



METALICE : The degree of returning salmon from smolt groups treated with anti-parasitic agent compared to untreated smolt groups – a systematic review and meta-analysis of Norwegian data

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Metalice - FHF project # 900932

Front page photo by Tore Wiers (Uni Research)

The photo is taken at one of the trap net locations in the migration route of the Vosso salmon in 2014. The salmon in the picture is a female approximately 16 kg. The fish is a second time spawner that was most likely released as a smolt in 2009, recaptured, tagged and released in 2012, and subsequently recaptured in 2014.

Final report: The degree of returning salmon from smolt groups treated with anti-parasitic agent compared to untreated smolt groups – a systematic review and meta-analysis of Norwegian data – MetaLice (FHF project # 900932)

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Illustration photo: Traditional capture method using trap nets in the migration route of the Vosso salmon for the recapture of adult salmon. Photo by Bjørn T. Barlaup

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Sammendrag (Extended Norwegian summary)

Bruk av forsøksgrupper med laksesmolt som er blitt behandlet eller er ubehandlet mot lakselus og fanget igjen som voksen laks er en utbredt metode for å evaluere populasjonseffekter av lakselus på vill laksefisk. Resultatene fra tidligere studier har vist relativt like gjennomsnittlig estimater (risk ratio = 1.14-1.41), men ha kommet til meget sprikende konklusjoner relatert til effekten av lakselus på bestandsnivå av villaks. I Norge er det blitt gjennomført en rekke slike studier med varierende omfang og varierende grad av rapportering og publisering. Det er knyttet flere usikkerhetsmomenter til resultatene fra slike studier. Årsaken til dette er til dels at mange av studiene har få gjenfangster og derfor stor usikkerhet knyttet til estimatene. Dette kan bøtes på ved å gjennomføre en overordnet meta-analyse av alle studiene. For å kunne gjøre dette må en være sikker på å få med seg alle studier slik at det ikke fører til publikasjonsbias (for eksempel at studier med liten effekt ikke blir inkludert). I dette arbeidet har vi derfor gjennomført en systematisk gjennomgang av alle slike studier i Norge («systematic review») og en meta-analyse av alle disse studiene, og prøvd å forklare hva som er kilden til variasjonen i effektstørrelse mellom de forskjellige forsøksgruppene (meta-regresjon).

Totalt ble det identifisert 118 forsøksgrupper. Av disse hadde 17 ingen gjenfangst og ble dermed ekskludert fra videre analyse. Den overordnede meta-analysen basert på gjennomsnittlig effektstørrelse resulterte i en estimert risk ratio (RR) på 1.18. Det var stor variasjon i resultater mellom forsøksgruppene, slik at effektstørrelsen (RR) varierte kraftig mellom gruppene. En mer detaljert studie (meta-regresjon) viste at 70 % av denne heterogeniteten kunne forklares av hvor fisken var blitt sluppet (elv/estuarier vs. fjord), i hvilken periode gruppen var blitt sluppet (1996-2003, 2004-2006, 2007-2008 and 2009-2012) og gjenfangst raten i den ubehandlede/kontroll gruppen (i meta-analyser blir denne variabelen ofte definert som «baseline risiko»). Den viktigste forklaringsvariabelen var gjenfangst raten. I forsøk med lav gjenfangst i kontroll gruppen synes effekt av behandling å være høy (RR = 1.7), mens i grupper med høy gjenfangst i kontroll gruppen var det ingen effekt av behandlingen (RR = 1.0).

Estimert lusemengde fra oppdrettsanlegg var ikke en signifikant forklaringsvariabel for effektstørrelsen (RR) mellom forsøksgruppene. Dette kan enten skyldes (1) at lusemengden i oppdrett ikke er årsaken til den observerte effekten av lusebehandling, at (2) metoden for å

estimere eksponering av lus fra oppdrettsanlegg ikke er presis nok til å kunne trekke konklusjoner om at lus fra oppdrettsanlegg fører til lavere overlevelse i de ubehandlete gruppene av fisk eller (3) at lusebehandlingen ikke beskytter disse gruppene av fisk godt nok til å observere en effekt.

Det er også gjennomført en generell vurdering av overførbarheten av de analyserte forsøksresultatene basert på kultiverte fisk til villfisk, samt en bias-analyse (analyse av feilkilder). Bias-analysen peker på flere forhold som kan føre til feil estimat av den reelle effekten av lakselus. Videre er det er knyttet mange usikkerhetsmomenter til i hvor stor grad disse studiene er direkte overførbare til villfisk. På den ene siden kan faktorer som påvirker eksponering variere mye, mens på den andre siden kan faktorer som påvirker hvordan individet takler det ekstra stresset fra lakselus variere mye.

Hovedkonklusjonene fra studien er:

- 1. Behandling av smolt med anti- parasittisk middel kan signifikant øke gjenfangsten av laks. Gitt at effekten vi estimerer av behandling er et resultat av beskyttelse mot lakselus, gir våre analyser utvetydig støtte til hypotesen at lakselus fører til økt dødelighet hos villaks. Dette er mest fremtredende i år med dårlig gjenfangst i den ubehandlete fiskegruppen.*
- 2. Effektstørrelsen (risk ratio – RR) er meget variable, og varierer mellom tidsperioder, og øker når gjenfangsten i den ubehandlete gruppen går ned. En estimert gjennomsnittsverdi for hvor stor andel av fisk som overlever på grunn av behandling har dermed relativt liten verdi i forhold til å si noen om potensialet for påvirkning på overlevelsen fra lakselus (Gjennomsnitts estimat = 1.18). Estimert RR varierer fra ca. 1.00 til i overkant av 1.7 avhengig av gjenfangsten i den ubehandlete fiskegruppen.*
- 3. Gjenfangst i kontrollgruppen er en funksjon av hvor langt fisken må vandre for å nå det åpne havet. Eksempelvis, øker gjenfangsten med over 7 ganger når fisken blir sluppet 50 km fra elvemunningen. I tillegg varierer gjenfangsten med elvens geografiske plassering.*
- 4. Estimert av modellert lakseluseksponering fra oppdrettsanlegg kunne ikke forklare variasjonen i effekt størrelse mellom slippene. Det var sammenheng mellom den estimerte lakseluseksponeringen og overlevelsen i den ubehandlete gruppen. Denne sammenhengen falt imidlertid bort når man brukte vandringsavstand som en forklaringsvariabel på grunn av korrelasjon mellom disse variablene.*

5. *Det er fremdeles mange uavklarte spørsmål relater til denne type metoder for å estimere bestandseffekter av lakselus på vill laks. Det er blant annet flere potensielle feilkilder som må avklares.*
6. *Slippene som er inkludert i studien er hovedsakelig konsentrert i ett område og meta-studiet legger derfor sterkt vekt på enkelte slipplokalteter i denne regionen som har relativt kort vandrings avstand gjennom områder som potensielt overlapper med produksjon av lakselus fra oppdrettsanlegg.*

Vi anbefaler at det gjennomføres flere studier på lokaliteter som har lengre migrasjonsruter og i tillegg kontrollstudier i områder med lite oppdrettsvirksomhet. I tillegg bør det gjennomføres studier for å avklare hvorfor meta-studien viser en så klar sammenheng mellom overlevelse i den ubehandlede gruppen og effektstørrelse.

Summary (in English)

A systematic review and meta-analysis on all Norwegian studies of release groups of Atlantic salmon smolt treated with an anti-parasitic agent compared to untreated smolt groups was conducted. In total, 118 release groups were identified. Of all the release groups, 17 did not contain any recaptures and were excluded from the analyses. Using meta-analysis techniques, we estimated the overall risk ratio to 1.18. This means that, on average, a treated fish has an 18% increased chance of being recaptured. However, the effect varied strongly between groups with a heterogeneity index, I^2 , of 39.2%. Meta-regression revealed that over 70% of the heterogeneity could be explained by release location (river/estuary versus fjord), period (1996-2003, 2004-2006, 2007-2008 and 2009-2012) and control group recapture rate (CGR). The most important predictor variable was CGR. Thus, in release groups with low recapture in the control group (most likely reflecting low marine survival) the effect of treatment was high (risk ratio = 1.7), while in release groups with high recapture in the control group there was no effect of treatment (risk ratio ~ 1.00). Assuming that the effect of treating smolt with anti-parasitic agent is a function of protection against salmon lice, and that recapture reflects survival, the results from this study gives unequivocal evidence to the hypothesis that salmon lice is a contributor to the mortality of salmon. However, this effect was not consistently present, but was especially evident in years and release groups where overall survival rates were low (as reflected by low CGR).

1. Introduction

Domestication of marine fishes is relatively new compared to land based food production, and the global exponential growth in marine finfish farming in recent decades reflects both the large marked demand and the fact that there are currently few limitations in areas that can be used for marine farming. In recent years the debate about the role of farmed marine fish as hosts and reservoir for diseases and parasites has spurred the debate about the sustainability of net pen farming and their subsequent effects on wild fish populations (Costello 2006, Torrissen et al. 2013). At the core of this scientific debate is the role of farmed Atlantic salmon as hosts of salmon lice and possible effects of this on wild salmonids. Farmed Atlantic salmon is mostly produced in open-net pen installations in coastal areas. Within the natural range of wild salmonids, these locations often overlap with the migration paths of young wild salmon smolt

migrating to sea, and the main concern is therefore whether the additional farm-generated production of diseases and parasites such as salmon lice, will inflict additional mortality on this vulnerable life stage.

The role of parasites in regulating host populations is a longstanding debate (May and Anderson 1978). While technically estimating the effects of parasites on populations is possible, in reality there are several issues related to quantifying such effects. This is perhaps especially true for marine fish populations, where survival is highly stochastic and strongly linked to variation in environmental condition during early life stages (Hjort 1914, Cushing 1975). For salmon, marine survival has been shown to correlate with the North Atlantic Oscillation (NAO; Friedland et al. 1993, Peyronnet et al. 2008), and effects of salmon lice likely correlates strongly with other effects on survival, making it hard to utilize correlative studies to separate the role of the parasite from other effects. One alternative approach is to use laboratory studies (Bjørn and Finstad 1998, Finstad et al. 2000, Wells et al. 2006, Wagner et al. 2008). However, the validity of these studies in natural systems is often questioned. Another method is to do experimental field trials with releases of control groups and groups treated with an anti-parasitic agent and compare the subsequent recaptures of adults in the two groups (randomized control trials, RCT¹). Such field experiments have become increasingly popular in recent years as they are believed to give unequivocal results regarding the relative role of the parasites on the marine survival of salmon (Gargan et al. 2012, Jackson et al. 2013a, Jackson et al. 2011a, Jackson et al. 2011b, Skilbrei et al. 2013, Vollset et al. 2014).

Studies conducted in Norway, Ireland, and Scotland have evaluated the effects of long-acting anti-parasitic treatment on growth and survival of salmon at the group-level (Gargan et al. 2012, Jackson et al. 2013a, Jackson et al. 2011a, Jackson et al. 2011b, Skilbrei et al. 2013, Vollset et al. 2014, Krkošek et al. 2013, 2014). Treatment of salmon smolt prior to release into the river or the fjord seems to give a significant increase in the number of recaptured returning adult fish. A positive effect of anti-parasitic treatment on length, weight, as well as effects on age of the returning salmon has also been reported (Skilbrei et al. 2013, Vollset et al. 2014). Some of the Norwegian trials have been conducted over a decade in the same river (Skilbrei et al. 2013,

¹ Also sometimes referred to as *randomized treatment control trials* or *randomized clinical trials*.

Vollset et al. 2014). However, in several trials, the number of recaptured fish has been low, and the power to detect differences has been considered to be poor.

A meta-analysis is a statistical method where data derived from a systematic review are weighted (proportional to the amount of evidence provided by the study) when computing an overall estimate of the effect (Borenstein et al., 2009; Higgins and Green, 2011). The objectives of the present study were to perform a meta-analysis of all available material both published and non-published, on anti-parasitic treatment trials in Norway to obtain an overall estimate of the treatment effect across studies, and to explore the potential effect of study- and trial-specific variables on that estimate by use of subgroup analyses and meta-regression. Trials were limited to Norway because of the availability of counts of salmon lice from fish farms and thus the possibility to explicitly analyze the contribution of salmon lice from fish farms. The systematic review rendered a dataset of 118 release groups making it by far the most extensive analysis of anti-parasitic treatment trials to date.

Organization

A project group was established to perform the data collection and analyses, and it consisted of researchers from the Norwegian University of Life Sciences (NMBU), the Norwegian Institute for Nature Research (NINA), the Norwegian Veterinary Institute (NVI), Uni Research, University of Prince Edward Island (UPEI), University of Toronto (UoT), and the Norwegian University of Science and Technology (NTNU).

List of research scientists

- Randi Ingebjørg Krontveit (NMBU), Arnfinn Aunsmo (NMBU) to 31.12.2013
- Bengt Finstad (NINA)
- Knut Wiik Vollset, Bjørn Torgeir Barlaup (Uni Research)
- Peder Jansen (VI)
- Ian Dohoo (UPEI)
- Martin Krkošek (UoT)
- Pål Romundstad (NTNU)

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Associate members of the project group were: Arnfinn Aunsmo (NMBU/AquaGen AS), Ove Skilbrei (Institute of Marine Research), and Arne J. Jensen (Norwegian Institute for Nature Research).

Project steering group

- Olav Breck, Marine Harvest ASA
- Ragnhild Aukan, Lerøy Midt
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- Tor Anders Elvegård, Nordlaks AS
- Arne Guttvik, SalMar ASA

Contact person in The Norwegian Seafood Research Fund (FHF) has been Kjell Maroni.

2. Materials and methods

Long-acting anti-parasitic agents for use in fish became available in Norway in the 1990s, and several experimental controlled trials have been conducted to evaluate the effect of anti-parasitic treatments applied to hatchery produced salmon smolt on the degree of returning after one, two or more years at sea. Groups of smolt have been assigned either to treatment or not treatment (controls). Two different anti-parasitic treatments have been used; emamectin benzoate (with marketing authorization, oral administration via feed or as intra-peritoneal injection) and Substance Ex (without marketing authorization, chitin synthesis inhibitor, topical bath treatment or injection). During outward migration from the river to the sea, salmon smolt are infected with salmon lice which are found near the surface in salinity above 20 (Heuch 1995). The infective stage of salmon lice conduct opposite dial vertical migration compared to their salmonid hosts to increase the encounter rate (Heuch et al. 1995). The hypothesis has been that long-acting anti-parasitic treatment would protect salmon smolt from salmon lice during outward migration and increase post-smolt survival and consequently the number of returning adult salmon.

