The Faculty of Biosciences, Fisheries and Economics

# Salmon lice (*Lepeophtheirus salmonis*) control method effectiveness in Atlantic salmon (*Salmo salar*) aquaculture

*A systematic literature review*

—

**Kristine Cerbule**

*Master's thesis in International Fisheries Management 15 May 2018 (30 ECTS)*

UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Photo: Kristine Cerbule

# Contents

# Abstract

**Background**: Salmon lice (*Lepeophtheirus salmonis*) is an increasing limiting factor of Atlantic salmon (*Salmo salar*) aquaculture development in Northern Hemisphere. Different types of treatments have been tested and used to control lice on farmed Atlantic salmon with varying results. The aim of this systematic review is to examine effectiveness expressed as the reduction of the number of lice and associated negative effects to fish health and welfare (Atlantic salmon and cleaner fish, if used) for three types of methods – chemical treatment, cleaner fish use and warm water treatment.

**Methods**: a systematic literature review was used to gather and analyse data related to each type of method reported in peer-reviewed documents.

**Results**: After applying inclusion criteria, 62 of 782 documents of two scientific databases combined were further analysed. Most of the documents described chemical treatment which showed decreasing effectiveness combined with increasing concentrations due to the significant development of resistance. Documents describing the use of cleaner fish showed effectiveness towards salmon lice in all studies with little or no negative associated effects, and did not show a decreased effectiveness over time. The lack of data related to warm water treatment did not allow to assess the effectiveness of this method.

**Conclusions**: Due to the development of resistance in lice selected by chemical treatments, those methods cannot be considered sustainable practices in aquaculture. Cleaner fish use is preferred if fish health and welfare criteria are met. A lack of data related to warm water treatment was noted, which is a research gap.

**Keywords**: Atlantic salmon, salmon lice, chemical treatment, warm water treatment, cleaner fish

# 1. Introduction

## 1.1. Background

Over the past decades, aquaculture has expanded remarkably and continues to be a growing industry providing food resources to the world. One of the largest cultured species in marine aquaculture is Atlantic salmon (*Salmo salar*) which is also the most produced species of salmonids – 66% in 2015 (Food and Agriculture Organisation of the United Nations, 2017). It is estimated that current production (as for year 2016) is 2 million tons (Marine Harvest, 2017). The largest Atlantic salmon culturing countries are Norway, Chile, Scotland and Canada (Marine Harvest, 2017). Norway is the leading producer of Atlantic salmon (54% of the 2016 harvest), leaving Chile as second largest producer (23% of the 2016 harvest) (Salmones Camanchaca, 2017). Annual income from the industry differs between countries. For example, in 2016 in Norway, income was 64 039 million Norwegian krone (NOK) (Statistik sentralbyrå, 2017), and in Scotland – 765 239 900 pounds (Kenyon & Davies, 2018) (equal to 8 356,04 million NOK).

Considering the growth of the industry and the increasing stocking densities, several disease outbreaks have taken place, causing serious economic losses as well as negatively affecting public acceptance of fish farming. There are several examples of such cases: Infectious Salmon Anaemia (ISA) outbreak in Chile and currently growing problem dealing with parasites during the rearing process. Salmonids in the Northern Hemisphere are affected by two lice species – *Lepeophtheirus salmonis* and *Caligus elongatus*. The topic of this thesis will focus on the main problem within aquaculture which is related to *L. salmonis*. The parasite is affecting Atlantic salmon aquaculture in most of the largest Atlantic salmon producing countries, with exception of Chile. There, the other lice species (*C. rogercresseyi*) is common which is not covered within this thesis, because this is not a problem in the Northern Hemisphere.

Salmon lice are described by numerous authors as a major threat to the Atlantic salmon aquaculture[1]. It reduces the income of aquaculture by 5 000 million NOK annually in Norway alone (Hjeltnes, Bornø, Jonson, Haukaas & Walde, 2017). However, the fact that parasite infestations are slowing down the expansion of the industry and causing problems with aquatic animal health and welfare issues needs to be fully considered. The infestation causes mortality in salmon, and it also reduces the market value and consumer acceptance. Increasing lice

---

[1] See, for example, Aaen & Horsberg, 2016, where lice are described as "the major obstacle facing a sustainable future for farmers of salmonids" (Aaen & Horsberg, 2016, p. 1213.)

*Figure 1 Female (on the right) and male (left) salmon lice observed by stereomicroscope in April 2018 in research station in Kårvika, Tromsø. Photo from personal archive.*

numbers in salmon aquaculture sites raise concern of possible effect to wild Atlantic salmon populations living in coastal areas. Wild fish can get more affected with increasing number of parasites in the area close to salmon farms. This suggests this problem is important for different sectors, ranging from producers and sellers, to consumers and environmentalists, and thus society at large.

The salmon louse (Figures 1 and 2) is a copepod ectoparasite – a small crustacean that attaches to salmon and feeds on the skin, blood and mucus, causing skin damage. It is a macroparasite, which means that it is mostly visible by eye (particularly in the case with adult salmon louse). The pathology it causes to their hosts "is tied to the number of parasites present" (Goater, Goater & Esch, 2014, p. 8). In high intensities, lice can cause damage leading to secondary infections. This happens particularly in farmed conditions where large numbers of salmon are stocked together.

Damage can range from small to large skin lesions on different body parts of salmon. The effect of



*Figure 2. Visual observation of a female louse with an egg string (on the right) (April, 2018, research station in Kårvika, Tromsø). Photo from personal archive.*

infestation is depending on several factors, such as fish health, life stage and number of parasites present. Skin lesions caused by lice may then result in viral or bacterial secondary infections because of the open wounds, stress (which negatively affects growth and health of

the salmon), and problems for salt and water balance for the fish. Salmon lice have a direct life cycle, involving ten life stages (2 life stages of salmon lice are shown in Figure 2). Four to five of these stages, are parasitic. Before parasitic stage, salmon lice have a free-swimming stage. That stage is infective to salmon. The earliest – nauplius – stage of the salmon lice is a free swimming planktonic larval stage (Goater et al., 2014). Number of lice are being reported by fish farms in the largest Atlantic salmon farming countries (expressed as number of lice per fish). Allowable number of lice per fish (as defined by particular institution in large Atlantic salmon farming countries, like Norway, Scotland, Canada and Chile) are ranging from 0,5 up to 3 adult female lice per fish. The aim of the measures is to control the infestation levels so that it is not expanding uncontrollably above a certain limit, jeopardizing the profitability of the enterprise.

To reduce the number of lice, different treatment methods have been used over the years. However, the chemical treatments effectiveness is negatively affected by both the increasing fish density and the developing resistance towards chemical treatments (see, for example, Aaen et al., 2015). High salmon density in sea pens increases disease transmission rates, and year-round production provides parasites with a year-round host availability, thereby increasing lice numbers. Therefore, current ability in the industry to deal with these parasites is limited.

Various chemical treatments have been used over time, such as pyrethroids, organophosphorus compounds, chitin synthesis inhibitors, avermectins and other therapeutic agents like hydrogen peroxide (Hjeltnes et al., 2017) each of which has different effects on lice (Table 1).

*Table 1 Most used chemical compounds in salmon lice treatment*

| Substance | Examples | Description |
|---|---|---|
| **Organophosphates** | Azamethiphos (bath treatment) | Paralysing substance |
| **Pyrethroids** | Cypermethrin Deltamethrin (bath treatments) | Paralysing substance |
| **Avermectin** | Emamectin benzoate (bath or oral treatment) | Reduces cell excitability, causes disruption of nerve impulses and rapid paralysis |
| **Hydrogen peroxide** | Hydrogen peroxide (bath treatment) | Creates gas bubbles within the body of lice making them unable to hold to a surface |
| **Benzoylurea** | Teflubenzuron (oral treatment) Diflubenzuron (oral treatment) | Chitin synthesis inhibition – lice cannot molt successfully |

Increasing resistance and stress caused to the fish are drawbacks for the use of chemical treatments. Lately, more of these treatments are supplemented or replaced by other methods, such as biological treatment (cleaner fish) and mechanical treatment (warm water treatment). The use of non-chemically based treatments tends to increase (Hjeltnes et al., 2017).

*Figure 3 Lumpfish breeding at the Centre for marine aquaculture in Kvaløya, Tromsø (Nofima). From: personal archive, 2016.*

Cleaner fish species used in Atlantic salmon aquaculture are lumpfish and wrasse species. Most common wrasse species used are the goldsinny wrasse (*Ctenolabrus rupestris*), corkwing wrasse (*Symphodus melops*) and ballan wrasse (*Labrus bergylta*). Due to larger tolerance of lower water temperature, lumpfish (*Cyclopterus lumpus*) is used as a cleaner fish in colder regions (Figure 3). Cleaner fish are being put in the same sea pens together with Atlantic salmon because of cleaner fish predation upon lice. Importantly, fish health and welfare must be ensured in both salmon and cleaner fish populations. Emergence of diseases common to both species may become a drawback for using this method.

Salmon lice do not tolerate higher water temperatures. For example, lice were reported to be absent from Norwegian farms when water temperature reached 18º C (Boxaspen, 2006). Therefore, their exposition to warmer water is used as a control method. The operational measures to deal with lice, one of them being warm water treatment, also have their possible drawbacks. The method must be applied in a way so that increased water temperature does not affect salmon negatively. Such negative effects were reported in Scotland fish farm in 2016, when because of too high water temperature, accidental death of Atlantic salmon reached the number of 95 000 (Fraser, 2017). Also, fish mortality after such mechanical lice treatments are caused by stress because of, for example, changed environment – "93% of fish health personnel had experienced 'significant mortality' because of non-chemically based de-licing treatment" (Hjeltnes et al., 2017, p. 5). Other damage can be possibly done by causing injuries to fish during, for example, transfer to the treatment tanks.

Apparently, in the current situation, the Atlantic salmon aquaculture industry is trying out any possible methods, and looking for the most effective way to solve this expensive and limiting factor. There is another opinion related to the increasing lice densities and search for the best treatment method. It states that the salmon aquaculture industry should focus on using only effective methods rather than trying several different ones, thereby possibly creating multi-

resistant "super-lice". Various treatment experiments may select the most resistant individuals of salmon lice, thereby, making an experiment on host and pathogen co-evolution (Ugelvik, Skorping, Moberg & Mennerat, 2017). Not all the parasite's population will be eliminated by the treatment: the most resistant individuals are surviving. They are creating a new, more resistant generation which then uses space and food resources left because individuals sensitive to treatment are eliminated. Moreover, infestation with lice taken from aquaculture site areas, are shown to cause more severe symptoms to fish than lice taken from wild fish populations (Ugelvik et al., 2017). The problem is thus to develop an effective method for salmon lice treatment without selecting for resistant parasites. Several methods have been used but their effectiveness has been decreasing over time, leading to search for new methods.

## 1.2. Scope of the research

The aim of this thesis is to evaluate the effectiveness of three different salmon lice treatments in Atlantic salmon aquaculture: chemical treatments, use of cleaner fish and warm water treatment. The three different treatment methods are the ones that are being the most used in the largest producing countries (BioMar, 2018). The aim of this thesis is to conduct a systematic review on each method, gathering and analysing data related to salmon lice treatment.

Research question for this thesis is as follows:

How effective is each of the three salmon lice treatment methods for Atlantic salmon population kept in aquaculture sea pens (in terms of number of lice compared to the number before treatment)?

The goal is to describe the overall tendency of the treatment effectiveness – whether lice number is reduced, whether the resistance to chemical treatments is statistically significant, and if there are any negative effects associated to each of the treatments. Effectiveness in this thesis is understood as assessing positive and negative effects of intervention in real life settings (adapted from Petticrew & Roberts, 2006). This question applies to health and welfare issues of Atlantic salmon. In case of use of cleaner fish, the analysis of negative effect applies also to these species.

Fish welfare definition is a controversial issue and different approaches can be applied on how we define welfare of fish – from a function based definition which includes fish adaption to the environment to a feelings-based approach (free from negative experiences). Noble et al.

operationalize welfare into four categories – access to food, sufficient environmental conditions and health status and natural behaviour (Noble, Nilsson, Stien, Iversen, Kolarevic & Gismervik, 2018). Large numbers of parasites, antagonistic behaviour between fish species that are stocked together and increased stress are examples that negatively affects fish welfare. The effect of salmon lice treatment methods on health and welfare of non-target organisms (for example small crustaceans) living in proximity to farming sites is not covered by this thesis.

The research question is answered by a systematic literature review assessing the effectiveness of each treatment. Farmed Atlantic salmon are typically held in higher densities than in the wild and, therefore, have a higher probability to spread salmon lice. The thesis is focused on a particular lice species – *L. salmonis* – not covering salmon lice species that are common in Chile (*C. rogercresseyi*). However, no geographical limitation for the review is applied. Differences in treatment responses caused by genetics of lice in different regions are not discussed as it would constitute a separate research in itself. This thesis is limited to the treatment effectiveness expressed as the reduction in lice abundance. Such topics as economical aspects and costs involved in each method, as well as selective breeding of salmon resistant to lice infestations are not being covered.

## 1.3. Outline

This thesis is structured according to the IMRaD format. It consists of the following sections: introduction, materials and methods, results and discussion/conclusions. This introductory part is followed by the materials and methods section which describes the criteria for the systematic literature review and the data synthesis strategy. Results are summarised under a separate section, which is followed by discussion that also contains conclusions of this systematic review.

## 2. Materials and methods

In this thesis, a systematic literature review of peer-reviewed documents was performed. This method was used to gather data about research question related to the effectiveness and possible negative effects of three different salmon lice treatment methods.

Systematic literature review which is used in this thesis is also applied in other evidence-based studies, including biology and medicine to understand what method, treatment or drug is proved to be effective in previous research experiments (called as effectiveness of interventions). These methods have been used to collect the best available clinical evidence in medicine and veterinary medicine, to detect "the accuracy and precision of diagnostic tests, the power of prognostic markers and the efficacy and safety of therapeutic, rehabilitative and preventive regimens" (Vandeweerd, Kirschvink, Clegg, Vandenput & Walde, 2012, p. 29) – which depends on the research question asked[2].

In this thesis, the systematic literature review is used because of its advantage to summarize in several researches over time carried out on different treatment methods. Thereby, it is potentially possible to access an impressive sample size. In addition, it is possible to map areas where the research is lacking. Searches for scientific articles concerning salmon lice in electronic scientific databases (like, for example, "Web of Science") usually gives several hundred hits. Therefore, one would expect to find evidence on salmon lice treatment interventions in aquaculture and to make use of it by integrating the scientific information in one report. The documents chosen for this review are, in a way, treated as respondents in an interview by developing and answering a questionnaire. All the documents are searched for information related to the effects of the treatment by following a pre-determined questionnaire. The questionnaire was specifically developed for this thesis.

Systematic literature reviews consist of several steps. The first step is to define the research question (for this review, see Introduction, p. 9), decide where to look for the sources and define search criteria to look for in published research documents. The next step is to define inclusion or exclusion criteria as well as study quality criteria – in other words, the characteristics for the document to be included as a "respondent" to this systematic literature review. Further step is to match documents found to those criteria to avoid a personal selection bias. Only from those

---

[2] For systematic literature review use in veterinary medicine, see, for example, Adel et al, 2016. The article uses systematic literature review in case of a parasitic disease in canines.

documents that met the defined characteristics, data were extracted (again, by pre-determining what kind of data one is interested in, depending on the research question).

Further in this section, materials and methods used to find data for this thesis are described. "Materials" covers the databases used to find the information. "Methods" provides a description of search terms, inclusion and exclusion criteria, data quality control and data extraction terms.

## 2.1.  Materials

The database to look for documents in the research was "Scopus". "Scopus" contains all the necessary tools for advanced search during data gathering process. It is the largest database of peer-reviewed literature and quality controlled web resources. The search was duplicated in the "Web of Science" database. This was done to find if there are any search results not covered by the search performed in "Scopus".

Documents were then evaluated by pre-determined criteria and either included or excluded from this systematic literature review. For criteria, see methods section of this thesis. Documents provided by "Scopus" included articles, book chapters, reviews, short surveys and conference papers. If the same article was found several times it was treated as one source. This applies only to identical articles with the same authors.

## 2.2.  Methods

This chapter includes information about the data gathering process. The outcome is summarised in the results section.

