one-way ANOVA, $P < 0.001$, Fig. 8).

Draft - do not

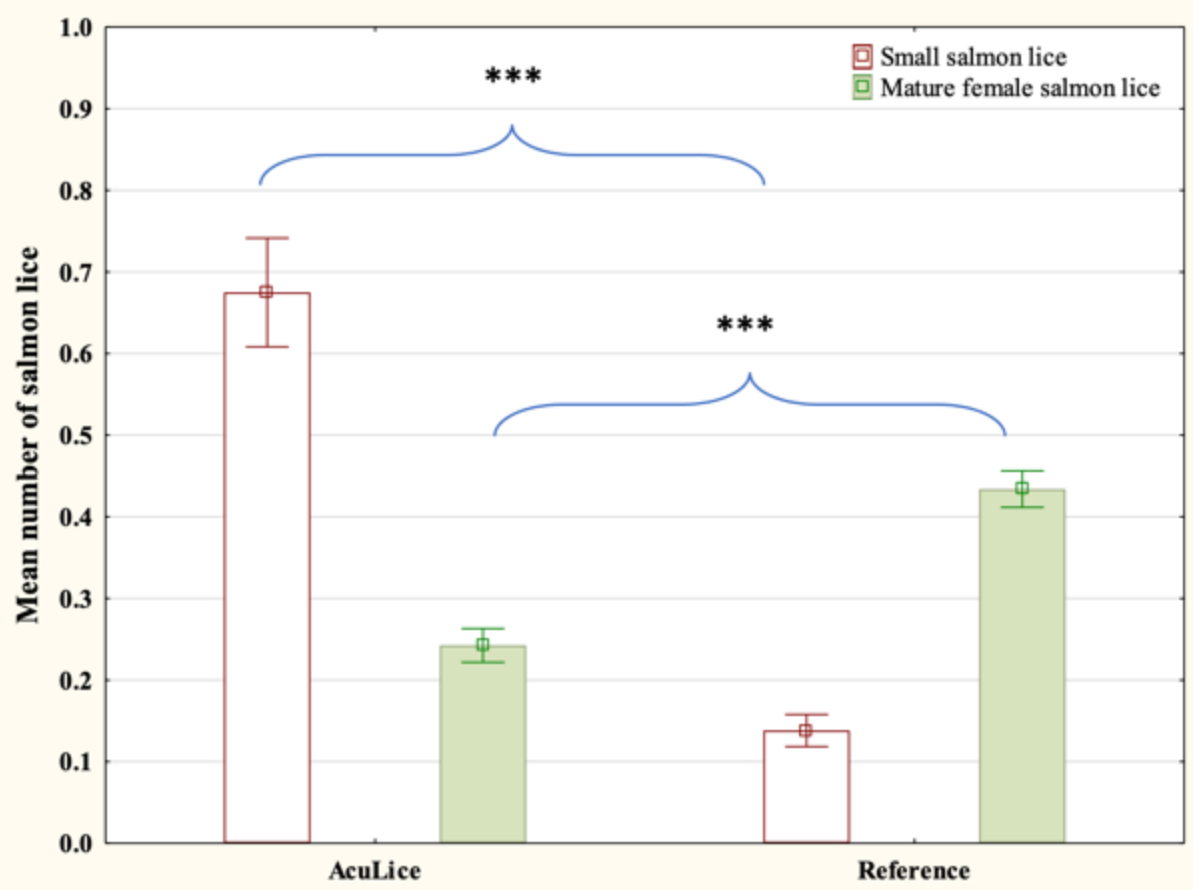


Fig. 8. Mean number of small salmon lice (red columns) and mature female lice (green columns) measured as per Atlantic Salmon between group exposed to AcuLice treatment compared to the reference group, during the period (week 30, 2019 to week 20, 2020). Asterisks indicates the level of significance between the two groups: *** P < 0.001. Data from each treatment group is present as a mean ± SEM.

