Antimicrobial Use Surveillance Indicators: Use and Practicality in Salmonid Aquaculture Settings

by

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ABSTRACT

Antimicrobial resistance (AMR) is a unique threat to One Health, and salmonid aquaculture industries are increasingly challenged to demonstrate responsible antimicrobial stewardship. Indicators and metrics to quantify antimicrobial use (AMU) data can comparatively enhance understanding of antimicrobial use patterns to inform stewardship policy. Global salmonid aquaculture growth and increased AMU creates the potential for the exchange of AMR between aquatic and terrestrial environments and humans, making AMU surveillance imperative for the salmonid aquaculture industry. The objectives of this thesis were to identify robust candidate AMU indicators for use in the salmonid aquaculture industry; apply robust AMU indicators to top salmonid producing regions using AMU and production datasets to summarize and describe annual AMU in each regions' salmonid aquaculture industry; and to analyze AMU in salmonid aquaculture using robust indicators and evaluate temporal and regional trends among top salmonid-producing regions. To identify current AMU metrics and indicators that could be applied to salmonid aquaculture AMU data, a systematic search strategy was applied to five databases: Medline, Embase, Agricola, CAB Abstracts, and Biosis. To be included, studies must have reported on at least one AMU surveillance indicator for use in animals. Total annual salmonid slaughter mass and AMU data were gathered from Norway, Chile, the United Kingdom (UK), and British Columbia (BC). Data for salmonid production and AMU were gathered for the comparison of unadjusted and biomass-adjusted AMU between 2004-2018. We applied the AMU indicator milligrams of active ingredient per adjusted and unadjusted Population Correction Unit (mg/APCU, mg/PCU) based on life-adjusted and non-life-adjusted annual salmonid slaughter and average production weights ($mg/PCU_{Slaughter/AW}$) using data from these regions for 2004-2018 (2005-2018 for Chile). Antimicrobial use between regions was analyzed

using descriptive statistics of unadjusted and adjusted AMU, ordinary least squares (OLS), and variance weighted least squares (VWLS) regression analysis.

Over the entire study period, Chile had the highest mg/$PCU_{slaughter}$ ($p<0.01$), followed by BC ($p<0.01$), and then the UK and Norway. The Chilean mg/$PCU_{Slaughter}$ was consistently 2-6 times higher than British Columbia and 300-500 times greater than Norway for the entire study period. Norway had among the lowest annual overall AMU based on mg/$PCU_{Slaughter}$ estimates. The mg/$PCU_{Slaughter}$ decreased in Chile from 2015-2018 despite increasing production, whereas BC AMU fluctuated over the same period. Norway and the UK have maintained annual mg/$PCU_{Slaughter/AW}$ levels below 50 mg/PCU throughout the study period. The BC drug-specific mg/$APCU_{Slaughter/AW}$ showed greater drops in drug-specific use when the total slaughter biomass species composition shifted away from Pacific salmon to Atlantic salmon in 2004-2006. The mg/$PCU_{slaughter}$ varied significantly between salmonid-producing regions when evaluating all AMU between 2004-2018 in the VWLS model, which was required to account for unequal variance in biomass-adjusted AMU by region. Individual OLS models were fit to each region using year (centered on 2004) as the temporal variable and found significant changes in mg/$PCU_{slaughter}$ over time. British Columbian biomass-adjusted salmonid aquaculture AMU followed a quadratic relationship, declining until 2011 followed by a subsequent increase into 2018 ($p<0.01$). Chilean biomass-adjusted salmonid aquaculture AMU rose and fell throughout the study period, with a decline towards the end of the study. Norwegian biomass-adjusted salmonid aquaculture AMU declined linearly over the entire study period ($p<0.01$). Biomass-adjusted salmonid aquaculture AMU in the United Kingdom declined from the first yearly quartile (2004-2007) to the second ($p<0.01$) and did not increase or decrease significantly afterwards. Indicators based on the PCU improve the comparability of AMU between regions

with different levels of salmonid production by standardizing annual AMU by measuring the size of the population. However, PCU-based indicators fail to account for differences in drug potency. If regions use different drugs with marked differences in mg/kg dosing, there can be large resulting differences in mg/$PCU_{Slaughter}$ or mg/APCU. However, indicators that account for drug dosages require a definition of an average treatment weight for aquaculture species. This research identified and described useful AMU indicators for reporting AMU data and determining how best to inform antimicrobial stewardship in the salmonid aquaculture industry. It also described possible reasons for differences in salmonid aquaculture AMU between regions. Future work will explore other AMU indicators and the use of AMR indicators in salmonid aquaculture, and the potential links between AMU and AMR/disease pressure in the context of salmonid aquaculture.

PREFACE

This thesis represents original work completed by Jacob A. Narbonne (JN) under the supervision of Dr. Simon Otto and thesis committee members Dr. Brian Radke, Dr. Derek Price, and Dr. Patrick Hanington. The conceptualization and design of this thesis was conducted by JN under the supervision of Dr. Simon Otto and the supervisory committee. This thesis is based on data provided directly from the government of British Columbia and publicly available information from the following countries: Norway, Chile, and the United Kingdom. No parts of this thesis have been previously published, although chapter one represents a modified version of a published manuscript[1] written by JN, Dr. Simon Otto, and the supervisory committee. This research was funded by the British Columbia Ministry of Agriculture.

Jacob Narbonne was responsible for the data collection, cleaning, sorting, and analysis under the supervision of Dr. Simon Otto. JN 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 structure of this thesis, as well as the analytical work in 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.

[1] Narbonne, J. A., Radke, B. R., Price, D., Hanington, P. C., Babujee, A., & Otto, S. J. G. (2021). Antimicrobial Use Surveillance Indicators for Finfish Aquaculture Production: A Review [Review]. Frontiers in Veterinary Science, 8(175). https://doi.org/10.3389/fvets.2021.595152

DEDICATION

"I'm a great believer in luck, and I find the harder I work the more I have of it."

-Thomas Jefferson

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I have been looking forward to the opportunity to acknowledge the people who supported me throughout the ups and downs of this master thesis. It is with great humility and happiness that I write these last words among thousands. I would first like to offer my deepest gratitude and thanks to my brilliant supervisor Dr. Simon Otto. It sometimes feels like yesterday we were having our first discussions on the outline of this graduate degree, and I knew from the get-go that I had a supervisor, a mentor, worth feeling grateful for. Dr. Otto's guidance and support throughout this degree has had transformational effects on both my academic skill set and confidence. Dr. Otto trained me on how to tackle an advanced degree such as this one and encouraged me to take steps outside my comfort zone. Never did I imagine I would be presenting original work at academic conferences, let alone winning abstract submission competitions. I sincerely appreciate Dr. Otto's candid and direct feedback throughout this degree and can safely say I have grown as a person thanks to his support.

I would also like to thank my incredible supervisory committee for their insightful feedback throughout this degree. I always felt accepted and welcomed when we met and wish it didn't have to be over zoom. Dr. Brian Radke amazed me with his incredibly rich feedback that was always right to the core of what needed to be done. I always felt better about a passage after incorporating recommendations he made. Dr. Derek Price generated such important discussions in every meeting and challenged me to think and understand the deeper analytical aspects of this thesis. Dr. Patrick Hanington was always kind and insightful to me and acted in my eyes as a neutral party in meetings, which had an immense calming effect for me when the discussion turned towards how I'd need to fix something. I truly appreciate the willingness of each member of my supervisory committee to take time away from their busy professional and personal lives to help an eager student develop their skills.

I would like to thank my parents for their encouragement and warmth throughout this dissertation. Chris and Amanda were always asking how I was with writing and were willing to hear me express some minor frustration affecting me that day. I'd like to thank my partner for her candid wisdom and personal experience concerning her own graduate degree. I greatly appreciate the private PowerPoint design lectures she put me through. However, I guarantee I still don't know how to properly use a master slide.

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LIST OF ABBREVIATIONS

ADD – Animal Daily Dose

AMD – Antimicrobial Drug

AMOX - Amoxicillin

AMU – Antimicrobial Use

AMR – Antimicrobial Resistance

APCU – Adjusted Population Correction Unit

$APCU_{AW}$ – Adjusted Population Correction Unit Average Weight

$APCU_{Slaughter}$ – Adjusted Population Correction Unit Slaughter

ATW – Average Treatment Weight

BC – British Columbia

BKD – Bacterial Kidney Disease

CIPARS – Canadian Integrated Program for Antimicrobial Resistance Surveillance

CL – Chile

DADD – Daily Animal Defined Dose

DANMAP – Danish Program for Surveillance of Antimicrobial Consumption and Resistance

DD – Daily Dose

DDD – Defined Daily Dose

DDD_{Vet} – Defined Daily Dose Animal

$nDDD_{Vet}$ – Number of Defined Daily Doses Animal

$nDDD_{VetCA}$ – Canadian Number of Defined Daily Doses Animal

DDDA – Defined Daily Dose Animal

nDDDA – Number of Defined Daily Doses Animal

DCD – Defined Course Dose

DCD_{Vet} – Defined Course Dose Animal

$nDCD_{Vet}$ – Number of Defined Course Doses Animal

DOE – Duration of Effect

EMA – European Medicines Agency

ERY - Erythromycin

ESVAC – European Surveillance of Antimicrobial Consumption

EU – European Union

FAO – Food and Agriculture Organization

FLOR – Florfenicol

FLU – Flumequine

IHN - Infectious Hematopoietic Necrosis

IPN – Infectious Pancreatic Necrosis

ISA – Infectious Salmon Anemia

KG – Kilograms

LINC - Lincomycin

MG – Milligrams

NW – Norway

OA – Oxolinic Acid

OIE – World Organization for Animal Health

OLS – Ordinary Least Squares

OTC – Oxytetracycline

PCU – Population Correction Unit

PCU_{AW} – Population Correction Unit Average Weight

$PCU_{Slaughter}$ – Population Correction Unit Slaughter

SIFA - Sistema de Fiscalización de la Acuicultura

SMOR – Sulfadiazine + Ormetoprim

SPD – Salmon Pancreas Disease

SRS – Salmon Rickettsial Syndrome/Septicemia

TF – Treatment Frequency

TI – Treatment Incidence

TMS – Trimethoprim + Sulfadiazine

UDDA – Used Daily Dose Animal

nUDDA – Number of Used Daily Doses Animal

UK – United Kingdom

UK-VARSS – United Kingdom Veterinary Antimicrobial Resistance and Sale Surveillance

VETREG – Veterinary Medicines Register

VETCAB-ID – Veterinary Consumption of Antibiotics – International Documentation

VWLS – Variance Weighted Least Squares

WHO – World Health Organization

CHAPTER 1

INTRODUCTION, INDUSTRY, LITERATURE REVIEW

1.1 INTRODUCTION

Aquaculture is the fastest-growing food-producing agricultural sector globally and is expected to continue growing year over year (Asche et al., 2013; Defoirdt et al., 2011). Within the industry umbrella of aquaculture, this thesis focused on salmonid aquaculture, which is among the most successful subsets of aquaculture when measured by recent production growth (Asche et al., 2013; Iversen et al., 2020). Salmonids are defined as any of a family (Salmonidae) of elongate bony fishes (such as a salmon or trout) that have the last three vertebrae upturned (Merriam-Webster, n.d). The global salmonid industry produces over two million tonnes of salmonid each year for consumers around the world (Love et al., 2020). With the exceptional growth of the salmonid aquaculture industry, diseases of farmed salmonids continue to represent an important challenge for producers around the world. As more treatments with antimicrobial drugs (AMD) are administered to meet the disease pressures of the growing industry, selective pressures for the development of resistant bacteria are likely to increase (Aarestrup, 2015; D. B. Morrison & S. Saksida, 2013). Antimicrobial drugs have been used to successfully manage many production diseases of salmonids, but it is now recognized that caution must be exercised when using antimicrobials due to the emergence of antimicrobial resistance (AMR) (Collineau et al., 2017; Fisheries & Oceans Canada, 2016). Animal agriculture utilizes more than twice the weight of AMDs worldwide than humans according to some estimates and can contribute to environmental contamination with resistant strains of bacteria and their genetic elements (Aarestrup, 2012; Brault, Hannon, Gow, Warr, et al., 2019; Cabello et al., 2013). A reduction in antimicrobial use (AMU) in salmonid aquaculture is possible via practices such as vaccination, improved husbandry practices involving enhanced biosecurity, and regulation and monitoring, as was seen in Norway's salmonid aquaculture systems over the last three decades (Lulijwa et al., 2019; Midtlyng et al., 2011). Ultimately, a reduction in AMU in salmonid aquaculture will most likely result from improved antimicrobial stewardship or prudent use. Prudent use here is defined as the optimal selection of drug, dose, and duration of an AMD treatment. Prudent can also mean the reduction in over-use while maintaining optimal clinical health outcomes in the target

populations (Scott Weese et al., 2013). While efforts are underway to reduce overall AMU in salmonid aquaculture, the continued development of monitoring systems and frameworks will be necessary monitor this reduction in AMU and manage the threat of AMR emergence.

The quantification of AMU is a critical factor in developing responsible antimicrobial stewardship policies and programs (Collineau et al., 2017). With the growing threat of AMR development in animal agriculture, governments and industry representatives have reinforced the need for ways to better monitor and report AMU in food animal agriculture (Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health, 2002; Mowi, 2020). In response to this need, organizations such as the World Health Organization (WHO) and World Organization for Animal Health (OIE) have encouraged the systematic collection of data to qualify and quantify AMU (World Health Organization, 2017; World Organisation for Animal Health, 2015). As a result, routine data collection and indicator development for AMU in terrestrial livestock has become a widely adopted strategy for monitoring animal use across many countries (AACTING, 2019; NORM/NORM-VET, 2018; Statens Serum Institut & National Food Institute, 2018). However, the level of data collection and analysis applied to terrestrial livestock has not seen high adoption to date in the salmonid aquaculture industry (AACTING, 2019).

It has been shown that AMR development in the environment can be attributed to a "resistome" of mobile genetic elements that can transfer from one space to another, including between aquatic and terrestrial environments (Boerlin & White, 2013; Heuer et al., 2009). As the growth in worldwide aquaculture products continues, so has the demand for AMDs for use in aquaculture grown, especially in developing countries (Cabello, 2006). As a result of this growth, robust indicators for analyzing and comparatively reporting salmonid aquaculture AMU between regions are becoming increasingly important tools to monitor AMU and combat the development of AMR. Monitoring AMU is an essential strategy for understanding the variability of AMU among different populations, assessing the relationship between AMU and AMR, and informing antimicrobial stewardship efforts (Brault, Hannon, Gow, Otto, et al., 2019). To maximize the effectiveness of AMU indicators in salmonid aquaculture, definitions of indicators must be easily understandable and transparent, especially when evaluating AMU in salmonid aquaculture between regions (Dupont et al., 2016).

Antimicrobial use data can either be quantitative (e.g., weight of antimicrobials) or include a qualitative component (e.g., type of antimicrobial, treatment route) (Collineau et al., 2017). However, there is no international consensus on the preferred AMU indicator(s) for such purposes. It is widely recognized that different indicators may be considered for other surveillance purposes (CCVO Antimicrobial Use in Animal Agriculture Committee – AMU Surveillance Working Group, 2016). Therefore, understanding the various AMU indicators and their strengths/weaknesses is essential when considering their use. One application of AMU indicators is comparing AMU in species between regions and comparing AMU between different species in the same region (NORM/NORM-VET, 2018; Statens Serum Institut & National Food Institute, 2018; Veterinary Medicines Directorate, 2018). Currently, indicators for analyzing, quantifying, and reporting AMU in food animal production range from non-normalized "total-kilograms of AMD" metrics to standardized AMU indicators. These standardized indicators allow for the comparison of AMU across various animal species in different regions using different AMDs. This is done by using defined AMU metrics such as the Defined Daily Dose Animal (DDD_{Vet}) (European Medicines Agency, 2015a), and Population Correction Unit (PCU) (European Medicines Agency, 2021).

When gathering data to generate robust AMU indicators, certain factors must be considered during data collection. First, data must be comparable between populations, as the level of detail of data for exposure to AMU increases, the level of comparability drops, and vice versa (Collineau et al., 2017). For example, if a region were to use a high-detail AMU indicator which can adjust for dose, route of administration, or even treatment length, this would reveal a large amount of information on the AMU of one population. Still, it would be difficult to use this indicator to compare against other populations using AMDs with different administration routes on various livestock species. Comparing total AMU in milligrams of active ingredient between populations is a relatively straightforward but simplistic way of comparing AMU. Another way AMU indicators and metrics can increase detail at the cost of comparability is by defining the data as farm-level/region level etc. Regions with developed surveillance and monitoring frameworks that include farm-level data may find that comparing farm-level AMU with other regions may be difficult if these other regions have not collected this level of data.

Antimicrobial use data meant for AMU indicators must also possess high spatial and temporal resolution. Spatial resolution relates to location data pertaining to AMU events, and temporal resolution relates to the frequency of AMU data collection (Collineau et al., 2017). High spatial resolution allows for AMU comparison between smaller units in a region, such as a farm or an aquaculture site. Low spatial resolution may be sufficient for AMU comparison between countries. Standard temporal resolution across most regions is generally monthly data collection, collated annually in surveillance reports in some regions (Collineau et al., 2017). Other recommendations for frequency of data collection in production animal systems is the collection of AMU data based on the length of a typical production cycle to match AMU to the exact period a particular animal population was exposed to AMDs (Collineau et al., 2017). Another essential trait of robust AMU indicators includes data comprehensiveness. In this context, comprehensive data generally relates to a regions capacity to collect AMU data on all units (e.g., farms) and relevant species within its borders (Collineau et al., 2017). Antimicrobial use data measurements meant for AMU indicators must also be stable over time. The stability of AMU data measurements is called into question when treatment practices shift over time; for example, the Average Treatment Weight (ATW) and treatment durations of animals may change year to year, or animal population demographics could have shifted drastically (Collineau et al., 2017). Analyzing AMU over time using AMU indicators that adjust for population demographics and treatment methodologies are ideal for the temporal analysis of AMU trends (Collineau et al., 2017).

The lack of consistency between AMU indicators renders it difficult to compare AMU between different countries and regions and over time. This is further compounded by differences in ATW, defined drug dose standards, and production practices related to production cycle lengths between regions (Bosman et al., 2019; Stephanie A. Brault et al., 2019). In addition to this lack of comparability, all AMU indicators suffer from their own respective limitations based on inadequate data availability, or lack of specificity due to factors such as standard animal weights and doses used (Waret-Szkuta et al., 2019). Regulators must be transparent in defining AMU indicator(s) to truly reflect the burden of AMU in a population and allow for fair and meaningful comparisons (Stephanie A. Brault et al., 2019). Different indicators require various data sources such as prescription and consumption numbers. Some indicators are more useful in specific cases, whereas others should be considered impractical.

1.2 **RESEARCH QUESTIONS AND OBJECTIVES**

This thesis aims to quantify AMU in the British Columbian (BC) salmonid aquaculture industry and compare AMU in BC salmonid aquaculture with AMU levels of other top salmonid producers (Norway, Chile, and the United Kingdom (UK)). This thesis will offer meaningful information concerning useful AMU indicators for salmonid aquaculture and provide insight into the AMU of varying top salmonid producers. This thesis hopes to aid in the development evaluation of antimicrobial stewardship for the BC salmonid aquaculture industry by outlining potential factors for varying AMU between top salmonid producers. The AMU indicators applied in Chapters 2 and 3 of this thesis were the milligrams of active ingredient per population correction unit (using final slaughter and average treatment weights) ($mg/PCU_{Slaughter/AW}$) and milligrams of active ingredient per adjusted population correction unit (using final slaughter and average treatment weights) ($mg/APCU_{Slaughter/AW}$). Defined dose indicators, and indicators such as treatment frequency or incidence were explored. Indicators that relied on defined doses for salmonids were not applied to AMU data in this thesis as there were no internationally agreed-upon standard weights nor dosages for common antimicrobial products used in salmonid aquaculture.

Research Objectives:

1. To identify robust candidate AMU indicators for use in the salmonid aquaculture industry.
2. To apply robust AMU indicators to top salmonid producing regions using AMU and production datasets to summarize and describe annual AMU in each regions' salmonid aquaculture industry.
3. To analyze AMU in global aquaculture using robust indicators and evaluate temporal and regional trends within among top salmonid-producing regions.

Research Question 1:

What are the most practical AMU indicators for summarizing and standardizing AMU data for salmonid aquaculture?

Chapter 1 Objective:

- Provide background for the global salmonid aquaculture industry.
- Characterize and describe the strengths and limitations of various AMU indicators currently in use worldwide and consider their applicability to salmonid aquaculture.

Research Question 2:

How does antimicrobial use between top salmonid-producing regions compare when robust antimicrobial use indicators are applied to each region?

Chapter 2 Objectives:

- Calculate AMU in BC and major global salmonid producers using defined AMU indicators.
- Evaluate the strengths and limitations of these AMU indicators.

Research Question 3:

Are there significant regional and temporal differences in AMU between and within salmonid-producing regions? Additionally, are there any temporal trends associated with AMU in top salmonid-producing regions from 2004-2018?

Chapter 3 Objective:

- Analyze biomass-adjusted AMU data from top salmonid producing regions using regression analysis to determine whether regional and temporal trends in AMU exist for top salmonid producing regions over the study period.

1.3 **INDUSTRY – BRITISH COLUMBIA AND OTHER MAJOR SALMONID PRODUCERS**

In Canada, Atlantic salmon (*Salmo salar*) are farmed in British Columbia (BC), Newfoundland, New Brunswick, and Nova Scotia, with pacific salmon exclusively produced in BC (Government of Canada, 2021a). Trout (*Oncorhynchus mykiss*) are farmed in both fresh and marine waters in Ontario, Quebec, New Brunswick, Nova Scotia, and BC (Government of Canada, 2021a). Outside Canada, Atlantic salmon are primarily produced in Norway, Chile, and the UK, while of these regions, Pacific salmon (*Oncorhynchus tshawytscha, kisutch*) are only produced in Canada and Chile (Government of Canada, 2021a; Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018). Other minor salmonid species such as Rainbow/Steelhead trout are produced by all top salmonid producers (British Trout Association, 2020; Directorate of Fisheries - Norway, 2020; Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018).

The primary production system discussed in this thesis will be cage/net-pen systems, which is the primary method of salmonid production for all major salmonid producers (Asche et al., 2013). Other minor production systems are used in each region, such as recirculating systems, raceways, etc., though these account for a minute portion of overall production for each region (Asche et al., 2018; Asche et al., 2013). The lifecycle of the average farmed salmonid begins in freshwater hatcheries, where eggs sourced from brood stock hatch and alevins (2 cm in length) emerge. After hatching, alevins are reliant on their yolk sacs for nutrition up until it is entirely absorbed, after which they are called fry (5-8 cm). Fry are transferred to freshwater tanks at the hatchery. They continue to grow for several months until they become parr (10-25 cm) (Fisheries & Oceans Canada, 2016; Marine Institute Foras na Mara, 2020). Salmonids are anadromous, meaning they spend the juvenile portion of their lives in freshwater and the adult portion in the ocean (Seafish, 2012, 2015). Parr in Canada are vaccinated against several common bacterial and viral pathogens and prepared for transfer from the hatchery to marine environments once they become smolts (Fisheries & Oceans Canada, 2016). A complete production cycle in Canada, including brood stock selection, hatchery production, and grow-out, can take up to five years (Fisheries & Oceans Canada, 2016).

The focus of this thesis is BC, which accounted for more than half of total Canadian salmonid production in 2018 (Government of Canada, 2019a). Salmonid aquaculture in BC began in the early 1970s in the town of Sechelt on the Sunshine Coast, where several minor species of pacific salmon such as Chinook and Coho were produced (Canada, 2013; Positive Aquaculture Awareness, 2020). Before this, hatchery operations were used to supplement diminishing stocks of wild pacific BC salmonid populations as long ago as the early 1900s (Farm Fresh Salmon, 2020; Fisheries and Oceans Canada, 2016). Today, due to increased production efficiency compared to other species of salmonid, the majority of farmed salmonids in BC and around the world are Atlantic salmon (Canada, 2011; Directorate of Fisheries - Norway, 2020; Food & Agriculture Organization, 2020c; Scottish Executive - Environmental and Rural Affairs, 2018; Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018).

Salmonid farming in the UK is concentrated in the west and northwest mainland coast of Scotland and raceways within England and Wales (British Trout Association, 2020; Monterey Bay Aquarium, 2017b). There, the production of Atlantic salmon has steadily increased since 2004, while trout production has not grown substantially, remaining under 16,000 metric tonnes for the entire study period (Eurostat, 2021). While production has increased over the years, the number of salmonid aquaculture operations in the UK has fallen as the industry began to consolidate towards fewer, larger active sea sites. A 2017 estimate put the number of active sites in Scotland at 87 freshwater and 254 marine sites (Monterey Bay Aquarium, 2017b). Salmonid production in the UK is not expected to proliferate over the next few years due to tight regulatory constraints, making it difficult for the industry to expand (Asche et al., 2013). Norway is currently the largest salmonid producer globally, harvesting over 1.35 million metric tonnes of Atlantic salmon and Rainbow trout in 2018 (Directorate of Fisheries - Norway, 2020). Before the relaxation of ownership regulations, there were hundreds of small salmonid aquaculture companies licensed in Norway. Today, there are only a small number of large, vertically integrated companies managing over 1000 active sea sites along the coast of Norway (Asche et al., 2013; Monterey Bay Aquarium, 2018). Chile is the second-largest salmonid producer globally, producing over 920,000 thousand metric tonnes of Atlantic and Pacific salmon and Rainbow trout in 2018 (Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018). Salmonid production is concentrated in Los Lagos, Aysen, and Magallanes (Monterey Bay Aquarium, 2017a). Chile is infamously known for a near-total collapse of its

salmonid aquaculture industry in 2010 due to extreme disease prevalence (Asche et al., 2013). Infectious Salmon Anemia outbreaks between 2008-2011 highlighted many aspects of poor performance in disease management in the area and has led to many changes at the production and regulatory levels (Monterey Bay Aquarium, 2017a).

1.4 **LITERATURE REVIEW**

A recent publication reviewed and categorized some commonly used AMU indicators for food animal production (Werner et al., 2018). A systematic search string to capture updated literature from this review was established with feedback from a librarian. The complete search strategy and results can be found in the Appendix (A.1) and our recent publication (Narbonne et al., 2021). Published articles were obtained via searches on five scientific databases on January 20, 2020 (A.1.1): Medline via Ovid®, Agricola® via ProQuest®, CAB Abstracts via Web of Science™, Biosis® via Web of Science™, and Embase via Ovid®. Keywords were broken into five categories to capture articles of interest: monitoring/surveillance, AMU metrics/indicators of interest, antimicrobials, and animal species of interest (A.1.2). Searches were limited to January 1, 2016, onwards to capture literature not covered by the recent publication that reviewed AMU indicators (Werner et al., 2018). Articles were then sorted and screened based on inclusion and exclusion criteria. To be included, studies must have reported on at least one AMU surveillance metric or indicator for use in animal agriculture. Studies were excluded if they did not contain discussion of an AMU surveillance metric or indicator, if they did not discuss AMU surveillance in animals, if they were not written in English, or if they were theses or dissertations. All articles were screened at two levels by JN (Figure 1.1).

All articles were managed, deduplicated, and screened using Endnote X9/X20 (Elsevier, 2020). First-level screening evaluated titles and abstracts. Second level screening evaluated full article text. Government and intergovernmental/international reports on AMU metrics and indicators in livestock and salmonid aquaculture settings were identified based on investigator knowledge. Articles on the integration of AMR data with AMU indicators were sourced from references of articles within this review, located via the database search. References from key articles for integration of AMU with AMR data were retrieved. Supplementary articles and reports were identified by hand-searching the reference lists of included articles and knowledge of the investigators. See the appendix for the complete results of database searches and article

screening. Figure 1.1 contains the detailed results of the search and screening. There were 1,660 articles after deduplication, of which 38 progressed to second-level screening. A total of 27 articles (20 peer-reviewed and seven governmental reports) were included in the final review. A complete list of articles with extracted data is included in our recent publication (*see Supplementary material 2*) (Narbonne et al., 2021).

