Servicio Nacional de Pesca y Acuicultura

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PROGRAMA PARA LA GESTIÓN SANITARIA EN LA ACUICULTURA

Summary Report for "Asesoría Especializada en Epidemiología de Piscirickettsia salmonis"

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SalmonChile

Prince Edward Island, Canada

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Contents

Summary

The objectives of the contract between Sernapesca and the University of Prince Edward Island (UPEI) were to 1) participate in group meetings to design and develop an epidemiological research center for the advancement of knowledge on Piscirickettsiosis (SRS) and sea lice; 2) propose a design to characterize the strains of *Piscirickettsia salmoni*s in Chile and maintain a repository for representative strains; 3) assess the research priorities identified by the industry and; 4) conduct a literature review on Piscirickettsia identifying the most likely sources of infection for farmed fish, and potential control strategies.

Our first report to Sernapesca was a proposal for creating a repository for *P. salmonis* (Appendix A). We presented this at the end of our initial meetings with the Sernapesca administrative team which occurred in late January 2016. At this meeting we also "brain stormed" potential frameworks for a research centre in epidemiology that could provide scientific support and advice for government policy (Appendix B). As a result of this initial meeting we reviewed the research questions identified by industry as priority for SRS and sea lice research (Appendix C).

A literature review of the potential sources of *P. salmonis* for farmed fish in Chile was conducted and submitted to Sernapesca in June 2016. This report was subsequently revised in June 2017 after new research was completed on SRS antibiotic treatment success (Appendix D). This report identified the need to better understand the biocapacity threshold to reduce pathogen transmission within aquaculture neighborhoods in Chile. As a result of this literature review, our research team conducted several independently funded studies to determine the reasons for SRS antibiotic treatment failure in Chile. We also with the remaining funds from this contract initiated a study in two neighborhoods to better understand the transmission relationship between infected and non-infected farms (Appendix E). The information from this project and the resulting dataset could be used for developing a biocapacity simulation model in the future. The report (Appendix E), a cleaned dataset, as well as a movie depicting the sequence of outbreaks investigated were provided to Sernapesca in June 2017.

Appendix A Proposal for *P. salmonis* repository

Proposal for the creation of a repository for *Piscirickettsia salmonis* in Chile.

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December 28, 2015

Introduction

Salmonid rickettsial septicemia (SRS), caused by *Piscirickettsia salmonis*, is the most prevalent infectious disease in farmed salmonids in Chile (Sernapesca 2013). In 2014, SRS mortality accounted for approximately 75% of all mortality due to infectious diseases in Atlantic salmon (Sernapesca, 2015). Control of this disease through the use of antibiotics is often unsuccessful, and results in repeated applications of chemotherapeutants (Price et al 2016). This has increased costs of production and a decline in market value due to public opinion of antibiotic use in food animals. The high incidence of SRS (Rees et al 2014; Sernapeca 2015) also inhibits sustainable growth of the industry.

In order for the industry to increase production and reduce its antibiotic usage, it needs to better understand how to prevent this disease and minimize the magnitude of outbreaks associated with this bacterium. Management strategies that are being investigated include vaccine development, immunostimulant therapies, and antibiotic assessments. All of these projects require the use of *P. salmonis* isolates that represent the situation in Chile. For example, it is unclear whether the poor treatment response to antibiotics is due to resistance of the bacteria to specific compounds, poor tissue concentrations of antibiotics due to pharmacokinetics or drug application, or a combination of all of these factors. To address the issue of poor treatment success we must answer these questions, which will requires the acquisition of representative *P. salmonis* isolates to conduct the research.

Currently, there is no library of isolates for this pathogen in Chile. Private laboratories have isolates from different sources, but these are not archived in a manner that is conducive to use by university and government researchers. The objective of this proposal is to design a sampling strategy to collect representative *P. salmonis* isolates from SRS cases in Chile for research purposes. This library will be updated annually with isolates that represent the temporal and spatial diversity of these bacteria in Chile. We will catalog basic information on each isolate in a manner that maintains the anonymity of individual farms and companies, but permits researchers to answer questions such as, 1) what is the genetic diversity of isolates in Chile? 2) Do vaccines have to be area specific? 3) Are there specific virulence factors associated with certain isolates that could explain variation in mortality within and between host species? and 4) Are some isolates less sensitive to certain antibiotics? The answers to these questions will help the industry manage SRS more effectively, and will inform policy-makers on the relevance of genotyping for control of SRS spread.

Methods

We will start by requesting private and university laboratories to donate historical isolates to our communal library. We will also start collecting *P. salmonis* isolates from new cases of SRS in the industry, using a sequential, cross-sectional study design. The specific methodologies for these two approaches of gathering representative isolates of *P. salmonis* from the industry are outlined below.

Historical samples

We will send a request to private and university laboratories conducting research on SRS for any archived *P. salmonis* isolates that have the following information available with the specimens: Source (laboratory) submitting the sample, date of isolation, neighborhood where the sample was collected, and species of the host sampled. All isolates donated to the library will be submitted directly to the Universidad Catolica de Valparaiso , where they will be sub-cultured as per that laboratory's protocol, cryopreserved, cataloged, and archived at -70 ^{0}C. This laboratory has been conducting research on bacterial agents of aquatic animal diseases for several decades, including a research program on *P. salmonis*. They have a formal arrangement with Sernapesca to archive samples under a new research initiative for aquatic animal disease prevention and control. A copy of all isolates will also be stored at the Universidad Austral de Chile in Valdivia as a back-up in the event of unforeseen losses of the original isolates. Isolates will be coded for storage purposes and all private information will be maintained separately with Sernapesca to ensure confidentiality of the origin of the samples.

New isolates of P.Salmonis:

We will actively collect *P. salmonis* isolates from farms that submit samples for culture confirmation in December, January, and February of every year. An effort will be made to stratify our sampling, such that we have representation from SRS cases in different neighborhoods and in different species over time. We will aim to collect 3 isolates from different farms in each neighborhood. When a neighborhood houses multiple species of fish, an effort will be made to collect an isolate from each species. If a neighborhood only has one species then all three isolates will originate from the same host species. We, therefore, anticipate receiving up to 165 isolates per year[1] . In addition, we will collect, using a targeted sampling protocol, up to 20 specimens from cases that are associated with high mortality or that occur at different times of the year than our sampling period.

To reduce duplication and costs of collecting *P. salmonis* isolates, we will request isolates from producers when they submit tissue samples to laboratories for culture confirmation and/or antibiotic sensitivity testing. Although not all producers test for antibiotic resistance, this is becoming more frequent amongst companies and is recommended in our new regulations (Sernapesca 2015). We anticipate that we will be able to gather a representative sample of *P. salmonis* isolates using this strategy.

We will recruit farms when we identify SRS mortality at their facility, using our mortality database. A formal email request, followed by a telephone call, will be made to the company of these farms once they declare they have mortalities associated with SRS, early in the outbreak to provide the company sufficient time to plan and to enable the capture of isolates before an antibiotic treatment has been applied on the farm. Contributions to our isolate repository will be voluntary. Given the benefits of

[1] 3 isolates from 55 neighborhoods

having a farm isolate represented in our library we do not anticipate that companies will refuse to submit samples.

To subsidize the cost of shipping bacteria to the repositories for cataloging and storage, and to reduce expenses associated with duplicate isolation from tissues, we will request the producers to have the isolates submitted to us directly from the private laboratories doing their cultures, after the primary isolation of the bacteria has been completed. We will follow-up with the laboratory doing the initial bacterial isolation with instructions on where to send a copy of the purified isolates. Participating laboratories will be asked to catalog basic information on the isolate (Siep code for the farm of origin and date of isolation) and store the pure culture at -70^0 C until the end of the survey period (March of each year). At that point, all isolates collected from participating farms will be shipped to our repository laboratory. Laboratories will be compensated for shipment and the temporary storage of isolates for this project. Once we have sufficient representation from each species within a neighborhood, we will terminate recruitment of isolates from those neighborhoods until the following year.

In the event that our passive random sampling strategy, described above, is insufficient to provide an adequate temporal and spatial representation of *P. salmonis* in Chile, we will employ targeted sampling strategies to collect bacterial isolates from the industry. Several targeted sampling options exist. If an insufficient number of farms are submitting for culture confirmation prior to treatment then we can request companies send tissues from affected fish directly to our laboratory partner at the Universidad Catolica de Valparaiso, who can do the primary isolation. Another option is that we utilize the extensive ongoing sampling program conducted by Sernapesca to collect, opportunistically, tissues for bacterial culture from SRS cases identified through our mortality databases. We would prefer not to resort to this type of sampling, as it will increase our costs and may bias cases of disease that are associated with higher mortality; however, they are available options, should our initial survey methods fail to produce an adequate number of samples for our library.

We may in fact need to use a more targeted sampling strategy for collecting isolates from coho salmon as companies may not be submitting samples for culture confirmation/ sensitivity testing in December, January, and February. This is late in the coho salmon production cycle and producers are likely starting to harvest their fish. A bacterial culture is usually done by companies if they are considering antibiotic treatment which they would not be if they are harvesting. We may also supplement our library of isolates with targeted sampling of isolates from exceptionally several cases or cases that occur at cold water temperatures. Isolates collected using a targeted non-random sampling strategy will be identified in our database to differentiate these from the randomly sampled isolates.