During the first step, data as scientific research documents were found using an electronic database. After that, these documents were checked against predetermined criteria related to their content and methodology. Finally, data were extracted according to developed data extraction form. The process is summarized in Figure 4.
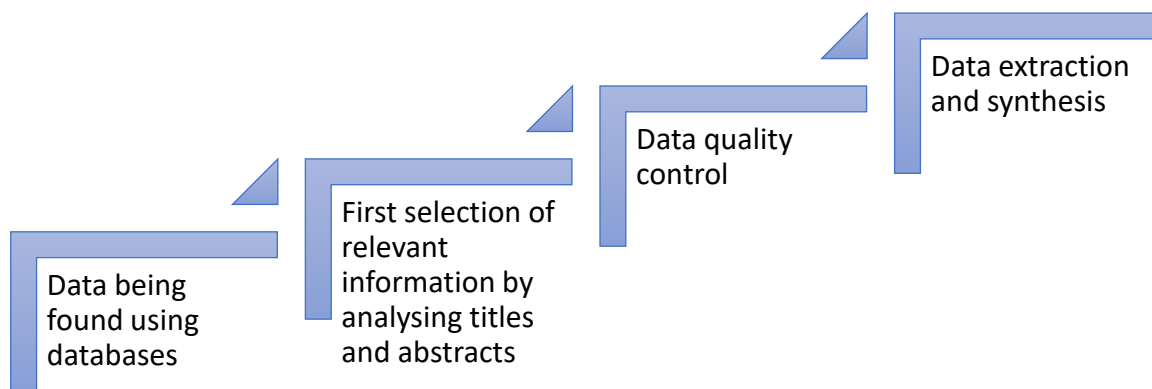
*Figure 4 Material selection and data gathering process*

### 2.2.1. Finding sources

The search was performed equally in both databases – "Scopus" and "Web of Science" by using the same search terms. Strategy included searching for the phrases "salmon lice" with alternatives or "salmon louse", "*L. salmonis*" and "*Lepeophtheirus salmonis*" within document title, abstract and keywords fields. The "" symbols were used to search for a whole phrase instead of looking for separate words. This search was then refined by adding one of following terms or phrases which describe treatment method of interest:

1. "chemical treatment", "medical treatment", drugs, "chitin synthesis inhibitor", "organophosphorus compound", "hydrogen peroxide", avermectin, pyrethroid, OR
2. "biological methods", "cleaner fish", lumpfish, lumpsucker, "*Cyclopterus lumpus*", wrasse, "*Ctenolabrus rupestris*", "*Labrus bergylta*", OR
3. "mechanical treatment", "operational methods", "warm water treatment", thermolicer.

Words and phrases in all three categories were further connected with a Boolean operator "OR" to find documents that contains information about salmon lice and at least one of the particular treatments.

Language chosen for the review was English, therefore, documents in other languages have been excluded. Time range for this review is from year $1^{st}$ of January 1991 (year of first publication of first document in "Scopus") until $3^{rd}$ of April 2018, thereby covering documents of a period of 27 years.

Keywords and their synonyms were generated by performing test searches in "Scopus" database. The final search on "Scopus" was performed at $26^{th}$ of February 2018. Checking for

additional documents published in 2018 was done on 3<sup>rd</sup> of April 2018. Search on "Web of Science" was performed on 3<sup>rd</sup> of April 2018. In order to avoid duplication, only documents that have not been included after searching the "Scopus" database, were considered as relevant. Search criteria were kept the same as for search in "Scopus". The exception was that the "Web of Science" do not allow to search within title, abstract and keywords fields. Therefore "document topic" was used to look for selected search terms.

### 2.2.2. Review of sources

First, documents were checked by title and abstract. Documents that did not contain data relevant for this research were excluded. To be included in further review, the abstract of each document was subjected to analysis according to the following criteria (Table 2):

*Table 2 Inclusion criteria*

| Criteria | |
|---|---|
| 1. **Population examined** | Farmed Atlantic salmon (*S. salar*) and salmon lice (*L. salmonis*) |
| 2. **Intervention method used (at least one of the list)** | Cleaner fish, warm water treatment, chemical treatment |
| 3. **Language** | English |
| 4. **Time period** | 01.01.1991. – 03.04.2018. |

If the examined abstract did not match those criteria, it was excluded from further review. All the criteria had to be met to include the document in the review. Intervention method (point 2) includes either documents where salmon lice attached to Atlantic salmon are treated, or bioassays where lice are being detached from salmon and, for example, immersed into a particular treatment substance. At least one method from point 2 of Table 2 must be used with a document. Documents describing data gathered from fish farms concerning treatment effectiveness were also included.

Second, the methodology section was examined for each document meeting the inclusion criteria. The aim of this step is to prevent serious systemic errors or selection bias. If the examined document contained serious errors within data quality control, then it was excluded, thus concentrating on high-quality documents.

A specific checklist was created for this review for the data quality control (Attachment 1). The form is based on the checklist created by Dawns & Black, 1998. Originally, this form contains

27 questions for the evaluation of human health care interventions. For this research, it was adapted to examine documents (defined as "studies" within the checklist) containing information of fish health and disease intervention. Human health care specific questions were excluded. The edited checklist contains questions to examine quality of reporting, (external and internal validity (bias and confounding), and statistical power). Other studies using the same checklist develop an evaluation system where each of source get a particular score depending on which the quality is evaluated. In an example which also using a modified Downs and Black checklist with 28-point evaluating system, scaling was as follows: "excellent" (28 – 24 points), "good" (23 - 19 points), "fair" (18 - 14 points) and "poor" (< 14 points) (O'Connor, Tully, Byan, Bradley, Baxter & McDonough, 2015). Maximum score depending on this checklist in this edition reaches 28. The same scaling is used for this review. Documents scaled as "poor" are going to be excluded from this review, as they may contain a "potentially serious flaw" (O'Connor S. R. et al., 2015, p. 2). At this stage, documents containing relevant but non-extractable information, were excluded from further review (such as descriptive articles that are not using any intervention method).

### 2.2.3. Data extraction

After data quality control was performed, the data were extracted and analysed by using a data extraction form (Attachment 2). The aim of this step was to provide information about both the effectiveness of the treatment method and its drawbacks. The following data were extracted: methodologies used in the experiment, characteristics of the sample, primary outcome - effect on lice number, and secondary outcomes - health impacts on fish and resistance, as well as the overall impression of validity, as specified in the data extraction form (see Attachment2). If no information was given on fields that do not cover primary outcome, they were left empty. If no information about primary outcome was given, then the documents was excluded because it did not meet inclusion criteria as stated in subsection 2.2.2. and Table 2 of this review.

### 2.2.4. Data synthesis strategy

Results containing number of documents found, being either included or excluded are showed using a QUORUM flow chart according to QUROUM statement (Moher, Cook, Eastwood, Olkin, Rennie & Stroup, 1999). The chart summarises the whole data searching process within this review, by showing, how many documents were found and retained in each step during the

research (Petticrew & Roberts, 2006). Finally, it shows the final number of documents on which this review is based.

Narrative analysis was performed by tabulating and describing data. Data from extraction forms were tabulated to form a summary. Tables include descriptions of documents, populations, methods and results. Chemical treatment groups were being used as categories to organize data within tables. Information on cleaner fish and warm water treatment events were each given a separate category to summarize the information. Data in each table were organized in chronological order. Data were also displayed graphically. Graphs summarize information extracted from several sources on each treatment method.

To summarize all chemical treatment results a separate table was made. It contains data from the first and the last documents about chemical treatment to compare treatment effectiveness changes over time. In some cases, other documents than the first and last ones have been chosen for this summary because of the need to make comparisons.

Meta – analysis was undertaken to measure:

(1) whether there is a statistically significant difference between resistant and non-resistant chemical treatment events, and

(2) whether there is a statistically significant relationship between resistance and intervention method used (particularly chemical treatment).

A goodness-of-fit test and Fisher's exact test were performed for first and second question respectively within R Commander software. Confidence level of 95% was used.

For first question, the null hypothesis was $H_0$= there is no statistically significant difference between the proportion of resistant and non-resistant treatment events. The research hypothesis was $H_1$= there is a statistically significant difference between the proportion of resistant and non-resistant treatment events.

The second question was about possible differences in resistance between chemical treatments, meaning that the resistance against one treatment is statistically significantly different than for another treatment. $H_0$= there is no statistically significant difference between chemical treatment groups and resistance. $H_1$= there is a statistically significant difference between chemical treatment groups and resistance.

Both statistical tests used are non-parametric and appropriate to analyse small sample sizes.

# 3. Results

This section is divided into two parts covering, first, results from data search and extraction and secondly, a summary of the gathered data during the data extraction process (using the data extraction form showed in Attachment 2).

## 3.1. Data gathering and extraction results

First search of data in "Scopus" database took place on 26th of February 2018. This search then gave 303 results. On 3rd of April the second search was carried out to find newly published documents in 2018 (after 26th of February 2018). This last search gave 2 more documents (305 in total). However, after first examination by title and abstract, both newly found documents were excluded as irrelevant for this review.

Search in "Web of Science" was performed once (on 3rd of April 2018). Search in this database contained 36% more results than in the last search in "Scopus" database (477 results in total).

Most of the documents that were found in both databases were scientific articles (Figures 5 and 6 for "Scopus" and "Web of Science", respectively).
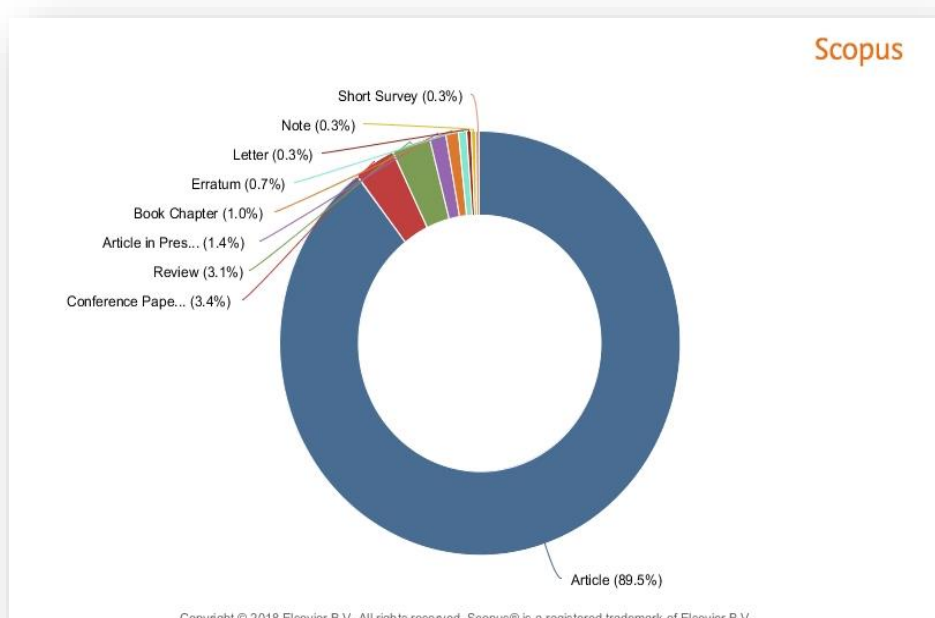


*Figure 5 Document types found in "Scopus" database (last search)*
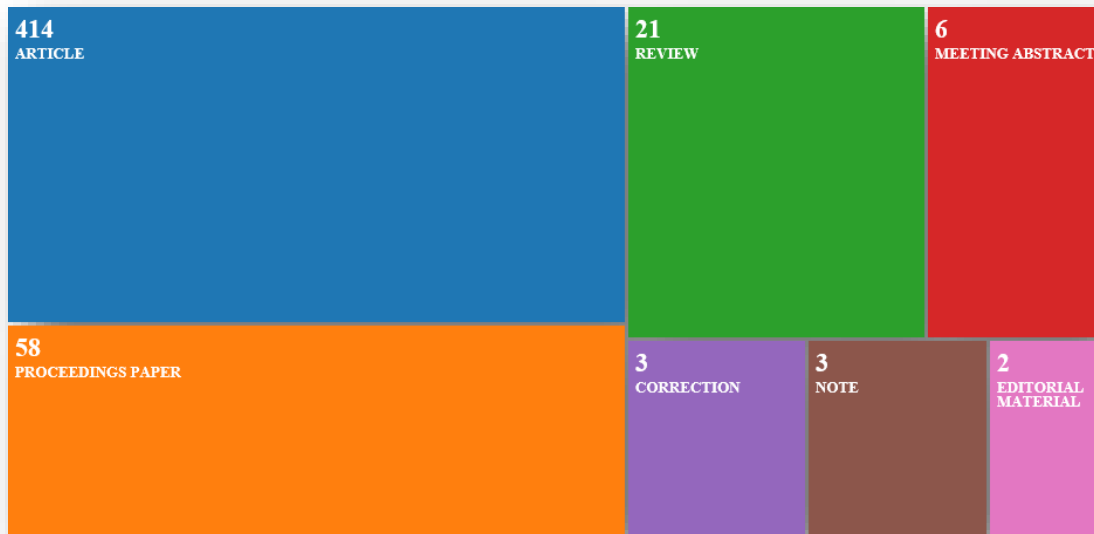
*Figure 6 Document types found in "Web of Science" database*

Articles constituted 89.5% of all results in "Scopus" and 86.8% in "Web of Science". Other types of documents were also found, the second largest group being conference/proceedings papers.

The result profile by country showed that most of the documents were from Norway (Figures 7 and 8 for "Scopus" and "Web of Science", respectively).



*Figure 7 Documents by country ("Scopus", last search)*

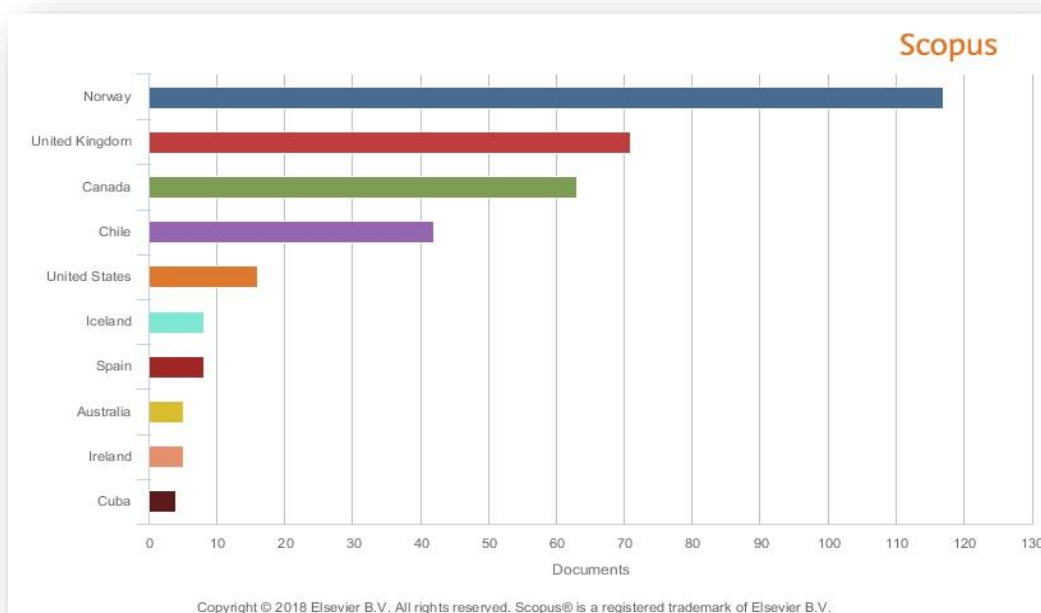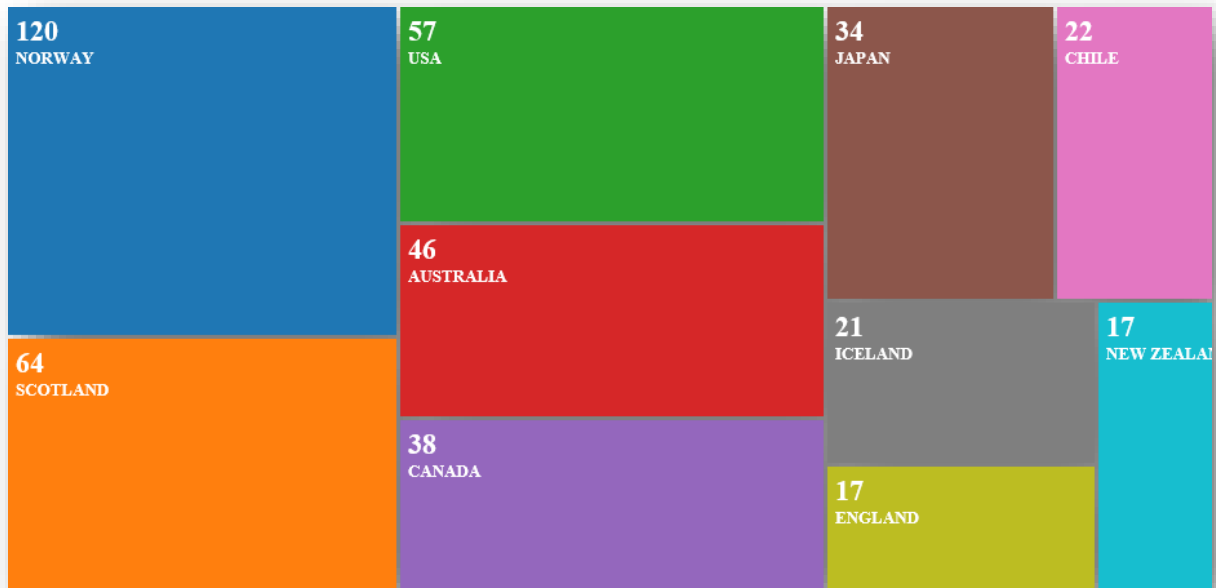*Figure 8 Documents by country ("Web of Science")*

The country profile is slightly different between the two databases. However, the largest countries are being represented similarly, the largest being Norway. The United Kingdom is being the second in both databases. In Figure 8, "Web of Science" data from Scotland and England must be summed up as it is in Figure 7. Canada and Chile are also between the largest represented countries. To sum up, all the largest Atlantic salmon farming countries are those that are producing the largest number of research in this field.

