Understanding Yellowmouth: Reeling in the Root of Antimicrobial Use in British Columbia Salmonid Production

by

Rebecca Mae Wassmuth

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University of Alberta

ABSTRACT

Food insecurity is a pressing global issue that has been further exacerbated by challenges such as a burgeoning global population and unforeseen crises like the COVID-19 pandemic. Salmonid aquaculture could mitigate these challenges by contributing to sustainable food systems. In British Columbia, Canada, the salmonid aquaculture industry is facing critical sustainability and economic challenges due to bacterial diseases, with yellowmouth disease caused by *Tenacibaculum maritimum* posing substantial concerns. This disease necessitates antimicrobial use (AMU), thereby increasing the risk of antimicrobial resistance (AMR), a problem that spans the One Health sectors of environmental, human, and animal health.

The current research delves into understanding and managing yellowmouth disease in salmonid farming operations to reduce AMU and mitigate AMR risks. By synthesizing existing research through a comprehensive scoping review and analyzing extensive data from an Atlantic salmon producer in British Columbia, this thesis aims to identify the management, production, and environmental factors that contribute to the incidence of yellowmouth disease. Understanding these factors is crucial for developing targeted interventions that can reduce disease.

Chapter 2 of the thesis is a scoping review aimed at synthesizing the available literature on factors associated with *T. maritimum* infection in both farmed and wild salmonids. The review highlighted the multifactorial nature of the disease, emphasizing the interplay of host biology, environmental factors, and pathogen characteristics. Key findings included the importance of considering fish age and size at sea entry, stocking density, minimizing physical abrasions, and reducing stress-inducing conditions to manage and prevent outbreaks effectively.

The chapter also identified an important gap in previous research regarding multivariable analyses of *T. maritimum* infections, underscoring a need for comprehensive studies that incorporate multiple interacting factors.

Chapter 3 examined data from a British Columbia Atlantic salmon producer (2015-2021) using random forest models and multivariable linear regression to identify factors associated with antimicrobial use (AMU), a proxy for yellowmouth disease incidence. Key findings included a significant protective effect of higher salinity levels, reducing AMU by 0.09 mg/kg biomass ($p < 0.05$). Temperature also acted as a confounding variable. The findings further underscored the importance of broodstock and days between weights of 100-500 grams (DBW) after placement at sea on AMU. Specific interactions between broodstock types and DBW showed differential impacts on AMU, indicating that genetic factors and growth rates are critical in disease management. There were seasonal and year-to-year trends that differed in magnitude and shape depending on the area of placement. Antimicrobial use was generally the greatest when fish were placed in the middle of the year (week 20), falling when fish were placed towards the end of the year, but these trends depended on the area of production. Additionally, site-level clustering was significant, with an intraclass correlation coefficient (ICC) of 0.30 ($p < 0.001$), emphasizing the need for site-specific management. The study also highlighted interactions between area and temporal variables, indicating the importance of regional and seasonal considerations in disease management strategies.

The findings of this thesis provide valuable insights into the factors influencing antimicrobial use (AMU) in the Atlantic salmon aquaculture industry in BC. As the industry faces increasing public pressure to adopt sustainable practices, understanding the specific roles

of environmental conditions, broodstock genetics, growth rates (DBW), and site-specific management is essential. Continued research is necessary, and the complexity of our findings highlight the necessity for targeted approaches to meet public demand for responsible aquaculture practices. Integrating the findings in this thesis and future research will support the health of both aquaculture systems and the broader environmental context in which they operate.

Preserving and improving aquatic health through informed management practices in BC finfish farms is crucial for reducing antimicrobial use (AMU) and slowing the development of antimicrobial resistance (AMR). This study embodies the One Health approach by exploring how management practices and environmental factors affect AMU in aquaculture. This highlights the idea that the management of *Tenacibaculum maritimum* impacts not only salmon health but also the broader marine ecosystem and human health by potentially contributing to the development of AMR, emphasizing the interconnected impact on animal, human, and environmental health.

PREFACE

This thesis represents original work completed by Rebecca M. Wassmuth (RW) under the supervision of Dr. Simon Otto, thesis committee member Dr. Patrick Hanington, and collaborator Dr. Carl Uhland. The conceptualization and design of this thesis was conducted by RW under the supervision of Dr. Simon Otto and the supervisory committee. This thesis is based on data provided directly from a salmonid production company after a privacy agreement signed in 2021. No parts of this thesis have been previously published.

This research was funded by the support from the industry partner, Public Health Agency of Canada, a grant from the Alberta Ministry of Technology and Innovation - the Major Innovation Fund Program for the AMR – One Health Consortium, a National Sciences and Engineering Research Council Discovery Grant, the HEAT-AMR (Human-Environment-Animal Transdisciplinary AMR) Research Group, and scholarships received through the University of Calgary Faculty of Veterinary Medicine, Calgary, AB, Canada, and the University of Alberta, Edmonton, AB, Canada.

The scoping review (Chapter 2) was completed by Rebecca Wassmuth with assistance from Dr. Carl Uhland and Etienne De Jongh. RW was responsible for developing the search strings and searching databases with assistance from the university librarian and Dr. Simon Otto. Kelsey Robertson helped in implementing the software tool (DistillerSR) used to manage the articles, screening, and data extraction. EDJ and CU acted as the secondary reviewers in both primary and secondary screening, RW performed final screening and data extraction individually. RW drafted the manuscript individually and revised it based on feedback from Dr. Simon Otto, Kelsey Robertson, Carl Uhland, and Etienne De Jongh. A manuscript for peer

review of this chapter is close to submission to *Frontiers in Veterinary Medicine* for peer reviewed publication.

Rebecca Wassmuth and Etienne De Jongh shared responsibility in data cleaning, and sorting prior to the data merger. RW was responsible for manually matching pens and assigning unique IDs between the antimicrobial use (AMU) dataset and fish biomass dataset and identifying unmatched pens, as well as matching new pens after requesting earlier 2015 data. Etienne calculated the final fish count-weighted mg/kg biomass AMU outcome variable for pens based on the AMU and biomass at time of treatment. RW and EDJ performed further data cleaning and analysis following the dataset merger under the supervision of Dr. Simon Otto. RW was also responsible for drafting and revising this thesis under the supervision of Dr. Simon Otto and the supervisory committee.

Dr. Simon Otto made substantial contributions to the structure of this thesis, as well as the revisions of each chapter and analysis of Chapter 3. Dr. Otto was responsible for obtaining funding for this project. Dr. Otto and the supervisory committee were responsible for substantial revisions and critical review of this thesis.

DEDICATION

“The more I read, the more I acquire, the more certain I am that I know nothing.”

- Voltaire

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Appreciation is also due to Dr. Carl Uhland for his meticulous feedback and assistance on numerous projects. His contributions have greatly enhanced my work. I am grateful to each committee member, including Dr. Patrick Hanington, for dedicating their time to providing me with valuable feedback.

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LIST OF ABBREVIATIONS

AMU - Antimicrobial Use

AMR - Antimicrobial Resistance

ARGs - Antimicrobial Resistance Genes

BC - British Columbia

BFCR - Biological Feed Conversion Ratio

CFIA - Canadian Food Inspection Agency

DBW - Days Between 100-500 g

DFO - Department of Fisheries and Oceans (Canada)

EFCR - Economic Feed Conversion Ratio

GCS – Unique pen identifier used by our industry partner

ICC - Intraclass Correlation Coefficient

IncNodePurity – Increase in node purity contributed by each variable in the random forest model

MSE - Mean Squared Error

OOB - Out of Bag (used in random forest models)

PCU - Population Correction Unit

PRISMA-ScR - Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews

RCT - Randomized Controlled Trial

Tmar - *Tenacibaculum maritimum*

CHAPTER 1

1.1 LITERATURE REVIEW

Food insecurity remains a global challenge, exacerbated by factors such as the rising global population and unforeseen crises like the COVID-19 pandemic. Currently, it is estimated that one in every nine individuals worldwide is undernourished, highlighting the need for solutions to improve food availability and adequacy across populations (1, 2). Aquaculture is an evolving sector that can improve food security, particularly in developing countries. By increasing incomes for individuals employed within the industry and improving access to nutritious, high-quality foods, aquaculture has the potential to make significant contributions to global nutrition and economic stability (3). Additionally, aquaculture employs a considerable percentage of the global workforce in local fisheries, thereby fostering community development and sustainable economic growth (2, 3). This review explores these themes, emphasizing the role of aquaculture-specifically salmonids- in addressing food security and the current challenges facing the industry.

Fish are the primary source of protein for 950 million people worldwide and account for 16.6 percent of global animal protein intake (1, 3). Demand for farmed finfish is growing and consumption of aquatic foods has expanded at an average of three percent per year between 1961 and 2019 (twice the rate of population growth) (1). The per capita consumption of aquatic animal products grew 1.4 percent per year in the same period (1). Despite rising demand, the volume of global fish catches saw minimal growth from 2000 to 2018 (2). This is attributed to the sustainable limits on fishing quotas, which cap the total catch to prevent overfishing and ensure marine biodiversity is maintained (2). Instead, there is a trend in production moving from

fisheries to aquaculture farms, which now account for 56 percent of total aquatic animal food production (1). This equates to US$250 billion in sales from aquaculture products out of US$401 billion in total initial sales of world fisheries products in 2018 (1).

Fish from the Salmonidae family are a highly sought-after food product (4, 5). Two of the genera in this family include *Salmo* – which encompasses Atlantic salmon and rainbow trout, and *Oncorhynchus* – which covers all species of Pacific salmon (4). Salmonidae are an anadromous fish that are unique to other families, because they hatch in freshwater, transition to saltwater to grow and mature, and then return to their home streams after one or more years to spawn (4, 6). Salmonids undergo a biological process known as smoltification, which allows them to survive in saltwater (4, 6). This process is signaled by temperature and photoperiod variations that indicate changing seasons (7). After salmonids smoltify, they may remain in freshwater but are not able to reach the same size due to limited energy sources (4).

Due to their unique life process, the farming of salmonids requires that eggs are produced and hatched in freshwater, where the fry emerge and are transferred to larger freshwater tanks to become juveniles (6). The smoltification process of these juveniles can be manipulated by strict photoperiod lighting protocols controlled in the hatcheries so that producers always have transfer-ready smolts (6, 7). After the juveniles undergo smoltification, they are moved to saltwater net pens floating in the ocean (6). These pens have historically been large volume (1000 m^3) anchored in sheltered bays or areas close to shore (6). This improves access to farm facilities and decreases the cost and environmental impact associated with shipping food and supplies (6). However, there is a trend where farms are moving further from shore, into areas with higher currents and less congestion between other sites (6). These currents are thought to

improve the oxygenation of water, and quickly distribute wastes to improve the health and productivity of fish (5, 6).

Atlantic salmon are the most produced salmonid species and the most valuable finfish species in the United States (U.S.) (5). Farming of these salmonids began in Norway in the 1960s and has now spread to many other countries across the world (8). Since 1990, worldwide production of Atlantic salmon has increased from 230 thousand metric tons to a staggering 2.2 million metric tons in 2018 (9). Conversely, rainbow trout which was the most-produced salmonid in the 1980s has now slowed production to 812,940 tons in 2012, being the second most-produced species (6). Norway still leads production of Atlantic salmon today (~60%), with Chile following (~30%) and other countries such as the UK, Canada, Australia, and the U.S. also contributing (5, 6, 9). With increased consumer demand and subsequent production from these systems, there are conversations surrounding the sustainability of aquaculture.

The United Nations has introduced the 2030 Agenda for Sustainable Development to steer countries and communities towards a sustainable future (10). In response, the Food and Agriculture Organization created a 'Blue Transformation' roadmap which aligns with the 2021 Declaration for Sustainable Fisheries, Aquaculture of the Committee on Fisheries (COFI) of the Food and Agriculture Organization of the United Nations (FAO), and FAO's Strategic Framework 2022–2031, to inform the transformation to more efficient and sustainable aquatic food systems (11). Assessing the sustainability of aquaculture systems has been difficult, especially considering the differing definitions of the term. The consensus on sustainability focuses on resource management—including economic, social, and environmental—to ensure the fulfillment of human needs both in the present and for future generations (12). Many indicators of sustainability have been proposed, with the environmental indicators evaluating the

efficiency with which resources are used and the minimum release of pollution or useless by-products, or the lowest risk to biodiversity (12).

Aquaculture systems can align well with these objectives, boasting an efficient feed conversion ratio (FCR)—approximately 1.3 kg of feed is needed to produce 1 kg of salmonid mass (13). In comparison, other animal protein sources such as chicken, pork, and beef have much larger FCRs of 2.5, 5, and 10 kg of feed/kg of live weight, respectively (14). Aquaculture products like finfish are vital for a balanced diet, providing crucial protein, micronutrients, and essential fatty acids that complement the predominantly carbohydrate-based diets prevalent in developing countries (3). Micronutrients present in fish such as vitamins A and D have high bioavailability, making them easily absorbed by the body (3). Other fish products such as fish oils are high in polyunsaturated fatty acids which are involved in bodily processes to reduce inflammation, mediate macrophage function, and inhibit platelet aggregation (3). After processing, about 60% of fish's live weight is defined as a by-product (15). By-products such as heads, backs, and skin can also be processed to obtain high-value bioactive compounds for use in collagen and oil products (2, 15). These compounds include proteins, amino acids, and gelatins (15). Recent industrial uses have included processing by-products for feed, fertilizers, biofuels, pharmaceuticals, dyes, and plastic alternatives (2). The diversity of uses for these by-products and continued research ensures that environmental sustainability indicators continue to improve (15).

A challenge in salmonid farming is the significant expense associated with their feed, which stems from their substantial protein needs, necessitating considerable quantities of fish meal and fish oil (6, 9). This accounts for more than half of the production costs, which is problematic because the prices of raw materials for feed are rising (6, 9, 16). There have been

attempts to replace raw materials with plant-based proteins, however, ~15% of protein is fishmeal due to high concentrations of micronutrients and difficulty in acceptance of feed (6, 16). This expansion has raised concerns regarding the sustainability of aquaculture and capture fisheries, as the supply of fishmeal, particularly from wild sources, has not kept pace with increasing demand (16, 17). Additionally, the aquaculture sector's growing consumption of wild fish resources exacerbates this issue (16, 17).

Due to the interconnected nature of net pen farms and the surrounding marine environment, there are concerns about environmental contamination. Effluents from aquaculture production systems include uneaten food and fish waste (6, 18). These waste products are made up of carbon, nitrogen, and phosphorus, and can change the makeup of the nutrients in marine sediments (19, 20). This change in nutrients can contribute to harmful natural events such as algal and phytoplankton blooms, hypoxia, and a change in faunal communities (6, 19, 21). Therefore, producers must work to keep organic effluents below an acceptable level to prevent any effects on the surrounding environment (22). Risk assessments are performed at the local level of each farm due to changes in hydrodynamics, farm husbandry practices, and the farm size (22, 23). In Norway, risk assessment is performed by the authorities on a random basis where chemical factors and presence of faunal indicators are evaluated to categorize the farm in terms of low to high organic loading (22). In 2013, the level of organic loading was found to be unacceptable in 2% of Norwegian farms, with the rest all within the threshold of acceptability (22). Regional impact can also be assessed by measuring the organic loading at the most likely area of distant accumulation (22). Farming practices to reduce nutrient loading, called Integrated Multitrophic Aquaculture (24),involves plant and filter-feeding organisms that extract nitrogen and phosphorous from the environment (24). Although not yet commercial, shellfish have been

tested with finfish in a marine environment and were shown to remove 54% of the particulates, and seaweed was demonstrated to remove 60% of nitrogen and phosphorous contaminants (24-27).

Another concern regarding salmon farming are escape events resulting in competition and genetic modification to wild salmon populations (22). Selective breeding of farmed salmonids can be used to improve growth rate, disease resistance, and FCRs in order to increase profitability and improve market supply (24). There are also initiatives to utilize selective breeding to decrease the impact of climate change (28) (24). This would improve the ability of farmed salmonids to adapt to wider ranges of temperature, salinity, and increase growth rates at higher temperatures (28). However, despite these advancements there has been limited (<10%) uptake of selective breeding and genetic modification techniques (24, 29). This may be due to the lack of public support or attraction of private investment (28, 30). Lack of public support could stem from the idea that escape events could result in competition between wild and farmed salmonids, and interbreeding resulting in genetic modification to wild salmonid strains (22). When Atlantic salmon escape, it is reported that many do not survive for long in the wild, or are recaptured easily due to their propensity to remain close to the farm (22, 31). However, there is still evidence of the persistence of escapees in the wild, including Atlantic salmon successfully spawning in the Pacific coast of North America, Northwest Atlantic, and Norway (22, 32, 33). The impacts of the introgression of farmed salmonids into the wild population is difficult due to large amounts of natural variation in these populations (22). However, some studies have shown lower fitness of wild and farmed salmonid offspring compared to wild origin alone (22, 34). Therefore, mitigation of escape events is an important component of sustainability within the industry.

As salmonid farming operations in British Columbia (BC), Canada, and across the globe increase output to meet consumer demands, subsequent challenges arise (35, 36). Bacterial and viral diseases are a major challenge affecting both the sustainability and economic stability of finfish farming (37). In BC, 95.7% of the aquaculture production biomass is attributed to Atlantic salmon (38). This is due to their efficient growth, feed preferences, and small FCR (38). In Canada, bacterial diseases impacting farmed Atlantic salmon are endemic to the area such as furunculosis, vibriosis, enteric redmouth, and stomatitis (yellowmouth) (39).Viral diseases such as infectious pancreatic necrosis, pancreas disease, heart and skeletal muscle inflammation, and cardiomyopathy syndrome are the largest causes of morbidity and mortality in Norway (22, 40). Viral infections are important causes of disease in farmed Atlantic salmonids, as vaccines have not been as efficacious in prevention as for bacterial diseases (22, 40). Therefore, prevention of viral diseases requires efforts to be focused on reducing the transmission of disease (40).

To prevent transmission of disease in aquaculture settings, factors like stocking density, stressful events, and environmental changes must be considered. Salmonids have a high level of innate immunity, therefore, they are most at risk for infection when under immunosuppressive conditions (40). These immunosuppressive conditions could include higher stocking densities, handling events, transfer from freshwater into saltwater, and infection with sea lice (40, 41). Temperature is a factor that may influence the rate of oxygen consumption, or metabolic demand of fish. In salmonids, the standard metabolic rate or oxygen consumption rate increases exponentially with temperature in resting fish (42). As temperatures increase from 6-18 ^{o}C, dissolved oxygen demands increase from 30-55% (42). Decreased oxygenation during events such as algal blooms have also been correlated with disease outbreaks due to immunosuppression (43, 44). Temperatures outside of the optimum rearing ranges for salmonids

subject salmonids to stress, which suppresses the immune system and can lead to dysbiosis (45, 46). Under experimental conditions, it has been demonstrated that temperature has a significant effect on the salmonid gut microbiota, skin mucous, and water microbiota (46). In salmonids, disease risk is lowest when reared at temperatures between 12-13 °C, increasing from 14-17°C and highest from 18-20°C (47).