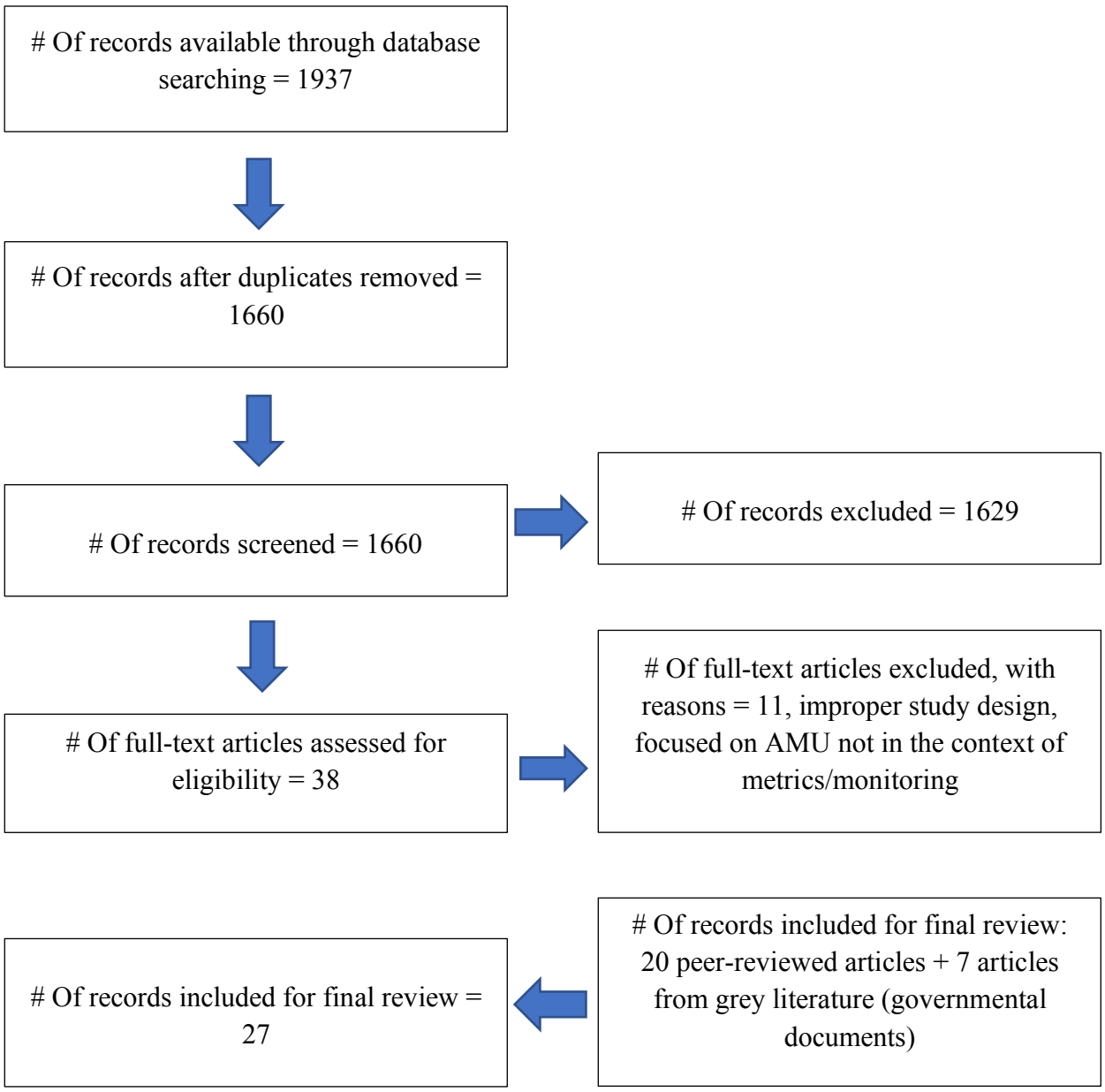

Figure 1.1. The compiled results from the search of five scientific databases and internet search engines, and screening of articles that included information about antimicrobial use surveillance metrics and indicators in animals (January 1, 2016, to January 20, 2020).

1.5 **OVERVIEW OF SALMONID DISEASES AND RELEVANT ANTIMICROBIAL DRUGS**

Bacterial diseases in salmonids are generally associated with stressful events such as low oxygen or crowding (Kelly, 2013). Some of the most important diseases of farmed salmonids include Bacterial Kidney Disease (BKD) (*Renibacterium salmoninarum)*, Vibriosis (*Vibrio anguillarum, V. oradlii, V. spp.),* Furunculosis (*Aeromonas salmonicida),* Yersiniosis (enteric red-mouth disease) (*Yersinia ruckeri*), Salmonid Rickettsial Septicemia/Syndrome (SRS) (*Piscirickettsia salmonis*), and yellow-mouth (*Tenacibaculum maritimum*) (Austin, 2016; Kent & Poppe, 1992). Bacterial diseases are generally the most prevalent disease challenge in salmonid farming in BC and Chile, whereas viral diseases are more common in Norway and the UK (Hossain & Shefat, 2018). Viral diseases of significant importance include Infectious Hematopoietic Necrosis (IHN), Infectious Pancreatic Necrosis (IPN), Salmon Pancreas Disease (SPD), and Infectious Salmon Anemia (ISA) (Kent & Poppe, 1992). The five most important principles of AMU in aquaculture, according to Reimschuessel et al. (2013) are: choosing the most appropriate drug at the most effective dose, avoiding toxicity in the animal, the safety of humans administering the antimicrobial or consuming the fish, avoidance of non-target species interactions and environmental damage, and legal restrictions. Salmonid aquaculture has a limited range of available AMDs, many of which are classified as highly or critically important AMDs by the World Health Organization (Lulijwa et al., 2019; World Health Organization, 2018; World Organisation for Animal Health, 2007). As a result of this limited selection, prudent use of approved antimicrobials is necessary to prevent the development of resistance against these AMDs as there are fewer options to replace them should they become ineffective. In salmonid aquaculture systems, AMDs are generally applied in feed as medicated pellets or are added directly to the water (immersion therapy) when the treated biomass is small (Henriksson et al., 2018; Kelly, 2013; Park et al., 2012).

Among the largest four producers of farmed salmonid, there is a limited number of previously and currently approved/utilized antimicrobials for salmonid aquaculture which vary from region to region (Table 1.1). Tetracyclines are a broad-spectrum bacteriostatic family of antimicrobials that interfere with protein translation, preventing protein synthesis (Hossain & Shefat, 2018; Park et al., 2012). Until recently, oxytetracycline (OTC) was the most used AMD

by weight in salmonid aquaculture in BC and is used to control many bacterial diseases such as furunculosis, vibriosis, BKD, and enteric red mouth disease (Animalytix LLC, 2021; Health Canada, 2019; Kent & Poppe, 1992). In addition, OTC is commonly used by other global producers to combat bacterial diseases in farmed salmonids (Government of Chile, 2018; Monterey Bay Aquarium, 2017b; NORM/NORM-VET, 2018). Unfortunately, the widespread use of OTC has led to bacterial resistance across much of the salmonid aquaculture industry to this drug (Park et al., 2012).

Interestingly, OTCs tendency to complex with positive cations such as calcium and magnesium render it generally unsuitable as an antimicrobial for salmonid aquaculture. It is poorly bioavailable to salmonids when administered as a medicated feed pellet (Park et al., 2012). The low bioavailability of OTC is circumvented by administering substantial doses compared to other AMDs (Park et al., 2012). OTC is classified as a highly important antimicrobial to human health by the WHO, and category three medium importance antimicrobial by the government of Canada (Government of Canada, 2009; World Health Organization, 2018).

Potentiated sulfonamides are a large class of antimicrobials that target different metabolic pathways by inhibiting different steps in the bacterial folic acid synthesis pathway (Armstrong et al., 2005). Potentiated sulfonamides (Trimethoprim + Sulfadiazine (TMS), Sulfadimethoxine + Ormetoprim (SMOR)) were commonly used to treat furunculosis, vibriosis, and enteric red-mouth disease in BC via in-feed delivery before 2009 (Armstrong et al., 2005; Bosse & Post, 1983; Kent & Poppe, 1992). However, potentiated sulfonamide antimicrobials have seen precipitous drops in usage in BC after 2009 due to poor tolerance in feed by salmonids and having a narrower therapeutic index than either OTC or florfenicol. Sulfonamides are particularly useful in water immersion therapy as they are well-absorbed through the gills (Park et al., 2012). Potentiated sulfonamides are classified as highly important antimicrobial by the WHO, and category three medium importance antimicrobial by the government of Canada (Government of Canada, 2009; World Health Organization, 2018).

Florfenicol (FLOR) is a broad-spectrum antibiotic with activity against gram-positive and negative bacteria by binding to the 50S ribosomal subunit – preventing bacterial protein synthesis (Armstrong et al., 2005). Florfenicol is used to treat furunculosis and yellow-mouth in

farmed Atlantic salmon around the globe (Animalytix LLC, 2021; D. B. Morrison & S. Saksida, 2013). As of 2018, FLOR is currently the most commonly prescribed drug for salmonid aquaculture in Canada (Government of Canada, 2018b) and the most used drug by weight in Chile (Government of Chile, 2018). It has become a staple drug in salmonid aquaculture due to low levels of AMR against it currently present. Additionally, there is a reduced likelihood of the development of cross-resistance to FLOR induced by other antibiotics such as OTC and potentiated sulfonamides. This is due to its highly dissimilar chemical structure to these other antimicrobials (Armstrong et al., 2005; Kent & Poppe, 1992). Florfenicol is classified as a highly important antimicrobial to human health by the WHO, and category three medium importance antimicrobial by the government of Canada (Government of Canada, 2009; World Health Organization, 2018).

Other AMDs used less commonly in salmonid aquaculture include the quinolone oxolinic acid (OA) and fluoroquinolone flumequine (FLU). These antimicrobials act primarily against gram-negative bacteria by inhibiting the bacterial enzyme DNA gyrase. Quinolones and fluoroquinolones are classified as critically important antimicrobials to human health by the WHO and category two high importance and category one very high importance antimicrobials respectively by the government of Canada (Government of Canada, 2009; World Health Organization, 2018). These drugs have high efficacy and low toxicity but are not susceptible to bacterial enzymatic degradation or transformation; thus, they can accumulate in aquatic environments (Armstrong et al., 2005). The beta-lactam amoxicillin (AMOX) was used briefly by Chile and the UK in the mid-2000s to control furunculosis. Amoxicillin interferes with enzymatic cross-linking of the bacterial cell wall of actively growing bacteria (Armstrong et al., 2005). Amoxicillin is classified as a critically important antimicrobial to human health by the WHO and is a category two high importance antimicrobial in Canada (Government of Canada, 2009; World Health Organization, 2018). The macrolide erythromycin (ERY) was used by BC and Chile briefly in the mid-2000s to control Rickettsial diseases and BKD (Park et al., 2012). Erythromycin disrupts protein synthesis in bacteria and is classified as critically important to human health by the WHO and listed as a category two high importance antimicrobial by the government of Canada (Government of Canada, 2009; World Health Organization, 2018). The lincosamide lincomycin (LINC) was also briefly used in BC. Lincomycin has a similar mechanism of action to macrolides such as ERY.

The future of antimicrobial therapy in salmonid aquaculture could include modalities other than AMDs, according to (Defoirdt et al., 2011). Bacteriophage therapy presents an opportunity for targeted treatments without the risk of antimicrobial resistance development against antimicrobials important to human health (Defoirdt et al., 2011; Lozano et al., 2018). Other methods include inhibiting various virulence factors and bacterial replication using targeted compounds such as polyhydroxyalkanoates and quorum disrupting compounds to inhibit gene transfer between bacteria (Defoirdt et al., 2011).

Table 1.1. Antimicrobials drugs previously, or currently used by each significant salmonid-producing region (Armstrong et al., 2005; Health Canada, 2010; NORM/NORM-VET, 2018; Veterinary Medicines Directorate, 2018). UK – United Kingdom.

Region	Antimicrobial	Status	Indications
Canada	Erythromycin	Approved	Used to treat BKD (*Renibacterium salmoninarum*) (Armstrong et al., 2005)
	Florfenicol	Approved	Used for treatment of furunculosis (*Aeromonas salmonicida*) in salmon Is active *in vitro* against *Vibrio anguillarum* and *Yersinia ruckeri (Animalytix LLC, 2021; Armstrong et al., 2005)*
	Oxytetracycline Hydrochloride	Approved	Used for treatment of furunculosis in salmonids Is active in vitro against *Vibrio anguillarum*, *Piscirickettsia salmonis* and *Yersinia ruckeri (Animalytix LLC, 2021)*
	Lincomycin	Not Approved	Used for treatment of furunculosis in salmonids (Animalytix LLC, 2021)
	Ormetoprim/ Sulfadimethoxine	Approved	Used for treatment of furunculosis in salmonids (Animalytix LLC, 2021)
	Trimethoprim/ Sulfadiazine	Approved	Used for treatment of furunculosis and vibriosis (*Vibrio anguillarum*) in salmonids (Animalytix LLC, 2021)
UK	Ormetoprim/ Sulfadimethoxine	Not Listed	Used for treatment of furunculosis in salmonids
	Oxytetracycline Hydrochloride	Approved	Used for treatment of furunculosis in salmonids Is active in vitro against *Vibrio anguillarum*, *Piscirickettsia salmonis* and *Yersinia ruckeri*
	Trimethoprim/ Sulfadiazine	Not Listed	Used for treatment of furunculosis and vibriosis in salmonids (Animalytix LLC, 2021)
	Florfenicol	Approved	Used for treatment of furunculosis in salmon Is active *in vitro* against *Vibrio anguillarum* and *Yersinia ruckeri (Animalytix LLC, 2021; Armstrong et al., 2005)*

	Oxolinic acid	Not Listed	Used to prevent furunculosis and enteric red mouth disease in salmonids (Austin, 2016)
	Amoxicillin	Approved	Used for treatment of furunculosis in salmonids
Norway	Oxolinic acid	Approved	Used to prevent furunculosis and enteric red mouth disease in salmonids (Austin, 2016)
	Florfenicol	Approved	Used for treatment of furunculosis in salmon Is active *in vitro* against *Vibrio anguillarum* and *Yersinia ruckeri (Animalytix LLC, 2021; Armstrong et al., 2005)*
	Flumequine	Not Approved	Used to treat BKD and vibriosis in salmonids Is active in vitro against *Aeromonas salmonicida*
	Oxytetracycline Hydrochloride	Approved	Used for treatment of furunculosis in salmonids Is active in vitro against *Vibrio anguillarum*, *Piscirickettsia salmonis* and *Yersinia ruckeri*
	Spectinomycin/ Lincomycin	Approved	Used for treatment of furunculosis in salmonids
Chile	Amoxicillin	Approved	Used for treatment of furunculosis in salmonids
	Oxytetracycline hydrochloride	Approved	Used for treatment of furunculosis. in salmonids Is active in vitro against *Vibrio anguillarum*, *Piscirickettsia salmonis* and *Yersinia ruckeri*
	Florfenicol	Approved	Used for treatment of furunculosis in salmon Is active *in vitro* against *Vibrio anguillarum* and *Yersinia ruckeri*
	Flumequine	Approved	Used to treat BKD and vibriosis in salmonids Is active in vitro against *Aeromonas salmonicida*
	Trimethoprim/ Sulfadiazine	Not Listed	Used for treatment of furunculosis and vibriosis in salmonids
	Erythromycin	Approved	Used to treat BKD (Armstrong et al., 2005)
	Oxolinic acid	Approved	Used to prevent furunculosis and enteric red mouth disease in salmonids (Austin, 2016)

1.6 **OVERVIEW OF ANTIMICROBIAL USE METRICS AND INDICATORS**

The terms AMU metric and indicator are used to describe measurements of AMU throughout this thesis. An AMU metric is described by some as a "technical unit of measurement" meant to quantify AMU according to AMD weights (mg, kg) or defined metrics such as Defined Daily Dose Animal (DDD_{Vet}, mg/kg/day) or Defined Course Dose Animal (DCD_{Vet}, mg/kg/treatment course) (Agunos et al., 2019; European Medicines Agency, 2015a, 2015b). An AMU indicator is an AMU metric in relation to a denominator such as animal biomass or an animal time unit (Agunos et al., 2019). Antimicrobial use indicators use measures of frequency and amount, adjusted by a defined denominator to estimate AMU in a standardized, comparable manner between populations, and that accounts for population size (Agunos et al., 2019). Different AMU metrics and indicators can be used depending on the requirements for surveillance. Werner et al. (2018) considered two overarching categories of AMU indicators based on the quantity of AMD used and the course of AMD application. Quantity-based indicators characterize the amount of AMU in terms of the weight of AMD distributed, sold, or administered/used per kg of body weight, standardized weight, or the number of doses used. Course-based indicators specify if and how often AMU occurred by estimating the number of drug treatments or courses an animal or group of animals received over time (Mills et al., 2018; Werner et al., 2018). Here, the terms AMD, "drug," and "active ingredient" are considered to mean a single active antimicrobial ingredient distinguished from antimicrobial products containing more than one active ingredient. A dose of active ingredient is the amount of AMD administered in a single application. In contrast, the dosage is the amount of AMD administered per kilogram of body weight per the drug label (Werner et al., 2018). However, the terminology in the literature is not consistent in the use of the dose versus dosage. A treatment is all administrations of an AMD given to one animal in one day (Werner et al., 2018). A course is a complete regimen (the number of days) of treatment with an AMD as outlined by the instructions on the drug label (Collineau et al., 2017). Table 1.2 includes examples of AMU and population metrics used to derive the resulting AMU indicators.

Table 1.2. Examples of antimicrobial use (AMU) metrics used alone or as part of indicators.

	AMU or Population Metrics	AMU Indicators
Quantity-Based Indicators	Weight of active ingredient	Total weight / PCU
	Biomass - Population Corrected Unit (PCU)	
	Biomass - Adjusted PCU (APCU)	Total weight / APCU
	Number of animals	Total weight / number of animals
	Defined Daily Dose Animal (DDD_{Vet})	Number of DDDA ($nDDD_{Vet}$)
	Used Daily Dose Animal (UDDA)	Number of UDDA (nUDDA)
	UDDA	Treatment Frequency (TF)
	DDD_{Vet} and PCU or APCU	Treatment Incidence (TI)*
Course-based Indicators	Defined Course Dose Animal (DCD_{Vet})	Number of DCDA ($nDCD_{Vet}$)
	DCD_{Vet} and PCU or APCU	Treatment Incidence (TI)*

*** Quantity or course-based definition of Treatment Incidence depends on the metric used to derive the indicator.**

1.7 **TOTAL WEIGHT OF ACTIVE INGREDIENT**

The total weight of active ingredients administered annually is a relatively rudimentary measure of AMU when used as a solitary metric (Mills et al., 2018). This metric is calculated by tabulating the total amount of AMDs sold or used over a period of the auditor's choosing and can generally be derived from records (if kept) or approximated from sales/prescription data. For example, the Canadian Integrated Program for AMR Surveillance (CIPARS) reported the total annual weight of AMDs distributed for animal use for over a decade (Government of Canada, 2020a). Denmark publishes the annual Danish Programme for Surveillance of Antimicrobial Consumption and Resistance (DANMAP) report, which has included the total amount of AMDs sold in kilograms to the aquaculture industry over the last decade (Statens Serum Institut & National Food Institute, 2018). Unfortunately, the total weight of the active ingredient is insufficient for AMU surveillance when used alone due to several problems inherent with the lack of standardization by drug dosage or population at risk. This metric can only be used to

meaningfully compare the AMU of two essentially identical salmonid aquaculture operations. Regions or countries using the same AMDs (with equivalent doses) for vastly differently sized livestock populations would appear to have either much higher or lower AMU depending on the size of their salmonid aquaculture populations (Agunos et al., 2017; Brault, Hannon, Gow, Otto, et al., 2019). This is undesirable as it fails to measure the AMU per unit of exposed/treated biomass – which is a useful indicator for prudent AMU (Brault, Hannon, Gow, Otto, et al., 2019). Measuring AMU using the total amount of active ingredients can also result in false comparisons of AMU between species of different sizes (e.g., humans vs. cattle) or with drugs of different dosages (e.g., tetracyclines vs. macrolides in cattle) (Brault, Hannon, Gow, Otto, et al., 2019). However, this metric is commonly used as a numerator in AMU indicators that standardize the total amount of active ingredient by various factors such as the number of doses, biomass exposed, or animal days at risk.

1.8 **POPULATION CORRECTION UNIT (PCU) AND MG/PCU INDICATOR**

The PCU is a theoretical unit of measurement of biomass (kg) of an animal potentially exposed to a certain weight of antimicrobials. A unit of biomass (kg) measured using the PCU is equal to one kilogram of biomass potentially exposed to antimicrobials (European Medicines Agency, 2021). The European Medicines Agency (EMA) developed the PCU in 2009 as part of the European Surveillance of Veterinary Antimicrobial Consumption group (ESVAC) (European Medicines Agency, 2021; Veterinary Medicines Directorate, 2016). It is used primarily as an indicator for reporting sales data of antimicrobials for a given animal population and comparing AMU in different populations using standard weights (European Medicines Agency, 2021). The PCU represents total animal biomass (kg) in a given year, as well as the estimated weight of each species at the time of treatment, denoted as the average treatment weight (ATW) (Table 1.3) (European Medicines Agency, 2021). The PCU for each species of livestock is calculated by multiplying the total estimated number of animals by a theoretical weight at treatment (Equation 1) (European Medicines Agency, 2016b, 2021). The number of animals included in the calculation consists of living and slaughtered animals and imported and exported livestock (Equation 2).

$$\boldsymbol{PCU_{species}\,(kg\ biomass) = number\ of\ animals\ x\ ATW\ (kg)} \qquad (\mathbf{1})$$

$$\textit{Number of animals} = \textit{animals present} + \textit{slaughtered} + \textit{imported} - \textit{exported} \quad (2)$$

The ATW is referred to as average treatment weight, average weight at treatment, or theoretical weight at the time most likely of treatment depending on the literature (European Medicines Agency, 2017, 2021). The agreed-upon ATW for each livestock species estimates the weight of animals in a respective livestock category when they are at the age when they would most likely be treated with antimicrobials. The specified animal ATW can vary from country to country and breed to breed, as demonstrated by Canadian vs. European ATWs for certain classes of bovine animals (European Medicines Agency, 2021; Government of Canada, 2019b; Lekagul et al., 2018). The ESVAC reports the weights they use (Table 1.3) and typically references the original publications for these values (Montforts, 1999; Montforts et al., 1999; Montforts & Tarazona Lafarga, 2003). These include body weights for categories of livestock, such as heifers, bulls, steers, etc. Montforts (1999) defined the term "averaged bodyweight" for animals that are reared from a starting weight onwards, compared to animals kept at their mature body weight for breeding and other purposes, for which maximum body weight is used. Montforts (1999) further proposed that body weights at treatment should be based on adult weights for mature animals and the mean of starting and slaughter weights for production animals.

Table 1.3. Examples of Average Treatment Weights for Population Correction Unit calculations.

Animal Category	Weight in Kg
Slaughtered cow	425
Slaughtered heifer	200
Slaughtered bullocks and bulls	425
Slaughtered calves and young cattle	140
Living dairy cow	425
Slaughtered pig	65
Living sow	240
Slaughtered broiler	1
Slaughtered turkey	6.5
Slaughtered sheep and goats	20

$$\boldsymbol{mg/PCU_{species}(mg/kg\ biomass) = \frac{total\ AMD\ used\ (mg)}{PCU_{species}}} \quad (\mathbf{3})$$

The AMU indicator milligrams/Population Correction Unit (mg/PCU) (Equation 3) is a quantity-based indicator that utilizes the total weight of antimicrobials (mg) as well as the PCU metric as a denominator. Canada and approximately 30 European countries currently use the PCU for some AMU surveillance purposes (European Medicines Agency, 2017; Government of Canada, 2018a). The mg/PCU indicator allows for the comparison of AMU between farms/countries with differing amounts of exposed animal biomass while controlling for animal demographics, specifically population size and species composition (European Medicines Agency, 2021; Radke, 2017). The mg/PCU indicator can also be used to evaluate the total overall AMU in a region and characterize trends in AMU over time by antimicrobial class (Government of Canada, 2018a). The mg/PCU indicator standardizes the amount of AMU (sold, administered, or distributed) by the biomass of a given animal population, whether by individual species or total livestock population under surveillance. Instead of using challenging to acquire actual animal weights at treatment with antimicrobials, animal weights are converted to "estimated standard weights"/ ATW according to a specific production class as defined by the ESVAC definition of the population correction unit (European Medicines Agency, 2021) (Table 1.3). An advantage of using assigned "estimated standard weights" to calculate the PCU includes the ability to separate antimicrobial sales and distribution data for specific production classes for each species. The mg/PCU AMU indicator can account for variations in animal numbers and weights across populations as long as the evaluations on AMU are conducted on the same species or animal populations with highly similar demographics (Mills et al., 2018). An important limitation of the mg/PCU indicator is that it cannot reliably evaluate how AMU can influence AMR development and persistence in different species. The mg/PCU indicator treats AMU in different species or risk categories equally, even when this may not be the case (e.g., 1 kg of poultry equivalent to 1 kilograms of cattle). For example, the milligrams of AMU per kilogram of poultry is potentially different from an AMR risk perspective than per kilogram of beef due to differing lifespans, production practices, and proximity in time of AMU to slaughter, processing, and human consumption (Government of Canada, 2018a; Radke, 2017). Also, the PCU metric assumes that the ATW of an animal at the likely time of treatment is constant and approximates field conditions.

When using this indicator to compare AMU of countries using total PCU values, auditors and other stakeholders should ensure that the regions being evaluated and compared have similar livestock demographics. This is because AMU intensity and duration can differ between species for reasons such as variations in production practices of the region or the varying length of life of different livestock species (Radke, 2017). The mg/PCU as an AMU indicator is limited when used on its own to compare AMU involving AMDs with varying dosages (e.g., the total mg of AMU will be less for a drug with a lower mg/kg dosage) (Brault, Hannon, Gow, Otto, et al., 2019). This is concerning with respect to salmonid aquaculture as some regions utilize more potent AMDs such as quinolones, which necessitate much lower doses than drugs such as OTC. As a result, analyzing and comparing the overall AMU in salmonid aquaculture of regions using different types of antimicrobials with vastly different potencies is not feasible using the indicator mg/PCU if one's goal is to determine antimicrobial stewardship based on overall adjusted use (Hyde et al., 2017; Mills et al., 2018).

The mg/PCU indicator is adept at identifying low and high users of AMDs due to its straightforward interpretation when the comparison groups have similar animal species demographics (Agunos et al., 2017). However, the comparison of AMU between farms/countries using the mg/PCU indicator can be problematic due to either under or over-representing AMU across operations with differing ATW (e.g., cattle in North America versus Europe) (Brault, Hannon, Gow, Otto, et al., 2019; Mills et al., 2018). Regions where production practices result in markedly different treated and mature animal weights are incentivized to develop animal weight standards of their own to make more accurate comparisons of AMU to other regions (Mills et al., 2018). For example, in 2017, Canada implemented their own ATWs for some livestock categories due to the possibility of Canadian livestock being heavier than their European counterparts (Government of Canada, 2020b). Unlike terrestrial agriculture, finfish production PCU is typically reported as the total annual slaughter weight and does not include reporting of animal numbers by any country or producing entity in the world (European Medicines Agency, 2021).

The PCU has been criticized for failing to account for drug potency and AMU at the species level (Brault, Hannon, Gow, Otto, et al., 2019; Hyde et al., 2017; Mills et al., 2018). Metrics like the DDD_{Vet} developed by ESVAC and co-opted by other nations have become

popular due to their abilities to account for varying drug doses/indications of AMU at more granular levels, such as at the species/breed level (Radke, 2017). Unfortunately, the DDD_{Vet} metric is highly data-dependent and, as such, has high resource demands such as species-specific AMD dose and indication information and animal ATWs (Lekagul et al., 2018; Radke, 2017). While the DDD_{Vet} has become a popular standard AMU metric in the European Union (EU), many countries still use the PCU as a means of AMU standardization (Radke, 2017). With this in mind, the PCU is likely to hold high importance until the development of more standardized indicators that account for drug potency, dosing levels, etc., across more countries (Radke, 2017).