After first examination by title and abstract for the first "Scopus" search on 26th of February, 2018, 203 documents were excluded as not meeting the inclusion criteria. This constitutes 67% of the results. After the next search steps, another 40 documents were excluded because they did not contain enough necessary primary data (i.e. the effect of treatment on the number of lice). There were no results excluded because of too low score within the data quality control. This left 60 documents from "Scopus" (20% from the total number) for this review.

During the "Web of Science" result overview, most of the documents were excluded during the title and abstract research. The reasons were either that these documents were not relevant for this review or that they were already been selected during "Scopus" database search. Only four documents were selected for further data quality control check and full text analysis. Of those, two did not contain sufficient primary data for extraction and, therefore, they were

excluded. Finally, two articles were included in this review and added to the total number of included "Scopus" documents.

A QUORUM chart containing information about data gathering process from "Scopus" is showed in Figure 9. This chart summarizes the information including both – first and second search results of "Scopus". "Web of science" added 2 more articles to the final number of 60 "Scopus" results. This gave basis for this review that consists of 62 documents.
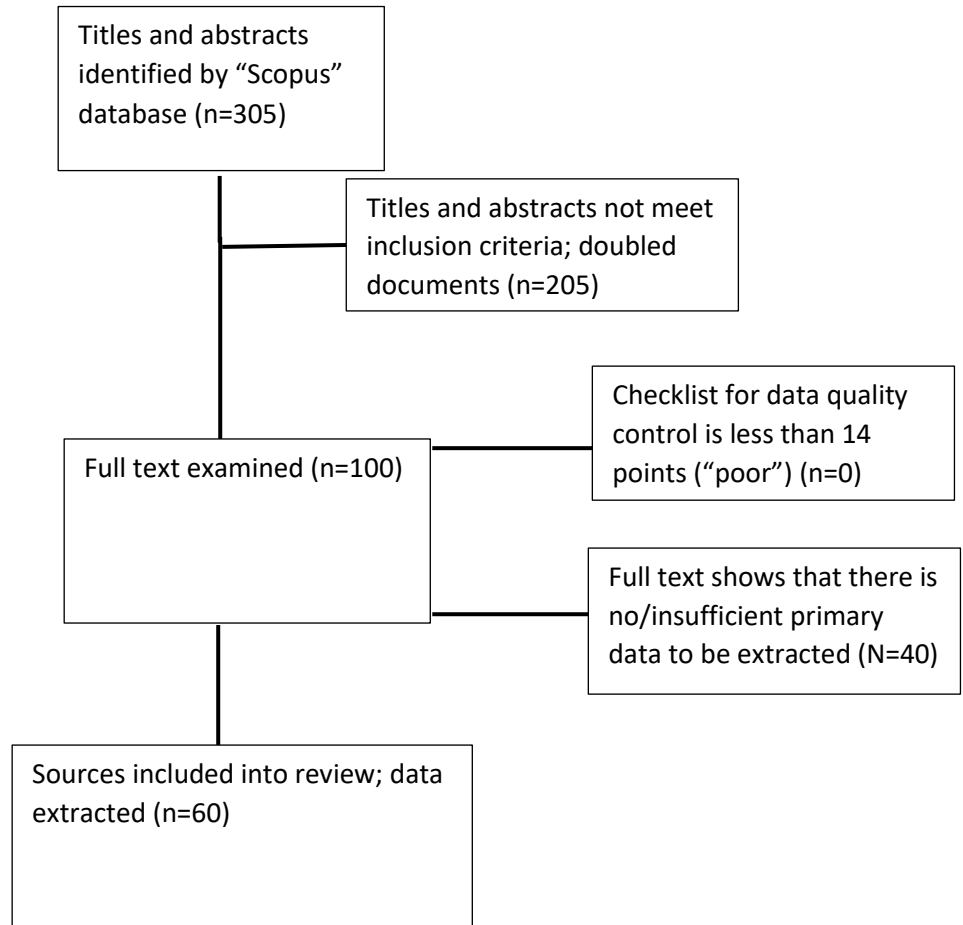


*Figure 9 QUORUM chart for total data extracted from "Scopus" database*

The included documents categorized by intervention method used showed such number per salmon lice treatment category (Figure 10):
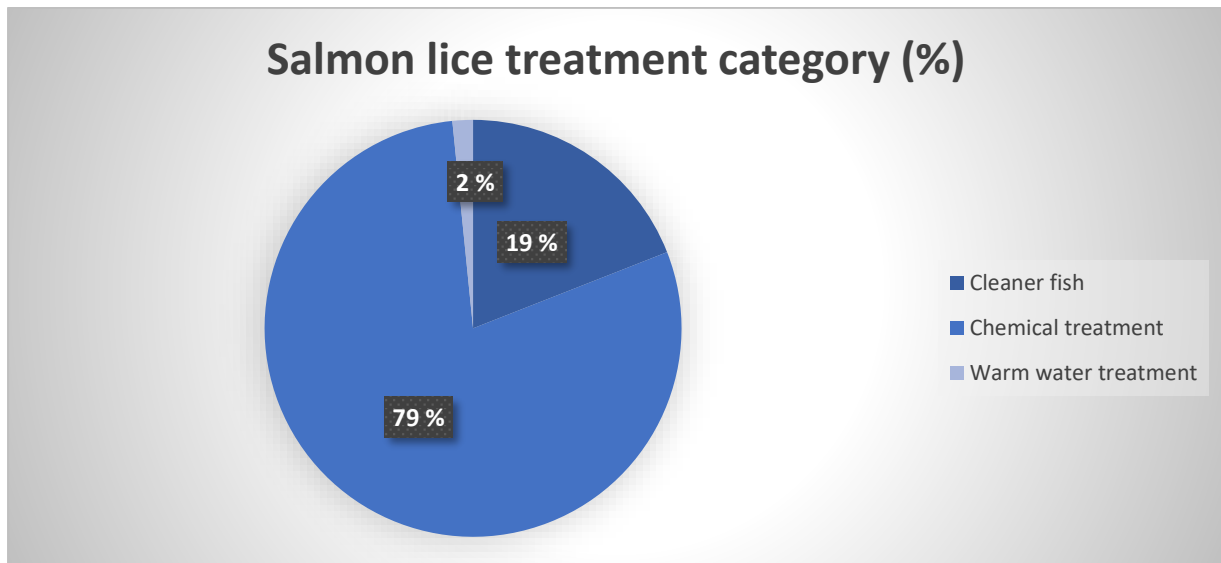


*Figure 10 Documents categorized by intervention methods*

The largest part of the documents assessed the effectiveness of chemical salmon lice treatments (79% of total number). The Avermectin treatment (with emamectin benzoate) was studied most – 26 out of 50 documents. It was followed by hydrogen peroxide (n=8) and combined chemical interventions (n=5). All others of the most used chemical lice treatment classes (as showed by Table 1) were described in at least 5 documents.

Cleaner fish species used in treatments were either wrasse (mostly goldsinny or ballan wrasses) or lumpfish. In total, 19% of the documents assessed the effectiveness of cleaner fish intervention. Within documents, the number of interventions using lumpfish was slightly higher (n=7) than the number of interventions using wrasse species (n=5). Most of the documents referring to the use of lumpfish as cleaner fish were more recent that those using wrasse species (those were performed at early 1990s with visual examination of fish by diving instead of using underwater cameras).

Less information related to warm water treatment was found in "Scopus" and "Web of Science" (2%).

Slight difference by country profile was detected (Figure 11). Most of the research was performed in Norway, Canada or Scotland with one document from USA, Faroe Islands and Ireland each.
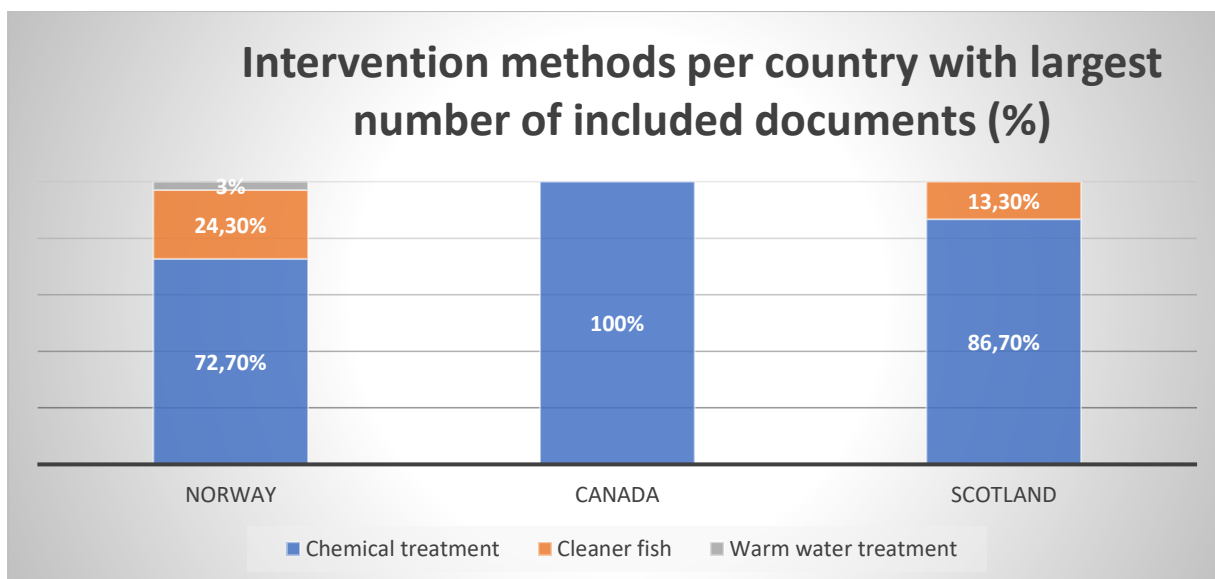
*Figure 11 Percentage of treatments described within documents in Norway, Canada and Scotland*

## 3.2. Data quality control results

Of all included documents, most were ranked as "good" or "excellent" according to the checklist for measuring study quality. None of the documents were excluded because of poor study quality. Only two documents were classified as "fair". "Good" and "excellent" were represented within the review sample with 24% and 73%, respectively. Most of the drawbacks detected included, for example, lack of actual probability values (exact value instead of "$p$<0.05") for statistical analysis. Some of the documents did not include the confidence level within the methods section, but it could be concluded from the research that a general confidence level of 95% had been used. Another example was the lack of description in the methodology section (for example, sample characteristics). None of these drawbacks were considered so serious that the document should be excluded from this review.

## 3.3.   Data extraction and analysis

Chemical treatment data are being categorized by chemical substance (i.e., hydrogen peroxide, pyrethroids, organophosphates, benzoylurea and avermectins) (as in Table 1). Relevant primary and secondary data about chemical treatments are summarized together (Attachments 3 – 7). It is followed by a subsection covering cleaner fish (with summary information within Attachment 8) and warm water treatment research data.

### 3.3.1.   Chemical treatment by treatment group

#### 3.3.1.1.   *Hydrogen peroxide*

The earliest research found was dated in 1993 and examined effectiveness of hydrogen peroxide treatment. Hydrogen peroxide treatments were studied overall using two methods – either *in vivo* interventions where lice were examined when attached to salmon which received the treatment (or not – in case of a control group), or as *in vitro* bioassays. *In vivo* treatments were performed as bath treatments at different hydrogen peroxide concentrations (many cases around 1500 ppm) for 20 minutes. In bioassays, lice were removed from salmon and put in a Petri dish to perform the experiment, usually adding a chemical substance and observing results after different contact times.

In hydrogen peroxide treatments, the first signs of resistance were described already in 1994 in Canada (Bruno & Raynard, 1994) (a year after it had first been used in this review). In Scotland (from 1993 to 1998), the resistance was not described. It was until 1999, that a research using hydrogen peroxide reported resistance of salmon lice towards this treatment (Treasurer, Wadsworth & Grant, 2000). In 2013, resistance towards hydrogen peroxide treatment was reported in Norway (Helgesen, Romstad, Aaen & Horsberg, 2015). Importantly, high pre-adult and adult lice survival was discovered since the beginning of the treatment use. Hydrogen peroxide, therefore, was most effective against chalimus stages of lice. Experiments reported in these documents showed that this chemical treatment is also toxic to Atlantic salmon (in 6 out of 9 documents). The adverse effects in several cases resulted into salmon mortality (documented by Johnson, Constible & Richard, 1993). In many cases, the hydrogen peroxide dosage that was reported toxic to Atlantic salmon, did not immobilize all the salmon lice. It was, however, also noted that mortality rates were temperature dependent and that salmon tolerates hydrogen peroxide better if it is administered in colder water temperatures ("no mortality (..) at concentrations of 4.0g $l^{-1}$ or less at 6⁰ C" (Johnson et al., 1993, p. 203)). At too

high concentrations, hydrogen peroxide caused damage to the gills of Atlantic salmon (Johnson et al., 1993 and Bruno & Raynold, 1994).

### *3.3.1.2.    Pyrethroids*

Pyrethroid treatment research covered experiments using cypermethrin and deltamethrin. These documents have been published since 1998 and performed as both – *in vivo* (bath treatment) and *in vitro* (bioassays) interventions. The first documentation of resistance to this drug was published in 2001. After that, the resistance had been documented in all other documents (Figure 12).