3.2.5 Numbers of salmon lice treatments in the experimental period

The AcuLice treated group had significant lower number (3.1) of salmon lice treatments (Student's t-test, $P < 0.05$) during the 43 weeks period (week 30, 2019 to week 20, 2020) compared to the reference group (6.3).

3.2.6 Numbers of weeks to first salmon lice treatment

For the AcuLice treated facilities the minimum number of weeks was 22 (Grimsholmen, Fig. 9) and the maximum number was 40 weeks (Loddetå). The reference group had a period of 16 to 25 weeks (Seglberget, Maradalen) before the first treatment was necessary. Overall, the mean number of weeks until the first salmon lice treatment increased significantly (Student's t-test, $P < 0.05$) from 20.3 weeks in the reference groups, to 33.2 weeks in the AcuLice treated groups.

Draft - do not

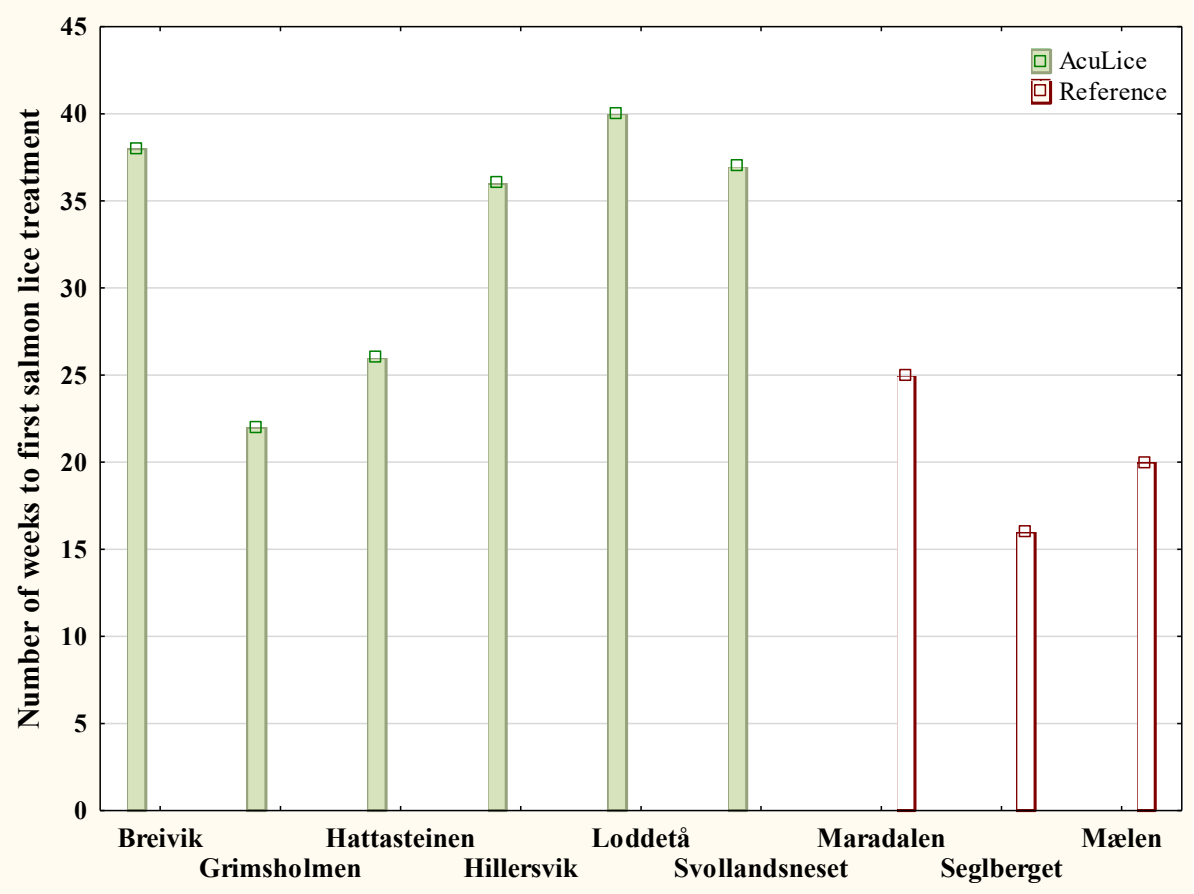


Fig. 9. *Mean* number of weeks to the first salmon lice treatment at the experimental facilities in Experiment 2. Green marked columns are facilities with AcuLice treatment and red columns are the reference facilities.

4. Discussion

4.1 Possible stress effects of AcuLice treatment

The cortisol results in Experiment 1 did not show any difference in concentration between the control sampling and after one hour with treatment. Cortisol has a central role in the stress response and homeostasis related to stress, in addition to impact other processes, such as growth, behavior, reproduction and osmoregulation (Wendelaar Bonga, 1997; Mommsen et al., 1999). The cortisol concentration will increase rapidly after the fish has been exposed to a stressor (Wendelaar Bonga, 1997) and go towards normal levels within one or more hours. In present experiment, samples were taken 1 h after AcuLice treatment was started. No behavior alteration was observed via camera in the moment the AcuLice treatment was started, which can support the findings that the fish was not stressed. If the fish were affected in this moment, it would have been a mild stress and there will be a possibility that the cortisol levels had already dropped to the normal levels when the sampling took place. However, present results show no significant difference in the concentration levels in blood between the two samplings. The observation with no behavior alteration can substantiate that the Atlantic salmon in Experiment 1 did not have a primary stress response.