1.9 **ADJUSTED POPULATION CORRECTION UNIT (APCU) AND MG/APCU INDICATOR**

Radke proposed the adjusted PCU (APCU) (Equation 4) alternative to the PCU (Radke, 2017). The APCU addresses an important limitation of the PCU in that the PCU does not correctly estimate the actual exposed biomass at risk of treatment over time (Brault, Hannon, Gow, Otto, et al., 2019; Radke, 2017). The risk of an animal's exposure to AMU is related to their weight and length of life (Radke, 2017). In addition to discrepancies in standard weights between nations, the risk of exposure to AMU between animals with vastly different lifespans should be accounted for when pooling animal biomass, making the mg/APCU an exciting option to supplement the mg/PCU indicator (Radke, 2017). The biomass denominator used as a standard for AMU adjustment in the mg/PCU indicator should reflect the length of life of livestock. Normalizing total active ingredient used/sold/prescribed using the APCU (mg/APCU) (Equation 5) accounts for the total weight of animals in a population and their length of life to calculate total exposed animal biomass, resulting in life-adjusted weights for animal categories (Radke, 2017). The length of life is calculated as being the inverse of the number of production cycles per year for each species of salmonid produced. The length of life variable is applied as a conversion factor to an animal category's AWT (Equation 6) (Radke, 2017). The consideration of an animal's average lifespan improves comparability between different species where length of life differs substantially, such as cattle, swine, and poultry. It also accounts for the increased possibility of exposure to AMD for animals as they live longer (Cuong et al., 2019).

Radke (2017) found that when comparing PCU calculations of 9 countries (Canada + 8 European countries) with APCU calculations using the same number of animals in both, there were differences between PCU and APCU values. The APCUs increased compared to PCU in cattle but decreased for pigs, poultry, sheep, and goats due to their longer or shorter respective production cycles (Radke, 2017). This thesis will apply the APCU to the BC salmonid aquaculture industry data using estimated ATWs and approximated species-specific production cycle lengths to evaluate how length of life and biomass-adjusted measurements influence AMU evaluation, and to facilitate future comparisons with terrestrial animal mg/PCU indicators. While the length of life is a worthwhile variable to look at for adjusting exposed biomass, it is still an estimate that relies on generalizations of salmonid production cycles that could vary from region to region.

$$\boldsymbol{APCU_{species} = number\ of\ animals\ x\ life-adjusted\ treatment\ weight\ (kg)} \quad (4)$$

$$\boldsymbol{mg/APCU_{species}(mg/kg\ adjusted\ biomass) = \frac{total\ AMD\ used\ (mg)}{APCU_{species}}} \quad (5)$$

$$\boldsymbol{Life-adjusted\ treatment\ weight\ (kg) = ATW\ x\ length\ of\ life\ (years)} \quad (6)$$

1.10 DEFINED DDAILY DOSE ANIMAL (DDD_{VET}) AND NUMBER OF DDD_{VET} ($nDDD_{VET}$) INDICATOR

The DDD_{Vet} metric (also known as the DDDA) is an adaptation of the human defined daily dose (DDD), defined as "the assumed average dose/day for a drug for its main indication in animals" with units mg/kg/day (Equation 7) (Bosman et al., 2019; European Medicines Agency, 2015b). The DDD_{Vet} metric is assigned for specific AMDs for specific indications in particular livestock animals. It was implemented by ESVAC (European Medicines Agency, 2015a) as part of a system for collecting harmonized AMU data in the EU (European Medicines Agency, 2015). The DDD_{Vet} for each AMD is specific to the EU. The DDD_{Vet} represents the average dosage (the arithmetic mean) of the daily dosages of that AMD based on the dosage labels from participating European countries (European Medicines Agency, 2015a, 2015b). Using the DDD_{Vet} metric, the $nDDD_{Vet}$ indicator can be derived and used to overcome the issue of other quantity-based indicators failing to account for varying dosage amounts of AMDs used in animal agriculture. The number of Defined Daily Doses Animal ($nDDD_{Vet}$) is calculated for an individual animal or

a population by dividing the total/individual quantity of AMD active ingredient used by the standardized DDD_{Vet} for that drug, indication, route of administration, and animal, multiplied by the individual standard ATW (Equation 8) (Brault, Hannon, Gow, Otto, et al., 2019).

$$\boldsymbol{DDD_{Vetdrug}(mg/kg/day) = \frac{mg/kg\ drug\ dose}{day(s)\ duration\ of\ effect}} \quad (7)$$

$$\boldsymbol{nDDD_{Vetdrug} = \frac{total\ AMD\ used(mg)}{DDD_{Vetdrug}(mg/kg/day)\ x\ ATW(kg)}} \quad (8)$$

This calculation is drug and animal specific, making its derivation comparable between populations using similar AMDs with similar livestock demographics. For mixed animal demographic comparisons, species-specific $nDDD_{Vets}$ can be calculated and summed to evaluate total AMU. Current, defined standardized daily doses (DDD_{Vet}) for AMDs exist for swine, cattle, and broilers as developed by ESVAC (European Medicines Agency, 2015b) (Table 1.4). The $nDDD_{Vet}$ also accounts for long-acting injectables by dividing the single administered dose of a long-acting injectable over the number of days of the duration of therapeutic effect (DOE) (European Medicines Agency, 2015). The DOE is an essential consideration when quantifying AMU and an especially important consideration for properly evaluating selective pressure and AMR development (Stephanie A. Brault et al., 2019). Indicators such as the $nDDD_{Vet}$ rely on estimated average treatment weights to calculate the estimated number of define doses applied per dosage (treatment). Currently, there are no defined ATWs for salmonids and there are no agreed upon DDD_{Vets} for approved aquaculture AMDs – thus $nDDD_{Vets}$ for salmonids cannot be applied. Defining standard weights for salmonids would require buy-in from stakeholders and producers – but reaching acceptable standard weights could be difficult due to the varying desired sizes of salmonids produced world-wide.

While the $nDDD_{Vet}$ as a defined-dose quantity-based indicator improves upon some areas of AMU evaluation over other quantity-based indicators such as mg/PCU, it is still difficult to interpret/compare between regions. The derivation of indicators like $nDDD_{Vet}$ exists in several variations based on which country is evaluated. These variations include the Defined Daily Dose (DDD) (Netherlands and Belgian), DDDA (ESVAC), the DDD_{VetCA} (Canada) (Bosman et al., 2019), as well as Animal Daily Dose (ADD), Daily Dosage (DD), or Daily Animal Defined Dose (DADD) by others (Stephanie A. Brault et al., 2019; Collineau et al., 2017). For example, the

DDD_{Vet} defined by ESVAC is singularly applied to an AMD regardless of how an oral administration of AMD is applied, while the DDD_{vetCA} derived by (Agunos et al., 2017) varies by stratification of AMD route of oral administration (e.g., feed and water). Defined dose indicators like the $nDDD_{Vet}$ can even vary by how the numerator and denominators are defined. Denmark evaluates AMU on a per species basis using the Defined Animal Daily Dose (DADD), a similar metric to the DDD_{Vet} (National Food Institute Statens Serum Institut, 2017). Instead of using the DADD as a component of the denominator, they evaluated Danish aquacultural AMU for 2017 by dividing the DADD of each AMD used in aquaculture by the estimated biomass at slaughter of salmonids. The result is the DADD per 1000 animals per day (DAPD) which is a statistical measure that estimated the proportion of animals treated daily with a maintenance dose of a particular AMD (National Food Institute Statens Serum Institut, 2017). The result of this Danish analysis was an estimate how much aquaculture AMU took place and the proportion of salmonids that received treatment.

Defined quantity-based indicators like the $nDDD_{Vet}$ allow for comparing AMU between nations using similar dosing standards/indications for treatment. However, inconsistent standard dosing values used (e.g., median, mean, highest, or indicated) and varying ATW estimates still create problems with comparability between different regions (Collineau et al., 2017). The $nDDD_{Vet}$ is a technical unit of measurement and does not reflect prescribed or used daily doses (European Medicines Agency, 2015). The $nDDD_{Vet}$ by itself does not provide any information as to the number of animals treated, the population at risk of treatment, the length of treatment (how many days of consecutive/total therapy was provided), the daily dose *actually* applied, or the total amount of AMDs used (Werner et al., 2018). The lack of defined dose-based indicators such as the $nDDD_{Vet}$ in salmonid aquaculture render analyzing prudent AMU based on the estimated number of doses applied infeasible. Knowing how many doses are applied per treatment allows for the evaluation of AMU frequency (dosing events) and thus stewardship in the context of treatments per salmonid.

Table 1.4. Cattle Defined Daily Dose (DDD_{Vet}) value examples (European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), 2016).

Substance	Route	DDD_{Vet} (mg/kg/day)
Amoxicillin	Oral	20
Cefalexin	Parenteral	7
Colistin	Oral	4.8
Ceftiofur	Parenteral	1

1.11 USED DAILY DOSE ANIMAL (UDDA) AND NUMBER OF UDDA (nUDDA) INDICATOR

The UDDA is the daily dose of an active ingredient that is typically administered to an animal (mg drug/animal/day - Equation 9) (Kasabova et al., 2019; Persoons et al., 2012; Timmerman et al., 2006; Werner et al., 2018). Alternatively, the $UDDA_{kg}$ (UDDA per kg – Equation 10) is the administered dosage of an active ingredient per day per kg of body weight of a treated animal (Werner et al., 2018). These indicators require the actual number of treated animals, their weights, and the number of days of treatment to be known. As a result, they are based on actual AMU data rather than the theoretical value presented by the consensus of several doses that make up the DDD_{Vet}. The UDDA allows for comparisons of AMU between populations using the same active ingredient.

$$UDDA_{drug}\,(mg/animal/day) = \frac{total\ AMD\ used\ (mg)}{\#\ treated\ animals\ x\ \#\ treatment\ days} \quad (9)$$

$$UDDA_{kg\ drug}(mg/kg/day) = \frac{total\ AMD\ used\ (mg)}{\#\ animals\ x\ weight\ (kg)\ x\ \#\ treatment\ days} \quad (10)$$

$$nUDDA = \#\ treated\ animals\ x\ \#\ active\ ingredients\ \#\ treatment\ days \quad (11)$$

Unlike the DDD_{Vet} metric, the UDDA metric is not a theoretical value reached by a consensus of several doses. It reflects the *actually* administered dose of AMD per day per kg of bodyweight of a treated animal species. It can only be calculated if the number of treated animals, their body weight, and the number and days of treatment are known (Kasabova et al., 2019; Werner et al., 2018). With the increased level of dosage specificity at the farm level,

metrics like UDDA become powerful tools for the non-theoretical estimation of actual AMU and benchmarking between farms (Kasabova et al., 2019). The number of UDDA (nUDDA) indicator is the sum of daily applications in a population (Equation 11) (Werner et al., 2018). It represents the amount of *actually* administered AMD doses for a given animal population (Lekagul et al., 2018; Werner et al., 2018). It does not indicate how much active ingredient is being used; it simply reflects the frequency of treatments with AMD (Hemme et al., 2018). It requires granular data such as the number of animals treated, the number of days treatment occurred, and the number of active ingredients administered (Hemme et al., 2018; Joosten et al., 2019). It is also specific for similar populations being analyzed at a point in time using the same active ingredients for treatment (Bosman et al., 2019; Werner et al., 2018). The use of this metric/indicator is limited compared to the DDD_{Vet} and mg/PCU.

1.12 DEFINED COURSE DOSE ANIMAL (DCD_{VET}) AND NUMBER OF DCD_{VET} ($nDCD_{VET}$) INDICATOR

The Defined Course Dose Animal (DCD_{Vet}) (Table 1.5) does not have a human medicine counterpart and is defined as the "average dose per kilogram of animal per species per treatment course," or the product of the treatment length and the DDD_{Vet} for that drug (Equation 12) (Collineau et al., 2017; European Medicines Agency, 2015b, 2016a; Mills et al., 2018). The number of DCD_{Vet} ($nDCD_{Vet}$) adjusts the total weight of active ingredient by the DCD_{Vet} and ATW (Equation 13).

$$\boldsymbol{DCD_{Vetdrug}(mg/kg_{course}) = DDD_{Vetdrug}\ (mg/kg/day)\ x\ treatment\ length\ (days)} \quad (12)$$

$$\boldsymbol{nDCD_{Vetdrug} = \frac{total\ AMD\ used\ (mg)}{DCD_{Vetdrug}(mg/kg_{course})\ x\ ATW\ (kg)}} \quad (13)$$

The recommended treatment/course length for the DCD_{Vet} can vary substantially between regions and on an individual case-by-case basis, depending on the prescriber of the treatment or the diagnosis. This influences the comparability between different populations (Collineau et al., 2017). Antimicrobial use would be underestimated if a recommended course is shorter than the assigned average course length for a drug's respective DCD_{Vet}, and vice versa if recommended treatment length is prolonged (Collineau et al., 2017). Regions with proprietary treatment practices that differ substantially from those outlined by the ESVAC DCD_{Vet} metric definitions

should consider designating their own DCD_{Vet} metrics that incorporate a proper treatment course length.

Norwegian researchers sought to evaluate AMU in the Norwegian aquaculture industry by looking at the total weight of AMDs prescribed for indications in aquaculture then dividing that amount by the defined course dose (DCD) metric similar to the DCD_{Vet} (Grave et al., 2008). The weight of prescribed active substances was not considered a suitable indicator as dosages of AMDs may vary considerably depending on potency, pharmacokinetics, and formulations (Grave et al., 2008). These researchers opted to use DCD as their AMU metric of choice due to unique properties related to how salmonids, as poikilothermic animals, consume feed based on water temperature. The total course dose per biomass of salmonid could be estimated from the prescribed treatment regimen. In their study, one DCD (for a respective AMD) represented the amount of AMD recommended to treat 1 kg of salmonid under standard aquatic conditions (Grave et al., 2008). The $nDCD_{Vet}$ for salmonid aquaculture is subject to the same limitations as the nUDDA and terrestrial species regarding the need for granular data and a defined ATW. Typical salmonid aquaculture operations do not report the numbers of animals treated or the course length for that treatment within AMU reporting programs. The Norwegian DCD_{Vet} presents an interesting concept but is highly data-dependent and may not generally apply to current AMU surveillance reporting for salmonid aquaculture as these data are not available. Interestingly, the Norwegian study also stated the limitation of brood stock being excluded from their biomass estimations, similar to using total slaughter weight for the mg/PCU indicator.

Table 1.5. Cattle Defined Course Dose (DCD_{Vet}) value examples (European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), 2016).

Substance	**Route**	**DCD_{Vet} (mg/kg/course)**
Amoxicillin	Oral	81
Cefalexin	Parenteral	32
Colistin	Oral	24
Ceftiofur	Parenteral	4

1.13 **TREATMENT FREQUENCY AND TREATMENT INCIDENCE INDICATORS**

Treatment frequency (TF) and treatment incidence (TI) can be equated to two epidemiological measures, cumulative incidence (risk of treatment) and incidence density/intensity (rate of treatment), respectively (Werner et al., 2018). On its own, TF is calculated as the product of the number of animals treated, the number of days of treatment and the number of active substances, divided by the number of animals in the population (Lekagul et al., 2018) (Equation 14). The TF indicator does not directly consider doses, body weights, or amounts of AMD used (Kasabova et al., 2019). Treatment Frequency does not indicate the rate of treatment, but rather how many days on average an animal in a population is treated with an active substance, from which the risk of treatment for that animal can be extrapolated (Werner et al., 2018). Suppose the TF of one population is drastically greater than the other. In that case, auditors should be evaluating disease pressure or AMU procedures of both populations to validate why the risk of treatment in one population is greater than another similar population.

$$\textbf{\textit{Treatment Frequency (doses/animal)}} = \frac{\textbf{\textit{nUDDA}}}{\textbf{\textit{number of animals in the population}}} \qquad (\mathbf{14})$$

Researchers studying AMU in broiler operations across nine European countries calculated treatment incidence (TI) in three different ways, using DDD_{Vet}, DCD_{Vet}, and UDDA metrics. All three variations were calculated in similar fashions, dividing the total quantity of an active substance by the product of either one of the above metrics, the number of animal days at risk (ADR) (referred to as production cycle length by others) and the total population of animals at risk (represented by the total weight of animals at risk) all multiplied by 1000 animals at risk (Equation 15) (Joosten et al., 2019). According to Joosten et al. (2019) this can also be interpreted as the number of days per 1000-animal days that a flock was receiving AMDs, reflecting the percentage of time spent an animal is being treated with AMDs in its life.

$$\textbf{\textit{nDDD}}_{\textbf{\textit{Vet}}}\ \textbf{\textit{per}}\ \mathbf{1,000}\ \textbf{\textit{ADR}} = \frac{\textbf{\textit{nDDD}}_{\textbf{\textit{Vet}}}}{\textbf{\textit{total animals x ATW x days at risk}}}\ \textbf{\textit{x}}\ \mathbf{1,000} \qquad (\mathbf{15})$$

Treatment incidence as an indicator was also discussed in the context of AMU on poultry farms in Vietnam compared to other indicators such as mg of active ingredient/kg of exposed biomass (Cuong et al., 2019). Researchers showed that TI was poorly correlated with indicators

such as mg AMD/kg at sale or mg AMD/kg at treatment, alluding to the possibility that TI as a course-based indicator can reveal trends not made apparent by quantity-based indicators. Using the TI indicator, researchers demonstrated that Vietnamese poultry flocks used on average three times the average global amount of AMDs for their size. In contrast, no significant deviation from international levels was made apparent by the quantity-based indicators in the study (Cuong et al., 2019). They speculated that the discrepancies observed between quantity-based indicators and the TI indicator could be explained by differences in the strengths of AMDs, the timing of AMU and variable mortality in flocks (Cuong et al., 2019). Treatment incidence was considered a more balanced indicator regarding its propensity to over-underestimate AMU compared to quantity-based indicators due to its incorporation of the variability of AMD dose into its calculation. However, it was noted that if using a defined dose in the calculation, there is the possibility that the TI indicator could be subject to similar weaknesses inherent to standardized metrics listed above in the DDD_{Vet} section, i.e., a potentially different used dose on-farm vs. the defined dose.

1.14 **CONCLUSION**

The accurate and efficient quantification of AMU for use in surveillance programs for salmonid aquaculture is a challenging but important task. The growing incidence of AMR in the industrialized world poses a threat to One Health worldwide, affecting animals, humans, and the environment. To combat AMR development in salmonid aquaculture, prudent antimicrobial use and stewardship will be important in reducing selective pressures on microbes prone to developing resistance traits. To measure AMU and inform stewardship programs, robust AMU metrics and indicators that can quantify, compare, and integrate AMU and AMR data within and between populations will be useful tools for combatting AMR development. Currently, no single AMU or AMR indicator can meet all possible surveillance objectives or criteria with 100% effectiveness (Agunos et al., 2019; Collineau et al., 2017). Different metrics and indicators achieve different surveillance goals depending on data availability and accuracy.

Of all the AMU metrics and indicators outlined in this review, two stand out as relatively robust indicators for use in salmonid aquaculture. The quantity-based indicator mg/PCU is a popular indicator for regional terrestrial agriculture AMU quantification due to its relatively straightforward calculation and interpretation. However, the mg/PCU suffers from a lack of

comparability between regions with different production practice, which can result in markedly different ATW. The lack of defined ATWs for salmonids renders AMU analysis of salmonid aquaculture limited to aggregate biomass AMU adjustments. Additionally, the mg/PCU indicator fails to account for the varying average lifespans of food-producing animals. These different lifespans can affect an animals risk of exposure to AMDs throughout its life, thus negatively influencing how comparable an AMU indicator is when adjusted using a denominator consisting of pooled animal biomass made up of many different species is (Radke, 2017). Different salmonid lifespans can impact the probability of exposure to AMDs when monitored on an annual basis. Accounting for the length of life of an animal when calculating the total biomass of animals exposed would be useful in generating more relevant normalized AMU data. With this in mind, quantity-based indicators based upon the APCU proposed by Radke (2017) offers a compelling balance of simplicity and relative accuracy over the standard PCU. Existing PCU calculations for salmonids can be converted into APCU values using the length of life variables for each species of major salmonid to adjust for their varying lifespans, making the APCU a familiar but robust AMU indicator.

The second AMU indicator recommended in the long-term by this review is the quantity-based indicator number of DDD_{Vets} ($nDDD_{Vet}$). This metric returns the total number of defined daily doses used on salmonids (within a specific study period) and complements the mg/APCU metric by adjusting for standardized AMD dosages. Country-specific standard dosages, as well as defined salmonid ATW would make this indicator a more accurate choice for comparing AMU between countries using AMDs for different indications in salmonids. It would also allow for more accurate region-specific AMU comparisons when measuring AMU using non-aggregated AMU data. The $nDDD_{Vet}$ indicator is simple to interpret and can be transformed into various other indicators (TI, TF) should the data become available. However, this data is difficult to generate and requires the buy-in of many stakeholders and producers to reach agreed-upon values for meaningful AMU comparison. Together, the $nDDD_{Vet}$ and PCU/APCU could allow salmonid producers to monitor the AMU of salmonids, adjusting for total biomass, drugs used, and species produced. This review highlights the variety of AMU indicators currently in use worldwide and expands upon the work done by Werner et al. (2018).

CHAPTER 2

APPLICATION OF METRICS AND INDICATORS TO ANTIMICROBIAL USE DATA FROM TOP GLOBAL SALMONID AQUACULTURE PRODUCERS

2.1 ABSTRACT

Monitoring antimicrobial use (AMU) in salmonid aquaculture is critical for controlling the development of antimicrobial resistance (AMR) and making it possible to quantify the effectiveness of disease control strategies and antimicrobial stewardship programs. Regions must monitor AMU in a standardized and harmonized fashion to compare AMU between different populations using different antimicrobial drugs (AMD). Using AMDs meant for food animals can lead to the development of AMR in select pathogens that threaten animal health and human health. This research sought to evaluate the AMU of Canadas' largest producer of farmed salmon and highest consumer of antimicrobials in salmonid aquaculture by weight, British Columbia (BC), using existing and novel biomass adjusted AMU indicators. Adjusting AMU by biomass is an important component of evaluating AMU comparatively when production varies heavily between producers. The AMU indicators used to evaluate AMU were "Milligrams of Active Ingredient per Population Correction Unit" (mg/PCU) and "Milligrams of Active Ingredient per Adjusted Population Correction Unit" (mg/APCU). Quantity-based indicators relying on defined doses were not applied here due to a lack of agreed-upon defined doses for AMDs used in salmonid aquaculture, as well as a lack of defined Average Treatment Weights (ATW). We applied the mg/PCU indicator to other top global salmonid producers to compare international relative AMU in salmonid aquaculture with BC. We found that northern European salmonid producers, including the UK and Norway, recorded the lowest biomass adjusted AMU, while Chile recorded the highest. British Columbia recorded AMU in between these regions and has shown modest decreases in AMU throughout the study period. Future research could include evaluating the prevalence of AMR in BC aquaculture operations via AMR indicators and integrating that data with AMU data.

2.2 **INTRODUCTION**

Salmonid aquaculture is the production of salmonids (*see Chapter 1*) under controlled conditions for commercial and recreational purposes. The most commonly farmed salmonids among the four regions studied were Atlantic salmon, Pacific salmon, and Trout for the entire study period (Food & Agriculture Organization, 2020d). Antimicrobial use in salmonid aquaculture is an important disease management tool but also imparts selection pressure for AMR development (Schar et al., 2020). Compared with AMU in terrestrial food animal production, the application of AMDs in salmonid aquaculture provides a potentially broader environmental exposure pathway for drug distribution through the surrounding waters and even linked terrestrial environments (Heuer et al., 2009; Schar et al., 2020). However, AMU in salmonid aquaculture is necessary to combat disease outbreaks when other husbandry methods fail. Antimicrobial use in BC salmonid aquaculture differs from other forms of terrestrial AMU in that all treatments with antimicrobial drugs (AMDs) are for existing clinical diseases (there are no prophylactic treatments), and the vast majority of AMDs are administered to grow-out (marine phase) salmonids as pelleted feed (D. B. Morrison & S. Saksida, 2013). Since its inception, most of the global salmonid aquaculture industry has generally avoided the need for expensive medicated feeds by employing husbandry methods such as routine vaccination, biosecurity protocols, and water management strategies. Unfortunately, when alternate measures are not implemented effectively or disease pressure is too high – disease outbreaks can occur, and antimicrobials may be needed for treatment.

Antimicrobial use in salmonid aquaculture exposes surrounding water columns, the seabed, and other biota to AMDs, which can have adverse outcomes on the immediate environment, especially the selection of resistant bacteria (Armstrong et al., 2005; Du et al., 2019; Miranda et al., 2018; Miranda & Zemelman, 2002). There are two potential risks associated with AMU in aquaculture: direct transmission of resistant bacteria from aquaculture to humans through the food chain, and the development and dissemination of resistance genes in aquatic bacteria and subsequent transfer to broader aquatic and terrestrial environments (Park et al., 2012). The two most common treatment methods in salmonid aquaculture are medicated feed and bath treatments (immersion therapy). However, injection is sometimes used for high-value individuals such as brood stock (Lunestad & Samuelsen, 2008). The former methods are

considered flock treatments and result in broad environmental application, which has the potential to impact a wide variety of non-target bacteria, unlike terrestrial agriculture, where common administration methods create barriers to broader environmental exposure (Heuer et al., 2009).

Reports of resistance against several antimicrobial drugs have been recorded in the salmonid aquaculture industry across major producers (Monterey Bay Aquarium, 2017a, 2017b, 2017c, 2018). The potential human health impact of AMR in aquatic bacteria is not inconsequential as many of the bacteria causing disease in salmonids belong to genera of bacteria known to cause human infections (Heuer et al., 2009). According to Heuer et al. (2009), the most effective means of controlling AMR spread in salmonid aquaculture is to reduce AMU in this space. Surveillance frameworks that gather information on AMU using tools such as AMU indicators may be necessary to monitor and validate a decline in AMU related to stewardship activities (Brault, Hannon, Gow, Otto, et al., 2019). Stakeholders and the public continue to express interest in alternate therapies to AMDs to reduce overall AMU in salmonid aquaculture (Defoirdt et al., 2011). Currently, vaccination and husbandry are two highly effective ways to reduce AMU (Grave & Hansen, 2009). However, there are only a limited number of commercially available vaccines for major salmonid diseases due to difficulties designing, manufacturing, and administering these vaccines (Hossain & Shefat, 2018).

Salmonid aquaculture operations in BC face several health management challenges associated with bacterial and viral diseases of salmonids. Intensive culture systems requiring rearing salmonids in net pens are generally economically efficient due to the minimal infrastructure needed (Hossain & Shefat, 2018). However, the high biomass of salmonids produced within the restricted volumes of net pens represents an optimal environment for disease transfer between salmonids (Armstrong et al., 2005). Bacterial diseases are the most prevalent disease challenge in salmonid production, while viral diseases are generally more difficult to control, depending on geographic location (Hossain & Shefat, 2018). Currently, vaccinations exist for several common bacterial infections of cultured salmonids (excluding trout), including, but not limited to: furunculosis (*Aeromonas salmonicida*), vibriosis (*Vibrio spp.*), bacterial kidney disease (BKD) (*Renibacterium salmoninarum*), and enteric-red-mouth disease (*Yersinia ruckeri*) (Hossain & Shefat, 2018). Reductions in AMU have been successful in northern

European countries such as Norway, where vaccination is common, and bacterial disease pressure is relatively low (Monterey Bay Aquarium, 2018). Several of the main drivers behind AMU in salmonid aquaculture are species vulnerability, production practices and technology, as well as varying regional vulnerability (Henriksson et al., 2018).

Monitoring AMU using metrics and indicators gives regulators, industry representatives, the scientific community, and the public the ability to track absolute and relative AMU for benchmarking purposes and comparing AMU with other regions and potentially other users/commodities. Antimicrobial use indicators are derived from combining defined AMU metrics with reported quantities of AMDs used, sold, or prescribed (Bosman et al., 2019). Antimicrobial use metrics are defined as technical units of AMU measurement, such as frequency/total amount of use (Agunos et al., 2019). Indicators generally estimate values such as animal weights and drug dosages, which are used to generate a standardized estimated measure of AMU (European Medicines Agency, 2015b). Our primary AMU metric of interest is the Population Correction Unit (PCU), first conceptualized in 2009 by the European Medicines Agency (EMA) European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) group (European Medicines Agency, 2013). The PCU is a theoretical unit of biomass (kg) measurement of an animal potentially exposed to a certain weight of antimicrobials. The PCU for each species is calculated by multiplying the total estimated number of animals by a theoretical Average Treatment Weight (ATW). (European Medicines Agency, 2016b, 2021). The agreed-upon ATW for each livestock species estimates the weight of animals in a respective livestock category when they're at the age when they would most likely be treated with antimicrobials; or the average and final weights for slaughter and breeding animals, respectively (European Medicines Agency, 2021; Montforts, 1999; Montforts & Tarazona Lafarga, 2003).