Sernapesca will match farm identification (Siep code) with the information on farm production and sanitary status at the time that the *P. salmonis* isolate was collected. Information will include date of submission, farm and company ID, species of fish, total mortality on the farm associated with SRS, treatment response, and vaccination status of the fish (see example Appendix A). The total mortality associated with SRS will be calculated based on the method used by Jakob et al (2014), and the treatment effect will be evaluated based on the methods used by Price et al (2016).

All information on isolates will be maintained by Sernapesca. Researchers will be able to request information on isolates through a formal process, but no information that identifies the source (i.e. laboratory, farm or company names) will be disclosed. To ensure confidentiality is maintained, all information will be aggregated at the macrozone level; however, in cases where this aggregation level is

not sufficient to protect the identity of the source of *P. salmonis* (i.e. when a macrozone has only one company or when researchers require information at the species level and there is only one company in the macrozone farming a specific species) we will combine information on adjacent macrozones to ensure that no farm or company is identifiable.

Protocol for researchers to use P. salmonis isolates from the collection

A formal request will be required to use any of the isolates in the library. All requests will have to include the following information: purpose of research, information required on isolates, method of containment for isolates, method of destruction for isolates after the project is completed, and plan for dissemination of the research findings to the industry (see example Appendix B). Although data will be aggregated at a level which does not permit identification of the source of the isolates, all researchers will also have to sign a confidentiality agreement with Sernapesca and an assurance that the receiving researchers will not maintain the isolates beyond the duration of their approved studies and will not share or use the isolates for other purposes. Only laboratories with proven biosecurity measures to contain bacterial agents will be granted access to the isolates. Sernapesca will review each request to ensure all research conducted on isolates from this library is done so in an ethical and safe manner. In some cases, Sernapesca may request external reviews of proposals.

Further, to recover the costs associated with each request (i.e. the laboratory cost associated with sub-culturing and Sernapesca's expenses associated with data extraction) there will be a nominal fee for using the isolates in the library. We anticipate this cost to be $20/isolate for revival and $3/isolate for the data query; however, these costs are likely to increase over time.

Cost of program per year for three years

	Service	Unit price (CAD)	Number of units	Cost year 1	Cost year 2	Cost year 3	Total cost
Historical isolates							
	Shipping costs	50	10	500			500
	Revival of frozen sample	15	25	375			375
	Cryopreservation	10	25	250			250
	Annual storage at -70	2	25	50			50
				$ 1,175			**$ 1,175**
New isolates							
	Cost of shipping and storage by industry labs	100	20	2000	2400	2800	**7200**
	Revival of frozen isolates	15	165	2475	2970	3465	**8910**
	Cryopreservation	10	165	1650	3960	6930	**12540**
	Annual storage at -70	2	165	330	792	1386	**2508**
Total				$ 7,630	$ 10,122	$ 14,581	**$ 32,333**
Notes: We assumed a 2% inflation per year. We assumed 10 and 20 laboratories participating in our historical isolate and new isolate surveys							

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Appendix A (part of Appendix A report)

Example of information collected on isolates

Year of isolation	Month of isolation	Random or targeted sample	SIEP	Barrio	Macrozona	Host species	Laboratory submitting the sample	Vaccine (name)	Total % morality associated with SRS **	Treatment success (yes or no) ***
2016	Dec	random	XXXX	1	1	trucha*	1	XXX	5	no
2016	Jan	random	YYYY	1	1	trucha	1	DDD	6	no
2016	Jan	targetted	ZZZZ	1	1	coho	2	XXX	8	yes
2016	Jan	random	MMMM	2	1	Salar	3	FFF	20	yes
2016	Jan	random	NNNN	2	1	trucha	2	GGGG	10	no
2016	Feb	random	OOOO	2	1	coho	2	none	12	no
2016	Dec	random	RRRR	3a	1	Salar	1	none	3	no
2016	Feb	random	SSSS	3a	1	trucha	3	DDD	5	yes
2016	Feb	random	TTTTT	3a	1	coho	3	FFF	9	yes

* because barrio 1 only has trout and coho the third sample could be from trout

** calculated based on method used by Jakob et al 2014

*** Determined based on method used by Price et al 2016

Appendix B (part of Appendix A report)

Request for use of *P. salmonis* isolates

Name and affiliation of person requesting isolates:

Purpose of research:

Information required on isolates:

Method of containment for isolates (include handling and storage protocols):

Method of destruction for isolates after the project is completed:

Plan for dissemination of the research findings to the industry:

Appendix B Framework for Research Centre

Notes from Jan 20, 2016 on Research center for SERNAPESCA

Mission statement for the Research Center for Aquatic Animal Diseases

Provide animal health research for the Chilean Aquaculture Industry that will directly support the regulatory mandate and the economic, social, and environmental sustainability of the industry

Goals

I) Provide a platform for multidisciplinary animal health research that 1) provides guidance for SERNAPESCA's regulatory framework and 2) supports the advancement of salmonid aquaculture production

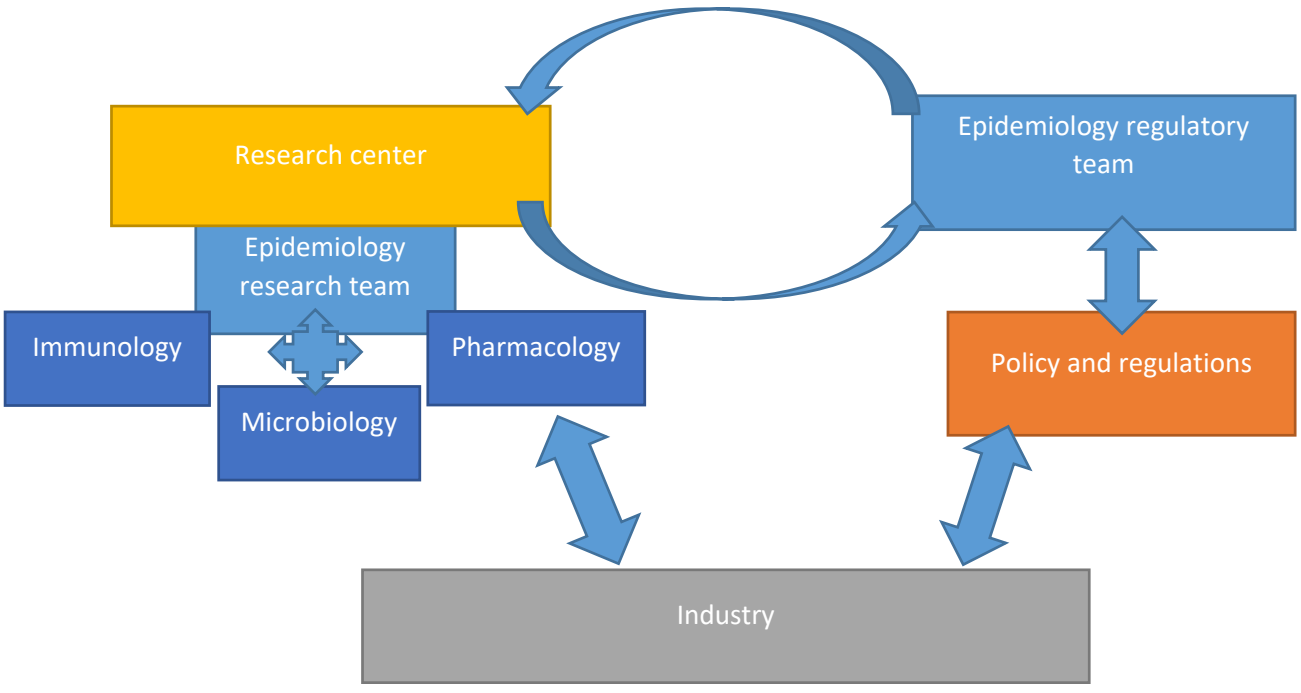
II) Reduce the incidence and the magnitude of communal infectious diseases that reduce the global competitive advantage of the Chilean aquaculture industry

First two years will focus on the two pathogens that are limiting the growth of the Chilean Industry (*P. salmonis* and *C. rogercresseyi*)

III) Create innovative and economically sound strategies for the control of infectious diseases that reduce the dependence on chemotherapuetants

IV) Provide a system to support continuous training and advancement of animal health knowledge in Chile

Operational strategy framework



The epidemiology research group will be part of SERNAPESCA and the other research clusters will be partnerships with the private or university research laboratories. The research center will have a director, a manager (Coni!), and an accountant +/- a lawyer! The epidemiology research group will have a lead researcher (with competencies in epidemiology and that can manage/motivate a multidisciplinary research team which will include a veterinarian/biologist/epidemiologist familiar with the industry, a statistician/ spatial modeler, an economist, and a database expert).

Specific Objectives for first two years

1) Create a registry of research in Chile and projects involving international collaborations
2) Integrate information on production, sanitary status, and the environment collected by government on the aquaculture industry into a central GIS system that:
    a. improves the efficiency of surveillance of infectious diseases in Chile
    b. facilitates early detection and implementation of control measures for emerging infectious diseases;
    c. permits industry-wide epidemiological research of health issues
3) Prevent SRS on salmon farms
4) Reduce the use or antibiotics on farms
5) Reduce the occurrence of sea lice on salmon farms
6) Reduce the use of sea lice treatments on salmon farms
7) Increase scientific capacity in fish health and epidemiology for Chile

Ideas for epidemiology group research projects that are consistent with the mission, goals and specific objectives of the center

Short term projects with immediate benefits that could be done once database is in place

1) Assessment of vaccines for SRS (outcomes time to disease and total mortality). This project could help advise the industry on how to improve resistance to *P. salmonis* and decrease magnitude and prevalence of SRS.
2) Assessment of treatment for SRS (3 years of data include injectable and oral treatments at different doses). This project could help advise the industry on which treatments work better and when treatment needs to be applied in order to be beneficial.
3) Sensitivity of using SRS mortality data to identify prevalence of *P. salmonis* infection vs the 2 month mandatory SRS PCR testing (gold standard comparison). This project could justify removing the active testing of fish or reducing sampling based on risk.
4) Publish data on fresh water testing to demonstrate the lack of infection during this life stage. This project could justify the removal of this sampling from this life stage.