Draft - do not

In Experiment 1, a significantly higher concentration of glucose was observed when the group had been exposed to AcuLice treatment for one hour. The plasma glucose concentration is affected by an increase in cortisol levels but can also be influenced by other factors such as diet and nutrient type (Mommsen et al., 1999). The elevations in plasma cortisol stimulate glycogenolysis (conversion of glycogen stored in the tissue to glucose that is released into the blood) and an increase will be a slow response to a stressor (Fast et al., 2008). According to Olsen et al. (2002), a maximum concentration of glucose in the blood will be achieved after approximately 3 – 6 hours after the salmon has been exposed to a stressor. Since the sampling took place one hour after the start of the treatment, it could indicate that the elevated concentration had either not reached the maximum concentration or that the glucose levels could be influenced by other factors, as feeding. Studies have shown that Atlantic salmon has a normal concentration of glucose in blood around 3.3 mmol L^{-1} (Fast et al., 2008) and values under 6 mmol L^{-1} is observed to be in the normal range (Skjervold et al., 2001). The mean values measured in the present study were 5.75 and 6.13 mmol L^{-1} for the control and AcuLice groups, respectively, so both can be considered to fall within the normal range for Atlantic salmon. Glucose levels in fish blood is also known to have a great variability and has been considered a poor indicator of secondary stress (Mommsen et al., 1999). In addition, the low values of lactic acid concentration in the plasma support the indication that the increase in glucose that has occurred is due to factors other than stress, such as diet. Based on this the increase in plasma glucose levels found in the present study does not have to be directly correlated with the AcuLice treatment.