Currently, international reporting of AMU for salmonid aquaculture does not use ATWs to calculate PCU for farmed finfish as is done for other terrestrial livestock using ESVAC methodology (European Medicines Agency, 2019; Narbonne et al., 2021; Norwegian Veterinary Institute, 2018). This is because ATWs for farmed salmonids have not been formally defined by the EMA or any other country (Table A.2.1) (European Medicines Agency, 2021; Government of Canada, 2020b; Radke, 2017). As most countries do not report the number of salmonids slaughtered or an ATW for salmonids, ESVAC uses the total annual live slaughter biomass of

farmed salmonids to estimate their aggregate PCU (European Medicines Agency, 2021). The AMU indicator milligrams/Population Correction Unit (mg/PCU) is a quantity-based indicator that utilizes the total weight of antimicrobials (mg) as well as the PCU metric as a denominator. The mg/PCU allows for comparing AMU between farms/countries with differing amounts of exposed animal biomass while controlling for animal demographics, specifically population size and species composition (European Medicines Agency, 2021; Radke, 2017).

Radke proposed the adjusted PCU (APCU) alternative to the PCU (Radke, 2017). The PCU does not correctly estimate the actual biomass at risk of treatment in a given period of time as it does not consider an animal's length of life (Brault, Hannon, Gow, Otto, et al., 2019; Radke, 2017). In addition to discrepancies in ATWs between regions, the risk of exposure to AMU between animals with vastly different lifespans should be accounted for when pooling animal biomass, making the mg/APCU an interesting option over the mg/PCU (Radke, 2017). The APCU accounts for the total weight of animals in a population and their length of life to calculate total exposed animal biomass, resulting in life-adjusted weights for animal categories (Radke, 2017). The consideration of an animal's average lifespan improves comparability between different species where length of life differs significantly, such as cattle, swine, and poultry. It does this by accounting for the increased possibility of exposure to AMD for animals as they live longer (Cuong et al., 2019).

Characterizing annual AMU and variations in absolute AMU (by weight) using a biomass-adjusted indicator such as the PCU or APCU is a yet untested method of reporting AMU in BC and other regional salmonid aquaculture operations. Norway has recently begun implementing the PCU in a limited fashion for its AMU quantification of its salmonid aquaculture industry (NORM/NORM-VET, 2018). While ESVAC defines a PCU for finfish, it is not production class, nor species-specific for the different species of salmonid produced among top producers. This is unlike certain terrestrial livestock species, where the same animal will have different PCU calculations depending on their production class (e.g. bulls vs. heifers) (Table A.2.1) (European Medicines Agency, 2021). Reports on surveillance and resistance in Canada currently outline AMU in cultured salmonids simply according to the absolute total quantities (kg) of AMDs used (Government of Canada, 2019b). Other major salmon-producing regions also report total absolute amounts of AMU in kilograms for their industries, allowing for

comparisons of AMU using the overall total weight of active ingredients. However, while it is apparent that AMU by total weight varies heavily between these countries and regions based on salmonid population size and numbers, there are to date no formal comparisons of AMU using recognized AMU metrics and indicators that account for salmonid population sizes or demographics. Accounting for population size is an important indicator for AMU stewardship when evaluating the prudent use of antimicrobials for every unit of biomass produced in a region.

The primary research question of this chapter is how does AMU between top salmonid producers compare when robust indicators are applied to each region? This chapter aims to 1) Calculate AMU in BC and other major global salmonid aquaculture producers using defined AMU indicators, 2) evaluate the strengths and limitations of these AMU indicators.

2.3 **MATERIALS AND METHODS**

2.3.1 **BRITISH COLUMBIA PRODUCTION AND ANTIMICROBIAL USE DATA**

Total annual slaughter mass for Atlantic, Pacific salmon and Rainbow/Steelhead trout were retrieved from publicly available documents from the BC Ministry of Agriculture via "Fast Stats" reports and "Seafood Industry Year in Review Reports," as well as Government of Canada aquaculture production values for BC (British Columbia Ministry of Agriculture, 2018; Government of British Columbia, 2018; Government of Canada, 2019a). Production data for BC included land-based and marine salmonid aquaculture. Steelhead trout (Rainbow trout grown in marine waters) production after 2013 was combined with the farmed Pacific salmon (Coho & Chinook) category as this is what was done in BC annual production reports. Steelhead trout produced before 2013 were attributed to the trout production category. These production categories were merged to avoid the risk of double-counting Steelhead within the Pacific salmon category in the event BC did this without reporting it. Various other farmed finfish species such as Sablefish (Black Cod), Arctic Char, Tilapia, Groundfish, etc., were excluded from analysis due to difficulties quantifying species-specific production levels for these species and the appropriate AMU attributable to these species. Production values for Atlantic and Pacific salmon were retrieved from reports generated by the Government of BC, whereas Rainbow/Steelhead trout production values were retrieved from Government of Canada data. Canadian data was

used to supplement BC data because BC reports did not treat trout production as a separate individual category. Land-based production (raceways, ponds etc.) of market-sized Atlantic salmon, Pacific salmon, and trout were included in this study. However, this production method is rarely used compared to marine production in BC.

The BC Ministry of Agriculture provided anonymized AMU data from BC feed mills that supply feed, including medicated feed, to BC salmonid aquaculture producers. These data included AMU by species and weight class during production. Antimicrobial use data meant for species other than Atlantic, Pacific salmon, and Steelhead/Rainbow trout were excluded from analysis. Due to the nature of the BC AMU data set, we were able to exclude all AMU meant for species other than Atlantic/Pacific salmon and trout in our analysis. The total annual amounts of AMDs, in kilograms and milligrams, for each prescription were calculated by multiplying the inclusion rate of active ingredients in the prescribed premix (g/kg) by the total amount of premix (kg) for that prescription. Annual amounts of overall AMU and drug-specific AMU were summed and aggregated by species and weight category (Atlantic Salmon, Pacific Salmon, trout). The AMDs analyzed (*see Chapter 1*) were: oxytetracycline (OTC), florfenicol (FLOR), Trimethoprim + Sulfadiazine (TMS), Sulfadimethoxine + Ormetoprim (SMOR), erythromycin (ERY), and lincomycin (LINC). These drugs were only considered for AMU analysis if prescribed for either Atlantic/Pacific salmon or trout.

2.3.2 **NORWAY PRODUCTION AND ANTIMICROBIAL USE DATA**

Annual production reports detailing total annual land-based and marine slaughter mass for Atlantic salmon and trout produced in Norway were retrieved for 2004-2018 (Directorate of Fisheries - Norway, 2020). We excluded slaughter biomass production data for minor species such as Halibut, Cod and Arctic Char from aggregate and species-specific biomass calculations to align with other regions. Data for AMD sales, and prescriptions were obtained from sales and prescription data from pharmacies, wholesalers, and the Veterinary Prescription Register (VETREG) (Norwegian Veterinary Institute, 2018). For 2004-2012, Norwegian AMU data represented AMU sales data from feed mills and wholesalers to salmonid farms, as reported by the Norwegian Institute of Public Health. For 2013-2018, data represented prescription data obtained from VETREG. Antimicrobial use data from Norway were not species-specific; thus, AMU meant for species other than Atlantic salmon and trout could not be excluded from our

analysis. We predicted that the impact of excluding minor species from Norwegian overall annual slaughter biomass calculations would have a minimal effect on biomass-adjusted AMU calculations. The proportion of Norwegian salmonid production attributable to species other than Atlantic salmon and trout varied between 0.21% in 2018 and 2.31% in 2010 (average 0.79% of production between 2004-2018). The exposed slaughter biomass of Atlantic salmon and trout production was combined for overall biomass-adjusted AMU calculations. Antimicrobial drugs (*see Chapter 1*) used in Norwegian aquaculture included OTC, FLOR, OA, FLU, and spectinomycin + lincomycin.

2.3.3 **CHILE PRODUCTION AND ANTIMICROBIAL USE DATA**

Chilean salmonid aquaculture AMU and production data detailing total annual land-based and marine slaughter mass for Atlantic salmon, Pacific Salmon and Trout were retrieved from government reports for 2005-2018, and 2004-2018 respectively (Government of Chile, 2018; Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018). Miscellaneous species of farmed salmonid were not included in total slaughter biomass AMU calculations to align with other regions. Chilean salmonid aquaculture AMU data were unavailable for 2004. Salmonid production records for 2006 detailing total slaughter mass produced were only reported up to November in that year's annual report. We were able to exclude AMU attributed to miscellaneous salmonid species as Chilean AMU reports only outlined AMU in Atlantic/Pacific Salmon and Trout. Chilean AMU data were drug-specific and species-specific based on estimated percentages of AMU instead of exact values. Inconsistencies in reported AMU and AMU totals in this report were due to percentage rounding in Chilean annual AMU reports. Antimicrobial drugs (*see Chapter 1*) in use in Chilean aquaculture included OTC, FLOR, FLU, amoxicillin (AMOX), OA, and ERY. Chilean AMU data represented actual use data as reported by producers to the Sistema de Fiscalización de la Acuicultura (SIFA) (Government of Chile, 2018).

2.3.4 **UNITED KINGDOM PRODUCTION AND ANTIMICROBIAL USE DATA**

Annual total land-based and marine salmonid slaughter mass production totals for Atlantic salmon and trout for the United Kingdom were sourced from Eurostat, an international database covering many countries in the European Union (Eurostat, 2021). Production data detailing the aggerate slaughter biomass of miscellaneous species such as Halibut, Cod, Char

etc., was excluded from biomass-adjusted AMU calculations to align with other regions. Annual aggregated salmonid aquaculture AMU data from the United Kingdom's Veterinary Antibiotic Resistance Sales and Surveillance reports (UK-VARSS) was gathered for 2004-2018 (Veterinary Medicines Directorate, 2018). These AMU data contained sales data for UK Atlantic salmon and trout farms as reported by the UK. We were not concerned with the UK AMU data underestimating exposed biomass as UK-VARSS data only covered aggregated Atlantic salmon and trout AMU. The AMU data from the UK-VARSS reports were not drug-specific nor species-specific for salmonid aquaculture. The UK AMU data did not contain exact values of AMU, instead rounding total AMU to the nearest hundred or thousand kilograms.

2.3.5 **ANTIMICROBIAL USE METRICS AND INDICATORS**

For the biomass-adjusted analysis of AMU of the top producing salmonid regions, we only considered the production of Atlantic/Pacific salmon and trout when generating regional annual PCU values. Atlantic/Pacific salmon and trout made up most of the production for each region. Also, defining AMU attributable to these species was deemed more accurate than attempting to attribute relevant AMU data for all salmonids produced. We defined the PCU metric for commonly farmed salmonids using two derivations. The $PCU_{slaughter}$ was defined as the total annual biomass (kg) of salmonids (Atlantic/Pacific salmon, trout) slaughtered for each region (Equation 1), as was done by ESVAC in defining the finfish PCU (European Medicines Agency, 2021). The $PCU_{Average\ Weight\ (AW)}$ was defined as the total annual salmonid slaughter biomass divided by two (Equation 2). The PCU_{AW} was used because, in PCU calculations, certain terrestrial livestock species raised for slaughter have their theoretical weight at treatment defined as the mean weight over the animal's lifetime. We sought to utilize a salmonid PCU definition that reflected similar methodologies used for terrestrial animals for salmonid aquaculture (European Medicines Agency, 2021; Montforts, 1999; Montforts et al., 1999; Montforts & Tarazona Lafarga, 2003). This method provides the equivalent result to using the formal derivation of PCU for terrestrial animals that multiplies the number of animals in the population by the ATW where the latter is the mean of the starting (approximately zero for salmonids) and slaughter weight for animals in the population as previously defined (European Medicines Agency, 2021; Montforts, 1999; Montforts et al., 1999; Montforts & Tarazona Lafarga, 2003).

By dividing the total annual slaughter weight of salmonids by two, the salmonid PCU_{AW} metric approximated a metric derived by multiplying a given number of salmonids by an average weight over production. Doing this rendered the PCU_{AW} more comparable to the PCU of terrestrial livestock where their pre-defined ATWs are used, rather than their final slaughter weights, as is done for finfish (Montforts et al., 1999; Montforts & Tarazona Lafarga, 2003; Radke, 2018). This relationship is highlighted by following finfish and terrestrial livestock PCU methodologies set out by ESVAC and the original description of determining the ATW (European Medicines Agency, 2021; Montforts et al., 1999). The $PCU_{Slaughter/AW}$ metrics were used in combination with the total amount of active ingredient (mg) used in salmonid aquaculture attributable to Atlantic/Pacific salmon and trout for the global top four salmonid producers to calculate biomass-adjusted AMU using the indicator $mg/PCU_{Slaughter/AW}$) (Equation 3, 4). Drug-specific AMU data for Norway, Chile, and BC were calculated using these indicators but were not available for the UK.

We also analyzed BC salmonid aquaculture AMU data using the APCU metric and milligrams of active ingredient per APCU (mg/APCU) indicator. The APCU was defined using the $PCU_{Slaughter}$, PCU_{AW} and a length of life conversion factor to generate the $APCU_{AW}$ and $APCU_{Slaughter}$ metrics (Equation 5, 6). The APCU is derived using a species-specific length of life conversion factor (*see Chapter 1*) to account for each species' production cycle length (Equation 7). The conversion factors applied to BC annual total salmonid slaughter data were 1.75 for Atlantic Salmon, 1.5 for Pacific salmon, and 1.58 for other minor species, representing an average grow-out cycle length of 21, 18, and 19 months for each species category, respectively (Food & Agriculture Organization, 2020a, 2020b, 2020c; Seafish, 2012, 2015). The APCU accounts for the total weight (or average weight throughout production) of animals in a population and their length of life to calculate total exposed animal biomass, resulting in life-adjusted weights for animal categories (Radke, 2017). The consideration of an animal's average lifespan improves comparability between different species where length of life differs greatly, such as cattle, swine, and poultry. It also accounts for the increased possibility of exposure to AMDs for animals as they live longer (Cuong et al., 2019). This is important to consider when animal populations slaughter mass is be calculated in a different year (i.e., the population has a production cycle > 1 year) than when the AMU attributable to that population is measured. A unit of biomass (kg) measured using the APCU is equivalent to one life-adjusted kilogram of

animal biomass potentially exposed to AMU. The total milligrams of antimicrobial per $APCU_{Slaughter/AW}$ indicators were applied to BC for overall and drug-specific AMU data (Equation 8, 9) (European Medicines Agency, 2021; Radke, 2017).

The APCU indicator was not applied to other top-producing regions reviewed, as determining region-specific length of life conversion factors for different salmonids in different salmon production systems and regions was beyond the scope of this project. Like the ATW, salmonid length of life can vary between regions for reasons such as market demands, environmental factors, and other husbandry factors. The hatchery phase and subsequent fresh-water phase of maturation until the smolt production stage for all salmonid producers was not included in the length of the grow-out phase of production for this analysis. This was done as the size and disease risk for salmonids at this production stage is insignificant compared to the marine phase of production (Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health, 2002; D. B. Morrison & S. Saksida, 2013).

$$\boldsymbol{PCU_{Slaughter} = Total\ Annual\ Salmonid\ Slaughter\ Biomass\ (kg)} \quad (1)$$

$$\boldsymbol{PCU_{AW} = Total\ Annual\ Slaughter\ Biomass\ (kg) \div 2} \quad (2)$$

$$\boldsymbol{mg/PCU_{Slaughter}}\textbf{(mg/kg adjusted biomass)} = \frac{\boldsymbol{total\ AMD\ used\ (mg)}}{\boldsymbol{PCU_{Slaughter}}} \quad (3)$$

$$\boldsymbol{mg/PCU_{AW}}\textbf{(mg/kg adjusted biomass)} = \frac{\boldsymbol{total\ AMD\ used\ (mg)}}{\boldsymbol{PCU_{AW}}} \quad (4)$$

$$\boldsymbol{APCU_{AW} = PCU_{AW} \times Lenght\ of\ Life\ Conversion\ Factor} \quad (5)$$

$$\boldsymbol{APCU_{Slaughter} = PCU_{Slaughter} \times Lenght\ of\ Life\ Conversion\ Factor} \quad (6)$$

$$\boldsymbol{Length\ of\ Life\ Conversion\ Factor} = \frac{\mathbf{1}}{\boldsymbol{(12/Production\ Cycle\ Length\ (months))}} \quad (7)$$

$$\boldsymbol{mg/APCU_{AW}}\textbf{(mg/kg adjusted biomass)} = \frac{\boldsymbol{total\ AMD\ used\ (mg)}}{\boldsymbol{APCU_{AW}}} \quad (8)$$

$$\boldsymbol{mg/APCU_{Slaughter}}\textbf{(mg/kg adjusted biomass)} = \frac{\boldsymbol{total\ AMD\ used\ (mg)}}{\boldsymbol{APCU_{Slaughter}}} \quad (9)$$

2.3.6 **STATISTICAL ANALYSIS**

Production and AMU datasets were summarized for each region: British Columbia, Norway, Chile and the United Kingdom using Excel® 2016 (Microsoft Corporation, 2020). Data were analyzed and summarized using Excel® 2016 (Microsoft Corporation, Redmond, WA) and STATA 17/BE (StataCorp LLC, College Station, TX).

2.4 **RESULTS**

Salmonid production in British Columbia increased throughout the study period, with production at its lowest in 2004 and highest in 2015 (Table 2.1). Most BC salmonid production was attributable to Atlantic salmon (96% in 2018), though some Pacific salmon production still occurred (3% in 2018) (Table A.2.2). Unadjusted AMU (mg) in BC salmonid aquaculture decreased throughout the study period, with the bulk of that reduction coming from the reduced use of OTC, TMS, and SMOR (Figure 2.3, Figure A.2.7, Table A.2.3). Unadjusted AMU in BC peaked in 2004 and was at its lowest in 2011 (Table A.2.3). Most of the prescriptions issued in BC salmonid aquaculture were prescribed for Atlantic salmon (1,377 total), increasing steadily throughout the study period, followed distantly by Pacific salmon (126 total) and trout (3 total) (Figure A.2.6). Pacific salmon received the greatest proportion of AMU by weight until 2006, after which most of the AMU by weight and frequency (# of prescriptions) was attributable to Atlantic salmon (Figures A.2.3, A.2.6). Within each species, the highest frequency (# of prescriptions) of AMU by production weight class was attributed to Atlantic salmon weighing 0-200 grams with 775 prescriptions overall (56%), followed by Atlantic salmon weighing 201-2,000 grams with 504 prescriptions overall (36%) (Figure A.2.1). Conversely, Pacific salmon weighing 201-2,000 grams received the bulk of prescriptions with 77 (61%), followed by Pacific salmon weighing >2,000 grams (21%) (Figure A.2.1). The majority of AMU (kg) within species belonged to Atlantic salmon weighing 201-2000g, receiving 50% of the overall AMU by weight throughout the study period (Figure A.2.2). For Pacific salmon, the most AMU by weight was attributed to salmon weighing 201-2000g, with 61% of overall AMU throughout the study period (Figure A.2.2).

When adjusting AMU by biomass using the $mg/PCU_{Slaughter}$ indicator for individual species, Pacific salmon were treated with between 68% in 2005 (466.09 vs. 150.90) to 93% in

2009 (597.21 vs 39.39) more AMU (mg/$PCU_{Slaughter}$) than Atlantic salmon (Figure A.2.4) This did not include the three years where there was no recorded AMU in Pacific salmon (2010, 2015, 2017), nor the years where Atlantic salmon saw drastically higher mg/PCUslaughter than Pacific salmon (2016, 2018). Trout in BC only received AMU in 2017; however, the biomass-adjusted AMU for this year for trout was 17,334.80 mg/$PCU_{Slaughter}$, but this was based on a very small number of prescriptions and relatively small biomass compared to the other two species groups. When we applied the same analysis of species-specific biomass-adjusted AMU using the mg/$APCU_{Slaughter}$ indicator, the annual relative differences in AMU between each species (Atlantic, Pacific salmon) increased, ranging from 72% in 2005 to 94% in 2009 (Figure A.2.5). This also did not include the three years where there was no recorded AMU in Pacific salmon (2010, 2015, 2017), nor the years where Atlantic salmon saw drastically more biomass-adjusted AMU than Pacific salmon (2016, 2018).

Table 2.1 shows the annualized production values for salmonids in Norwegian aquaculture. Salmonid aquaculture production in Norway increased the most of all salmonid producers throughout the study period in terms of absolute tonnage, with production peaking in 2015 (Figure 2.1). Most salmonid production in Norway is attributable to Atlantic salmon (95% production in 2018), with trout production remaining consistent throughout the study period (Table A.2.4). Overall Norwegian unadjusted AMU appeared to decrease throughout the study period, with spikes in use matching closely with years of increased OA use (kg), which peaked in 2012 (Table 2.2). Unlike BC and Chile, OTC use in Norwegian salmonid aquaculture was minimal throughout the study period. Still, FLOR has recently seen an increase in use in Norway in line with BC and Chile, with a sharp increase in use in 2018 (Figure A.2.9). Norway used the greatest proportion of OA compared to any other antimicrobial of any other producer in this study, with 2004 (89%) and 2012 (88%) being the years with the greatest OA use relative to other AMDs. There were no AMU data on production class allocation of antimicrobials for Norway during the study period, though some sources specify that most of the AMU used in Norwegian salmonid aquaculture is meant for smaller production weight classes of salmonids (Grave & Hansen, 2009). Biomass-adjusted AMU did not exceed 4 mg/$PCU_{Slaughter/AW}$ throughout the study period (Figure A.2.10).

Salmonid aquaculture production in Chile appeared to increase throughout the study period, with production at its minimum in 2010 and peaking in 2014 (Figure 2.1). The bulk of salmonid output in Chile for 2004-2018 was attributed to Atlantic salmon production (72% in 2018), followed by a large portion of Pacific salmon (19% in 2018) and Rainbow trout production (9% in 2018), highlighting the differences in species production breakdown between the regions under study (Table A.2.5). 2010 was the only year where Pacific salmon production eclipsed Atlantic salmon production. Chilean biomass-adjusted AMU increased and decreased in a cyclical pattern over the study period with two peaks, one in 2007 and 2014 (Figures 2.2, A.2.11, A.2.12). The first peak was due to a mixture of several AMDs in use at the time, with moderate FLOR use (37%). The second peak in 2014 was almost entirely attributable to increased FLOR use (63%) and moderate OTC use (35%) (Figure A.2.11). Florfenicol was the primary AMD used in Chile after 2007, overtaking other antimicrobials such as ERY, FLU and others (Figure A.2.11). Since 2009 the two most dominant AMDs were by far FLOR and OTC, accounting for on average 91% and 8% of overall use, respectively. The use of quinolones and macrolides in Chilean salmonid aquaculture declined to <2% in 2008-2009 and never recovered. Figure A.2.13 shows Chilean species-specific AMU for 2005-2018. Antimicrobial use in Atlantic salmon increased until 2007 before declining until 2010, followed by a sharp increase until 2015. Pacific Salmon and trout AMU appears to have remained level or decline slightly throughout the study period. Chilean species-specific biomass-adjusted AMU (mg/$PCU_{Slaughter}$) showed Atlantic salmon received on average 39% more AMU (mg/$PCU_{Slaughter}$) than Pacific salmon throughout the study period. For 2005-2006, Pacific salmon had the higher biomass-adjusted AMU, though Atlantic salmon received the most biomass-adjusted AMU after 2006, peaking in 2018 at 74%.

Salmonid aquaculture production in the UK appeared to moderately increase throughout the study period, with the lowest production level occurring in 2005 and peak production occurring in 2017 (Figure 2.1). The bulk of production in the UK is attributable to Atlantic salmon, which on average made up 92% of UK salmonid production throughout the study period (Table A.2.6). Unadjusted salmonid aquaculture AMU in the UK appeared to decrease over the study period, peaking at 4000 kg in 2006 and 2007 and dropping to 700 kg in 2015 (Table 2.2). Drug, species, and production class-specific AMU data were not available from the UK. Overall

biomass-adjusted AMU for UK salmonid aquaculture peaked in 2006 and 2007 and was lowest in 2015.

Tables 2.3 and 2.4 present the biomass-adjusted AMU (mg/$PCU_{Slaughter/AW}$) values for Norway, Chile, the UK, and BC. The producer with the highest annual mg/$PCU_{Slaughter/AW}$ appeared to be Chile, followed by BC. Total AMU evaluation using mg/$PCU_{Slaughter}$ and mg/PCU_{AW} AMU indicators showed Norway to be the lowest consumer of AMDs throughout the study period (Figure 2.2). Norwegian drug-specific biomass-adjusted AMU indicated that the use of all AMDs except FLOR appeared to decrease throughout the study period, with OA showing the greatest reduction in use throughout the study period (Figure A.2.9). Total AMU evaluation using mg/$PCU_{Slaughter}$ and mg/PCU_{AW} AMU indicators found the UK to be the second-lowest consumer of AMDs (Table 2.3, 2.4). Tables A.2.6, A.2.7 shows BCs annual salmonid aquaculture drug specific mg/$PCU_{Slaughter}$, mg/PCU_{AW}, mg/$APCU_{Slaughter}$, and mg/$APCU_{AW}$ values. All indicators appeared to show decreases in biomass-adjusted AMU throughout the study period, with a spike in biomass-adjusted use in 2015. Figures 2.3, 2.4 shows drug-specific breakdowns of the annual mg/$PCU_{Slaughter}$ and mg/PCU_{AW}, mg/$APCU_{Slaughter}$ and mg/$APCU_{AW}$ estimates for BC, which showed possible decreasing trends in biomass-adjusted AMU for all drugs except FLOR.

An expanded array of data tables and figures are included in the Appendix for a complete breakdown of AMU and production by salmonid species, drug, and prescription patterns in BC and Chilean salmonid aquaculture for 2004-2018. Norway and the UK lacked the requisite data for AMU breakdowns by species, with the latter region also lacking the requisite data for drug-specific AMU breakdowns. Adjusted and unadjusted drug-specific AMU for Chile and Norway are included in the appendix.

Table 2.1. Annual salmonid aquaculture production levels for top global salmonid producers (kg). BC - British Columbia, UK - United Kingdom. Includes data for Atlantic Salmon, Pacific Salmon, and trout.