Infection with sea lice may be associated with an increased risk of disease, either as a stressful stimulus or as a direct vector for disease (6, 22, 48-50). At high levels, sea lice can cause damage to the epithelium, leading to opportunity for secondary infection and decreased growth (18). The impact of these sea lice on wild salmonids is however, controversial. Salmon farming in British Columbia (BC) is unique since they are one of the only salmon farming regions where farmed salmon are interspersed within areas that five species of wild Pacific salmon are found (51, 52). These salmon have both ecological and cultural significance in the region (51, 52). Previously, sea lice infestations were not considered a health risk to farmed salmon in the BC region (52). However, following a decline in pink salmon (*Oncorhynchus gorbuscha*) populations, the government implemented sea lice monitoring and control protocols onto all farms (52). The connection between lice in farmed salmonids and wild salmonid mortality is still debated with some studies concluding that sea lice could destroy wild salmonid populations (53) and that wild production is limited in areas around farms (54), which could result in pink and chum salmon stock declines if lice induced mortality rates reach 20-30% (55). However, other analyses contest that increased levels of lice surrounding farms is not sufficient to cause increased mortality, and that production of wild salmon is not reduced in surrounding farms (56). Regardless, the reputation of salmon farms operating in British Columbia has been damaged by these claims (52). In Norway and Canada, the number of sea lice on each salmon is

tightly controlled to less than 3 mobile lice per fish in order to reduce the spread to wild salmonid populations (22). In Norway, lice counts are performed every 2 to 4 weeks after transfer to seawater, where delousing treatments are performed if more than 0.5 adult female lice or more than 3 mobile lice are found per fish in a pen (51). Sea lice control by means of hydrogen peroxide treatment baths may also result in stressful handling events that predispose salmonids to disease (52). Treatment also comes in the form of veterinarian prescribed feed pellets which include 0.2% emamectin benzoate, known as SLICE (51). The continual use of SLICE may lead to the development of resistance, however this has not yet been observed in British Columbia (57). Cleaner fish such as lumpsuckers have also been employed as natural biological controls for lice (58). However, salmonids and lumpsuckers have differing optimal rearing temperatures, where lumpfish cannot survive at warmer temperatures around 18 °C, when this is within the optimum range for Atlantic salmon (58, 59). Therefore, the welfare of these biological control cleaner fish should also be considered in the context of the environmental factors at each farm (58).

Vaccines have been an invaluable tool in reducing disease outbreaks from most bacterial and some viral causes (39). This has reduced the need for antimicrobials, as vaccines can be formulated to target diseases specific to each production site (39, 60). Therefore, producers have been successful in reducing the need for antimicrobials in most bacterial diseases such as vibriosis, furunculosis, enteric redmouth, with the exception of yellowmouth (39). The causative agent of this disease is *Tenacibaculum maritimum* - an opportunistic bacteria associated with many fish species worldwide (61). Infection with *T. maritimum* can also result in tenacibaculosis; a more widespread and clinically unique disease characterized by frayed fins, tail rot, and ulcerative lesions (61, 62). Clinical signs of tenacibaculosis may be attributable to

several *Tenacibaculum* species, making strain and species selection for vaccine development a challenge (63, 64). In contrast to tenacibaculosis, yellowmouth is specific to Western Canada and Washington state (61). Clinical signs, when present, are limited to small yellow plaques in the mouth (61). Because of this, bacterial culture on KABAMA or MSSM plates (specialized *T. maritimum* agar), gross pathology, or PCR are commonly needed to confirm the presence of this disease (65). Despite the rarity of clinical signs, mortality rates in BC farmed salmon attributed to yellowmouth can be as high as 15% (66) with an economic burden that has been estimated to be $1.6 million per year for a single company (65).

Producers utilize antimicrobials to combat outbreaks of bacterial diseases and administer them to fish by medicated feed (39). In Canada, all salmonid farming operations require a veterinarian's prescription to administer antimicrobials (39). Medication records must then be reported to Fisheries and Oceans Canada (DFO) (39). Antimicrobials approved for use in Canadian-farmed salmon include florfenicol, sulfadimethoxine and ormetoprim, sulfadiazine and trimethoprim, and oxytetracycline (39, 65). Due to the lack of a yellowmouth vaccine, florfenicol and potentiated sulfonamides are prescribed to treat and control the disease (39). Antimicrobial use (AMU) in salmonid production in BC is largely attributed to the treatment and control of yellowmouth (39). For example, in 2011, 98% of the antimicrobials prescribed for BC aquaculture operations were written for bacterial stomatitis (39). This suggests that AMU could be dramatically reduced in Western Canadian aquaculture if the disease could be prevented by other means, but little is known about management, production, environmental, and other factors contributing to its incidence, and subsequent risk reduction strategies (65).

Antimicrobial resistance (AMR) is a problem that exists at the interface of humans, animals, and the environment, therefore, we must consider it from a One Health perspective (67).

The World Health Organization (WHO), Food and Agriculture Organization (FAO), and World Organization for Animal Health (WOAH), have partnered to develop One Health action plans to combat AMR (67). One of the five pillars of the WHO Global Plan is to optimize the use of antimicrobial medicines in human and animal health (68). With AMU in aquaculture, there is the risk of AMR bacterial strains and genes developing in the aquatic environment and spreading to the terrestrial environment (67). Although AMR is a naturally occurring process, there is concern surrounding the development of extensive drug-resistant and mutridrug-resistant bacteria due to imprudent AMU (69). Due to the interconnected nature of AMR, it is essential that all relevant sectors promote antimicrobial stewardship to ensure the future health of humans, animals, and the environment (70).

It is difficult to quantify and compare AMU in aquaculture internationally due to the lack of standardized monitoring and varying prescription and use reporting regulations across countries (69). Where data are available, there is high variability in AMU across countries, for example, Chile uses approximately 660 g of antibiotics per tonne of salmon produced, in comparison to AMU in Norway which is estimated at 0.02-0.39 g/tonne (69). More research is required to understand differing disease pressures across countries and to determine appropriate AMU guidelines to ensure prudent use.

There is no readily available vaccine for yellowmouth and many of the antimicrobials prescribed in BC are a response to control it, therefore, we must begin to elucidate the factors associated with disease development. It has been postulated that several environmental factors such as water salinity and temperature are associated with yellowmouth in salmonids (65). Decreasing the salinity and/or temperature in a pen has previously been shown to reduce yellowmouth mortality in affected salmonids (71), however, subsequent studies found that

freshwater treatments had no significant effect on the presence of the bacteria (72). Outbreaks of yellowmouth are significantly correlated with seasonality, with increasing prevalence in the summer, followed by a decline in outbreaks over the winter months (72). Specifically, water temperatures over 15 degrees Celsius have been suggested as a risk factor for tenacibaculosis (73). Pen sediments and water previously exposed to infected fish could also serve as reservoirs for *T. maritimum* as the bacterial undergoes horizontal transmission (61). It has also been hypothesized that bacterial vectors such as jellyfish or sea lice could introduce *T. maritimum* into the aquaculture environment, or that gill or skin abrasions from jellyfish stings could provide an opportunity for infection with the bacteria (74). Although, previous experiments have found no evidence that gill abrasion or co-infection with amoebic gill disease has an additive effect on mortality when fish are infected with *T. maritimum* (72, 75).

In conclusion, the rising challenges of food insecurity, exacerbated by a growing global population and unforeseen crises like the COVID-19 pandemic, underscore the need for new and sustainable solutions (1, 2). Aquaculture, particularly through the cultivation of salmonids, has emerged as a significant player in this arena (3). By providing a source of nutritious food and supporting economic stability through job creation, aquaculture has the potential to substantially enhance global food security (3). However, as the industry expands, addressing potential challenges, including feed sourcing, disease management, and environmental impacts, will be critical. Moving forward, integrating sustainable practices and new technologies into aquaculture will be essential to combat global food insecurity while maintaining ecological health.

1.2 RESEARCH QUESTIONS AND OBJECTIVES

Thesis Aim:

The aim of this thesis work is to understand the management, production, environmental, and other factors that contribute to the incidence of yellowmouth in Atlantic salmon production caused by *Tenacibaculum maritimum*, and to identify opportunities to reduce the incidence of yellowmouth in BC farmed Atlantic salmon.

Research Questions and Objectives:

Research Question 1

What are the factors reported in the scientific literature to be associated with disease in salmonids caused by the bacterium *Tenacibaculum maritimum*?

Objective 1

1. To synthesize available knowledge through a scoping review to identify factors (management, production, environmental, other) associated with disease in farmed and wild salmonids caused by *Tenacibaculum maritimum*.
2. To identify factors for focus in the analysis for Research Question 2.

Research Question 2

What factors specific to farmed BC Atlantic salmon are associated with the development of yellowmouth disease from *Tenacibaculum maritimum* in Western Canadian Atlantic salmon production?

Objective 2

1. To utilize random forest models and multivariable linear regression to identify factors (management, production, environmental, other) associated with yellowmouth disease in BC farmed Atlantic salmon.

CHAPTER 2

Factors Associated with Disease in Farmed and Wild Salmonids Caused by *Tenacibaculum maritimum*: a scoping review

2.1 ABSTRACT

Introduction: Yellowmouth disease, caused by *Tenacibaculum maritimum* (Tmar), is an important disease of farmed salmonids. Disease management currently necessitates the use of antimicrobials, raising concerns about antimicrobial resistance (AMR) in aquatic and potentially terrestrial environments. Identifying management, production, environmental, and other factors associated with the development of yellowmouth in salmonids will help to elucidate disease control strategies and decrease the economic and environmental burden of its treatment. The objective of this scoping review was to synthesize the available literature to identify factors associated with disease in farmed and wild salmonids from Tmar.

Methods: The scoping review followed the framework outlined in the Joanna Briggs Institute Reviewer's Manual and PRSIMA-ScRT reporting guidelines. The protocol was developed *a priori* in consultation with a librarian and was used to search Environment Complete®, Earth, Atmospheric, and Aquatic Science®, Scopus®, and Web of Science™ databases on July 21, 2022, and again on April 27, 2023. Articles were included if they focused on Tmar infection in salmonids and discussed factors (environmental, management, or other) that impacted the disease and/or organism of interest.

Results: Twenty-five articles were included for review. Over half of the included articles were published within the last five years (n=14/25). The included articles revealed a complex interplay

of salmonid (host)-specific factors (age/size), management practices (vaccination, marine transfer, stocking density, gill/body abrasion), environmental conditions (water temperature, oxygenation, salinity, algal blooms, vectors), and microbial dynamics (load, co-infections, strain, biofilms, microbiome) influencing Tmar infections.

Discussion: The review highlights the complex, multifactorial nature of Tmar infections, including the interplay of host biology, environmental factors, and pathogen characteristics. A comprehensive approach incorporating both management and environmental components is essential to mitigate Tmar infections in salmonid production.

2.2 INTRODUCTION

Food insecurity is growing due to the increasing global population and challenges like the COVID-19 pandemic (1). As the world's population continues to grow, demand for food also rises (1). Subsequently, the demand for seafood is rising and finfish is becoming a popular source of protein, accounting for 16.6 percent of global animal protein intake (1). Consumption of aquatic foods has expanded at an average of three percent per year between 1961 and 2019 (twice the rate of population growth) (1). Due to the environmental limitations associated with wild-capture fisheries, aquaculture farms are increasing production to meet the growing demand, and account for 56 percent of total aquatic animal food supply (1). These production systems are uniquely suited to meet the goals of the United Nations 2030 Agenda for Sustainable Development (10). As salmonid farming operations across the globe increase output to supply the growing demand, subsequent challenges such as bacterial diseases emerge (35, 36).

Bacterial diseases are a major challenge affecting both the sustainability and economic stability of finfish farming (37). To combat these bacterial diseases, producers administer antimicrobials through medicated feed (39). Vaccines have been successful in reducing the need for antimicrobials in common bacterial diseases in finfish, but are inefficacious against yellowmouth (39). The causative agent of this disease is *Tenacibaculum maritimum* (Tmar), an opportunistic bacteria associated with many fish species worldwide (61). Infection with Tmar can also result in tenacibaculosis (formerly known as marine flexibacteriosis); a more widespread and clinically unique disease characterized by frayed fins, tail rot, and ulcerative lesions (61, 62, 76). In contrast to tenacibaculosis, yellowmouth is specific to the western Pacific coast in British Columbia (BC), Canada, and Washington state (61). Mortality rates in BC farmed salmon attributed to yellowmouth can be as high as 15% (66), with an economic burden

that has been estimated to be $1.6 million per year for a single company (65). Since there is no commercially available vaccine to protect salmonids from yellowmouth, antimicrobials such as florfenicol and potentiated sulfonamides are prescribed to treat and control the disease (39). Antimicrobial use (AMU) in salmonid production in BC is largely attributed to the treatment and control of yellowmouth (39). For example, in 2011, 98% of the antimicrobials prescribed for BC aquaculture operations were written for bacterial stomatitis (39). This suggests that AMU could be dramatically reduced in western Canadian aquaculture if the disease could be prevented by other means, but little is known about management, production, environmental, and other factors contributing to its incidence, and subsequent risk reduction strategies (65).

Although many factors may be associated with the development of disease caused by *T. maritimum*, a search of Web of Science, Scopus, Environment Complete, and Earth, Science & Aquatic Collection on June 15th, 2022, and April 27, 2023, did not identify a systematic or scoping review on this topic. The objective of this scoping review study was to synthesize the range of existing research on the factors associated with disease in farmed and wild salmonids caused by Tmar infection.

2.3 MATERIALS AND METHODS

2.3.1 Protocol, search, and information sources

This scoping review follows the framework outlined in the Joanna Briggs Institute Reviewer's Manual (77) and was reported according to the PRISMA-ScR guidelines (77, 78). A comprehensive search strategy was developed with the assistance of Janice Kung - a librarian at the University of Alberta - to identify articles that reported factors that contribute to Tmar infection in farmed and wild salmonids. This *a priori* review protocol and all amended protocols

were time-stamped and are accessible through Open Science Framework Search strings (79) (Table 2.1, Appendix 2.1) were used to search Environment Complete®, Earth, Atmospheric, and Aquatic Science®, Scopus®, and Web of Science™ databases on July 21, 2022, and again on April 27, 2023.

2.3.2 **Eligibility Criteria**

Eligibility for inclusion in each stage of screening was determined by two independent reviewers. In the first stage, article titles, abstracts, and key words were screened, followed by full text retrieval and screening in the second stage. To be included, studies had to report that they were focused on Tmar infection in salmonids and factors (environmental, management, or other) that impact the disease and/or organism of interest. Factors were defined as observations that were hypothesized or measured to have a relationship with infection from Tmar. The search strings did not include a factor component; instead, this was assessed during screening to ensure that all relevant articles were captured for screening. No search restrictions were placed on language, publishing date, or geography. Review articles, conference abstracts, preprints, books, book chapters, theses, dissertations, commentaries, opinion pieces, editorials, and newspaper articles were excluded.

Articles were screened for eligibility via a two-stage process by two independent reviewers. Article titles, abstracts, and keywords were screened in the first stage, with articles proceeding to secondary screening if both reviewers determined that they fully met the inclusion criteria or were unclear. Secondary screening utilized a “1 in 2 out” procedure, where all articles were screened by a primary reviewer. If the reviewer included the article, it was automatically included in the review, if the reviewer excluded the article, it was screened by a secondary reviewer to confirm exclusion. This second screening round protocol amendment was reflected

in the protocol which was uploaded onto Open Science Framework on September 19, 2022. After the second search was conducted on April 27, 2023, the amended protocol with search results was uploaded on May 3, 2023. Screening conflicts were resolved by discussion between reviewers, with a third independent reviewer available to resolve conflicts if required. Google translate was used to translate any non-English article for screening.

2.3.3 **Data collection and synthesis**

All articles retrieved from the database search were downloaded into EndNote X9 for automatic and manual removal of duplicates. The remaining articles were uploaded to DistillerSR® (Evidence Partners, Ottawa, ON, Canada) and automatically removed/deduplicated at a confidence level of 80%; manual deduplication was also performed during screening. Data extraction was performed by a single primary reviewer after consultation. Citation data, study location, year(s) of data collection, study type, characteristics of host (salmonid) and bacteria (Tmar), disease diagnosis technique, clinical presentation of infection, description of factors identified, and how those factors impact the disease and/or bacteria of interest were all extracted. Article information was collected in DistillerSR® and exported to a pre-developed data extraction form within Excel (Microsoft, Redmond, WA) for further interpretation. A narrative approach was used for data synthesis.

2.4 **RESULTS**

2.4.1.0 **Selection of information sources**

The primary search on July 21, 2022, identified 1,974 articles. A secondary search April 27, 2023, identified an additional 79 articles. After deduplication, 1,555 articles went to primary screening, where 1,437 articles were excluded, with the main reasons being that the research did

not focus on Tmar (n=1,214) or salmonids (n=170). Of the 125 full-text articles identified for secondary screening, 7 were excluded during full text retrieval. Three of these were grey literature or book chapters. Three non-English articles did not meet the screening criteria. One article (a one-page abstract) was inaccessible after an extensive search through our institutional library and interlibrary loan program. After secondary screening, 93 articles were excluded for not pertaining to research on Tmar (n=45), salmonids (n=6), factors of interest (n=27), or was not primary research (n=15). Following all stages of screening, 25 studies were eligible for data extraction (Figure 2.1).

2.4.1.1 **Characteristics of included articles**

Most of the 25 included articles were published between 2017 to 2023 (n=14/25), but dated back to 1994 (Table 2.2). The study designs ranged from experimental (n=10/25) to longitudinal (n=7), randomized controlled trials (n=3), cross-sectional (n=2), and case reports (n=3). All included articles originated from high-income countries (80) with Australia (n=7) and Canada (n=8) representing the highest proportions. Most studies reported Atlantic salmon as one of the salmonid species of interest (n=21). Disease caused by Tmar was defined as yellowmouth (n=9), tenacibaculosis (n=9), marine flexibacteriosis (n=2), acute gill disease (n=1), or not defined (n=3).

2.4.1.2 **Synthesis of Results**

Several thematic categories were reported for factors that related to Tmar infection, including salmonid (n=6), management (n=15), environmental (n=16), and microbial factors (n=15) (Table 2.3). Most studies reported more than one factor of interest. Out of the 25 studies included in the review, 14 did not run formal statistical comparisons. Multivariable analysis was

conducted in only one study to examine the impact of temperature, salinity, and oxygenation at different depths with florfenicol use (48). The remainder of the studies used statistical analysis to characterize a change in one parameter with a significant increase or decrease in another outcome variable.

2.4.1.3 **Salmonid Factors**

Six articles reported on one or more salmonid factors that contributed to infection with Tmar (Table 2.3) (72, 81-85). Two studies reported an increased prevalence of infection during the first year of production at sea when the fish are smaller (72, 83), with a possible factor being the softness of scales at a young age (81). In one study, the size of fish was investigated by comparing the mass of wild fish (kg) to their current length (cm) during their first year at sea (85). Authors reported that fish with a higher bacterial load of Tmar had a lower-than-expected mass for their length, which was identified as a function of decreased feeding rates and feed consumption in fish infected with the bacteria (85).

A challenge trial to assess the fish species effect found that a Tmar challenge concentration of 1.6×10^6 cells/mL was significantly ($p<0.05$) associated with higher mortality in Atlantic salmon (74.9%) compared to Rainbow trout (50.0%); there were no differences at other challenge concentrations: (1.8×10^3, 2.3×10^4, 2.3×10^5, and 1.6×10^7 cells/mL) (82). When salmonids were compared to non-salmonids such as greenback flounder, there was significantly higher mortality (10% vs 2%) and morbidity with lesions (20% vs 0%) in Atlantic salmon (82). Another study reported species-specific variability in the response to infection with Tmar (84). Greenback flounder showed mild to moderate erosions on the fins and tail with minimal histological lesions compared to the higher susceptibility and more severe lesions observed in rainbow trout and Atlantic salmon (84).