2.1. Literature review

A systematic review of all published and non-published studies using anti-parasitic agents on release groups of Atlantic salmon smolt was conducted to identify Norwegian studies that could be defined as randomized control trials (RCT) trials to evaluate whether salmon returns would be enhanced by treatment against parasites/salmon lice. The review was initiated by inviting Norwegian scientists who had been identified to have expertise within such experiments

(Bengt Finstad, Bjørn Barlaup, Ove Skilbrei, Knut Wiik Vollset) to participate in the project “Metalice” together with international and national expertise on salmon lice and biostatistics (Arnfinn Aunsmo, Randi Krontveit, Peder Jansen, Martin Krkošek, Ian Dohoo, Pål Romunstad).

The group first presented all studies (published and non-published) where any of the members had been participating (by 29.10.2013). A librarian assisted in the set-up of the literature search across databases. The following databases were used in the literature search: ASFA (Aquatic Sciences and Fisheries Abstracts) and CAB abstracts. The search was conducted by first classifying categories of synonymous words by OR statements which created exhaustive searches within one category. These categories were then used to create search combinations that were relevant for the studies. The search categories were as follows:

- A) lepeophtheirus or louse or lice or lakselus or caligus or skottelus or parasite*²
- B) treatment* or emamectin or slice or substance ex or lakselusmiddel or protection
- C) smolt* or hatchery or laksesmolt or salmon or *Salmo salar* or post-smolt*
- D) return* or recapture* or survival or mortality or recruitment or impact

Searching the title, abstract and keywords we combined the search strings with an AND statement (A and B and C and D). The first 50 results were then examined to identify search words in the title that could be used as exclusion criteria yielding the following search string:

- E) poultry or turkey or chicken or nematode* or rabbit* or catfish or bream or carp or sheep or lamb or bug* or cod or fluke or squirrel or swine or pig* or lumpfish or hydrogen peroxide or sea bass or mussel or perch or h₂O₂ or cucumber* or elephant* or cricket* or tilapia

This search string was used to exclude any results from the original search that included one of the search words in the title using the NOT statement ((A and B and C and D) not E). To refine the search we checked that all the published articles that were identified from the group of experts were present in the final search results. The search results from each combination for each database were imported into standard software for managing references (Endnote®) where each search was organized in a group. At this point no limitation to either country or time frame was

² * allows for alternative endings to words such as plural. For example, the search word “return*” will return both results with the words “return” and “returns”

done. Following the import of all articles from the search, the publication list from the search was hand searched by first reading through the titles, then reading through the abstract and finally reading through the full texts, and at each step excluding non-relevant articles. For all articles for which the full text was read, the list of references was reviewed and relevant articles added to the reference database.

Because the study was restricted to Norwegian studies, all publications were in either English or Norwegian. Efforts were also made to include non-published data from scientists not present in the project group by sending a formalized letter requesting information to the following institutions: Norsk institutt for vannforskning (NIVA), Rådgivende Biologer AS, and Pharmaq AS. The letter (in Norwegian) is available from the first author upon request.

The inclusion criteria consisted of 1) the design setup incorporated treatment and control groups of released smolt, 2) the number of released smolt in each treatment group was given, 3) the type of anti-parasite treatment was given, 4) there were recapture efforts with number of recaptures registered, 5) recaptures the following two years after release had been conducted and 6) the year, date, and location of release was given.

The following exclusion criteria were applied: lack of recapture figures for either treatment or control group, species other than salmon, or that the trial was performed outside Norway.

2.2. Data extraction

The project group identified factors that potentially could influence the number of recaptured adult salmon, and this information was extracted from the reports and publications or by contacting the responsible scientist for the particular trial. A list of these variables with definition of relevant categories is outlined in Appendix I (Table A1). The variables were grouped in trial-specific variables at smolt release, migratory route-related variables, and recapture related variables. Missing data were given specific numerical codes depending on the reason for being missing, in order to separate them during statistical analysis.

2.2.1 Salmon lice exposure from fish farms

As part of sea lice control in marine salmon farms in Norway, it is mandatory to monitor and monthly report salmon lice abundances, total number of fish on the farms and mean fish

weight. From 2002 – 2011, farmers were instructed to report the highest abundance of sea lice encountered during a month (Jansen et al. 2012). These reporting-requirements for sea lice were changed from a monthly pen maximum abundance to a weekly mean abundance from January 2012. Hence, comparable numbers after 2012 were derived by selecting the highest weekly abundance of adult female lice reported during a month. These data were available from 2002 and onwards and formed the basis for infection pressure modelling along the Norwegian coast in different months. The quantities for the given month were calculated by multiplying adult female lice abundance with the reported number of fish per farm. To derive an expression for the intensity of farm infections of reproductive female lice along the coast, lice numbers were interpolated by kernel density functions in ArcGIS, Spatial analyst. Two variants of the kernel density interpolations were undertaken, using search radii of 50 and 200 km, respectively. From the location of release sites, the shortest migratory path to the open sea was estimated. Furthermore, statistics for this pathway intersecting the grid-layers on adult female lice were extracted. These statistics were the accumulated sum of grid-cells intersected, the mean or the maximum of grid cells. These were then used as a proxy for exposure of migrating salmon smolt to salmon lice of farm origin.

2.3. Statistical analysis

The data was compiled in Microsoft® Excel (Microsoft Corporation, Redmond, WA, USA) and subsequently imported into Stata (SE/ 13 for Windows, Stata Corporation, College Station, TX 778445, USA) which was used for all statistical analyses.

2.3.1. Descriptive statistics

The data extracted from the releases was quality checked for extreme values by summary statistics with means, medians, maximum and minimum values and graphically by histograms. Extreme values were checked against original data and corrected if necessary.

2.3.2. Meta-analysis

Three main outcomes were evaluated. In the first, the number of released, treated, and control fish and the number of recaptured, treated, and control fish were used to calculate the risk ratio³ (RR) for recapture in each trial. For the second and third outcomes, the mean difference in

³ Risk ratio (RR) can in these studies be defined as the probability of being recaptured in the treated group divided by the probability of being recaptured in the control group.

recapture weight and length between treated and control fish were calculated and the effect size was the weighted mean difference (WMD). Meta-analyses of WMD of recapture weight and length were performed separated by sea-winter.

Random effects meta-analyses of the described outcomes were performed using the method of DerSimonian and Laird. The estimate of heterogeneity was taken from the inverse-variance of random-effect model using the metan command in Stata (Borenstein et al., 2009; Dohoo et al., 2009; Sterne, 2009).

The metan command in Stata generates an estimate of the Cochran's Q which tests for differences in true effect sizes across studies, an estimate of the true variance of effect sizes between studies (τ^2), and Higgins I^2 (hereafter denoted I^2) which is an estimate of the proportion of the observed variance that reflects real differences in effect size (Borenstein et al., 2009; Sterne, 2009):

$$I^2 = (Q - \frac{d.f.}{Q}) * 100$$

where Q is the Cochran's Q statistic and d.f. is the degrees of freedom (number of studies minus 1). If I^2 is close to zero, then the observed variation between studies is assumed to be attributable to random variation as opposed to variance in true effect sizes. If I^2 is large then reasons for the observed variance should be evaluated (Borenstein et al., 2009; Dohoo et al., 2009; Higgins and Green, 2011; Rothman et al., 2008; Sterne, 2009).

There were several occurrences where multiple releases of paired control-treatment groups occurred in the same river and year. Whereas other meta-analyses have aggregated such observations (Krkošek et al. 2013) into single river-year observations, our analysis treated these releases as separate (independent) observations. In our dataset the estimated RR varied strongly between release groups released at the same location the same year. This indicates that a presentation of these as an average value, pooling data from multiple releases was not appropriate for these data. For comparability with aforementioned studies we replicated the method from previous meta-analysis analysis where data were aggregated into unique river-year observations, which resulted in 42 trials that had non-zero returns and found only a slight increase in both RR and heterogeneity, but not a larger standard error on the RR.

2.3.3. Meta-regression

Trial-level random effects meta-regression models using the metareg command in Stata were used to identify sources of heterogeneity in log(RR) and WMD estimates among releases and to evaluate the association between the selected variables (Table A1) and log (RR) and WMD estimates. Restricted maximum likelihood (REML) methods were used to estimate the additive (between-study) component of the variance τ^2 .

Each variable was screened and defined as either a continuous variable or categorical variables. Some continuous variables were redefined as categorical variables if their relationship with the log (RR) were clearly non-linear. This was assessed using lowess curves and by adding polynomial terms to the regression models. An updated list of variables evaluated in the final models is presented in Table 1.

Table 1. Variables used in meta-regression

Predictor variable	Grouping/response	Type	Pooling
Publication type	Peer-review, other	Categorical	
Release location	Fjord, river/estuary	Categorical	River and estuary releases pooled
Release river	Southern rivers (Imsa, Årdal, Suldalslågen), Vosso, Dale, Matre and Northern rivers (Eira, Surna, Orkla, Halselv)	Categorical	Rivers pooled into 5 groups
Period	1996-2003, 2004-2006, 2007-2008, 2009-2012	Categorical	Release years pooled into four periods (release quartiles)
Release day	Days after May 1 st	Continuous	
Treatment type	Emamectin in feed, Emamectin injected, EX	Categorical	
Lice burden	Density kernel 50 meter (sum)	Continuous	
Lice burden	Density kernel 50 meter (max)	Continuous	
Lice burden	Density kernel 200 meter (sum)	Continuous	
Lice burden	Density kernel 200 meter (max)	Continuous	
Distance	Distance migrated from release to 200 km boarder (m)	Continuous	
Temperature	Average temperature in migration path (C°)	Continuous	
Release weight	Average weight of smolt group at release (g)	Continuous	
Control group recapture rate (CGR)	Natural log of percent recaptured in control group	Continuous	

The variables were first assessed by univariable meta-regression, and variables with p-value <0.20 were considered candidates for multivariable meta-regression. In the multivariable analyses only variables with a p-value < 0.05 were retained. The proportion of variance explained was estimated as

$$R^2 = 1 - \frac{\tau^2_{\text{unexplained}}}{\tau^2_{\text{total}}}$$

where $\tau^2_{\text{unexplained}}$ was estimated from the null model without any variables and τ^2_{total} was unexplained between-trial variance with a given variable in the model. Control group recapture rate (CGR) was initially evaluated in the same manner as other potential causes of heterogeneity, but because there is a structural relationship between CGR and RR for the effect of treatment (the proportion of fish recaptured in the control group is the denominator of the RR for treatment effect), alternative methods of evaluating this effect were undertaken (see section 2.3.5.4).

By including CGR as predictor variable we assume that the variation in recapture in the control group reflects survival variation between release groups (see Results for a test of this assumption). In standard meta-analysis control group survival is sometimes referred to as “baseline risk” (e.g. Dohoo et al. 2007). However, we have chosen to use the terminology control group recapture rate (CGR) rather than “baseline risk” for clarity. The general reasoning for including “baseline risk” or CGR is that it reflects the “[...] summary of the effects of unmeasured population characteristics” (Dohoo et al. 2007).

2.3.5. Assessment of potential bias

2.3.5.1. Publication bias

The Begg’s and Egger’s tests were used in combination with a funnel plot to assess potential publication bias (Borenstein et al., 2009; Dohoo et al., 2009; Sterne, 2009). An influence plot was used to identify any influential trials.

2.3.5.2. Information bias

Information bias relates to having the “wrong information” about study subjects. While there was not likely any confusion as to which fish had been treated or not, fish that were classified as treated may not have been sufficiently protected against salmon lice. This would constitute a form of information bias and may have happened for several reasons which will be discussed in more length in the discussion. To consider this bias a quantitative bias analysis

(QBA) was conducted. Three scenarios were explored where protection was defined as having only 50% efficacy, 75% efficacy, or 90% efficacy. In addition, a fourth scenario was also tested where the likely efficacy across trials was a consensus value from four individuals in the expert group (BTB, PJ, AA, KWV). The four individuals were asked to define based on their own experience a trapezoidal distribution within which they thought the real efficacy ranged. The suggested values were averaged to one trapezoidal distribution (50-75-90-98%). This efficacy was termed “consensus” and a probabilistic QBA was conducted in which values of efficacy was picked randomly from this distribution. As CGR was shown to have large impact on RR (see results), bias was calculated and presented for each quartile of CGR.

2.3.5.3. Selection bias

A bias may also occur if the treated and untreated group had different likelihood of being recaptured. It has been shown both that treated fish grow faster (Skilbrei et al. 2013) and/or mature earlier (Vollset et al. 2014). Thus size selective sampling methods may lead to differential selection between treated and untreated group, which would consequently bias the risk ratio. For example, a large proportion of the fish in the dataset were caught with large trap net installation. These are known to catch small fish insufficiently since the large mesh size will allow smaller individuals to pass through without being caught. If treated 1SW fish are larger than control 1SW fish this would bias the risk ratio upwards, while if control fish were more likely to return as larger multiple seawinter fish this would bias the risk ratio downwards. To test for selection bias two QBA analyses were conducted in which the recapture rates (i.e., number of fish caught of total returning) was assumed to be 10% in the control group and then decreased to 9% or increased to 11% in the treated group (i.e., a 10% differential recapture rate).

2.3.5.4. Structural bias of introducing control group recapture (CGR) as a predictor variable

As noted above, CGR is a component of the RR for treatment effect and consequently, standard meta-regression techniques will produce biased estimates of the effect of CGR on the RR (Dohoo et al. 2006). A model was developed by Sharp and Thompson (2000) which models the log odds of recapture and which contains two correlated random effects terms to account for variation across studies. The random intercept accounts for variation in recapture rates across studies and a random slope for treatment allows the effect of treatment to vary across studies. The

correlation between these two random terms describes the manner in which CGR affects the RR for treatment. This model functions on the log odds scale as opposed to the log risk scale used in the standard meta-regression, but with recapture rates being so low, the two scales are virtually indistinguishable and were treated as the same⁴.

Two models were fit. The first replicated the final model determined from the standard meta-regression procedures and included treatment, fjord and release year as predictors. Subsequently, a model with treatment as the sole predictor was fit in order to obtain an estimate of the role of CGR on treatment effect averaged over release locations and year groups.

2.3.6 Analysis of control group recapture (CGR)

Since CGR appeared to be a very important predictor variable in the meta-regression analyses, it was important to understand what variables affected CGR. The variables were first assessed by univariable linear regression, and variables with p-value <0.20 were considered candidates for multivariable linear regression. In the multivariable analyses only variables with a p-value < 0.05 were retained.