Year	BC	Norway	Chile	UK
2004	61,915,000	627,128,479	575,169,640	173,384,000
2005	70,571,000	645,080,335	601,876,910	142,281,000
2006	78,274,000	692,346,398	557,500,000	144,954,000
2007	79,405,000	821,798,636	602,769,590	145,232,000
2008	82,035,000	822,820,244	630,647,000	141,833,500
2009	76,823,000	936,609,293	474,176,000	159,592,000
2010	79,300,000	994,211,278	466,857,000	168,226,600
2011	83,958,000	1,123,422,309	649,492,000	170,461,000
2012	78,993,000	1,306,772,714	826,949,000	177,139,000
2013	62,462,000	1,239,875,885	786,091,000	175,984,000
2014	67,744,000	1,327,342,047	955,181,000	192,103,820
2015	93,000,000	1,376,353,162	883,102,000	186,984,870
2016	93,936,000	1,321,470,715	727,812,000	176,985,580
2017	85,733,000	1,303,352,095	855,326,000	202,748,358
2018	88,290,000	1,350,348,012	923,901,000	167,884,170

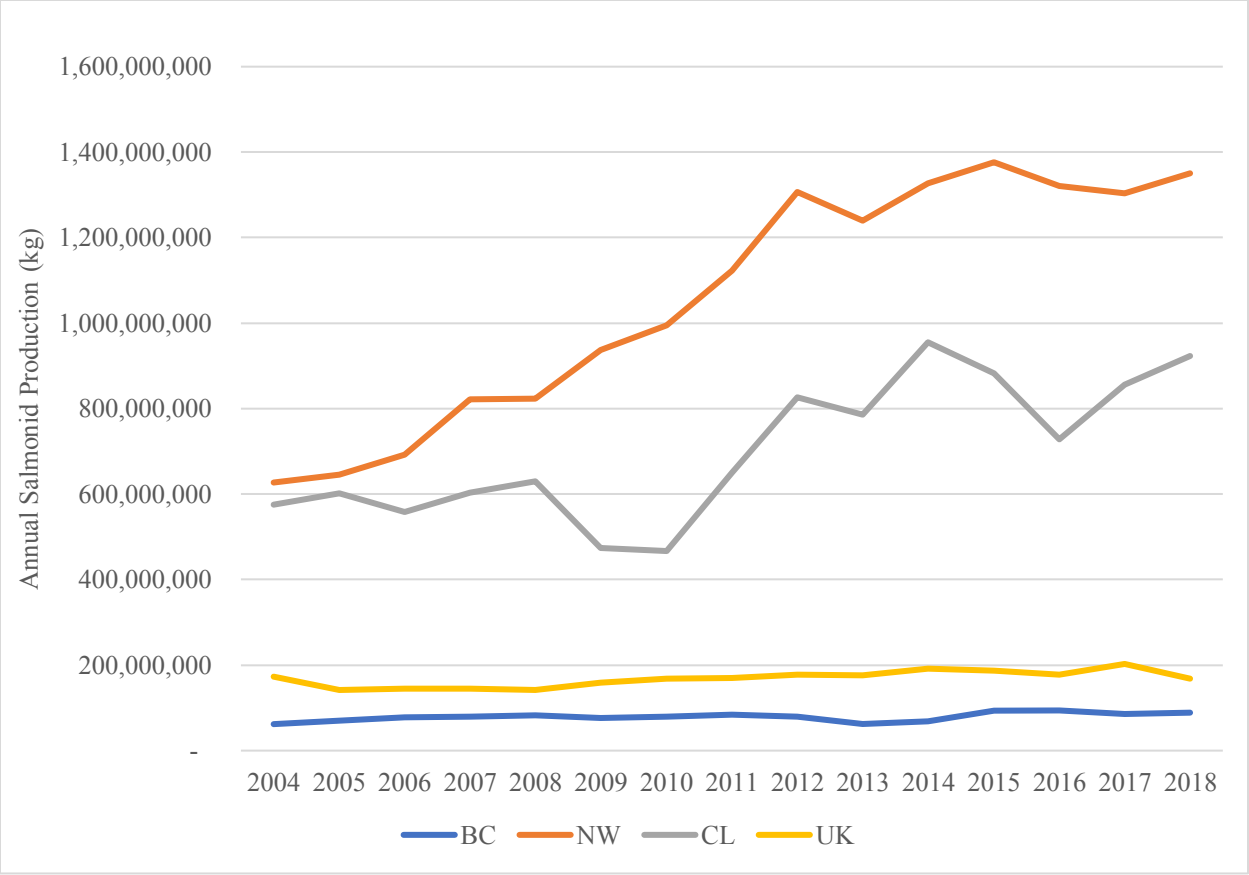

Figure 2.1. Annual salmonid aquaculture production levels for top global salmonid producers (kg). BC - British Columbia, NW - Norway, CL - Chile, UK - United Kingdom. Includes data for Atlantic Salmon, Pacific Salmon, and trout.

Table 2.2. Overall annual unadjusted salmonid aquaculture antimicrobial use (AMU) levels for top global salmonid producers (kg). BC – British Columbia, UK – United Kingdom.

Year	BC	Norway	Chile	UK
2004	20,368.87	1,159.00	-	4,000.00
2005	15,855.20	1,215.00	239,146.08	3,000.00
2006	8,079.12	1,478.00	343,810.00	4,000.00
2007	8,471.30	648.00	385,668.56	4,000.00
2008	5,490.10	941.00	325,620.00	1,000.00
2009	5,013.46	1,313.00	184,488.45	3,000.00
2010	5,664.06	650.00	144,059.20	1,000.00
2011	3,426.07	549.00	207,420.40	2,100.00
2012	4,976.84	1,591.00	337,900.00	2,100.00
2013	4,038.48	835.00	450,700.00	800.00
2014	5,547.94	523.00	563,200.00	2,400.00
2015	14,732.40	273.05	557,200.00	700.00
2016	5,094.43	201.05	382,117.50	1,600.00
2017	5,481.07	612.05	393,506.10	3,400.00
2018	11,765.06	932.00	322,700.00	1,200.00

Table 2.3. Overall annual biomass-adjusted salmonid aquaculture antimicrobial use (AMU) levels for top global salmonid producers (mg/$PCU_{Slaughter}$). BC – British Columbia, UK – United Kingdom.

Year	BC	Norway	Chile	UK
2004	328.98	1.85	-	23.07
2005	224.67	1.88	397.33	21.09
2006	103.22	2.13	616.70	27.59
2007	106.68	0.79	639.83	27.54
2008	66.92	1.14	516.33	7.05
2009	65.26	1.40	389.07	18.80
2010	71.43	0.65	308.57	5.94
2011	40.81	0.49	319.36	12.32
2012	63.00	1.22	408.61	11.86
2013	64.65	0.67	573.34	4.55
2014	81.90	0.39	589.63	12.49
2015	158.41	0.20	630.96	3.74
2016	54.23	0.15	525.02	9.04
2017	63.93	0.47	460.07	16.77
2018	133.25	0.69	349.28	7.15

Table 2.4. Overall annual biomass-adjusted salmonid aquaculture antimicrobial use (AMU) levels for top global salmonid producers (mg/PCU$_{AW}$). BC – British Columbia, UK – United Kingdom.

Year	BC	Norway	Chile	UK
2004	657.96	3.70	-	46.14
2005	449.34	3.77	794.67	42.17
2006	206.43	4.27	1,233.40	55.19
2007	213.37	1.58	1,279.66	55.08
2008	133.85	2.29	1,032.65	14.10
2009	130.52	2.80	778.14	37.60
2010	142.85	1.31	617.14	11.89
2011	81.61	0.98	638.72	24.64
2012	126.01	2.44	817.22	23.71
2013	129.31	1.35	1,146.69	9.09
2014	163.79	0.79	1,179.25	24.99
2015	316.83	0.40	1,261.92	7.49
2016	108.47	0.30	1,050.04	18.08
2017	127.86	0.94	920.13	33.54
2018	266.51	1.38	698.56	14.30

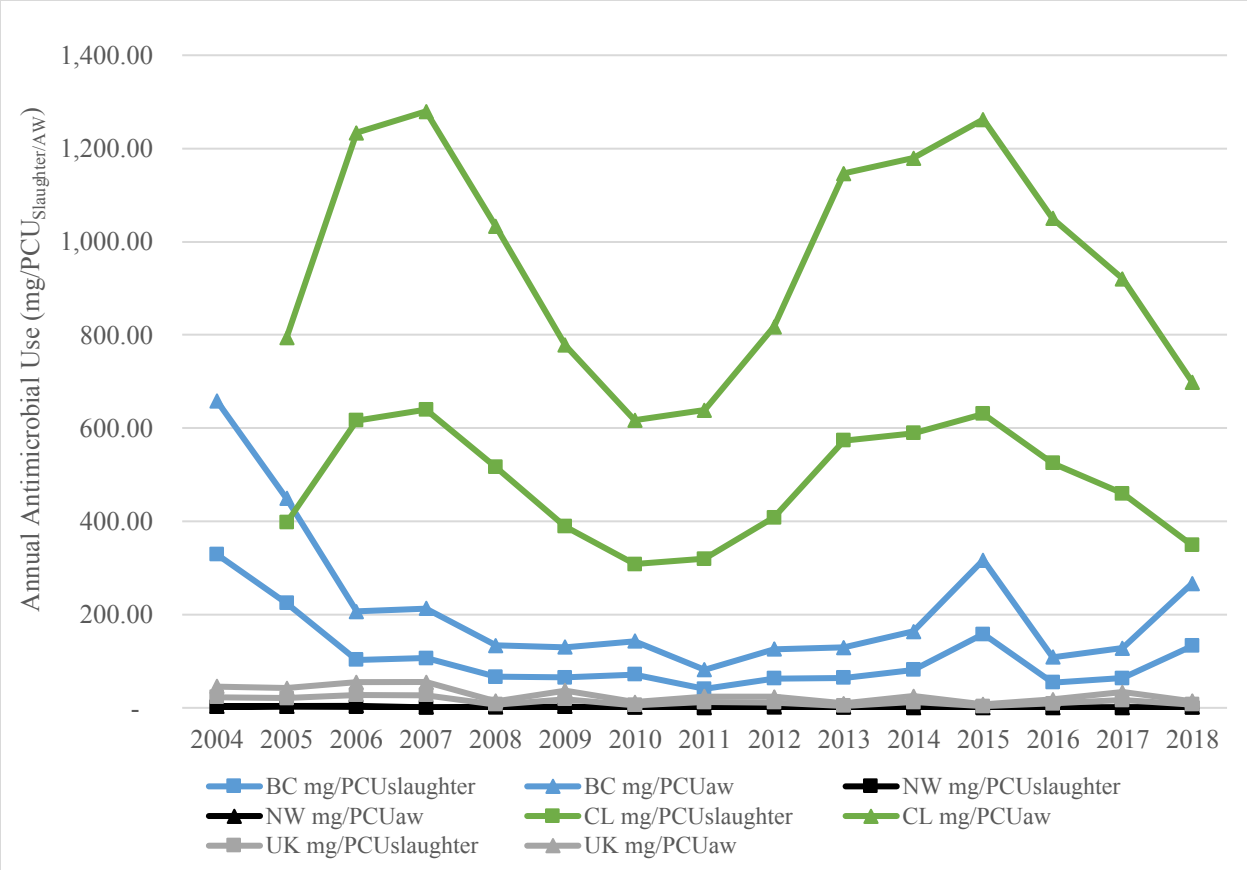

Figure 2.2. Overall annual adjusted salmonid aquaculture antimicrobial use (AMU) levels for top global salmonid producers (mg/PCU$_{Slaughter, AW}$). PCU – Population Correction Unit, AW – Average weight, BC – British Columbia. NW – Norway, CL – Chile, UK – United Kingdom.

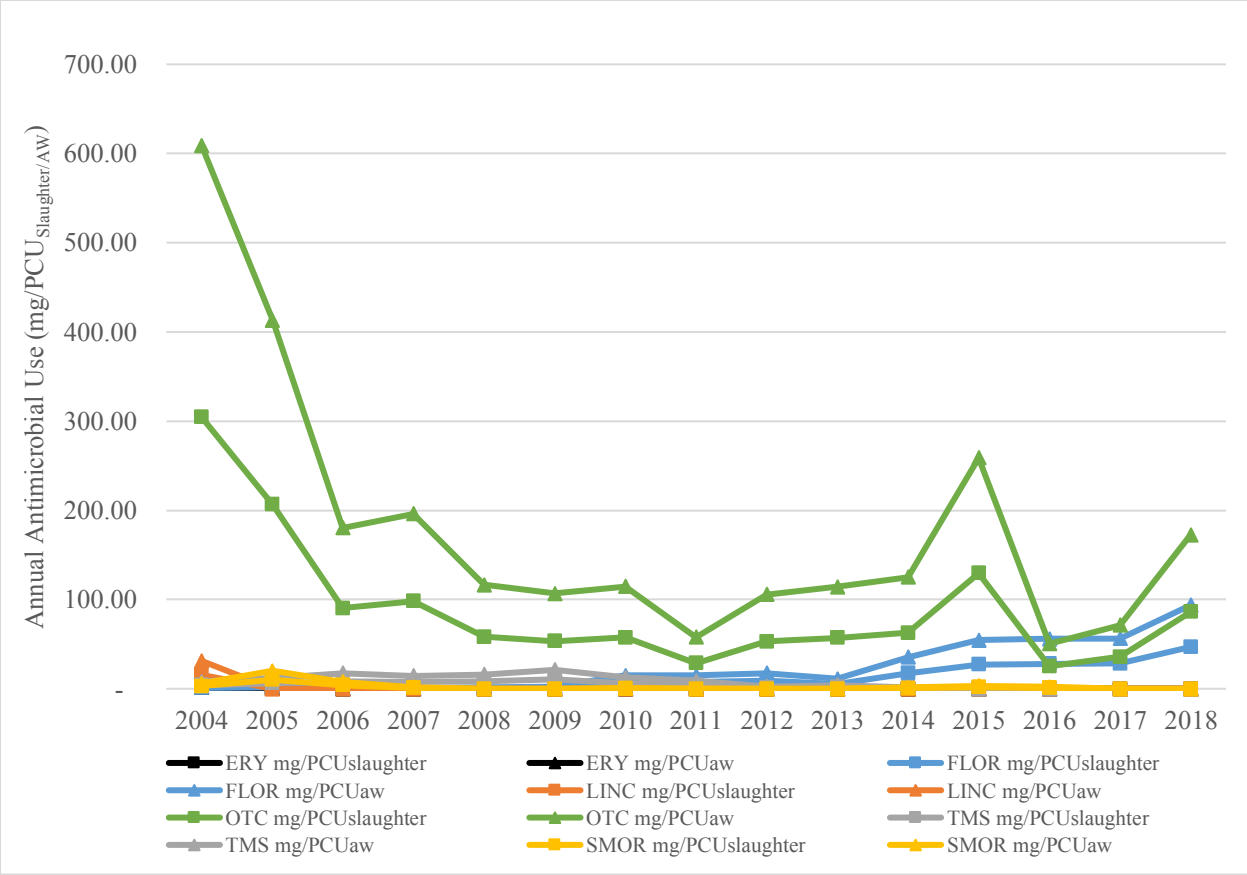

Figure 2.3. Drug-Specific annual biomass-adjusted salmonid aquaculture antimicrobial use (AMU) levels for British Columbia (mg/PCU$_{Slaughter, AW}$). PCU – Adjusted Population Correction Unit, AW – Average weight, FLOR – Florfenicol, OTC – Oxytetracycline, ERY – Erythromycin, LINC – Lincomycin, TMS – Trimethoprim + Sulfadiazine, SMOR – Sulfadimethoxine + Ormetoprim.

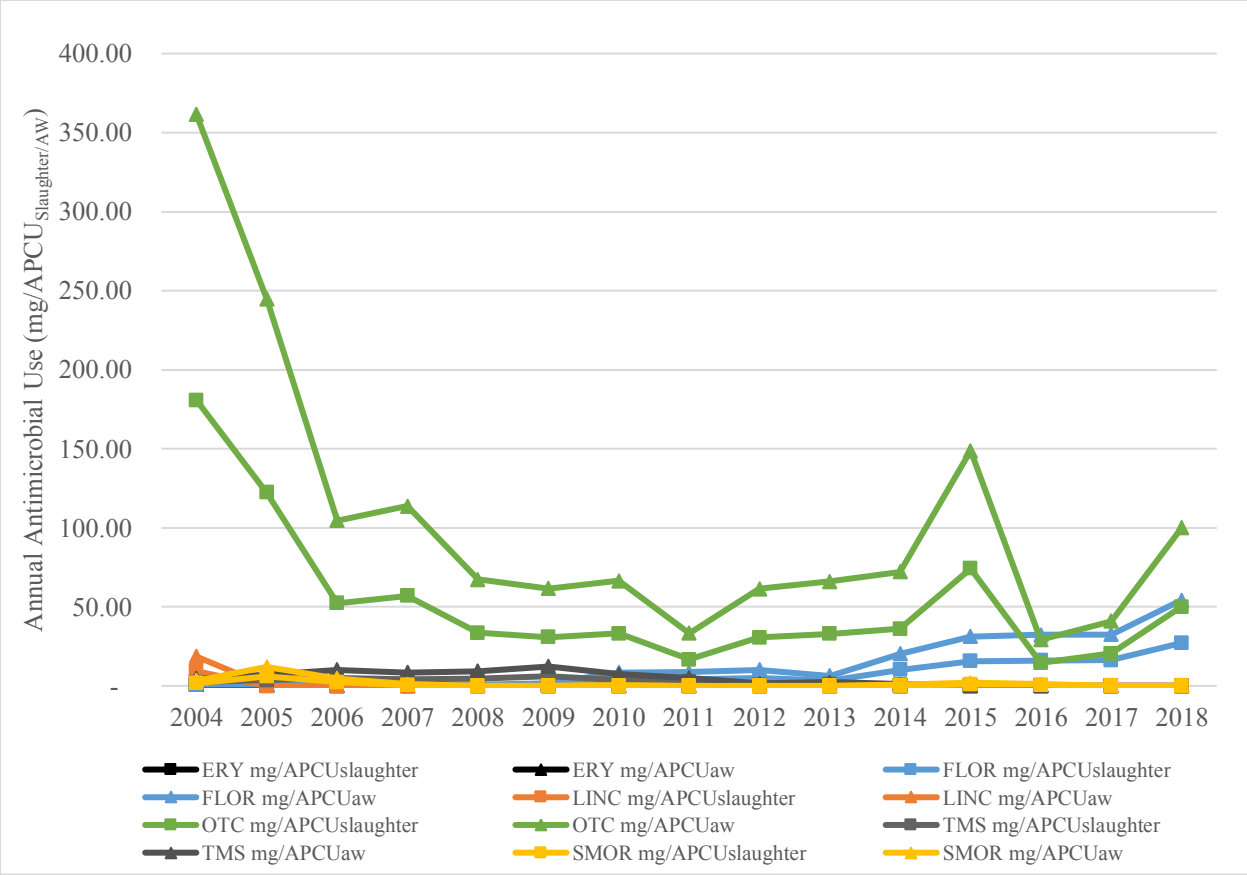

Figure 2.4. Drug-Specific annual biomass-adjusted salmonid aquaculture antimicrobial use (AMU) levels for British Columbia (mg/APCU$_{Slaughter, AW}$). APCU – Adjusted Population Correction Unit, AW – Average Weight, FLOR – Florfenicol, OTC – Oxytetracycline, ERY – Erythromycin, LINC – Lincomycin, TMS – Trimethoprim + Sulfadiazine, SMOR – Sulfadimethoxine + Ormetoprim.

2.5 **DISCUSSION**

We compared the annual AMU in salmonid aquaculture in BC to other major salmonid-producing regions (Chile, Norway, and the UK) for 2004-2018 using different AMU metrics and indicators. We found that the AMU indicators mg/$PCU_{Slaughter/AW}$ could adjust the annual AMU of a region over the total and average annual biomass of salmonids (Atlantic/Pacific salmon, trout) slaughtered. To our knowledge, this was the first study that leveraged the PCU metric as defined by ESVAC and modified it to mimic terrestrial animal PCU guidelines for use in salmonid aquaculture AMU measurements. These indicators alone presented a similar story concerning absolute AMU (total weight of active ingredients) in the evaluated regions with these data. Chile was the highest relative and absolute user of antimicrobials throughout the entire study period, and Norway was the lowest user. Interestingly, these weight-based AMU indicators drastically reinforce the discrepancy in use when adjusting for annual biomass produced. Norway produced on average 32% more salmonid biomass throughout the study period than Chile but used up to 510x less AMU per unit of biomass than Chile. Standardizing AMU using biomass-adjusted indicators is essential when analyzing AMU to demonstrate the differences in use relative to population size. Total AMU is not necessarily a good measure of antimicrobial stewardship, rather how much each unit of an antimicrobial drug is used for a given population is (Scott Weese et al., 2013).

Reporting and comparing AMU for global salmonid aquaculture producers using the biomass-adjusted AMU indicators mg/$PCU_{Slaughter}$ and mg/PCU_{AW} has not been done before. Standardizing salmonid aquaculture AMU by total annual production of primary salmonid species facilitated meaningful comparison of AMU between the major global salmonid producers with varying population demographics. Global salmonid production is currently dominated by Norway and Chile, with Scotland and BC also being major producers (Asche et al., 2013; Lees et al., 2008). Comparing AMU between top salmonid producers using biomass-adjusted indicators relies on excellent public data availability to make meaningful comparisons of AMU. Among the producers studied, each offered varying degrees of data completeness and comparability. No single region offered both completely comprehensive production and AMU data reports. However, some reported more comprehensive data than others. For example, The UK did not have their own governmental production reports for all salmonids produced in the

region, instead relying on the European Union's collective database, Eurostat, to present aggregated production data (Eurostat, 2021). The UK also reported estimates of AMU sales data, rounding to the nearest hundred kilograms instead of reporting exact values (Veterinary Medicines Directorate, 2018). Chile reported detailed breakdowns of production by salmonid species in their fisheries statistical yearbooks (Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018). Chile also reported graphical estimates of species-specific, drug-specific, and phase-specific (marine/freshwater) AMU. In addition to inter-governmental inconsistencies in data reporting, there were annual inconsistencies between production and AMU reports. These annual inconsistencies required further cross-referencing data with other governmental agencies as well as third-party reports.

Despite variations in availability and level of detail of data between the top salmonid producers, biomass-adjusted AMU indicators aggregated over all AMDs and species of salmonids calculations were possible for each region over time. Due to data limitations, estimates of drug-specific or species-specific AMU evaluation were only possible for BC and Chile. British Columbia was the only producer to report detailed AMU data by AMD derived from feed mill prescriptions that allowed for the species, age of administration, and drug-specific evaluation of AMU throughout the study period. It should be noted that the BC AMU data were sourced from the provincial government and were not publicly reported. Some regions reported total slaughter weights of salmon and total kilograms of active antimicrobial ingredient used/sold/prescribed (NORM/NORM-VET, 2018; Statens Serum Institut & National Food Institute, 2018; Veterinary Medicines Directorate, 2018). Chilean AMU data were almost as detailed as BC but reported graphical results without absolute values and failed to include exact breakdowns of AMU by species and drug, instead relying on graphical representations (Government of Chile, 2018). Norwegian drug-specific AMU data were available for all years of the study period but did not offer a species-specific breakdown (NORM/NORM-VET, 2018). This was less important for a region like Norway, whose production consisted almost entirely of Atlantic salmon. The UK AMU data were the least detailed in terms of drug type and species specificity and did not allow for a drug or species-specific comparison of AMU (Veterinary Medicines Directorate, 2018).

Deriving the mg/$PCU_{Slaughter, AW}$ indicators from available data was straightforward following ESVAC and our methodology (European Medicines Agency, 2021; Narbonne et al., 2021). Since the $PCU_{slaughter}$ for finfish relied on annual total slaughter weights of salmonids, the mg/$PCU_{Slaughter}$ is similar to indicators that are recommended for AMU quantification in animal livestock by the World Health Organization (WHO), such as grams of AMU per tonne of production on an annual basis (World Organisation for Animal Health, 2015). However, there are no defined ATWs assigned for salmonids, nor are there reported numbers of salmonids produced among top-producing regions – making the derivation of a consistent PCU difficult across regions if population demographics are vastly different. Furthermore, the finfish $PCU_{slaughter}$ definition from ESVAC does not allow for the easy comparison of AMU between salmonids and terrestrial livestock (European Medicines Agency, 2021). To address this, our derivation of mg/PCU_{AW} presented a valuable alternative to the existing finfish PCU to allow for potential terrestrial animal comparison of AMU as the PCU_{AW} for finfish is approximately equivalent to terrestrial livestock PCUs. Using mg/$PCU_{slaughter}$ relies on reliable, published data that do not make any assumptions about ATW or number of animals slaughtered. While it does not allow for direct terrestrial animal comparisons using the mg/PCU indicator, it is superior for comparing AMU in salmonid production between different regions because it accounts for the size of the population based on robust annual production and AMU data. The PCU_{AW} indicator, as derived, simply provides a scaled effect of the $PCU_{Slaughter}$ to allow for a more robust comparison to terrestrial animal AMU. The AMU indicators based on the PCU metric are robust in that they can adjust AMU by a region's biomass produced – alleviating the bias against large producers. However, these indicators do not allow for the evaluation of AMU in other ways such as frequency of AMU (number of applications), or duration of AMU treatments (number of courses).

We evaluated BC AMU using an APCU based on slaughter and average weights. We did this because of the limitation inherent in the standard PCU to adjust for length of life (Narbonne et al., 2021; Radke, 2017). The PCU fails to accurately reflect the exposed salmonid biomass to AMU in a given year because it considers biomass treated outside a given year being studied as belonging to the year being evaluated (Narbonne et al., 2021; Radke, 2017). The annual BC salmonid $APCU_{Slaughter, AW}$ calculated represents the estimated average daily mass of salmonids throughout each year that is at risk of exposure to antimicrobials using two different variations

on the same indicator. The $APCU_{Slaughter}$ simply increases the accuracy of the already existing finfish PCU methodology used by ESVAC to consider finfish PCU as total annual slaughtered biomass (European Medicines Agency, 2021). The $APCU_{AW}$ builds on the PCU_{AW} for salmonids in that it adjusts the biomass to reflect the population biomass at risk of treatment based on an ATW. This biomass estimate includes the juvenile salmonids that will be slaughtered in a subsequent year along with the adults that will be slaughtered in a given year – forgoing the assumption that all treatments are at final slaughter weight while attempting to adjust annual biomass to accurately reflect the growth that took place outside the evaluation year (Radke, 2017). The necessity of this adjustment was demonstrated using BC production-class specific treatment data. Most treatments with AMDs went to Atlantic salmon weighing <200 grams, while most treatments for Pacific salmon went to salmonids weighing between 201 grams and 2kg. The proportion of treatments given to salmon weighing >2kg (approaching slaughter biomass) was minimal. The APCU improves the accuracy of biomass-adjusted AMU calculations for species that live longer than a year. It also allows for a better comparison of annual biomass adjusted AMU between food animal species with relatively long or short life cycles and differing weights (e.g., chickens versus finfish versus cattle). As Atlantic salmon spend the most time at sea during grow-out, their APCU adjustment from their PCU was larger than that of Pacific salmon and trout. During years where BC salmonid production consisted primarily of Atlantic salmon, the corresponding overall $APCU_{Slaughter/AW}$ for that year would be larger than a year with a more balanced mix of Atlantic/Pacific salmon and trout production. This difference in contribution to the annual overall $APCU_{Slaughter/AW}$ led to smaller differences in overall APCU and PCU when the proportion of Pacific salmon and other species cultured was highest, particularly in 2004 and 2005. The implication of this difference in life-adjusted contribution to biomass when performing annual AMU analysis is how population demographics can influence the actual annually exposed annual biomass for a given population – despite being essentially the same type of livestock.

The $mg/APCU_{Slaughter}$ and $mg/APCU_{AW}$ indicators were straightforward to derive, as data availability on the length of life of salmonids in the marine phase of production were available for BC, however inconsistent. Salmonid length of life can vary year to year, as well as between regions and species, largely due to marine temperature differences and market demands for harvest size (Mowi, 2020; Thyholdt, 2014). Unlike terrestrial production cycles for poultry and

cattle, where production cycle lengths are consistent year to year, salmonids' grow-out (marine phase of production) length can vary with environmental conditions. In BC, the grow-put phase of production for Atlantic salmon averaged 20-24 months, whereas Pacific salmon varied between 15-18 months (Government of Canada, 2016). Norwegian grow-out lengths for Atlantic salmon are generally 16-24 months (Thyholdt, 2014). The Food and Agriculture Organization (FAO) listed Atlantic salmon as having a grow-out phase lasting 24 months, whereas the Pacific salmon grow-out phase was cited as lasting between 10-12 months (Food & Agriculture Organization, 2020a, 2020c). Data availability for grow-out lengths for trout was limited from individual regions, but the FAO listed trout as having a grow-out phase of 18 months (Food & Agriculture Organization, 2020b). These data suggest that an overall average grow-out time for each salmonid species would have to be used to reach an acceptable middle-ground for AMU analysis of salmonid aquaculture operations across all regions if using the APCU metric. The grow-out lengths selected for BC were 21, 18, and 19 months for Atlantic and Pacific salmon and trout. The resulting conversion factors applied to BC data were 1.75 for Atlantic Salmon, 1.5 for Pacific salmon, and 1.58 for other minor species. The grow-out lengths selected for BC data attempted to represent a global average of the different salmonid species grow-out lengths. A global average was chosen as these values could approximate grow-out lengths from other regions without requiring extensive knowledge of regional production practices. The length of life conversion factor is used to adjust the estimated annual biomass exposed to antimicrobials. As salmonids take more than one year to reach harvest size, any AMU attributed to salmonids in one year will miss out on use in another. This has important implications concerning over or underestimating AMU when using biomass-adjusted indicators such as mg/$PCU_{Slaughter, AW}$. If total slaughter weight data for 2018 is used in conjunction with AMU data from 2018, the denominator, i.e., the biomass used to adjust AMU, will be all attributed to 2018, whereas it was spread out over 21 months in the case of Atlantic salmon.