Medium term projects

1) Establish a library of *P. salmonis* isolates that represents the temporal and spatial diversity of these bacteria in Chile and that can be used to answer specific questions such as the genetic diversity of isolates in Chile, whether strains cluster by host, whether genetically different isolates have different resistance patterns and whether these cluster in space, etc.

2) Determine the mortality threshold on farms that indicates when a farm is at high risk of transmitting *P. salmonis* to its neighbors located at different seaway distances. This project will help set thresholds for action by SERNAPESCA.
3) Cluster analysis of antibiotic resistance . This project will require that the information on resistance is capture by SERNAPESCA but could help advise the industry on choice of antibiotics to use in different areas.
4) Development of a risk tool that identifies farms at high risk of SRS based on their risk factors. This project will help the industry determine when they should be increasing their surveillance for early detection and management
5) Assess the role of acopios in disease transmission. This will provide information that helps guide policy on Acopios.

Long term projects

1) Establish biocapacity of farming areas to reduce SRS outbreaks. This will help guide the biomass restrictions used by SERNAPESCA. This could simplify the regulations to enable easier management of smolt entry by industry.
2) Conduct an economic model to evaluate the cost of treatments and losses of SRS given different management strategies. This will help motivate industry to use certain management strategies.

Appendix C Review of Industry Question 2016

Prepared by S. St-Hilaire based on discussion by working group (AusVet and UPEI).

The following is the list of original research questions by the Salmon Industry. Although all research questions were interesting and should be pursued, we (the working group) had to prioritize the questions from the list that directly aligned with the mission and strategy of the "Plataforma para la Gestión sanitaria en la Acuicultura industry". Our guiding principles when we set the research priority list was that the research conducted under this program had to be directly related to the control and prevention of SRS and Caligus and or lead to a reduction in antimicrobial use. We focused on projects that require industry-wide participation and that benefited the entire industry (i.e. assessment of treatments and management strategies under field conditions). We were asked to focus on SRS research, but many of the concepts identified could be applied to Caligus investigations.

Although we recognize the importance of developing new drugs and vaccines we excluded these types of studies from the priority list for this particular research initiative for a number of reasons. First this type of study does not require participation or the use of industry wide data which was the initiative for establishing this type of research body. Secondly, although chemotherapeutant and vaccine development would benefit the industry overall, the commercial incentive for the producer of the product suggests this should be led by the private sector more efficiently. Where we see the role of this research program is as a non-bias third party in the evaluation of the performance of these new products under field conditions. Lastly, there are other sources of funding for the development of commercial products that are better suited for funding these projects.

We have identified in italic below each question which section or pillar of the proposed research program would address the issue identified within the question.

1. What are the sensitivity and specificity of diagnostic tests for P. salmonis detection?

Detection of P. salmonis on a farm is relatively easy to do using PCR, tissue imprint, and or bacterial culture. The disease caused by this bacteria has characteristics that are pathonomonic, so the sensitivity of detecting this bacteria on a farm with clinical disease is high. Therefore, this question was deemed a lower priority than others that are more important for the direct control and prevention of the disease.

The issue of early detection of this bacteria on farms came up during discussion but this is not because we do not have sensitive diagnostic tests; rather, it is because it is difficult to detect a

few subclinically infected fish within the large populations on farms. We have provided some suggestions to address this particular issue under the topic of 4.1as we feel it is directly affecting the producers ability to control SRS.

2. Can the results obtained be replicated if the test is repeated? (this is related to the reliability or repeatability of the tests)

Again, we gave this question a lower priority as it does not relate directly to the control and prevention of SRS, and the diagnosis of SRS on farms is relatively easy due to clinical signs and the diagnostic tests available.

3. Which factors can influence validity and reliability of diagnostic test, and how to use such information for better disease diagnosis and management?

The issue of early pathogen detection is addressed in 4.1

4. Which factors (risk or protective) can modulate the time to infection, clinical type and magnitude of SRS outbreaks? If any, how they act or interact?

This question was given priortity and will be addressed 2.1

5. Which factors are associated with salmon farms that may shed P. salmonis in a higher rate compared to others?

This question will be addressed in 2.1. One of the risk factors for SRS on a farm is infected neighbors. By refining this predictor we may be able to determine what the mortality threshold is for neighbors to be a risk to others. The proposed risk factor study in 2.1 will address this issue.

6. How these factors can contribute to developing optimal management strategies at the farm and neighbor levels?

By understanding the mortality threshold for transmission to other farms the industry can implement critical control points more effectively. The first stage of this is addressed in 2.1, and improving management of SRS is included in section 3.0.

7. Which mechanisms influence the re-emergence of P. salmonis in a farm and Caligus infestation, and which biological and non-biological mechanisms participate in the transmission and further spread of the bacteria within a farm?

SRS is spread via the water, so once it is on a farm it is impossible for a farmer to prevent the spread between pens.

8. How P. salmonis and Caligus spread from an infected farm to others (between-farm spread) and neighborhoods?

Several studies have confirmed that these two pathogens are spread via water between sites (Kristofferson et al 2013; Rees et al 2014). Other mechanisms of transmission may include poor fallowing for SRS. This is addressed in 4.3. The only other potential source of infection for both of these pathogens is wild fish. We will address some of the issues in this question in section 2.3 (study onbiocapacity).

9. Are within- and between-farm spread related? How these two mechanisms can be coupled in an explanatory/predictive model for the use of the industry/government?

We address this question with a risk tool that can help producers identify when they are at risk of infection; see section 7.1

10. Can a disease spread model(s) be designed and implemented as a tool for an early warning system, and to evaluate management strategies or treatments schemes that ultimately support the decision-making process?

We propose this in section 7.1

11. What is the role of vector diversity and (non) salmonid reservoirs in the P. salmonis dynamics?

This will be indirectly covered in section 2.1, on risk factors.

12. What is the role of diversity, abundance and proximity of wild fish in the infestation process of Caligus, and what are these infestation mechanisms?

Given the number of wild fish vs the number of farmed fish in the fish farming areas of Chile and the results from Kristoffersen et al. 2013, which suggested that the sea lice prevalence was very well predicted by lice transmission within and between farms, this question was given lower priority. Further, producers can not manage this particular source of pathogen, so we focused the research priorities on factors that could be manipulated or altered by the industry.

13. What biological interaction (predation, competition) might influence the abundance of Caligus?

This would be part of the risk assessment for Caligus; however, we focused this analysis on SRS to start with in section 2.1. We felt, given the research already done on sea lice, the industry should focus on improving its treatment strategies and reducing biomass to control this parasite. Research on these strategies are covered in 3.1 and 2.3.

14. What are the consequences of nutrient superabundance on pathogens, parasites, and disease?

This could be covered under pillar 5; however, this information is not currently collected and difficult to acquire from the industry as it changes over time.

15. What attributes of the ecosystem might predispose it toward stabilizing negative feedback (SRS outbreaks reducing the likelihood of subsequent disease), versus positive feedback (SRS outbreaks facilitating subsequent pathogen attacks)?

This question would be answered with the risk factor study in section 2.1. However, it may be difficult to collect all the information at the industry level.

16. How the seascape structure (including oceanography and abiotic factors) influences population density and movement patterns of agents, vectors, and transmission stages?

This question would be answered in the biocapacity study section 2.3

17. How can knowledge of seascape structure be used to improve quantitative predictions about disease (infestation) spread and persistence?

This question would be answered in the biocapacity study section 2.3. We currently do not have all the hydrodynamic information, but as part of the biocapacity project we would evaluate the use of proxies for hydrodynamic information.

18. Which mechanisms are involved in the vertical transmission of P. salmonis?

The surveillance information thus far suggests there is no vertical transmission of P. salmonis. A study analysing the data from freshwater surveillance, section 6.3, will help clarify this issue.

19. What is the role of fresh and salt water in the life stage, viability and virulence of P. salmonis?

This question will be answered through a number of studies, including the summary of fresh water surveillance and the genetic typing of isolates (sections 6.3 and 1.2, respectively).

20. How P. salmonis strains differ in terms of pathogenicity and virulence, and how the host responds to these differences and signals?

This will be addressed in section 1.2

21. How P. salmonis interact with surfaces, how they survive outside of their hosts, how signals are relayed between the microorganism and the host?

We have suggested focusing on the survival of the bacteria over the fallow period to begin to address this question (section 4.3). A recent study by Price et al. will be published on this topic in 2016.

22. How P. salmonis strains differ in terms of host susceptibility and geographical zones? How is the population structure?

This will be addressed in section 1.2

23. What is the frequency of P. salmonis resistant strains, if any, before antimicrobial use in a farm?

This will be addressed in section 3.3 and 3.4

24. What are the processes related to the emergence of drug resistance, and timing the emergence of resistance of P. salmonis?

Although this is important, we did not make it a priority in this initial round of research projects because it is not directly related to the control of SRS in Chile.

