Does exposure to sea lice from aquaculture have a population-reducing effect on wild Atlantic salmon? A systematic review protocol

Rytwinski T[1], Larsen, ML[2,3], Lennox RJ[4], Gargan PG[5], Harper M[1], Cooke SJ[1], Vollset KW[6]

[1] Canadian Centre for Evidence-Based Conservation, Department of Biology and Institute of Environmental and Interdisciplinary Sciences, Carleton University, 1125 Colonel By Drive, Ottawa, Canada

[2] The Fridtjof Nansen Institute, Fridtjof Nansens vei 17, 1366 Lysaker, Norway

[3] Department of Biological Sciences, University of Bergen, Thormøhlensgate 53 A, 5006 Bergen

[4] Ocean Tracking Network, Faculty of Science, Dalhousie University, 1355 Oxford St, Halifax, Nova Scotia, B3H 3Z1 Canada

[5] Inland Fisheries Ireland, 3044 Lake Drive, CityWest Business Campus, Dublin 24, Ireland

[6] Laboratory for Freshwater Ecology and Inland Fisheries, NORCE Norwegian Research Centre, Nygardsgaten 112, 5008 Bergen, Norway

Correspondence: T. Rytwinski; Email: Trina.Rytwinski@carleton.ca

Abstract

Background: Wild Atlantic salmon are increasingly threatened throughout their distribution in north Atlantic rivers. Ectoparasitic sea lice have the potential to cause severe damage to salmon, especially the smolts early in the marine migration; these lice proliferate in areas where fish farming is prevalent due to the high density of hosts. Many studies have attempted to investigate how lice infestation pressure affects salmon population recruitment, however, there has not been a compelling systematic review of this topic. Here, we present a protocol for a systematic review that will collate and synthesize the evidence base related to population-level effects of sea lice (both *Caligus* and *Lepeoptherius*) from aquaculture on wild Atlantic salmon (*Salmo salar*). This review will include a comprehensive assessment of the comparative validity of the selected studies (i.e., critical appraisal), a step that is currently lacking from previous synthesis efforts, but one that is essential to improve our understanding of the existing evidence base and ensure review results are reliable.

Methods: This systematic review will use published and grey literature without date restriction. Searches will be performed using three bibliographic databases, two search engines, and networking tools to identify relevant literature. Eligibility screening will be conducted at two stages: 1) title and abstract, and 2) full text. Studies written in English or Norwegian that evaluate the impact of sea lice exposure from copepod salmon louse (*Lepeophtheirus salmonis*) and *Caligus elongatus* on the abundance, marine survival, or adult returns of wild and/or hatchery produced Atlantic salmon will be included. Included studies will undergo critical appraisal of internal and external study validity. We will extract information on study characteristics, study design, exposure and comparator details, measured outcomes (abundance, survival, adult returns), and potential sources of heterogeneity (e.g., release location, time period). A narrative synthesis will be used to describe the quantity and characteristics of the evidence base, while quantitative synthesis (i.e., meta-analysis) will be conducted to estimate an overall population-level effect of sea lice from aquaculture on wild Atlantic salmon when sufficient numbers of similar studies are available.

Keywords: Emamectin benzoate, evidence synthesis, fish farming, management, parasite, salmonid, smolt, substance EX

Background

The parasitic copepod salmon louse is one of the biggest concerns in Atlantic salmon producing countries in the world (Forseth et al., 2017). At high density, the parasite can cause direct physical and physiological harm to an individual Atlantic salmon as well as being a mechanism causing secondary infections (Nolan et al., 1999; Finstad et al., 2000; Wagner et al., 2003), raising concerns about animal welfare and conservation of the wild Atlantic salmon. High densities of salmon in farms support epidemic proportions of lice and in recent decades, concerns about the collateral effect of farm-origin lice on wild salmon have amplified (Vollset et al., 2017). Among other concerns about the impacts of fish farms is the proliferation of sea lice and the potential for epidemics because a dramatic increase in host densities increases infestation pressure of lice during outmigration of young of the year (i.e., post-smolts) from the rivers (Krkosek, 2017). This infestation from natural sources to farmed fish and re-infestations from farms back to wild fish is termed "spillback" (Bouwmeester et al., 2021). The effect of parasites has been curtailed by treatment using chemicals, but the development of resistant strains of salmon lice in almost all places of the world and few new chemicals allowed on the market the last 10-15 years (Fjørtoft et al., 2019). Although multiple non-chemical treatments have been developed (e.g., use of cleaner fish, various flushing techniques), there is limited information available about their delousing efficiency and it is generally agreed that they can cause large animal welfare and conservation issues (Gismerviket al., 2019; Nilsson et al., 2019; Overton et al., 2019).