2.3.7 Assessment of risk difference and attributable fraction values

In order to evaluate the effect of treatment in terms of numbers of fish “saved” by treatment, two additional measures were computed. The risk difference (RD – the difference in recapture rates between treated and control groups) was determined by a meta-analysis of the individual study values. The attributable fraction (AF – the proportion of surviving fish in the treated group which could attribute their survival to having been treated) was computed for each trial individually from the observed recapture rates in the treated and non-treated groups. The distribution of these AF values was plotted and an overall average was estimated by using the weights derived from the meta-analysis of recapture rates to compute the weighted average AF.

2.4 Work allocation

- Project manager: NMBU (Randi Krontveit, Arnfinn Aunsmo until January 1st 2014)
- Literature search and data retrieval responsibility: NINA (Bengt Finstad), Uni Research (Knut Wiik Vollset, Bjørn Barlaup), Ove Skilbrei and NMBU (Randi Krontveit)
- Collection of historic data of lice abundance in the fish farms on the migratory route:

⁴ Consequently, in the results sections the first model refers to risk ratio (RR) while the model taking account of the structural bias refers to the odds ratio (OR)

- NINA (Bengt Finstad) and Uni Research (Bjørn Barlaup / Knut Wiik Vollset prepared lists of geographical areas of the post-smolt migratory route per river
- VI (Peder Jansen) retrieved the relevant data from the Havbruksdata database
- Evaluation of data quality and validity: NMBU (Randi Krontveit), VI (Peder Jansen)
- Statistical analysis: NMBU (Randi Krontveit), UPEI (Ian Dohoo), Uni Research (Knut Wiik Vollset)
- Publication and reports: the whole project group did actively participate in this part, with Uni Research and NMBU being main responsible partner.

2.5 Deviations from the project plan

Data collection and finalizing of the dataset was delayed by approximately one month, but this did not influence the final project progress. Work allocation was changed during the project period, and Uni Research was given the responsibility of finalizing the planned deliverables.

3. Results

3.1. Literature review and data material

From the studies that contained relevant data, four published articles and two editorial comment/response were excluded because they were from countries other than Norway (Gargan et al. 2012, Jackson et al. 2013b, Jackson et al. 2011a, Jackson et al. 2011b, Krkošek et al. 2013, 2014). Two releases performed in Norway were excluded because they included sea trout. Finally, a total of 118 smolt releases were identified by the systematic review and included in the study. These releases were extracted from four published international peer-reviewed scientific papers (84 releases), four national reports (10 releases), and releases from four non-published reports/assignments (26 releases) as a result of the literature search inclusion and exclusion criteria described. Table 2 gives an overview of the studies and some key figures from the different studies. Year of release ranged from 1996 until 2012 from the following Norwegian rivers (located from south to north): Imsa, Årdal, Suldalslågen, Vosso, Dale, Matre, Eira, Surna, Orkla and Halselv. Figure 1 presents a map of Norway with location of the release-rivers.

Table 2. Summary of the 118 Norwegian trials/releases used in the meta-analysis of the effects of anti-parasitic treatment of smolt at release on degree of adult salmon recapture.

River	Author	Publication year	Release groups	Smolt releases (N)		Recaptures (N)	
				Controls	Treated	Controls	Treated
Halselv	Hazon et al 2006 + Finstad_Unpublished	2007	3	6156	5958	21	17
Halselv	Strand og Finstad	2010	1	3365	4426	0	0
Orkla	Hvidsten et al. 2007	2007	2	5913	5901	32	62
Surna	Hvidsten et al. 2007	2007	1	2985	3000	51	66
Eira	Jensen et al 2013	2013	4	12112	11796	33	34
Matre	Skilbrei_Unpublished	n.a.	16	31965	32045	98	111
Vosso	Barlaup et al. 2013	2013	37	158366	160826	947	1058
Dale	Skilbrei et al. 2012	2012	44	73068	77200	498	615
Dale	Skilbrei_Unpublished	n.a.	3	8165	8115	92	125
Suldalslågen	Finstad_Unpublished	n.a.	3	15995	15497	1	3
Imsa	Hazon et al 2006 + Finstad_Unpublished	2006	2	6000	4000	65	44
Årdal	Lehmann_Unpublished	n.a.	2	6385	6385	13	9

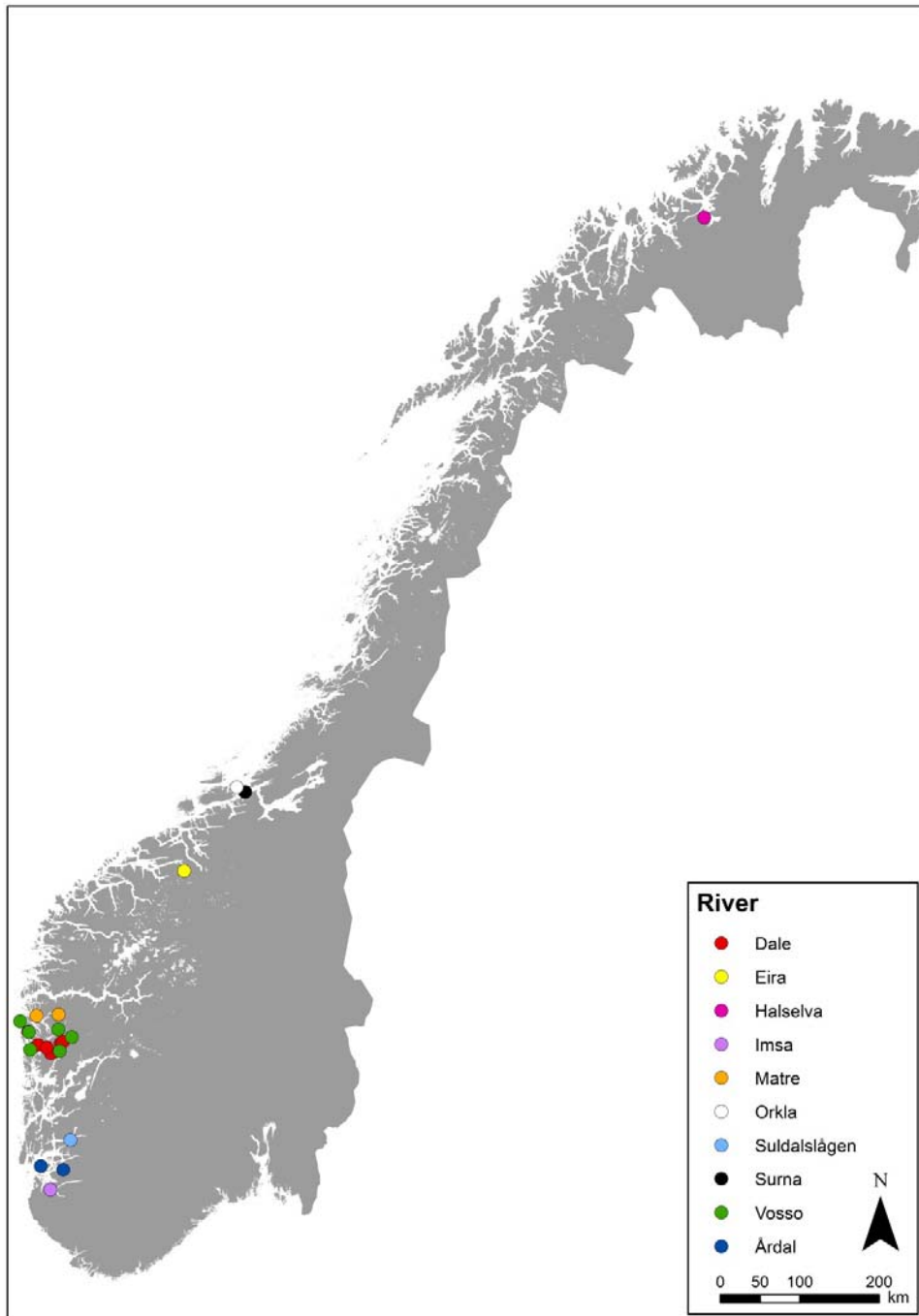


Figure 1 Figure of release locations with fish from different rivers along the coastline of Norway

A total of 17 releases had zero recaptured fish in both treatment and control group - eight were from Vosso, seven from Dale and two from Halselv. These provided no information and were consequently excluded from all analyses. Of the remaining 101 releases 14 contained

release groups were either the control group or the treated group had zero recapture. These were retained in the final dataset but 0 was substituted with 0.5 However, after exploring the weights of these release groups in the overall meta-analysis they were all found to have very low weights and they contributed very little to the final results.

Weight and length data was available from a smaller subset of releases from Vosso, Dale, Matre, Eira, Årdal, Imsa and Halselv. Lice exposure estimates, migratory distance and mean temperature along the migratory route were not available for releases performed before 2002 (n=15).

3.2. Meta-analysis

The overall random effects meta-analysis of all the studies including 103 release groups estimated an overall RR of 1.18 (95 % CI : 1.07-1.30) and a significant effect of treatment on returns of recaptures (P=0.001). However, there was a substantial amount of heterogeneity in the data revealed by an I^2 of 40.1% (P-value<0.001). The estimated between study variance τ^2 was 0.0719.

The meta-analyses of weight and length measurements of recaptured fish, indicated that treated 1 SW fish were significantly heavier than the controls (WMD, 123 grams, 95% CI : 45 - 200 , P=0.002), but there were no significant treatment effects on length in any of the sea winter groups. There was considerable variation between releases in terms of weights of 1SW fish (I^2 = 78%). Thus, meta-regression was used to explore factors that might contribute to this heterogeneity.

3.3. Meta-regression

3.3.1 Model for overall estimates of survival

The following variables rendered a P-value <0.20 and were included in the multivariable analysis: release location, release-period, temperature and CGR. In the final model, temperature along the migration route was not significant and was not retained. The variables release location, period and CGR were all significant and the results with coefficients, standard error, P-values and 95% confidence interval is outlined in Table 3. (Note that these results have not been adjusted for the structural bias between CGR and observed RR).

Table 3. Results from the multivariable random effects meta-regression

Variable and level	Log risk ratio	P	95% confidence interval	
Release location				
River/estuary	Baseline	-	-	
Fjord	0.185 (0.09)	0.036	.013	.357
Release year period				
1996-2003	Baseline	-	-	
2004-2006	-0.512 (0.16)	0.002	-.833	-.191
2007-2008	-0.231 (0.14)	0.094	-.502	.040
2009-2012	-0.116 (0.10)	0.249	-.315	.083
Control group recapture (CGR)	-0.241 (0.05)	<0.001	-.337	-.144
Intercept	-0.893 (0.25)	<0.001	-1.384	-.402

In the final model ($F_{5,97}=7.69$, $p<0.001$) I^2 was reduced to 13.9% and the three retained variables explained 70.6% of the between-study variation. CGR was a major predictor, and for a one unit increase in baseline survival the log (RR) dropped by 0.24 units. CGR is however both a function of actual variation in survival and recapture efforts. To evaluate the impact of recapture effort we ran a new model only including data from Vosso and Dale which has had a relatively constant recapture effort over the years. This did not alter the final model ($F_{5,63}=6.04$, $p<0.0001$) except that I^2 was now 28.8 % and the variance explained was 67.9 %. In short, the effect of baseline survival suggests that risk ratio is high when survival in the control group is low and low when survival in the control group is high.

After accounting for the effect of CGR, the risk ratio was highest in the first period (1996-2003) and then dropped to almost no effect of treatment in the second period (2004-2006), but increased again in the third period (2007-2008), and was almost back to the same level as in first period in the last period (2009-2012). The risk ratio was higher in groups released in the fjord compared to groups released in the river or estuary.

The effect of one outlier with a very high risk ratio (Release group in Dale River, 1997, Skilbrei et al. 2013) was tested by running the model excluding this datapoint. This did not alter the final result ($F_{5,96}=6.73$, $p<0.0001$, adjusted- $R^2=68.2$, $I^2=10.58$).

Figure 2 display the relationship between the RR and CGR in the four different release periods for the river/estuary releases and fjord releases, respectively. (Note that this graph has not taken into account the structural relationship between CGR and RR – see below).

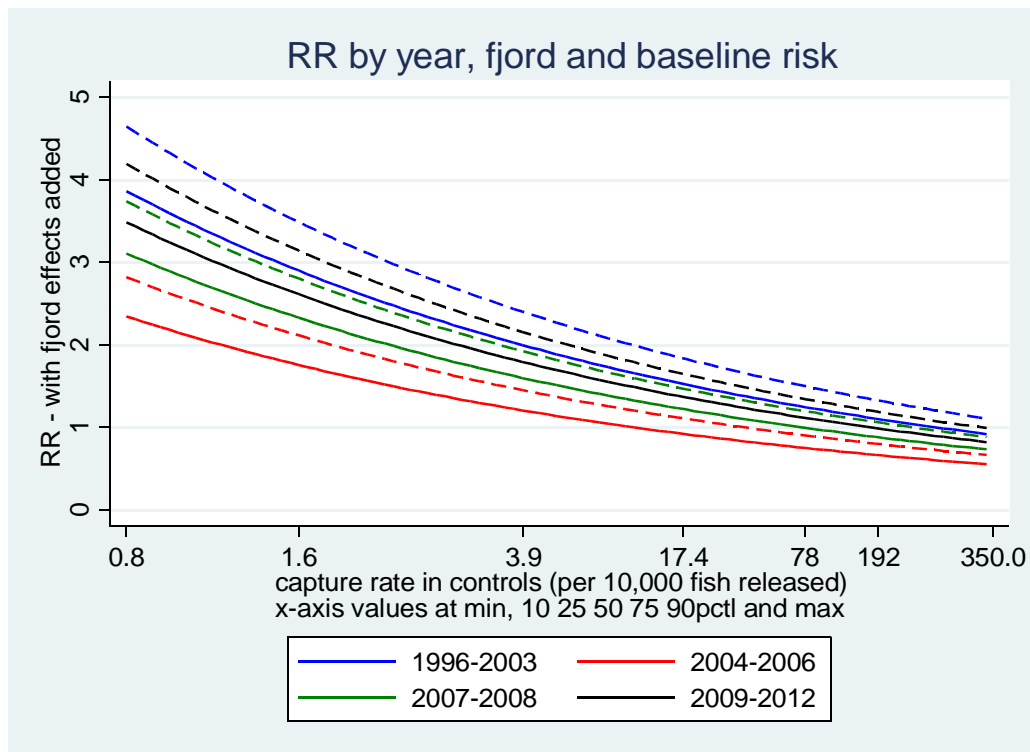


Figure 2 Relationship between CGR (recapture in control groups) and estimated risk ratio for each period (see color panel). Solid line indicates releases in the river and estuary, while dashed lines indicate releases in the fjord.

3.3.2 Model for overall estimates of weight

Meta-regression of factors contributing to heterogeneity ($I^2 = 78\%$) of the effects of treatment on weights in 1SW fish were not very productive. The distance the smolt travelled was the only significant ($P=0.03$) factor and it only explained 11% of the unexplained variation. Variation in treatment effects on weights remained largely unexplained.