25. What are the best drugs, schemes and strategies to be implemented in order to avoid drug resistance?

This will be addressed under the treatment efficacy pillar 3.0.

26. Which mechanisms are involved in the transmission of Caligus?

This was not a clear research question. We know that lice are transmitted via the water.

27. What stages of the life cycle are key in the infestation process?

It is well known that the fish are infected with the juvenile life stage of the parasite and that it develops into the adult stage. All life stages play key roles in the process. It is not clear what else is being asked in this question.

28. How the duration and behavior of planktonic stages of Caligus increases the risks of infestations?

Although this is interesting it is unlikely to lead to effective control strategies, so we did not prioritize this in the initial round of research projects.

29. Which are the population-level genetic differences increasing the success of Caligus? How is the population structure?

This was not made a priority as it is not directly related to the control of sea lice

30. What is the frequency of Caligus resistant individuals in wild and farmed salmons?

This would be answered partially in a geographic analysis of caligus resistance, similar to what was proposed in 3.4 for SRS. We were initially requested to focus on SRS, but many of the studies could apply to Caligus as well.

31. What are the processes related to the emergence of drug resistance of Caligus?

We focused on SRS for most of our research priorities; however, this questions would be addressed in section 3, if the same approach is applied to Caligus

32. What are the best drugs, rotation schemes and strategies to be implemented in order to avoid drug resistance?

This is important and should be added to section 3.0 for caligus.

33. What are the conditions that specify whether P. salmonis will establish a persistent infection or will be cleared by the fish immune response? What are the immune mechanisms of salmonids to lower the adverse effects of caligus?

This did not make the first round of research priorities, but is interesting and should be pursued at a later date.

34. What is the correlation between bacterial dynamics, damage and inflammation to the fish, and the fish response? the correlation between bacterial load (abundance of P. salmonis) and the magnitude of the antibacterial immune response?

This did not make the first round of research priorities, but is interesting and should be pursued at a later date.

35. How the use of vaccines or alternative products aimed to enhance the force of the immune response will decrease fish susceptibility and decreased shedding rates?

This is addressed in section 5.0.

36. What are the mechanisms involved in the host-multipathogen responses? What is the role of stress and immunity threshold?

It is well known that stress promotes disease. Identifying factors that cause stress would be beneficial, but are likely farm specific and not captured in a central database. For this reason we did not focus on this question; however, some stressors would be captured in the risk factor analysis in section 2.1.

37. Is there any temporal succession pattern of different diseases?

This will be answered in the risk factor analysis in section 2.1.

38. What is the most effective antibiotic/antiparasite currently available to treat infected salmon? How are their sensitivity?

This will be answered in section 3.0.

39. How different are the pharmacokinetics and pharmacodynamics of these drugs? Which factors can modulate these processes?

This will be answered in section 3.0.

40. How to measure effective plasmatic concentrations? and how correlated are the antimicrobial susceptibility in vitro and in vivo with treatment success?

This will be answered in section 3.0.

41. How to develop of antimicrobials/antiparasites? How to ensure their optimal performance (safety, effectiveness, low cost, easy to apply, etc.)?

This will be answered in section 3.0.

42. How to develop and use alternatives drugs (probiotics, additives, other immunostimulant, etc.)? How to ensure their optimal performance?

Some of these questions will be answered in section 3.0; however, drug development was left for the private sector to undertake, as there will be economic benefits for the individual companies developing the drugs and there are already sources of funding for this type of research.

43. How to achieve the prescribed dose in each treatment?

This will be answered in section 3.0.

44. What rotation scheme of different drugs minimizes the resistance of Caligus and to P.salmonis to drugs?

This will be answered in section 3.0; however, more emphasis should be made on the schemes for minimizing resistance.

45. Is the spatial scale of salmon farming "barrios" appropriate? Do they need to be coordinated in the control of Caligus or P. salmonis?

This will be addressed in section 2.3.

46. What are the ecological impact and costs of these rotation schemes?

This will be addressed in section 7.2 and 7.3.

47. What are the areas of the genome (genetic markers) that encode for fish resistance mechanisms to Caligus and P. salmonis?

This is interesting but unlikely to lead directly to management changes that reduce the occurrence of these diseases and the use of antibiotics, so it was not included in the priority projects and themes.

48. Are the genetic resistance mechanisms described the same in experimental versus natural infections? How to standardize such evaluation?

We felt the industry should focus on schemes that reduce resistance (i.e. section 3) under field conditions instead of experimental studies.

49. How genetic selection for disease resistance could interfere with desirable production and health-related characteristics?

This is important and should be carried out by the companies that are profiting from these genetic resistant fish. The goal of the research platform is to provide research that helps guide policy and benefits the industry as a whole, so we did not prioritize projects that could commercially benefit individual companies.

50. What are the areas of the genome (genetic markers) that encode for agent resistance mechanisms to drugs (antibacterials, antiparasites)?

This is interesting but not related directly to the management of disease, so it did not make our initial priority list.

51. Are the genetic resistance mechanisms described the same in experimental versus natural infections? How to standardize such evaluation?

Repeated question; see #48

52. How to develop an interdisciplinary research framework to optimize approaches and interventions needed to reduce disease risk to farmed salmon?

This is the goal of this research platform. One of the first studies will be a risk factor analysis which will, in part, address this question (section 2.1).

53. How to measure the impacts and effectiveness of animal health decisions including cost-benefit analysis, cost-effectiveness, welfare measures, externalities, risk, asymmetric information, strategic behavior, and others?

This question will be addressed in part in sections 7.2 and 7.3.

54. How to develop indicators for scientific-based norms?

This question is unclear.

55. What is the interplay between environmental norms and policy?

This question is unclear.

56. How much policy coherence is found in the salmon aquaculture sector?

This will be somewhat addressed in section 1.3 on communication.

57. How can stakeholder perceptions inform the policy-making process?

This is not directly related to science and disease control.

58. What are the benefits and costs of implementing such policies?

The costs and benefits of some policies (i.e. related to SRS and Caligus) will be addressed in sections 7.2 and 7.3.

59. What are the most relevant agent factors that may be useful to develop an efficacious vaccine against P. salmonis?

This is not directly addressed in this initial round of research priorities, because it was felt that the type of research to answer this question should be undertaken by the company that will directly benefit commercially from the vaccine development. We focused the research priorities on the issues that would help guide policy and or that would benefit the industry as a whole and could not be conducted by individual companies.

60. How to enhance the uptake, processing and presentation of the antigen to the fish innate immune system at the mucosal level as a booster for vaccination strategies?

We focused the research priorities on the issues that would help guide policy and or that would benefit the industry as a whole and could not be conducted by individual companies. We will, however, evaluate the efficacy of vaccines under section 5.0.

61. How to define a standard and adequate method to assess vaccine safety, efficacy and transmissibility?

This will be addressed in section 5.0.

Appendix D SRS Literature review

Control and prevention of salmon rickettsia septicemia

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Submitted June 2016 finalized June 2017

Salmon rickettsial septicemia (SRS), a chronic bacterial disease caused by *Piscirickettsia salmonis*, is increasing in prevalence in Chile (Rees et al 2014). It was estimated that in the summer of 2013 the prevalence of SRS infected farms was as high as 70% (Rees et al 2014). This bacterial disease is causing high mortality and, perhaps equally as important, it is resulting in an increase in antimicrobial use in Chile. Today, the marketplace is becoming more critical of the use of antibiotics and, although there are no drug residues in the fish when they reach the marketplace, consumers do not want antibiotics used in food production due to issues with the development of antibiotic resistance globally. For this reason, it is important to find ways to reduce the use of antibiotics in aquaculture.

The first approach to reducing antibiotic use associated with the treatment of SRS is to prevent the occurrence of SRS on farms. To do this, the industry needs to reduce or eliminate the sources of *P. salmonis* and or improve the immunity of the host so it is less susceptible to the pathogen and, thus, does not succumb to disease even when exposure is not be fully prevented.

There are very few potential sources of *P. salmonis* for aquaculture fish. It is unlikely that this bacteria is acquired in fresh water given the vast amount of negative screening conducted by SERNAPESCA. For example, in 2015 almost 6000 fish from freshwater were evaluated for *P. salmonis* using PCR and none were found to be positive (Sernapesca 2016). Once in salt water, the fish are likely either infected from residual bacteria from the previous production cycle at their own site (e.g. failed fallow), from infected neighbors, or from infected wild fish. The latter is difficult to assess, but other researchers have found from survey results approximately 8% of wild fish are positive by PCR for *P. salmonis* (Contreras-Lynch et al 2015; Garcia et al 2016). The prevalence in wild fish seems too low to account for the high prevalence of positive aquaculture sites in Chile; however, wild fish may play a role in pathogen transmission. The other two sources of this bacteria have been, or are currently being, evaluated for their significance in propagating and or maintaining *P. salmonis* in fish farming areas.

The efficacy of fallowing farms to remove *P. salmonis* is currently under investigation. Preliminary results suggest the risk of developing SRS post salt water entry is similar for farms that fallowed for more than 3 months; however farms that fallow for less than 3 months may have a higher risk of SRS within the first three months of salt water entry (Price et al 2017). . Laboratory studies also suggest that the bacteria do not survive longer than 60 days in salt water without a host (Olivares and Marshall 2010). Given that most farms fallow for 3 months or more, it is unlikely that *P. salmonis* from previous production cycles on farms can account for very many cases of SRS. A more likely source of infection is other infected fish in close proximity to the farm.