2.4.1.4 **Management Factors**

Most of the 15 articles that mentioned management factors (Table 2.3) investigated gill/body abrasion (n=8) (43, 48, 74, 75, 81, 84, 86, 87) or pen elements (netting, cleaning systems) (n=3) (48, 81, 88). Abrasion of the gills was reported to disturb respiration, which ultimately enhanced the progression of disease from Tmar (43, 64, 86). Bodily abrasion, which could be a result of contact with netting or other pen elements, was reported to enhance the rate and severity of infection (81, 87). Skin lesions were commonly found in areas that were more prone to abrasion or movement, such as dorsal and pectoral fins (81). Other studies reported that abrasion allowed bacterial proliferation below the epidermis (81) and that the infiltration of Tmar was restricted to necrotic tissue (84). One article reported that infection with Tmar appeared to occur only after abrasion of the skin (87). However, another study reported that at high concentrations, the fish became infected with Tmar and died after 72 hours with no prior abrasion to the epithelium, and little sign of erosion (81).

Contact with pen elements (81), other fish (81), or jellyfish (74), or net pen cleaning (48), could have introduced Tmar, as it was reported that Tmar exists in several reservoirs such as tank walls, net pens, and water samples (48, 81, 88). Studies also reported on transfer from freshwater to saltwater (48, 74), and time since transfer to saltwater between three to eight weeks (89), and one week and one year, as factors for Tmar infection (48, 72). The prevalence of Tmar in dead and dying salmon (from all causes) was also found to be the highest in the first year after ocean entry (83). Other management factors such as vaccines were examined (61, 90). A vaccine developed for yellowmouth using isolates from western Canada was reported to be unsuccessful in protecting fish under experimental challenge conditions (61). In another vaccination study on marine flexibacteriosis, authors reported that naïve Atlantic salmon had significantly better

survival rates when injected with vaccine and adjuvant than the control group or vaccine only group (90).

2.4.1.5 **Environmental Factors**

Environmental factors were reported by 16 of the included studies (Tables 2.3-2.5). One study reported that infection with Tmar did not always result in clinical signs, therefore other environmental factors might be necessary to result in clinical disease (91). Water temperature was the most frequently reported environmental factor (n=10) (Tables 2.3 & 2.4) (43, 46, 48, 49, 72, 81, 84, 87, 89, 91). An increased water temperature was reported to result in a greater prevalence of Tmar in the gill arches of farmed salmon (72) or an increased frequency of application of florfenicol treatments (48). The remaining eight articles reported a potential connection between water temperature and Tmar (43, 46, 49, 81, 84, 87, 89, 91). Some studies reported an increased number of disease outbreaks of disease caused by Tmar during periods of warmer water temperatures (49, 72, 87). One study reported that infection during periods of warmer water temperature was the result of increased stress on fish and increased bacterial growth (81). In contrast, one study reported that the prevalence of Tmar did not appear to have a strong correlation with warmer water, although no statistical analysis was presented (91). Another study reported an overgrowth of *Tenacibaculum* species in the fecal microbiota of salmonids undergoing low-temperature water treatment, however, the species could not be confirmed as Tmar (46). Increases in water temperature were reported to be associated with algal blooms (43, 81), which have been hypothesized to be a risk factor for Tmar infection.

Seasonality was reported in numerous studies and relationships to other factors such as water temperature, salinity, and dissolved oxygen were considered (n=7) (Tables 2.3 and 2.5)

(48, 49, 72, 81, 85, 89). Ultraviolet irradiation from the sun was reported as a possible cause of skin lesions that then propagate the growth of Tmar (84). One study found that increased numbers of all *Tenacibaculum* spp., higher fish mortality, and increased tenacibaculosis outbreaks were recorded in the spring and summer compared to the fall and winter months (48). Mortalities and antimicrobial applications to treat yellowmouth outbreaks during the spring and summer months were also identified to be indirectly correlated with increased temperature and decreased dissolved oxygen (48). Another study reported decreased dissolved oxygen to be an environmental stressor (43) that could result from events such as algal blooms, contributing to an increased prevalence of infection with Tmar. The prevalence of Tmar found in sea lice surrounding farmed salmonid pens was also highest in the summer at times with the highest water temperature and lowest dissolved oxygen (48). Another study stated that decreased levels of Tmar in Chinook salmon were associated with decreased mortality in fall and winter (85).

An increase in water salinity was reported as another factor associated with the application of antimicrobials (48). Outbreaks have been described to occur during periods where salinity levels were between 29-32% (89). One study reported that they found no association between the bacterial load of Tmar in salmonid-parasitizing sea lice and changes in water salinity, however, the statistical significance of these results was not reported (49).

Vectors of Tmar have also been reported as factors of infection. Horizontal transmission of Tmar between smolts has been experimentally reported (61). Other proposed vectors include sea lice (48-50), lumpsuckers (61), and from farmed onto wild salmonids (92). One study reported that salmon infected with sea lice *(Lepeophtheirus salmonis*) did not have increased levels of Tmar compared to control fish (50). Jellyfish have also been proposed as a vector, however, two studies reported that they were not suspected as the original source of Tmar

infection (72, 74). However, one article reported that jellyfish species do have the ability to carry Tmar and deposit it on or into the epidermis of fish upon contact (93).

2.4.1.6 **Microbial Factors**

Microbial factors were reported by 14 of the included studies (Tables 2.3 and 2.6). Several studies reported that Tmar is a natural part of the microbial community on the surface of salmonid skin, mucosal layer, and the oral cavity (46, 48, 64, 81, 94). One study also reported that Tmar was able to form a biofilm on different surfaces such as tank walls (61). Reports also suggested that endotoxins may be involved in the pathogenesis of disease from Tmar, since there was a lack of inflammatory markers found at the site of lesions (81, 84).

Fish microbiota has been reported to shift according to stressors such as temperature or co-infection (94). The amount of Tmar that a fish is exposed to, or bacterial load, has been reported as a possible factor for infection (81, 86). In two experimental studies, higher concentrations (2.3×10^5 cells/mL & 1×10^8 cells/mL) of Tmar in a bath challenge at constant salinity and temperature were reported to result in 100% mortality from yellowmouth within 3 days, whereas lower concentrations ($< 2.3 \times 10^5$ cells/mL) only resulted in mortalities after days to weeks (81, 82).

In skin lesions on the dorsal and pectoral fins, it was observed that Tmar was restricted to the necrotic areas of the epithelium and did not infiltrate the musculature of salmon at any concentration (81). The size of skin lesions was reported to be smaller in challenges with higher doses, and larger as doses decreased, although in the highest dose (1×10^8 cells/mL) lesions across dorsal, lateral, and pectoral areas were found in similar percentages as the other doses (81). This finding was similarly reported in another study, which stated that superficial lesions

were common in early mortalities with a higher challenge concentration ($2.3 \times 10^5 – 1.6 \times 10^7$ cells/mL) and later mortalities had eroded ulcers (82). Another study reported that wild salmon with a higher Tmar load had reduced lower-than-expected mass for their measured length (85).

Co-infection with other pathogens such as salmonid alphavirus (SAV) has been reported to increase the bacterial load of *Tenacibaculum* species on the skin of fish in a dose-dependent manner (94). This may be a response to a change in the microbial makeup of the skin, which allowed for Tmar to act as an opportunistic pathogen (94). One study also found higher levels of Tmar in dead and dying fish when compared to live fish collected from various salmon farm locations across British Columbia (83). In cases of yellowmouth, it has been reported that Tmar is the dominant bacteria found in the oral cavity, although it is also one of the common bacteria in the mouths of unaffected or recovered salmon as well (64). Co-infection with Tmar and *Vibrio* species was significantly associated only in salmon with yellowmouth, where *Vibrio* spp. load significantly increased in fish with clinical signs of yellowmouth when compared to healthy fish (64). It was also reported that amoebic gill disease (AGD) in salmon was not found to be a significant risk factor for the development of yellowmouth (72).

2.5 DISCUSSION

2.5.1.1 Summary of evidence

This study synthesized the range of existing research from 25 studies on salmonid management, environmental, and microbial factors associated with disease in farmed and wild salmonids caused by Tmar infection. Most articles were published within the last five years, with study designs ranging from experimental to case reports. Most articles (n=16) reported more than one factor, which speaks to the multifactorial nature of the disease. In general, many articles

identified salmonid factors such as age and size, environmental factors such as higher temperatures and salinities, management factors such as stress from transfer, and microbial traits as a risk factor for infection with Tmar*,* along with stress and abrasion, and risk of co-infection due to its role as an opportunistic pathogen. However, most studies did not conduct multivariable analyses to understand the interplay between factors in disease caused by Tmar. This speaks to a knowledge gap and area for future research to elucidate the multifactorial etiology of the disease and identify areas for future research into management and control options. With continued research regarding the multifactorial etiology of yellowmouth, there is the potential to reduce AMU through well informed management solutions.

2.5.1.2 **Salmonid Factors**

The age and size of salmonids appear to be a contributing factor of Tmar infection. Younger fish, particularly during their first year at sea when they are smaller and have softer scales, are reported as more susceptible to infection (72, 81, 83). Due to its lack of host specificity, disease from Tmar has been described in many other species such as dover sole, sea bass, red/black sea bream, and turbot, with a wide geographic range (95). In red/black sea bream, younger and smaller fish are reported to have more severe clinical signs than older and larger (>60 mm long) fish (96). Infection in these fish only occurred between 1-2 weeks following transfer from freshwater to saltwater (96). In Dover sole, the condition has also been described to be more common in younger fish, specifically during 60-100 days after hatching (97). Decreased size as a function of increased Tmar load was also reported by a study, where it was concluded that this was a result of a decreased feeding rate (85). This is consistent with previous research indicating that Tmar infected fish become anorexic, making treatment with oral antimicrobials difficult (73, 98).

The comparison between different salmonid species (Atlantic salmon and Rainbow trout) and non-salmonids (like greenback flounder) highlights variations in susceptibility, with Atlantic salmon showing significantly higher mortality and morbidity rates than greenback flounder, and similar morbidity/mortality to Rainbow trout (82, 84). There were no studies comparing morbidity and mortality in Atlantic and Pacific (including Chinook and Coho) salmon. However, in one study comparing Atlantic salmon and Rainbow trout at different bacterial concentrations, there was only a significant difference in mortalities at a concentration of 1.6×10^6 cells/mL, not at any of the other concentrations, including a higher concentration of and 1.6×10^7 cells/mL (82). This result was not discussed by the authors, and based on the results of the other challenges, Rainbow trout and Atlantic salmon are assumed to show similar patterns of infection; consistent with other studies (82, 84). This information is crucial to understand the vulnerability of fish of different species, sizes, and ages to Tmar, which can inform infection risk.

2.5.1.3 **Management Factors**

Management practices likely play a pivotal role in Tmar infection and subsequent prevention. Tmar has been shown to be a part of the microbial community in both healthy and yellowmouth-affected salmonids (64). This emphasizes its role as an opportunistic pathogen that could cause disease in immunocompromised fish (64, 83). Management events such as transfer from fresh to saltwater, pen cleaning resulting in abrasion, and aggression from high stocking densities could all be stressful events resulting in infection (64). Although at high enough concentrations, disease from Tmar has been demonstrated in the absence of abrasion (81), many studies report that abrasion of the gills and body results in an increased severity of infection and mortality (43, 64, 81, 86, 87). This was reported in another study involving sea bass, when scarified and smeared with *Flexibacter maritimus* broth culture, total mortality occurred within

four days, with no mortality occurring in fish injected with the bacteria (95). The potential role of pen cleaning events in introducing Tmar emphasizes the need for careful maintenance of aquaculture facilities (81). Higher stocking densities and improper feeding practices could result in aggressive behaviour in Atlantic salmon (81) who have been shown to bite and charge, resulting in abrasions (99). Reduced mortality due to Tmar has been demonstrated in Tasmania, where changed management procedures such as feeding practices and stocking densities were decreased (84).

Transfer from freshwater to saltwater was also identified as a factor (48, 74), especially during the first year at sea (48, 72, 89). This transfer is a stressful event that can pre-dispose fish to infection with Tmar (61, 96). This change has also been shown to alter the microbial community of the salmonid gut (100), which could lead to dysbiosis and the overgrowth of opportunistic pathogens such as Tmar. Future research investigating microbial indicators could identify salmonids at risk for dysbiosis and disease (100).

Vaccine development is a crucial management tool that could decrease Tmar outbreaks and thus antimicrobial use. AMR is a problem that exists at the interface of humans, animals, and the environment, therefore, we must consider it from a One Health perspective (67). With AMU in aquaculture, there comes the risk of AMR bacterial strains and genes developing in the aquatic environment and spreading to the terrestrial environment (67). By developing vaccines to prevent disease outbreaks or reduce morbidity/mortality of fish due to yellowmouth, the environmental and economic damages associated with yellowmouth can be modulated. Currently, the only vaccine approved for use against Tmar is for turbot in Spain (63). In a vaccination study conducted in Tasmania and published in 2009, naïve Atlantic salmon had significantly better survival rates when injected with vaccine and adjuvant (Freund's incomplete

adjuvant), than the control group or vaccine-only group when challenged with Tmar (90). This suggests the necessity for an adjuvant to demonstrate protection, however, the adjuvant group developed areas of melanin with granulomas and cysts focused in the fundic region (90). These side-effects could lead to growth impairment and feed impaction (90). A more recent study developed a vaccine for yellowmouth using isolates from western Canada that was able to elicit an antibody response, however, in a challenge scenario protection was not observed (61). A difficulty in the development of a vaccine for salmonids may be due to the lack of repeatable and reliable challenge models (63). Further, clinical signs of tenacibaculosis may be attributable to several *Tenacibaculum* spp., making strain and species selection a challenge (63, 64). This emphasizes the need for a reliable challenge model for Tmar in salmonids, and further vaccine development research.

2.5.1.4 **Environmental Factors**

Environmental conditions could significantly impact the amount of Tmar in the environment and therefore increase the risk of Tmar infection. Water temperature was reported as a factor of infection in many studies, with a consistent theme being that warmer water increases the prevalence of Tmar. The optimum growth range for Tmar is between 15-30°C, with temperatures above 15°C and higher salinities between 30-35% described as risk factors for tenacibaculosis (72, 73). Outbreaks in both salmonids and other farmed fish such as wedge sole have been reported to occur at water temperatures between 15-20°C, which may be the result of an increased stress response in fish and increased bacterial growth (81, 101). In adult Chinook salmon, warmer water temperature (16-24°C) has been associated with decreased growth and impaired smoltification (47). In salmonids, it is suggested that risk from all diseases is limited at temperatures between 12-13°C, with risk increasing from 14-17°C and high from 18-20°C (47).

Exposure to temperatures outside of optimum rearing ranges may result in increased stress, which has immunosuppressive action (45). There is an observed suppression of the immune system when fish are exposed to cold stress due to overwintering strategies (45). This could explain why outbreaks of tenacibaculosis have also been observed in the winter months, why another study described an overgrowth of *Tenacibaculum* spp. in the fecal microbiota of salmonids undergoing low-temperature water treatment (46), or why decreased levels of Tmar in Chinook salmon in the fall and winter months were associated with decreased mortality (85). This further highlights the multiplicity of considerations at play even within one environmental factor of interest.

It has been postulated that factors such as UV irradiation, changes in salinity, and dissolved oxygen levels are associated with yellowmouth in salmonids (65). Decreasing the salinity and/or temperature in a pen has previously been shown to reduce yellowmouth mortality in affected salmonids (71), however, subsequent studies found that freshwater treatments had no significant effect on the presence of the bacteria (72). Outbreaks of yellowmouth are significantly correlated with seasonality, with increasing prevalence in the summer, followed by a decline in outbreaks over the winter months (72). The predisposing source of tissue damage by UV irradiation and subsequent Tmar infiltration has been supported by several outbreak cases where spongy changes, as reported by Bullock *et al*. 1988, were observed to be the likely result of UV damage (84, 102). In natural infections, eye and dorsal surface lesions were more common in comparison to experimental conditions, which could also confirm the importance of UV irradiation (84). However, this is not the sole predisposing factor, since disease with similar lesions in different locations has been found in fish in settings with controlled lighting (84). Algal blooms have been suggested as a factor in Tmar infection outbreaks, since algal blooms

decrease oxygenation and increase stress on fish (43, 44). Higher water temperatures which are conducive to the growth of Tmar also lead to algal blooms, which could result in a multiplicity of stressors leading to infection (43, 44). Recognizing these environmental factors can aid in predicting and mitigating Tmar outbreaks, especially in regions where aquaculture is prevalent.

Tmar has no host specificity and can transmit horizontally, therefore vectors are a factor of interest. Potential vectors for transmission in salmonids could be sea lice (48-50), jellyfish (72, 74), and lumpsuckers (61). However, none of the included studies were able to demonstrate that the vector of interest was the original source of Tmar. Instead, vectors such as jellyfish or plankton may play a role in tissue damage leading to abrasion, which could allow Tmar to proliferate (43, 93). Tmar has been detected in *Pelagia quadtrata* and *Muggiaea atlantica* and *Pelagia notiluca* jellyfish species which are known to cause gill damage leading to disease in salmon (103, 104). The transmission of Tmar between wild and farmed salmonids in BC has also been a concern in the recent years, due to the decline of Sockeye salmon in the region (92). In a study screening Sockeye salmon smolts as they migrate past salmon farms in the Discovery Islands region, there was a peak of 12.7 times the background level of Tmar prevalence (92). However, this could not be confirmed because of interaction with farmed fish, as the region is described as a hydrographic funnel that forces migrating salmon into a higher density and could magnify all sources of Tmar pressure (92).

2.5.1.5 **Microbial Factors**

Tmar is part of the natural microbial community on the surface of salmonids (46, 48, 64, 81, 94). Based on previous bacterial culture reports, Tmar is difficult to culture in non-sterile seawater, which may suggest that its growth is inhibited in the natural aquatic environment due to inhibition of other bacteria (105). Understanding the microbial makeup of fish skin, mucus,

and oral cavity is essential in assessing the risk of Tmar infections. Fish microbiota can shift and undergo dysbiosis because of challenges from stress such as temperature, fresh to saltwater transfer, and co-infection (46, 94). Under experimental conditions, it has been demonstrated that temperature has a significant effect on the salmonid gut microbiota, skin mucous, and water microbiota (46). This dysbiosis allows for pathogenic and opportunistic bacteria to proliferate, for example, salmonid alphavirus infection can increase the bacterial load of *Tenacibaculum* spp. on the skin of infected fish (94). Studies have also reported strong and significant positive correlations between Tmar and many other infectious agents in farmed Atlantic salmon in BC (83). *Tenacibaculum dicentrarchi* and *Tenacibaculum finnmarkense* have also been linked to tenacibaculosis outbreaks in Chile and Canada (48, 106). These bacteria are often found together and may result in disease displaying similar clinical signs such as mouth erosions and frayed fins (48, 64, 101). Therefore, it is becoming increasingly important to understand the natural microbial community of salmonids and the pathogenic source of outbreaks, to further develop treatment and control strategies.