3.4. Bias

3.4.1 Publication and influence bias

Publication bias was not expected given that we included both published and non-published data in the meta-analyses. Neither Begg's nor Egger's test for publication bias showed significant evidence of publication bias. When individual studies were examined, one release

group in the Vosso river in a study by Barlaup et al. (2013) (also included in Vollset et al. 2014) did have considerable influence on the overall RR estimate (which would have been higher without this release group, 95 treated vs 142 control \rightarrow RR = 0.69). However, these data were from just one release group of 29 in this report and there was no reason why these results should be doubted so the data were retained.

3.4.2 Information bias

A probabilistic QBA⁵ was carried out to evaluate the effect of treatment efficacy on the observed RR for treatment. As the observed RR depended strongly on CGR, effect of QBA was also strongly dependent on the CGR. In the first quartile of CGR (i.e., low control group survival), the effect of poor efficacy of treatment was strongly negative (i.e., the RR for treatment would have been underestimated). For example based on the estimates for the last release period (2009-2012) and fjord releases, the RR in the lowest quartile of CGR would increase from 3.1 to 3.4, 3.9, and 45.2 if efficacy were 90, 75 or 50%, respectively. In the second and third quartile the potential for underestimation was less prominent but still present, increasing the risk ratio from 1.6 to 1.7, 1.9 and 2.3 in the second quartile and from 1.3 to 1.3, 1.4 and 1.6 in the second quartile if efficacy were 90, 75 or 50%, respectively. In the last quartile of CGR (i.e., high control group survival) the RR would decrease from 0.95 to 0.94, 0.93 and 0.90 if efficacy were 90, 75 or 50%, respectively. The “consensus” distribution of efficacy increased the risk ratios from 2.9, 1.6 and 1.3 to 3.6, 1.8 and 1.4 in the first to third quartile of baseline survival while it decreased the baseline survival from 0.95 to 0.93 in the fourth quartile.

3.4.3 Selection bias

The two scenarios with an increase and decrease in recapture rates in the treated group versus the control group rendered a bias estimate of 11% positive bias and a 9% negative bias across baseline survival. This indicated that differences in recapture rates between treated and untreated fish did not have a major effect on the estimates of the RR.

3.4.4 Structural bias of introducing control group recapture (CGR) as a predictor variable

Output from the two models which account for the structural bias between CGR and risk ratio (RR) of treatment effect are presented in Appendix II. The full model which included CGR

⁵ Quantitative bias analysis

as well as release period and location produced very similar estimates of effects for release location and period. However, the coefficient for CGR dropped from 0.248 to 0.147 suggesting that approximately ½ of the effect observed in the standard meta-regression was attributable to the structural bias. In the model with treatment as the sole predictor, the coefficient for CGR was 0.105 (per unit log CGR). The estimated OR at a low CGR (low control group recapture = 0.01%) was 1.7, and at a high CGR (high control group recapture = 2 %) was 0.99.

In figure 3 and 4 modelled data are plotted on top of scatter plots between risk ratio and CGR (recapture in control group) from the bias corrected model on a normal and log-transformed scale, respectively. In figure 5 modelled estimates are plotted together with confidence intervals. In the scatter plot two outliers are removed for clarity. The plots including outliers are presented in Appendix III.

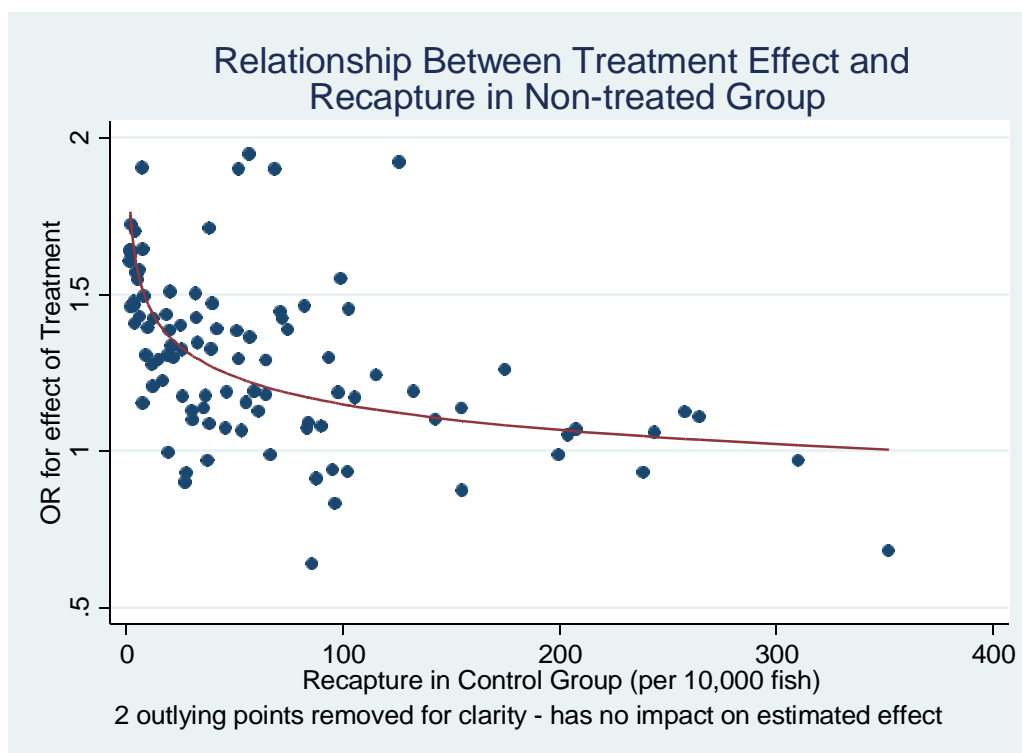


Figure 3 OR for the effect of treatment plotted against recapture in control group on a normal scale.

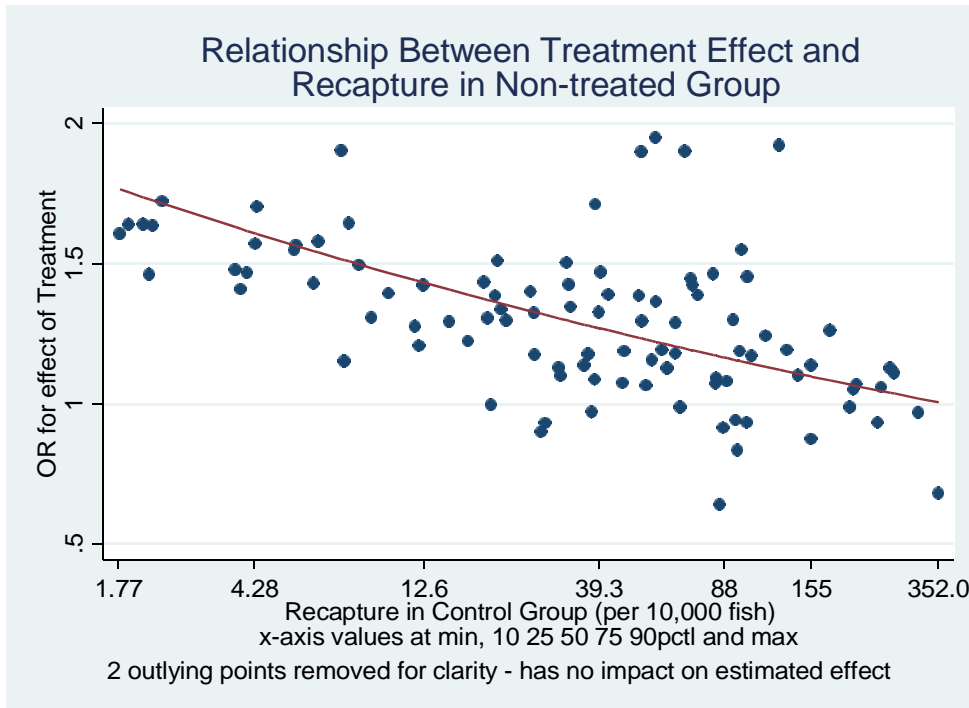


Figure 4 OR for the effect of treatment plotted against recapture in control group on a log scale.

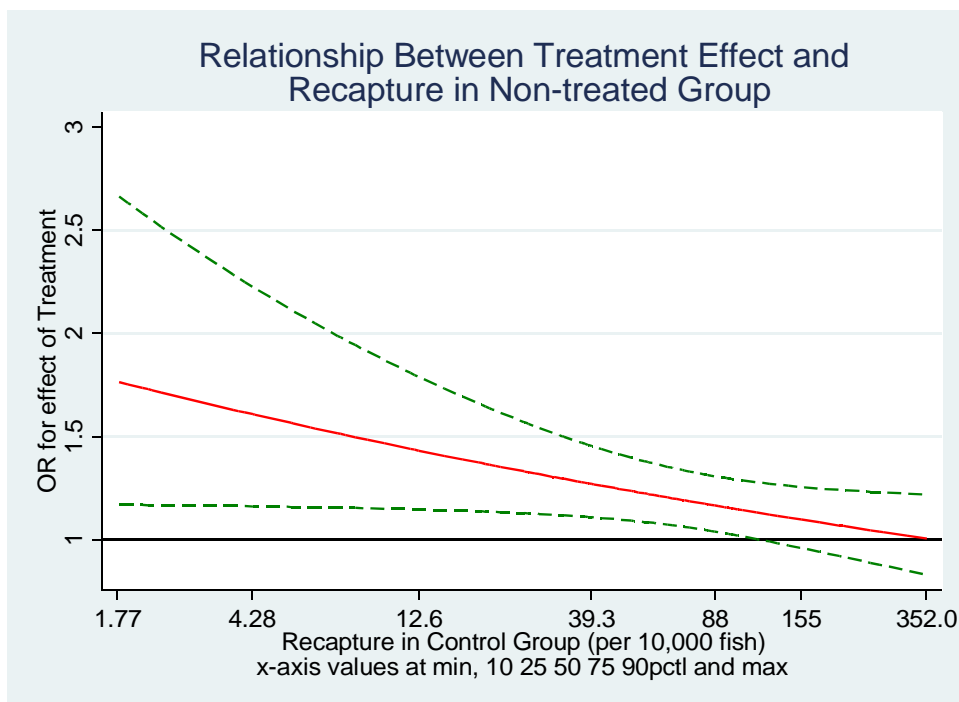


Figure 5 OR for the effect of treatment plotted against recapture in control group on a log scale with 95% confidence intervals.

3.4.5 Factors affecting control group recapture (CGR)

The following variables rendered a P-value <0.20 and were included in the multivariable analysis: release location (fjord versus river/estuary), river location, temperature, release day, lice burden (200, sum), and distance migrated.

In the final model lice burden and release day were not significant and were consequently omitted from the final model. The reason that lice burden became insignificant in the final model was partially due to correlation with distance ($\rho=0.448$). This was also the case for release location and distance migrated ($\rho=0.72$). Thus, release location was dropped from the model since migration distance was a better predictor. The final model included river location and migration distance ($F_{5,83} = 8.56$, adjusted $R^2=0.34$, $P<0.0001$). The model predicted that CGR would decrease with 0.04 units (on a log scale) for every km migrated. This means that groups of non-treated fish that are released for example 50 km from the river outlet (i.e., will have to migrate 50 km less to reach the ocean) will have a 7.1 times higher survival than non-treated fish released in the river or river outlet.

3.5 Evaluating the impact of treatment

The question, “what proportion of fish are killed by sea lice?” inevitably ensues from research of the type that has been reviewed in this meta-analysis. This parameter is referred to as the population attributable fraction (PAF). PAF is the proportional reduction in mortality that would occur in a population if exposure to salmon lice were eliminated. The PAF can be calculated as follows

$$PAF = P_e(RR_e - 1) / [1 + P_e(RR_e - 1)]$$

Where RR_e is the relative risk of mortality for the fraction of the population that is exposed, and P_e is the fraction of the population that is exposed (to a level of salmon lice adequate to have a detrimental effect on the smolt). However, the RR calculated in our meta-analysis is not the relative risk of mortality in an exposed group, but the relative risk of dying due to not being treated. We have no data on the prevalence of disease (i.e. the fraction of the population that was exposed to salmon lice during migration) and it is therefore not possible to calculate RR_e or PAF. However, there are two parameters that measure the effect of the intervention studied (anti-parasitic treatment of smolts) in terms of number (or proportion) of fish

“saved” by the intervention in the treated group. These are risk difference (RD) and attributable fraction (AF).

Risk Difference (RD)

The risk difference is the difference in the mortality risks in the treated and no-treated groups. It is computed as:

$$RD = p(D+|E+) - p(D+|E-)$$

where $p(D+|E+)$ and $p(D+|E-)$ are the disease risks in the exposed and non-exposed groups respectively. In this study this means recapture rates in the treated and control groups.

The RD will be positive if more treated fish survived, and negative if more control fish survived. The overall estimate (weighted average - derived from a random effects meta-analysis of RD values) from the studies used in this meta-analysis was 0.001 meaning that, on average there is 1 extra survivor per 1000 fish released in the treated groups compared to the control groups. A histogram of the RD estimates for the individual releases is shown in figure 6.