Rees et al. (2014) found *P. salmonis* was spread up to 10 km between salt water farms in Chile (average size of farms in the study was 900,000 fish) during the period from 2011 to 2013. As farms increase in number and in size this infective distance may be reduced. To prevent spread of *P. salmonis* between farms we have to either 1) increase the distance between farms, 2) decrease the number of hosts on the farm, or 3) control the number of infected fish on the farm before the infectious load reaches the threshold level for infection of other farms. The Rees study did not capture the level of mortality required on a farm to infect other farms at different distances, nor did it examine whether different species shed more bacteria than others.

Currently, SERNAPESCA uses the cut-off farm level mortality rate of 0.35% to mandate an action by the producer to reduce the spread of *P. salmonis*. Given the high incidence of cases in the industry, this cut-off does not appear to be controlling the size of outbreaks; however, without more information it is difficult to justify changing this action point. A more refined estimate of the connection between farms is necessary to better understand how to reduce farm-to-farm transmission. Given the proximity of farms to one another in Chile, and the results from at least two statistical analyses identifying an association between infected neighbors (Rees et al 2014; Price et al 2017), this source of infection is likely responsible for maintaining *P. salmonis* in aquaculture areas.

Although an action threshold that reduces farm-to-farm transmission would be beneficial to the industry, without effective management strategies to control this disease on farms the cut-off action point cannot reduce pathogen load in the environment on its own. One strategy is to improve the host resistance to this bacterial infection, through better vaccines, immunostimulant feeds, genetic selection, reduction of stressors, etc. However, these strategies take time to improve and thus far have been inadequate to control mortality levels. The other options for controlling the level of mortality associated with SRS are early harvest, if the fish are of an appropriate market size, or the use of antibiotics. Currently, antibiotic treatments are not working as effectively as expected (Price et al. 2016). In fact, one of the reasons antibiotic use is increasing is that current antibiotic treatments are not effective, which results in repeated treatments. Investigation of treatment failures will enable us to identify the reasons and mitigation strategies for the poor performance of these products, which should lead to a reduction in the overall volume of antibiotics used in Chile and the secondary spread of *P. salmonis* between farms.

In general, antimicrobial treatments fail for a number of reasons, including 1) misdiagnosis or co-infection with non-bacterial diseases, 2) bacteria resistant to the specific drug used, 3) inadequate tissue therapeutic concentrations , and 4) inadequate duration of treatments to eliminate the pathogen. There are a number of known and unknown scenarios that can result in these situations; however, in Chile, with regards to SRS treatment, our knowledge of the reasons for treatment failure is limited. Identifying and, more importantly, finding solutions to address these issues will help reduce treatment failures.

Assuming producers are not misdiagnosing co-infections with other pathogens that cannot be treated with antimicrobials, the first step in understanding treatment failure is determining the minimum inhibitory concentration (MIC) and therapeutic tissue concentrations (TTC) required to eliminate *P. salmonis* in Chile. A recent study by Henriquez et al. (2015) suggests there is a wide range of MICs for isolates in Chile, but for the most part the MIC for florfenicol and oxytetracycline were usually lower than what was expected for resistant strains of the bacteria. To enable the continued assessment of

MICs in a systematic manner that represents isolates from a number of different areas and species in Chile over time, creation of a central repository of isolates should be initiated. A proposal was submitted in 2016 that outlined a sampling scheme that would ensure a representative collection of isolates from Chile was captured for research purposes. The MICs will help determine if there are resistant strains of *P. salmonis* emerging in the industry, and the TTC will help define how much of each antimicrobial is required in tissues to treat or eliminate the pathogen. Establishing a correlation between these two values in the lab will help farmers to estimate, using their own isolate MIC values, what concentration of antimicrobial in the tissues is required for treatment. The research team has identified the MIC and isolate repository as essential initial research projects to the Plataforma para la Gestión sanitaria en la Acuicultura.

The second step to understanding treatment failure is determining whether the TTC is attained in the entire fish population when medicated feed is delivered, under field conditions. A recent study suggests there are wide ranges of tissue concentrations with both oxytetracycline (OTC) and florfenicol treatments in pens of fish (Price et al submitted). Further, many fish (i.e. ~50%) did not reach the epidemiological cut-off concentration for florfenicol determined by Henriquez et al. (2015) for isolates found in Chile. Given this observation, it is necessary to identify the reasons why tissue concentrations are so low in a large number of fish. Reducing the variability in TTC in a population and ensuring that the majority of fish attain the TTC for an adequate period of time is critical for a successful treatment. This is likely a function of concentration of medication in the feed, duration of the treatment, feeding strategies, number of fish chronically infected before the treatment is initiated, and the water temperature (which affects the half-life and metabolism of the antibiotics, especially florfenicol, which does not accumulate in fish tissues due to its short half-life). The research priorities to determine management strategies that will reduce treatment failures are outlined in Appendix A.

Although improving treatment efficacy will reduce mortality on farms and the spread of *P. salmonis* between sites it is not likely to prevent all secondary cases of SRS, given the complicated structure of the industry (i.e. close proximity of some sites and interconnections between farms), the limited duration of treatment effect, and that the incubation period of this disease is longer than the duration of most treatments. It is, therefore, important to identify factors that can increase the resistance of fish to SRS. A study conducted on a limited number of farms identified several factors that may predispose fish to SRS (Jakob et al. 2014). These factors included sea lice treatments and poor smolt quality. The authors also assessed the effect of vaccines and suggested that the use of boosters may delay the onset of infection; however, many potential confounders in this study were not controlled, due to the lack of information and the small study size.

There are also certain practices that likely increase the risk of SRS, based on biological plausibility and infectious disease principles. These include, but are not limited to, the following: stressors such as bath treatments for sea lice, which bring fish in close proximity to one another and increase the potential for skin-to-skin transmission of pathogens; predator attacks, which stress fish; poor smoltification; co-morbidities (i.e. other infectious diseases including sea lice infestations); poor environmental conditions such as algal blooms; and low oxygen. Although a comprehensive study could be conducted to evaluate these factors, many of these events are not captured at an industry level and would be cumbersome to collect. If we accept that stressors increase the risk of disease then any factor that has been associated with stress on fish farms may increase the risk of SRS. Further, most producers are already doing their best to avoid these situations.

Understanding the efficacy of different vaccinations and immune-stimulants may have a higher impact on management practices. Aside from Jakob et al. (2014), there have not been many published studies evaluating vaccines under field conditions. The issue with these types of studies is that because producers do not usually have unvaccinated fish or even two or three different types of vaccines on their farms, analyses require a large number of farms to control for other factors that confound vaccination. As part of the new Plataforma para la Gestión sanitaria en la Acuicultura, this type of study will be feasible because the entire industry shares information on the types of vaccines used in salt water. An analysis similar to Jakob et al. (2014), which assessed the time to disease and total SRS mortality in pens of fish vaccinated with different vaccines, controlling for other factors, will now be feasible. If the industry shared information on when they administered booster vaccines and immunostimulants with SERNAPESCA, it would also be possible to evaluate these management strategies.

It is not possible to avoid all risks associated with SRS and, as the number of active farms and sizes of farms increase, it becomes impossible to avoid exposure to *P. salmonis* from neighboring sites. Reduced connectivity between farms can be accomplished through a number of strategies that target either the number of fish on farms, the size of the farm, or the distance between farms. Determining the optimal biocapacity of farming areas where pathogen transmission is minimized and economic return is optimized is complicated, as the system is dynamic, water currents are unknown, and management strategies (such as treatments) that reduce disease are not consistent across farms. Although several research groups have tried to establish the threshold biocapacity for different aquaculture regions, none have yet succeeded.

Currently, the regulations in Chile penalize farms with high mortality by reducing the number of fish permitted in the subsequent production cycle. Despite this new regulation, the incidence of SRS continues to increase. Interestingly, as the biomass produced in Chile increases, so does the incidence of disease (i.e. sea lice intensity and SRS incidence) and the frequency of treatments for these pathogens (Sernapesca, 2014, 2015a,2015b).

We propose to initially investigate the optimal biocapacity for reducing SRS outbreaks in Chile, while maintaining the economic integrity of the industry, for a small relatively contained area in Chile (i.e. Barrio 17a and 17b). If successful this model could be used to evaluate different biocapacity scenarios and farm configurations within this contained area, and it could be scaled-up to incorporate large interconnected areas and regions in Chile. .

The working group helped to identify and prioritize SRS research needs based on the industry's 61 research (Appendix B) questions and the perceived needs of the industry. The working group organized the questions into broad categories and then sorted through the list to determine which were consistent with the mission and strategy of the Plataforma para la Gestión sanitaria en la Acuicultura [2]. Any research questions that were not relevant to the direct management of SRS were not included in the

[2] The mission of the Fish Health Industry Research Platform is to improve the global competitiveness of the Chilean Salmon Industry by improving the health of fish and reducing the use of antimicrobials. The strategy to achieve this mission is to conduct fish health research that directly improves the sanitary state of the industry with regards to the two most problematic pathogens occurring in the industry today, and use of antimicrobials to control these pests.

initial proposed research initiative for this disease, as they do not directly address the issue of controlling this pathogen and reducing the use of antimicrobials.