No significant difference in the plasma lactic acid concentration between the control and treated group in the Experiment 1 was observed. Lactic acid is a result of a limited amount of oxygen accessible for aerobe cell metabolism and can be achieved by hard physical activity or low oxygen levels in the water (Milligan and Girard, 1993). In relation to a stressor, lactic acid indicates that there has been a high muscle activity which can be correlated with a fish being exposed to a stressor (Iversen et al., 2003). As a result of a stressor the lactic acid concentrations is observed to be over 6 mmol L^{-1} in blood plasma (Iversen et al., 2003). This can indicate that present results, with concentration levels around 2.7 mmol L^{-1}, is in the normal range of lactate concentration. It does also correspond to the schooling behaviour observed trough camera, showing no changes in swimming behaviour during the treatment period. There were also not found a significant difference in plasma chloride concentration between the control sampling and one hour after the AcuLice was started in the present study. In SW the plasma chloride concentration will increase when an acute stressor occurs due to leakage through the tight junctions of the epithelium (McDonald and Milligan, 1997). For a non-stressed Atlantic salmon in SW the plasma chloride concentration has been reported to be around 135 ± 2.5 mmol L^{-1} (Fivelstad et al., 1998). Present observations are thus lower and indicates no elevated values associated with a stressor.

There were not found any differences in the magnesium concentration between the two experimental groups. Previous studies have shown that there is a high connection between increased plasma magnesium and mortality after a fish is undergoing a stressor (Liebert and Schreck, 2006; Iversen and Eliassen, 2009). Changes in the magnesium concentration are a

Draft - do not copy or cite

good indicator of acute stress (Stewart et al., 2016). Normal plasma magnesium concentration is typically less than 1 mmol L^{-1} for salmonids (Liebert & Schreck, 2006; Iversen and Eliassen, 2009), which is consistent with the current values in this experiment.

Overall, the findings of current trial indicates that the secondary stress response was not activated during the one-hour treatment with AcuLice. The glucose levels did increase during the experiment, but in relation to the other parameters as well as results from previous studies, this is potentially based on factors other than the treatment.

4.2 Effect of AcuLice treatment in field

No differences in specific growth rate was found between the AcuLice and reference production facilities during the 42 week trial period. This indicates no tertiary stress response in the treatment group. A chronic stress factor will negatively affect the growth, reproductive ability and immune system (Schreck, 2010) and present data indicate that this was not the case in the current study.

To find out if AcuLice treatment has a salmon lice removal effect, the number of salmon lice was counted weekly and categorized. In the salmon farming context, the two categories small salmon lice and mature female salmon lice, are the most relevant in connection with accumulation and the delicing limit (Torrissen et al., 2013; Mattilsynet, 2021). Therefore, these main categories were analyzed. The results showed that there was a difference in the number of salmon lice between the two experimental groups in the 42-week study period. The AcuLice sites had a significantly larger proportion of small salmon lice in their facilities. This may indicate that the salmon lice pressure at the sites with AcuLice treatment was higher and thus has a significantly greater salmon lice impact on these facilities compared to the reference group. Based on the results that the AcuLice sites had a significantly higher number of small salmon lice, this would lead to that the other salmon lice stages would be accumulated in a larger number than at the reference sites (Stien et al., 2005). However, the results showed that the AcuLice sites had a significantly lower number of mature female salmon lice than the reference sites. This is contrary to the expected development where a larger number of small salmon lice should lead to more mature female salmon lice (Kristoffersen et al., 2014). The lower proportion of mature female salmon lice thus indicates that salmon lice are removed or disappeared during the salmon lice life cycle at the localities using AcuLice.

A previous study by Heuch and Karlsen (1997) has observed the anterolateral flow field from a swimming salmonids is one of the most important factors for successful infestation with a host for a salmon lice. The flow field is derived from water being moved when the salmonid is swimming and is in a low frequency range of 1 – 5 Hz (Kalmijn 1988, 1989; Heuch and Karlsen, 1997). Therefore, low frequencies in this range can be used to mask the water pressure signature from a potential host. 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. It is conceivable that salmon lice that have infected the salmon become unsure whether it is in the right species and therefore choose to jump off while waiting for the apparently correct host where the sound frequency comes from. Another possible reason is that the

Draft - do not copy or cite

salmon lice were disturbed by the constant frequency which causes them to stop eating on the salmon skin and thus end up dying.