**Resistance towards plyrethroid treartments (n=7)**
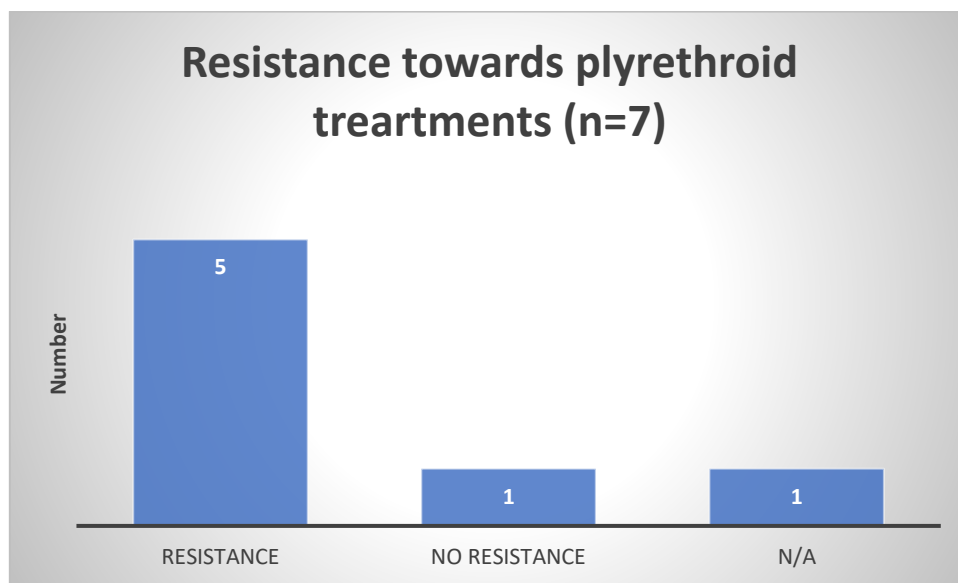
*Figure 12 Resistance towards pyrethroids*

 Most of the documents were reporting experiments performed in Norway. One intervention was performed in Scotland but did not conclude about resistance to pyrethroid treatment ("N/A" in Figure 12). A shift in effectiveness was noted. For example, in 2001, deltamethrin in concentration of 1.3ppb caused 50% lice immobilization (Sevatdal, Copley, Wallace, Jackson & Horsberg, 2004). In 2016, the same medicament with dose of 2.0 ppb caused 13.2% immobilization for lice that were characterized as resistant strain – i.e. developing resistance towards the chemical (Jensen, Sevatdal, Bakke, Kaur & Horsberg, 2017). These documents did not discuss the effects of this treatment on salmon health. In seven out of eight documents, the effects of the treatment on the health of salmon were not discussed because salmon did not receive the treatment (bioassays were performed and lice were removed prior to the experiment).

### 3.3.1.3. Benzoylurea

Benzoylurea treatment was described from 1995 onwards, but there have been relatively few documents covering this treatment group (five being included into this review) compared to other chemical treatments. The benzoylurea treatment group covers such chemical treatments as teflubenzuron and diflubenzuron, both being administered in feed, usually for period of 7 days. Resistance was first described in 2016 (Aaen & Horsberg, 2016). However, no effect on adult lice stage was documented in 2000 (Branson, Rønsberg & Ritchie, 2000) – only the chalimus stages were affected by the treatment. The effectiveness had a tendency to decrease over time. In 2000 (Branson et al., 2000), effectiveness towards chalimus stages were reduced to 86.3% from 92% in year 1995 (Ritchie, Rønsberg, Hoff & Branson, 2002). During those trials, no negative effect on the health of fish was documented. However, it was mentioned that the high concentration of benzoylurea treatment that had been used when performing bioassays could have negative effect on the health of fish.

### 3.3.1.4. Organophosphates

Organophosphate treatment experiments were performed using azamethiphos as a bath treatment. Research data were available since 1996 for both – *in vivo* treatment and bioassays. The first resistance towards the treatment was documented in 2012 (Kaur, Jansen, Aspehaug & Horsberg, 2016) and has been described in all documents onwards. One intervention performed in 1996 in Canada, did not report resistance in lice towards organophosphate treatment (O'Halloran & Hogans, 1996). In 2016, a large difference between sensitive and resistant lice strain towards immobilization was documented. Organophosphate-sensitive strains were still being 100% eliminated while only 19,1% of resistant strains were being immobilized - both at azamethiphos concentration of 100ppb (Jensen et al., 2017). Again, no document described possible adverse effects to salmon health – with one exception that concluded that concentration of azamethiphos used in bioassay could be too high for salmon bath treatments.

### 3.3.1.5. Avermectins

Treatments by emamectin benzoate were described the most – by 27 documents about avermectins where emamectin benzoate was the only described substance. This treatment was

administered to salmon in feed but two experiments used also intra-peritoneal injection. Some bioassays were performed. Resistance towards emamectin benzoate was first documented in 2002 - 2006 (Lees, Bailie, Gettinby & Revie, 2008b). Later, it was mentioned in every document related to emamectin benzoate treatments (performed either *in vivo* or *in vitro*). Because of the large sample, development of resistance was seen most clearly for this treatment over the years, starting from 2004 in Norway (Skilbrei, Glover, Samuelsen & Lunestad, 2008). Since then, the shift in resistance towards emamectin benzoate is evident (Figure 13). Resistance began to be develop in interventions performed in 2006 in both Canada and Scotland. The number of documents (as shown in Figure 13) was fluctuating over the years with most interventions performed around year 2006 – 2010.
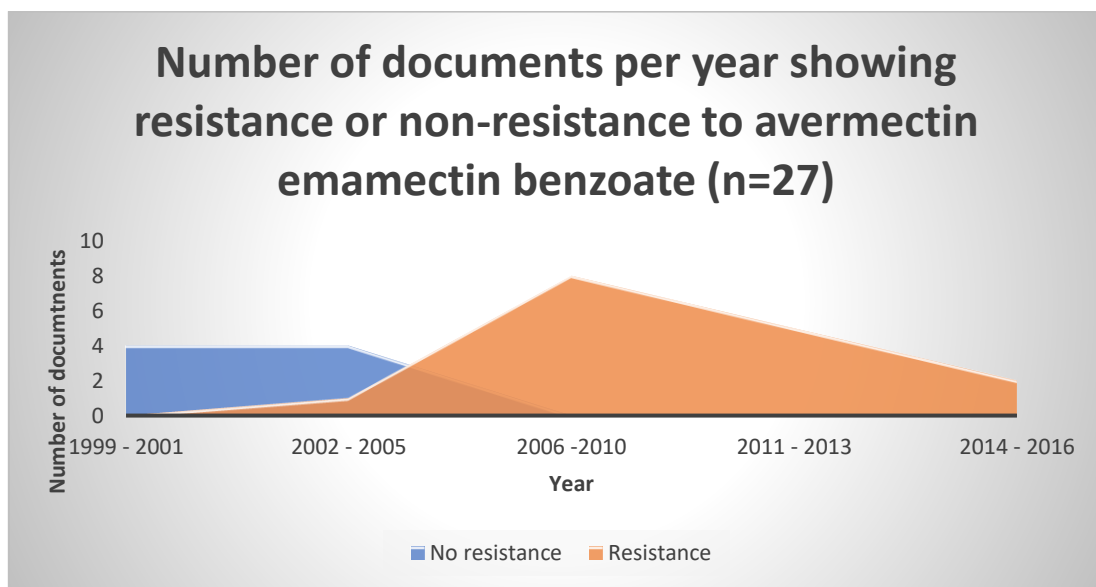


*Figure 13 Resistance towards emamectin benzoate treatment and number of documents published during the period*

The maximum effectiveness of the treatment that had been documented during 1999 to 2000 was around 90% (from 89% to 95% of lice immobilized with dose of 50µg kg$^{-1}$ biomass d$^{-1}$). In 2012, using a triple dose of 150µg kg$^{-1}$ biomass d$^{-1}$ of emamectin benzoate, the maximum effectiveness was 77% in females and 73% in males. There was no negative effect registered on the health of Atlantic salmon. There were 2 documents out of 27 that noted a slight negative effect which was associated with a reduction of feed intake. In 9 documents, negative effects were not examined because experiments including Atlantic salmon were not performed. Instead, bioassays were carried out, in which only salmon lice were involved. In experiments including Atlantic salmon, 9 out of 11 did not note negative effect on salmon health. This pattern did not change according to the year when the intervention was performed.

### 3.3.2. Overall chemical treatment results

Overall treatment effectiveness over the years are summarized within a table (Table 3).

*Table 3 Chemical treatment effectiveness over examined period*

| Treatment group | Year of first intervention | Maximum effectiveness reported for the first document | Year of the resistance first documented | Maximum effectiveness reported for the last document |
|---|---|---|---|---|
| **Hydrogen peroxide** | 1993 | 20% pre-adult survival (conc. 1.5 g/l) (Johnson et al., 1993) | 1994 | 16% pre-adult survival (conc. 1.5 g/l) (Overton, Samsing, Oppedal, Stien & Dempster, 2017) |
| **Pyrethroids** | 1998 | 50% immobilization at 1.03 ppb deltamethrin (Sevatdal et al., 2004) | 2001 | 2.0 ppb immobilization for resistant strain 13.2%, for sensitive strain 70.3% (Jensen et al., 2017) |
| **Benzoylurea** | 1995 | 10 mg kg$^{-1}$: 69,4% and 77.5% effectiveness (2 trials) (Ritchie et al., 2002) | 2000/2016 | In year 2000, the same maximum effectiveness but no effect to adult lice (Brenson et al., 2000). No data for 2016. |
| **Organophosphates** | 1996 | 100% gravid female reduction; 98.3% pre-adult reduction; 68% chalimus reduction (0.1 mg/l) (O'Halloran & Hogans, 1996) | 2012 | 28% mortality for 0.4 ppb and 43% mortality for 2 ppb in 2012 (Kaur et al., 2016). 2016 (bioassay): 19.1% of the resistant strain immobilized at 100 ppb (Jensen et al., 2017). |
| **Avermectins** | 1999 | 68 – 98% immobilization at concentrations of 50μg kg$^{-1}$ biomass d$^{-1}$ (Stone, Sutherland, Sommerville, Richards & Varma, 1999). | 2002 | 77% female and 73% male immobilization using a triple dose (150μg kg$^{-1}$ biomass d$^{-1}$) in 2012 (Poley, Purcell, Igboeli, Donkin, Wotton & Fast, 2013). |

Table 3 contains data (in percentages) from the first and the last documents to compare treatment effectiveness changes over time. Percentages were the most commonly used in the different documents and, therefore, they were retained here instead of numbers of lice.

Indications of resistance at some point of time in all documents were reported. Overall, there were 17 no resistance and 33 resistance events within the chemical treatment document sample. However, as mentioned before, in the benzoylurea treatment group, resistance is only reported in 2016. It is important to note that another intervention done earlier (year 2000) concluded that the treatment has no effect to adult lice (Branson et al. 2000). Less effect to adult lice had been documented previously, but none of the interventions performed before 2000 documented zero effect in adult lice. Less development of resistance is shown when looking to the hydrogen peroxide example. The first intervention showed that there was about 20% adult lice survival, but in 2017, it was 16%. This document mentions that resistance has emerged to the treatment in some regions (Overton et al., 2017).

Statistical analysis showed that the difference between resistance and no resistance in this sample is statistically significant (goodness-of-fit test; $x^2$ (1, N = 50) = 5.12; $p$ = 0.024). This applies to all treatment groups without describing this difference by each of five chemical treatment groups (Table 1). Resistance data according to chemical treatment group are summarised in Table 4.

*Table 4 Resistance per chemical treatment group*

| Chemical treatment | Resistance | No resistance | N/A | Percentage of resistant events |
|---|---|---|---|---|
| Hydrogen peroxide | 5 | 3 | 1 | 55.5% |
| Avermectins | 18 | 8 | 1 | 66.7% |
| Pyrethroids | 5 | 1 | 2 | 62.5% |
| Organophosphates | 4 | 1 | 0 | 80% |
| Benzoylurea | 1 | 4 | 0 | 20% |

Analysis on whether there is a statistically significant relationship between resistance and a chemical treatment group in use was performed. Results showed that there is no statistically

significant relationship between resistance/no resistance and the chemical treatment method used within this sample (Fisher's exact test, $p = 0.246$).

At some point in time, some of the treatments are showing high effectiveness which are close to 100% mortality of salmon lice. However, this effectiveness tends to decrease in all treatment groups in interventions performed in different countries.

### 3.3.3. Cleaner fish

Of 12 documents related to cleaner fish treatment, five used different wrasse species while seven used lumpfish (see summarised results of cleaner fish treatment in Attachment 8). Wrasse species used for interventions included goldsinny wrasse ballan wrasse and corkwing wrasse (*Symphodus melops*). All the wrasse interventions were performed during 1993 to 2013. Since 2014, researches about lumpfish as cleaner fish were performed. Different stocking densities (or salmon: cleaner fish ratio) were used. In half of the documents 10% or 10% and 15% stocking densities of cleaner fish were used (Figure 14). In earlier interventions with wrasse species, stocking density determined was highly approximate because of large losses of wrasse during the intervention (for example, 200 to 300 wrasses per week were reported to be disappearing in the document by Kvenseth, 1993) due to small size of the fish, allowing them to escape from the pens.
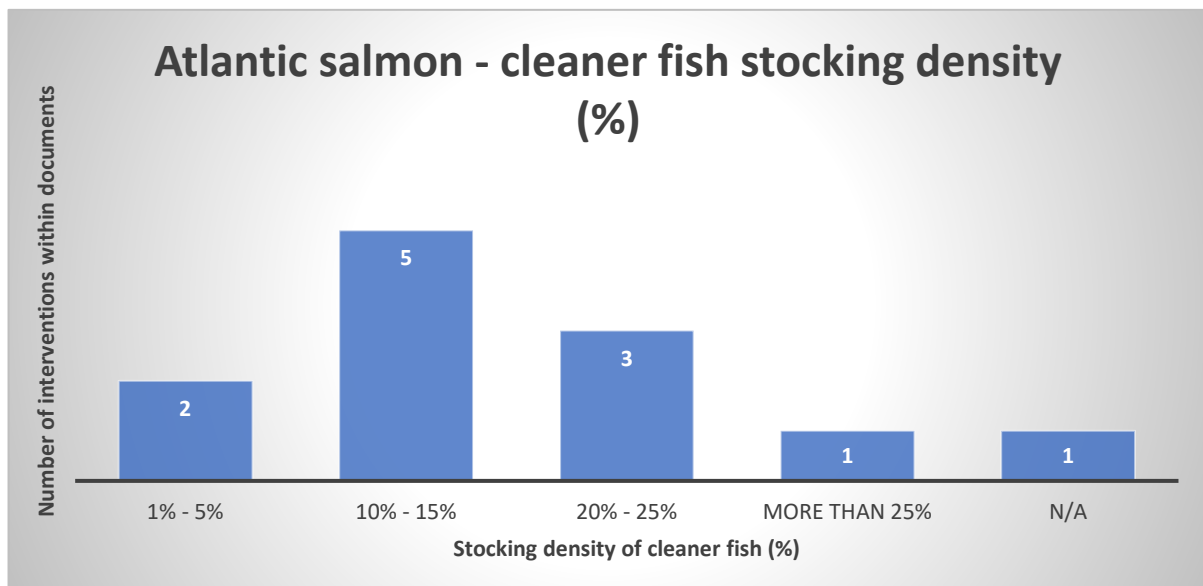


*Figure 14 Stocking densities in trials with cleaner fish*

Most of the interventions were performed in Norway (8 out of 12 documents). Different methods were used to observe cleaning effectiveness. In most cases (11 of 12), lice counting on salmon were performed several times during the trial but not less than two times (i.e. before and after the experiment). Stomach contents of cleaner fish were analysed to determine, whether they had ingested salmon lice. This analysis was performed either by dissection or as a gastric lavage. In some documents, fish behaviour was observed to detect whether there was any antagonistic behaviour between both species (biting or chasing, for example) and whether cleaner fish are eating lice. In interventions performed since 2013, underwater camera technology was used, while wrasse behaviour in earlier documents was examined by diving and visual observation. Feeding was also examined during the intervention. In two experiments, specific growth rate for both species were calculated (see Figure 15 for frequency of each method).
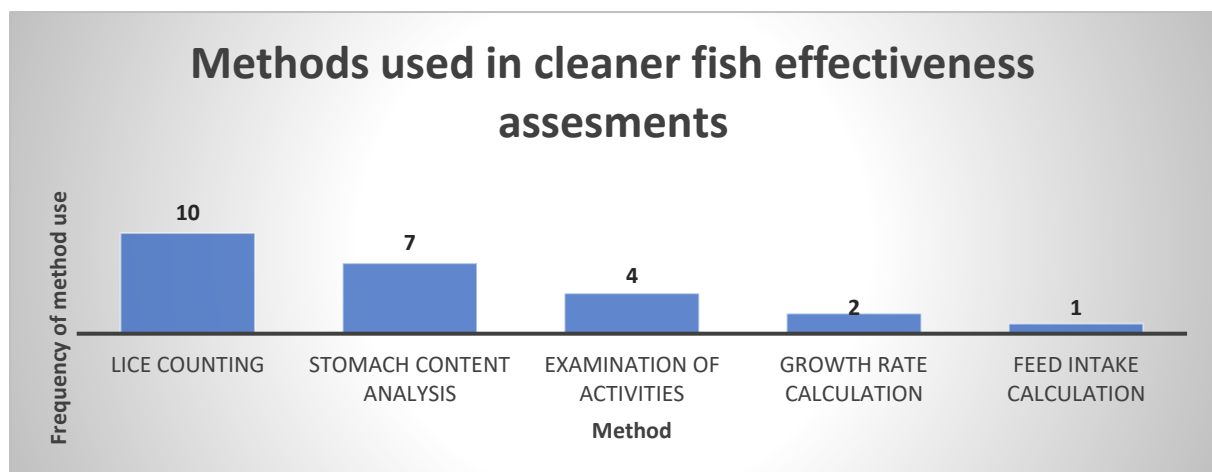


*Figure 15 Frequency of assessment methods used in documents describing cleaner fish efficiency in lice control*

Because of the different methods used, the cleaning effectiveness was measured differently within the documents. However, all of them allowed to conclude about treatment effectiveness.