Briefly, we outlined the following as research priorities for the Plataforma para la Gestión sanitaria en la Acuicultura:

1) Create a repository of *P. salmonis* isolates;
2) Evaluate why treatments are failing (See appendix A for more detail) :
    a. Determine the MIC for a representative group of isolates;
    b. Conduct a spatial analysis of the MIC to determine if resistance is occurring and if it clusters;
    c. Determine whether the therapeutic tissue concentrations are achieved in a large number of fish under field conditions using different antibiotic application strategies;

3) Conduct a risk factor analysis to help determine the level of mortality on neighboring farms when they become infective to other farms;
4) Assess vaccines for SRS using industry database;
5) Create a risk tool that identifies when farms are at risk of SRS so they can increase surveillance and treat fish earlier in the disease process;
6) Create a simulation model that can be used to evaluate different industry configurations on the incidence of SRS. UPEI will explore a small simulation project in barrio 17a and 17b;
7) Determine the micro (farm level) and macro (industry level) economic impact of delayed treatments, interruptions in treatment, and farm to farm spread of SRS under differnet industry configurations to inform producers of the impact of their management decisions;
8) Assess the need for fresh water surveillance;
9) Align regulations with research findings on critical treatment thresholds, appropriate antibiotic treatment strategies, fallowing, biocapacity thresholds for areas, etc.

For more detailed information on these proposed projects see the report "Plan de Acción" drafted by the research group.

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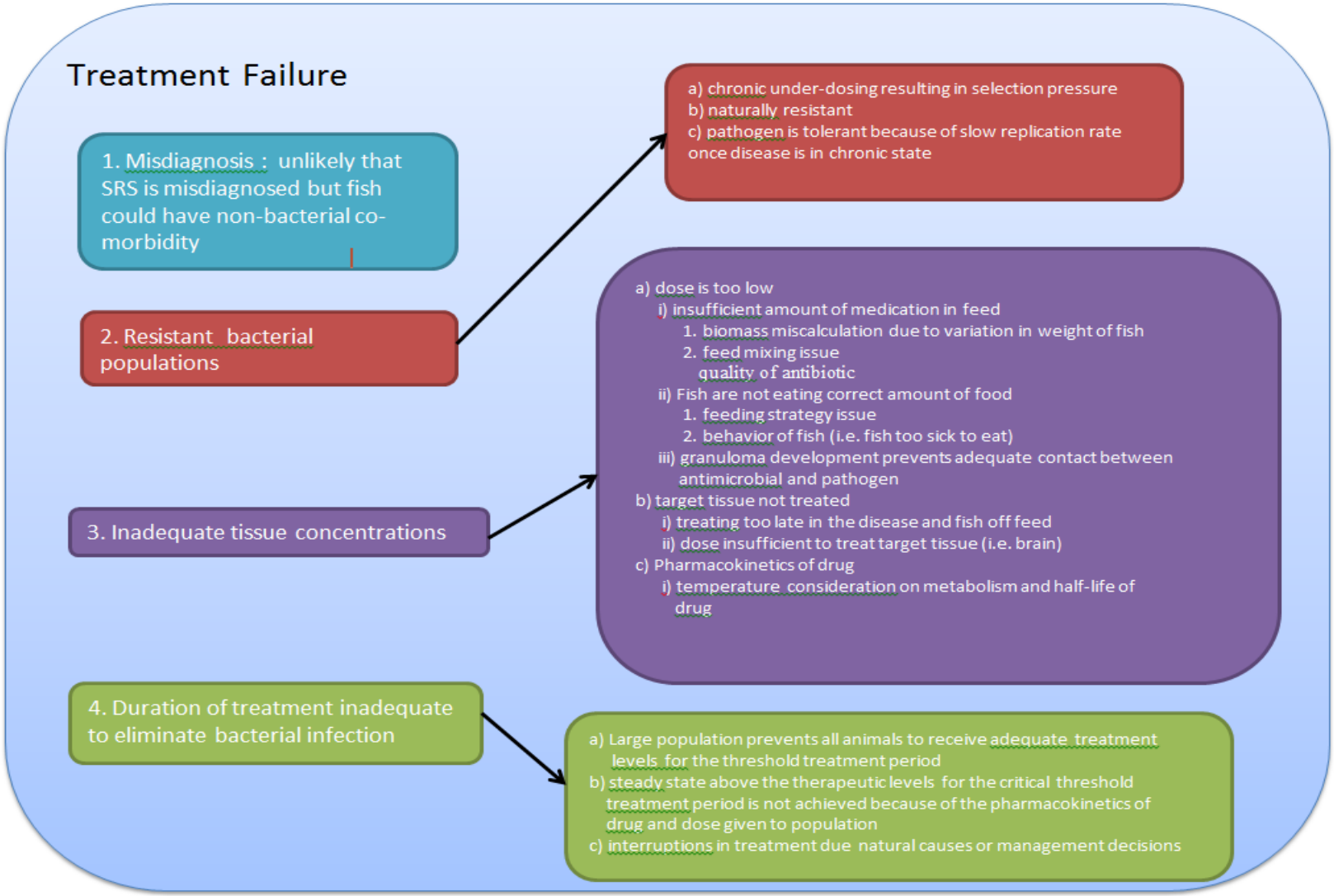
Appendix A (part of report in Appendix D)



Research:

a) Continuous evaluation of the minimum inhibitory concentration (MIC) of *P. salmonis* isolates in Chile (laboratory and field study)
b) Annual spatial analysis of *P. salmonis* MIC levels to determine if there is spatial clustering of high and low MICs (statistical analysis)
c) *In vivo* analysis of **therapeutic** inhibitory concentration for different isolates of P. salmonis and for chronic and acute presentation of SRS (laboraotry study)
d) *In vivo* analysis of the necessary duration of treatments for chronic presentation of SRS (laboratory study)
e) establish the dose to acheive therapeutic concentrations under labortory conditions.
Outcome: Provide information to help guide veterinarians on how to treat this disease (necessary tissue levels to acheive success)

a) Evaluate tissue concentrations of antimicrobials at the lowest and highest point during a treatment in populations of fish with SRS.
- goal is to determine if 95% of the fish population achieves therapeutic levels of the antimicrobials (based on laboratory studies) for an adequate amount of time (based on the laboratory studies)

b) Evaluate different antibiotic application strategies under field conditions to determine tissue concentrations within the population. Example strategies may include:
- early treatment
- injectables followed by in feed antimicrobial treatment
- grading to reduce competition and improve biomass estimation
- pre-feeding to reduce uneven levels of antimicrobials in population
- different doses and duration of treatments applied at different feeding frequency depending on water temperature

Outcome: Provide information on how to administer antibiotics under field conditions for better treatment success

a) Evaluate , under field conditions, different management strategies to improve antimicrobial treatments in large animal populations (i.e. even and sufficient antimicrobial tissue levels to eliminate bacteria) Example strategies may include
- culling
- new delivery systems
- comparison of treatment success at different population densities
- many of the strategies identified above may address this issue

b) Evaluate the cost of interruptions in treatments

Appendix B (part of report in Appendix D)

List of research questions

1. What are the sensitivity and specificity of diagnostic tests for P. salmonis detection?

2. Can the results obtained be replicated if the test is repeated? (this is related to the reliability or repeatability of the tests)

3. Which factors can influence validity and reliability of diagnostic test, and how to use such information for better disease diagnosis and management?

4. Which factors (risk or protective) can modulate the time to infection, clinical type and magnitude of SRS outbreaks? If any, how they act or interact?

5. Which factors are associated with salmon farms that may shed P. salmonis in a higher rate compared to others?

6. How these factors can contribute to developing optimal management strategies at the farm and neighbor levels?

7. Which mechanisms influence the re-emergence of P. salmonis in a farm and Caligus infestation, and which biological and non-biological mechanisms participate in the transmission and further spread of the bacteria within a farm?

8. How P. salmonis and Caligus spread from an infected farm to others (between-farm spread) and neighborhoods?

9. Are within- and between-farm spread related? How these two mechanisms can be coupled in an explanatory/predictive model for the use of the industry/government?

10. Can a disease spread model(s) be designed and implemented as a tool for an early warning system, and to evaluate management strategies or treatments schemes that ultimately support the decision-making process?

11. What is the role of vector diversity and (non) salmonid reservoirs in the P. salmonis dynamics?

12. What is the role of diversity, abundance and proximity of wild fish in the infestation process of Caligus, and what are these infestation mechanisms?

13. What biological interaction (predation, competition) might influence the abundance of Caligus?

14. What are the consequences of nutrient superabundance on pathogens, parasites, and disease?

15. What attributes of the ecosystem might predispose it toward stabilizing negative feedback (SRS outbreaks reducing the likelihood of subsequent disease), versus positive feedback (SRS outbreaks facilitating subsequent pathogen attacks)?

16. How the seascape structure (including oceanography and abiotic factors) influences population density and movement patterns of agents, vectors, and transmission stages?

17. How can knowledge of seascape structure be used to improve quantitative predictions about disease (infestation) spread and persistence?

18. Which mechanisms are involved in the vertical transmission of P. salmonis?

19. What is the role of fresh and salt water in the life stage, viability and virulence of P. salmonis?

20. How P. salmonis strains differ in terms of pathogenicity and virulence, and how the host responds to these differences and signals?

21. How P. salmonis interact with surfaces, how they survive outside of their hosts, how signals are relayed between the microorganism and the host?

22. How P. salmonis strains differ in terms of host susceptibility and geographical zones? How is the population structure?

23. What is the frequency of P. salmonis resistant strains, if any, before antimicrobial use in a farm?

24. What are the processes related to the emergence of drug resistance, and timing the emergence of resistance of P. salmonis?