Lice numbers on wild fish in areas with fish farms can be much higher than lethal thresholds that have been established as national guidelines. In Norway, for example, estimates of salmon lice induced mortality is based on a threshold level of lice on individual fish, which has over several years been estimated to be more than 30% of all out-migrating salmon smolts in the most intensive farming areas (Taranger et al., 2015). The total biomass of farmed fish remains too high, despite adherence to site-level regulations regarding the average number of sea lice per farmed fish. This is putting pressure on management around the world to reduce farmed biomass or regulate farmers to take steps to reduce impacts on wild fish. This will however have large economic consequences for the local economy, and to fish welfare (Costello, 2009; Overton et al., 2019; Iversen et al., 2020; Larsen and Vormedal, 2021). Consequently, the evidence that sea lice have an effect on populations of salmon is an extremely divisive and politically charged question.

Estimating population-level effects of sea lice on wild salmon populations is inherently challenging. This is because, for instance, obtaining quantitative estimates of overall stock abundance would require full trapping facilities operated year-round, of which, there are very few in existence in Europe (Thorstad et al., 2014), and salmonid survival is highly variable and strongly linked to environmental conditions during early life stages (Cushing, 1975; Friedland et al., 2014). Furthermore, the sublethal effects of sea lice likely interact with other components of survival, such as competition or predation risk (Godwin et al., 2015), making it difficult to use certain types of data (e.g., observational) to separate the role of the parasite from other effects (Vollset et al., 2016). Additionally, there are very few observations/samples of salmon after they leave the coast where we expect the virulent effect of sea lice to occur. Salmon are infested with lice during their first few weeks at sea but the development to mobile lice, which is the stage that does the most damage, may take several days and weeks depending on temperature (Hamre et al., 2019). Consequently, studies on the population-level impacts of lice have been limited to (1) field based experimental studies where groups of hatchery produced (or in some cases wild fish) are released as smolts and recaptured as adults to estimate survival and compare the survival of differentially treated groups that are (a) given anti-parasitic drugs or not, (b) directly infested with salmon lice or not, or (c) towed out to the coast/sea to avoid or reduce lice exposure or not, and (2) field based observational studies where observations between adult returns or marine survival has been correlated with an estimate of lice infestation pressure.

Previous efforts to describe and/or synthesize the literature evaluating the population-level impacts of salmon lice on wild Atlantic salmon have focused primarily on experimental studies on the mortality impact of salmon lice on out-migrating smolts, using chemically treated and untreated fish groups in Norway and/or Ireland (e.g., Jackson et al., 2013; Krkošek et al., 2013; Vollset et al., 2016). To our knowledge, there has not been a rigorous, systematic review that included (1) studies from multiple countries, (2) studies using experimental and/or observational study designs to evaluate population-level effects of salmon lice on wild salmon, and (3) a comprehensive assessment of the comparative validity of the included studies (i.e., critical appraisal). Critical appraisal is an assessment of how well the studies included in the review are conducted and thus, how reliable their findings are. Two aspects of study validity are generally assessed: (1) internal validity (study reliability): is there potential for error and bias in the methodology employed to generate the study data, and (2) external validity (generalisability): how transferable is the study to the context of the question (Collaboration for Environmental Evidence [CEE], 2022). If bias is present in primary studies, their results will be incorrect. Subsequently, if a systematic review is based on incorrect evidence, the results of the meta-analysis will also be incorrect, resulting in misleading conclusions (Boutron et al., 2019). Systematic reviews are particularly attractive to decision makers when evidence from different sources conflicts, especially if accompanied by a quantitative synthesis that can weigh the conflicting evidence according to some measure of its reliability (e.g., the inverse of the effect size variance) or partition studies into subgroups for separate analyses (Rytwinski et al., 2021). Systematic reviews can also provide transparent and objective assessments where topics are controversial or high profile (Haddaway and Pullin, 2014), as in the case here with the impacts of salmon lice on wild salmon populations.

Our proposed systematic review will be complementary to previous efforts, but broader in scope, and include a critical appraisal of the included studies. Also, to improve the rigor, transparency, and repeatability of our methods, this synthesis is developed adopting best practices from the Collaboration for Environmental Evidence (CEE, 2022).

Identification of review topic and stakeholder engagement

At their annual meeting in June 2022, the North Atlantic Salmon Conservation Organization (NASCO) Council identified the need for a State of Knowledge paper on the impacts of sea lice on Atlantic salmon (and other related topics, e.g., escaped farmed salmon on wild salmon stocks). During early planning of this review, a group of topic experts from academia and government was established to lay out project needs, goals, and potential challenges (i.e., the initial advisory team). This group then collaborated with evidence synthesis experts from the Canadian Centre for Evidence-Based Conservation (CEBC) with the ultimate goal of conducting a systematic review that allowed for a rigorous and comprehensive examination of the impacts of sea lice from aquaculture on wild Atlantic salmon. In February of 2023, this group of topic and evidence synthesis experts held a workshop in Scotland to lay out the project plan, including preliminary development of the risk of bias assessment. Following this workshop, additional topic experts were invited to join the advisory team for consultation during the development of this protocol. The advisory team consists of fisheries ecologists, and molecular biologists/ecological epidemiologists, and includes academics (2 members; University of Bergen, University of Maine), staff from Inland Fisheries Ireland (1 member), Department of Fisheries and Oceans Canada (1 member) and Marine Scotland Science, and researchers from the Institute of Marine Research in Norway (1 member). The role of the advisory team is crucial to many aspects of the systematic review, including suggestions on where to search for relevant evidence (especially grey literature), generation of the benchmark list, the development of the inclusion criteria for article screening and data extraction strategy, and identifying risks of bias. The advisory team will continue to participate in the systematic review through to completion.