Stomach content analysis from wrasse species showed large variations in lice ingestion. The number of ingested lice varied between 7 to 46 per wrasse stomach on average. In lumpfish, percent of fish that had ingested lice varied from 15% to 38%, without specifying the number of lice per stomach content.

All the documents showed lower lice number on salmon when stocked together with cleaner fish – either wrasses or lumpfish. These numbers were compared with average lice numbers before the trial or with a control group of salmon, stocked without cleaner fish. For example, in an intervention with ballan wrasse, lice abundance decreased from 1.2 to 0.5 lice per Atlantic

salmon (Leclercq, Davie & Migaud, 2014). This difference when using lumpfish, varied from 10% to 100%. It was documented that lumpfish was most effective in capturing large mature female lice. When lice numbers were counted after the intervention in the document by Imsland, the number of mature females was decreased by 97% while the chalimus stage by 10% (Imsland et al., 2014). However, the average number of lice was significantly lower after the intervention with cleaner fish in all the documents.

Negative effects of stocking both species together in sea pens should be looked separately for wrasse and lumpfish species.

For wrasses, a large number of disappearance was noted. In some interventions, new wrasses were added during because of the disappearance. In a case when no additional wrasses were added, only 5.7% of goldsinny wrasse and 10.2% of corkwing wrasse were found at the last fish count (during an approximately 4 months' period from 18th of June to 23rd October) (Deady, Varian & Fives, 1995). As only a smaller part of fish was found dead (for causes not related to Atlantic salmon), it was concluded that wrasses had been escaping the sea cages because of their relatively small size. Apart from wrasse disappearance from the cages, possible antagonistic behaviour was noted to three Atlantic salmon individuals which were found dead in the cage with an eye missing (Leclercq et al., 2014). In this case, Atlantic salmon were stocked together with large ballan wrasses. Otherwise, no mortality in salmon was detected that could be associated with wrasses.

No antagonistic behaviour was detected when interventions included lumpfish. In one of the documents, a lower feed conversion ratio was detected when salmon was stocked in sea pens with large lumpfish (>350 g) (Imsland et al., 2014a). Lumpfish are actively competing with salmon for salmon feed pellets which is also noted within documents were fish behaviour was examined (Powell et al., 2017). It was concluded that large lumpfish have a better opportunity to compete with salmon. However, if salmon is stocked with smaller lumpfish, there was no effect of the presence of cleaner fish on the growth of salmon. It was also concluded that smaller lumpfish display higher grazing effectiveness (Imsland et al., 2014b), meaning a larger predation upon salmon lice. In one document, some lumpfish mortality was reported because of bacterial disease due to *Pasteurella spp.* (Imsland et al., 2016). No salmon mortality was noted.

### 3.3.4. Warm water treatment

Only one document related to warm water treatment was found that met the inclusion criteria (Ljungfeldt, Quintela, Besnier, Nilsen & Glover, 2017). This intervention was performed in 2017 in Norway by using a heat application to lice which were previously removed from Atlantic salmon. Lice counting was performed before and after the experiment. Two temperature challenges were applied at temperatures around $22^{\circ}$ C for 3.5 hours and $24^{\circ}$ - $26^{\circ}$ C for 30 minutes. Mortality rates with full heat challenge were below 50% and varied between the tanks. Survival ranged between 58% and 81.4% per tank (Ljungfeldt et al., 2017). Female lice performed better than males within this experiment.

No possible effect on salmon was discussed because lice were de-attached prior to the intervention and, therefore, salmon was not involved in the heat challenge.

# 4. Discussion

This systematic literature review showed that it is possible to draw conclusions about effectiveness of two of three salmon lice treatment methods: chemical treatment and use of cleaner fish. There was no sufficient information related to warm water treatment that would meet inclusion criteria for this review.

## 4.1. Chemical treatment

Decrease in effectiveness is showed in most chemical treatment groups combined with increase in resistance. Resistance in latest interventions is detected for all chemical treatment groups and is statistically significant.

A similar pattern in resistance development can be seen in all countries in which these experiments were performed. Resistance appeared after a certain period of particular treatment usage. In some cases, resistance was first reported in one country and was discovered later in another. This was the case with, for example, hydrogen peroxide (development of resistance is described in 1994 in Canada and 1999 in Scotland).

Increase in resistance towards drugs that previously were effective has happened several times with different chemical treatments. Therefore, it is expected to happen also if another new drug would emerge. It was not common to include resistance as a problem concerning salmon lice treatments in 1990s documents. It started later as more drugs for chemical treatment were introduced. Resistance development is common around salmon farms using a particular chemical treatment, thereby creating a resistant lice strain. There is a large response difference with the organophosphate azamethiphos treatment where sensitive lice strains are being eliminated by almost 100% while in resistant strain, only 19,1% mortality was detected. In addition, the drug concentrations tend to increase while the effectiveness decreases (as in the cases of pyrethroid and organophosphates). One would conclude that it cannot be advised to use this treatment on sensitive lice strains because this chemical treatment will likely select resistant lice. This would only help temporarily until resistance develops and would thus lead to the selection of multi-resistant salmon lice.

Finally, it is concluded that effectiveness of different chemical treatment groups expressed in percentage of lice immobilized is varying. The common trend is that this effectiveness is decreasing after some time of treatment application which is a direct effect of resistance

development in lice. This resistance development along with some health issues associated with increasing drug concentrations, are considered as negative effects for this treatment.

## 4.2. Cleaner fish

Different cleaner fish species were used for lice treatment. All of them were effective in delousing in all cases with no decreasing tendency as it was with chemical treatment. On the contrary, efficiency could be increased over time by selection and breeding of most effective cleaner fish families (Imsland et al., 2016).

Huge losses of wrasses took place during the trials. Currently this would be a fish welfare issue, as well as a threat to fish health because of escapees and disease transmission to the wild populations. However, lately because of larger low temperature tolerance, lumpfish are the preferred cleaner fish species and they do not show such escaping rate.

Tendency to avoid using wild caught fish as cleaner fish in aquaculture is now also emerging. Wild caught wrasses were used in earlier experiments (including Scotland). This approach has changed lately. For example, in Scotland, "all the lumpfish deployed are farmed and the production of farmed wrasse is increasing" (Scottish Salmon Producers Organization, 2017, p. 6). In 2016, in Scotland hatcheries produced 3.3 million lumpfish and 5.2 million wrasse ova being laid to hatch (Scottish Government, 2017).

Lumpfish were found competing with salmon for salmon pellets (small effect on salmon growth detected) (Imsland et al., 2015). This happened in a case when large lumpfish were stocked with Atlantic salmon. In cases where lumpfish size did not reach 350 g in weight, no effect on salmon growth rate was detected (Imsland et al., 2015). Small lumpfish were found to be more effecting in lice grazing during trials. Therefore, use of small (<350 g) lumpfish would be preferable. This, however, creates an ethical issue as to the use of lumpfish after they have reached the size limit (e.g. possible use of fish after slaughter).

Little or no direct negative effect such as biting by cleaner fish towards Atlantic salmon (or other way around) was noted. In other research, a larger percentage of lumpfish were found to be resting when were stocked without Atlantic salmon (Imsland et al., 2014a). However, this did not cause a significant difference in feeding and growth in both species. Cleaning behaviour as performed by lumpfish or wrasse species towards Atlantic salmon, can be considered as mutualism as both species benefit from those interactions (Goater et al., 2014). Lumpfish were

find to graze especially on mature female lice (with 97% effectiveness comparing to 10% effectiveness in chalimus (Imsland et al., 2014). This is most preferable to decrease the lice abundance.

However, a disease spread that is common to both – salmon and cleaner fish species – would be considered as a serious drawback for using this method. For example, some of the cleaner fish during trials died because of infection with the bacteria *Pasteurella* spp. which can also infect Atlantic salmon. Other disease spread is possible. Lumpfish can become vectors for transfer of amoebic gill disease (caused by *Paramoeba perunans*). This means that lumpfish could spread this disease to Atlantic salmon (Haugland, Olsen, Rønneseth & Andersen, 2017).

Overall, use of cleaner fish can be considered efficient for salmon lice control in fish farms. The effect of the cleaner fish is not decreasing over time as it is with chemical treatment and it also does not cause a risk of selecting resistant lice because of chemical treatment. Lumpfish and salmon share the same feeding grounds in wild conditions, so it is possible to stock them together in sea pens. Some drawbacks are detected for wrasse stocking with Atlantic salmon which is connected to large escape rate.

## 4.3. Warm water treatment

Considerably less data sources were found for this research concerning warm water treatment in lice elimination. However, information about this method is available in other sources, like, for example, reports from the industry or salmon producers. This review could have more results about this method if a number of reports and description from these sources would be included into this review. However, this would affect study quality control and cause a possible bias which is less likely if peer reviewed documents are being examined. Because of these reasons, it was concluded that scientific research with this method is currently lacking and it is not possible to answer research questions for this review concerning warm water treatment. Instead, a gap in research is noted.

## 4.4. Evaluation of research question

This review showed that effectiveness of chemical and biological salmon lice control treatments is varying and none of the treatment events describes the possibility to eliminate lice completely. In addition, there is a necessity to look for alternative ways for extensive chemical treatment use because of both – reduced effectiveness and increasing resistance.

Therefore, the focus must be on sustainable ways to reduce lice infestation compatible with aquaculture expansion and by mitigating its increasing effect on the aquatic environment. Therefore, all the negative effects such as contamination of the environment by drug residues must be kept at a minimum level. Biological methods are not selecting resistance and, if administered properly, have less negative effect on environment in proximity of the aquaculture sites.

Susceptibility of lice infestations are dependent on the condition (health and welfare) of the Atlantic salmon. Already diseased, inappropriately fed and stressed fish are more subject to infestations. Therefore, salmon lice infestation, health and welfare of Atlantic salmon are inter-related aspects that must be considered in a holistic approach. Importantly, new trends are emerging when it comes to increasing salmon tolerance against lice infestations by selective breeding of Atlantic salmon. Selective breeding implies that less treatment would be needed for lice outbreak control because the parasite load will decrease by salmon selection (Gharbi et al., 2018). In my opinion, appropriate fish welfare conditions, possibly combined with selective breeding, and when necessary supplemented with use of cleaner fish would be the most effective and sustainable way in dealing with salmon lice.

Possible further research can be directed to examination the effect of lice and secondary infections in triploid Atlantic salmon, which is important for future development of aquaculture in Northern Norway. Triploid salmon are beneficial in aquaculture because they are not able to interbreed with wild populations in case of escapes and because early sexual maturation is avoided. A study documented that triploid salmon susceptibility to salmon lice is the same as the susceptibility of diploid Atlantic salmon (Franzl et al., 2014). However, they may have different susceptibility to secondary infections after lice infestation and different response to treatment methods, which warrants further investigations.

# References

**Literature**

1. Aaen, S. M., Helgesen, K. O., Jørgensen Bakke, M., Kaur, K. & Horsberg, T. E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology, 31* (2), 72 – 81.

2. Aaen, S. M. &Horsberg,T. E. (2016). A screening of multiple classes of pharmaceutical compounds for effect on preadult salmon lice *Lepeophtheirus salmonis*. *Journal of Fish Diseases, 39*, 1213 – 1223.

3. Adel, A., Berkvens, D., Abatih, E., Soukehal, A., Bianchini, J. & Saegerman, C. (2016). Evaluation of Immunofluorescence Antibody Test Used for the Diagnosis of Canine Leishmaniosis in the Mediterranean Basin: A Systematic Review and Meta-Analysis. *PLoS ONE 11*(8): e0161051. https://doi.org/10.1371/journal.pone.0161051

4. Armstrong, R., MacPhee, D., Katz, T. & Endris, R. (2000). A field efficacy evaluation of emamectin benzoate for the control of sea lice on Atlantic salmon. *Canadian Veterinary Journal, 41*, 607 – 612.

5. BioMar (2018). *What are salmon lice?* Retrieved from http://www.biomar.com/en/uk/articles/products/cleaner-fish/what-are-salmon-lice/

6. Boxaspen, K. (2006). A review of the biology and genetics of sea lice. *ICES Journal of Marine Science, 63* (7), 1304 – 1316.

7. Branson, E. J., Rønsberg, S. S. & Ritchie, G. (2000). Efficacy of teflubenzuron (Calicide®) for the treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer 1838), infestations of farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture Research, 31*, 861 – 867.

8. Bruno, D. W. & Raynard, R. S. (1994). Studies on the use of hydrogen peroxide as a method for the control of sea lice on Atlantic salmon. *Aquaculture International, 2*, 10 – 18.

9. Campbell, P. J., Hammell, K. L., Dohoo, I. R. & Ritchie, G. (2006a). Randomized clinical trial to investigate the effectiveness of teflubenzuron for testing sea lice on Atlantic salmon. *Diseases of Aquatic Organisms, 70*, 101 – 108.

10. Campbell, P. J. Hammell, K. L., Dohoo, I. R. & Ritchie, G. (2006b). Historical control clinical trial to assess the effectiveness of teflubenzuron for treating sea lice on Atlantic salmon. *Diseases of Aquatic Organisms, 70,* 109 – 114.

11. Carmona-Antoñanzas, G., Humble, J. L., Carmichael, S. N., Heumann, J., Christie, H. R. L., Green, D. M., Bassett, D. I., Bron, J. E. & Sturm, A. (2016). Time-to-response toxicity analysis as a method for drug susceptibility assessment in salmon lice. *Aquaculture, 464*, 570 – 575.

12. Deady, S., Varian, S. J. A. & Fives, J. M. (1995). The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. *Aquaculture, 131*, 73 – 90.

13. Downs, S. H. & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *J Epidemiol Community Health, 52*, 377 - 384.

14. Eliasen, K., Danielsen, E., Johannesen, Á., Joensen, L. L. & Patursson, E. J. (2018). The cleaning efficiency of lumpfish (*Cyclopterus lumpus* L.) in Faroese salmon (*Salmo salar* L.) farming pens in relation to lumpfish size and seasonality. *Aquaculture, 488*, 61 – 65.

15. Espedal, P. G., Glover, K. A., Horsberg, T. E. & Nilsen, F. (2013). Emamectin benzoate resistance and fitness in laboratory reared salmon lice (*Lepeophtheirus salmonis*). *Aquaculture, 416 – 417*, 111 – 118.

16. Food and Agriculture Organisation of the United Nations, 2017. *Globefish Highlights: A Quarterly Update on World Seafood Markets*. Retrieved from http://www.fao.org/3/a-i7095e.pdf.

17. Franzl, B., Migaud, H., Fjelldal, P. G., Shinn, A. P., Taylor, J. F. Richards, R. H., Glover, K. A., Cockerill, D. & Bron, J. E. (2014). Triploid and diploid Atlantic salmon show similar susceptibility to infection with salmon lice *Lepeophtheirus salmonis*. *Pest Management Science, 70* (6), 982 – 988.

18. Fraser, D. (2017). Scottish salmon farming's sea lice 'crisis'. *BBC*. Retrieved from bbc.com/news/uk-scotland-38966188.

19. Gautam, R., Vanderstichel, R., Boerlage, A. S., Revie, C. W. & Hammell, K. L. (2017). Evaluating bath treatment effectiveness in the control of sea lice burdens on Atlantic salmon in New Brunswick, Canada. *Journal of Fish Diseases, 40*, 895 – 905.