25. What are the best drugs, schemes and strategies to be implemented in order to avoid drug resistance?

26. Which mechanisms are involved in the transmission of Caligus?

27. What stages of the life cycle are key in the infestation process?

28. How the duration and behavior of planktonic stages of Caligus increases the risks of infestations?

29. Which are the population-level genetic differences increasing the success of Caligus? How is the population structure?

30. What is the frequency of Caligus resistant individuals in wild and farmed salmons?

31. What are the processes related to the emergence of drug resistance of Caligus?

32. What are the best drugs, rotation schemes and strategies to be implemented in order to avoid drug resistance?

33. What are the conditions that specify whether P. salmonis will establish a persistent infection or will be cleared by the fish immune response? What are the immune mechanisms of salmonids to lower the adverse effects of caligus?

34. What is the correlation between bacterial dynamics, damage and inflammation to the fish, and the fish response? the correlation between bacterial load (abundance of P. salmonis) and the magnitude of the antibacterial immune response?

35. How the use of vaccines or alternative products aimed to enhance the force of the immune response will decrease fish susceptibility and decreased shedding rates?

36. What are the mechanisms involved in the host-multipathogen responses? What is the role of stress and immunity threshold?

37. Is there any temporal succession pattern of different diseases?

38. What is the most effective antibiotic/antiparasite currently available to treat infected salmon? How are their sensitivity?

39. How different are the pharmacokinetics and pharmacodynamics of these drugs? Which factors can modulate these processes?

40. How to measure effective plasmatic concentrations? and how correlated are the antimicrobial susceptibility in vitro and in vivo with treatment success?

41. How to develop of antimicrobials/antiparasites? How to ensure their optimal performance (safety, effectiveness, low cost, easy to apply, etc.)?

42. How to develop and use alternatives drugs (probiotics, additives, other immunostimulant, etc.)? How to ensure their optimal performance?

43. How to achieve the prescribed dose in each treatment?

44. What rotation scheme of different drugs minimizes the resistance of Caligus and to P.salmonis to drugs?

45. Is the spatial scale of salmon farming “barrios” appropriate? Do they need to be coordinated in the control of Caligus or P. salmonis?

46. What are the ecological impact and costs of these rotation schemes?

47. What are the areas of the genome (genetic markers) that encode for fish resistance mechanisms to Caligus and P. salmonis?

48. Are the genetic resistance mechanisms described the same in experimental versus natural infections? How to standardize such evaluation?

49. How genetic selection for disease resistance could interfere with desirable production and health-related characteristics?

50. What are the areas of the genome (genetic markers) that encode for agent resistance mechanisms to drugs (antibacterials, antiparasites)?

51. Are the genetic resistance mechanisms described the same in experimental versus natural infections? How to standardize such evaluation?

52. How to develop an interdisciplinary research framework to optimize approaches and interventions needed to reduce disease risk to farmed salmon?

53. How to measure the impacts and effectiveness of animal health decisions including cost-benefit analysis, cost-effectiveness, welfare measures, externalities, risk, asymmetric information, strategic behavior, and others?

54. How to develop indicators for scientific-based norms?

55. What is the interplay between environmental norms and policy?

56. How much policy coherence is found in the salmon aquaculture sector?.

57. How can stakeholder perceptions inform the policy-making process?

58. What are the benefits and costs of implementing such policies?

59. What are the most relevant agent factors that may be useful to develop an efficacious vaccine against P. salmonis?

60. How to enhance the uptake, processing and presentation of the antigen to the fish innate immune system at the mucosal level as a booster for vaccination strategies?

61. How to define a standard and adequate method to assess vaccine safety, efficacy and transmissibility?

Appendix E Descriptive study on SRS outbreaks

Descriptive study of SRS in neighborhoods 17a and 17b

Background:

Over the last decade, the salmon aquaculture industry has expanded by 26% in Chile (http://www.salmonchile.cl/en/produccion.php). Farms have increased in size and number. The net result has been an increase in biomass in designated farming regions, which has led to an increase in the risk of host dependent pathogen spread between farms. Several recent studies have described density dependent farm-to-farm spread of pathogens, including *P. salmonis* and *Caligus rogercresseyi* in Chile, over large distances (Kristoffersen et al 2013;Rees et al 2014). At a certain point, the loss of fish from infectious diseases or the cost of treatments to control diseases outweighs the potential economic benefit of producing more biomass per square km.

Defining an area's optimal production capacity, in which disease is minimized and economic viability is maintained, has historically been difficult for a number of reasons. First, in many instances, farms in shared areas may be owned by different companies and, therefore, do not consider the biocapacity of the entire area in their economic assessments. These companies may also have different economic risk thresholds, so that even if they were to consider area level factors when stocking farms, they may not reach consensus on the optimal biomass per square km. Second, the constantly changing number of active farms in defined areas makes it difficult to predict area density from one production cycle to the next. Further, different areas may have different density thresholds due to their hydrodynamic characteristics, making it difficult to reach an industry-level consensus. However, as the aquaculture industry continues to expand, it is essential to define biological mass thresholds in areas that share communal resources, to ensure the economic sustainability of the industry and welfare of the fish.

In order to determine the optimal biomass, that minimizes pathogen spread, for hydrologically connected farms we need to understand the relationships between sites. In two previous studies, we determined that there was *P. salmonis* transmission between farms, but the neighbor effect in our models (Rees et al 2014; Price et al 2017) did not explain as much of the variance in SRS as we initially expected, and much of the variance remained unexplained at the neighborhood level. We believe this occurred because we did not capture the neighbor effect appropriately in these studies.

Other sources of *P. salmonis* for aquaculture fish in Chile, such as wild fish and carry over bacteria from the previous cycle on sites, appear less important in the epidemiology of SRS. Our study on fallow effect suggests the hazard of SRS in the new year class of fish on sites is similar regardless of the length of the fallow period beyond 3 months, and this hazard was not different for farms that had a recent history of SRS and those that did not (Price et al 2017). Other researchers have suggested that wild fish could be a source of *P. salmonis* for farmed fish; however, the infection rate in wild fish surveys is relatively low (Contreras-Lynch, 2015; García et

al., 2016) and would not explain the majority of cases. The only remaining source of infection for farm fish are neighboring infected farms.

The objective of this study was to begin to explore the transmission of *P. salmonis* between neighboring farms in a small, relatively isolated area of Chile. The results of this study will inform a future statistical model to determine the association between neighboring farms and incidence of SRS. The results of the latter will be used to develop a simulation model for determining the effect of different farm configurations (size and locations) on the incidence of the disease in these neighborhoods.

Methods:

We explored the spatial-temporal relationships between the onset of SRS on different farms in neighborhood 17a and 17b to better understand the relationships between cases using a video depicting the onset and duration of cases in the area. We identified the first report of SRS on farms in these neighborhoods, between 2011 and 2016, based on data provided to us by SERNAPESCA. We created a video to depict the time and location of SRS in these neighborhoods, by species. Based on the space-time disease trends we observed, we created predictors that captured different measures of infection pressure from neighboring farms. The different predictors are listed in Table 1. These can now be used in a multivariate survival analysis, to determine the statistical association between the onset of SRS and the neighbor infection pressure.

Results:

Description of patterns observed in the video (see attached fileSRS outbreaks 2011_2016.wmv)

Observations:

2011

There were several occurrences where fish were diagnosed with SRS on the first week that they appeared in the database (see 2011-02-26, 2011-07-23); however, in all cases, the fish were larger than 480g (Table 2), which suggests either they were transferred from another salt water (i.e. smoltification) site, or the fish were already present in the area, but producers did not report to the database, or there was an error in the data download process.

The first outbreak, in 2011, was in a relatively isolated farm of Atlantic salmon, and it took 10 weeks until the next case occurred. The second case was in trout. There were very few Atlantic salmon in close proximity to the first report of infected Atlantic salmon in our study area. After the first report of SRS in rainbow trout, it took 5 weeks for the next farm to report SRS. This farm was the closest neighbor to the infected trout farm (see 2011-05-21). After this case, several farms in close proximity report SRS within 3 to 4 weeks of each other (see 2011-06-26), suggesting that infection pressure in the area was increasing, and resulting in a shorter incubation period. Within 4 weeks all trout farms in this small cluster of sites report SRS (see 2011-09-10). Once all farms (4 to 5sites) in close proximity were infected, it took approximately 9 weeks for farms farther away to report SRS (see 2011-08-20).

Eventually, Atlantic salmon farms in 17b declared SRS, 7 weeks after the report of SRS in Atlantic salmon on 2011-07-23. Several Atlantic farms in close proximity to the cluster of infected trout farms did not report SRS (see 2011-10-01), which may indicate that Atlantic salmon are more resistant to the pathogen, as has been suggested by other researchers (Jacob et al 2014; Rees et al 2014), or Atlantic producers do not report this disease as consistently as trout farmers, or the trout SRS strain is more virulent and pathogenic to that species than to Atlantic salmon.

2012

There was a long period of time when there appeared to only be 2 trout farms in neighborhoods 17a and 17b. At least one of these farms introduced fish while SRS was present on a neighboring farm. Both farms reported the disease at the same time; however, it took one farm 12 and the other 5 weeks from salt water entry (or first week in the dataset) to report SRS (see 2011-11-19 to 2012-02-04). Note: there was elevated mortality in the first farm to stock fish 6 weeks prior to the declaration of SRS.

There were not many active farms in 17a in 2012, due to a fallow from May to July 2012.