The results indicated that salmon lice disappeared in the period from when they are defined as small salmon lice to the stage of mature female salmon lice. Due to the fact that the study includes localities that produce fish during ordinary operation, these must follow national regulations with delice at the limit of 0.5 mature female salmon lice. An average of 3.1 delice operations per cage has been carried out in the AcuLice facilities, which is a significant lower number of treatments compared to the reference group with an average of 6.3 delice during the period from week 30, 2019 to week 20, 2020. This suggests that delicing is not the cause of lower number of mature female salmon lice in AcuLice facilities. Furthermore, it supports previous findings that AcuLice has a lower number of mature female lice which leads to fewer salmon lice treatments.

Overall, the results indicate that the AcuLice sites have had a greater salmon lice pressure with a significantly larger number of small lice during the period. In addition, the results suggest that salmon lice are removed from the fish during the salmon lice life cycle at the AcuLice sites and that the number of delice treatments compared to the reference sites is significantly lower. Based on these results, it appears that AcuLice influences the removal of salmon lice and has a significant effect on reduction of the salmon lice burden in Atlantic salmon commercial production.

5. Conclusion

The Atlantic salmon group reared with low frequent sound treatment (AcuLice) for one hour in commercial open sea cages showed minor or no acute stress response compared to the control. Long term field study showed changes in salmon lice composition, number of salmon lice treatments and in the number of weeks until the first needed treatment, indicating that AcuLice treatment had a significant effect on reduction of the salmon lice burden in Atlantic salmon commercial production.

Acknowledgements

The authors would like to thank the technical staff at the involved salmon production sites for valuable assistance during the experimental period. The financial assistance of the Norwegian Seafood Fund (FHF 901567) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the funding bodies.

Author statement

Bibbi Maria Kállay Hjelle, Investigation; Visualizaiton; Writing – original draft; Albert Kjartan Dagbjartarson Imsland, Conceptualization¸ Funding acquisition; Project administration; Supervision; Writing – original draft; Writing – review & editing; Pablo Balseiro, Supervision; Writing – review & editing; Sigurd Handeland, Conceptualization¸ Funding acquisition; Project administration; Supervision; Writing – original draft.

Draft - do not copy or cite

Conflict of interest

There is no conflict of interest in relation to this study.

ORCID

A.K.D. Imsland http://orcid.org/0000-0003-0077-8077

Draft - do not copy or cite

References

Abolofia, J., Wilen, J.E., Asche, F., 2017. The cost of lice: Quantifying the impacts of parasitic sea lice on farmed salmon. Marine Resource Economics 32, 329–349. doi:10.1086/691981

Bowers, J.M., Mustafa, A., Speare, D.J., Conboy, G.A., Brimacombe, M., Sims, D.E., Burka, J.F., 2000. The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. Journal of Fish Diseases 23, 165–172. doi:10.1046/j.1365-2761.2000.00225.x

Burrin, J. M., Price, C.P., 1985. Measurement of blood glucose. Annals of Clinical Biochemistry 22, 327–342. doi:10.1177/000456328502200401

Dawson, L.H.J., Pike, A.W., Houlihan, D.F., McVicar, A.H., 1999. Changes in physiological parameters and feeding behaviour of Atlantic salmon (*Salmo salar*) infected with sea lice (*Lepeophtheirus salmonis*). Diseases of Aquatic Organisms 35, 89–99. doi:10.3354/dao035089

Fast, M.D., Hosoya, S., Johnson, S.C., Afonso, L.O.B., 2008. Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short- and long-term stress. Fish and Shellfish Immunology 24, 194–204. doi:10.1016/j.fsi.2007.10.009

Fivelstad, S., Haavik, H., Løvik, G., Olsen, A.B., 1998. Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): Ion regulation and growth. Aquaculture 160, 305–316. doi:10.1016/S0044-8486(97)00166-X