20. Gharbi, K., Matthews, L., Bron, J., Roberts, R., Tinch, A. & Stear, M. (2018). The control of sea lice in Atlantic salmon by selective breeding. In: *Journal of Royal Society Interface, 12*, pp. 1 – 8.

21. Glover, K. A., Samuelsen, O. B., Skilbrei, O. T., Boxaspen, K. & Lunestad, B. T. (2010). Pharmacokinetics of emamectin benzoate administered to Atlantic salmon, Salmo salar L., by intra-peritoneal injection. *Journal of Fish Diseases, 33*, 183 – 186.

22. Goater, T. M., Goater, C. P. & Esch, G. W. (2014). Parasitism. Cambridge: Cambridge University Press, pp. 1 – 10; 263 – 334; 411 – 427.

23. Gustafson, L., Ellis, S., Robinson, T., Marenghi, F. & Endris, R. (2006). Efficacy of emamectin benzoate against sea lice infestations of Atlantic salmon, *Salmo salar* L.: evaluation in the absence of an untreated contemporary control. *Journal of Fish Diseases, 29*, 621 – 627.

24. Haugland, G. T., Olsen, A. – B., Rønneseth, A. & Andersen, L. (2017). Lumpfish (*Cyclopterus lumpus* L.) develop amoebic gill disease (AGD) after experimental challenge with *Paramoeba perunans* and can transfer amoebae to Atlantic salmon (*Salmo salar* L.). *Aquaculture, 478*, 48 – 55.

25. Helgesen, K. O., Romstad, H., Aaen S. M. & Horsberg, T. E. (2015). First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. *Aquaculture Reports, 1*, 37 – 42.

26. Hjeltnes, B., Bornø, G., Jonson, M. D., Haukaas, A. & Walde, C. (eds.) (2017.). The Health Situation in Norwegian Aquaculture 2016. *Norwegian Veterinary Institute*, 1 – 127.

27. Igboeli, O. O., Fast, M. D., Heumann, J. & Burka, J. F. (2012). Role of P-glycoprotein in emamectin benzoate (SLICE®) resistance in sea lice, *Lepeophtheirus salmonis*. *Aquaculture, 344 – 349*, 40 – 47.

28. Igboeli, O. O., Purcell, S. L., Wotton, H., Poley, J., Burka, J. F. & Fast, M. D. (2013). Immunostimulation of *Salmo salar* L., and its effect on *Lepeophtheirus salmonis* (Krøyer) P-glycoprotein mRNA expression following subsequent emamectin benzoate exposure. *Journal of Fish Diseases*, *36*, 339 – 351.

29. Igboeli, O. O., Burka, J. F. & Fast, M. D. (2013). Sea lice population and sex differences in P-glycoprotein expression and emamectin benzoate resistance on salmon farms in the Bay of Fundy, New Brunswick, Canada. *Pest Management Science, 70*, 905 – 914.

30. Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Foss, A., Vikingstad, E. & Elvegård, T. A. (2014). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture, 424 – 425*, 18 – 23.

31. Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Vikingstad & E., Elvegård, T. A. (2014a). Notes on the behaviour of lumpfish in sea pens with and without Atlantic salmon present. *Journal of Ethology, 32*, 117 – 122.

32. Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Vikingstad, E. & Elvegård, T. A. (2014b). Assessment of growth and sea lice infestation levels in Atlantic salmon stocked in small-scale cages with lumpfish. *Aquaculture, 433*, 137 – 142.

33. Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Vikingstad, E. & Elvegård, T. A. (2015). Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.). *Aquaculture, 436*, 47 – 51.

34. Imsland, A. K., Reynolds, P., Nytrø A. V., Eliassen, G., Hangstad, T. A., Jónsdóttir, Ó. D. B., Emaus, P.-A., Elvegård, T. A., Lemmens, S. C. A., Rydland, R. & Jonassen, T. M. (2016). Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. *Aquaculture, 465*, 19 – 27.

35. Imsland, A. K., Reynolds, P., Eliassen, G., Morten, A., Hansen, Ø. J., Puvanendran V., Hangstad, T. A., Jónsdóttir, Ó. D. B., Emaus, P. – A., Elvegård, T. A., Lemmens, S. C. A., Rydland, R., Nytrø, A. V. & Jonassen, T. M. (2016). Is cleaning behaviour in lumpfish (*Cyclopterus lumpus*) parentally controlled? *Aquaculture, 459*, 156 – 165.

36. Jansen, P. A., Grøntvedt, R. N., Tarpai, A., Helgesen, K. O. & Horsberg, T. E. (2016). Surveillance of the Sensitivity towards Antiparasitic Bath – Treatments in the Salmon Louse (*Lepeophtheirus salmonis*). *PLoS ONE 11* (2): e0149006. https://doi.org/10.1371/journal.pone.0149006

37. Jensen, E. M., Sevatdal, S., Bakke, M. J., Kaur, K. & Horsberg, T. E. (2017). A selection study on a laboratory-designed population of salmon lice (*Lepeophtheirus salmonis*) using organophosphate and pyrethroid pesticides. *PLoS ONE 11* (2): e0149264. https://doi.org/10.1371/journal.pone.0149264

38. Jimenez, D. F., Revie, C. W., Hardy, S. P., Jansen, P. A. & Gettinby, G. (2013). Multivariate evolution of the effectiveness of treatment efficacy of cypermethrin against sea lice (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar*). *BMC Veterinary Research*, *9*:258, 1 – 9.

39. Johnson, S. C., Constible, J. M. & Richard, J. (1993). Laboratory investigations on the efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis* and its toxicological and histopathological effects on Atlantic salmon *Salmo salar* and

chinook salmon *Oncorhynchus tshawytscha. Diseases of Aquatic Organisms, 17*, 197 – 204.

40. Jones, P. G., Hammell, K. L., Dohoo, I. R. & Revie, C. W. (2012). Effectiveness of emamectin benzoate for treatment of *Lepeophtheirus salmonis* on farmed Atlantic salmon *Salmo salar* in the Bay of Fundy, Canada. *Diseases of Aquatic Organisms*, 102, 53 – 64.

41. Jones, P. G., Hammell, K. L., Gettinby, G. & Revie, C. W. (2013). Detection of emamectin benzoate tolerance emergence in different life stages of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*, *36*, 209 – 220.

42. Kaur, K., Jansen, P. A., Aspehaug, V. T. & Horsberg, T. E. (2016). *Phe362Tyr* in AchE: A major Factor Responsible for Azamethiphos Resistance in *Lepeophtheirus salmonis* in Norway. *PLoS ONE 12* (5): e0178068. https://doi.org/10.1371/journal.pone.0178068

43. Kenyon, W., Davies, D. (2018). Salmon Farming in Scotland. *SPICe Briefing*, pp. 1 – 36.

44. Kvenseth P. G. (1993). Use of wrasse to control salmon lice. In: Reinertsen, H., Dahle, L. A., Jørgensen, L., Tvinnereim, K. (eds.), *Fish Farming Technology* (227 – 232). Rotterdam: A. A. Balkema.

45. Leclercq, E., Davie, A. & Migaud, H. (2013). Delousing efficiency of farmed ballan wrasse (*Labrus bergylta*) against *Lepeophtheirus salmonis* infecting Atlantic salmon (*Salmo salar*) post-smolts. *Pest Management Science, 70*, 1274 – 1282.

46. Lees, F., Bailie, M., Gettinby, G. & Revie C. W. (2008a). The Efficacy of Emamectin Benzoate against Infestations of *Lepeophtheirus salmonis* on Farmed Atlantic Salmon (*Salmo salar* L) in Scotland, 2002 – 2006. *PLoS ONE 12* (5): e1549. https://doi.org/10.1371/journal.pone.0001549

47. Lees, F., Bailie, M., Gettinby, G. & Revie, C. W. (2008b). Factors associated with changing efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* in Scottish salmon farms. *Journal of Fish Diseases, 31*, 947 – 951.

48. Ljungfeldt, L. E. R., Espedal, P. G., Nilsen, F., Skern-Mauritzen, M. & Glover, K. A. (2014). A common-garden experiment to quantify evolutionary processes in copepods: the case of emamectin benzoate resistance in the parasitic sea louse *Lepeophtheirus salmonis*. *BMC Evolutionary Biology*, *14*:108, 1 – 17.

49. Ljungfeldt, L. E. R., Quintela, M., Besnier, F., Nilsen, F. & Glover, K. A. (2017). A pedigree-based experiment reveals variation in salinity and thermal tolerance in the salmon louse, *Lepeophtheirus salmonis*. *Evolutionary Applications, 10*, 1007 – 1019.

50. Marine Harvest (2017). *Salmon Farming Industry Handbook*. Retrieved from: http://marineharvest.com/globalassets/investors/handbook/salmon-industry-handbook-2017.pdf.

51. McAndrew, K. J., Sommerville, C., Wooten, R. & Bron, J. E. (1998). The effects of hydrogen peroxide treatment on different life-cycle stages of the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837). *Journal of Fish Diseases*, *21*, 221 – 228.

52. Moher, D., Cook, D. J., Eastwood, S., Olkin, I., Rennie, D. & Stroup, D. F (1999). Improving the quality of reports of meta-analyses of randomised controlled trials: The QUORUM statement. *Lancet, 354,* 1896 – 1900.

53. Noble, C., Nilsson, J., Stien, L. H., Iversen, M. H., Kolarevic, J. & Gismervik, K. (2018).Velferdsindikatorer for oppdrettslaks: Hvordan vurdere og dokumentere fiskevelferd, 1 - 32. Retrieved from: www.nofima.no/fishwell.

54. O'Connor, S. R., Tully, M. A., Byan, B., Bradley, J. M., Baxter, G. D. & McDonough, S. M. (2015). Failure of numerical quality assessment scale to identify potential risk of bias in a systematic literature review: a comparison study. *BMC Research Notes*, *8*, 1 – 7.

55. O'Halloran, J. & Hogans, W. E. (1996). First use in North America of azamethiphos to treat Atlantic salmon for sea lice infestation: Procedures and efficacy. *Canadian Veterinary Journal, 37*, 610 – 611.

56. Overton, K., Samsing, F., Oppedal, F., Stien, L. H. & Dempster, T. (2017). Lowering treatment temperature reduces salmon mortality: a new way to treat with hydrogen peroxide in aquaculture. *Pest Management Science, 74* (3), 535 – 540.

57. Overton, K., Samsing, F., Oppedal, F., Dalvin, S., Stien, L. H. & Dempster, T. (2018). The use and effects of hydrogen peroxide on salmon lice and post-smolt Atlantic salmon. *Aquaculture, 486*, 246 – 252.

58. Petticrew, M. & Roberts, H. (2006). Systematic Reviews in the Social Sciences: A Practical Guide. Oxford: Blackwell Publishing.

59. Poley, J. D., Purcell, S. L., Igboeli, O. O., Donkin, A., Wotton, H. & Fast, M. D. (2013). Combinatorial effects of administration of immunostimulatory compounds in feed and follow-up administration of triple-dose SLICE® (emamectin benzoate) on Atlantic

salmon, *Salmo salar* L., infection with *Lepeophtheirus salmonis*. *Journal of Fish Diseases, 36*, 299 – 309.

60. Poley, J. D., Igboeli, O. O. & Fast, M. D. (2015). Towards a consensus: Multiple experiments provide evidence for constitutive expression differences among sexes and populations of sea lice (*Lepeophtheirus salmonis*) related to emamectin benzoate resistance. *Aquaculture, 448*, 445 – 450.

61. Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K. & Garcia de Leaniz, C. (2017). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. *Reviews in Aquaculture, 0*, pp. 1 – 20.

62. Ramstad, A., Colquhoun, D. J., Nordmo, R., Sutherland, I. H. & Simmons, R. (2002). Field trials in Norway with SLICE® (0.2% emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms, 50*, 29 – 33.

63. Ritchie, G., Rønsberg, S. S., Hoff, K. A. & Branson, E. J. (2002). Clinical efficacy of teflubenzuron (Calicide®) for the treatment of *Lepeophtheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures. *Diseases of Aquatic Organisms*, *51*, 101 – 106.

64. Salmones Camanchaca (2017). *Annual Report 2016*. Retrieved from http://www.camanchaca.cl/en/wp-content/uploads/2017/06/Camanchaca-Annual-Report-2016.pdf.

65. Saksida, S. M., Morrison, D. & Revie, C. W. (2010). The efficacy of emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L., in British Columbia. *Journal of Fish Diseases*, *33*, 913 – 917.

66. Saksida, S. M., Morrison, D., McKenzie, P., Milligan, B., Downey, E., Boyce, B. & Eaves, A. (2013). Use of Atlantic salmon, *Salmo salar* L., farm treatment data and bioassays to assess for resistance of sea lice, *Lepeophtheirus salmonis*, to emamectin benzoate (SLICE®) in British Columbia, Canada. *Journal of Fish Diseases, 36*, 515 – 520.

67. Scottish Government (2017). *Scottish Fish Farm Production Survey 2016*. Retrieved from www.gov.scot/Publications/2017/09/5208.

68. Scottish Salmon Producers Organisation (2017). *Fish Health Management Annual Report*. Retrieved from http://scottishsalmon.co.uk/wp-content/uploads/2017/06/sspo-fhmar-2016-june-final.pdf.

69. Sevatdal, S., Copley, L., Wallace, C., Jackson, D. & Horsberg, T. E. (2004). Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modelling. *Aquaculture, 244*, 19 – 27.

70. Sevatdal, S, Magnusson, Å., Ingebrigtsen, K., Haldorsen, R. & Horsberg, T. E. (2005). Distribution of emamectin benzoate in Atlantic salmon (*Salmo salar* L.). *Journal of Veterinary Pharmacology and Therapeutics*, *28*, 101 – 107.

71. Sevatdal, S., Fallang, A., Ingebrigtsen, K. & Horsberg, T. E. (2005). Monooxygenase mediated pyrethroid detoxification in sea lice (*Lepeophtheirus salmonis*). *Pest Management Science, 61*, 772 – 778.

72. Sevatdal, S. & Horsberg, T. E. (2003). Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Krøyer) against the pyrethroid deltamethrin using bioassays and probit modelling. *Aquaculture, 218*, 21 – 31.

73. Skiftesvik, A. B., Bjelland, R. M., Durif, C. M. F., Johansen, I. S. & Browman H. I. (2013). Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). *Aquaculture, 402 – 403*, 113 – 118.

74. Skilbrei, O. T., Glover, K. A., Samuelsen, O. B. & Lunestad, B. T. (2008). A laboratory study to evaluate the use of emamectin benzoate in the control of sea lice in sea-ranched Atlantic salmon (*Salmo salar* L.). *Aquaculture, 285*, 2 – 7.

75. Skilbrei, O. T., Espedal, P. G., Nilsen, F., Garcia, E. P. & Glover, K. A. (2015). Evaluation of emamectin benzoate and substance EX against salmon lice in sea-ranched Atlantic salmon smolts. *Diseases of Aquatic Organisms*, *113*, 187 – 194.

76. Statistik sentralbyrå (2017). *Aquaculture*. Retrieved from https://www.ssb.no/en/fiskeoppdrett.

77. Stone, J., Sutherland, I. H., Sommerville, C., Richards, R. H. & Varma, K. J. (2000a). Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann, infestations in Atlantic salmon *Salmo salar* L. *Aquaculture, 186*, 205 – 219.

78. Stone, J., Sutherland, I. H., Sommerville, C., Richards, R. H. & Varma, K. J. (2000b). Commercial trials using emamectin benzoate to control sea lice *Lepeophtheirus salmonis* infestations in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms, 41*, 141 – 149.

79. Toovey, J. P. G. & Lyndon, A. R. (2000). Effects of hydrogen peroxide, dichlorvos and cypermethrin on subsequent fecundity of sea lice, *Lepeophtheirus salmonis*, under fish

farm conditions. *Bulletin – European Association of Fish Pathologists, 20* (6), 224 – 228.

80. Treasurer, J. W., Wadsworth, S. & Grant, A. (2000). Resistance of sea lice, *Lepeophtheirus salmonis* (Krøyer), to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar* L. *Aquaculture Research*, *31*, 855 – 860.