Species-specific AMU in BC salmonid aquaculture varied heavily, with Pacific salmon consuming the most AMU by weight in 2004 and 2005, despite making up less than a third of overall production. This was likely due to the high use of OTC in Pacific salmon relative to Atlantic salmon. One reason is the prevalence of bacterial kidney disease (*Renibacterium salmoninarum*) in adult Pacific salmon, being much higher than in Atlantic salmon (D. Morrison & S. Saksida, 2013). Oxytetracycline has a much higher label dosage (70-100 mg/kg) than all

other approved drugs for aquaculture (D. B. Morrison & S. Saksida, 2013). Varying drug dosages between commonly used AMDs in salmonid aquaculture are not accounted for in the mg/PCU, mg/APCU indicators. Interestingly, while absolute OTC use (mg) was the most used AMD in BC throughout the study period, the number of prescriptions for FLOR far surpassed the number of prescriptions for OTC after 2008, where FLOR overtook all other AMDs to become the most prescribed and used drug in salmonid aquaculture in BC. Florfenicol is administered at a dosage of 10 mg/kg while OTC is administered at a dose of roughly 75 mg/kg (Animalytix LLC, 2021; Canadian Food Inspection Agency, 2018). Varying salmonid population demographics could play a role in disease susceptibility as well as management procedures. These factors could influence the necessity of AMU in a region (Henriksson et al., 2018; D. B. Morrison & S. Saksida, 2013).

Northern European countries such as the UK and Norway have reduced AMU ($mg/PCU_{Slaughter/AW}$) to levels several magnitudes lower than producers in Chile and BC. One major reason for this includes varying species vulnerability between different salmonids (D. Morrison & S. Saksida, 2013). This was seen in the shift away from Pacific salmon production, favouring Atlantic salmon in BC and Norway. This shift resulted in reducing the incidence of bacterial kidney disease, which led to a nearly 10-fold decrease in FLOR use between 2004-2007 (Henriksson et al., 2018; D. B. Morrison & S. Saksida, 2013). Production practices and technology, and varying regional vulnerability are also crucial (Henriksson et al., 2018). Atlantic salmon farms in BC and Chile are subject to Salmon Rickettsial Syndrome (SRS) (*Piscirickettsia salmonis*), which can cause large-scale morbidity and mortality if left untreated (Austin, 2016). Atlantic salmon farms in northern Europe are generally unaffected by SRS and other bacterial outbreaks (Henriksson et al., 2018). Reasons for this could include environmental conditions such as water temperature that are not conducive to bacterial growth or better overall salmonid aquaculture management practices (Henriksson et al., 2018; Thyholdt, 2014).

Factors that may have contributed to Norway's low AMU throughout the study period include the propensity for greater viral disease challenges rather than bacterial disease challenges, as well as strict regulatory oversight and relatively excellent production practices (Grave & Hansen, 2009; Henriksson et al., 2018; Midtlyng et al., 2011; Watts et al., 2017). These have been credited for the 99% reduction in salmonid aquaculture AMU (kg) between

1987 and 2013 in Norway. Also, AMU in salmonid aquaculture is heavily influenced by legislation and regulation (Watts et al., 2017). The Norwegian government strictly regulates the prescription of antimicrobials approved for salmonids. This includes medicated feed for the treatment of bacterial and parasitic diseases. All drugs for use in salmonid aquaculture in Norway must be dispensed from an authorized pharmacy or feed mill, like Canada (Bangen et al., 1994; Grave et al., 2008). In Canada, some medically important antimicrobials were available for use in terrestrial food animals by over-the-counter access, but as of December 2018, this access was restricted to all be under veterinary prescription, in line with restrictions on AMD access in Canadian aquaculture (Government of Canada, 2021b). While Norwegian salmonid aquaculture AMU levels are noteworthy for being so low, another factor that must be considered is the types of antimicrobials used. For most of this study period, the primary antimicrobial used in Norway was oxolinic acid (OA), followed by FLOR in later years (Norwegian Veterinary Institute, 2018). These two drugs require relatively small dosages to reach effective concentrations in salmonids compared to an antimicrobial such as OTC. The potency of antimicrobials such as OA, which are considered critically important to human health by the WHO, render a region's apparent overall AMU lower than a region using a high dose drug such as OTC, but it remains a question as to whether this is “better” AMU stewardship. Using an AMU indicator that adjusts or drug dosage, such as the $nDDD_{Vet}$ would help remedy this issue in AMU surveillance. Drugs such as OA still impart selection pressures that are not necessarily less than other AMDs because they carry a lower mg/PCU. Total AMU is an important indicator for how a region is performing concerning its prudent use of AMDs, though the composition and potency of that AMU must also be considered.

British Columbian annual salmonid aquaculture AMU appeared to decrease over the study period, followed by a sudden increase in 2015 linked to an outbreak of SRS in Atlantic salmon due to warmer water temperatures that year (Monterey Bay Aquarium, 2017c). This sudden increase in AMU highlighted the vulnerability of the BC salmonid aquaculture industry to environmental variability. While overall BC AMU appears to be decreasing when considering annual mg/PCU, FLOR use increased while OTC use decreased. Any indicator relying on knowing the milligrams used/sold/prescribed of a given AMD, such as $mg/PCU_{Slaughter/AW}$, would not be suitable to compare the trend of reduced AMU due to the use of an alternative antimicrobial such a FLOR. As FLOR requires a dosage about seven times lower than OTC in

salmonids on an mg/kg basis, it would undoubtedly appear that AMU would be lower when it is being used. An indicator such as $nDDD_{Vet}$ would be more suitable for this type of analysis as it accounts for dosage (Narbonne et al., 2021). The limitation in BC to only two commonly used AMDS among slightly more approved products limits the ability to combat disease without the fear of AMR development against these popular drugs (Love et al., 2020).

These data appear to show limitations associated with evaluating salmonid aquaculture AMU according to aggregated biomass and antimicrobial use. British Columbian and Chilean salmonid production include both Atlantic and Pacific salmon – with the overall proportion of these two species varying annually. According to species-specific AMU trends, evaluating BC salmonid aquaculture AMU according to only aggregated production data would fail to reflect the possibility of species-specific vulnerability to certain diseases and the resulting increase in necessary AMU. Regions such as Norway and the UK, which produce almost entirely Atlantic salmon and no Pacific salmon may not suffer from AMU analysis using aggregate species AMU data as they do not produce other species in large proportion compared to Atlantic salmon. Further compounding the issue of species-specific vulnerability is the drug-specific and treatment variability that comes with varying species treatment needs. We found that in BC, Pacific salmon necessitated more AMU per unit biomass due to needing treatment for a disease requiring a high dosage of antimicrobials, at heavier weights compared to Atlantic salmon (D. Morrison & S. Saksida, 2013). This raises the question of how to best evaluate AMU in regions with varying salmonid aquaculture population demographics. While global salmonid production continues to move towards primarily Atlantic salmon (Asche et al., 2013), this research shows the pitfalls associated with using AMU indicators relying on aggregated biomass to evaluate AMU.

Each of the largest salmonid-producing regions in the world has differing methods to report and evaluate AMU and AMR development within the context of salmonid aquaculture. The farm-level AMU monitoring systems vary for some of the largest salmonid producers. Chile publicly reports salmonid aquaculture AMU in an integrated manner (AMU by species, age, location) and total annual biomass of salmonids slaughtered (Government of Chile, 2018). In contrast, the UK does not publish exact values for overall or drug-specific salmonid aquaculture AMU (Veterinary Medicines Directorate, 2018). The Canadian Integrated Program for

Antimicrobial Resistance Surveillance (CIPARS) monitors AMU and AMR in humans, animals, and crops in an integrated manner (Government of Canada, 2020b). Unfortunately, CIPARS only has surveillance in select voluntary sentinel farms for grower-finisher pigs, broiler chickens and turkeys, but not for salmonid aquaculture (Government of Canada, 2020a). Beginning in 2017, the Department of Fisheries and Oceans Canada provided quantities of AMDs used in marine and freshwater salmonid aquaculture to the Public Health Agency of Canada but still failed to integrate AMU findings and salmonid PCU in any form (Government of Canada, 2018a, 2020a). Separate from CIPARS, Fisheries and Oceans Canada collects AMU data from all licensed freshwater and marine aquaculture operations in Canada. However, data are only publicly available for the 2016-2018 production years in this study. These data include information on AMU by date, exact farm, as well as for what specific disease event necessitated treatment. At this time, aquaculture AMU data are not analyzed further or used for formal benchmarking activities in any major producing countries (AACTING, 2019; Government of Canada, 2018a).

Norway's national AMU monitoring system, the Veterinary Medicines Register (VETREG), was established first for farmed finfish in 2011, then terrestrial animals in 2012 (AACTING, 2019; NORM/NORM-VET, 2018). This program is operated by the Norwegian Food Safety Authority and operates based on mandatory legislation that applies to all veterinarians, feed mills, and pharmacies. As part of VETREG, all prescriptions written for farmed finfish are required to be reported as either AMU in amounts of AMD (ml, g, etc.) or the number of packages prescribed. Analysis of aquaculture AMU is performed on a milligram of active ingredient basis. Indicators for benchmarking AMU have not been defined or implemented as a tool for aquaculture antimicrobial stewardship as part of VETREG (AACTING, 2019). Since 1987, the use of AMDs in Norwegian salmonid aquaculture has declined from 48 tonnes to less than 1 tonne annually (Midtlyng et al., 2011), while production has increased by over 100% between 2004 and 2018. Several factors associated with this extremely low AMU are the development and use of high-quality vaccines against diseases like furunculosis, adopting an all-in-all-out production strategy, and the mandatory fallowing periods between year classes of salmonids (Midtlyng et al., 2011).

The UK does not have a farm-level monitoring system implemented for salmonid aquaculture (AACTING, 2019). The United Kingdom's national AMU/AMR monitoring system

is the Veterinary Antimicrobial Resistance and Sales Surveillance system (VARSS) (Veterinary Medicines Directorate, 2018). While no analysis, benchmarking, or surveillance is done at the farm level, VARSS still annual reports total aquaculture antimicrobial sales data (and actual use data in later years) for various drugs in weights of active ingredient (Veterinary Medicines Directorate, 2018).

Chilean Salmonid AMU is reported annually by the Aquaculture Branch of the Department of Animal Health Chile (Government of Chile, 2018). While no formal national surveillance systems dedicated to the surveillance of AMU/AMR exist in Chile, some international participation in veterinary AMU surveillance by Chile does exist. The VetCAb-ID (Veterinary Consumption of Antibiotics – International Documentation) based in Germany is a scientific project that collects and analyses data on antimicrobial usage in animals in different countries, founded in 2018 with two partners from Chile (AACTING, 2019; VetCAb ID, 2021). Unfortunately, VetCAb-ID only reports AMU on farm animals such as swine and poultry and does not perform any benchmarking, leaving a large gap in aquaculture AMU monitoring in Chile (VetCAb ID, 2021). Antimicrobials are mainly used to treat Atlantic salmon in Chilean salmonid production, which accounted for 80% of the total AMU in 2015, followed by 11% for Coho salmon and 9% for trout (Lozano et al., 2018). The most common disease treated in Chilean salmonid aquaculture is SRS, which accounted for 77% of all AMDs in 2005, and 89.3% of all AMDs in 2016 (Lozano et al., 2018). Of the six currently approved antimicrobials for use in Chilean salmonid aquaculture, FLOR and OTC have dominated overall use since 2005, though FLOR has since overtaken OTC use (Miranda et al., 2018). This is because FLOR is considered the first choice AMD against the causative agent of SRS, *P. salmonis* (Miranda et al., 2018).

Limitations of this study resulted primarily from data availability and comprehensiveness. Chilean salmonid aquaculture AMU data could not be found for the year 2004 – and consisted primarily of actual use data according to the agency responsible for collecting it (Government of Chile, 2018). This is unlike the AMU data from BC, Norway, and the UK, which all had either prescription and sales data available or a combination of both (NORM/NORM-VET, 2018; Veterinary Medicines Directorate, 2018). This difference in AMU data type could have influenced the accurate representation of Chilean AMU data versus the

others as actual AMU data may differ from sales and prescription data. Moreover, Norwegian and UK AMU data for salmonid aquaculture lacked detailed breakdowns by species and production class. Other limitations of this study include the relatively short period of the study (2004-2018).

Accurately defining the annual total slaughter biomass of salmonids for each region varied based on how extensively each region reported separated production statistics for each salmonid species. We focused on collating Atlantic salmon, Pacific salmon (Coho, Chinook), as well as trout production data – as these were among the most commonly produced species of salmonid worldwide (Food & Agriculture Organization - Fisheries and Aquaculture Information and Statistics Branch, 2020). With this in mind – some regional production data may be inconsistent with others based on how the region reported production levels. British Columbia reported annual breakdowns of Atlantic and Pacific salmon production but did not report Rainbow trout or steelhead trout production individually, instead grouping it with other minor salmonid species. We had to rely on governmental data sources to align trout production with what could have been expected in BC based on BC annual production reports. The UK produces salmonids in Scotland, Ireland, and England, but no production data for England after 2012 was found, nor was Irish data considered numerous enough to warrant inclusion. As a result, we relied on Eurostat database reports for UK production data. This study attempted to accurately attribute the proper AMU associated with a given annual biomass – but assumptions were made concerning how AMU was categorized regarding use in aquaculture (i.e., did AMU account for the production of salmonids not meant for human consumption, did AMU data include species not included in production biomass totals).

2.6 **CONCLUSION**

Comparing AMU using surveillance indicators to standardize AMU for varying population sizes of global salmonid aquaculture producers is a relatively novel effort in the fight against AMR development. Countries such as Norway and the UK have shown success in reducing AMU in salmonid aquaculture via vaccination and legislative restrictions on AMU and enhanced biosecurity and environmental management. These successes are essential models for improvement for regions such as BC and Chile, where AMU is still relatively high. However, varying regional vulnerability and disease pressures play a large role in AMU and must be

considered when designing stewardship programs and assigning long-term targets for AMU. This is one of the first applications of using pooled salmonid biomass to adjust total AMU in salmonid aquaculture for comparison of biomass-adjusted AMU between regions. Additionally, adjusting AMU using a life-adjusted biomass denominator is a promising method for enhancing the accuracy of potentially exposed biomass estimates of salmonids. Monitoring and evaluating AMU in all aspects of animal agriculture is a long-term project. It requires adequate tools and detailed data to do the job in a fair and accurate manner for all regions involved. We demonstrated the difficulties in acquiring detailed data for the derivation of biomass-adjusted indicators. We recommended open and public access and detailed recording and reporting of AMU and production data in all salmonid aquaculture operations. This would lead to the transparent and honest use of AMDs and reduce the likelihood of misuse of AMDs in marine environments.

CHAPTER 3

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

3.1 ABSTRACT

Antimicrobial use (AMU) surveillance in salmonid aquaculture is an essential step towards reducing the overall usage of antimicrobials in the salmonid aquaculture industry. Salmonid production and AMU data was gathered from top salmonid-producing regions to analyze differences in biomass-adjusted AMU between regions and over time. The objective of this study was to evaluate and compare biomass-adjusted AMU for the four top salmonid producing regions – British Columbia (BC) (Canada), Chile, Norway, and the United Kingdom (UK), and to evaluate changes in AMU from 2004-2018 (2005-2018 for Chile). Annual AMU data and salmonid aquaculture production data from each regions' annual production and AMU surveillance reports were used to generate the biomass-adjusted AMU indicator milligrams of active ingredient per Population Correction Unit (PCU) using annual salmonid slaughter biomass ($mg/PCU_{Slaughter}$). We built a variance weighted least squares regression (VWLS) model using AMU data from the top four salmonid producing regions in the world to evaluate regional biomass-adjusted AMU differences in salmonid aquaculture from 2004-2018. In addition to VWLS, we ran four ordinary least squares regression (OLS) models to analyze temporal biomass-adjusted AMU trends within each region. Descriptive trends from Chapter 2 pointed towards Chile and BC having the greatest AMU per $PCU_{Slaughter}$, while Norway and the UK appeared to record the lowest. The $mg/PCU_{slaughter}$ varied significantly between salmonid-producing regions when collating all AMU between 2004-2018 in the VWLS model. Over the entire study period, Chile had the highest $mg/PCU_{slaughter}$ ($p<0.01$), followed by BC ($p<0.01$), and then the UK and Norway. Antimicrobial use in the UK and Norway did not differ significantly over the study period in the OLS model ($p=0.61$) but did differ significantly in the VWLS model ($p<0.01$). Due to the different trends within regional AMU, we could not find a suitable model to analyze region and time together. As a result, individual OLS models were fit to each region using year centered on 2004 as the explanatory variable. Significant changes in $mg/PCU_{slaughter}$ over time were found within each region. British Columbian biomass-adjusted salmonid

aquaculture AMU followed a quadratic relationship, with a substantial decline until 2011 and a smaller subsequent increase into 2018 ($p<0.01$). Chilean biomass-adjusted salmonid aquaculture AMU rose and fell throughout the study period, with a decline towards the end of the study period. Norwegian biomass-adjusted salmonid aquaculture AMU declined linearly over the entire study period ($p<0.01$). Biomass-adjusted salmonid aquaculture AMU in the UK declined from the first annual quartile (2004-2007) to the second ($p<0.01$) and remained stable until the final annual quartile (20016-2018). This analysis highlighted the disparity in unadjusted and biomass-adjusted AMU between top global salmonid producers. Using the $mg/PCU_{slaughter}$ AMU indicator accounts for the relative salmonid population sizes of these different regions, meaning that the differences in AMU could be due to other potentially influential and unmeasured factors. These results show how these AMU trends differ and speak to possible differences in environmental, species, and, critically, husbandry factors such as vaccination and biosecurity protocols as potential reasons for varying regional AMU that warrant further exploration.

3.2 **INTRODUCTION**

Aquaculture is the fastest growing food-producing agricultural sector globally and is expected to continue growing year over year (Asche et al., 2013; Defoirdt et al., 2011; Schar et al., 2020). As more treatments with antimicrobial drugs (AMDs) are administered to meet disease pressures in this growing industry, antimicrobial resistance (AMR) development is likely to increase (Aarestrup, 2015; D. B. Morrison & S. Saksida, 2013). Compounding the issue of potentially increased antimicrobial use (AMU) driving increased AMR development is the limited selection of AMDs used in salmonid aquaculture. Bacterial diseases are generally the most prevalent disease challenges in salmonid aquaculture, while viral diseases are generally more difficult to control, depending on geographic location (Hossain & Shefat, 2018). A reduction in AMU in salmonid aquaculture has been shown to be possible via practices such as vaccination, improved husbandry, enhanced biosecurity, and regulation and monitoring, as was seen in Norway's salmonid aquaculture systems over the last three decades (Lulijwa et al., 2019; Midtlyng et al., 2011). Ultimately, a reduction in AMU in salmonid aquaculture could result from improved antimicrobial stewardship or prudent use. Antimicrobial stewardship is the process of using antimicrobial drugs prudently. Prudent AMU can be broadly defined as using the right drug, at the correct dose, at the right time, to the right animals (Scott Weese et al.,

2013). Evaluating prudent use can be a complex task when the goal is to assess an entire country or sectors' AMU – which is why several nations worldwide have developed advanced AMU surveillance frameworks.

Within these frameworks exist tools such as AMU indicators, discussed in previous chapters of this thesis. Monitoring salmonid aquaculture AMU using metrics and indicators allows for the ability to track absolute and relative AMU for benchmarking purposes and comparing domestic AMU with other regions and potentially other types of livestock. Antimicrobial use indicators are the resulting values of estimated AMU derived from combining defined metrics with reported quantities of AMDs used, sold, or prescribed (Bosman et al., 2019). As discussed in Chapter 2, the types and use of advanced AMU indicators in salmonid aquaculture are limited. Of the largest salmonid producers globally, the United Kingdom (UK) measures salmonid aquaculture AMU using a biomass-adjusted indicator milligrams of active ingredient per kilogram of salmonid produced (Veterinary Medicines Directorate, 2018). However, the UK does not publicly report drug-specific or species-specific AMU using this indicator. Norway reports overall drug-specific use (kg) for salmonid aquaculture in annual reports, as well as overall biomass-adjusted AMU in some but not all annual reports (NORM/NORM-VET, 2018). In Canada, BC collects but does not yet report drug-specific or species-specific AMU for their salmonid aquaculture operations. Canada has begun reporting drug and farm-specific AMU in 2016 (Government of Canada, 2018b). Chile reported drug-specific and species-specific AMU in annual reports from 2005-2018 (Government of Chile, 2018).

The PCU is a theoretical unit of biomass measurement (kg) of an animal at risk of exposure to a certain weight of antimicrobials. The biomass-adjusted AMU indicator milligrams of active ingredient per PCU using annual slaughter weight ($mg/PCU_{Slaughter}$) standardizes AMU by the approximate total exposed biomass to those antimicrobials. Global leading salmonid producers (Iversen et al., 2020) report annual salmonid slaughter weights, making the $PCU_{Slaughter}$ a reliable estimate of population size to calculate biomass-adjusted AMU. This methodology aligns with European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) group rules for defining a yearly national finfish PCU (European Medicines Agency, 2021). The $mg/PCU_{Slaughter}$ allows for the comparison of AMU between farms/countries with differing

amounts of exposed animal biomass while controlling for animal demographics, specifically population size (Radke, 2017). Following the ESVAC approach to PCU calculation, species composition can also be adjusted for, if species production breakdowns are known. Measuring AMU in salmonid aquaculture using a biomass-adjusted indicator like the mg/$PCU_{Slaughter}$ is important because it reduces the influence of production population size on overall unadjusted AMU. Large producers are generally more likely to use more antimicrobials on a total milligram basis than smaller producers. It is essential to standardize overall AMU using a metric such as the PCU to adjust AMU by the underlying population biomass. As discussed in previous chapters, until the international community agrees upon defined dose standards for common antimicrobial drugs used in salmonid aquaculture, AMU indicators such as the mg/$PCU_{Slaughter}$ are among the first line AMU indicators that should be used to compare relative AMU between top producers in salmonid aquaculture (Narbonne et al., 2021). The PCU_{AW} was not considered for regression analysis. Despite its usefulness in adjusting AMU by the biomass of salmonids at a period when they would most likely receive antimicrobial treatment, it would likely not show different regression trends as it is simply a quotient of two of the $PCU_{Slaughter}$. The research question considered for this chapter was whether there exist regional and temporal significance for AMU between and within salmonid-producing regions. Additionally, are there any temporal trends associated with AMU in top salmonid-producing regions from 2004-2018? The objective of this chapter was to analyze biomass-adjusted AMU data from top salmonid producing regions using regression analysis to determine whether regional and temporal trends in AMU exist for top salmonid producing regions over the study period.

3.3 **METHODS**

Annual antimicrobial use and salmonid production data (*see Chapter 2*) from each region were obtained from government reports and a government representative in the case of BC salmonid AMU (British Columbia Ministry of Agriculture, 2018; Directorate of Fisheries - Norway, 2020; Eurostat, 2021; Government of Chile, 2018; NORM/NORM-VET, 2018; Sernapesca - National Fisheries and Aquaculture Service - Government of Chile, 2018; Veterinary Medicines Directorate, 2018). The annual AMU for all antimicrobial drugs (mg) was calculated and divided by the annually reported slaughter weights (kg) of Atlantic salmon, Pacific salmon, and trout for each region for 2004-2018 to derive the annual mg/$PCU_{Slaughter}$ for

each region, apart from missing AMU data for Chile in 2004. The BC AMU dataset included annual prescriptions filled for salmonid aquaculture by feed mills in the province, with data reported to the BC Ministry of Agriculture. These data included drug-specific prescription line listings for AMDs meant for salmonids from 2004-2018, with information on grams of active ingredient per kilogram of premix administered in feed and total kilograms of premix. Chilean AMU data were collated from annual reports by the Government of Chile and included estimates of absolute drug-specific antimicrobial use (kg) (Government of Chile, 2018). Norwegian AMU data were retrieved from annual surveillance reports from the Government of Norway and was composed of drug-specific antimicrobial prescriptions and sales (NORM/NORM-VET, 2018). Antimicrobial use data from the UK were collected from annual surveillance reports by the UK Government and included information on estimated total antimicrobial sales and use (not drug-specific) in salmonid aquaculture (Veterinary Medicines Directorate, 2018).

Summary statistics of annual biomass-adjusted AMU (mg/$PCU_{Slaughter}$) for all antimicrobials by region, scatter plots, and lowess fit lines for temporal trends were used to identify potential regional differences and temporal AMU trends. The outcome for all models in this chapter was overall annual mg/$PCU_{slaughter}$ for all AMDs used in salmonid aquaculture by region. The explanatory variable for the overall regional comparison model was region – set as an indicator variable with BC, Chile, and Norway set as referents in three versions of the model. The explanatory variable for the temporal models was year centered on 2004. To assess overall differences between regions, ordinary least squares (linear) regression (OLS) and variance weight least squares regression (VWLS) models were compared (StataCorp, 2017, 2021). The region-specific standard deviations in mg/$PCU_{slaughter}$ over 2004-2018 (2005-2018 for Chile) were used to weight the VWLS model variance. Bonferroni (Bonferroni, 1936) correction for multiple comparisons was applied to regional OLS and VWLS models, as well as individual temporal models with variables containing >3 factors. Regional and temporal significance did not change after Bonferroni correction (data not shown). Multiple regression models were explored that included region and time (year centered on 2004) as predictors, but regression diagnostics revealed the multivariable models to be unsuitable due to the heteroskedastic distribution of the residuals and other challenges with model fit. We attempted to model the multivariable relationship between region and time using variance weighted least squares, but VWLS does not support continuous predictor variables (StataCorp, 2021). Separate OLS

temporal models were fit for each region to the annual mg/$PCU_{Slaughter}$ for all AMDs. We assessed potential linear or quadratic relationships between annual biomass-adjusted AMU and year for each region using lowess and lintrend plots (Cleveland, 1981; Joanne M. Garrett, 2017), testing the significance of a quadratic term for year, and assessing the changes in coefficients for year quartile indicators. Variables were tested for inclusion in all models using extra sum of squares F-tests, and differences between indicator variables were assessed with *t*-tests, with $p \leq 0.05$ considered statistically significant. Multiple comparisons were accounted for where needed using the Bonferroni method (Bonferroni, 1936).

Model fit for the regional and temporal OLS models was assessed using cumulative density function (p-norm) and quantile function (q-norm) normality plots of standardized residuals and standardized residual vs. linear prediction plots to check for normality of residuals. Also, we evaluated the histogram of standardized residuals to further determine normality of residuals. We confirmed our findings of non-normality in the residuals in the regional OLS, and normality in the residuals for temporal OLS models from the q-norm plots, p-norm plots, and histogram using the Shapiro Wilks test for normality of residuals (Shapiro & Wilk, 1965). The equal variance assumption for the overall regional trends OLS model was evaluated using the Breusch-Pagan test for equal variance (Breusch & Pagan, 1979). Model fit for the VWLS model was assessed using a chi-squared goodness of fit (GOF) test. Where appropriate, we also used Akaike's and Bayesian Information Criteria (AIC/BIC) to compare the fit of non-nested models, with lower values of each indicating a better fit (Akaike, 1998; Schwarz, 1978). We first checked for outliers and influential observations descriptively via box plots, scatter plots, and summary statistics for the OLS models. After running OLS models for the combined regional analysis and separate temporal analyses, we used leverage-versus-residual-squared plots to assess outliers and influential values. Standardized and studentized residuals were used as discrepancy measures to adjust residuals for their standard errors. Observations with standardized and studentized residuals >3 or <-3 were considered problematic and further reviewed. We evaluated high-leverage observations using the leverage option (Chatterjee & Hadi, 1986) in STATA® and considered any observations with leverage >3k/n (k=number of explanatory variables, n = number of observations) to be problematic and were reviewed further. We used Cook's distance (Cook, 1977) to evaluate influential observations, with observations with Cook's distance greater than 4/N (within each region for temporal models) considered problematic and checked for

inclusion into the final models. Ultimately, all observations were included in all models, regardless of their outlier or influence status. All data were analyzed using Excel® (Microsoft Corporation, Redmond, WA) and STATA® 17.0 BE (17.0, StataCorp LLC, College Station, TX).