2.5.1.6 **Data Gaps**

The identification of multivariable models that simultaneously account for several factors simultaneously associated with Tmar infections in salmonids is notably absent in the literature, representing a significant gap in our understanding of the disease's multifactorial etiology. Although the articles identified individual factors—such as environmental conditions, management practices, and host-specific traits—that influence Tmar infections, the interactions among these variables remain poorly characterized. To better understand the nature of yellowmouth outbreaks, the antagonistic or synergistic effects of a combination of factors must be examined.

2.5.1.7 **Limitations**

To reduce the risk of not capturing all eligible articles, this review followed a systematic approach (107). The search strategy did not include any restrictions regarding language, however, a few articles were excluded because of difficulty with translation. This review was specific to salmonids and infection with Tmar resulting in yellowmouth disease. Due to the complex interactions of pathogens that may result in tenacibaculosis, and the lack of common naming standards, it is possible that some articles were missed. Also, species other than salmonids were outside of the scope of this review, therefore, some factors that impact other species of fish which could give insight to salmonid disease may not have been included and are a topic for further study.

2.5.1.8 **Conclusions**

These results suggest a complex interplay of factors contributing to Tmar infections in salmonids. Effective management and prevention strategies should consider the age and size of fish, minimize gill and body abrasion, environmental conditions, and account for the microbial composition of fish and their surroundings. Future research to conduct experiments and observational studies that allow for assessment of the interplay between factors is crucial to fill this data gap. This will be crucial for developing targeted approaches to reduce the impact of Tmar in farmed and wild salmonids.

Table 2.1 Example search string used to search Web of Science. All Databases for articles about *Tenacibaculum maritimum* in farmed and wild salmonids. Complete search strings for all databases are included in the Supplementary Materials.

Keyword	Search String
Tenacibaculum maritimum	TOPIC: Tenacibaculum OR Tmar OR "T. mar" OR "T. maritimum" OR "Flexibacter maritimus" OR "Tenacibaculum maritimum" **OR**
Yellow Mouth	TOPIC: Yellowmouth OR (Yellow AND Mouth) OR (Mouth AND Rot) OR Tenacibaculosis OR "Salt Water Columnaris Disease" OR "Bacterial Stomatitis" OR "Eroded Mouth Syndrome" OR "Black Patch Necrosis" OR "Gill Disease" **AND**
Salmonids	TOPIC: Salmon OR salmonid* OR smolt* OR Grayling* OR Thymallus* OR Whitefish* OR Sockeye* OR (Salmo AND (Salar OR Gairdneri)) OR (Trout AND (Rainbow OR Redband OR Steelhead*)) OR (Oncorhynchus AND (tshawytscha OR keta OR kisutch OR nerka OR Mykiss))

Table 2.2 Key characteristics of peer-reviewed articles included in the scoping review of factors related to salmonid infection with *Tenacibaculum maritimum*.

Study Design [a]	Author and year	Location [b]	Species [c]	Disease Presentation [d]	Factor Categories
Case Report n=3					
	Apablaza *et al*. 2017	Chile	F Atlantic salmon	Tenacibaculosis	Mg, E
	Wynne *et al*. 2020	Canada	F Atlantic salmon	Yellowmouth	Mi
	Ferguson *et al*. 2010	Scotland	F Atlantic salmon	Tenacibaculosis	E
Experimental n=10					
	Van Gelderen *et al*. 2011	Australia	F Atlantic salmon	Marine Flexibacteriosis	S, Mg, E, Mi
	Powell *et al*. 2005	Australia	F Atlantic salmon	Yellowmouth	Mg
	van Gelderen *et al.* 2009	Australia	F Atlantic salmon	Marine Flexibacteriosis	Mg
	Soltani *et al*. 1996	Australia	F Atlantic salmon	N/A	S, Mi
	Handlinger *et al.* 1997	Australia	F Atlantic salmon	Tenacibaculosis	Mg, E
	Olsen *et al*. 2011	Norway	F Atlantic salmon	Tenacibaculosis	Mg, E, Mi
	Llewellyn *et al.* 2017	Canada	F Atlantic salmon	Tenacibaculosis	E, Mi
	Jones *et al.* 2007	Australia	F Atlantic salmon	Tenacibaculosis	Mg
	Reid *et al*. 2017	Norway	F Atlantic salmon	N/A	Mi
	Rud *et al*. 2017	Norway	F Atlantic salmon	N/A	Mg, Mi
RCT n=3					
	Frisch *et al*. 2018	Canada	F Atlantic salmon	Yellowmouth	Mg, E, Mi
	Powell *et al*. 2004	Australia	F Atlantic salmon	Acute gill disease	Mg, E. Mi
	Ghosh *et al*. 2022	Japan	F Chum salmon	N/A	E, Mi
Cross-Sectional n=2					
	Nowlan *et al*. 2021	Canada	F Atlantic salmon & rainow trout	Yellowmouth	Mg, E, Mi
	Smage *et al*. 2017	Norway	F Atlantic salmon	Tenacibaculosis	Mg, E
Longitudinal n=7					

Downes *et al*. 2018	Ireland	F Atlantic salmon	Tenacibaculosis	S, Mg, E, Mi
Bateman *et al*. 2022	Canada	W Sockeye salmon	Yellowmouth	E
Brosnahan *et al.* 2019	New Zealand	F NZ Chinook salmon	Tenacibaculosis	E
Bateman *et al*. 2021	Canada	F Atlantic salmon	Yellowmouth	S, Mi
Barker *et al*. 2009	Canada	F Atlantic salmon	Yellowmouth	E
Bass *et al*. 2022	Canada	W Chinook and Coho salmon	Yellowmouth	S, Mg, E, Mi
Frelier *et al*. 1994	United States	F Atlantic salmon	Yellowmouth	Mg, E

[a] When a study design was not specified in the article, it was determined by the first author during data extraction based on the reported methods.

[b] When the country of study and the country source of isolates did not match, the source of isolates was used.

[c] F = farmed salmonid, W= wild salmonid.

[d] Disease presentation as reported in the article. N/A= when the disease presentation was not specified or not applicable.

[e] The factor categories that each article examined, as determined during data analysis after extraction: S=salmonid factors, Mg = management factors, E=environmental factors, Mi=microbial factors

Table 2.3 Thematic breakdown of all articles included in the scoping review of factors related to salmonid infection with *Tenacibaculum maritimum* according to their corresponding factors of interest.

Factor of Interest	# of Articles (n=25)	Article Reference ID
Salmonid factors (n=6)		
Species	2	(82, 84)
Age/size	4	(72, 81, 83, 85)
Management factors (n=15)		
Stocking density	1	(81)
Vaccination	2	(61, 90)
Fresh to saltwater transfer	2	(48, 74)
Feeding rate	2	(85, 108)
Time since transfer	3	(48, 72, 89)
Pen elements	3	(48, 81, 88)
Gill/body abrasion	8	(43, 48, 74, 75, 81, 84, 86, 87)
Environmental factors (n=16)		
UV irradiation	1	(84)
Algal blooms	1	(43)
Dissolved oxygen	2	(43, 48)
Water salinity	3	(48, 49, 89)
Seasonality	6	(48, 49, 72, 81, 85, 89)
Vectors	8	(48-50, 61, 72, 74, 92, 93)
Water temperature	10	(43, 46, 48, 49, 72, 81, 84, 87, 89, 91)
Microbial factors (n=15)		
Endotoxin production	2	(61, 84)
Strain	2	(61, 86)
Biofilm formation	2	(61, 88)
Co-infection	4	(64, 72, 83, 94)
Skin/GI microbiota	5	(46, 48, 64, 81, 94)
Bacterial load	8	(48, 50, 81, 82, 85-87, 94)

Table 2.4 Key findings and significance of temperature as an environmental factor from articles included in the scoping review of salmonid infection with *Tenacibaculum maritimum* (Tmar).

Article	Species [a]	Outcome	Assessment/Evidence
Apablaza *et al*. 2017	F Atlantic salmon	Tmar grew on agar at 8,16, 19, 25, and 30°C for 7d. Limited at 8°C. Higher water temperature contributed to algal bloom and overgrowth of Tmar.	Hypothesis/ Experimental Evidence
Van Gelderen *et al*. 2011	F Atlantic salmon	Above normal water temperatures decreased fish immune response resulting in Tmar infection	Hypothesis
Nowlan *et al*. 2021[b]			
Midsummer site	F Atlantic salmon	↑Temperature = ↑ florfenicol use at 0, 5, 10 m depth (SLR[c])	p = <0.001
		↑Temperature = ↑ florfenicol use at 0, 5 m depth (MLR[d])	p = 0.012, 0.042
Larson Island site	F Atlantic salmon	↑Temperature = ↑ florfenicol use at 0 m depth (SLR)	p = 0.077
		Interaction between temperature, salinity, oxygenation at all depths (MLR)	p = 0.046
Downes *et al*. 2018	F Atlantic salmon	↑Temperature = ↑ prevalence of Tmar in the gill arches of farmed salmonids. Increased Tmar prevalence in the summer /autumn, decline during winter.	r = 0.48, p<0.05
Brosnahan *et al*. 2019	F NZ Chinook salmon	Warm seawater temperatures do not correlate with increased prevalence of Tmar	No statistical evidence
Handlinger *et al*. 1997	F Atlantic salmon	Outbreak of Tmar in 1995 attributed to extended periods of warm sunny cloudless days and water temperatures as high as 21°C	Hypothesis
Barker *et al*. 2009	F Atlantic salmon	Prevalence of total bacteria was higher in the warmer months in sea lice externally and internally. *T.maritimum* appeared at 100% prevalence externally in all months, authors did not comment on internal prevalence pattern.	Prevalence
Olsen *et al*. 2011	F Atlantic salmon	A lengthened exposure time to *Tenacibaculum* species led to ulceration and keratitis, authors speculate that an increased	Hypothesis

		temperature (12°C compared to 9.5°C) could have contributed to the result.	
Frelier *et al*. 1994	F Atlantic salmon	Outbreaks occurred from April to July when water temp was between 8-12 °C.	Observation
Ghosh *et al*. 2022	F Chum salmon	Overgrowth of tenacibaculum species in the fecal microbiota of salmonids undergoing low-temperature water treatment.	Observation

[a] F = farmed salmonid

[b] = Results from binomial logistic regression. Non-significant results were not included.

[c] = Simple logistic regression

[d] = Multivariable logistic regression

Table 2.5 Key findings and significance of other environmental factors from articles included in the scoping review of salmonid infection with *Tenacibaculum maritimum* (Tmar).

Article	Species [a]	Factor	Outcome	Significance
Handlinger *et al*. 1997	Atlantic salmon	UV irradiation	Likely the initial cause of lesions that could provide growth opportunity of Tmar	Hypothesized
Bass *et al*. 2022	W Chinook salmon & W rainbow trout	Seasonality	Decreased levels of Tmar in Chinook salmon associated with decreased mortality in fall and winter	Posterior probability = 0.87
	F Atlantic salmon	Dissolved oxygen	Described as environmental stressor	Hypothesized
Nowlan *et al*. 2021				
Midsummer site	F Atlantic salmon	Dissolved Oxygen	Significant relationship between dissolved oxygen and application of florfenicol at 0, 5 m when independently tested in a binomial logistic regression	p = 0.000051, 0.0001
Larson Island site	F Atlantic salmon	Salinity	Significant relationship between salinity and application of florfenicol at 0, 5, 10 m when tested in an additive binomial logistic regression	p= 0.04, 0.047, 0.018
Frelier *et al*. 1994	F Atlantic salmon		Outbreaks occurred during periods where salinity was between 29-32%	Observation
Barker *et al*. 2009	F Atlantic salmon	Vectors & Salinity	No 'apparent' association found between bacterial load of Tmar in sea lice and salinity	Observation
Frisch *et al*. 2018	F Atlantic salmon	Horizontal Transmission / Vectors	Evidence of horizontal transmission in cohabitation experiment between shedders and cohabitants. Could allow transmission between lumpsuckers and farmed salmon.	Experimental evidence & Hypothesis

Llewellyn *et al*. 2017	F Atlantic salmon	Vectors	The abundance of *Tenacibaculum* species in salmonid mucous was not different in those parasitized by lice vs controls	Kruskal-Wallis test indicated no significant difference
Ferguson *et al.* 2010	F Atlantic salmon	Vectors	Jellyfish can carry and deposit Tmar onto fish gills	Samples showing only 1 bp difference between Tmar on salmonid gill arch lesion and jellyfish
Bateman *et al*. 2022	W Sockeye salmon	Vectors	Sharp peak in Tmar detections in wild sockeye salmon in the Discovery Islands region of BC.	Modelled in many ways

[a] F = farmed salmonid, W= wild salmonid

Table 2.6 Key findings and significance of microbial factors from articles included in the scoping review of salmonid infection with *Tenacibaculum maritimum* (Tmar).

Article	Species [b]	Factor	Outcome	Significance
Frisch *et al*. 2018	F Atlantic salmon	Biofilm formation	Visible Tmar biofilm detected in experimental tank 24 hours after water flow.	Experimental Evidence
Reid *et al.* 2017	F Atlantic salmon	Co-infection	Increased incidence and amount of tenacibaculum species on skin of salmonid alphavirus infected fish.	Dose-dependent relationship
Ghosh *et al*. 2022	F Chum salmon	Microbiota	Temperature had significant effect on chum salmon gut microbiota, skin mucous, water microbiota.	P <0.0001, <0.0009, <0.0001
Wynne *et al*. 2020	F Atlantic salmon	Microbiota & Co-infection	Tmar was dominant bacteria in the oral cavity in diseased and healthy fish. *Vibrio* load significantly increased in fish with clinical signs of yellowmouth.	P<0.05
Brosnahan *et al*. 2019	F NZ Chinook salmon	Bacterial load	Higher challenge concentrations of (2.3 x 10^5 cells/mL) lead to mortalities. Lower concentrations lead to lesions formed after a week.	Experimental Evidence
	F Atlantic salmon	Bacterial load	Higher challenge concentrations of Tmar (1 x 10^8 cells/mL) lead to 100% mortality in 2-3 days. Lower concentrations took days to weeks to cause mortalities.	Experimental Evidence
Downes *et al*. 2018	F Atlantic salmon	Co-infection	Ameobic gill disease was not found to be a significant risk factor for the development or yellow mouth.	Experimental Evidence

[a] F = farmed salmonid, W= wild salmonid

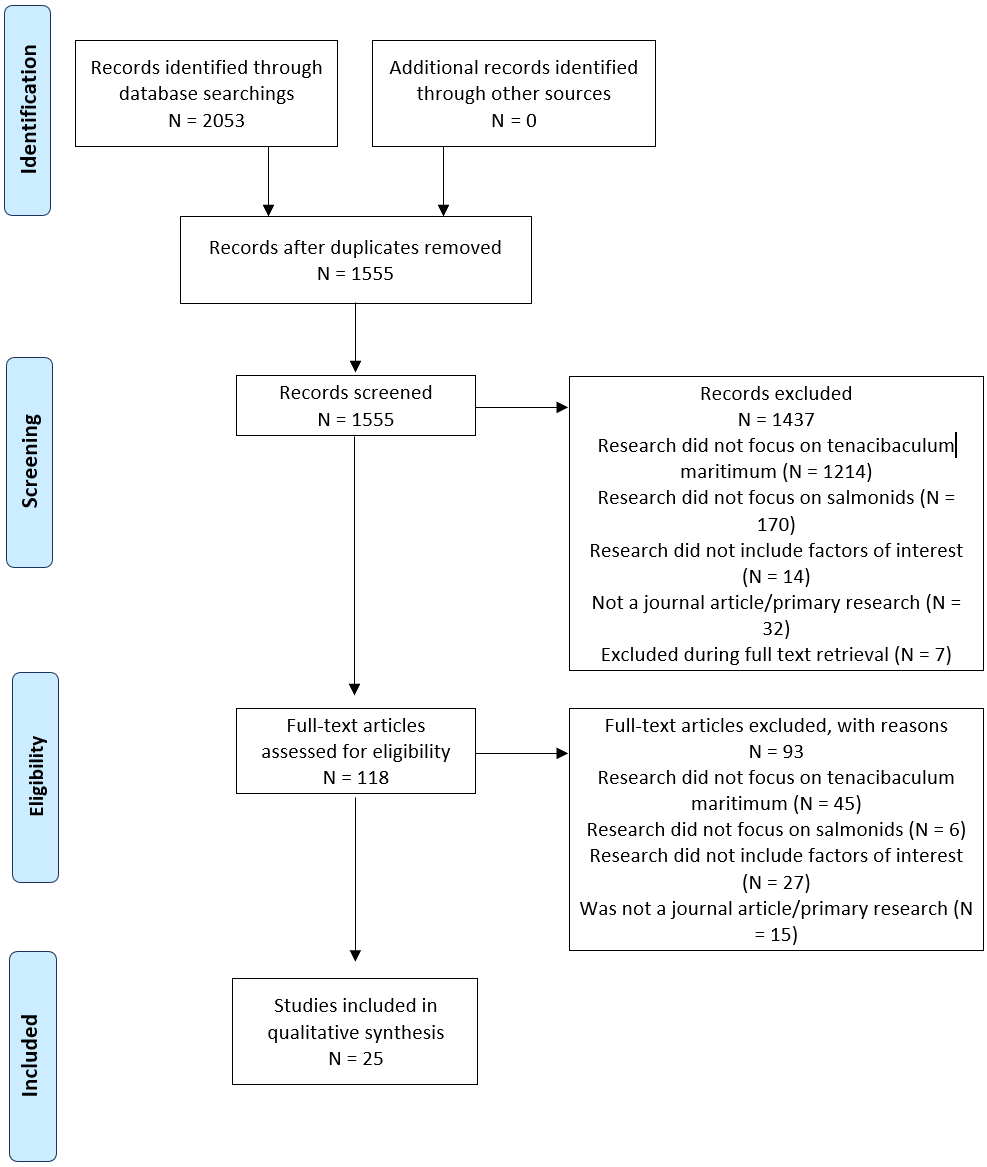

Figure 2.1 PRISMA-ScR flow diagram of study selection process for the systematic scoping review of the factors associated with *Tenacibaculum maritimum* infection in farmed and wild salmonids.

CHAPTER 3

REGIONAL AND TEMPORAL STATISTICAL ANALYSIS OF BIOMASS-ADJUSTED ANTIMICROBIAL USE IN THE TOP SALMONID PRODUCING REGIONS

3.1 ABSTRACT

Introduction: The sustainability of finfish aquaculture faces challenges, notably bacterial and viral diseases. While advancements in vaccine development have reduced outbreaks of common bacterial diseases, yellowmouth disease caused by *Tenacibaculum maritimum* (Tmar) remains a major disease of concern. Tmar is an opportunistic bacterium that affects young fish upon transfer from freshwater to saltwater. Yellowmouth is the primary reason for antimicrobial use (AMU) in BC Atlantic salmon aquaculture. Antimicrobial resistance (AMR) driven by antimicrobial use (AMU) remains a critical problem, posing risks to animal, environmental, and human health.

Methods Data were collected from a commercial Atlantic salmon production company in British Columbia, Canada, between 2015-2021 involving environmental, production, and management variables from 339 pen placements across 17 sites and 5 areas. AMU data, specifically florfenicol use, served as a proxy for the incidence of yellowmouth disease, which was the outcome variable (mg/kg biomass). A random forest regression model was first used to identify variables of interest for the final multivariable linear regression model. Manual, backward, stepwise selection was used to build the final multivariable model ($p<0.05$) for which site was included as a random effect and area as a fixed effect. Variables excluded were assessed for confounding (>25%) and biologically plausible interactions were tested for inclusion in the final model.