81. Treasurer, J. (1994). Prey selection and daily food consumption by a cleaner fish, *Ctenolabrus rupestris* (L.), on farmed Atlantic salmon, *Salmo salar* L. *Aquaculture, 122,* 269 – 277.

82. Treasurer, J. W. & Grant, A. (1997). The efficacy of hydrogen peroxide for the treatment of farmed Atlantic salmon, *Salmo salar* L. infested with sea lice (Copepoda: Caligidae). *Aquaculture*, *148*, 265 – 275.

83. Tribble, N. D., Burka, J. F. & Kibenge, F. S. B. (2007). Evidence for changes in the transcription levels of two putative P-glycoprotein genes in sea lice (*Lepeophtheirus salmonis*) in response to emamectin benzoate exposure. *Molecular & Biochemical Parasitology, 153*, 59 – 65.

84. Ugelvik, M. S., Skorping, A., Moberg, O. & Mennerat, A. (2017). Evolution of virulence under intensive farming: salmon lice increase skin lesions and reduce host growth in salmon farms. *Journal of Evolutionary Biology, 30*, 1136 – 1142.

85. Vandeweerd, J. – M., Kirschvink, N., Clegg, P., Vandenput, S., Gustin, P. & Saegerman, C. (2012). Is evidence-based medicine so evident in veterinary research and practice? History, obstacles and perspectives. *The Veterinary Journal, 191,* 28 – 34.

86. Westcott, J. D., Stryhn, H., Burka, J. F. & Hammell, K. L. (2008). Optimization and field use of a bioassay to monitor sea lice *Lepeophtheirus salmonis* sensitivity to emamectin benzoate. *Diseases of Aquatic Organisms, 79*, 119 -131.

87. Whyte, S. K., Westcott, J. D., Elmoslemany, A., Hammell, K. L. & Revie, C. W. (2013). A fixed-dose approach to concluding emamectin benzoate tolerance assessments on field-collected sea lice, *Lepeophtheirus salmonis*. *Journal of Fish Diseases, 36,* 283 – 292.

# Attachments

## Attachment 1 Checklist for measuring study quality (based on Downs, Black, 1998)

**Reporting**

1. Is the hypothesis/aim/objective of the study clearly described?

yes 1    no 0

2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?

*If the main outcomes are first mentioned in the Results section, the question should be answered no.*

yes 1    no 0

3.Are the characteristics of the population included in the study clearly described?

*In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case-definition and the source for controls should be given.*

yes 1    no 0

4. Are the interventions of interest clearly described?

yes 1    no 0

5.Are the distributions of principal confounders in each group of subjects to be compared clearly described?

*A list of principal confounders is provided.*

yes 2 partially 1 no 0

6. Are the main findings of the study clearly described?

*Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions. This question does not cover statistical tests which are considered below.*

yes 1 no 0

7. Does the study provide estimates of the random variability in the data for the main outcomes?

*In non-normally distributed data the interquartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should*

*be reported. If the distribution of the data is not described, it must be assumed that the estimates used were appropriate and the question should be answered yes.*

yes 1 no 0

8. Have all important adverse events that may be a consequence of the intervention been reported? *This should be answered yes if the study demonstrates that there was a comprehensive attempt to measure adverse events. A list of possible adverse events is provided.*

yes 1 no 0

9. Have the characteristics of population lost to follow-up been described? *This should be answered yes where there were no losses to follow-up or where losses to follow-up were so small that findings would be unaffected by their inclusion. This should be answered "no" where a study does not report the number lost to follow-up.*

yes 1 no 0

10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?

yes 1   no 0

## External validity

All the following criteria attempt to address the representativeness of the findings of the study and whether they may be generalised to the population from which the study subjects were derived.

11. Were the subjects in the study representative of the entire population from which they were recruited?

*The study must identify the source population and describe how the sample were selected. Sample would be representative if they comprised the entire source population, an unselected sample, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists.*

yes 1   no 0    unable to determine 0

12. Were those subjects representative of the entire population from which they were recruited?

*Validation that the sample was representative would include demonstrating that the*

*distribution of the main confounding factors was the same in the study sample and the source population.*

 yes 1   no 0     unable to determine 0

13. Were the staff, places, and facilities where the sample were treated, representative of the treatment the majority of population receive?

*For the question to be answered yes, the study should demonstrate that the intervention was representative of that in use in the source population.*

yes 1    no 0     unable to determine 0

## Internal validity - bias

14. If any of the results of the study were based on "data dredging", was this made clear?

*Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes.*

yes 1    no 0     unable to determine 0

15. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of sample, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?

yes 1    no 0     unable to determine 0

16. Were the statistical tests used to assess the main outcomes appropriate?
*The statistical techniques used must be appropriate to the data. For example, non- parametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described it must be assumed that the estimates used were appropriate and the question should be answered yes.*

yes 1    no 0     unable to determine 0

17. Were the main outcome measures used accurate (valid and reliable)?
*For studies where the outcome measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes.*

yes 1    no 0     unable to determine 0

## Internal validity / confounding (selection bias)

18. Were the samples in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?

yes 1    no 0    unable to determine 0

19. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?

yes 1    no 0    unable to determine 0

20. Were study subjects randomised to intervention groups?

yes 1    no 0    unable to determine 0

21. Was there adequate adjustment for confounding in the analyses from which the main findings                                        were                                        drawn?
*In non-randomised studies if the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses the question should be answered as no.*

yes 1    no 0    unable to determine 0

22. Were losses to follow-up taken into account?

yes 1    no 0    unable to determine 0

23. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?

Insufficient power 0                Medium power 3                Sufficient power 5

**Total:**

## Attachment 2 Data extraction form

| Data to be extracted | Notes to reviewer | Data |
|---|---|---|
| Title of study | | |
| Author | | |
| Year of publication | | |
| Place | | |
| Study on salmon lice treatments in Atlantic salmon aquaculture (yes/no) | If "no" – exclude | |
| Intervention method used (either chemical or cleaner fish, or warm water treatment) | If "no" – exclude | |
| Methodologies used for measurements | | |
| Period | | |
| Data source | | |
| Sample size | | |
| Age of the individuals in the sample | | |
| Size of the individuals in the sample | | |
| Other relevant details of the sample | If they have some bearing on the results of the study | |
| Number of lice before treatment | If applicable | |
| Reported effect on number of lice | Include details of significance testing, if reported | |

| | | |
|---|---|---|
| *Specific information regarding effect* | Other useful information given (e.g. how long time after intervention to effect) | |
| *Control group (yes/no)* | | |
| *Results compared to a control group* | If "yes" to the question above | |
| *Health impacts on Atlantic salmon (diseases, mortality)* | Other than number of parasites (if specified) | |
| *Health impacts on cleaner fish (diseases, mortality)* | If cleaner fish is used as intervention method | |
| *Resistance to chemical treatment detected (yes/no/no information)* | If chemical treatment is used as intervention method | |
| *Effect of temperature changes on Atlantic salmon detected (yes/no/no information)* | If warm water treatment is used as intervention method | |
| *Other impacts associated with the treatment* | | |
| *Overall impression of internal validity (low, medium, high)* | Assessment based on the quality of the sampling and response, and the treatment of confounding factors | |
| *External validity* | For example, only effect on particular age of fish or special conditions observed | |

Attachment 3 Summary of hydrogen peroxide treatment research

**Summary of data about hydrogen peroxide treatments**

| Number of studies | Reference | Year | Place | Methodology | Concentrations | Time of intervention (min) | Sample size (n of Atlantic salmon) per treatment | Effect on lice number | Resistance (Y/N) | Negative effect on Atlantic salmon (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Johnson et al.,1993 | 1993 | Scotland | *In vivo* study - effect on lice number while attached to salmon, and salmon health | 1.5 g/l⁻¹ | 20 | 50 | 96% larvae survival; 20% pre-adult and adult survival | N | Y |
| 2 | Bruno & Raynard, 1994 | 1994 | Canada | *In vivo* study - effect on lice number while attached to salmon, and salmon health | 0.25%, 0.50%, 1.25%, 2, 3% | 20 | 10 to 50 | 33% lice dead at 0.5% hydrogen peroxide; 98% dead at 2%. | Y | Y |
| 3 | Treasurer et al., 1997 | 1997 | Scotland | *In vitro* study - effect on lice number (bioassay) | 1500 ppm | 20 | - | 100% inactive after the treatment, but recovery by 35% after 1 h; 85% after 24h. | N | N/A |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | McAndrew, Sommerville, Wooten & Bron 1998 | 1998 | Scotland | *In vitro* study - effect on lice number (bioassay) | 1500ppm | 20 | - | Only nauplii and copepodite stages affected by treatment (percentage not given) | N | N/A |
| 5 | Treasurer et al., 2000 | 1992 - 1999 | Scotland | *In vivo* study - effect on lice number while attached to salmon, and salmon health | 1500 - 1700 ppm (1992). 2000 - 2350 ppm (1999). | 20 | 10 fish per cage sampled | 1992: 89% and 76% reduction in lice number (two cages). 1999: 7% reduction in lice number (2000 ppm) and 70% reduction (2350 ppm) | Y | Y |
| 6 | Toovey & Lyndon, 2000 | 1998 - 2000 | Scotland | *In vitro* study - effect on lice egg viability (bioassay) | 1500 ppm | 20 | - | Treatment group produced significantly lower hatching. Resulting larvae was less able to proceed to the copepodite stage | N/A | N/A |
| 7 | Helgesen et al., 2015 | 2013 | Norway | *In vivo* study - effect on lice number while attached to salmon, and salmon health | 0 - 5 g/l$^{-1}$ | 30 | - | 51% immobilized (susceptive strain); 21% immobilized (resistant strain) | Y | Y |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8 | Overton, Samsing, Oppedal, Dalvin, Stien & Demster, 2018 | 2012 - 2015 | Norway | *In vivo* study - effect on lice number while attached to salmon. | 0, 1, 1.25, 1.5, 1.75, 2 and 2.25 g/C | 20 | 20 | Up to 95% immobilized. No difference in lice removal across concentrations 1 - 2 g/l⁻¹ | Y Y |
| 9 | Overton et al., 2017 | 2017 | Norway | *In vivo* study - effect on lice number while attached to salmon, and salmon health | 1.5 g/L | 20 | 40 | On average 16% pre-adult lice stage survival | Y Y |

* Y -yes
N - no
N/A – not applicable

Attachment 4 Summary of pyrethroid treatment research

**Summary of data about pyrethroid treatments**

| Number of documents | Reference | Year | Place | Methodology | Concentrations | Time of intervention (min) | Sample size (n of Atlantic salmon) per treatment | Effect on lice number | Resistance (Y/N) | Negative effect on Atlantic salmon (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Toovey & Lyndon, 2000 | 1998 - 2000 | Scotland | *In vitro* study - effect on lice egg viability (bioassay) | 5 ppb | 60 | - | Treatment group produced significantly lower hatching. Resulting larvae was less able to proceed to the copepodite stage | N/A | N/A |
| 2 | Sevatdal et al., 2004 | 2001 - 2003 | Norway | Bioassays using cypermethrin and deltamethrin | High-cis-cypermethrin: 0, 0.15, 0.5, 1.5, 5, 15 ppb. Deltamethrin: 0, 0.03, 0.1, 0.3, 1, 3 ppb | 30 | - | 50% immobilization by dosage of high-cis-cypermethrin of 0.22 ppb and deltamethrin of 1.03. | Y | N/A |

| # | Reference | Year | Country | Method | Concentrations | | | Results | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **3** | Sevatdal & Horsberg, 2003 | 2003 | Norway | Deltamethrin bioassay and *in vivo* treatment. | 0, 0.1, 0.25, 0.5, 1.0, 3.0 ppb (for in vivo); 0, 0.03, 0.1, 0.25, 0.3, 0.5, 1.0, 3.0 ppb (bioassay) | 30 | 2 | Immobilization in in vivo test: control group up to 15%; 0.1 ppb - 37 - 55%; 0.25 ppb - 57 - 85%; 0.5 ppb 70 - 95%, 1 and 3 ppb up to 100%. Bioassays: control up to 10%; 0.1 ppb 0 - 30%; 0.25 ppb - 26 - 50%; 0.5 ppb 33 - 63%; 1ppb - 50 - 76%; 3 ppb - 96 - 100%. | Y | N/A |
| **4** | Sevatdal, Fallang, Ingebrigtsen & Horsberg, 2005 | 2005 | Norway | Bioassays using cypermethrin and deltamethrin | 0, 0.15, 0.5, 1.5, 5.0, 15.0 ppb (cypermethrin); 0, 0.03, 0.1, 0.3, 1.0, 3.0 ppb (deltamethrin) | 30 | - | For the most sensitive strain 50% immobilization in concentrations of 0.26 (cypermethrin) and 0.12 (deltamethrin). | N/A | N/A |
| **5** | Jimenez, Revie, Hardy, Jansen & Gettinby, 2013 | 2012 | Norway | *In vivo* treatment with cypermethrin | Not given | 5d | 455 before and 412 after the treatment | Reduced abundance of pre-adult and adult lice (90% effectiveness). Less chalimus reduction (49%). | N | N/A |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **6** | Jansen, Grøntvedt, Tarpai, Helgesen & Horsberg, 2016 | 2013 - 2014 | Norway | Bioassays with deltamethrin | 0.2 ppb and 1 ppb | 1440 | | Varying mortality from 90% to 20% depending on previous treatment | Y | N/A |
| **7** | Aaen & Horsberg, 2016 | 2016 | Norway | Bioassays with cypermethrin | 50 mg l$^{-1}$ | 30 | - | Lethal to >70% larvae. Reduced hatching by 50%. | Y | N/A |
| **8** | Jensen et al., 2017 | 2016 | Norway | Bioassays with deltamethrin | 2 ppb | 60 | - | Sensitive strain: 70.3% immobilization; resistant strain 13.2% immobilization | Y | N/A |

* Y -yes
N - no
N/A – not applicable

## Attachment 5 Summary of organophosphate treatment research

**Summary of data about organophosphate treatment**

| Number of documents | Reference | Year | Place | Methodology | Concentrations | Time of intervention (min) | Sample size (n of Atlantic salmon) per treatment | Effect on lice number | Resistance (Y/N) | Negative effect on Atlantic salmon (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | O'Halloran & Hogans, 1996 | 1996 | Canada | *In vivo* treatment with azamethiphos. | 0.1 mg/l | 30 | 3000 | 100% reduction of gravid females; 98.3% reduction of pre-adults; 68% reduction chalimus | N | N |
| 2 | Kaur et al., 2016 | 2012-2014 | Norway | Bioassays with azamethiphos. | 0, 0.4, 2 ppb | 1440 | - | 28% mortality for 0.4 ppb and 43% for 2 ppb | Y | N/A |
| 3 | Jansen et al., 2016 | 2013 - 2014 | Norway | Bioassays with azamethiphos | 0.4 ppb and 2 ppb | 1440 | | Varying mortality from 90% to 20% depending on previous treatment | Y | N/A |
| 4 | Aaen et al., 2016 | 2016 | Norway | Bioassays with azamethiphos | 50 mg l$^{-1}$ | 30 | - | Lethal to >70% larvae. No significant effect on hatching. | Y | Such dose would be too high for fish |

| 5 | Jensen et al., 2017 | 2016 | Norway | Bioassays with azamethiphos | 100 ppb | 60 | - | Immobilization. Sensitive strain: 100% heterozygous resistant strain: 80%, resistant strain 19.1% | Y | N/A |