3.4 **RESULTS**

The largest salmonid producer by annual biomass produced throughout the study period was Norway, followed by Chile, the UK, and BC. Scatter plots, and Lowess fit plots of annual mg/$PCU_{slaughter}$ for each region are shown in Figure 3.1 and 3.2. The OLS and VWLS model comparisons for overall biomass-adjusted AMU and region are shown (Table 3.2). The explanatory indicator variable for region was significant ($p<0.01$) in both the OLS (with either BC or Chile set as referents) and VWLS models (all comparisons significant). Chile had a higher overall mg/$PCU_{Slaughter}$ than all other regions (VWLS $p<0.01$ for each comparison). After Chile, BC was the next largest user, with significantly higher mg/$PCU_{Slaughter}$ than Norway and the UK (VWLS for both $p<0.01$). Biomass-adjusted AMU differed significantly between the UK and Norway (VWLS $p<0.01$) but did not differ significantly ($p=0.61$) when regional variance was not weighted in the OLS model. For the regional VWLS model, we were unable to evaluate the model for outliers, high-leverage, and influential values due to the limitations of this regression type in STATA®. These model characteristics were approximated using the OLS version of the overall regional model. British Columbia was the only region with observations containing biomass-adjusted AMU studentized/standardized residuals >3 in the year 2004 for the overall regional model. There were no observations with leverage >3k/n in the overall regional model. Observations in the overall regional model with cook's distance greater then 4/n included BC in 2004, and Chile in 2006, 2007, 2010, 2011, 2015, 2018. No observations were removed from the overall regional model. The VWLS model of biomass-adjusted AMU including region and year as explanatory variables did not return significant results and had poor model fit (data not shown). This, combined with the apparent different temporal relationships for each region (Figure 3.1, 3.2), prompted us to evaluate temporal trends in AMU for each region using separate OLS models for each region individually.

The annual AMU trends for each region had different temporal relationships in the OLS models (Figures 3.3-3.5). All regional temporal models had homoscedastic, normally distributed

standardized residuals. The temporal AMU trend in BC had a significant quadratic relationship ($p < 0.01$) (Table 3.3) with predicted AMU (Figure 3.3) decreasing from the beginning of the study period to 2013 followed by a subsequent increase through 2018. Chilean AMU data over time followed a bimodal distribution (Figure 3.4). As a result, OLS models using linear (2005-2007, 2008-2010, 2011-2015, 2016-2018) and quadratic (2005-2010 and 2011-2018) splines were considered, with the linear spline model having the best fit (based on AIC/BIC – data not shown). This model found significant linear trends for each of the four time periods in the data (all $p < 0.01$) (Table 3.4). Predicted salmonid aquaculture AMU ($mg/PCU_{slaughter}$) in Chile increased from 2005 to 2007, decreased to 2010, increased to 2014, and decreased to the end of the study period (Figure 3.4). The plots for Norway suggested a linear relationship between adjusted AMU and year. The coefficient for linear year was significant ($p<0.01$), while the quadratic was not (Table 3.5). Predicted salmonid aquaculture $mg/PCU_{slaughter}$ in Norway decreased from 2004 to 2018 (Figure 3.5). Lowess plots for the UK suggested a linear relationship between biomass-adjusted AMU and year (Figure 3.2). A quadratic relationship between year and adjusted AMU was not significant, and quartiles of year (2004-2007, 2008-2011, 2012-2015, 2016-2018) indicated that the relationship was not linear, and they were significant as a group in the model ($p<0.01$). Biomass-adjusted AMU in the UK was significantly higher in the first quartile (2004-2007) compared to all other quartiles ($p < 0.01$ for all comparisons), while AMU in all other quartiles did not differ significantly (Table 3.6). This suggests that AMU in the UK decreased from 2004-2007 and then stayed relatively stable when analyzing biomass-adjusted AMU ($mg/PCU_{Slaughter}$) for the next three quartiles (2008-2011, 2012-2015, 2016-2018).

The BC temporal model did not contain observations of biomass-adjusted AMU with standardized and studentized residuals >3 or <-3. The BC temporal model contained observations of biomass-adjusted AMU with leverage > 3k/n in 2004 and 2018. The BC temporal model contained an observation of biomass-adjusted AMU with cook's distance >4/n in the year 2004. No observations were removed from the BC temporal model. The Chilean temporal model did not contain observations of biomass-adjusted AMU with standardized and studentized residuals >3 or <-3. The Chilean temporal model did not contain observations of biomass-adjusted AMU with leverage > 3k/n. The Chilean temporal model contained observations of biomass-adjusted AMU with cook's distance >4/n in the years 2005, and 2006.

No observations were removed from the Chilean temporal model. The Norwegian temporal model did contain observations of biomass-adjusted AMU with standardized and studentized residuals >3 or <-3. The Norwegian temporal model contained observations of biomass-adjusted AMU with leverage > 3k/n in 2004 and 2018. The Norwegian temporal model contained observations of biomass-adjusted AMU with cook's distance >4/n in the year 2006, and 2018. No observations were removed from the Norwegian temporal model. The UK temporal model did not contain observations of biomass-adjusted AMU with standardized and studentized residuals >3 or <-3. The UK temporal model did not contain observations of biomass-adjusted AMU with leverage > 3k/n. The UK temporal model contained observations of biomass-adjusted AMU with cook's distance >4/n in the year 2009, and 2017. No observations were removed from the UK temporal model.

Table 3.1. Mean, minimum, and maximum unadjusted (tonnes) and biomass-adjusted ($mg/PCU_{Slaughter}$) Antimicrobial Use (AMU) for British Columbia (BC), Chile (CL), Norway (NW), and the United Kingdom (UK). Includes data for all salmonid aquaculture (Atlantic Salmon, Pacific Salmon, trout) between 2004-2018 (2005-2018 for Chile).

Region	Antimicrobial Use (AMU)	Mean AMU	Minimum AMU	Maximum AMU
BC	Unadjusted AMU (tonnes)	8.27	3.43	20.37
	Adjusted AMU ($mg/PCU_{slaughter}$)	108.49	40.81	328.98
CL	Unadjusted AMU (tonnes)	345.54	144.06	563.20
	Adjusted AMU ($mg/PCU_{slaughter}$)	480.30	308.57	639.83
NW	Unadjusted AMU (tonnes)	0.86	0.20	1.59
	Adjusted AMU ($mg/PCU_{slaughter}$)	0.94	0.15	2.13
UK	Unadjusted AMU (tonnes)	2.29	0.70	4.00
	Adjusted AMU ($mg/PCU_{slaughter}$)	13.93	3.74	27.59

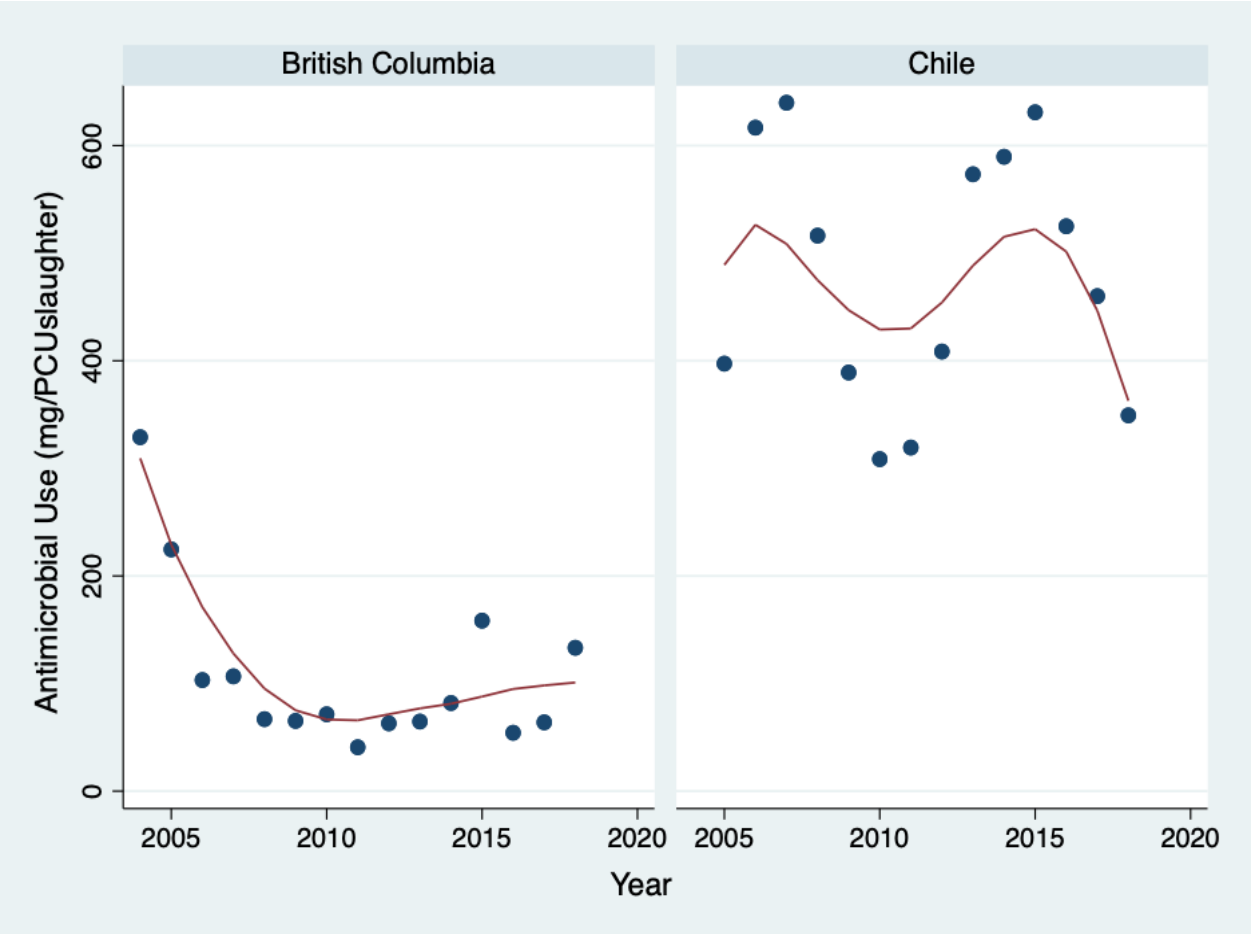

Figure 3.1. Lowess smoothed curves for annual antimicrobial use of all antimicrobials (mg per Population Correction Unit based on slaughter weight – mg/$PCU_{Slaughter}$) for salmonid aquaculture (Atlantic Salmon, Pacific Salmon, and trout) in British Columbia and Chile (2004-2018, 2005-2018 for Chile).

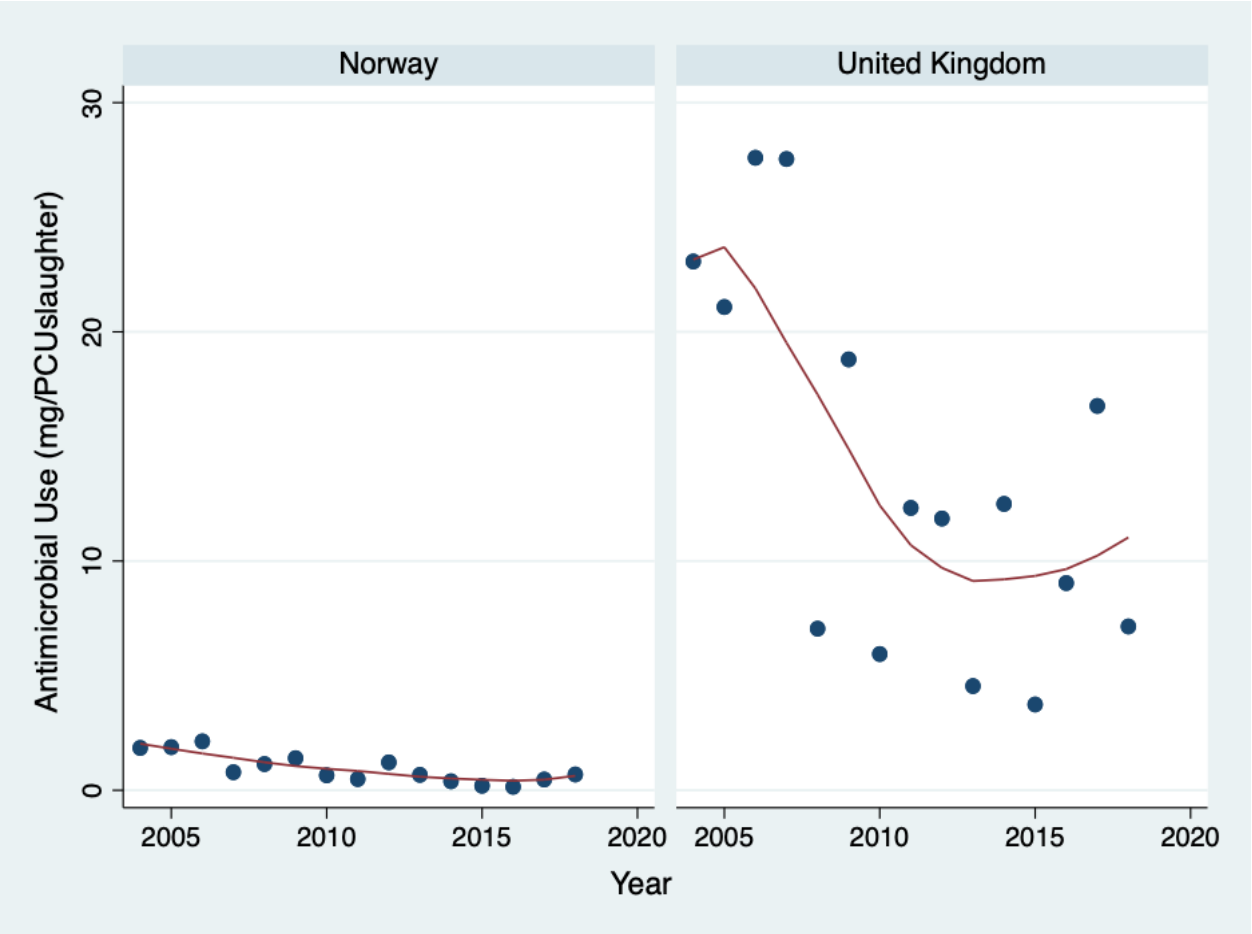

Figure 3.2. Lowess smoothed curves for annual antimicrobial use of all antimicrobials (mg per Population Correction Unit based on slaughter weight – mg/PCUSlaughter) for all salmonid aquaculture (Atlantic Salmon, Pacific Salmon, and trout) in Norway and the United Kingdom (2004-2018).

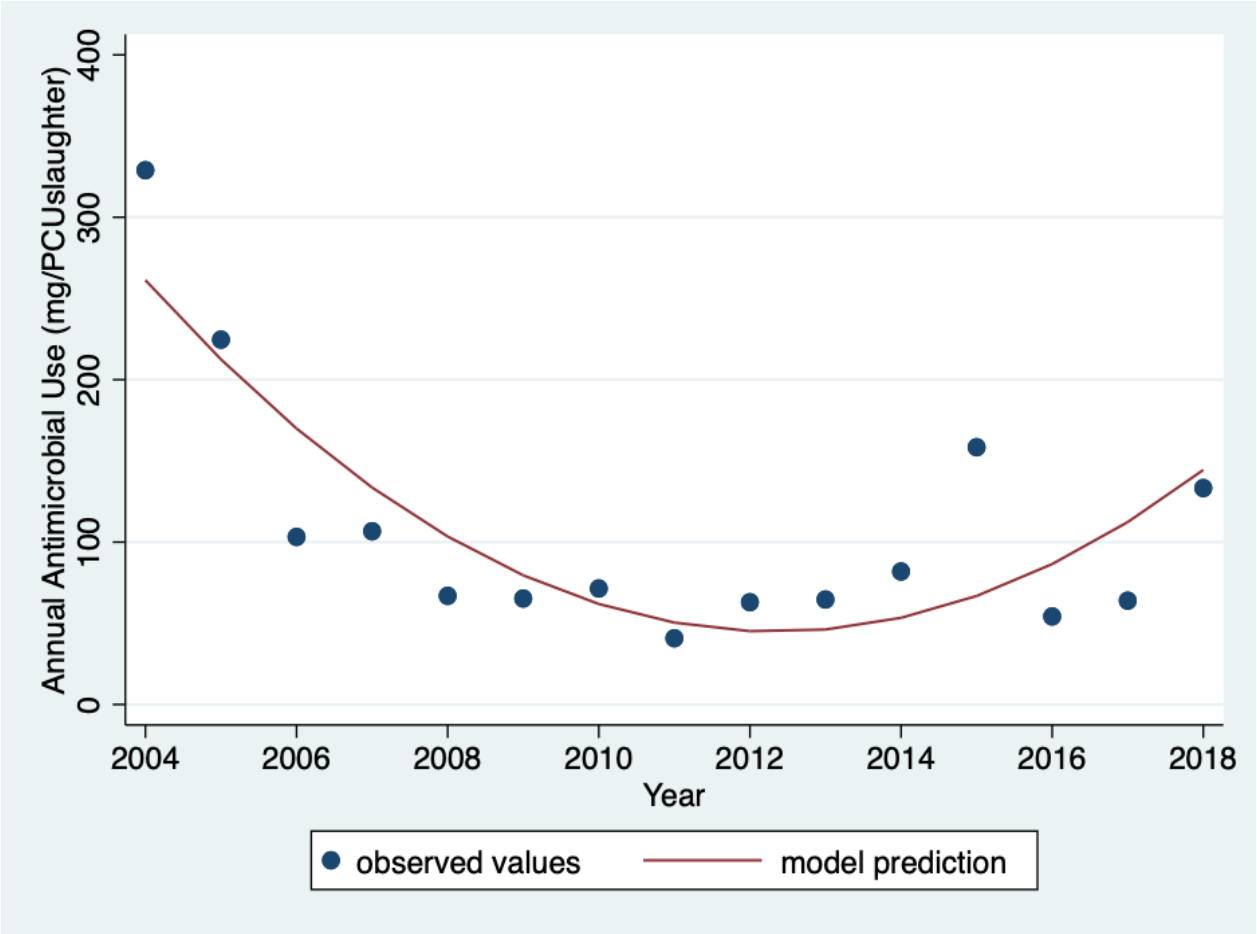

Figure 3.3. Predicted temporal trend Ordinary Least Squares model output compared to observed values for annual antimicrobial use of all antimicrobials (mg per Population Correction Unit based on overall annual slaughter weight – mg/$PCU_{Slaughter}$) for all salmonid aquaculture (Atlantic Salmon, Pacific Salmon, and trout) in British Columbia (2004-2018).

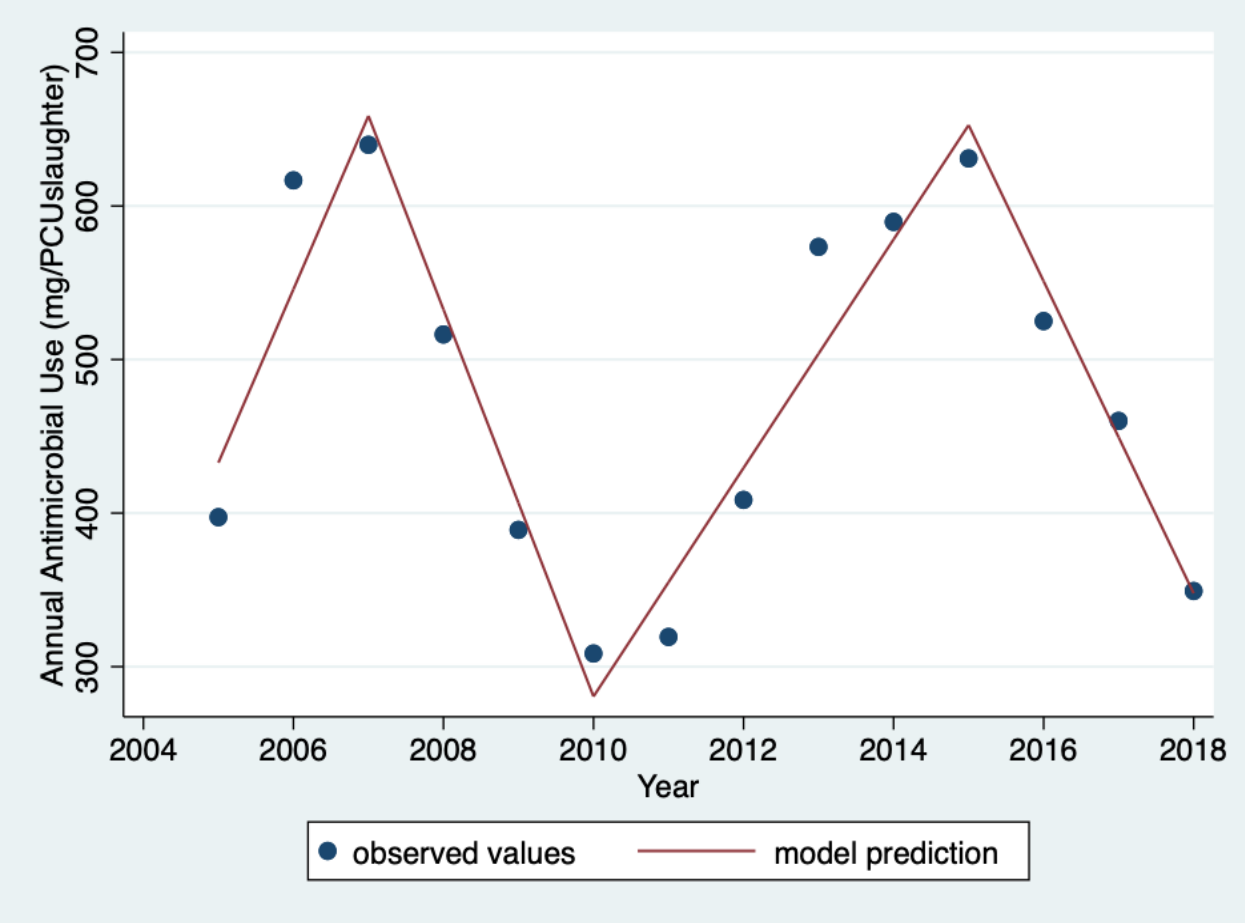

Figure 3.4. Predicted temporal trend Ordinary Least Squares model output compared to observed values for annual antimicrobial use of all antimicrobials (mg per Population Correction Unit based on overall annual slaughter weight – mg/$PCU_{Slaughter}$) for all salmonid aquaculture (Atlantic Salmon, Pacific Salmon, and trout) in Chile (2005-2018). Chilean data unavailable for 2004.

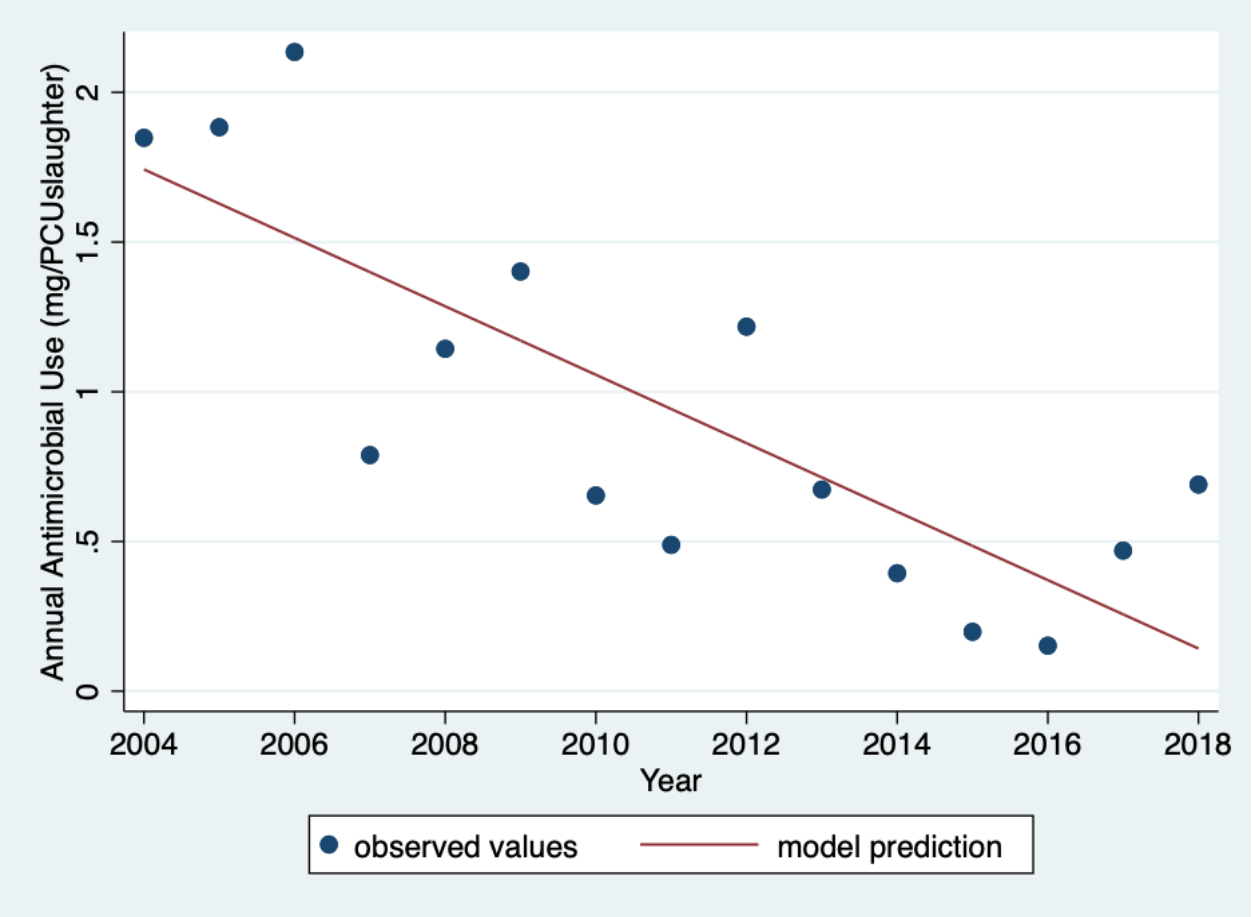

Figure 3.5. Predicted temporal trend Ordinary Least Squares model output compared to observed values for annual antimicrobial use of all antimicrobials (mg per Population Correction Unit based on overall annual slaughter weight – mg/$PCU_{Slaughter}$) for all salmonid aquaculture (Atlantic Salmon, Pacific Salmon, and trout) in Norway (2004-2018).

Table 3.2. Outputs of the linear regression (ordinary least squares - OLS) and variance-weighted linear regression (variance-weighted lease squares - VWLS) models comparing overall annual antimicrobial use (mg per Population Correction Unit based on overall annual slaughter weight – mg/$PCU_{Slaughter}$) for 2004-2018 in salmonid aquaculture for major salmonid-producing regions (British Columbia – BC, Chile – CL, Norway – NW, United Kingdom - UK).

Region (all possible contrasts)	OLS model			VWLS model		
	Coefficient (SE)	***P*-value**	**95% CI**	**Coefficient (SE)**	***P*-value**	**95% CI**
BC	Referent			Referent		
CL vs BC	371.80 (26.00)	<0.01	319.69-423.92	371.80 (37.59)	<0.01	298.13-445.47
NW vs BC	-107.55 (25.55)	<0.01	-158.76--56.34	-107.55 (20.05)	<0.01	-146.85--68.25
UK vs BC	-94.56 (25.55)	<0.01	-145.76--43.35	-94.56 (20.16)	<0.01	-134.07--55.04
Constant	108.49 (18.07)	<0.01	72.28-144.70	108.49 (20.05)	<0.01	69.19-147.79
CL	Referent			Referent		
NW vs CL	-479.35 (26.00)	<0.01	-531.46--427.23	-479.35 (31.79)	<0.01	-541.67--417.03
UK vs CL	-466.36 (26.00)	<0.01	-518.47--414.25	-466.36 (31.86)	<0.01	-528.81--403.91
Constant	480.29 (18.70)	<0.01	442.81-517.77	480.29 (31.79)	<0.01	417.98-542.61
NW	Referent			Referent		
UK vs NW	12.99 (25.55)	**0.61**	-38.22-64.20	12.99 (2.10)	<0.01	8.88-17.11
Constant	0.94 (18.07)	**0.96**	-35.27-37.15	0.94 (0.16)	<0.01	0.62-1.26
Region indicators	F-test	<0.01		F-test	<0.01	
Observations	59			59		
R-squared	0.89			N/A		
Model Significance	F-test	<0.01		Chi-squared	<0.01	
Model Goodness-of-Fit		N/A		Chi-squared	0.47	

Regional Standard deviation in mg/$PCU_{Slaughter}$ used in the VWLS model	
BC	77.66
CL	118.96
NW	0.63
UK	8.11

Table 3.3. Outputs of the linear regression (ordinary least squares - OLS) model evaluating temporal trends in British Columbia (BC) salmonid aquaculture antimicrobial use using the indicator mg of active ingredient per Population Correction Unit based on overall annual slaughter weight – $mg/PCU_{Slaughter}$ and a quadratic transformation of year.