Objective of the review

The objective of this systematic review is to evaluate the existing literature base to determine whether exposure to sea lice from aquaculture has a population-reducing effect on wild adult Atlantic salmon.

Components of the review question

Population (subject): Wild and/or farmed Atlantic salmon (*Salmo salar*) in all regions of the North Atlantic.

Exposure: Exposure of Atlantic salmon to sea lice i.e., *Lepeophtheirus salmonis* and *Caligus elongatus*.

Comparator: No exposure to sea lice which could include a comparison between (1) groups of fish in experimental studies (i.e., a group(s) of anti-parasitically treated salmon, a shame group(s) not exposed to sea lice, group(s) of displaced/towed salmon to avoid exposure to sea lice), (2) study sites (control/impact design with sites with no fish farming), (3) over time (before/after or time-series with time periods with no fish farms or low infestation pressure), or (4) the combination of 2 and 3 above (before/after/control/impact). Additionally, this could include comparative studies of an alternative level(s) of exposure (e.g., low versus high infestation pressure) or antiparasitic treatment. However, no studies will be excluded based on the presence or absence of a comparator.

Outcomes: Studies reporting measured effects that indicate a potential change in population status using either a direct metric of adult salmon abundance (e.g. abundance, density, CPUE) or marine survival, or an indirect metric related to adult returns (e.g., proportion of adult returns in relation to the number of out migrating smolts, or number of returning adults or a subset of returns i.e., raised data).

Methods

The proposed review will follow standards and guidelines from the CEE (2022) and the reporting standards from ROSES (Haddaway et al., 2018) (see Supporting Information 1).

Searching for articles

Search terms and language

A list of potentially relevant search terms in English and Norwegian was generated by the review team (i.e., a subset of the advisory team including both topic and evidence synthesis experts) and divided into two components: the population and exposure. The review team then developed a set of search strings that were modified and refined iteratively through a scoping exercise using Web of Science Core Collections which evaluated the sensitivity of the search terms and associated wildcards (Table 1). Search terms and associated strings were also vetted by librarians at the University of Bergen and the Norwegian Institute for Nature Research (NINA) in Norway.

No document type, date, or language restrictions will be applied during the search (i.e., if an abstract was in English but the article was in a different language), but only English and Norwegian language literature was included during the screening stage.

All bibliographic databases will be accessed using University of Bergen's institutional subscriptions. When complex search strings are not accepted, search strings will be customized and included in the final report.

Table 1. Proposed search string that will be used to execute searches formatted for Web of Science Core Collection.

Component	String
Population	TS=("atlantic salmon" OR salmo)
	AND
Exposure	TS=(lepeophtheirus OR "salmon louse" OR "salmon lice" OR "sea louse" OR "sea lice" OR lakselus OR caligus OR skottelus)

Publication databases
The following online bibliographic databases will be searched:

1. ISI Web of Science Core Collection — multidisciplinary research topics including journals, books, proceedings, published data sets and patents.
2. Scopus — abstract and citation database of peer reviewed literature including journals, books, and conference proceedings.
3. ProQuest Dissertation & Theses Global — collection of dissertations and theses from around the world, spanning from 1743 to present.

Search engines
Internet searches will be performed to identify additional published and grey literature using two search engines: (1) Google Scholar, and (2) Oria (https://en.uit.no/ub/searchhelp/oria-search). Results will be sorted by relevance and a reasonably sized subset will be screened for appropriate fit with the review question. If the reviewer determines that the level of relevance of each article significantly declines (i.e., no new inclusions for 50 consecutive titles) before reaching that point, the reviewer will stop (Livoreil et al., 2017).

Supplemental searches
To supplement the above searches, the bibliographies of relevant review articles identified during the search will be manually searched for any additional relevant articles or datasets that are not already captured by the search strategy. Authors of any unpublished references will be contacted to request access to the full article. Additionally, a call for grey literature will be circulated via professional networks to solicit articles for inclusion in this synthesis. Furthermore, social media and email will be used to alert the community of this synthesis and to reach out to recognized experts and practitioners for further recommendations and provision of relevant unpublished material, with no date or language restrictions. Sources of information retrieved through these supplemental searches will be recorded in the database.