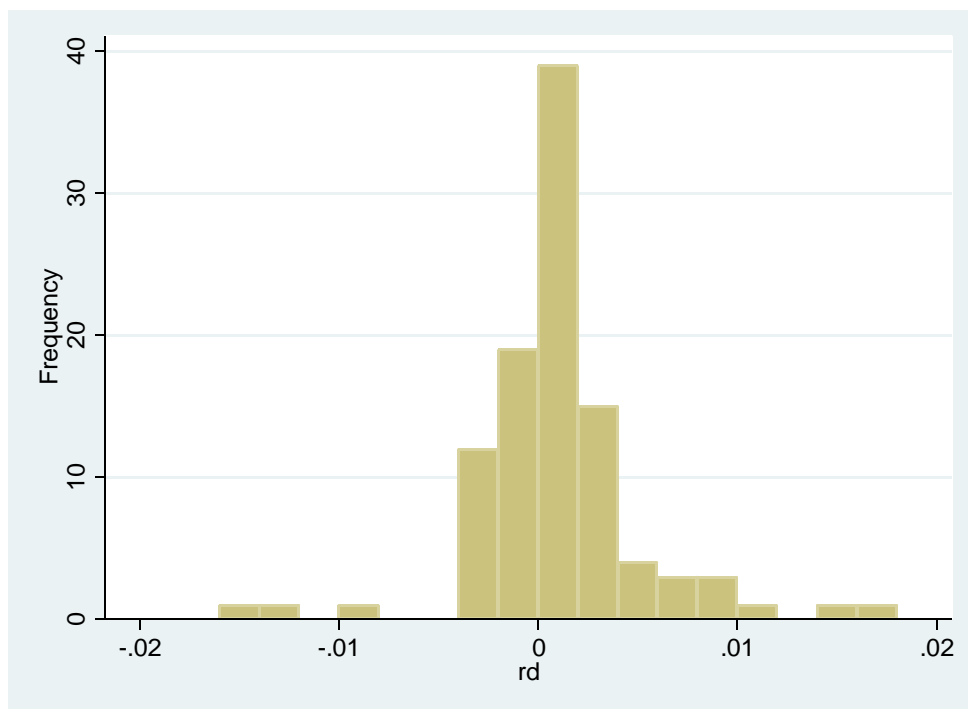


Figure 6 Histogram of risk difference calculated for all release groups

Attributable Fraction (AF)

The attributable fraction (AF - also called attributable fraction in the exposed or AF_e) is the proportion of disease or mortality in an exposed group that can be attributed to the exposure. The AF relates the additional fish surviving in the treated group (i.e., the RD) to the number surviving in the treated group (i.e., it expresses RD as a proportion) and consequently, removes the influence of factors such as recapture effort. The attributable fraction is derived from the RR calculated from our release groups as:

$$\begin{aligned}
 AF_e &= RD/p(D+|E+) \\
 &= (RR - 1) / RR \\
 &\approx (OR - 1) / OR \quad (\text{approximate } AF_e)
 \end{aligned}$$

where AF_e is attributable fraction, RR is risk ratio, OR is odds ratio, RD is risk difference and $p(D+|E+)$ is recapture rate in the control group. We computed the AF directly for each study and a histogram of those values is shown in figure 7.

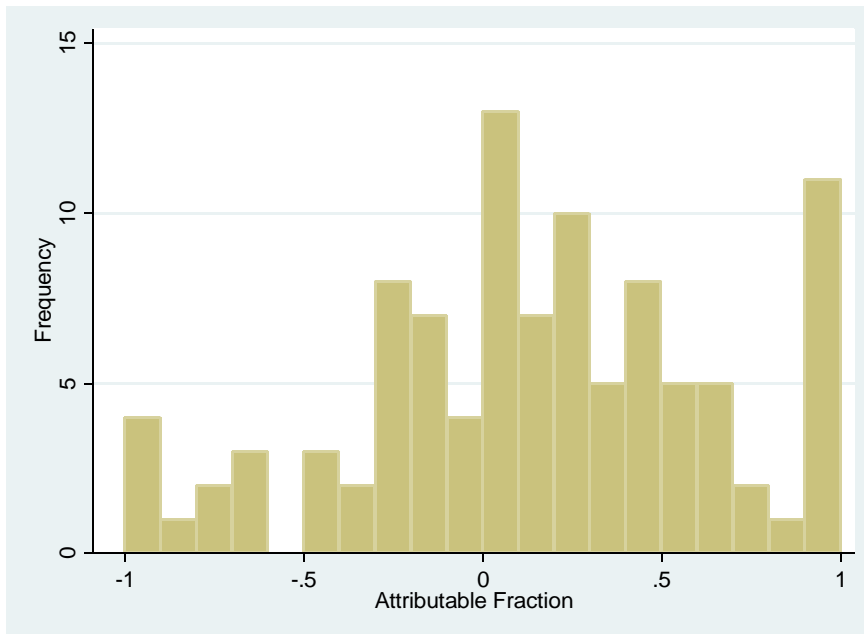


Figure 7 Histogram of attributable fractions calculated for all release groups

Studies with a value >0 were showing a protective effect from treatment while those with values <0 were showing a detrimental effect. It is not possible to do a meta-analysis of AF values, so we computed a weighted average value by weighting the individual values by the weights generated by the meta-analysis of the risk ratio (RR). It produces an overall average value of 11.3% (CI: 4.5 – 18.0 %). This suggests that on average, of all the fish that survived in the treated group, 11.3% could attribute their survival to the fact that they had been treated. Alternative we would have expected 11.3% of this fish to not have been recaptured if no treatment had been applied to this group. The estimates for the four separate time periods (1996-2003, 2004-2006, 2007-2008 and 2009-2012) were 21%, -4% (effectively 0), 15% and 8 %, respectively. (In a previous study (Krkošek et al. 2013), the AF was estimated from the summary RR instead of making use of the individual release AF values, This approach to estimation would have produced a slightly higher AF of 15.2%.)

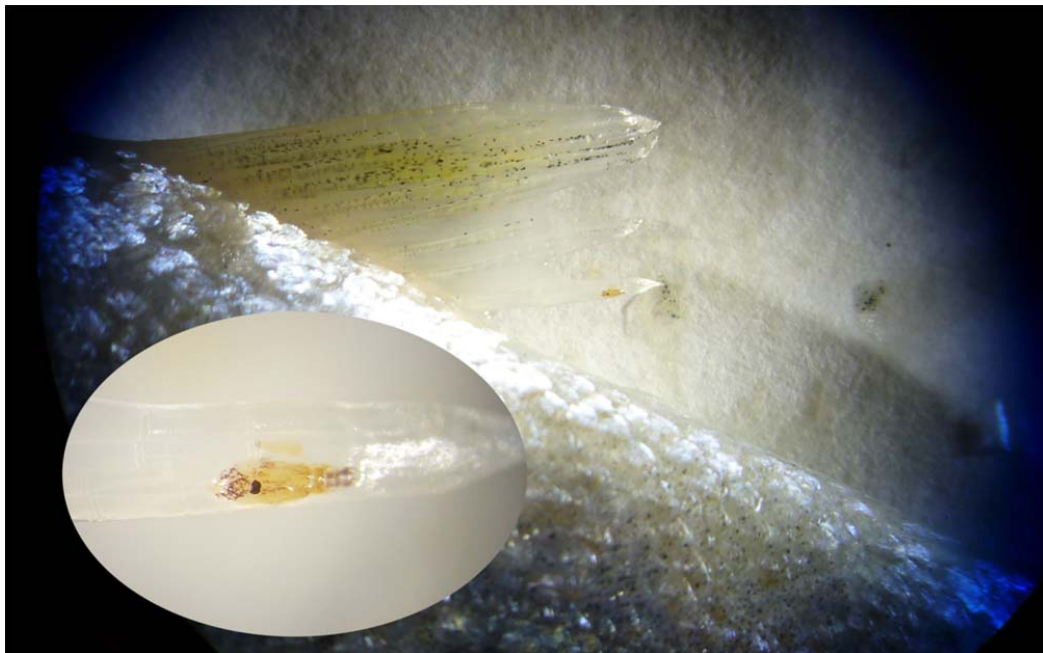


Illustration photo: Copepodite of salmon lice, *Lepeoptheirus salmonis*, on the anal fin of a cultivated Atlantic salmon smolt.
Photo by Knut Wiik Vollset

4. Discussion

Overall the results from this meta-analysis suggest that treatment increases survival in the release groups (mean risk ratio = 1.18, 95% CI : 1.07-1.3). That is, on average, fish in treatment groups were 1.18 times more likely to be recaptured than control groups, or conversely control groups had $1/1.18 = 0.85$ (95 % CI: 0.93-0.76) the recapture rate of treated groups. This is lower than what Krkošek et al. (2013) reported from a meta-analysis (1.39, 95% CI: 1.18 -1.42) based on mostly Irish and some Norwegian studies (the Norwegian studies were also included in this meta-analysis). However, our analyses included more trials than previous studies, and also contain a larger heterogeneity than in previous meta-analyses. Thus, for our data, an average risk ratio is an incomplete representation of the effect of treatment on recapture of returning adult salmon. Indeed, the dependency of the treatment effect on control group recapture rate (CGR) suggests large variation among trials ranging from a relative risk of 1.7 when control group recapture is low to no effect on treatment when control group recapture is high. Thus, although our main conclusion is that exposure to sea lice (measured by a lack of treatment) is a significant contributor to marine survival of Atlantic salmon, our secondary conclusion is that in some release groups treatment was very beneficial, while in others there was clearly no effect. This variation in treatment effect could be explained by where the fish was released, what period they were released and the CGR. The CGR was by far the most import source of heterogeneity.

4.1 Effect of control group recapture rate (CGR) on estimate of treatment effect

After correcting for the structural dependency between CGR and RR, the estimated risk ratio at a high CGR was 1.7, while at low CGR it was 0.99. This suggests that if survival in the control group is generally good then risk ratio is low, while if survival is poor, the risk ratio is high. Dohoo et al. (2008) defined baseline risk (CGR in this report) as the «[...] summary of the effects of unmeasured population characteristics». There are two main potential hypotheses to why we observe this strong relationship with CGR: (1) the detrimental effect of lice is exacerbated in situations when the salmon smolt also have to cope with increased pressure from other causes of mortality, and (2) there is large unmeasured variation in the exposure to lice between release groups. In this scenario, release groups with low survival will also be associated with high exposure of lice.

The first hypothesis can be broken down into two non-mutually exclusive hypotheses:

(1a) the CGR effect is driven by a biological interaction between salmon lice and other risk factors the salmon encounters during their marine sea sojourn. For example, in years where prey conditions are poor, salmon lice can be detrimental for a starving smolt, while in years where prey conditions are good, the smolt will have fewer problems coping with the additional stress posed by the parasite. This is in line with the study by Connor et al. (2012), who found that the decline of pink salmon could be explained by a synergetic effect of climate, predation and salmon farm exposure.

(1b) the CGR effect is driven by a biological interaction between salmon lice and the variation in some inherent traits in the smolt (e.g., smolt size or quality). For example, fish that are inherently in poor state due to poor cultivation practice during migration will cope poorly with salmon lice exposure. For example, Finstad et al. (2007) showed experimentally that smolt with a prior exposure to suboptimal water quality were more affected by salmon lice than smolt without such exposure.

The second hypothesis (2) suggests that baseline survival itself may, in part, be driven by salmon lice exposure. This means that in release groups with high exposure to salmon lice, survival in the control group would be relatively low and risk ratio would be high and vice versa. If salmon lice exposure is mainly driven by production of lice in fish farms we would expect a correlation with CGR and lice burden estimation from fish farms. There was a correlation between salmon lice burden from fish farms and the log survival in control group ($\rho=-0.25$), but salmon lice burden could not explain the heterogeneity in risk ratio (see below). Furthermore, lice burden fell out of the final model when including distance the fish had to migrate to reach the ocean. However, it seems reasonable that there is a large variation in exposure between release groups as they are released into a highly stochastic environment with a multitude of potential biological and physical interactions that may affect their exposure to salmon lice. For example, the variation in migration distance also reflects that a large proportion of the release groups were towed in containers to the outer perimeter of the fjord system before release (in for example the Vosso and Dale data). These fish have a high survival, but also a low exposure (shorter migration) and can therefore explain some of the relationship between a low risk ratio and high survival in some of the groups.

Statistically it is not possible separate these hypothesis. The results thus calls for more robust and properly designed field experiments to for example test how prior experience such as poor water quality or general smolt quality will affect the treatment effect.

4.2 Absence of observed effect of sea lice burdens estimated from fish farms

None of the salmon lice burden estimates from the production of lice from fish farms had any significant effects on the risk ratio estimates. This could either be explained by (1) the additional salmon lice from fish farms do not affect the release groups, (2) the salmon lice burden estimates do not replicate the exposure of lice from fish farms appropriately or (3) the efficacy of treatment is reduced for lice from fish farms due to resistance to treatment. The salmon lice burden estimate based on a density kernel in combination with assumed migration path of smolt used in this study is the best available and operational estimate to date. Similar methods have recently been used to model associations between farm origin lice and lice burdens on samples of wild sea trout along the Norwegian coast, as well as that of the development of lice infections on naïve farmed fish from the onset of marine production (Serra-Llinares et al. 2014; Kristoffersen et al. 2014). Both of these studies argue that farm production of lice probably is an important driver of lice transmission to wild sea trout and naïve farmed salmon, respectively. These studies tend to put more emphasis on explanations (1) and (3). However, extrapolating this method to calculate exposure of migrating salmon smolts to farm origin lice may not suffice. For example, the vertical distribution of smolt (Thorstad et al. 2012) and avoidance of low salinity waters by salmon lice (Heuch 1995, Heuch et al. 1995) will strongly affect their interaction. Furthermore, while fish farms aggregate salmon lice over a longer time period, the exposure of salmon smolt most likely depends strongly on whether or not smolt encounter denser patches of salmon lice (Penston et al. 2008). Several other difficulties and complications of estimating exposure of salmon lice on migrating smolt can also be mentioned, but we will not spend more time on speculations here. Using more detailed hydrodynamic models (e.g. Johnsen et al. 2014) to estimate the spread and patchiness of infectious lice stages could potentially give better explanatory power, and should be explored. However, even though an appropriate model of distribution of salmon lice can be set up, the question of where the salmon smolt migrate and how the release groups distribute in the fjord system will still be uncertain.

4.3 Change in effect of treatment over time

The effect of treatment also changed with years. In the first period from 1996 to 2003 the risk ratio was relatively high, but fell to almost no effect in the second period from 2004 to 2006. In the last two periods the risk ratio rose again and was in the last period (2009-2012) similar to the first period. Our compartmentalization of the time changes in treatment effect into various year spans was not due to external information on how treatment efficacy or louse exposure changed with time, but rather divided into quartiles based on number of release groups (after identifying that the temporal trends were clearly not linear). This was done as the data was unbalanced (few release groups some years and several release groups other years), and it was therefore not possible to include year as a categorical variable. Thus, the temporal effect does not necessarily represent a meaningful biological process but may instead reflect a spurious organization of the temporal data into categorical variables. Nonetheless it is worthwhile considering the biological mechanisms. The production of salmon lice from fish farms is mainly driven by the number of fish and female lice per fish. During the last 10-15 years there has been an increased focus on lowering the production of infective stages of salmon lice (copepodites) during the wild Atlantic smolt run in spring time in Norway. A coordinated spring delousing has been implemented and is today mandatory across all regions in Norway. This has manifested itself as a decreased abundance of female lice during spring time in the mandatory sea lice counts data that started in 2002 (Jansen et al. 2012). Studies from other regions have suggested that spring delousing is an effective tool to alleviate wild migratory salmon smolt from salmon lice given that effective treatment is used and sufficiently coordinated (Peacock et al. 2013). Meanwhile, however, the number of farmed fish (and consequently number of hosts) in most regions has increased steadily during the same period. A combination of these two patterns may explain the decreasing risk ratio from the first period to the second period and subsequent increased risk ratio in the last two periods. However, as mentioned above the risk ratio was not a function of estimated lice burden from fish farms from 2002-2012, and there are most likely also other environmental factors contributing to this pattern.