Results Random Forest regression identified 9 variables for inclusion in the linear regression model. The final multivariable linear regression revealed that AMU varied across different sites and areas with an intraclass correlation coefficient for site of 0.3 ($p<0.001$). Significant interactions ($p<0.001$) were found between area and week quadratic, and area and year quadratic. Pens with high salinity (>30.83 ppt) had significantly lower AMU (-0.09 mg/kg biomass) compared to low salinity. Temperature at 5m was included in the model as a confounder. Significant interactions were also found between DBW and broodstock type.

Discussion This study provides one of the first multivariable analyses of factors that are associated with AMU as a representation of Tmar incidence in farmed Atlantic salmon aquaculture sites from one production company in BC from 2015 to 2021. This study underscores the heterogeneity and interplay between environmental, management, and biological factors that are linked to AMU as a measure of Tmar. The findings indicate that area-specific and site-specific conditions affect disease management and AMU patterns. Managing these factors effectively is essential for reducing the incidence of diseases like yellowmouth and addressing AMR concerns, ultimately contributing to the sustainability of aquaculture practices.

3.2 INTRODUCTION

Currently, fish account for 16.6 percent of global animal protein intake, with the demand for aquatic foods continuing to expand at an average of 3 percent per year (1, 3). To meet these growing needs, there is a trend in production moving from fisheries to aquaculture farms, which now account for 56 percent of total aquatic animal food production, with outputs projected to at least double by 2050 (1, 109, 110). With increased consumer demand and subsequent production from these systems, there are an increasing number of challenges impacting the sustainability of finfish aquaculture.

Bacterial and viral diseases are a major challenge affecting the economic stability of finfish farming (37). The advancement in vaccine development has reduced disease outbreaks from most bacterial diseases such as vibriosis, furunculosis, and enteric redmouth (39). This has reduced the need for antimicrobials, which are prescribed by a veterinarian and administered to fish by medicated feed to treat and control disease (39). Antimicrobials approved for use in Canadian-farmed salmon include florfenicol, sulfadimethoxine and ormetoprim, sulfadiazine and trimethoprim, and oxytetracycline (39, 65).

Antimicrobial resistance (AMR) is a natural phenomenon that can be driven by increased antimicrobial use (AMU) (109). The use of antimicrobials in aquaculture may contribute to the selection for antimicrobial resistance genes (ARGs), which could move between the aquatic and terrestrial environment on mobile genetic elements (111-116). This may pose a risk to human health, as clinically important antimicrobials used to prevent and treat human infections could be ineffective against antimicrobial-resistant bacteria (67).

In British Columbia (BC), Canada, 95.7% of the aquaculture production biomass is attributed to Atlantic salmon (38). Although vaccines exist for many bacterial diseases of

salmonids, there is no commercially available vaccine to that is effective against *Tenacibaculum maritimum* (Tmar) infection in salmonids, the causative organism of bacterial stomatitis (yellowmouth disease) (39). Therefore, when an outbreak occurs, florfenicol or potentiated sulfonamides are prescribed for disease treatment and control (39). It is estimated that 98% of the antimicrobials prescribed for BC aquaculture operations were prescribed for yellowmouth disease, with the annual cost estimated as $1.6 million per company (39). Without a vaccine, understanding and addressing risk factors associated with the disease is essential to mitigate the economic and environmental impacts (65).

The objective of this study is to assess the management, production, environmental, and other factors that are associated with increases in AMU in BC farmed Atlantic salmon production, which is used as a proxy for incidence of yellowmouth caused by *T. maritimum*. Identification of these factors will highlight potential opportunities to reduce the incidence of the disease.

3.3 METHODS

Data were obtained from a commercial Atlantic salmon production company with sites off Vancouver Island, BC, Canada. The variables examined included environmental, production, management and other factors which are further detailed in Appendix 2, Table A2.1. Farm staff and veterinarians often diagnose yellowmouth disease following clinical examination of fish, and antimicrobials are prescribed to the whole pen to combat the disease. Since lab-confirmed diagnoses are not available for all suspected cases, florfenicol AMU data were used as the proxy for yellowmouth disease incidence, as this is the only reason for the prescription of florfenicol according to the industry partner. The onset date of treatment was considered the start of the disease. Data were acquired for net cage-raised salmonids under their care from Jan 1, 2015, to

February 28, 2021. All analyses were conducted at the level of the pen, as that is the level at which AMU interventions were applied and all factor measurements taken. Pens were clustered within different production sites (n=17), which were organized within broader production areas (n=5).

Dataset Description and Modifications

Dataset 1: 120-Day Overview

The primary dataset comprised data for multiple variables, detailed below), that were collected over the first 120-day period after placement at sea from 339 uniquely identified pens. Each pen had a unique pen identifier (GCS ID) to distinguish different fish placements in the same pen, site, and area over the course of the study period. The variables included in the dataset are detailed in Appendix 2 (Table A2.1).

Dataset 2: AMU and Biomass Records

The AMU dataset captured both the biomass metrics, such as the weight of the fish in the pen at the time of treatment, and the pharmacological prescription details, including the specific antimicrobials utilized at the time of each AMU treatment, along with the duration of these interventions (Appendix 2, Table A2.2). In contrast to the primary 120-day dataset, which typically contained a singular row of data per pen placement, this dataset featured multiple entries per pen to accommodate various treatment instances. Pens were identified using "unit numbers" that persisted through several cycles of fish placements across different years.

Biomass at the time of each antimicrobial treatment was calculated for all treatments for each pen placement using the daily fish count and the average biomass (weight in kg) per fish.

The outcome variable defined as "AMU" in mg/kg biomass was the total mg of florfenicol used during the placement divided by the sum of the fish count-weighted biomass (kg) of fish at the time of treatment for the placement. The count-weighted biomass for each treatment was calculated by multiplying the biomass (kg) per fish by the weighted count, where the weighted count was determined by dividing the count (number of fish at the time of treatment) by the total number of fish across all treatments.

The AMU dataset did not include GCS identifiers for these placements, which posed a challenge in correlating unit numbers with specific pen placements. This is because each unit ID could represent multiple placements over the years. To address this, each fish placement within a unit was assigned a distinct identifier, derived from the treatment dates and fish counts within the unit. Changes in the fish count within a unit or significant alterations in treatment dates necessitated the assignment of a new identifier. This adjustment was crucial in the later merging steps between the datasets. Each unique identifier was matched with the pen placements from the 120-day dataset, where a new row was created for the unique ID number.

Dataset 3: Daily Environmental and Mortality Data

This dataset contained daily records for each pen, detailing 52 variables that included environmental measures and mortality counts, some of which overlapped with the previous two datasets (Appendix 2, Tables A2.3 and A2.4). Daily measures were available for each pen during the first 120 days of placement. These daily environmental measures were temperature, salinity, and oxygenation at 0, 3, 5, 10, 15, 20, and 25m. To streamline the data upon merging, these variables were reduced to eliminate redundant columns and to specifically include only environmental measurements at 5 and 10 meters. Following discussions with the industry

partner, we decided to focus solely on environmental readings at these depths, as they are both accurate and most sensitive to subtle environmental changes. The primary goal was to compute a 3-day average of key environmental factors (temperature, salinity, oxygenation) preceding the beginning of each AMU treatment noted in the AMU dataset as this was the timing recommended by the industry partner for when changes would be impactful on yellowmouth incidence. Another daily variable included mortality count, which was used to update the counts of fish in each pen. This mortality count along with the average weight of fish in the pen was used to calculate the biomass at the end of each day. This value is also present in the AMU set. This dataset also utilized GCS identifiers that allowed linking with the identifiers used in the 120-day dataset.

Data Merge

These three datasets were merged into a final sheet using Microsoft Excel and Access **(**Microsoft Office® for Microsoft 365 MSO (Version 2406 Build 16.0.17726.20078) 64-bit**).** The merging process involved two key phases, with the initial phase focusing on integrating the 120-day primary dataset with the AMU data. Due to the absence of a common pen identifier across the datasets, pens from the 120-day dataset were aligned with those in the AMU dataset by assigning the same 120-day unique identifier, which served as the basis for merging the sheets.

During the integration, some pens could not be matched based on the unique identifier. This discrepancy was primarily due to some fish being divided from one placement into multiple placements, thus generating multiple final placements originating from a single initial one. For analysis, all fish were considered according to their initial pen placement as it was not possible to track split movements within the data. Additionally, certain pens from Barnes Bay were

excluded due to their involvement in a trial involving larger smolts, which could potentially distort the data. Furthermore, a few treatment records related to salmon rickettsia identified in the AMU dataset were removed. Despite diligent efforts to align these placements, 14 could not be matched and were subsequently omitted, resulting in a total of 325 unique placements.

In the final stage of merging, the consolidated 120-day and AMU dataset was merged with the daily dataset. Given that both sheets utilized GCS identifiers, this identifier facilitated the merging process. This integration produced an extensive master sheet encapsulating daily environmental data and AMU data for each pen placement.

Random Forest Regression Model

A random forest regression model was constructed using R Studio (RStudio 2023.06.1 +524 "Mountain Hydrangea" Release) and the randomForest package, which is a type of decision tree analysis that has been described to identify important variables from a larger set of potential variables for subsequent linear regression analysis (117). The main objective of the model was to examine the outcome variable AMU (mg/kg biomass) using 18 predictor variables (Table 3.1). These 18 predictor variables were all considered and selected from the merged datasets. The importance of each variable was assessed based on the increase in Mean Squared Error (MSE) and the decrease in model accuracy resulting from random permutations of the variables (117). This process quantifies the impact of each variable on the predictive accuracy of the model (117). Variables were ranked in descending order of importance based on the IncNodePurity measure, which reflects the increase in node purity contributed by each variable (Table 3.1 and Appendix 2, Figure A2.1). Default values for ntree (500) and mtry ($\sqrt{p}$) were used where p is defined as the number of variables.

The selection of the least important variables involved setting a cutoff at a purity score of 0.5 where there was a substantial drop between higher and lower purity scores. This method led to the retention of ten primary variables. Additionally, 'area' and 'broodstock' were also included as potential variables of interest for our regression modeling despite their relatively lower purity scores (IncNodePurity). Area was included to control for this level of clustering, along with site. The type of broodstock was identified by the industry partner as a variable of interest. The Out-of-Bag (OOB) R-squared and MSE were calculated for the models before and after the removal of less important variables to assess the impact on model accuracy. The results showed a decrease in OOB R-squared from 0.78 to 0.66, indicating a reduction in model accuracy. However, there was a negligible change in MSE (0.00114 before and after variable removal), suggesting that the predictive error of the model remained consistent. Twelve variables were selected for analysis, and two of those variables; days between 100-500g (DBW) and input grams had high node purity, however, due to their biological relatedness and collinearity, DBW was selected and input grams was removed from the list for final analysis. Ultimately, 11 variables were selected for further evaluation in a multilevel linear regression analysis.

Model Building

Descriptive statistics were calculated using STATA18.0/IC (StataCorp LLC, College Station, Texas, United States). All eleven predictors were assessed for collinearity using Pearson (continuous) and Spearman (categorical) correlation. Associations between the primary variable of interest AMU (mg/kg biomass) and the 11 predictor variables were assessed using multilevel linear regression with a random intercept for pen site (118). Variables considered for inclusion in the linear regression model, after initial screening by the random forest models, were initially screened with the outcome using single-variable linear regression. Continuous variables were

assessed for linearity with the outcome by assessing a quadratic (squared) term, lowess and linear trend plots, and assessing the change in coefficients for quartile indicators. Final decisions on how to model continuous variables were based on t-test $P \leq 0.05$ and lower Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (118). Variables with an extra sum-of-squares F-test (F-test) $P \leq 0.20$ were considered for inclusion in the final, multivariable model. The indicators for area were kept in the multivariable model during screening and in the final model regardless of significance to control for clustering at that level.

Manual, backward, stepwise selection was used to build the final multivariable model. Variables with F-test $P \leq 0.05$ were kept in the final model. Biologically plausible, two-way interactions were tested between significant final model variables inclusion in the final model and included if F-test $P \leq 0.05$, including assessment of interaction with the area indicators. Variables excluded from the final model were assessed for a confounding effect on final model variables if the inclusion of the potential confounding variable altered the value of any model coefficient by >25%. Final model fit was assessed by considering the normality of the Best Linear Unbiased Predictors (BLUPS) of the errors at the site level. Models were checked for influential observations using standardized residuals to determine if any observations had large impacts on model outcomes. All linear regression analyses were conducted in STATA18.0/IC (StataCorp LLC, College Station, Texas, United States).

3.4 **RESULTS**

Salmon placements occurred in five areas and within these areas, there were 17 sites with a range of total placements (4 – 41) (Table 3.2). Total AMU per placement ranged from a minimum of zero (no treatment) to a maximum of 1.19 mg/kg biomass in the first 120 days

across all sites (Table 3.3). Temperature measurements taken five meters below the surface, three days prior to AMU treatment, recorded a low of 6.47 ℃ at Site 5.2 and a high of 16.43 ℃ at Site 3.1 across all locations (Table 3.2). Similarly, salinity levels measured three days before AMU application were lowest at 21.36 ppt (parts per thousand) at Site 5.2 and highest at 33.67 ppt at Site 4.4, spanning all sites (Table 3.2). Days between 100-500 grams ranged between 26.6 and 198 days (Table 3.4), and broodstock supply were mostly from multiple sources (Table 3.5).

Random Forest Regression Model

Eleven primary variables were retained upon examination of node purity scores above 0.5 (Table 3.1, and Appendix 2 - Figure A2.1), after excluding input grams and including area and broodstock. Temperature at 5m and salinity at 5m were selected to be modeled instead of 10m values for each due to their higher node purity and collinearity with the 10m measurements. Upon removal of other variables, there was a decrease in OOB R-squared from 0.78 to 0.66, however, there was a negligible change in MSE (0.00114 before and after variable removal).

Linear Regression Model

Pearson and Spearman correlations of the final eleven variables did not identify two-way variable comparisons with high correlation other than temperature and salinity 10m values with 5m values. The results of single-variable analyses (Table 3.6 and Table 3.7) found that all nine variables (after removal of temp 10m and salinity 10m) were significant ($p<0.001$).

The final multivariable model (Table 3.8) included broodstock (Atlantic versus other sources), salinity 5m (high or low), DBW (high or low), a quadratic for week and for year, indicators for area, and interactions between area and each of the week quadratic and year quadratic, and between broodstock and DBW (Table 3.8). Temperature at 5m was included as a

confounding variable and BFCR was excluded. In the final multivariable model, the intraclass correlation coefficient for site as a random intercept was 0.30 ($p<0.001$). The plots of BLUPS for the final model demonstrated a normal distribution of the site errors/residuals. Evaluation of standardized residuals did not have any major impact on the final model.

The predictive AMU margins for the significant interactions between area and week quadratic ($p<0.001$) (Figure 3.1) and area and year quadratic ($p<0.001$) (Figure 3.2) demonstrated that there are seasonal and year-to-year trends that differ in magnitude and shape depending on the area of placement. The predictive margins plots show that AMU is generally the greatest when fish are placed in the middle of the year (week 20) and then falls at the end of the year (Figure 3.1). This is true for all areas except for Area 4 and Area 2, which show relatively consistent use independent of placement week. The AMU margins plots for year describe relatively stable AMU over the years, except for Area 3 and Area 2 (Figure 3.2). Area 3 illustrates the largest confidence intervals and an inverted U-shaped curvature. Area 2 on the other hand, depicts an exponential shaped curve with AMU highest in 2021. This area also had the highest maximum (1.19 mg/kg biomass) and mean (0.58 mg/kg biomass) AMU. Including these interactions allowed for adjustment of the impacts of other model variables for these trends.

Pens with high salinity (>30.83ppt) had significantly lower AMU (-0.09 mg/kg biomass) compared to low salinity. Linear combinations between broodstock and DBW were conducted holding all other variables in the model constant (Table 3.9). All combinations: other broodstock and high vs low DBW, Atlantic broodstock and high vs low DBW, Atlantic vs other broodstock when DBW is high, and Atlantic vs other broodstock when DBW is low are significant ($p <0.05$). Pens with other broodstock and high DBW had significantly higher AMU (0.10 mg/kg

biomass) compared to those with low DBW. The trend was reversed in pens with Atlantic and high DBW, which had significantly lower AMU (-0.21 mg/kg biomass) compared to those with low DBW. Pens with high DBW and Atlantic broodstock had significantly lower AMU (-0.17 mg/kg biomass) compared to those with other broodstock. Conversely, pens with low DBW and Atlantic broodstock had significantly higher AMU (0.14 mg/kg biomass) compared to those with other broodstock.

3.5 DISCUSSION

Summary of Evidence

This study characterized the variability in AMU for Atlantic salmon production by one company in BC, Canada in the first 120 days of placement across 5 different areas and their 17 different sites between 2015 and 2021. The AMU in these pens was used as a proxy for yellowmouth incidence, as this is the only reason for the prescription of florfenicol in the first 120 days according to the industry partner. The analysis represents one of the first in-depth multivariable analyses of such AMU data for Tmar incidence based on this cohort of pen placements. Findings underscore the heterogeneity in AMU across seasons and years that differ between the area and site placement. Environmental factors such as salinity also varied across the area and site placements, with higher salinity demonstrating a protective effect. The interactions between DBW and broodstock and the confounding impacts of temperature demonstrate the importance of examining the multifactorial etiology of yellowmouth disease when model building. The results of the multivariable model suggest that AMU patterns are not only influenced by environmental and temporal factors but also by the biological characteristics of the salmon stocks, and that seasonal, temporal, and area variables must be included to assess

their impact on AMU and subsequently Tmar incidence. Much of the previous literature has not conducted multivariable analyses to understand the interplay between disease factors of Tmar (Chapter 2).

Our analysis also highlighted the importance of site-level clustering in this analysis. The ICC of 0.3 is relatively high, meaning that 30% of the variation in the data is accounted for by site-level clustering. This relatively high level of clustering at the site level indicates that pens within sites are more like each other than between pens at different sites. The degree of clustering was also influenced by how environmental variables were tracked.

Environmental Factors

The results highlighted the role of environmental conditions, particularly temperature and salinity at 5m depth, in influencing AMU levels. Temperature and salinity variations were different across sites, suggesting that local environmental conditions may impact disease prevalence and treatment approaches. The significantly lower AMU (-0.09 mg/kg biomass) in pens with high salinity (>30.83 ppt) vs low salinity when controlling for area and site effects may indicate a potential protective effect of certain salinity levels against yellowmouth disease. This contrasts with previous research that has reported higher salinity (30-35%) as a risk factor for tenacibaculosis (72, 73). Decreasing the salinity in a pen has also been reported to reduce yellowmouth mortality in affected salmonids (71), however, other studies showed that freshwater treatments had no significant effect on the presence of Tmar (72). Our results do not examine the severity of infection, only when infection occurred, which was inferred by proxy based on AMU. However, it may be assumed that a longer or more severe infection may result in higher levels of AMU due to multiple treatments, which occurred during periods of lower salinity in our study.