Estimating comprehensive of the search
The comprehensiveness of the search string was tested using a list of benchmark papers (Supporting Information 2) that were identified as relevant for this review by the advisory team.

Search record database

All articles found by database and search engine searches will be exported into the EndNote database into separate groups. Once all searches are complete and references from each strategy have been compiled, individual databases will be exported into MS-excel for backup. Then duplicates will be identified and merged in EndNote. All references, regardless of their likely relevance to the systematic review, will be included in the database so that it can act as an archive. The merged database will be exported into MS-excel as a single database. This database is a direct product of the search strategy and will not be changed during the review process. The database will therefore be useful when updating the systematic review archive in the future.

Article screening and study eligibility criteria

Screening process

Articles will be screened at two stages: (1) title and abstract, and (2) full text. Documents found through databases and search engines will be screened at title and abstract. Before screening begins, two reviewers using a random subset of 10% of all articles will undertake consistency checks at both stages to ensure consistent and repeatable decisions are being made. The results of the consistency checks will be compared between reviewers and all discrepancies will be discussed to understand why an inclusion/exclusion decision was made. Revisions to the inclusion criteria will be made as necessary. Following the consistency check, articles will be screened by one experienced reviewer. Articles or datasets found by other means (i.e., searching bibliographies of relevant reviews, social media, etc.) will enter at the second stage of this screening process (i.e., full text) but will not be included in consistency checks. If the reviewer is uncertain whether to include an article at any screening stage, they will tend toward inclusion to the next stage. If there is further doubt, the review team will discuss those articles as a group and come to a decision. If a decision could not be made by the review team, the query will be brought to the broader advisory team for discussion until a decision can be made. A list of studies excluded at full text will be provided in an additional file together with reason for exclusion. Digital media will be screened, when they are available online without the need for purchasing the media or having specialized pay-for-use software to view it. The Interlibrary Loans program at University of Bergen or Carleton University will be used to acquire hard or digital, full-text copies of any articles that are included once the title and abstract screening has occurred. Reviewers will not screen studies (at title and abstract or full text) for which they are an author.

Eligibility criteria

All articles will be screened according to the predefined criteria developed in consultation with the advisory team (Table 2) and will only be included when all criteria are met.

Table 2. Article inclusion and exclusion criteria

Included	Excluded
Population	
Wild or farmed/hatchery produced Atlantic salmon (*Salmo salar*) in any regions.	Any fish species other than Atlantic salmon e.g., Brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*), Sockeye salmon (*Oncorhynchus nerka*). **Note, during screening, studies that meet all other criteria except they did not mention Atlantic salmon in the title or abstract but related to another marine fish species, were moved forward for further consideration at full text in case it contained relevant information on Atlantic salmon.*
Exposure	
Articles that include Atlantic salmon exposure to sea lice, including the species of copepod salmon louse (*Lepeophtheirus salmonis*) and *Caligus elongatus*. All life stages of copepods will be considered (planktonic nauplius, copepodid, chalimus, preadult and adult).	Any other copepod or parasitic species e.g., cod louse (*Caligus curtus*), *Gyrodactylus* sp. Salmon diseases not related to sea lice (e.g., cardiomyopathy syndrome, pancreas diseases, salmonid rickettsial septicaemia, infectious pancreatic necrosis, heart and skeletal muscle inflammation, infectious salmon anaemia and gill diseases.
Comparator	
Relevant comparators include: (1) no exposure to sea lice (a) for fish groups in experimental studies (i.e., a group(s) of anti-parasitically treated salmon, a shame group(s) not exposed to sea lice, group(s) of displaced /towed salmon to avoid exposure to sea lice), (b) between study sites, and/or (c) over time, or (2) comparison with an alternative level(s) of exposure (e.g., low versus high infestation pressure) or antiparasitic treatment.	No studies were excluded based on a comparator (or lack thereof).
Outcome	
Studies must report measured effects that indicate a potential change in population status using either a direct metric of adult salmon abundance (e.g. abundance, density, CPUE) or marine survival, or an indirect metric related to adult returns (e.g., proportion of adult returns in relation to the number of out migrating smolts, or number of returning adults or a subset of returns i.e., raised data).	Studies only reporting individual-level measured effects such as body size (e.g. length, weight), growth rate, body condition, swimming speed, changes in maturation, physiological outcomes, behavioral outcomes (e.g. changes in feed behaviour, activity patterns), visible injuries (tissue damage), and osmoregulation.
Study design	
Field-based experimental or observational studies. Studies that used a randomized [RCT] or non-randomized control trial [CT]), control/impact (CI), before/after (BA), before/after/control/impact (BACI), or time series (TS).	Purely theoretical or laboratory studies, review or expert opinion papers, and policy discussions.
Language	
English or Norwegian at full text.	Any study that was not in English or Norwegian at full text.