2013

There was a sudden increase in active farms in January 2013, and many of them were positive for SRS as soon as they appeared in the dataset (see 2012-12-29). Some fish were over 1kg and all fish were above 450g. We believe this is due to an issue of underreporting of activity by the farms or an issue with the data extraction step or the database. Regardless, it appears that SRS on Atlantic salmon farms clusters together (see 17b 2012-12-29). No trout farms in this neighborhood were affected for a long time, despite Atlantic farms reporting SRS. Note: this is the opposite of what was observed in 2011 where the rainbow trout succumb to SRS more than the Atlantic salmon.

Once SRS started in the trout it was not clear if it was related with the Atlantic farms or other the trout farms (see 2013-01-05 to 2013-04-20), but this could be explored further with statistical models. All farms in 17b eventually succumbed to SRS, but it took 16 weeks to appear in 17a once fish were reintroduced to this neighborhood (see 2013-03-30 to 2013-07-20). Interestingly, it was not the farms in 17a, neighboring 17b, that reported SRS first (see 2013-07-20). Once SRS started in 17a it spread to all farms relatively quickly (see 2013-07-20 forward).

2014

New fish were introduced to 17b after a fallow period while infected fish were in neighborhood 17a. It appears to have taken 14 weeks before the first case of SRS was reported in 17b (See 2014-11-15). This case was not close to any farms in neighborhood 17a. (It was at the opposite end of 17b close to an estuary.) The second case in neighborhood 17b was on a site very close to neighborhood 17a (see 2014-11-29), and then all farms in this neighborhood reported SRS within ~3 months.

2015

New fish (mostly Atlantic salmon) were introduced into neighborhood 17a and within 11 weeks (see 2015-06-20) the first case of SRS was reported. It took another 11 weeks for the next case of SRS (see 2015-08-08) to appear. Eventually, all farms in 17a reported SRS (see 2015-09-26), but the single trout farm in the neighborhood took 15 weeks to declare SRS (see 2015-12-12), despite having infected Atlantic salmon in close proximity (in 2011, it only took 3 weeks for the trout farms in this neighborhood to all declare SRS). This isolate, in 2015, seems to have taken longer to cause disease in trout than in previous years, and was quicker to cause disease in Atlantic salmon than in previous years. The majority of farms in neighborhood 17a in 2015 held Atlantic salmon, whereas in 2011 they held rainbow trout. It is possible that the reporting on farms has changed over time, but it would be worthwhile, given that other researchers have found strain differences that cluster by species (Saavedra et al 2017), to investigate whether the neighbor effect is species dependent. It is possible that, with sufficient dosage, all strains can infect any species, but the risk of infection from farms varies depending on the strain of *P.salmonis*. It is also possible that the dose of *P.salmonis* necessary to trigger an outbreak of disease on a farm is temperature dependent. The effect on temperature was not captured in this descriptive analysis.

Overall observations:

- Some fish may already be infected with *P. salmonis* when transferred to salt water sites, or farmers are not reporting farming activity to SERNAPESCA prior to the diagnosis of SRS.
- SRS cases in 17a and 17b clustered in time and space. The spread of this disease within the neighborhoods appeared to be faster than between neighborhoods, but there is strong evidence to suggest that these two neighborhoods are connected and *P. salmonis* moves between them relatively freely (i.e. when farmers reintroduced smolts into either neighborhoods while the other neighborhood had active infections, the new smolts become diseased within 12 to 16 weeks, and this occurred more than once in both 17a and 17b).
- The time between the reports of SRS appears to be related to the distance between farms and to the number of infected farms around a site (i.e. infectious dose). Given this pattern, we should explore, in a statistical model, the mortality level on farms as a potential factor for the risk of disease on neighboring farms. We should also explore whether the dose relationship is temperature dependent.
    - Incubation period appears to be related to the exposure dose from neighbors. This period seems to vary from 4 to 12 weeks.
- There may be a species specificity to strains of *P. salmonis,* because SRS seems to cluster by species; however, it is difficult to separate this effect (in the video) from the clustering of farms by species within the area. This relationship should be explored in a statistical model.

Based on our observation of the pattern of disease spread within these two neighborhoods, we created several predictors to capture the infection pressure from neighbors (see attached datasets listed in Table 3). We believe the distance between neighbors, the level of mortality, the temperature, and the species of fish on the neighboring farms will affect the time to onset of SRS. The fact that this relationship is so complicated is likely the reason why we have been unable to fully explain cases of SRS in the Chilean salmon industry with previous statistical models. It is necessary that we better capture this relationship in order to assist in the development of a simulation model to determine the effect of biocapacity on SRS incidence for interconnected farms in this area.

Future research

Using a survival analysis, with the onset of SRS as the outcome, explore which of the predictors of the neighbor effect best explains the variation in cases of SRS, adjusting for time and environmental factors that affect onset of disease.

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Table 1. List of the names of the predictors and an explanation of their meaning.

	VariablesID	Variables	Description	Codes/Units
	1	fmcode	Farm ID	
	2	date2	1st day of the corresponding week	
	3	year	Year	
	4	n1	Sequential week of the production cycle	
	5	fmyrcls	The production cycle ID	
	6	newfishsrs	Whether the 1st week of production cycle is srs positive	1= yes; 0=no
	7	species2	species type (re-caterogrized)	1= Atlantic Salmon; 2= Rainbow trout or Cohoo
	8	meanwgt	Weekly avergage weight aggregated from pen(s) on the same farm at the nth week (n1)	gram
	9	wgtCorr	Single pen weight before aggregation from the original data	gram
	44	longitude		
	45	latitude		
	46	region		
	47	municipality	neighborhood	
	49	meantemp	Weekly avergage weight aggregated from pen(s) on the same farm at the nth week (n1)	
	50	meansalnity	Weekly avergage weight aggregated from pen(s) on the same farm at the nth week (n1)	
	51	srsstart	Which week is the start week of the production cycle	
	52	ifsrsstart	Whether the current week is the start week of the production cycle	1= yes;0=no
	53	n3	the week number when srs postive on the farm	
	54	Totalnumber	the total number of fish on the farm	
	55	Totalmort	the total number of dead fish on the farm	
	56	Totalsrsmort	the total number of dead fish due to srs on the farm	
	10	Totalymortality2	100* Totalmort over Total number of fish aggregated for pens from pen(s) on the same farm at the nth week (n1)	
	16	fmsrs2	Whether the farm is srs positive in the current week	1= yes; 0=no
Neighbor distance predictors numb next to neibr refers to distance; number at the end refers to the lag period back thes are repeated for species; for count of farms; count of high farms; and count of fish with SRS				
	28	neibr2_num	the number of farms within 2 km	
	29	neibr2_act6	the number of active farms within 2 km during the previous 6 weeks	
	30	neibr2_ifatls6	the number of active atlantic salmon farms within 2 km during the previous 6 weeks	
	31	neibr2_iftrt6	the number of active trout/cohoofarms within 2 km during the previous 6 weeks	
	32	neibr2_inf6	the number of srs-positive farms within 2 km during the previous 6 weeks	
	33	neibr2_ifatlssrs6	the number of srs-positive atlantic salmon farms within 2 km during the previous 6 weeks	
	34	neibr2_iftrtsrs6	the number of srs-positive trout/cohoo farms within 2 km during the previous 6 weeks	
	35	neibr2_ifmorthigh6	the number of high-mortality farms within 2 km during the previous 6 weeks	
	36	neibr2_ifatlssrsmh6	the number of high-mortality srs-positive atlantic salmon farms within 2 km during the previous 6 weeks	
	37	neibr2_iftrtsrsmh6	the number of high-mortality srs-positive trout/cohoo farms within 2 km during the previous 6 weeks	
	38	Totalmort2_srs6	the total mort number of fish of srs srs-positive farms within 2 km during the previous 6 weeks	
	39	Totalmort2_atlssrs6	the total mort number of fish of srs atlantic salmon srs-positive farms within 2 km during the previous 6 weeks	
	40	Totalmort2_trtsrs6	the total mort number of fish of srs srs-positive trout/cohoo farms within 2 km during the previous 6 weeks	
	41	Totalnumb2_srs6	the total mort number of fish of srs srs-positive farms within 2 km during the previous 6 weeks	
	42	Totalnumb2_atlssrs6	the total mort number of fish of srs atlantic salmon srs-positive farms within 2 km during the previous 6 weeks	
	43	Totalnumb2_trtsrs6	the total mort number of fish of srs srs-positive trout/cohoo farms within 2 km during the previous 6 weeks	

Table 2. Date, farm code, species(species2; 1- Atlantic and 2- trout), and weight (mean_wt_corr) of the fish that were positive on the first week in the database.

Date	fmcode	num_neibr_within_10km	newfishsrs	species2	mean_wt_Corr	meantemp	meansalnity	Total%mortalit
2/26/2011	103944	0	1	1	465	14	33	0.0
7/23/2011	102459	2	1	1	4797	10.2	30	0.2
8/20/2011	101284	5	1	2	2474	10	30	0.0
9/3/2011	102013	1	1	1	3809	11.9	13.5	0.2
1/1/2013	100649	1	1	2	2557	16	27	1.2
1/1/2013	101326	7	1	1	643	15.4	27	0.2
1/1/2013	102765	4	1	1	1127	15.6	25.4	0.1
1/1/2013	102813	6	1	2	485	14.5	28	0.0
1/1/2013	103418	5	1	1	459	13.6	27	0.1
1/1/2013	103944	1	1	1	466	13.2	20.5	0.0
5/13/2016	103923	2	1	1	3464	11	NA	0.2

Table 3. Flow chart and list of the names of the datasets with predictors of neighbor effect.