The dichotomized temperature (high > 9.38 ℃) at 5m was a confounder in the model. When tested in the single variable analysis, higher temperature (>9.38 ℃) was significantly associated with decreased AMU (-0.25 mg/kg biomass). The optimum temperature range for Tmar growth has been reported between 15-30℃ (72, 73). Reports identified associations between Tmar detection in fish and temperature, demonstrating seasonal patterns of increasing levels during the summer and fall of the first year of production, which decrease during the winter (72). However, outbreaks have still been reported to occur during the winter months (46, 82, 85, 87). Our data showed that seasonal AMU varied greatly between sites, and with different patterns, with higher AMU in the first 20 weeks of the year, the middle of the year, or the last 20 weeks. These differing trends suggest that there are other unmeasured factors that contribute to the seasonal variations between areas, and that the interplay between all the factors included in the model are likely very complex.

Winter outbreaks of Tmar could be due to immunosuppression and gut dysbiosis, which is described to occur when fish are exposed to low-temperature water (45, 46). *Tenacibaculum* overgrowth in chum salmon fecal microbiota has been reported in fish exposed to low water temperature treatment (8℃) but not high water temperature (18℃) (46). The temperature range (6.47 ℃ - 16.43 ℃) for the placements in this study is lower than the higher challenge temperatures of up to 21℃ examined in previous studies (119, 120). The maximum water temperature is still within the lower range of the optimum growth zone for Tmar and the optimum growth range for Atlantic salmon between 14 - 16 ℃ (121-123). This study also examines AMU as a proxy for the clinical incidence of Tmar infection, instead of the prevalence in the environment or on salmonids based on culture or genomic detection. Therefore, the previously observed relationship between high temperature and Tmar infection may not be the

same as was detected in this study. Previous studies performed in the Discovery Island region reported a high prevalence of Tmar in Sockeye salmon fish caught further south compared to other years of study (92). These high levels of Tmar detections were attributed to higher ocean temperatures in 2015 (83, 124). However, our study did not identify higher levels of AMU in any area in 2015. This may further indicate that the prevalence of Tmar may not translate to clinical infection or outcome.

The seasonal patterns of AMU found in our study are consistent with previous reports which identified a seasonal pattern of Tmar prevalence with Tmar detected first in July and peaking in September (72). The disconnect between environmental variables such as salinity and temperature and the subsequent variation in AMU over the season, between years, and between areas, suggest that it is difficult to make uniform recommendations for salmon placement to reduce Tmar risk or with respect to environmental variables that have been reported to increase risk of Tmar outbreaks. Since salmonids are most at risk when they are young during their first 120 days at sea and Tmar load has been demonstrated as higher during the summer months, this may indicate that Atlantic salmon smolts should be placed during the winter months. However, decreased temperatures during the winter months may stress salmonids if previously exposed to higher temperatures in freshwater. In this scenario, their growth rate during the winter months may also be lower, as temperatures fall further from the optimum growth range (121-123). This would result in a longer period at risk (i.e., more days between 100-500 g) which was one of the variables captured by our DBW variable.

Biological Factors

The influence of broodstock type on AMU, particularly the differential effects observed between broodstock types and days between 100-500 grams as a measure of the rate of growth, points to the genetic status of different salmon stocks playing a critical role in disease susceptibility and subsequent AMU requirements. DBW is not only a measure of growth in this case but also captures the weight of fish at sea input. Pens stocked with broodstock lineages classed as other (MCXMOWI, Mowi, & Mixed Mowi) had lower AMU than Atlantic broodstock when they grew quickly or were placed at a higher weight (low DBW), but this trend was reversed when they grew slowly or were placed at a lower weight (other broodstock had higher AMU). The trends within broodstock groups were also conflicting. Fish from broodstock classed as other that grew more slowly or placed at a lower weight had higher AMU than those that grew quickly or were placed at a higher weight, with the opposite being the case for Atlantic broodstock.

Our study findings indicate the importance of broodstock selection and DBW in yellowmouth disease outbreaks that result in antimicrobial treatment. These results highlight the complicated interplay between underlying genetics with a secondary factor such as DBW that is impacted by a variety of other environmental, management, and biological factors. For example, the growth rate (but not feed conversion efficiency) of Atlantic salmon is increased in higher temperatures of up to 16℃ (121-123). Optimum growth occurs at increasing temperatures (from 12.8 to 16 ℃) as Atlantic salmon increase in size (70 – 300g) (122). It may also be that DBW represents the impact of another unmeasured factor. Selective breeding of farmed salmonids has been reported to increase disease resistance and growth rate, leading to more efficient resource use and a lower carbon footprint (24, 28). While some reports estimate the use of genetically improved stocks for aquaculture at 10% (24, 28, 29), another study in Europe found that the

uptake of genetic selection is closer to 80%, with most companies focusing on improved growth efficiency (125). Genetic selection and genetic modification (GMO) are two separate techniques. Genetically engineered Atlantic salmon such as AquAdvantage produced by AquaBounty have integrated a growth hormone gene that allows them to grow a third heavier and be ready to harvest in 18 months (vs three years) (126). The industry claims that these salmonids may be more sustainable and disease-resistant, however, there are many objectors (126). The FDA approved these salmon for human consumption in 2010, with Health Canada following in 2016, however, they are still not farmed in Canada (126, 127). Despite the high level of innate immunity in salmonids, they remain susceptible to opportunistic infections under immunosuppressive conditions (40). The risk of immunosuppression increases with high stocking densities and during the transfer from freshwater to saltwater (40, 41). Therefore, ongoing research into the genetic traits associated with disease resistance in Atlantic salmon could alleviate the economic and environmental impacts of outbreaks.

Implications for Management and Policy

Aquaculture has the potential to sustainably meet growing food demands while promoting improved human and environmental health (2, 3, 110, 128, 129). While some aquatic species rely on agricultural sources for food, aquaculture generally requires less feed crops and land than other animal protein production (110). Atlantic salmon are both the most produced salmonid species and the most valuable finfish species in the United States (U.S.) (5). However, the use of antimicrobials, especially in open pen farms, is a controversial topic that impacts the sustainability of the system and consumers' views of the industry.

In Canada, producers utilize veterinarian-prescribed antimicrobials to combat bacterial diseases such as yellowmouth, where all records are then reported to Fisheries and Oceans Canada (DFO) (39). Antimicrobials approved for use in Canadian-farmed salmon include florfenicol, sulfadimethoxine and ormetoprim, sulfadiazine and trimethoprim, and oxytetracycline [39, 65]. These drugs are listed as "highly important" in human medicine by the World Health Organization (5). Therefore, the concern of resistant bacteria spreading from animals into the environment and to humans makes this a One Health issue (5). There is also concern regarding the consumption of salmonid products treated with antimicrobials, however, a recent risk assessment performed by experts in public and animal health fields concluded that the risk of AMR spread by human consumption of salmon is low (130).

Growing concern surrounding AMR highlights the need for continued investigation into disease prevention strategies such as vaccination and husbandry practices (5). This is especially true for diseases such as yellowmouth, where no vaccine is available and most prescribed antimicrobials in BC are a response to control it (39).

3.6 **LIMITATIONS**

In this study, AMU was used as a proxy for yellowmouth disease incidence. Farm managers classify all cases of mortality daily and are quick to alert the veterinarians if an outbreak of yellowmouth is suspected. These outbreaks are treated based on clinical signs, and not confirmed culture due to a long turnaround time and risk of further spread. Due to this, our data represents suspected cases of yellowmouth. Previous studies have measured the prevalence of Tmar bacteria, however, since Tmar is ubiquitous in marine environments, those may not correlate to clinical signs of disease or the need for treatment. The benefit of our study is that it represents incident cases in that these pen placements represent a cohort, allowing for the

measurement of potential factors prior to the onset of clinical signs. Comparatively, most published literature represents cross-sectional studies which can be subject to reverse causation (131). This study provides insight into the environmental, management, and other factors that may impact the actual treatment of yellowmouth, not only the prevalence of bacteria. The other strength of our study design was the ability to account for clustering in the data at the site and area levels, which no published study has accounted for to date.

3.7 CONCLUSIONS

Salmon aquaculture has the potential to contribute to global sustainability goals by promoting economic, social, and environmental health. However, managing disease outbreaks remains a challenge. Our results highlight the need for aquaculture management practices that consider both site-specific and area-specific ecological factors. This study provides one of the first multivariable analyses of factors that are associated with AMU across farmed Atlantic salmon aquaculture sites from one production company in BC from 2015 to 2021. The findings reveal the challenges of management for yellowmouth disease, including the complexity of the interplay between environmental, management, and biological factors, as well as the need to account for site and area-level clustering when considering these associations. Continued research into genetic traits for disease resistance and careful monitoring of environmental conditions is essential to optimize health outcomes, reduce disease outbreaks' economic and ecological impacts, and promote the sustainable growth of the salmon aquaculture industry.

Table 3.1 Results for the random forest regression model of antimicrobial use for *Tenacibaculum maritimum* infection in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Test	Result
Number of trees (ntree)	500.000
No. of variables tried at each split (mtry)	6.000
Mean of squared residuals	0.007
% Var explained	91.710
# trees for lowest MSE*	268.000
RMSE** of best model	0.083
Variable Importance	**IncNodePurity**
week ***	5.785
year ***	4.819
temp5m ***	2.511
sal5m ***	1.923
sal10m ***	1.889
bfcr ***	1.847
dbw ***	1.678
input_grams	1.551
temp10m ***	1.543
startsite ***	0.525
mr_120	0.477
mr90	0.475
denscount_av120d	0.450
denskg_av120d	0.349
mr30	0.293
broodstock ***	0.282
area ***	0.280

supplier	0.113
Model before and after factor removal:	**Result**
MSE	0.001
OOB	0.781
After removal - MSE	0.001
After removal - OOB R-squared	0.658

*MSE: mean squared error

**RMSE: root of the mean squared error

*** Variables selected for examination in final linear regression model

Table 3.2 Summary statistics of area-level and site-level environmental conditions from averaged 3-day pre-treatment environmental values from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Area	Start Site	Temp 5m (°C)					Salinity 5m (ppt[a])				
		Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
Area 1	**Overall (n=57)**	**10.72**	**1.19**	**10.87**	**8.42**	**12.52**	**30.09**	**2.30**	**30.83**	**25.63**	**32.83**
	Site 1.1 (n=35)	10.23	1.06	10.60	8.42	11.38	30.97	1.76	31.00	25.67	32.83
	Site 1.2 (n=22)	11.50	0.94	10.87	10.30	12.52	28.70	2.39	30.08	25.63	30.83
Area 2	**Overall (n=64)**	**10.00**	**1.30**	**9.47**	**8.41**	**12.91**	**30.54**	**1.32**	**30.30**	**27.81**	**32.83**
	Site 2.1 (n=17)	10.54	0.95	10.65	9.35	12.19	30.01	0.74	30.17	28.67	31.25
	Site 2.2 (n=18)	9.53	0.51	9.47	8.82	10.21	31.19	1.80	32.08	28.20	32.83
	Site 2.3 (n=29)	9.99	1.68	9.00	8.41	12.91	30.45	1.09	30.33	27.81	32.06
Area 3	**Overall (n=28)**	**13.03**	**1.43**	**12.39**	**11.52**	**16.43**	**25.08**	**0.60**	**25.17**	**24.17**	**26.00**
	Site 3.1 (n=4)	14.10	2.70	14.10	11.76	16.43	24.75	0.10	24.75	24.67	24.83
	Site 3.2 (n=6)	12.30	0.00	12.30	12.30	12.30	24.17	0.00	24.17	24.17	24.17
	Site 3.3 (n=10)	13.89	0.91	13.81	13.03	14.84	25.24	0.14	25.17	25.03	25.45
	Site 3.4 (n=8)	11.97	0.49	11.94	11.52	12.55	25.73	0.29	25.73	25.44	26.00
Area 4	**Overall (n=141)**	**10.05**	**1.14**	**10.07**	**8.17**	**14.96**	**28.05**	**2.82**	**28.14**	**21.36**	**33.67**
	Site 4.1 (n=18)	11.21	1.99	11.78	8.17	14.96	27.69	1.38	28.21	25.17	29.83
	Site 4.2 (n=32)	9.04	0.57	8.80	7.88	10.87	25.30	2.92	26.79	21.36	29.25
	Site 4.3 (n=8)	8.66	0.06	8.64	8.60	8.79	29.77	1.62	29.47	28.14	31.53
	Site 4.4 (n=41)	10.27	0.58	10.23	9.23	11.08	30.19	2.04	30.83	27.40	33.67
	Site 4.5 (n=14)	10.95	0.10	10.99	10.74	11.03	25.47	0.66	25.50	24.67	26.33
	Site 4.6 (n=28)	10.06	0.40	10.14	9.38	10.65	29.08	1.37	29.58	25.89	31.43
Area 5	**Overall (n=35)**	**11.94**	**2.67**	**12.26**	**6.47**	**15.29**	**26.16**	**1.69**	**26.58**	**21.67**	**28.33**
	Site 5.1 (n=17)	12.94	1.35	12.84	11.34	15.29	26.39	1.25	26.34	24.83	28.33
	Site 5.2 (n=18)	11.00	3.26	11.67	6.47	15.28	25.94	2.04	26.78	21.67	27.78

[a] = parts per thousand

Table 3.3 Summary statistics of area-level and site-level total antimicrobial use from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Antimicrobial use (mg/kg biomass[a])						
Area	**Start Site**	**Mean**	**SD**	**Median**	**Min**	**Max**
Area 1	**Overall (n=57)**	**0.28**	**0.16**	**0.30**	**0.00**	**0.70**
	Site 1.1 (n=35)	0.33	0.14	0.33	0.12	0.70
	Site 1.2 (n=22)	0.20	0.16	0.26	0.00	0.35
Area 2	**Overall (n=64)**	**0.58**	**0.30**	**0.49**	**0.00**	**1.19**
	Site 2.1 (n=17)	0.56	0.38	0.25	0.13	1.03
	Site 2.2 (n=18)	0.64	0.28	0.49	0.41	1.19
	Site 2.3 (n=29)	0.55	0.26	0.51	0.00	1.12
Area 3	**Overall (n=28)**	**0.15**	**0.14**	**0.09**	**0.00**	**0.39**
	Site 3.1 (n=4)	0.20	0.06	0.20	0.14	0.25
	Site 3.2 (n=6)	0.09	0.00	0.09	0.09	0.09
	Site 3.3 (n=10)	0.19	0.20	0.19	0.00	0.39
	Site 3.4 (n=8)	0.12	0.13	0.12	0.00	0.24
Area 4	**Overall (n=141)**	**0.55**	**0.25**	**0.45**	**0.12**	**1.02**
	Site 4.1 (n=18)	0.50	0.27	0.44	0.12	0.99
	Site 4.2 (n=32)	0.56	0.26	0.44	0.16	0.86
	Site 4.3 (n=8)	0.89	0.14	0.89	0.67	1.03
	Site 4.4 (n=41)	0.52	0.23	0.43	0.22	0.85
	Site 4.5 (n=14)	0.31	0.02	0.31	0.29	0.34
	Site 4.6 (n=28)	0.65	0.23	0.64	0.33	0.99
Area 5	**Overall (n=35)**	**0.14**	**0.13**	**0.12**	**0.00**	**0.45**
	Site 5.1 (n=17)	0.11	0.12	0.12	0.00	0.37
	Site 5.2 (n=18)	0.17	0.15	0.12	0.00	0.45

[a] = mg/kg biomass: total mg antimicrobial used / fish count-weighted kg of fish biomass at time of treatment)

Table 3.4 Summary statistics of days between 100-500 grams (DBW) once fish are placed at sea in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Variable	Mean	SD	Median	Min	Max
DBW (100-500g)	121.65	26.62	126.00	19.00	198.00

Table 3.5 Summary statistics of broodstock in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Variable	# of placements
Other [a]	299
Atlantic	26

[a] = Other: MCXMOWI, Mowi, & Mixed Mowi.

Table 3.1 Final linear regression model variable definitions and p-values during single-variable screening with antimicrobial use (mg/kg biomass) as the outcome.

Variable	Definition	P-Value
Temperature 5m	3-day pre-treatment average of temperature at 5m. Dichotomous variable. High (>9.38).	<0.001
Salinity 5m	3-day pre-treatment average of salinity at 5m. Dichotomous variable. High (>30.83).	<0.001
Broodstock	Brood of smolts, classified into 2 dichotomized groups "Atlantic" vs "other". Other: MCXMOWI, Mowi, & Mixed Mowi.	<0.001
BFCR	Biological feed conversion ratio (kg feed/kg weight). Dichotomous variable. High (>1.21).	<0.001
DBW	Days between 100-500 grams. Dichotomous variable. High (>108).	<0.001
Week	Input week of year (#1-52). Quadratic variable.	<0.001
Year	Year of input (2015-2021). Centered quadratic variable.	<0.001
Site	Input site. Included as a random intercept	-
Area	Input area. Included as a fixed effect	-

[a] = mg/kg biomass: total mg antimicrobial used / fish count-weighted kg of fish biomass at time of treatment

Table 3.2 Single-variable results for the pen-level linear regression models of antimicrobial use for *Tenacibaculum maritimum* infection in farmed Atlantic salmon in British Columbia, Canada (2015-2021). Each observation represents a new pen placement of fish with the model including a random intercept for pen site. Site area is modeled as a fixed effect. See Table 3.6 for variable definitions.

Variable	mg/kg (95% CI) *	T-test p-value	F-test p-value
Temperature 5m (ºC)			
Low (n=85)	Referent		<0.001
High (n=240, >9.38)	-0.25 (-0.32, -0.19)	<0.001	
Broodstock			
Other**	Referent		<0.001
Atlantic	-0.38 (-0.50, -0.27)	<0.001	
Year	0.25 (0.21, 0.29)	<0.001	<0.001
$Year^2$	-0.03 (-0.034, -0.021)	<0.001	
Week	-0.023 (-0.032, -0.013)	<0.001	<0.001
$Week^2$	0.00056 (0.00038 , 0.00072)	<0.001	
Salinity 5m (ppt)			
Low (n = 241)	Referent		<0.001
High (n = 84, >30.83)	-0.20 (-0.26, -0.13)	<0.001	
DBW 100-500g (days)			
Low (n = 85)	Referent		<0.001

High (n = 240, >108)	0.15 (0.074, 0.22)	<0.001	
Area			
Area 1	Referent		<0.001
Area 2	0.31 (0.15, 0.48)	<0.001	
Area 3	-0.12 (-0.29, 0.053)	0.18	
Area 4	0.29 (0.14, 0.44)	<0.001	
Area 5	-0.13 (-0.31, 0.056)	0.17	

95% CI - 95% confidence interval. ppt - parts per thousand.

DBW: days between 100-100g; median 126 days, range 19-198, high ≥108 days.

* Coefficient for mg/kg biomass: total mg antimicrobial used / fish count-weighted kg of fish biomass at time of treatment)

** Other broodstock categories: MCXMOWI, Mowi, Mixed Mowi & MCXMOWI

Table 3.3 Results for the pen-level multivariable linear regression model of pen-level antimicrobial use for *Tenacibaculum maritimum* infection in farmed Atlantic salmon in British Columbia, Canada (2015-2021). Each observation represents a new pen placement of fish with the model including a random intercept for pen site. Site area is modeled as a fixed effect. See Table 3.6 for variable definitions.