Study validity assessment

Articles that are found to be relevant to this review at the full-text screening stage will then undergo a study validity assessment. This critical appraisal will be carried out on a study-by-study basis rather than article-by-article; meaning if a single article reported more than one experiment/observation, these will be regarded as separate studies. For example, if a single article reported more than one release location and/or used different designs/experimental setups (e.g., described a case study using an RCT and a time series), separate study validity assessments will be recorded. The focus of the assessment will be on the internal study validity (i.e., reliability/susceptibility of each study to bias). External validity (study generalizability) will be captured during screening, and with a single external validity question described further below.

Data will be extracted from each relevant study in a detailed and transparent manner and entered in a MS-Excel worksheet. The information from the assessments will be used to describe studies in the narrative synthesis for both internal and external validity, and in sensitivity analyses during the quantitative synthesis (if performed, and only in relation to internal validity). Critical appraisal will be done by at least two reviewers on a subset of articles to ensure consistency in assessments and, when unsure, the reviewers will come together to discuss. Final decisions regarding doubtful cases will be taken by the review team as a whole. No studies will be rejected based on validity assessment; however, a sensitivity analysis will be carried out to investigate the influence of internal validity categories in the quantitative analysis when the evidence-base allows. Reviewers will not assess studies for risk of bias for which they are an author.

Critical appraisal will incorporate FEAT (focused, extensive, applied and transparent) principles as recommended by Frampton et al. (2022) and the recommendation guidelines from the CEE (2022). To define the initial criteria of this appraisal, the advisory team held a 2-day workshop in February 2023 to discuss the gold standard protocol of an ideal study answering our primary question with unlimited resources. Subsequently, using these initial criteria, a critical appraisal tool was developed by modifying the Critical Appraisal Tool version 0.3 (prototype) (Konno et al., 2021; CEE, 2022). This tool was further refined in consultation with additional topic experts added to the advisory team to ensure that it accurately identified and reflected the risks of bias for this topic/evidence base. A draft of the critical appraisal tool can be found in Supporting Information 3. For internal validity, studies will be classified on three levels of overall risk of bias: low risk of bias, medium risk of bias, or high risk of bias. To meet these criteria, articles will be assessed on up to eight criterion depending on the study design used, including: (1) risk of confounding biases prior to the exposure (i.e., baseline imbalances due to the selection of fish or sites into studies before exposure to sea lice) (all study designs), (2) risk of post-exposure selection biases (i.e., non-random or non-systematic sampling) (all study designs), (3) risk of misclassified comparison biases (i.e., lacking well defined exposure and comparators) (observational studies only), (4) risk of information and performance biases (i.e., deviations from intended antiparasitic treatment) [(R)CTs with antiparasitic treatment studies only], (5) risk of performance bias (i.e., deviations from the intended exposure) [(R)CTs designs only], (6) risk of detection biases (i.e., use of an inappropriate outcome for the intended assessment of causal effect) (all study designs), (7) risk of outcome reporting bias (i.e., missing data) (all study designs), and (8) risk of outcome assessment bias (i.e., inappropriate statistical methods) (all study designs). Questions within each criterion will be answered as “Yes”, “No”, “Unclear” or “Not applicable”, and the combined influence of these questions will be used to determine if each criterion has high, medium or low risk of bias. To determine the overall risk of bias (internal validity), if any of the criteria for a given study design type are identified as having a high risk of bias, the overall risk of bias for the study will be *high*. If no criteria are at high risk of bias, but at least one criterion is identified as having medium risk of bias, the overall risk of bias for the study will be *medium*. Studies with *low* risk of bias will therefore have no criteria with high or medium risk of bias (Konno et al., 2021).

External validity will be assessed separately from internal validity using a single study generalizability question: Are the subjects (fish population) representative of the wild population? Three answer categories will be used to define external validity:

- *Green (high external validity)*: A) Subjects used in the study (for both exposure and comparator groups) were all wild Atlantic salmon (i.e., the phenotype, genotype, and length distributions representative of the wild population); B) for observational studies, population is not supplemented by hatchery fish or if supplementation occurred, these data were removed from analysis;
- *Yellow (medium external validity)*: A) Subjects used in the study were a mixture of wild Atlantic salmon and hatchery origin (whether from wild sourced brood stock or not); B) Subjects were all hatchery origin but from wild sourced brood stock (i.e., similar genetic composition but possibly not representative of size of wild fish);
- *Red (low external validity)*: A) Subjects used in the study were all hatchery origin Atlantic salmon not from brood stock wild populations (i.e., not representative of wild population); B) unreported - author(s) do not state where subjects used in the study came from.

The information from this external validity assessment will be used to describe studies in the narrative synthesis only.