4.4 Bias

While studies from RCTs are often thought to give unequivocal answers to treatment effects, applying such methods to study effects of parasites on wild fish is complex. While in traditional RCTs the treatment effects are under scrutiny, the efficacy of treatment in studies with

treated and untreated salmon smolt are assumed to be 100%, and any variation in treatment effect is treated as either natural variation or heterogeneity. In reality, there is a suit of potential reasons why the results from release groups do not necessarily reflect the mortality patterns in wild fish. On one hand there are several sources of biases that need to be addressed, and on the other hand, survival and risk ratio calculated from cultivated or handled fish may not be the same as for wild fish.

The sources of such biases can be divided into two categories: (a) the efficacy of treatment is less than 100 % (information bias) and (b) the recapture rate is biased either towards the treated or untreated group (selection bias).

4.5 Information bias – treatment efficacy

The treatment effect (a) is perhaps the easiest factor to control for as it is possible to measure the uptake of the anti-parasitic agent in the tissue of fish and compare these to threshold levels in laboratory studies. This is however seldom or at best only sporadically applied. Skilbrei et al. (2008) documented that when oral administration of emamectin benzoate is used, the resulting levels in tissue samples were very variable, with a proportion of the fish having levels below the recommended level already a week after administration. Similarly, Gargan et al. (2012) reported that 35 % of the sampled fish had tissue levels below the limit of detection ($9 \mu\text{g}\cdot\text{kg}^{-1}$). This resulted in a change from oral to inter-peritoneal injection in the study by Skilbrei et al. (2013). It must therefore be expected that treated groups which were given treatment through oral administration were not 100 % protected during the first weeks after release (more than 50% of the release groups had oral administration).

Even when administration of treatment is done correctly, anti-parasitic agents may still not render 100% protection. For example, resistance or lowered sensitivity to emamectin benzoate has been reported for the entire Norwegian coast in the recent years (Grøntvedt et al. 2014, report). Whether resistance has affected the results in our study is not known. However, it is assumed that resistance to emamectin benzoate in fish farms was not present in the beginning of the study period and possibly more prevalent in the latest years. This may explain why some of the largest treatment effects were observed in the beginning of our data series. However, we found no differential effects of EX and emamectin. However, it is difficult to draw inference

since the dataset is unbalanced. The potential effect of resistance on these results should be studied in more detail.

Another assumption is that the effect of the treatment will last for 6-8 weeks and that this will protect fish from salmon lice (Stone 2000). This assumes that most exposure to salmon lice happens during near shore migration and that salmon smolt will migrate quickly from near shore habitat. However, while the estuary and fjord migration of Atlantic salmon smolt has been documented thoroughly by the use of different tagging equipment (e.g., acoustic transmitters; Thorstad et al. 2012), there is little data that can document how the fish migrates after leaving the fjord. One possibility is that the fish follows the coastal current northwards before entering the North Sea. If this is the case then exposure to salmon lice produced in fish farms can be decoupled from the fjord migration, and the treatment effect may not protect the fish during the entire period of exposure. In our results there was larger estimated effect size for groups released in the fjord compared to groups released in the river or estuary. If the exposure of lice is mostly in the outer part of the fjords and exposure is most effective the first period after release, the difference we observe between the two groups could be that the release groups in the outer fjord encounters lice when they have an effective treatment, while release groups in the river encounters lice when they have a less optimal treatment.

Another possible bias is that the anti-parasitic agents may affect other parasites other than salmon lice. Emamectin benzoate belongs to the group avermectins which are broad-spectrum anti-parasitic agents (Jansson et al. 2007). If the smolt encounter other parasites during outward migration, the protection which emamectin benzoate provides may give a beneficial effect on survival irrespective of salmon lice exposure. For example, salmonids can in some samples have 100 % prevalence of endo-parasites such as parasitic nematodes (*Anisakis* sp., Urquhart et al. 2013). However, to date there is no documentation that could support or refute this hypothesis. The other anti-parasitic treatment used is Substance EX which is a chitin-inhibitor, and will unlikely have effect on parasites which do not change chitin-shell in their life-cycle.

In the bias analysis applied in this study, different scenarios of lowered efficacy were tested. In general the results suggested that lowered efficacy will result in an underestimation of the risk ratio and the underestimation will be stronger when CGR is low. Although the bias analysis did not alter our general conclusions, the risk ratio seems to be underestimated when

trying to assess the effect of salmon lice on migrating salmon smolt. This underestimation was important when treatment efficacy was assumed to be only 50%, but was close to negligible when treatment efficacy was assumed to be 90%.

4.6 Selection bias – differential recapture rates

Differential recapture between the two groups may also be a source of bias. For example, efforts to recapture fish can for example be higher the first years after releases or methods may target certain size and age classes. Results from recent publications from Vollset et al. (2014) suggest that treatment may affect the age at maturation of salmon. In the present study there was a significant average size difference of 123 grams between treated and untreated 1SW salmon. This in line with findings from Skilbrei et al. (2013) performed on a subset of the data in this analysis. Given these results, a differential targeting of size classes, for example by using too large mesh size to capture small individuals, would bias results. However, the bias analysis suggested that even very strong selection bias would not alter the results to a substantial degree.



Illustration photo: Adult female salmon lice, *Lepeoptheirus salmonis*, on an adult salmon.

4.7 Extrapolation of results to wild smolt runs

Looking past the potential issues relating to biases in the method it is still not straightforward to extrapolate the results from release groups of cultivated smolt to wild smolt

migrating from rivers. Studies using release groups of cultivated smolt usually attempt to mimic the migration time of wild fish from a river, but in most cases the time of release is mostly controlled by the growth and physiological state of the fish in the hatchery rather than when the optimal time to release them would be. In some studies multiple releases are done across the season to be able to study the seasonal effect. Skilbrei and Wennevik (2006) demonstrated that the effect size in release groups was much higher in groups release later in the season. However, salmon smolt are also known to desmoltify (Stefansson et al. 1998) and holding back fish may lead to suboptimal smolt quality which may lead to overestimation of the effect of salmon lice (see below). Moreover, cultivated smolt may in addition behave differentially than wild fish. Jonsson et al. (1991) concluded that the survival and the ability to cope with different environmental challenges are much lower for cultivated fish compared to wild fish. Consequently one source of the large variation in CGR may be attributed to variation in quality of the cultivated smolt and its ability of the smolt to cope with environmental challenges.

4.8 Geographic limitation

The results are also limited due to that most of the data and the weight of the analysis come from limited region just north of Bergen (Vosso, Dale & Matre). The results also weighted heavily on release groups that have been released in the outer region of the fjord, because these have higher survival (and will therefore have higher weights in the meta-analysis). The high survival in these groups can partially be explained by that they avoid predation during transition through estuaries (Thorstad et al. 2012). Consequently, if exposure is predominantly during fjord migration the weight of the dataset is on release groups with relatively low exposure.

4.9 Comparing results to other meta-analysis

Recent publications, such as Jackson et al. (2013a) and Krkošek et al. (2013) have reported results from meta-analyses based on mostly Irish studies (but also some Norwegian studies included in this metaanalysis). The conclusions in these two meta-analyses are quite different, even though the effect size calculated from the data seems fairly similar depending how the data are presented (odds ratio 1.14, Jackson et al. (2013a) and 1.39, Krkošek et al. (2013)), and have also spurred a debate about how to interpret results from such studies (comment by Krkošek et al. (2014), reply by Jackson et al. (2014)). The large heterogeneity observed in our

study and the potential differences in sources of heterogeneity between this study and earlier studies make a direct comparison of impact estimates complicated. The disagreement between the earlier studies is partly due to a debate on whether one should emphasize on the risk difference or the attributable fraction when reporting the impact of treatment in such studies, and this debate is therefore relevant for the interpretation of our results (see below for a discussion).

4.10 Evaluating the impact of sea lice

Even though reporting averages in meta-analysis with strong heterogeneity is problematic, we have chosen to report estimated overall effect of treatment calculated as risk differences (RD) and attributable fractions (AF). This is done because it highlights some of the issues related to calculating such estimates based on the data in our study. The first, risk difference (RD), has been reported in some RCT studies to describe the effect of salmon lice on overall marine survival (Jackson et al. 2011a, 2011b and 2013b). There is a problem of misinterpretation with RD that it does not actually equal the difference in survival between control and treatment groups. This has been detailed in Krkošek et al. (2014), but to reiterate, by example, a RD of 0.001 would equate to a difference in survival between control and treatment groups of 1 in 1000. However, if the RD comes from species with overall low survival so that the recapture in control groups is 1/1000 and in treatment groups is 2/1000, this reflects a relatively large risk ratio (RR=2). Furthermore, if nothing changed except that the recapture effort was doubled, we would expect to catch 4 and 2 fish in the treated and control groups - producing a RD of 0.002, while the risk ratio would remain the same (RR= 2, 4/1000 divided by 2/1000). This limits the usefulness of the RD as a measure of treatment effect.

The second measure of effect computed was the AF, which was computed individually from each release groups and then averaged to produce an overall estimate of 11.3% (CI: 4.5 – 18.0 %) However, given that there is an indication that the effect of treatment is not 100% effective in all trials and there is evidence from lice on salmon farms that lice are tolerant or resistant to the treatment, then the estimated AF may be regarded as an underestimate of the potential effect of a 100% effective treatment. Furthermore, as mentioned before, an average value is in our study an incomplete representation of how the treatment effects the survival of cultivated fish due to the large heterogeneity in treatment effect among studies. For example,

estimating the AF for the four different time periods the effect would range from ~0 to 23 %, while it would vary even more if CGR was factored in.

Another caveat of calculating average AF also needs mentioning. An AF represents the impact of the treatment in the treated group, because the effects of all other factors that affect survival of smolts are held constant (by comparing the treated group to an identical control group). However, under natural conditions, factors that affect smolt survival are highly variable. In these conditions, evaluating the effect of one factor in isolation (and computing an AF for that factor) is very likely to overstate its importance. If multiple factors are evaluated in isolation, it is almost certain that the sum of the % mortality that they explain collectively will exceed 100%. As a result, an estimate of a single factor in isolation should be considered as an upper limit of its potential effect. See Appendix IV for further explanation of this phenomenon.

Extrapolation of the estimates mentioned above to a measure of impact of lice in *wild smolt populations* is also problematic. Some of these have been discussed above (e.g. extrapolation of results from cultivated smolt to wild fish). Another relates to calculating an average when the size of wild populations is not known. If a large study was done in a river or region in which the wild populations were small, the study would be given more weight in the analyses than it should have if we wanted to evaluate an overall population impact across regions and rivers. The converse is true if a small study was done in a river or region with large population. As a consequence, the estimate may be a strong over- or under- estimate of the effects of lice on wild smolt across regions.

5. Conclusions

1. Treating released cultivated smolt with an anti-parasitic agent significantly increase recapture-rates of adult salmon. Assuming that the effect of treatment is due to a protection against salmon lice, the results from this study gives unequivocal evidence to the hypothesis that salmon lice is a component cause to the mortality of salmon. This is especially evident in years and release groups where recapture is low in the control group.
2. The effect size (risk ratio - RR) is highly variable, varies between years and increases with decreasing recapture in the control group (CGR). An average value of percent fish “saved” by treatment holds little value related to the potential effect of treatment (due to

large heterogeneity). The estimated RR ranged from approximately 1.0 (i.e. No effect at all) to 1.7, depending on the CGR.

3. Recapture in the control group (CGR) is a function of how long the fish have to migrate to reach the ocean. This means that if a fish is released 50 km from the river outlet the recapture will increase 7-fold (relative to its expected recapture if it was released at the river outlet). In addition the CGR varies between river locations.
4. Lice burden estimates based on density kernel distribution of salmon lice from fish farms could not predict the variation in treatment effect (RR). There was a tendency that lice burden could explain some of the variation in CGR. However, lice burden is a function of migration distance and is a poorer predictor of CGR than distance migrated.
5. There are still many unanswered questions related to the use of the RCTs to study the effect of salmon lice on the survival of smolt. Several issues related to potential biases needs to be resolved.
6. The studies conducted in Norway are mostly concentrated in one area with a strong weight on data from release groups that had a relatively short migration distance through potentially critical areas overlapping with fish farms.

Future recommendations. It is highly recommended that studies on locations with longer migration routes in addition to studies in areas without fish farms be conducted in the future. Furthermore, the finding of a strong relationship between CGR and effect of treatment against salmon lice is novel and warrants further attention. Scale reading from the historic dataset from these trials may give important insight into to marine growth conditions. In addition, studies using individual tagging methods such as PIT can be used to test potential interactions between smolt quality and effect sizes of treatment.

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Appendix I**Table A1.** Description of information extracted from the trials considered in the review of anti-parasitic treatment of smolt at release on degree of adult salmon recapture

Variable	Definition and description of relevant categories
Trial-specific variables at smolt release	
Study #	Unique study identification
Pub year	Publication year, 9999 if unpublished
Author	Name of first author or responsible researcher
Sponsor	Sponsor of the study: power company, fish farmers, Directorate of
Pub type	Type of publication: peer reviewed, report, unpublished data
Full data in report	No if contact with author/responsible scientist was necessary
Release fjord	Name of fjord
Release category	Release site category: river, estuary, fjord
Hatchery production	Smolt production: produced in tanks, produced in net-pen in lake
Release river	River of smolt-release
Release place	Geographical place of release
Latitude	Latitude at the river outlet
Longitude	Longitude at the river outlet
Municipality	Municipality of release
County	County of release
Release year	Year of smolt release
Release date	Date of smolt release
Origin	River specific salmon strain
General origin	Origin of smolt: cultivated from wild broodstock, cultivated from gene
Age smolt	Age of smolt at release: 1 year smolt, 2 year smolt
Treatment	Treatment categories: emamectin benzoate feed, emamectin benzoate
Treatment dose	Recorded if available otherwise missing
Tag	Type of tag on smolt: code wire tag (CWT), Carlin, other
N control	Number of released control (untreated) smolt
N treated	Number of released treated smolt
Release length	Mean length of smolt at time of release, in cm
Release length sd	Standard deviation of mean length at release
Release weight	Mean weight of smolt at time of release, in grams
Release weight sd	Standard deviation of mean weight at release
Migratory route related variables	
Migration river	Distance from release site to river outlet
Migratory distance	Distance from river outlet to 12 mile border
Migratory temp	Temperature from river outlet to 12 mile border
Lice abundance	Estimated exposure to farm sea lice at 50 and 200 km search radius,
Feeding area	Feeding area in the sea: south, north

Recapture related variables

Recapture method	Main method of recapture: organized recaptures by trap nets, organized
N recaptured control	Number of recaptured control (untreated) fish
N recaptured treated	Number of recaptured treated fish
N 1SW control	Number of 1-SW recaptured control (untreated) fish
N 1SW treated	Number of 1-SW recaptured treated fish
N 2SW control	Number of 2-SW recaptured control (untreated) fish
N 2SW treated	Number of 2-SW recaptured treated fish
N 3SW control	Number of 3-SW recaptured control (untreated) fish
N 3SW treated	Number of 3-SW recaptured treated fish
Recapture length control 1SW	Mean length of recaptured control (untreated) 1SW fish in cm
Recapture length treated 1SW	Mean length of recaptured treated 1SW fish in cm
Recapture weight control 1SW	Mean weight of recaptured control (untreated) 1SW fish in grams
Recapture weight treated 1SW	Mean weight of recaptured treated 1SW fish in grams
Recapture length control 2SW	Mean length of recaptured control (untreated) 2SW fish in cm
Recapture length treated 2SW	Mean length of recaptured treated 2SW fish in cm
Recapture weight control 2SW	Mean weight of recaptured control (untreated) 2SW fish in grams
Recapture weight treated 2SW	Mean weight of recaptured treated 2SW fish in grams
Recapture length control 3SW	Mean length of recaptured control (untreated) 3SW fish in cm
Recapture length treated 3SW	Mean length of recaptured treated 3SW fish in cm
Recapture weight control 3SW	Mean weight of recaptured control (untreated) 3SW fish in grams
Recapture weight treated 3SW	Mean weight of recaptured treated 3SW fish in grams

Appendix II Output from STATA – Random effects logistic models for estimating the effect of baseline survival (CGR) on treatment effects.