Variable	mg/kg biomass (95% CI)*	T-test p-value	F-test p-value
Area			
Area 1	Referent		<0.001
Area 2	-0.14 (-0.53, 0.25)	0.49	
Area 3	-1.17 (-2.54, 0.21)	0.10	
Area 4	0.30 (-0.081, 0.67)	0.12	
Area 5	-0.17 (-0.55, 0.21)	0.38	
Week	0.070 (0.033, 0.10)	<0.001	<0.001
$Week^2$	-0.0031 (-0.0041, -0.0021)	<0.001	
Year (centered on 2015)	0.050 (-0.052, 0.15)	<0.001	<0.001
$Year^2$	-0.00010 (-0.016, 0.014)	0.90	
DBW 100-500g (days)			<0.001
Low	Referent		
High	0.10 (0.068, 0.14)	<0.001	
Broodstock			<0.001
Other**	Referent		

Atlantic	0.14 (0.052, 0.23)	0.002	
Salinity 5m (ppt)			<0.001
Low (n=241)	Referent		
High (n=84, >30.83)	-0.094 (-0.13, -0.058)	<0.001	
Area*Week			<0.001
Area 2	-0.03 (-0.65, 0.0048)	0.09	
Area 3	0.10 (-0.061, 0.26)	0.23	
Area 4	-0.10 (-0.13, -0.06)	<0.001	
Area 5	-0.019 (-0.060, 0.020)	0.34	
Area*Week^2			
Area 2	0.0025 (0.0015, 0.0036)	<0.001	
Area 3	-0.0013 (-0.006, 0.0030)	0.55	
Area 4	0.0040 (0.0028, 0.0050)	<0.001	
Area 5	0.0015 (0.00032, 0.0027)	0.01	
Area*Year			<0.001
Area 2	-0.082 (-0.20, 0.034)	0.17	
Area 3	0.30 (-0.014, 0.61)	0.06	
Area 4	0.050 (-0.067, 0.16)	0.40	
Area 5	0.020 (-0.10, 0.14)	0.74	
Area*Year^2			
Area 2	0.050 (0.026, 0.070)	<0.001	
Area 3	-0.065 (-0.11, -0.016)	0.01	
Area 4	0.001 (-0.016, 0.018)	0.89	
Area 5	-0.0062 (-0.025, 0.012)	0.51	
DBW*broodstock			<0.001
High*Atlantic	-0.31 (-0.41, -0.22)	<0.001	

Temperature 5m (ºC)			0.09
Low (n=85)	Referent		
High (n=240, >9.38)	-0.036 (-0.078, 0.0057)	0.09	
Constant	-0.052 (-0.40, 0.30)	0.77	
Between site variance	0.0045 (0.0019, 0.0099)	ICC	<0.001
Within site variance	0.0069 (0.0059, 0.0081)	0.30	

95% CI - 95% confidence interval. ppt - parts per thousand.

DBW: days between 100-500g; median 126 days, range 19-198, high ≥108 days.

ICC: intraclass correlation coefficient = (between-site variance)2 / ((between site variance)2+(within site variance)2)

* Coefficient for mg/kg biomass: total mg antimicrobial used / fish count-weighted kg of fish biomass at time of treatment)

** Other broodstock categories: MCXMOWI, Mowi, Mixed Mowi & MCXMOWI

Table 3.4 Contrasts of the interaction between broodstock source and days between (DBW) 100 and 500 grams at sea from the final pen-level multivariable model for antimicrobial use for *Tenacibaculum maritimum* infection in farmed Atlantic salmon in British Columbia, Canada (2015-2021). Comparisons are limited to within broodstock source and within DBW levels.

Broodstock source * DBW (100-500g) Contrast	**Coefficient (mg/kg biomass)***	**P-Value****	**95% CI**	
Other broodstock*** (high vs low)	0.10	<0.001	0.07	0.14
Atlantic broodstock (high vs low)	-0.21	<0.001	-0.30	-0.12
Atlantic vs Other broodstock (both high)	-0.17	<0.001	-0.24	-0.10
Atlantic vs Other broodstock (both low)	0.14	0.002	0.05	0.23

95% CI - 95% confidence interval.

* Coefficient for mg/kg biomass: total mg antimicrobial used / fish count-weighted kg of fish biomass at time of treatment)

** $p < 0.05$ for contrast while holding all other variables constant.

*** Other broodstock categories: MCXMOWI, Mowi, Mixed Mowi & MCXMOWI

DBW: median 126 days, range 19-198, high ≥108 days.

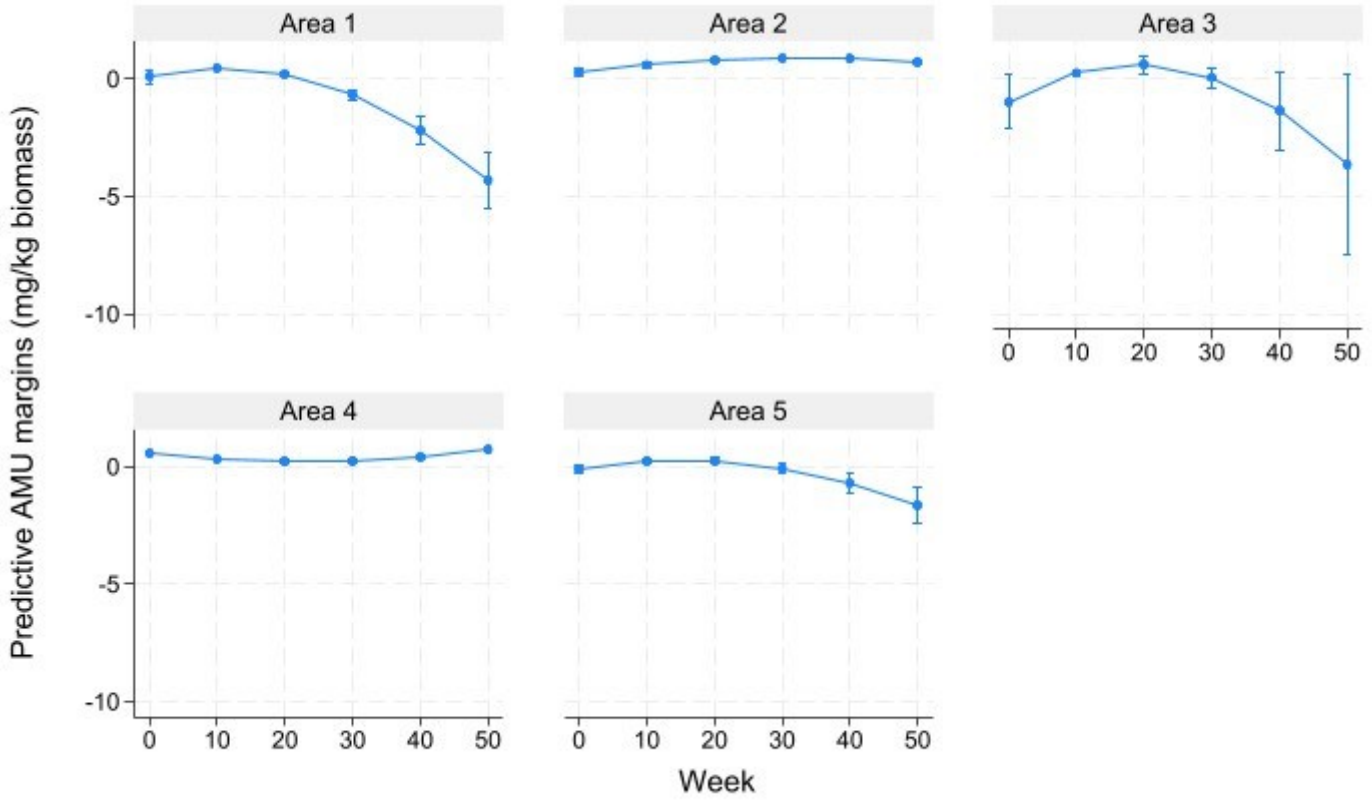

Figure 3.1 Predictive antimicrobial use (AMU) (mg/kg biomass) margins for the significant interactions between area and week quadratic ($p<0.001$) from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

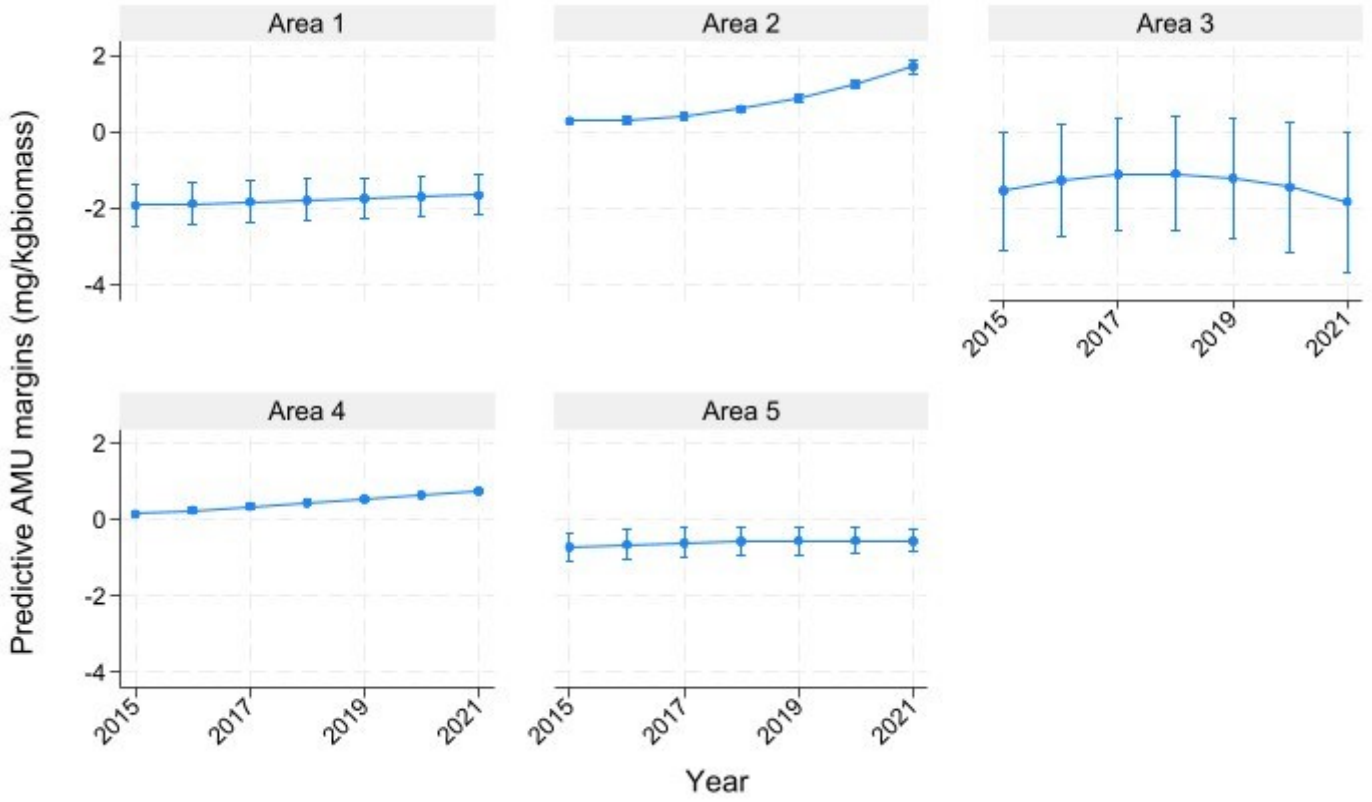

Figure 3.2 Predictive antimicrobial use (AMU) (mg/kg biomass) margins for the significant interactions between area and year quadratic ($p<0.001$) from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

CHAPTER 4

CONCLUSION

This thesis aimed to understand the management, production, environmental, and other factors that contribute to the incidence of yellowmouth in Atlantic salmon production caused by *Tenacibaculum maritimum* (Tmar), and to identify opportunities to reduce the use of antimicrobials for the treatment of yellowmouth in BC-farmed Atlantic salmon. The findings highlighted the multifactorial etiology of yellowmouth disease and provided insight into the factors associated with outbreaks in BC-farmed Atlantic salmon between 2015 and 2021. The main research questions proposed in this thesis were: 1) what are the factors reported in the scientific literature to be associated with disease in salmonids caused by the bacterium *Tenacibaculum maritimum* and 2) what factors specific to farmed BC Atlantic salmon are associated with the development of yellowmouth disease from *Tenacibaculum maritimum* in Western Canadian Atlantic salmon production?

The main objectives of this were to: 1) synthesize available knowledge through a scoping review to identify factors (management, production, environmental, other) associated with disease in farmed and wild salmonids caused by *Tenacibaculum maritimum,* and 2) utilize random forest models and multivariable linear regression to identify factors (management, production, environmental, other) associated with yellowmouth disease in BC farmed Atlantic salmon. The first objective was achieved by performing a scoping review to synthesize the available literature and identify factors associated with disease in farmed and wild salmonids from Tmar (*see Chapter 2*). Production data from a BC Atlantic salmon producer between 2015-2021 were utilized to achieve the second objective (*see Chapter 3*).

The scoping review highlighted the multifactorial nature of Tmar infection in salmonids, including the interplay of host biology, environmental factors, and pathogen characteristics. The results suggested that management and prevention strategies may consider the age and size of fish at the time of sea input, stocking density, minimizing gill and bodily abrasion, and minimizing stressful events. It is also necessary to understand the environmental conditions in the area and account for the microbial composition of fish and their surrounding environments. This review also identified the lack of multivariable analyses regarding Tmar infections in salmonids. This helped to inform the analysis and identified factors for focus in the second objective. Overall, the published literature suggested that one or even a small number of factors do not contribute to Tmar infection alone and that studies and subsequent analyses need to be designed and conducted to account for this, which were largely lacking.

The search strategy employed in the scoping review was broad and had little restrictions on language. However, some articles were still excluded due to translation difficulties which may have limited the information gathered. Reducing the scope to salmonids specifically enabled the gathering of relevant data for Chapter 3, however, certain factors impacting other species that may be relevant could have been missed. This review achieved the stated objectives and identified factors for consideration in the analysis of Research Question 2.

Chapter 3 included data from a BC industry partner on cage-raised salmonids under their care from Jan 1, 2015, to February 28, 2021. The multivariable analysis considered antimicrobial use (AMU) at the pen-level as a proxy for yellowmouth in farmed salmonids to understand the interplay and at interactions between variables contributing to Tmar infection; something that was notably absent in previous literature. This study utilized random forest decision trees and multilevel, multivariable linear regression models of AMU across 17 farmed Atlantic salmon

aquaculture sites from 5 areas in British Columbia from 2015 to 2021. The merging of three different datasets with different numbers of lines of data for each pen and no common unique identifier was a difficulty. This resulted in some manual matching of pens, where there was some room for error. Furthermore, managing analysis that dealt with clustering at two levels (area and site) was extremely important, as we saw the high intraclass correlation coefficient (ICC) at the site level and important interactions between season and time with the fixed effect for area. The importance of including multiple variables, interactions, and confounders was also emphasized, as the significance of variables differed throughout single to multivariable inclusion in models.

The findings reveal the complexity of disease management, influenced by temperature, salinity, and broodstock type factors. The importance of site-level clustering and the different AMU trends between areas was also elucidated, indicating the relevance of local conditions and management practices. High salinity levels demonstrated a protective effect against yellowmouth treatment, after adjusting for other variables and interactions in the model, which contrasted with the results of previous studies. Past reports have stated that higher salinity (30-35%) is a risk factor for tenacibaculosis, and that freshwater treatments may reduce mortality in affected salmonids (71-73). Another study however, did not find a significant effect of freshwater treatments on the presence of Tmar, but a significant effect of temperature, where both of these factors were considered in the model (72). Similarly, in this study when accounting for other factors, a protective effect of higher salinity was indicated. This indicates that the relationship between salinity and AMU as a proxy for Tmar outbreaks is complex.

This highlights the nuanced relationship between environmental factors and disease dynamics. The differential impacts of broodstock type and days between 100-500 grams (DBW)

on AMU emphasize the role of genetic selection and broodstock management in mitigating disease outbreaks. Continued research into genetic traits for disease resistance and careful monitoring of environmental conditions is essential to improve fish health, reduce the economic and ecological impacts of the disease, and promote the sustainable growth of the salmon aquaculture industry with less dependence on AMU as a disease management tool.

This study used antimicrobial use (AMU) as an indirect measure of yellowmouth disease incidence among salmon. Unlike previous research which measured the prevalence of *Tenacibaculum maritimum* (Tmar) bacteria, this study accounted for the actual clinical signs of yellowmouth disease, recognizing that Tmar is commonly found in marine environments and its presence doesn't always indicate disease. The study uniquely tracked a cohort of pen placements over time, minimizing issues of reverse causation often seen in cross-sectional studies. Additionally, this study enhances understanding by considering how environmental and management factors influence the actual treatment practices. A significant strength of this research was that it accounted for data clustering at both the site and area levels, an aspect not typically addressed in published literature.

Salmon farms on the coast of BC have long been contested, with scientists, government, non-governmental organizations, and other special interest groups debating the reasons for the declining population of wild Pacific salmon (127, 132). Wild Pacific salmon are a species with important economic, ecological, and cultural significance in the region (51, 52). The issue of salmon farming becomes more contentious as many operations are in the water surrounding the traditional territories of Indigenous groups (133). Due to substantial concerns regarding the decline of Fraser River sockeye salmon, the Cohen Commission was established in 2009 to

collect evidence and make recommendations for future conservation (127). The Cohen report suggested over 75 reasons for the decline, including climate change, commercial overfishing, manufacturing, logging, and development along the Fraser River (134). Unfortunately, many industries such as forestry, mining, fishing, terrestrial agriculture, housing, and flood control have led to salmonid habitat destruction (132, 134). One of the recommendations from the report concluded that if there was more than minimal risk to Fraser River sockeye salmon by Atlantic salmon farms in the Discovery Islands area, those farms should be closed (92, 134). In response, the Canadian Science Advisory Secretariat produced nine reports to conclude that aquaculture posed no more than a minimal risk of harm to the Fraser River Sockeye salmon (135, 136). However, following consultation with the Indigenous peoples in the area and lack of consideration of the perspectives of other stakeholders including salmon farm operators, the Department of Fisheries and Oceans (DFO), Canada, still announced the immediate closure of the farms starting in 2020 with all operations closing by June 2022 (137, 138). All other open-net aquaculture operations are to be phased out by 2029 (139). After these decisions, the future of the farmed salmonid aquaculture industry in Canada remains uncertain.

Due to conflicting evidence and the lack of consensus among stakeholders, these groups depend on the media to promote their agendas (127, 140). In a study examining the media coverage of aquaculture in 68 countries, Canada had the highest proportion of negative sentiment towards all kinds of aquaculture, and the most polarized opinions on the industry (127, 141). This could be attributed in part to oversimplified messaging by the campaigns of non-governmental organizations (NGOs), which tend to focus the aquaculture conversation only on the risks (127, 142).