Data coding and extraction strategy

Meta-data from studies included at full text will be extracted by the review team and recorded in a MS-Excel spreadsheet. When sufficient, good quality data exists, the information will be used in meta-analysis. The following main categories of descriptive data will be extracted for all included studies and used in narrative synthesis: (1) bibliographic information; (2) study location (e.g., country, province/state, release river); (3) study design details (e.g., study/release dates, study type [e.g., experimental, observational], study design [e.g., [R]CT, BA, CI, BACI, time series]); (4) population details (e.g., source [wild and/or hatchery Atlantic salmon], origin of smolts [cultivated from wild broodstock, farmed origin from genetically improved strain, wild caught], age and size of smolts); (5) intervention/exposure (e.g., species of parasitic copepod [salmon louse, Scottish louse]; level of exposure/infestation [e.g., number of lice, number of fish on farms, out-migration infestation pressure]; chemical treatment type and dose) and comparator details (e.g., untreated [no chemical treatment], no exposure [to sea lice], alternate level of treatment or exposure, no comparator); (6) outcome details (e.g., abundance of adult salmon [e.g. abundance, density, CPUE), marine survival, adult returns; age of adult salmon [e.g., 1 sea winter, 2 sea winter, 3 sea winter]) and sampling methods (e.g., type, size); (7) effect modifiers (see below); and (8) study validity assessment results. This list may be expanded depending on the type and variety of included studies. Coding options within these key variables will be compiled in a partly iterative process, expanding the range of options as they are encountered during extraction.

Some quantitative data that will be recorded include: (1) experimental studies: number of released control and treatment smolts, number of recaptured control and treatment fish; (2) observational studies: sample size (e.g., study sites (CIs), time periods (BA, time series), outcomes (e.g., adult returns [e.g., totals, means, subsets (raised data)], number of out-migrating smolts, correlation coefficients), and measures of variation (e.g., standard deviation, standard error, confidence intervals). When information is present in tables or graphs, all information will be extracted; if it is not possible to interpret the information from graphs, the corresponding author will be contacted (via email) if time permits or imaging software (i.e., WebPlotDigitizer; Rohatgi 2022) will be used. When only raw data are included in the article, the review team will calculate summary statistics and will record how the calculations were conducted and what information was used. All extracted data will be made available with the final report. Reviewers will not extract data from studies for which they are an author.

To ensure that quantitative data are being extracted in a consistent and repeatable manner, reviewers will extract information from a random subset of articles included at full text at the beginning of the process. The information will be compared, and any inconsistencies will be discussed with the review team. If any disagreements occur the entire review team will discuss them, and modifications to the extraction codebook will be made where needed to ensure reviewers are extracting and interpreting data in the same manner.

Potential effect modifiers and reasons for heterogeneity

Potential reasons for heterogeneity will be identified to obtain a better understanding of the factors that are associated with variation in effects among studies. The review team will extract data on potential effect modifiers from articles that are included at the full-text level of screening. All information will be recorded in the MS-Excel database. The following list is not exhaustive but includes potential effect modifiers and reasons for heterogeneity which will be recorded where available. Further factors will be identified, defined, and included throughout the process, through consultation with the advisory team.

- Publication type (peer-reviewed, grey literature)
- Release location (Fjord, river/estuary)
- Release period (release years pooled into categorical time periods, release quartiles)
- Temperature (average temperature in migration path)
- Treatment type (Emamectin, Substance EX)

Data synthesis and presentation

A narrative synthesis of all eligible articles in the systematic review will be generated. The synthesis will aim to be as visual as possible, describing the validity of the results and summarizing findings in figures and tables. The goal of this review is to determine the overall population-level effect of sea lice from aquaculture on wild Atlantic salmon, as such, we will aim to conduct quantitative synthesis. Meta-analyses of effect sizes will be conducted when study designs and data formalizations of the included studies are sufficiently homogeneous. Separate subgroup analyses will be conducted for experimental and observational study types, as well as outcome types (e.g., abundance, survival). In the case that meta-analysis is possible, study effect sizes will be standardized and weighted appropriately, and analysis will take the form of random-effects models. Depending on the availability of the data, meta-regression or subgroup analysis of studies will also be performed. Risk of publication bias will be assessed through funnel plots and Egger's regression test. Sensitivity analysis we also be carried out to investigate the influence of study validity categories when possible.

Supporting information

Supporting Information 1. ROSES form for Systematic Review Protocols.

Supporting Information 2. **Benchmark list**. Includes the list of benchmark articles used to test the comprehensiveness of the search string.

Supporting Information 3. Draft critical appraisal form. Includes a draft of the critical appraisal tool for internal and external study validity assessment.

Acknowledgements

The authors would like to sincerely thank advisory team members who provided valuable insight to strengthen this systematic review protocol, especially in relation to the development of the draft critical appraisal tool, including Sussie Dalvin, Sam Shephard, Sandy Murray, and Frank Nilsen.

Authors' contributions

All authors conceived the ideas and designed methodologies. TR and KWV led the writing of the manuscript. All authors provided comments and revisions. All authors contributed critically to the drafts and gave final approval.