1. Model with all covariates (-tx-, -fjord-, and -river-)

```
. gllamm r tx txfjord txry1 txry2 txry3, fam(bin) denom(n) i(id) adapt
> eqs(int slope) nrf(2)
```

Running adaptive quadrature

...

number of level 1 units = 202

number of level 2 units = 101

Condition Number = 6.4876843

gllamm model

log likelihood = -697.63931

r	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
tx	.4247604	.1134834	3.74	0.000	.2023371	.6471838
txfjord	.2267414	.10758	2.11	0.035	.0158885	.4375943
txry1	-.5711461	.1672888	-3.41	0.001	-.8990261	-.2432662
txry2	-.2269951	.1520386	-1.49	0.135	-.5249852	.070995
txry3	-.1825213	.1195694	-1.53	0.127	-.4168729	.0518303
_cons	-5.741532	.152242	-37.71	0.000	-6.039921	-5.443144

Variances and covariances of random effects

***level 2 (id)

var(1): 2.0371634 (.34901527)

cov(2,1): -.29964089 (.13209208) cor(2,1): -.60703448

var(2): .11960506 (.04520978)

Coefficient for CGR derived from above model is:

Beta: -.14708731

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2. Model with only -tx-

(to get estimate of effect of treatment averaged over values of -fjord- and -river-)

```
. gllamm r tx , fam(bin) denom(n) i(id) adapt eqs(int slope) nrf(2)
```

```
Running adaptive quadrature
```

```
...
```

```
number of level 1 units = 202
```

```
number of level 2 units = 101
```

```
Condition Number = 3.751165
```

```
gllamm model
```

```
log likelihood = -705.82549
```

```
-----
```

r	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
tx	.2514987	.0744299	3.38	0.001	.1056188	.3973787
_cons	-5.752134	.1504659	-38.23	0.000	-6.047042	-5.457226

```
-----
```

```
Variances and covariances of random effects
```

```
***level 2 (id)
```

```
var(1): 1.986172 (.33895296)
```

```
cov(2,1): -.20795473 (.12272031) cor(2,1): -.39998044
```

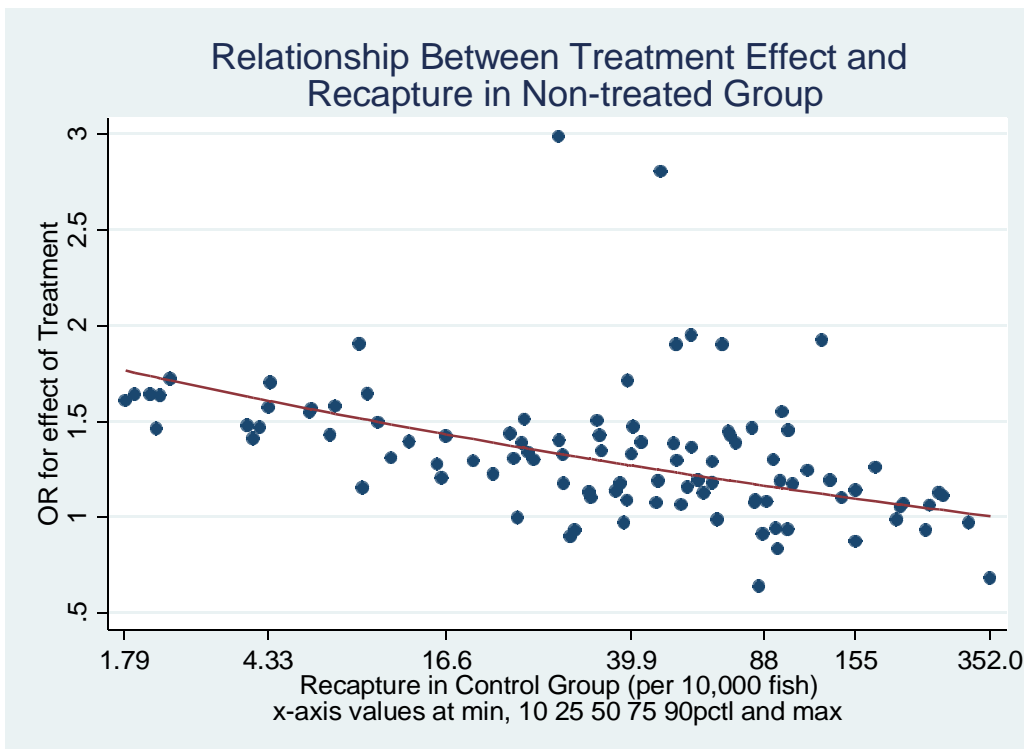
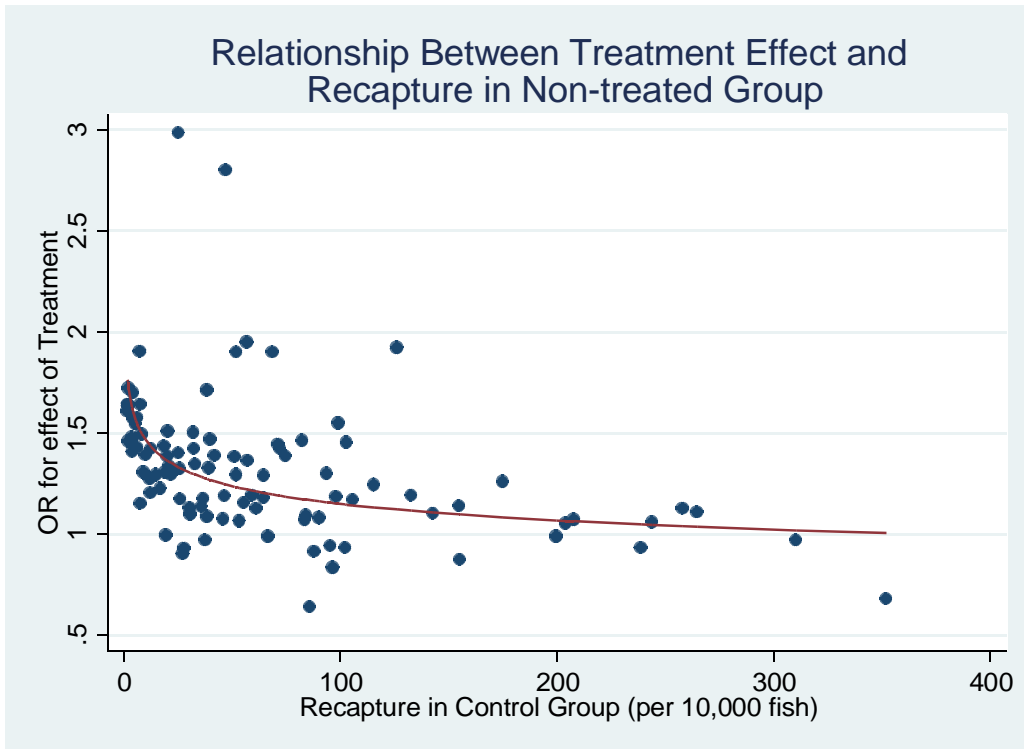
```
var(2): .13609533 (.04527396)
```

```
-----
```

```
Coefficient for CGR derived from above model is:
```

```
Beta: -.10470127
```

Appendix III Figures including outliers excluded in main text



Appendix IV

Attributable fraction and population attributable fraction

Given that RR and OR say nothing about the effect of a risk factor on the amount of disease (survival) in a population, the attributable fraction AF and the population attributable fraction (AF_p) are of interest. The AF expresses the proportion of survival in the treated group that can be attributed to the treatment. The AF_p expresses the proportion of survival in the whole population that is due to the exposure (treatment). It is based on the AF and the prevalence of the exposure (sea lice). Since we are working with the average AF , it seems that the AF would be a good estimate of the AF_p (assuming all fish are exposed to the average level of sea lice).

However, when multiple factors affect survival, and only one of them is studied, an estimate of AF_p will virtually always over-estimate the effect of the factor, as explained in the following section.

Estimating attributable fractions for outcomes with multiple contributing factors

When multiple factors contribute to an outcome (such as mortality), the factors are known as “**component causes**”. When a combination of component causes comes together in an individual in a manner that causes the outcome (e.g. mortality), the specific combination of component causes is referred to as a “**sufficient cause**”. For example, a moderate sea lice burden, when combined with a level of physiological stress associated with smoltification which exceeds some threshold may be sufficient to kill the smolt. This combination would be referred to as a sufficient cause. Similarly, insufficient prey species combined with non-optimal water temperature may also be sufficient to kill smolt and would be another sufficient cause. In reality, we never know what all of the component or sufficient causes are, however, a simple representation of 4 component causes (sea lice and 3 unidentified component causes) combining to make up 4 sufficient causes (I - IV) is presented in Table 3. Sufficient cause I accounts for 35% of mortalities, II for 25% etc.

If sea lice are the only factor studied, it would appear that 60% of all mortality could be prevented by eliminating sea lice because sufficient causes I and II would be eliminated. However, if x1 was studied, it also accounts for 60% of mortality, etc. With only 4 sufficient causes listed (in reality there are probably many more) we can “account for” 210% of all mortalities.

Table 3.

Component Causes	Sufficient Causes				AFp
	I	II	III	IV	
sea lice	+	+			60%
x1	+		+		60%
x2		+	+	+	75%
x3				+	15%
prevalence of sufficient cause among all mortalities	35%	25%	25%	15%	210%

In order for an estimate of AF_p to truly reflect the impact of a single factor (e.g. sea lice) 2 conditions must be met:

- the prevalence and impact of all of the other factors must be constant (over time and space), and
- the intervention used to bring about the elimination of sea lice must have no impact on any of the other factors.

These conditions would be met if: 1) lice exposure was constant (equal to the average exposure), 2) all other factors affecting smolt survival remained constant, and 3) it was possible to treat all wild smolt without any adverse consequences. Given the very wide range of survivals observed in treated fish (presumably protected from sea lice) in the studies reviewed, the first two assumptions seem extremely unlikely to be met. Unless the intervention to be used to eliminate sea lice is specified (it is not possible to treat wild smolt), it is impossible to speculate as to the validity of the 3rd assumption.

Consequently, although it would be desirable to answer the question “what proportion of fish are killed by sea lice?” it is impossible to provide such an estimate. A recent review of the issue of estimating attributable fractions has been published (Levine 2008). Although the article describes the issue in terms of estimating the impact of obesity on various health conditions, the principles are the same.

References

Levine, B.J. 2008. The other causality question: estimating attributable fractions for obesity as a cause of mortality. *Int J Obesity* **32**: S4-S7.

Appendix V. Description of the included releases with geographical location, treatment type, number of released fish in treated and control group, recapture method and summary recapture numbers of control and treated fish.

Release ID	Reference	River	Place	County	Year	Date	Treatment	N control	N treated	Recapture method ^a	Recaptured control	Recaptured treated
1	Barlaup et al. 2013	Vosso	Vosso_river	Hordaland	2000	15.5.00	Emamectin benzoate feed	4370	4370	1	0	0
2	Barlaup et al. 2013	Vosso	Bolstad_river	Hordaland	2000	15.5.00	Emamectin benzoate feed	4370	4370	1	0	1
3	Barlaup et al. 2013	Vosso	Vosso_river	Hordaland	2001	9.5.01	Emamectin benzoate feed	2840	2775	1	0	0
4	Barlaup et al. 2013	Vosso	Bolstad_river	Hordaland	2001	9.5.01	Emamectin benzoate feed	2820	3060	1	0	0
5	Barlaup et al. 2013	Vosso	Fedje	Hordaland	2001	9.5.01	Emamectin benzoate feed	2740	2840	1	3	5
6	Barlaup et al. 2013	Vosso	Vosso_river	Hordaland	2002	14.5.02	Emamectin benzoate feed	4700	4700	1	0	0
7	Barlaup et al. 2013	Vosso	Bolstad_river	Hordaland	2002	14.5.02	Emamectin benzoate feed	4700	4700	1	0	1
8	Barlaup et al. 2013	Vosso	Fedje	Hordaland	2002	13.5.02	Emamectin benzoate feed	4700	4700	1	12	16
9	Barlaup et al. 2013	Vosso	Vosso_river	Hordaland	2003	13.5.03	Emamectin benzoate feed	3625	4035	1	0	0
10	Barlaup et al. 2013	Vosso	Bolstad_river	Hordaland	2003	14.5.03	Emamectin benzoate feed	3900	3890	1	0	0
11	Barlaup et al. 2013	Vosso	Manger	Hordaland	2003	13.5.03	Emamectin benzoate feed	4100	4025	1	13	59
12	Barlaup et al. 2013	Vosso	Sørfjorden	Hordaland	2005	8.5.05	Substance EX bath	3603	3500	1	1	1
13	Barlaup et al. 2013	Vosso	Osterfjorden	Hordaland	2005	10.5.05	Substance EX bath	3500	3500	1	1	3
14	Barlaup et al. 2013	Vosso	Manger	Hordaland	2005	11.5.05	Substance EX bath	3500	4334	1	20	27
15	Barlaup et al. 2013	Vosso	Bolstad_river	Hordaland	2006	10.5.06	Emamectin benzoate feed	3250	3250	1	0	0
16	Barlaup et al. 2013	Vosso	Sørfjorden	Hordaland	2006	10.5.06	Emamectin benzoate feed	3250	3250	1	1	1
17	Barlaup et al. 2013	Vosso	Osterfjorden	Hordaland	2006	11.5.06	Emamectin benzoate feed	3250	3250	1	0	0
18	Barlaup et al. 2013	Vosso	Manger	Hordaland	2006	12.5.06	Emamectin benzoate feed	3250	3250	1	2	4
19	Barlaup et al. 2013	Vosso	Sørfjorden	Hordaland	2007	14.5.07	Emamectin benzoate feed	4800	5000	1	5	2
20	Barlaup et al. 2013	Vosso	Osterfjorden	Hordaland	2007	15.5.07	Emamectin benzoate feed	4800	5000	1	1	9
21	Barlaup et al. 2013	Vosso	Manger	Hordaland	2007	18.5.07	Emamectin benzoate	4800	5130	1	41	46