Pressure from NGOs and the media has pushed aquaculture operations to develop in a positive direction as well (127). This messaging creates public demand that results in the aquaculture industry being held to higher standards than nearly any other major sector in the Canadian economy (127). For example, antimicrobials are not used prophylactically and all treatments are reported to and monitored by DFO (39). All AMU data have been publicly available at a granular (e.g., farm) level since 2010 (143), in contrast to other agriculture such as beef, swine, or poultry, which is not publicly available at the farm level (39). Public pressure has also led to research and development into more sustainable practices and a great reduction in the number of antimicrobials prescribed for finfish aquaculture in Canada (39). For example, in BC between 1995 and 2009 there was an 87.5% reduction in antimicrobial use (39). This is due to improved biosecurity, husbandry, broodstock disease screening, vaccine efficacy, improved pen placement, and usage of antimicrobials that require a lower effective dose (39). Research shows that residents of coastal BC generally support aquaculture when the discussion is separated from the contentious topic of salmon farming (127). This suggests there is a path forward for aquaculture development with public backing if there is open dialogue and responsible management (127).

By tracking how antimicrobials are used to manage suspected outbreaks of yellowmouth disease and identifying the factors associated with AMU in farmed salmonids in BC, this thesis shed light on antimicrobial application in aquaculture. Understanding the factors associated with use is crucial because indiscriminate or excessive AMU can lead to the development of resistant strains of bacteria, which is a major concern in both veterinary and human medicine (67). Identifying AMU patterns helps in developing strategies to inform prudent use, thereby mitigating the risk of AMR development (67).

The development of AMR involves the health of people, animals, and the environment, making it a One Health concept (67). This study exemplifies the One Health approach by examining how management practices and environmental factors influence antimicrobial use in aquaculture. Since *Tenacibaculum maritimum* is ubiquitous in marine environments, its management through antimicrobials not only affects the health of salmon but potentially impacts the broader marine ecosystem and may contribute to AMR.

Future research should continue to investigate area and site-specific factors associated with yellowmouth incidence. Analyzing these data in multifactorial analysis will provide deeper insight into area-specific management recommendations. Previous studies indicate that the integration of traditional ecological knowledge (TEK) by engaging local Indigenous within production systems supports sustainability (133). This local knowledge may also provide insight into area and site-specific factors that may contribute to disease incidence. Currently, 78% of farmed salmon in BC is under a beneficial partnership with a First Nation with twenty First Nations holding partnership agreements for farming in their territory (144). Beginning in 2022 the Federal government also announced that permits would only be granted to fish farms with First Nations agreements in the proposed territory of operation. The future of salmon aquaculture in BC also includes reconciliation and Indigenous partnerships, as the industry potentially transitions towards land-based systems and hybrid marine aquaculture (145). The DFO also acknowledges the substantial barriers to the implementation of these production systems (145).

It is imperative to continue to investigate ways to reduce AMU in salmonid aquaculture, and for BC, this involves the prevention of yellowmouth. The industry has faced heavy scrutiny surrounding disease on farms (particularly from Tmar) and the use of antimicrobials. Due to the interconnectedness of these farms, the environment, and human health, along with social and

economic considerations, this topic must be considered through a One Health lens. There needs to be an emphasis on research, but also improving communication between industry and the public. By fostering open communication and knowledge-sharing pathways, meaningful and sustainable recommendations can be made to maintain the health of the animals, humans, and the environment.

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APPENDIX 1

SCOPING REVIEW PROTOCOL

Complete protocol available at: https://doi.org/10.17605/OSF.IO/NCQ56

Table A1. 1 Yellowmouth salmonid scoping review data extraction table

Table available in separate excel file

APPENDIX 2

Table A2.1 Summary of pen-level and site-level overall variables in the original 120-day dataset from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Variable name	Description	Level of measurement [a]
GCS	Identification of pen placement	O, P
Unique ID	Manually matched from AMU dataset	O, P
Site Name	Site name sea	O, S
Longitude	Longitude at site placement	O, S
Latitude	Latitude at site placement	O, S
Area Name	Area name sea	O, S
Input Date	Input date sea	O, P
Input Month	Input month sea	O, P
Avg Input Weight (grams)	Avg Input Weight (grams)	O, P
Florfenicol treatments 120 first days	Florfenicol treatments 120 first days	O, P
Mortality Ratio 30 first days	Mortality Ratio 30 first days sea	O, P
Mortality Ratio 120 first days	Mortality Ratio 120 first days sea	O, P
Mortality Ratio	Mortality ratio (mortality per input count)	O, P
Days since vaccination(min)	Days since vaccination when put in sea	O, P
Superior Ratio	Superior ratio sea when harvested (qualtiy of fish)	O, P
EFCR	Economic feed conversion ratio sea ($ feed / $ weight)	O, P
BFCR	Biological feed conversion ratio sea (kg feed / kg weight)	O, P
Broodstock	Broodstock name (genetic strain)	O, P
Supplier	Supplier name (who produced the fish in freshwater)	O, P
Nr unique suppliers	Number of unique suppliers	O, P
Input Count Sea	Input count sea	O, P
Day Degrees Freshwater	Day degrees freshwater	O, P
Freshwater Avg Temperature	Freshwater average temperature °C	O, P

Freshwater Avg Salinity	Freshwater average salinity (parts per thousand)	O, P
Freshwater Sum Salinity	Freshwater sum salinity (parts per thousand)	O, P
Freshwater Salinity 90 Last Days	Freshwater salinity 90 days before input to sea (parts per thousand)	O, P
Freshwater days Under 8 degrees ratio	Freshwater days under 8 degrees ratio	O, P
Salinity 15m 120 first days	Salinity 15m 120 first days sea (parts per thousand)	O, P/S
Salinity 10m 120 first days	Salinity 10m 120 first days sea (parts per thousand)	O, P/S
Salinity 5m 120 first days	Salinity 5m 120 first days sea (parts per thousand	O, P/S
Temperature 15m 120 first days	Temperature 15m 120 first days sea ℃	O, P/S
Temperature 10m 120 first days	Temperature 10m 120 first days sea ℃	O, P/S
Temperature 5m 120 first days	Temperature 5m 120 first days sea ℃	O, P/S
Oxygen 15m 120 first days	Oxygen 15m 120 first days sea (mg/L)	O, P/S
Oxygen 10m 120 first days	Oxygen 10m 120 first days sea (mg/L)	O, P/S
Oxygen 5m 120 first days	Oxygen 5m 120 first days sea (mg/L)	O, P/S
Nr of production days sea	Nr of production days sea	O, P
Nr of production days freshwater	Nr of production days freshwater	O, P
Days between 100 and 500 grams sea	Days between 100 and 500 grams sea	O, P
Density Biomass Kg pr. M3 120 first days	Density Biomass Kg pr. M3 120 first days sea (avg)	O, P
Density Count Pr. M3 120 first days	Density Count Pr. M3 120 first days sea (avg)	O, P

[a] = O: overall measurement, P: pen level, S: site level

Table A2.2 Summary of pen-level and site-level daily and overall variables in the AMU dataset from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Variable name	Description	Level of measurement [a]
Start Date	Start of treatment date	D , P
End Date	End of treatment date	D , P
Type	Type identifier	D , P
Order	Order identifier	D , P
Registration	Treatment type	D , P
Site	Site identifier	O , S
Unit	Unit identifier	O , P
Unique Placement ID	Unique ID generated manually	O , P
Count	Number of fish	D , P
Avg. weight [g]	Avg. weight [g] per fish	D , P
Biomass [kg]	Total biomass of fish [kg]	D , P
Temperature	Temperature at treatment ℃	D , P/S
Medicament	Medication type	D , P
Method	Administration method	D , P
Active substances used [kg]	Active substances of antimicrobial [kg]	D , P
Treatment type	Medication	D , P
Reason for treatment	Bacterial, viral, etc.	D , P

[a] = O: overall measurement, D: daily measurement, P: pen level, S: site level

Table A2.3 Summary of pen-level and site-level daily and overall variables in the environmental dataset from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Variable name	Description	Level of measurement [a]
StartGCS	Identification of pen placement	O , P
ClosingBalanceCount	Fish count at end of day	D , P
ClosingBalanceKgPrFish	Average weight of fish at end of day (kg)	D , P
ClosingBalanceKg	Total weight of fish at end of day (kg)	D , P
CurrentUnit	Unit identifier of fish that day	D , P
CurrentSite	Site name of fish that day	D , S
CurrentSiteID	Site ID identifier of fish that day	D , S
StatusDate	Date of measures	D , P
MortalityCountPrTotalCount	Daily mortality percentage	D , P
MortalityCount	Daily mortality count	D , P
startunitid	Part of GCS ID	O , P
startgen	Part of GCS ID	O , P
startcagetankname	Unit ID of fish when placed	O , P
startsitename	Site name of fish when placed	O , S
startsiteid	Start site ID of fish when placed	O , S
startgcs	Start GCS ID of fish when placed	O , P
Sensor temperature5m	Temperature of pen 5m	D , P/S
Sensor temperature10m	Temperature of pen 10m	D , P/S
Sensor salinity5m	Salinity of pen 5m	D , P/S
Sensor salinity10m	Salinity of pen 10m	D , P/S
Sensor oxygen5m	Oxygen of pen 5m	D , P/S
Sensor oxygen10m	Oxygen of pen 10m	D , P/S

[a] = O: overall measurement, D: daily measurement, P: pen level, S: site level

Table A2.4 Summary of variable inclusions in the random forest model from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

Variable ID	Include in Random Forest?
startGCS_id	No (unique identifier)
Area	Yes (cluster variable)
startpen_daily_fix	No (identifier)
startsite	Yes (cluster variable)
current_unit	No
current_site	No
date_msmt	No
date_placement	Yes (seasonality variable)
amu_start_date	No
amu_end_date	No
length_of_treatment	No
days_between_(next)_treatment	No
temp5m_3d_pen_average	Yes
temp10m_3d_pen_average	Yes
sal5m_3d_pen_average	Yes
sal10m_3d_pen_average	Yes
oxyg5m_3d_pen_average	Yes
oxyg10m_3d_pen_average	Yes
flor120	No (part of outcome)
closing_balance_count	No (part of outcome)
closing_balance_kg_pr_fish	No (part of outcome)
closing_balance_kg	No (part of outcome)
mortality_count	No (nonsensical for per pen data)
uniqueID	No

longitude	No (overlaps with cluster and temp)
latitude	No (overlaps with cluster and temp)
week_placement	Yes (seasonality variable)
year_placement	Yes
input_grams	Yes
mr30	Yes
mr90	Yes
Mr	Yes
days_since_vacc	Yes
Bfcr	Yes
broodstock	Yes
supplier	Yes
input_count	Yes
frtemp_avg	No (only freshwater data for some pens)
frsal_avg	No (only freshwater data for some pens)
frsal_sum	No (only freshwater data for some pens)
frsal90	No (only freshwater data for some pens)
frd8	No (only freshwater data for some pens)
freshnr	No (only freshwater data for some pens)
120d_av_sal10	No (already have 3d env. variables)
120d_av_sal5	No (already have 3d env. variables)
120d_av_temp10	No (already have 3d env. variables)
120d_av_temp5	No (already have 3d env. variables)
120d_av_ox10	No (already have 3d env. variables)
120d_av_ox5	No (already have 3d env. variables)
Dbw	Yes
denskg_av120d	Yes
denscount_av120d	Yes
collective_pen_mg _active	No
collective_average_biomass	No

collective_average_weighted_biomass	No
mg_pcu_avg	No
Amu	outcome

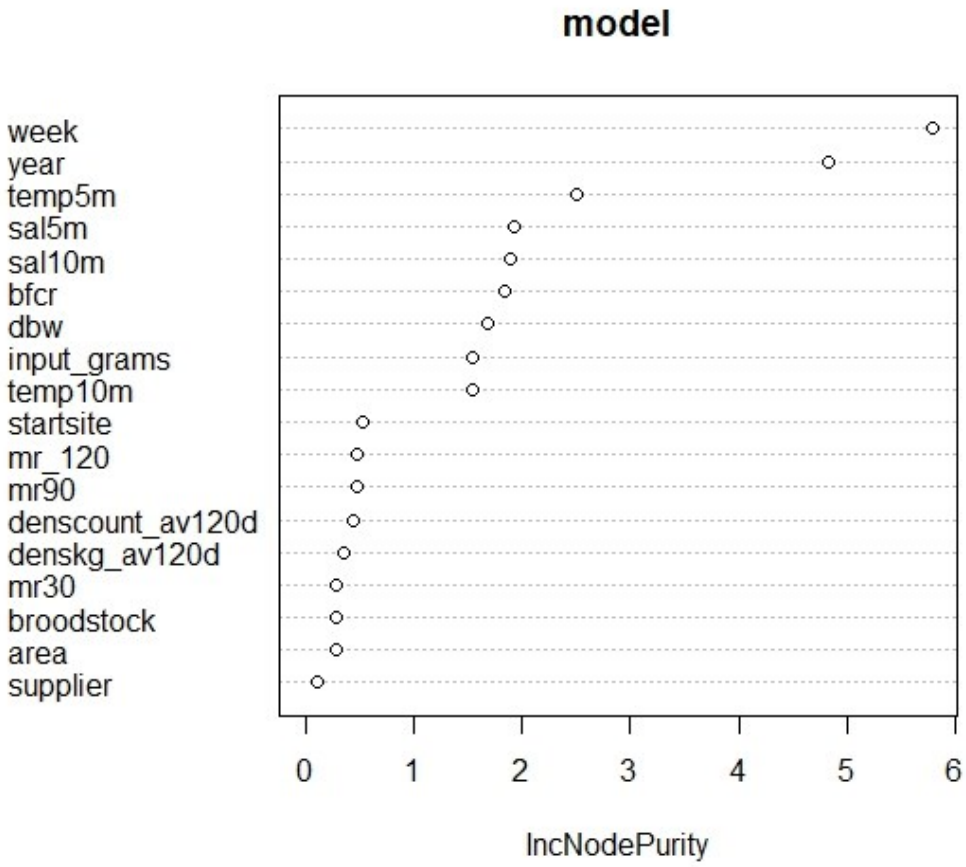

Figure A2.1 Random forest regression model output of pen-level and site-level variables and respective increase in node purity from each pen placement in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

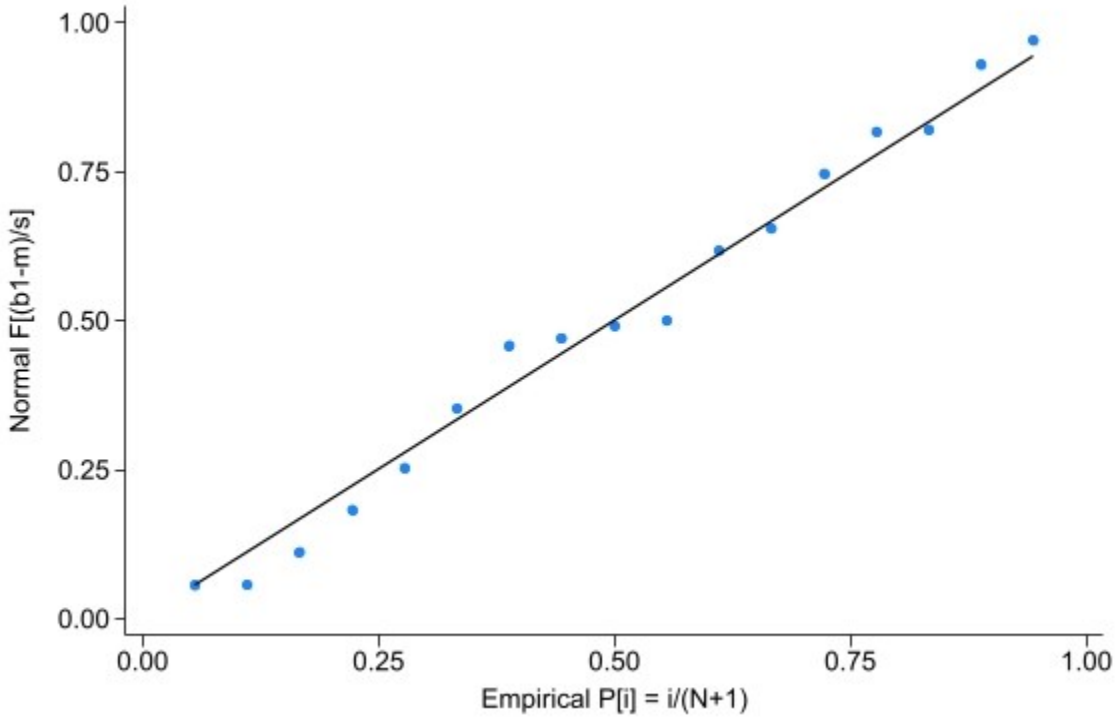

Figure A2.2 Pnorm plot of the normality of the Best Linear Unbiased Predictors (BLUPS) of the errors at the site level of the linear regression model of antimicrobial use (mg/kg biomass) in farmed Atlantic salmon in British Columbia, Canada (2015-2021).

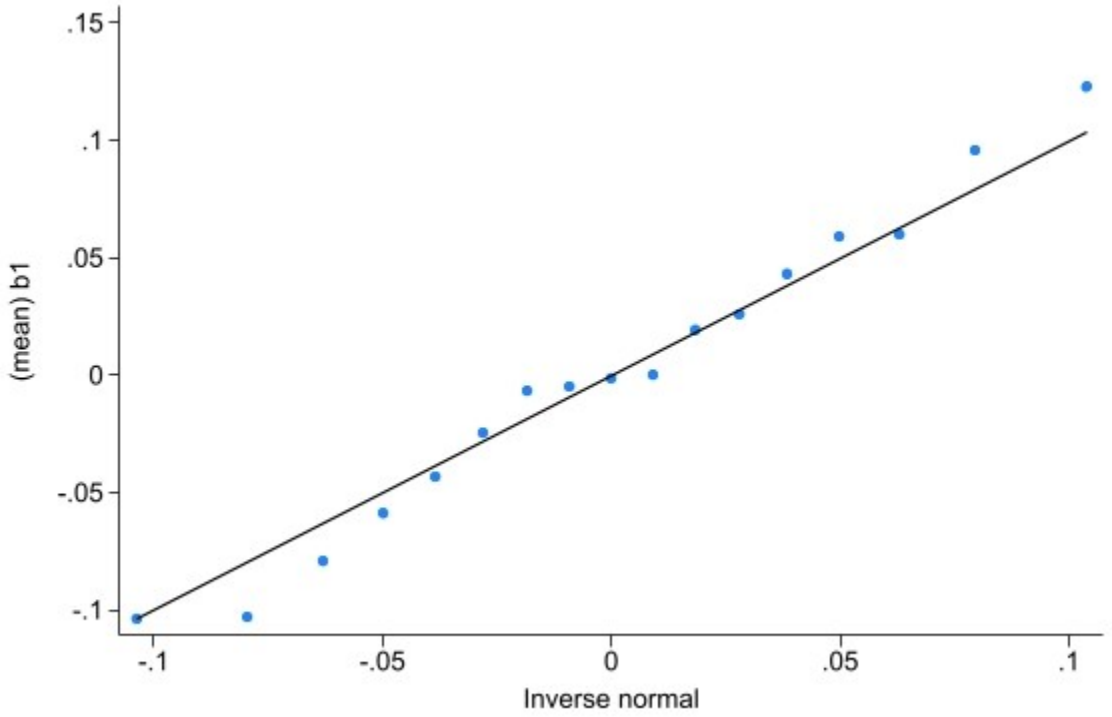

Figure A2.3 Qnorm plot of the normality of the Best Linear Unbiased Predictors (BLUPS) of the errors at the site level of the linear regression model of antimicrobial use (mg/kg biomass) in farmed Atlantic salmon in British Columbia, Canada (2015-2021).