Funding

The review will be primarily supported by the North Atlantic Salmon Conservation Organization. Additional support will be provided by the Natural Science and Engineering Research Council of Canada and Carleton University, Research Council of Norway.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent of publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

Boutron, I., Page, M.J., Higgins, J.P.T., Altman, D.G., Lundh, A., Hróbjartsson, A. (2019). Chapter 7: considering bias and conflicts of interest among the included studies. In: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (Eds.), Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated August 2023). http://www.training.cochrane.org/handbook.

Bouwmeester, M. M., Goedknegt, M. A., Poulin, R., & Thieltges, D. W. (2021). Collateral diseases: aquaculture impacts on wildlife infections. Journal of Applied Ecology, 58(3), 453-464.

[CEE] Collaboration for Environmental Evidence. (2022). Guidelines and Standards for Evidence Synthesis in Environmental Management. Version 5.1. https://environmentalevidence.org/information-for-authors/

Costello, M.J. (2009). The global economic cost of sea lice to the salmonid farming industry. Journal of Fish Disease, 32, 115–118. https://doi.org/10.1111/j.1365-2761.2008.01011.x.

Cushing, D.H. (1975). Marine Ecology and Fisheries. Cambridge University Press, Cambridge.

Finstad, B., Johnsen, B. O., Hvidsten, N. A. (2000). Laboratory and field investigations of salmon lice *[Lepeophtheirus salmonis* (Kroyer)] infestation on Atlantic salmon (*Salmo salar* L.) post-smolts. Aquaculture Research, 31(11), 795−803.

Fjørtoft, H. B., Nilsen, F., Besnier, F., Stene, A., Bjørn, P. A., Tveten, A.-K., Aspehaug, V. T., Finstad, B., Glover, K. A. (2019). Salmon lice sampled from wild Atlantic salmon and sea trout throughout Norway display high frequencies of the genotype associated with pyrethroid resistance. Aquaculture Environment Interactions, 11, 459–468. https://www.jstor.org/stable/26920856

Forseth, T., Barlaup, B.T., Finstad, B., Fiske, P., Gjosater, H., Falkegard, M., Hindar, A., Mo, T.A., Rikardsen, A.H., Thorstad, E.B., Vollestad, L.A., Wennevik, V. (2017). The major threats to Atlantic salmon in Norway. ICES Journal of Marine Science, 74, 1496–1513. https://doi.org/10.1093/icesjms/fsx020.

Frampton, G., Whaley, P., Bennett, M., Bilotta, G., Dorne, J. L. C., Eales, J., James, K., Kohl, C., Land, M., Livoreil, B., Makowski, D. (2022). Principles and framework for assessing the risk of bias for studies included in comparative quantitative environmental systematic reviews. Environmental Evidence, 11(1), 12. https://doi.org/10.1186/s13750-022-00264-0

Friedland, K.D., Shank, B.V., Todd, C.D., McGinnity, P., Nye, J.A. (2014). Differential response of continental stock complexes of Atlantic salmon (*Salmo salar*) to the Atlantic Multidecadal oscillation. Journal of Marine Systems 133, 77–87. [In English].

Gismervik, K., Gasnes, S.K., Gu, J., Stien, L.H., Madaro, A. (2019). Thermal injuries in Atlantic salmon in a pilot laboratory trial. Veterinary and Animal Sciences 8, 100081. https://doi.org/10.1016/j.vas.2019.100081.

Godwin, S.C., Dill, L.M., Reynolds, J.D. and Krkošek, M. (2015). Sea lice, sockeye salmon, and foraging competition: lousy fish are lousy competitors. Canadian Journal of Fisheries and Aquatic Sciences 72, 8.

Haddaway, N.R., Pullin, A.S. (2014). The policy role of systematic reviews: past, present and future. Springer Science Reviews 2, 179–183.

Haddaway, N.R., Macura, B., Whaley, P., Pullin, A. S. (2018). ROSES RepOrting standards for systematic evidence syntheses: Pro forma, flow-diagram and descriptive summary of the plan and conduct of environmental systematic reviews and systematic maps. Environmental Evidence, 7, 7. https://doi.org/10.1186/s13750-018-0121-7

Hamre, L.A., Bui, S., Oppedal, F., Skern-Mauritzen, R., Dalvin, S. (2019). Development of the salmon louse *Lepeophtheirus salmonis* parasitic stages in temperatures ranging from 3 to 24^{o}C. Aquaculture Environment Interactions, 11, 429-443. https://doi.org/10.3354/aei00320

Iversen, A., Asche, F., Hermansen, O., Nystoyl, R. (2020). Production cost and competitiveness in major salmon farming countries 2003–2018. Aquaculture 522, 735089. https://doi.org/10.1016/j.aquaculture.2020.735089.