Gallardo-Escárate, C., Valenzuela-Muñoz, V., Núñez-Acuña, G., Carrera, C., Gonçalves, A. T., Valenzuela-Miranda, D., Benavente, B.P., Roberts, S. (2019). Catching the complexity of salmon-louse interactions. Fish and Shellfish Immunology 90, 199–209. doi:10.1016/j.fsi.2019.04.065

Handeland, S.O., Järvi, T., Fernö, A., Stefansson, S.O., 1996. Osmotic stress, antipredatory behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. Canadian Journal of Fisheries and Aquatic Sciences 53, 2673-2680. doi:10.1139/cjfas-53-12-2673

Handeland, S.O., Stefansson, S. O., 2001. Photoperiod control and influence of body size on off-season parr-smolt transformation and post-smolt growth. Aquaculture 192, 291–307. doi:10.1016/S0044-8486(00)00457-9

Handeland, S.O., Geitung, L., Calabrese, S., 2018. Dokumentasjon av tilvekst og velferd hos postsmolt i forbindelse med bruk av lavfrekvent lyd. Uni Research Report, Bergen, Norway (in Norwegian).

Heuch, P.A, Karlsen, H.E., 1997. Detection of infrasonic water oscillations by copepodids of *Lepeophtheirus salmonis* (Copepoda: Caligida). Journal of Plankton Research 19, 735–747. https://academic.oup.com/plankt/article/19/6/735/1521406

Iversen, A., Hermansen, Ø., Nystøyl, R., Hess, E.J., 2017. Kostnadsutvikling i lakseoppdrett–med fokus på fôr- og lusekostnader (Report nr. 24/2017). Tromsø: Nofima. Retrieved from https://nofima.no/publikasjon/1523319/ (in Norwegain).

Iversen, M., Finstad, B., McKinley, R.S., Eliassen, R.A., 2003. The efficacy of metomidate, clove oil, Aqui-STM and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture 221, 549–566. doi:10.1016/S0044-8486(03)00111-X

Iversen, M.H., Eliassen, R.A., 2014. The effect of allostatic load on hypothalamic-pituitary-interrenal (HPI) axis before and after secondary vaccination in Atlantic salmon postsmolts (*Salmo salar* L.). Fish Physiology and Biochemistry 40, 527–538. doi:10.1007/s10695-013-9863-x

Kalmijn, A.J., 1988. Hydrodynamic and Acoustic Field Detection. In: Atema J., Fay R.R., Popper A.N., Tavolga W.N. (Eds) Sensory Biology of Aquatic Animals. Springer, New York, pp. 83–130. doi:10.1007/978-1-4612-3714-3_4

Kalmijn, A.J., 1989. Functional Evolution of Lateral Line and Inner Ear Sensory Systems. In: Coombs, S., Görner, P., Münz, H. (Eds) The Mechanosensory Lateral Line. Springer, New York, pp. 187-215. doi:10.1007/978-1-4612-3560-6_9

Kristoffersen, A.B., Jimenez, D., Viljugrein, H., Grøntvedt, R., Stien, A., Jansen, P.A., 2014. Large scale modelling of salmon lice (*Lepeophtheirus salmonis*) infection pressure based on lice monitoring data from Norwegian salmonid farms. Epidemics 9, 31–39. doi:10.1016/j.epidem.2014.09.007

Draft - do not copy or cite

Kvadsheim, P.H., Sivle, L.D., Hansen, R.R., Karlsen, H.E., 2017. Effekter av menneskeskapt støy på havmiljø (Report nr. 17/00075). Kjeller: Norwegian Defence Research Establishment (FFI). Retrieved from https://publications.ffi.no/nb/item/asset/dspace:2657/17-00075.pdf (in Norwegain).