Release ID	Reference	River	Place	County	Year	Date	Treatment	N control	N treated	Recapture method ^a	Recaptured control	Recaptured treated
22	Barlaup et al. 2013	Vosso	Arna	Hordaland	2008	19.5.08	Emamectin benzoate feed	4347	4604	1	26	32
23	Barlaup et al. 2013	Vosso	Ramsøy	Hordaland	2008	22.5.08	Emamectin benzoate feed	5305	5306	1	37	33
24	Barlaup et al. 2013	Vosso	Manger	Hordaland	2008	24.5.08	Emamectin benzoate feed	5296	5287	1	18	37
25	Barlaup et al. 2013	Vosso	Arna	Hordaland	2009	5.6.09	Emamectin benzoate feed	4050	4200	1	142	95
26	Barlaup et al. 2013	Vosso	Ramsøy	Hordaland	2009	5.6.09	Emamectin benzoate feed	4400	4400	1	114	126
27	Barlaup et al. 2013	Vosso	Manger	Hordaland	2009	3.6.09	Emamectin benzoate feed	4400	4400	1	134	128
28	Barlaup et al. 2013	Vosso	Arna	Hordaland	2010	2.6.10	Emamectin benzoate feed	3500	3500	1	37	43
29	Barlaup et al. 2013	Vosso	Manger	Hordaland	2010	2.6.10	Emamectin benzoate feed	3500	3500	1	46	55
30	Barlaup et al. 2013	Vosso	Bolstad_river	Hordaland	2010	13.5.10	Emamectin benzoate feed	3500	3500	1	0	3
31	Barlaup et al. 2013	Vosso	Arna	Hordaland	2010	19.5.10	Emamectin benzoate feed	3500	3500	1	24	37
32	Barlaup et al. 2013	Vosso	Manger	Hordaland	2010	19.5.10	Emamectin benzoate feed	3500	3500	1	70	67
33	Barlaup et al. 2013	Vosso	Arna	Hordaland	2011	28.5.11	Emamectin benzoate feed	6100	6100	1	39	51
34	Barlaup et al. 2013	Vosso	Manger	Hordaland	2011	28.5.11	Emamectin benzoate feed	6100	6100	1	34	48
35	Barlaup et al. 2013	Vosso	Evanger_lake	Hordaland	2011	13.5.11	Emamectin benzoate feed	10000	10000	1	1	2
36	Barlaup et al. 2013	Vosso	Arna	Hordaland	2011	17.5.11	Emamectin benzoate feed	5000	5000	1	79	65
37	Barlaup et al. 2013	Vosso	Manger	Hordaland	2011	17.5.11	Emamectin benzoate feed	5000	5000	1	46	61
38	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	1997	5.5.97	Substance EX bath	2978	2975	1,2	1	29
39	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	1997	6.5.97	Substance EX bath	2983	2985	1,2	46	52
40	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	1999	20.5.99	Substance EX bath	2959	2940	1,2	29	34
41	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2001	23.5.01	Emamectin benzoate feed	2294	6302	1,2	25	47
42	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2002	11.5.02	Emamectin benzoate feed	1698	1836	1,2	16	29
43	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2002	25.5.02	Emamectin benzoate	1761	1771	1,2	20	15

Release ID	Reference	River	Place	County	Year	Date	Treatment	N control	N treated	Recapture method ^a	Recaptured control	Recaptured treated
44	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2002	7.6.02	Emamectin benzoate feed	1650	1755	1,2	17	46
45	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2003	4.5.03	Emamectin benzoate feed	2039	2019	1,2	7	13
46	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2003	18.5.03	Emamectin benzoate feed	2082	2023	1,2	10	5
47	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2003	2.6.03	Emamectin benzoate feed	1479	1575	1,2	4	8
48	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	7.5.04	Emamectin benzoate feed	1857	1858	1,2	7	10
49	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	21.5.04	Emamectin benzoate feed	1859	1866	1,2	21	5
50	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	4.6.04	Emamectin benzoate feed	1750	1777	1,2	13	23
51	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2005	7.5.05	Substance EX bath	1750	1750	1,2	0	0
52	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2005	20.5.05	Substance EX bath	1750	1750	1,2	0	0
53	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2005	4.6.05	Substance EX bath	1742	1740	1,2	3	1
54	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2006	7.5.06	Emamectin benzoate feed	1945	1941	1,2	6	1
55	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2006	21.5.06	Emamectin benzoate feed	1940	1940	1,2	8	2
56	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2006	4.6.06	Emamectin benzoate feed	2271	2262	1,2	9	9
57	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2007	9.5.07	Emamectin benzoate injection	2500	2500	1,2	0	0
58	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2007	23.5.07	Emamectin benzoate injection	2500	2500	1,2	1	0
59	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2007	6.6.07	Emamectin benzoate injection	2500	2500	1,2	0	0
60	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2008	7.5.08	Emamectin benzoate injection	1475	1475	1,2	0	1
61	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2008	21.5.08	Emamectin benzoate injection	1500	1500	1,2	3	4
62	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2008	3.6.08	Emamectin benzoate injection	1275	1270	1,2	0	0
63	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2009	3.6.09	Emamectin benzoate injection	2020	2020	1,2	9	7
64	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2009	24.6.09	Emamectin benzoate injection	1320	1290	1,2	6	10
65	Skilbrei et al. 2012	Dale	Manger	Hordaland	2007	18.6.07	Emamectin benzoate	2200	2110	1,2	9	27

Release ID	Reference	River	Place	County	Year	Date	Treatment	N control	N treated	Recapture method ^a	Recaptured control	Recaptured treated
66	Skilbrei et al. 2012	Dale	Arna	Hordaland	2008	20.5.08	Emamectin benzoate injection	1500	1500	1,2	17	22
67	Skilbrei et al. 2012	Dale	Manger	Hordaland	2008	26.5.08	Emamectin benzoate injection	2000	2000	1,2	21	16
68	Skilbrei et al. 2012	Dale	Stanghelle	Hordaland	2009	13.5.09	Emamectin benzoate injection	2020	2020	1,2	20	14
69	Skilbrei et al. 2012	Dale	Stanghelle	Hordaland	2009	27.5.09	Emamectin benzoate injection	2020	2020	1,2	49	43
70	Skilbrei et al. 2012	Dale	Kvisti	Hordaland	2009	13.5.09	Emamectin benzoate injection	2020	2020	1,2	49	51
71	Skilbrei et al. 2012	Dale	Kvisti	Hordaland	2009	27.5.09	Emamectin benzoate injection	2020	2020	1,2	18	33
72	Skilbrei et al. 2012	Dale	Vikane	Hordaland	2009	30.5.09	Emamectin benzoate injection	2020	2020	1,2	42	44
73	Skilbrei unpublished	Dale	Vikane	Hordaland	2010	29.5.10	Emamectin benzoate injection	2805	2810	1,2	71	80
74	Skilbrei unpublished	Dale	Kvisti	Hordaland	2010	22.5.10	Emamectin benzoate injection	2805	2805	1,2	11	17
75	Skilbrei unpublished	Dale	Kvisti	Hordaland	2010	13.6.10	Emamectin benzoate injection	2555	2500	1,2	10	28
76	Skilbrei et al. 2012	Dale	Dale river	Hordaland	2004	May	Substance EX bath	66	64	1,2	1	0
77	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	June	Substance EX bath	879	871	1,2	2	2
78	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	May	Substance EX bath	151	150	1,2	0	0
79	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2005	June	Substance EX bath	555	518	1,2	0	0
80	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2005	May	Substance EX bath	443	392	1,2	7	6
81	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2005	June	Substance EX bath	107	178	1,2	0	3
82	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	May	Substance EX bath	398	405	1,2	1	2
83	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	May	Substance EX bath	476	480	1,2	1	0
84	Skilbrei et al. 2012	Dale	Dale_river	Hordaland	2004	June	Substance EX bath	316	312	1,2	0	1
85	Skilbrei unpublished	Matre	Matre_river	Hordaland	2005	27.5.05	Emamectin benzoate feed	968	968	3	4	2
86	Skilbrei unpublished	Matre	Matre_river	Hordaland	2005	10.6.05	Emamectin benzoate feed	1001	1001	3	10	9
87	Skilbrei unpublished	Matre	Matre_river	Hordaland	2006	29.5.06	Emamectin benzoate	2520	2520	3	9	7

Release ID	Reference	River	Place	County	Year	Date	Treatment	N control	N treated	Recapture method ^a	Recaptured control	Recaptured treated
88	Skilbrei unpublished	Matre	Matre_river	Hordaland	2007	1.6.07	Emamectin benzoate injection feed	2000	2000	3	3	7
89	Skilbrei unpublished	Matre	Matre_river	Hordaland	2007	5.6.07	Emamectin benzoate injection	2000	2000	3	4	3
90	Skilbrei unpublished	Matre	Matre_river	Hordaland	2007	3.7.07	Emamectin benzoate injection	1818	1818	3	0	3
91	Skilbrei unpublished	Matre	Fensfjord	Hordaland	2007	5.6.07	Emamectin benzoate injection	2000	2000	3	1	1
92	Skilbrei unpublished	Matre	Matre_river	Hordaland	2008	16.5.08	Emamectin benzoate injection	1850	1850	3	2	1
93	Skilbrei unpublished	Matre	Matre_river	Hordaland	2008	26.6.08	Emamectin benzoate injection	1000	1000	3	2	6
94	Skilbrei unpublished	Matre	Matre_river	Hordaland	2008	14.8.08	Emamectin benzoate injection	1000	1000	3	2	2
95	Skilbrei unpublished	Matre	Matre_river	Hordaland	2009	15.5.09	Emamectin benzoate injection	1999	1998	3	6	5
96	Skilbrei unpublished	Matre	Matre_river	Hordaland	2009	18.6.09	Emamectin benzoate injection	2000	1999	3	0	1
97	Skilbrei unpublished	Matre	Matre_river	Hordaland	2010	21.5.10	Emamectin benzoate injection	1998	1993	3	13	13
98	Skilbrei unpublished	Matre	Matre_river	Hordaland	2010	29.6.10	Emamectin benzoate injection	1900	1900	3	10	8
99	Skilbrei unpublished	Matre	Matre_river	Hordaland	2011	21.6.11	Emamectin benzoate injection	2000	2000	3	13	22
100	Skilbrei unpublished	Matre	Matre_river	Hordaland	2011	2.8.11	Emamectin benzoate injection	1911	1998	3	17	17
101	Hvidsten et al. 2007	Surna	Agdenes	Sør-Trøndelag	1996	21.5.96	Substance EX bath	2985	3000	1,2,3	51	66
102	Hvidsten et al. 2007	Orkla	Agdenes	Sør-Trøndelag	1997	22.5.97	Substance EX bath	2936	2935	1,2,3	15	20
103	Hvidsten et al. 2007	Orkla	Agdenes	Sør-Trøndelag	1998	20.5.98	Substance EX bath	2977	2966	1,2,3	17	42
104	Finstad_Unpublished	Suldalslågen	Suldal estuary	Rogaland	1997	12.5.97	Emamectin benzoate feed	5999	5000	1,2,3	0	2
105	Finstad_Unpublished	Suldalslågen	Suldal estuary	Rogaland	1998	11.5.98	Substance EX bath	4998	5498	1,2,3	0	1
106	Finstad_Unpublished	Suldalslågen	Suldal estuary	Rogaland	1999	6.5.99	Substance EX bath	4998	4999	1,2,3	1	0
107	Jensen et al 2013	Eira	Eira River	Møre og Romsdal	2008	15.5.08	Emamectin benzoate feed	2916	2999	1,2,3	14	16
118	Jensen et al 2013	Eira	Eira River	Møre og Romsdal	2009	15.5.09	Emamectin benzoate feed	2999	2999	1,2,3	11	4
109	Jensen et al 2013	Eira	Eira River	Møre og	2010	15.5.10	Emamectin benzoate	3200	2800	1,2,3	1	3

Release ID	Reference	River	Place	County	Year	Date	Treatment	N control	N treated	Recapture method^a	Recaptured control	Recaptured treated
110	Jensen et al 2013	Eira	Eira River	Romsdal	2011	15.5.11	Emamectin benzoate feed	2997	2998	1,2,3	7	11
111	Lehmann_Unpublished	Årdal	Årdal river	Møre og Romsdal	2010	28.5.10	Emamectin benzoate feed	3197	3189	1,2,3	4	3
112	Lehmann_Unpublished	Årdal	Ertensøy	Rogaland	2010	30.5.10	Emamectin benzoate feed	3188	3196	1,2,3	9	6
113	Hazon et al 2006 + Finstad_Unpublished	Imsa	River Imsa	Rogaland	2003	15.5.03	Substance EX bath	3000	2000	1,2,3,4	61	42
114	Hazon et al 2006 + Finstad_Unpublished	Imsa	River Imsa	Rogaland	2004	15.5.04	Substance EX bath	3000	2000	1,2,3,4	4	2
115	Hazon et al 2006 + Finstad_Unpublished	Halselv	River Halselva	Finnmark	2003	25.6.03	Substance EX bath	2199	1987	1,2,3,4	13	10
116	Hazon et al 2006 + Finstad_Unpublished	Halselv	River Halselva	Finnmark	2004	1.6.04	Substance EX bath	1972	1983	1,2,3,4	8	7
117	Hazon et al 2006 + Finstad_Unpublished	Halselv	River Halselva	Finnmark	2008	26.6.08	Emamectin benzoate feed	1985	1988	1,2,3,4	0	0
118	Strand og Finstad	Halselv	River Halselva	Finnmark	2007	26.6.07	Emamectin benzoate feed	3365	4426	1,2,3,4	0	0