Jackson, D., Cotter, D., Newell, J., McEvoy, S., O'Donohoe, P., Kane, F., McDermott, T., Kelly, S., Drumm, A. (2013). Impact of *Lepeophtheirus salmonis* infestations on migrating Atlantic salmon, *Salmo salar* L., smolts at eight locations in Ireland with an analysis of lice-induced marine mortality. Journal of Fish Disease, 36, 273−281.

Konno, K., Livoreil, B., Pullin, A. (2021). Collaboration for Environmental Evidence Critical Appraisal Tool version 0.3 (prototype). Collaboration for Environmental Evidence. https://environmentalevidence.org/cee-critical-appraisal-tool/

Krkošek M. 2017. Population biology of infectious diseases shared by wild and farmed fish. Canadian Journal of Fisheries and Aquatic Sciences, 74 (4), 620-628.

Krkošek, M., Revie, C. W, Gargan, P. G, Skilbrei, O. T, Finstad, B., Todd, C. D. (2013). Impact of parasites on salmon recruitment in the Northeast Atlantic Ocean. Proceedings of the Royal Society B, 280(1750), 20122359. http://dx.doi.org/10.1098/rspb.2012.2359

Larsen, M. L., Vormedal, I. (2021). The environmental effectiveness of sea lice regulation: Compliance and consequences for farmed and wild salmon. Aquaculture 532, 736000. https://doi.org/10.1016/j.aquaculture.2020.736000

Livoreil, B., Glanville, J., Haddaway, N. R., Bayliss, H. R., Bethel, A., de Lachapelle, F. F., Robalino, S., Savilaakso, S., Zhou,W., Petrokofsky, G., Frampton, G. (2017). Systematic searching for environmental evidence using multiple tools and sources. Environmental Evidence, 6(1), 1–14. https://doi.org/10.1186/s13750-017-0099-6

Nilsson, J., Moltumyr, L., Madaro, A., Kristiansen, T.S., Gasnes, S.K., Mejdell, C.M., Gismervik, K., Stien, L.H. (2019). Sudden exposure to warm water causes instant behavioural responses indicative of nociception or pain in Atlantic salmon. Veterinary and Animal Sciences, 8, 100076. https://doi.org/10.1016/j.vas.2019.100076.

Nolan, D. T., Reilly, P., Wendelaar Bonga, S. E. (1999). Infection with low numbers of the sea louse *Lepeophtheirus salmonis* induces stress-related effects in post-smolt Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences, 56 (6), 947–959. https://doi.org/10.1139/f99-021.

Rohatgi, A. (2022). WebPlotDigitizer: HTML5 based online tool to extract numerical data from plot images.

Rytwinski, T., Cooke, S. J., Taylor, J. J., Roche, D. G., Smith, P. A., Mitchell, G. W., Smokorowski, K. E., Prior, K. A., Bennett, J. R. (2021.) Acting in the face of evidentiary ambiguity, bias, and absence arising from systematic reviews in applied environmental science. Science of the Total Environment, 775, 145122. https://doi.org/10.1016/j.scitotenv.2021.145122

Taranger, G. L., Karlsen, Ø., Bannister, R. J., Glover, K. A., Husa, V., Karlsbakk, E., Kvamme, B. O., Boxaspen, K. K., Bjørn, P. A., Finstad, B., Madhun, A. S., Morton, H. C., Svåsandl, T. 2015. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES Journal of Marine Science, 72, 997–1021.

Thorstad, E. B., Todd, C. D., Bjørn, P. A., Gargan, P. G., Vollset, K. W., Halttunen, E., Kålås, S., Uglem, I., Berg, M., Finstad, B. (2014). Effects of salmon lice on sea trout - a literature review. NINA Report 1044, 1-162.

Vollset, K. W., Krontveit, R. I., Jansen, P. A., Finstad, B., Barlaup, B. T., Skilbrei, O. T., Krkošek, M., Romunstad, P., Aunsmo, A., Jensen, A. J. and Dohoo, I. (2016). Impacts of parasites on marine survival of Atlantic salmon: a meta-analysis. Fish and Fisheries, 17, 714−730. https://doi.org/10.1111/faf.12141

Vollset, K. W., Dohoo, I., Karlsen, Ø., Halttunen, E., Kvamme, B. O., Finstad, B., Wennevik, V., Diserud, O. H., Bateman, A., Friedland, K. D. and Mahlum, S. (2018). Disentangling the role of sea lice on the marine survival of Atlantic salmon. *ICES Journal of Marine Science*, *75*(1), 50-60. https://doi.org/10.1093/icesjms/fsx104

Wagner, G. N., McKinley, R. S., Bjorn, P. A., Finstad, B., 2003. Physiological impact of sea lice on swimming performance of Atlantic salmon. Journal of Fish Biology, 62(5), 1000-1009. https://doi.org/10.1046/j.1095-8649.2003.00091.x.