Liebert, A.M., Schreck, C.B., 2006. Effects of acute stress on osmoregulation, feed intake, IGF-1, and cortisol in yearling steelhead trout (*Oncorhynchus mykiss*) during seawater adaptation. General and Comparative Endocrinology 148, 195–202. doi:10.1016/j.ygcen.2006.03.002

Mackinnon, B.M, 1998. Host factors important in sea lice infections. ICES Journal of Marine Science 55, 188–192. doi:10.1006/jmsc.1997.0361

Mattilsynet., 2021. Lakselus. Mattilsynet (29 January 2018). Retrieved 10.05.21 from https://www.mattilsynet.no/fisk_og_akvakultur/fiskehelse/fiske_og_skjellsykdommer/lakselus/ (in Norwegian).

McDonald, G., Milligan, L., 1997. Ionic, osmotic and acid-base regulation in stress. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.) Stress and Health in Aquaculture. Cambridge University Press, Cambridge, pp. 119-145.

Milligan, C.L., Girard, S.S., 1993. Lactate metabolism in rainbow trout. Journal of Experimental Biology 180, 175–193. doi:10.1242/jeb.180.1.175

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9, 211–268. doi:10.1023/A:1008924418720

Mustafa, A., MacWilliams, C., Fernandez, N., Matchett, K., Conboy, G.A., Burka, J.F., 2000. Effects of sea lice (*Lepeophtheirus salmonis*) infestation non-specific defence mechanisms in Atlantic salmon (*Salmo salar* L.). Fish and Shellfish Immunology 10, 47–59. doi:10.1006/fsim.1999.0229

Olsen, R.E., Sundell, K., Hansen, T., Hemre, G.I., Myklebust, R., Mayhew, T.M., Ringø, E., 2002. Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study. Fish Physiology and Biochemistry 26, 211–221. doi:10.1023/A:1026217719534

Pike, A.W., Wadsworth, S.L., 1999. Sealice on salmonids: Their biology and control. Advances in Parasitology 44, 233–337. doi:10.1016/s0065-308x(08)60233-x

Saligaut, C., Linard, B., Breton, B., Anglade, I., Bailhache, T., Kah, O., Jego, P., 1999. Brain aminergic systems in salmonids and other teleosts in relation to steroid feedback and gonadotropin release. Aquaculture 177, 13–20. doi:10.1016/S0044-8486(99)00065-4

Schreck, C.B., 2010. Stress and fish reproduction: The roles of allostasis and hormesis. General and Comparative Endocrinology 165, 549–556. doi:10.1016/j.ygcen.2009.07.004

Skjervold, P.O., Fjæra, S.O., Østby, P.B., Einen, O., 2001. Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 192, 265–280. doi:10.1016/S0044-8486(00)00447-6

Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). Journal of Experimental Biology 207, 427-435. doi:10.1242/jeb.00755

Sterling, P., 2012. Allostasis: A model of predictive regulation. Physiology and Behavior,106, 5–15. doi:10.1016/j.physbeh.2011.06.004

Stien, A., Bjørn, P.A., Heuch, P.A., Elston, D.A., 2005. Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. Marine Ecology Progress Series, 290, 263-275. doi:10.3354/meps290263

The Veterinary Institute, 2009. Veileder for nasjonal bekjempelse av lakselus. Retrieved from https://sjomatnorge.no/wp-content/uploads/importedmedia/akselusveilederen_web_231109.pdf (in Norwegian).

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., Horsberg, T.E., Jackson, D., 2013). Salmon lice - impact on wild salmonids and salmon aquaculture. Journal of Fish Diseases 36, 171–194. doi:10.1111/jfd.12061

Tort, L., 2011. Stress and immune modulation in fish. Developmental and Comparative Immunology 35, 1366–1375. doi:10.1016/j.dci.2011.07.002

Wendelaar Bonga, S.E., 1997. The Stress Response in Fish. Physiological Reviews 77, 591–625. doi:10.1152/physrev.1997.77.3.591

Wendelaar Bonga, S.E., 2011. Hormonal Responses to Stress. Encyclopedia of Fish Physiology 2, 1515–1523.

Draft - Do not copy or cite