Attachment 6 Summary of benzoylurea treatment research

**Summary of data about benzoylurea treatments**

| Number of documents | Reference | Year | Place | Methodology | Concentrations | Time of intervention (days) | Sample size (n of Atlantic salmon) per treatment | Effect on lice number | Resistance (Y/N) | Negative effect on Atlantic salmon (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ritchie et al., 2002 | 1995 - 1996 | Norway | *In vivo* treatment with teflubenzuron | 10 mg kg$^{-1}$ d$^{-1}$ | 7 | 10 to 20 per sampling even. In total, 167 000 - 299 357 | Maximum effectiveness 69.4% and 77.5% | N | N |
| 2 | Branson et al., 2000 | 2000 | Scotland (trial 1), Norway (trial 2) | *In vivo* treatment with teflubenzuron | 11 mg kg$^{-1}$ d$^{-1}$ | 7 | Several sea pens within aquaculture facilities. Exact number not given. | Maximum effectiveness at day 15 in trial 1 (83.4%), and at day 14 in trial 2 (86.3%). Maximum effectiveness toward chalimus, preadult. No effect to adult lice. | N | N |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **3** | Campbell, Hammell, Dohoo & Ritchie, 2006b | 2006 | Canada | *In vivo* treatment with teflubenzuron | 10 mg kg$^{-1}$ d$^{-1}$ | 7 | 100 fish per sampling event. In total 6 cages containing from 18 000 - 40 000 | Chalimus and mobile stages reduces by 92% and 74%, respectively on the first week; 41% and 61% in second. Increase of mobile stage in third week. | N | N |
| **4** | Campbell Hammell, Dohoo & Ritchie, 2006a | 2006 | Canada | *In vivo* treatment with teflubenzuron | 11 mg kg$^{-1}$ d$^{-1}$ | 7 | 1st site:14 cages with 3 000 salmon each; 2nd site - 10 cages, 20 000 salmon each; 3rd cage - 18 cages, 3 000 salmon each. | Chalimus stages first week after treatment: 79% lower. Second week: 53%. Mobile stages reduced by 69% in first week and 40% in second. | N | N/A |
| **5** | Aaen & Horsberg, 2016 | 2016 | Norway | Bioassays with diflubenzuron | 50 mg l$^{-1}$ | 30 | - | Diminished the ability of nauplii developing to copepodites. No statistically significant reduction in hatching and development of embryos. | Y | Such dose would be too high for fish |

* Y -yes
N - no
N/A – not applicable

## Attachment 7 Summary of avermectin treatment research

| | Summary of data about avermectin treatments | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of documents | Reference | Year | Place | Methodology | Concentrations | Time of intervention | Sample size (n of Atlantic salmon) per treatment | Effect on lice number | Resistance (Y/N) | Negative effect on Atlantic salmon (Y/N) |
| 1 | Stone, Sutherland, Sommerville, Richards & Varma, 2000a | 1999 | Scotland | Administered via feed, lice counts performed | $50\mu g\ kg^{-1}$ biomass $d^{-1}$ | 7 days | 1st trial: 180 salmon x 2 cages. 2nd trial: 149 salmon x 2 cages. 3rd trial: 360 salmon x 2 cages. | Lice number decreased in treatment group by 68 - 98% while increased in control group by 87% - 284% | N | N |
| 2 | Stone, Sutherland, Sommerville, Richards & Varma, 2000b | 2000 | Scotland | Administered via feed, lice counts performed | $50\mu g\ kg^{-1}$ biomass $d^{-1}$ | 7 days | 8 pens with 14000 - 17000 salmon each. Four of them - treatment group | 89% reduction in lice numbers. | N | N |
| 3 | Armstrong et al., 2000 | 2000 | Canada | Administered via feed, lice counts performed | $50\mu g\ kg^{-1}$ biomass $d^{-1}$ | 7 days | 151351 salmon, 76210 received treatment | Effectiveness of the treatment: 70% (week 1); 88% (week 3); 95% (week 4); 61% (week 6) | N | N |

| # | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | Ramstad, Colquhoun, Nordmo, Sutherland & Simons, 2002 | 2001 - 2002 | Norway | Administered via feed, lice counts performed | 50μg kg$^{-1}$ biomass d$^{-1}$ | 7 days | 561000 received treatment; 10 fish per each sampling event | 3 weeks after the treatment reduction by 94% | N | Y |
| 5 | Sevatdal et al., 2005 | 2002 | Norway | Administered via feed, lice counts performed | 50μg kg$^{-1}$ biomass d$^{-1}$ | 7 days | 20 fish per sampling event | No lice on sampled fish before day 123. | N | N |
| 6 | Westcott, Stryhn, Burka & Hammell, 2008 | 2002 - 2005 | Canada | 38 bioassays with different EMB concentrations | 0, 1, 3, 10, 30, 100, 300 ppb | 1 day | - | The effective concentration leading to 50% immobilization was 21 ppb | N | N |
| 7 | Gustafson, Ellis, Robinson, Marenghi & Endris, 2006 | 2002 - 2005 | USA | Administered via feed, lice counts performed | 50μg kg$^{-1}$ biomass d$^{-1}$ | 7 days | 13 farms, each held from 150 000 to 600 000 salmon. | Maximum effect ranged from 68% to 100% depending on site. | N | N |
| 8 | Lees, Bailie, Gettinby & Revie, 2008a | 2002 - 2006 | Scotland | Administered via feed, lice counts performed | 50μg kg$^{-1}$ biomass d$^{-1}$ | 7 days | 50 commercial salmon farms involved; sample size between 10 to 30 fish | 2002: <1% abundance at day 34 and 12% until day 83 after EMB; 2003: 5% and 40%, respectively; 2004 and 2005: 17% and 30%, respectively; 2006: lowest abundance 35%. | Y | N |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 9 | Lees et al., 2008b | 2002 - 2006 | Scotland | Administered via feed, lice counts performed | 50μg kg⁻¹ biomass d⁻¹ | 7 days | 56 commercial salmon farms involved; sample size between 10 to 30 fish | 2002, 2003: 10% abundance at day 20 after EMB; 2004: 6%; 2005: 23%; 2006: 19% | Y | N |
| 10 | Skilbrei et al., 2008 | 2004 | Norway | Administered via feed, lice counts performed | 50μg kg⁻¹ biomass d⁻¹ | 7 days | 100 Atlantic salmon, representing 3 treatment groups | No statistically significant effect of treatment with EMB | Y | N |
| 11 | Saksida, Morisson &, Revie, 2010 | 2003, 2007, 2008 | Canada | Examination of lice abundance records (15 - 39 farms depending on year) | 50μg kg⁻¹ biomass d⁻¹ | 7 days | - | Lice abundance in all years fell below 20%. In 2008, drop of the number was observed later. | N | N |
| 12 | Jones, Hammell, Dohoo & Revie, 2012 | 2004 - 2008 | Canada | Examination of lice abundance records (54 sites; 114 treatment episodes) | 50μg kg⁻¹ biomass d⁻¹ | 7 days | Mean of 40 fish per sampling event | Max. effectiveness decreased. Post treatment mean abundance - 2004: 0.9%; 2005: 6.8%; 2006: 15.3%; 2007: 22.6%; 2008: 75.7%. | Y | N |

| # | Study | Year(s) | Country | Method | Concentrations | Duration | Sample | Results | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 13 | Jones, Hammell, Gettinby & Revie, 2013 | Dataset 1: 2002 - 2006; Dataset 2: 2004 - 2008 | Dataset 1: Scotland; Dataset 2: Canada | Examination of lice abundance records | No data | No data | 54 sites in Canada; 47 sites in Scotland | Canada - 2004 - 2007: <1 adult female lice/fish; 2008 - increase in post-treatment abundance, lowest level: 2.4 mobile lice/fish and no decrease in adult female lice. Scotland - adequate removal of lice except 2006 when female lice number decreased <1 lice/fish only after 9 weeks (comparing to 4) | Y | N |
| 14 | Glover, Samuelsen, Skilbrei, Boxaspen & Lunestad, 2010 | 2007 | Norway | Intra-peritoneal injection | 50, 100, 200, 400 µml$^{-1}$ (pilot experiment to find dosage); 438 µg kg$^{-1}$ (experiment) | - | 6 fish per concentration sampled | Injection concentrations of 100, 200, 400, 800 µg kg$^{-1}$ protects fish for 6, 7, 9, 10 weeks, respectively | N/A | N |
| 15 | Tribble, Burka & Kibenge, 2007 | 2007 | Canada | EMB bioassay | 0, 1, 3, 10, 30, 100, 300 ppb | 1 day | - | 0, 1, 3 ppb high survival rates. 10 ppb - 60% survival, 30ppb - 26% survival. Higher - 100% mortality. | Y | N/A |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 16 | Espedal, Glover, Horsberg & Nilsen, 2013 | 2008 - 2009 | Norway | EMB bioassay | 200 μm/L | 1 day | - | Resistant strain lice displayed high survival at 400 400 μm/L. Sensitive strain displayed 50% survival at 100 400 μm/L concentration | Y | N/A |
| 17 | Ljungfeldt et al., 2014 | 2008 - 2010 | Norway | EMB bioassay | 50 ppb | 20 - 23 hours | - | Differences between lice families - from 7.9% to 74% survival. In total, 37% lice survived the treatment. | Y | N/A |
| 18 | Whyte, Westcott, Elmoslemany, Hammell & Revie, 2013 | 2008 - 2011 | Canada | EMB bioassay | 400 and 800 ppb | 1 day | - | Proportion of dead lice was higher for female than male lice. | Y | N/A |
| 19 | Saksida et al., 2013 | 2010 - 2012 | Canada | EMB bioassay | 0, 10, 100, 200, 400, 800 ppb and 0, 31.3, 62.5, 125, 250, 500 ppb | 1 day | - | No exact numbers given. Female lice more sensitive. Lice collected from the farming area had significantly higher $EC_{50}$ (204.6 ppb instead of 126.9 ppb) | Y | N/A |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | Igboeli, Fast, Heumann & Burka, 2012 | 2011 | Canada | EMB bioassay | 0, 10, 100, 300, 1000, 3000 ppb | 1 day | - | $EC_{50}$ values in March: female 399.50 ppb, male 457.20 ppb. In July: male 315.30.ppb, female 279.30 ppb. In 2002 - 2004 $EC_{50}$ values were 4 - 26-fold lower. | Y | N/A |
| 21 | Poley et al., 2013 | 2012 | Canada | EMB bioassays and *in vivo* experiment | *In vivo* treatment: $150\mu g\ kg^{-1}$ biomass $d^{-1}$. Bioassay concentrations: 0.1, 25, 300, 1000 ppb | *In vivo*: 7 days. Bioassay: 24 h | 21 Atlantic salmon | Higher survival of resistant strain in all experiments. Bioassay with sensitive strain showed 100% survival at 100 ppb EMB | Y | N/A |
| 22 | Poley, Igboeli & Fast, 2015 | 2012 - 2013 | Canada | *In vivo* treatment with EMB | $150\mu g\ kg^{-1}$ biomass $d^{-1}$. | 7 days | 8 tanks with 50 - 55 salmon (2 units) | Mortality without immunostimulat feed given to fish: 73% males, 77% females. CPG immunostimulant and EMB: 42% males, 62% females. Aquate immunostimulant and EMB: 41% males, 74% females. | Y | N |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 23 | Igboeli, Purcell, Wotton, Poley, Burka & Fast, 2013 | 2012 - 2013 | Canada | *In vivo* treatment with EMB | 150µg kg⁻¹ biomass d⁻¹ | 7 days | 30 fish per tank (8 tanks) | Lice survival. EMB treatment only: 26.5% males and 22.8% females. EMB+SLX immunost. 57.7% males and 29.6% females. EMB+CpG: 58.1% males, 37.7% females. EMB+Aquate: 58.7% males, 25.8% females. | Y | N |
| 24 | Igboeli, Burka & Fast, 2013 | 2012 - 2013 | Canada | EMB bioassays, *in vivo* treatment | 5 concentrations 0 - 1000 µg l⁻¹ for bioassays. 150µg kg⁻¹ biomass d⁻¹ for *in vivo* treatment. | 24 hours (bioassay), 7 days (*in vivo*) | 20 salmon per treatment tank | All lice survived EMB bioassay at conc. less/= to 100 µg l⁻¹. EC₅₀ values were 329 and 304 µg l⁻¹ for adult male and pre-adult female lice, respectively. In vivo: mean number of lice decreased from 17.0 to 10.0 for resistant strain and from 15.7 to 1.2 for resistant strain cross, and from 11.0 to 2.6 for sensitive strain. | Y | N |
| 25 | Skilbrei et al., 2015 | 2013 | Norway | Intra-peritoneal injection | 400 µg kg⁻¹ fish | - | 254 salmon smolts | Effectiveness of EB was 7.8% - 25.4% | Y | Y |

| # | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 26 | Carmona – Antoñanzas et al., 2016 | 2015 - 2016 | Scotland | EMB bioassay | 400 µg l$^{-1}$, 800 µg l$^{-1}$, 1200 µg l$^{-1}$ | 1 day | - | Higher EMB concentrations required to provoke similar response in resistant strain. EC 50 for adult males ranged from 74.3 to 159.3 µg l$^{-1}$ for sensitive strain, from 553 to 780 µg l$^{-1}$ for EMB-resistant strain and 445 to 675 µg l$^{-1}$ for multi-resistant strain. | Y | N/A |
| 27 | Aaen & Horsberg, 2016 | 2016 | Norway | Bioassays with EMB | 50 mg l$^{-1}$ | 30 | - | Lethal to >70% larvae. Reduced hatching by 50%. | Y | Such dose would be too high for fish |

* Y -yes

N - no

N/A – not applicable

## Attachment 8 Cleaner fish treatment

**Summary of data about cleaner fish treatments**

| No. | Reference | Year | Place | Cleaner fish species | Stocking density | Method | Effect on lice number | Negative effect (salmon, cleaner fish) |
|---|---|---|---|---|---|---|---|---|
| 1 | Kvenseth, 1993 | 1993 | Norway | Wrasse | 31.2% at the beginning | Lice counting on salmon, stomach content analysis CF | 7 lice per wrasse stomach. | Large disappearance of wrasse (200 – 300 wrasse/week) |
| 2 | Treasurer, 1994 | 1994 | Scotland | Wrasse | 25% | Food content analysis. Lice count on salmon. | 26 – 46 lice per wrasse stomach. | No |
| 3 | Deady et al., 1995 | 1995 | Ireland | Wrasse | 1% | Examination of activities. Lice counting on salmon. Stomach content analysis. | Mean lice number on salmon lower than 5. | Large disappearance of wrasse |
| 4 | Skiftesvik, Bjelland, Durif, Johansen & Browman, 2013 | 2013 | Norway | Wrasse | 20% | Lice counting | Low number of lice on salmon. Approx. 4000 lice consumed by wrasse/week. | Low mortality, high number of escapes by wrasse. |
| 5 | Leclercq et al., 2014 | 2014 | Scotland | Wrasse | 5% | Lice counting | Lice number decreased below 0.5 per salmon (1.2 lice/fish before the treatment). | Biting to salmon when stocked with large wrasse (noted on 3 salmon within the trial). |
| 6 | Imsland et al., 2014 | 2014 | Norway | Lumpfish | 10% and 15% | Lice counting, stomach content analysis. | Significantly lower lice number than control. Chalimus by 10%, pre-adult by 40%, mature males by 58%, mature females by 97% | No |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 7 | Imsland et al., 2014a | 2014 | Norway | Lumpfish | 10% | Behaviour observation. Lice counting. Feed intake calculation. | Less lice than control group (difference by 0.5 to 1 lice per salmon) | No |
| 8 | Imsland et al., 2014b | 2014 | Norway | Lumpfish | 10% and 15% | Lice counting. Specific growth rate calculation. | First trial: 60% and 56% less lice than controls. Second trial: 1 – 1.5 lice per salmon compared to control with 2.4 lice/salmon | Lower salmon feed conversion ratio when stocked with large lumpfish. |
| 9 | Imsland et al, 2015 | 2015 | Norway | Lumpfish | 10% and 15% | Behavioural observation. Stomach content analysis. | 33 – 38% of lumpfish had ingested lice. | No |
| 10 | Imsland et al., 2016 | 2016 | Norway | Lumpfish | 20% | Lice counting. Behavioural observation. Specific growth rate calculation. Lice counting. | 15% lice consumption by lumpfish. Average number of lice on salmon 43 – 92% lower than in sea pens without lumpfish. | No. Some lumpfish mortality because of *Pasteurella* spp. |
| 11 | Imsland, et al., 2016 | 2016 | Norway | Lumpfish | 10% | Lice counting. Stomach content analysis. | Percentage of consumed lice from 0 to 25%. Total lice number on salmon 40% lower in lumpfish than control group. | No |
| 12 | Eliasen, Danielsen, Johannesen, Joensen & Pattursson, 2018 | 2018 | Faroe Islands (Denmark) | Lumpfish | - | Stomach content analysis | 743 of 5511 lumpfish had sea lice in their stomachs. | No |

*CF – cleaner fish