Variables	BC Temporal Model		
	Coefficient (SE)	***P*-value**	**95% CI**
Year	-51.90 (10.32)	<0.01	-74.37--29.42
Year squared	3.11 (0.71)	<0.01	1.56-4.66
Constant	261.28 (31.11)	<0.01	193.49-329.07
Year + Year-squared	F-test	<0.01	
Observations	15		
R-squared	0.70		
Model Significance	F-test	<0.01	

Table 3.4. Outputs of the linear regression (ordinary least squares - OLS) model evaluating temporal trends in Chilean salmonid aquaculture antimicrobial use using the indicator mg of active ingredient per Population Correction Unit based on overall annual slaughter weight – $mg/PCU_{Slaughter}$ and linear splines.

Variables	Chilean Temporal Model		
	Coefficient (SE)	***P*-value**	**95% CI**
Spline 1 (2005-2007)	112.90 (27.10)	<0.01	51.60-174.19
Spline 2 (2008-2010)	-126.01 (15.52)	<0.01	-161.12--90.91
Spline 3 (2011-2015)	74.41 (8.78)	<0.01	54.54-94.28
Spline 4 (2016-2018)	-101.62 (16.21)	<0.01	-138.29--64.96
Constant	-319.92 (62.15)	<0.01	-179.33--460.52
Spline 1-4	F-test	<0.01	
Observations	15		
R-squared	0.91		
Model Significance	F-test	<0.01	

Table 3.5. Outputs of the linear regression (ordinary least squares - OLS) model evaluating temporal trends in Norwegian salmonid aquaculture antimicrobial use using the indicator mg of active ingredient per Population Correction Unit based on overall annual slaughter weight – $mg/PCU_{Slaughter}$ and a linear relationship with year.

Variables	**Norwegian Temporal Model**		
	Coefficient (SE)	***P*-value**	**95% CI**
Year	-0.11 (0.02)	<0.01	-0.16--0.06
Constant	1.74 (0.19)	<0.01	1.33-2.15
Observations	15		
R-squared	0.65		
Model Significance	F-test	<0.01	

Table 3.6. Outputs of the linear regression (ordinary least squares - OLS) model evaluating temporal trends in the United Kingdom (UK) salmonid aquaculture antimicrobial use using the indicator mg of active ingredient per Population Correction Unit based on overall annual slaughter weight – mg/$PCU_{Slaughter}$ and indicator variables for year using quartiles: 2004-2007-Quartile 1, 2008-2010-Quartile 2, 2011-2014-Quartile 3, 2015-2018-Quartile 4.

Variables	UK Temporal Model		
	Coefficient (SE)	***P*-value**	**95% CI**
Quartile 1	Referent		
Quartile 2 vs. 1	-13.79 (3.39)	<0.01	-21.26--6.33
Quartile 3 vs. 1	-16.66 (3.39)	<0.01	-24.12--9.20
Quartile 4 vs. 1	-13.84 (3.66)	<0.01	-21.90--5.78
Constant	24.82 (2.40)	<0.01	19.55-30.10
Quartile 2	Referent		
Quartile 3 vs. 2	-2.87 (3.39)	**0.42**	-10.33-4.59
Quartile 4 vs. 2	-0.04 (3.66)	**0.99**	-8.10-8.02
Constant	11.03 (2.40)	<0.01	5.75-16.30
Quartile 3	Referent		
Quartile 4 vs. 3	2.83 (3.66)	**0.46**	-5.23-10.86
Constant	8.16 (2.40)	<0.01	2.88-13.44
Observations	15		
R-squared	0.73		
Model Significance	F-test	<0.01	

3.5 **DISCUSSION**

This study evaluated regional and temporal differences in salmonid aquaculture biomass-adjusted AMU (mg/$PCU_{Slaughter}$) for the four largest salmonid-producing regions in the world. Overall, we found that biomass-adjusted AMU from 2004-2018 in salmonid aquaculture differed significantly between regions. Chile was the highest user of AMDs in its salmonid aquaculture industry, followed by British Columbia, the UK, and Norway. Interestingly, adjusting AMU using total annual salmonid biomass only further exasperated the disparity in AMU between regions. Antimicrobial use reduction has become a global initiative – with wealthier nation's leading the charge in AMU reduction (World Health Organization, 2015). As a result, we were curious to know if every region in this study experienced a reduction in biomass-adjusted AMU, which was true but with differing patterns of decline. Chilean annual AMU rose and fell in an apparent cyclical fashion, which was in sharp contrast to a region such as Norway, which showed linear annual declines in AMU. Biomass-adjusted AMU in BC appeared to decline quickly at first, then leveling out later in the study period with a small increase through the last three years. Antimicrobial use in the UK decline sharply in the beginning of the study period, followed by a steady AMU trend for the rest of the study period. Any outliers, influential observations, and observation with high leverage in the overall regional, and region-specific temporal models were kept in all models. We did not exclude these observations as they were not reporting errors were considered to be valuable observations concerning the level of AMU by region or by year within regions. Outliers, leverage points, and influential observations could indicate years of extreme (high or low) AMU relative to other years. Reasons for relatively high AMU between regions and as well as within regions (on an annual basis) are discussed below.

This analysis presented results concerning how advanced some regions are ahead of others in the race to minimize AMU in salmonid aquaculture. For example, despite producing on average 32% more salmonid biomass annually than Chile, Norway used approximately 400x less unadjusted AMU (tonnes) and 510x less biomass-adjusted AMU (mg/$PCU_{Slaughter}$) between 2004-2018 (Table 3.1). We found that all regions underwent an overall decline in AMU from 2004-2018 (2005-2018 for Chile), but with variations within this overall study period. The magnitude of overall AMU decline varied between regions. This variation was part of the reason these data were so difficult to analyze for temporal trends when building a model for all regions

and years combined. The variability in absolute AMU and the direction of yearly AMU trends between regions made it extremely difficult to model the relationship between year and overall biomass adjusted antimicrobial use when all regions were considered together. The variability in regional and temporal AMU trends indicated the biological reality of varying disease pressures worldwide and how different regions manage their salmonid aquaculture industries to minimize the necessity of AMU.

Variability in antimicrobial usage between regions and over time at levels seen here can be traced back to several important considerations concerning why disease pressure may be higher in some areas or why annual AMU may be lower in some years despite prominent disease challenges. Henriksson et al. (2018) described several main drivers of AMU relevant to salmonid aquaculture among the largest producers, including species vulnerability, production practices, regional vulnerability, and institutional vulnerability. Separate from these drivers is the selection of antimicrobial drugs used in a regions salmonid aquaculture industry. Antimicrobial selection can greatly influence the apparent unadjusted and biomass-adjusted AMU of a region depending on the potency and necessary dosage of the antimicrobials being used. Unfortunately, as discussed in Chapter 2, the PCU metric, and AMU indicator mg/$PCU_{Slaughter}$ is unable to adjust AMU for drug dosage. This has the undesirable implication of preventing any evaluation of a regions AMU in ways other than AMU weight. Antimicrobial stewardship aims to not only reduce overall use, but also minimize the use of potent antimicrobials important to human health and improve the quality of AMU per unit of livestock exposed.

For example, oxytetracycline (OTC) is a bacteriostatic protein synthesis inhibitor in bacteria that performs poorly as an antimicrobial drug in seawater due to undesirable chemical properties relating to its tendency to bind cations in the water rendering it less bioavailable to salmonids (Park et al., 2012). As a result, OTC requires dosages up to 75 mg/kg of biomass per day to reach therapeutic levels in salmonids (Animalytix LLC, 2021; Canadian Food Inspection Agency, 2012). This is much higher than comparable drugs such as florfenicol (FLOR) and oxolinic acid (OA), both necessitating dosages of ~10 mg/kg per day (Animalytix LLC, 2021; Canadian Food Inspection Agency, 2018). Oxytetracycline use was and still is much higher in BC and Chile than Norway (Government of Chile, 2018; NORM/NORM-VET, 2018). While antimicrobial drug selection does relate to production practices of a region, it also highlights the

limitation of the mg/$PCU_{Slaughter}$ indicator.

We speculate that some negative temporal trends in absolute AMU (by weight) in BC and Chile are the result of a decline in the use of drugs necessitating a high dosage such as OTC in favor of drugs requiring a lower dosage, such as FLOR. As discussed in Chapter 2, D. B. Morrison and S. Saksida (2013) found that between 2003 and 2006, OTC accounted for 90% of total AMU by Marine Harvest in BC, which was almost exclusively used to treat BKD in Pacific salmon. As producers moved entirely to Atlantic salmon production, OTC use dropped, corresponding with declines in biomass-adjusted AMU in our study. This analysis showed the greatest drops in biomass adjusted AMU in BC were between 2004-2006, coinciding with the largest shifts in production population demographics away from Pacific salmon. An additional large increase in biomass-adjusted AMU in BC in 2015-2016 was attributed to disease outbreaks caused by unusually warm waters (Monterey Bay Aquarium, 2017c). This indicates how important the environment, and population demographics are to quantifying and interpreting AMU data and how shifting salmonid population demographics can create the illusion of responsible, prudent AMU in terms of reduced overall use because of direct legislative or other stewardship related activities. As BC moved away from OTC use to primarily FLOR, the total amount of AMU dropped (both overall mg and mg/$PCU_{Slaughter}$, but the number of annual prescriptions for FLOR rose from 18 in 2004 to 120 in 2018 (*see Chapter 2*). We cannot infer identical species driven AMU trends in Chile as AMU there seemed to primarily follow trends in Atlantic salmon production. The possibility of an opposite trend (with respect to species-specific vulnerability) in Chile also exists, with a top Chilean producer commenting that the Pacific salmon stock in Chile was heartier and more disease resistant than their Atlantic salmon counterparts (Cermaq, 2013).

The first driver of AMU according to Henriksson et al. (2015) is species vulnerability to infection, which is highly relevant in salmonid aquaculture. Variable species vulnerability to bacterial diseases affects two commonly farmed salmonids, Atlantic and Pacific salmon. Henriksson et al. (2018) speculated that the main driver for shifting population demographics of salmonid production in BC and in minor part the United States was due to varying salmonid species vulnerability to bacterial pathogens. Farmed Pacific salmon are much more susceptible to diseases such as bacterial kidney disease (BKD) compared to Atlantic salmon (D. B. Morrison &

S. Saksida, 2013). Also, disease such as BKD generally occur at later stages of life where the salmon are heavier, necessitating a larger dose of AMDs for treatment. This example of variable species vulnerability shows how the compounding effect of species-specific diseases along with tendencies for heavier treatment weights of Pacific Salmon can lead to greater AMU on a milligram basis in a region producing this species of salmonid. This is relevant in Chile and BC, with the former currently standing as the largest producer of Pacific salmon worldwide – and the latter having once produced a sizable number of pacific salmon before switching to primarily Atlantic salmon production.

Our analysis supports the hypothesis that salmonid population demographics can potentially influence annual AMU. D. B. Morrison and S. Saksida (2013) conducted a study on the AMU the largest salmonid-producing company in BC (Marine Harvest) between 2003 and 2011. They found that as Marine Harvest phased out Pacific salmon in 2003 towards 100% Atlantic salmon production in 2008, there was a tenfold drop in annual AMU (D. B. Morrison & S. Saksida, 2013). This drop can mostly be seen in our data between 2004 and 2006. D. B. Morrison and S. Saksida (2013) also found that Atlantic salmon generally needed treatment for bacterial diseases at much lighter body weights than Pacific salmon, resulting in smaller dosages administered, a trend also seen in Norway (Brun & Grave, 2016; D. B. Morrison & S. Saksida, 2013). This corresponds with the trend we detected with the $mg/PCU_{Slaughter}$ indicator data for BC, which decreased significantly from >300 $mg/PCU_{Slaughter}$ in 2004 to <50 $mg/PCU_{Slaughter}$ in 2011. Based on data in Chapter 2, prior to the switch to primarily Atlantic salmon production in BC, most AMD prescriptions in 2003 were meant for Pacific salmon weighing between 500g-3.5kg. In 2006, approximately half of the AMD prescriptions written were for Atlantic salmon weighing less than 500g (*See Chapter 2*). We can only speculate as to the reason why producers still choose to farm Pacific salmon in Chile – though we acknowledge that potential AMU is a relatively minor determinant in the decision for which species to farm. Reasons such as market demand and biological advantages of Pacific salmon in Chile are cited by the company Cermaq, the third-largest global salmonid producer (Cermaq, 2013).

Alongside species-specific disease vulnerability, production practices and technology can also greatly influence regional salmonid aquaculture AMU (Henriksson et al., 2018). Cage/net-pen production is the most common form of production for farmed Atlantic and Pacific salmon

worldwide (Henriksson et al., 2018). Benefits of this production system include natural water exchange for oxygen as well as consistent waste removal. However, the exposed nature of these production systems allows for greater exposure to disease-causing agents from wild fish and surrounding water (Henriksson et al., 2018). Other production systems such as land-based recirculating operations have been designed and implemented at smaller scales to reduce environmental exposure while maintaining the benefits of cage/net-pen systems. Antimicrobial use is also affected by production practices related to how often and for what purpose antimicrobial drugs are used. Production practices among the four regions studied here are similar concerning the limitation of AMU to treatment and non-prophylactic use. Chile has previously used AMDs prophylactically to prevent mass mortality events, but this is related more to regional vulnerability than commonplace production practice (Henriksson et al., 2018). Vaccination against common finfish pathogens has been shown to directly reduce AMU when applied correctly (Henriksson et al., 2018; Hossain & Shefat, 2018). Norway's extensive use of vaccination, coupled with the fact that most disease challenges for salmonids in Norway are not bacterial in origin, has allowed them to reduce AMU levels drastically over the past 40 years (Brun & Grave, 2016; Grave & Hansen, 2009). Conversely, vaccine development against bacterial pathogens such as *P. Salmonis* have yielded very limited results – reducing the positive impact vaccines have on AMU in Chile, and presumably BC (Hossain & Shefat, 2018)

Regional vulnerability to disease also affects how AMU can vary between regions (Henriksson et al., 2018). Taking Chile and Norway as examples, the magnitude of the difference in AMU over the study period is so massive that despite adjusting use by biomass, Chile currently dominates the global salmonid aquaculture industry concerning overall AMU. We have discussed how potential species vulnerability differences, as well as production practices, could play a role as to why Chile requires greater AMU to manage disease outbreaks. Arguably the most important factor at play is regional differences between Chile and Norway that greatly influence the intensity and duration of bacterial challenges in and around salmonid aquaculture sites. Salmon farms in Chile are far more susceptible to disease challenges from *Piscirickettsia salmonis (Henriksson et al., 2018; Leung & Bates, 2013)*. This bacterium causes Salmon Rickettsial Syndrome/Septicemia (SRS), leading to massive die-offs if left untreated (Henriksson et al., 2018; Leung & Bates, 2013). It was partly regional disease vulnerability that led to the near collapse of the salmonid aquaculture industry in Chile between 2008-2011 – partially

explaining the notable drop in AMU during that time (Monterey Bay Aquarium, 2017a). Notably, SRS outbreaks are far less common in salmon farms in northern Europe (Henriksson et al., 2018; Leung & Bates, 2013). This is potentially due to environmental factors that are less conducive to *P. salmonis* growth or its vectors (Leung & Bates, 2013). Also *P. Salmonis* generally affects older salmonids, necessitating larger treatment doses than a disease that affects younger salmonids (Jakob et al., 2014). This contrasts with diseases such as yellow mouth (*Tenacibaculum maritimum*) which is common in BC. Primarily younger salmonids are affected by yellow mouth, compared to older salmonids affected by SRS (Frisch et al., 2018).

Leung and Bates (2013) found that controlling for governmental policies and management practices, aquaculture disease intensity and duration was greater at lower latitudes. They speculated that this increase in disease pressure was due to environmental factors, including warmer average water temperatures – promoting the proliferation and transmission of finfish pathogens such as *P. salmonis* (Leung & Bates, 2013). Combatting factors associated with regional vulnerability will be crucial for regions such as Chile if they hope to decrease AMU meaningfully in the next decade. Improved management of juvenile production stages is cited often to better improve survivability in the face of disease outbreaks (Leung & Bates, 2013). Proactive management solutions such as lowering net-pens to greater depths and reducing stressful handling events as temperatures are forecasted to rise is a management practice that could reduce mortality rates and subsequent reduction in the necessary AMU (Leung & Bates, 2013). Additionally, disease surveillance efforts for diseases such as SRS have been shown to be successful in reducing the impact of SRS and as a result AMU (Chile, 2012). Alternatively, perhaps the goal of reduced AMU in favor of prudent AMU in salmonid aquaculture should not apply equally to all regions. As discussed, there exist several factors that influence salmonid aquaculture AMU, and these factors can have unequal influence depending on which region is being analyzed. Perhaps prudent AMU, and the goal of lowest achievable AMU levels must become more flexible to accommodate the regions where this may be more inherently difficult.

Understanding the factors that influence AMU and how it can vary between regions is crucial when making meaningful comparison of AMU between regions. We eliminated the influence of overall production size on AMU by implementing the biomass-adjusted $mg/PCU_{Slaughter}$ indicator. This revealed that relative AMU trends are comparable to absolute

AMU trends across the studied regions in this instance despite accounting for production differences. It also highlighted the disparity in AMU between some regions, though it is important to consider the impact of other factors that are possible reasons for these differences. Importantly, metrics such as the PCU and corresponding indicators such as the $mg/PCU_{Slaughter}$ are just the start when it comes to adjusting salmonid aquaculture AMU between regions. Along with salmonid production levels, drug potency and treatment durations, factors that the $mg/PCU_{Slaughter}$ cannot adjust for can also impact a regions perceived antimicrobial stewardship. This paper presents the first steps in meaningfully adjusting AMU to reduce the bias when comparing prudent use across regions. Future analyses may attempt to adjust for some of the regional and management factors discussed here to better evaluate what qualifies as prudent use.

Limitations of this study include the difficulty of modelling the regional and annual data gathered for this project. Extreme variability in AMU between regions and over time rendered common regression techniques inappropriate for modeling region and time together, forcing us to model regions separately. Model fits could not be achieved when analyzing region and annual trends together. As a result, we could not comment on which regions underwent the largest or smallest declines/increases in AMU on an annual basis. We could only discuss temporal trends for individual regions and make assumptions about AMU trends using descriptive statistics and graphics. Our data set was gathered using publicly available resources for all regions except for BC (AMU data). Regional reporting of AMU and salmonid production were inconsistent between regions and resulted assumptions being made concerning the magnitude of AMU for a given value of exposed biomass. For example, some regions defined their AMU data as actual use (Chile), whereas others described it as sales data and prescription. While industry representatives have specified that AMDs prescribed are generally used, we acknowledge that prescription and sales data may be an over-representation of actual AMU. An important next step will be analyzing temporal trends of drug-specific and species-specific AMU data by region to determine if our hypotheses regarding changing drug preference are an underlying factor for changing biomass-adjusted AMU over time in these regions.

3.6 **CONCLUSIONS**

These statistical analyses of regional and temporal biomass-adjusted AMU trends of top salmonid producing regions revealed vast differences in AMU between regions as well as varying temporal trends within each region. This analysis reinforced the disparity in salmonid aquaculture AMU between the lowest and highest users, which may be linked to drug selection/potency, underlying disease pressures, environmental conditions, and overall management. Antimicrobial use in any salmonid aquaculture production system is an important management tool for disease challenges that threaten the health and welfare salmonids. The need for monitoring systems to promote prudent use is recommended for all top salmonid producers. This study is, to our knowledge, among the first to utilize a biomass-adjusted indicator such as the $mg/PCU_{Slaughter}$ to quantify and characterize the AMU of top salmonid-producing regions. Future research in this space could include the integration of AMU indicators such as the $mg/PCU_{Slaughter}$ with AMR indicators for pathogens of concern for the industry. The integration of this data would be of great importance to the salmonid aquaculture industry to further assess resistance development and stewardship. Future application of dose-based indicators such as the Defined Daily Dose Animal (European Medicines Agency, 2015a, 2015b; Narbonne et al., 2021) in salmonid aquaculture could normalize absolute AMU by accounting for the potencies of drugs being used.

CHAPTER 4

CONCLUSION

The global salmonid aquaculture industry has seen incredible growth in the last three decades and is expected to continue growing year-over-year (Asche et al., 2013; Park et al., 2012). With this growth comes concerns regarding the potential for increased antimicrobial use (AMU) in aquatic environments. Antimicrobial drugs (AMDs) are important tools to combat disease and maintain healthy salmonid stocks. However, AMU in aquaculture is the number one driver of antimicrobial resistance (AMR) development (Heuer et al., 2009). Antimicrobial resistance has become a growing threat in salmonid aquaculture due to the development of resistance against some commonly used antimicrobials (Miranda et al., 2018; Miranda & Zemelman, 2002; Watts et al., 2017). This threat of AMR development has spurred industry stakeholders and the public to monitor AMU in salmonid aquaculture and has reinforced the need for the prudent AMU in salmonid aquaculture. Among what this thesis revealed is the biological reality of varying disease pressure that renders the playing field of AMU among top-salmonid producers unequal. Monitoring AMU is a key method in validating AMU patterns around the globe and allows for the evaluation of antimicrobial stewardship programs being implemented in various salmonid-producing regions.

This thesis aimed to quantify and characterize AMU in the British Columbian (BC) salmonid aquaculture industry and compare it to other top salmonid producers (Norway, Chile, and the United Kingdom). It presented meaningful information concerning useful AMU indicators for salmonid aquaculture and provided insight into the AMU profiles of top salmonid producers. The main objectives of this thesis were to 1) identify robust candidate AMU indicators for use in the salmonid aquaculture industry; 2) to apply robust candidate AMU indicators to top salmonid producing regions using AMU datasets to summarize and describe annual AMU in each regions' salmonid aquaculture industry, and 3) to analyze AMU in BC aquaculture using robust indicators and evaluate temporal and regional trends within and between BC and other top salmonid-producing regions. A systematic search string to capture updated literature on antimicrobial use indicators and metrics was established to achieve the first objective (*see Chapter 1*). The second objective was achieved using salmonid production and

AMU data from Norway, Chile, the United Kingdom, and BC. These data were collated, described, and compared (*see Chapter 2*). The third objective was achieved via regional and temporal statistical analysis (*see Chapter 3*) of salmonid production and AMU data gathered in Chapter 2.

The main research questions proposed in this thesis were: 1) what are the most practical AMU indicators for summarizing and standardizing AMU data for salmonid aquaculture; 2) how does AMU between top salmonid-producing regions compare when robust indicators are applied to each region, and 3) are there significant differences in AMU between salmonid-producing regions and over time from 2004-2018? Question one was described and answered in Chapter 1. This thesis discussed and identified several candidate AMU metrics and indicators suitable for use in salmonid aquaculture operations without delay. Additionally, Chapter 1 discussed a broad array of metrics and indicators in use in terrestrial agriculture – but still currently unsuitable for use in salmonid aquaculture. Among acceptable metrics and indicators, the Population Correction Unit (PCU) metric was considered foundational it its ability to standardize AMU according to animal biomass. All candidate AMU indicators identified in Chapter 1 and applied in Chapter 2 were based on the PCU metric – owing to its relatively simplistic derivation. The milligram (mg) of active ingredient numerator was identical for all metrics, with the denominator modifying the PCU to some extent. The $mg/PCU_{Slaughter}$ indicator for salmonid aquaculture was the most suitable indicator identified in Chapter 1 due to its relative simplicity and straightforward interpretation. The $mg/PCU_{Slaughter}$ was defined in line with the European Surveillance Veterinary Antimicrobial Consumption Group (ESVAC) definition for the finfish PCU – being the total annual slaughter biomass of finfish.

A modified salmonid PCU referred to as the PCU Average-Weight (AW) was also defined using an alternative definition – the annual total slaughter biomass divided by two (mg/PCU_{AW}). This indicator was suggested because it was postulated that the mg/PCU_{AW} may better represent actual animal biomass exposed to antimicrobials at the time of treatment with antimicrobials. This is because salmonids are generally not treated at their maximum weights (*see Chapter 2*); thus, the $mg/PCU_{Slaughter}$ indicator could underestimate biomass-adjusted AMU due to an inflated biomass denominator. Additionally, the mg/PCU_{AW} closely aligned with the terrestrial mg/PCU AMU indicator as many groups of terrestrial livestock were assigned average

weights at treatment (European Medicines Agency, 2019). Using an average weight throughout production, the salmonid mg/PCU_{AW} does a better job of approximating a salmonid PCU that approximates how terrestrial livestock PCU is derived. This is important from an AMU comparison standpoint between terrestrial aquatic livestock. As salmonids do not have defined standard or average treatment weights (ATW), and countries do not report the number of salmonids produced, using the number of salmonids multiplied by an ATW instead of overall slaughter mass to compare AMU between terrestrial and aquatic livestock would be unfeasible. Quantity-based indicators relying on defined doses of AMDs such as the Defined Daily Dose Animal (DDD_{Vet}) were discussed in Chapter 1 but not implemented in Chapter 2. This is because there are no agreed-upon defined ATWs for salmonids, nor are there defined doses for AMDs used in salmonid aquaculture. One of the challenges of defining average treatment weights for salmonid aquaculture is that: 1) it is impractical to treat different salmonid species as one (this is like using one defined average treatment weight for chickens/pigs/cattle); and 2) the different growth rates/average slaughter weights of salmonids around the globe are affected by water temperature in different regions of the world, making constant average treatment weight definitions challenging between regions. Deriving defined doses for antimicrobials in salmonid aquaculture would be simpler than defining ATW as there are relatively fewer AMDs used in aquaculture – and most regions use similar dosing regimens for certain drugs.

Chapter 2 implemented some of the candidate AMU indicators discussed in Chapter 1 to AMU data from the top four salmonid-producing regions. Research question two was answered after subsequent descriptive analyses of these AMU data. Chile was found to be the highest user of antimicrobials in salmonid aquaculture in absolute and biomass-adjusted terms. Following Chile was BC, the United Kingdom and Norway, with the latter two having exceptionally lower AMU than the former two regions. Descriptive analyses of BCs salmonid population demographics over the study period revealed interesting trends associated with AMU frequency and absolute use (by weight) related to the proportion of Pacific salmon production in a given year. This was the first indication that a region's AMU could vary according to the species composition of the salmonid population. Others have found that species-specific disease susceptibility coupled with the tendency to face disease challenges at different body weights leads to regions producing Pacific salmon requiring more AMU per overall unit of biomass produced (Henriksson et al., 2018; D. B. Morrison & S. Saksida, 2013). Challenges associated

with the descriptive data analysis of Chapter 2 centered around the extreme variability of available AMU and salmonid production data from each region. Not only was regional production data variable, but annual data within regions also varied between reports. Norway was considered to have excellent data availability concerning reporting AMU and salmonid aquaculture production. Excellent data availability here was considered as comprehensive data that could be searched online in a user-friendly format. This level of data availability was not shared among all regions analyzed. Antimicrobial use data from the UK was reported as estimates of AMD sales data rather than exact values, unlike all other regions studied. Additionally, the UK did not have an easily accessible governmental resource for quantifying their salmonid aquaculture production levels from 2004-2018. Instead, production data for the UK was gathered from the European Union's Eurostat database. Chile had among the most detailed AMU and production data of the top producers. Chile reported AMU estimates (graphical representations) of AMU by drug, species, disease treated, and production phase. This level of data represented an exceptional tool for third parties to evaluate Chilean salmonid aquaculture AMU. Unfortunately, Chilean data was extremely difficult to access – owing to archived websites, dead links, and inexplicably unorganized online databases. British Columbia had relatively acceptable production data records but did not report AMU its salmonid aquaculture operations. While BC is not a national entity, the government of Canada also did not report AMU in BC salmonid aquaculture until 2016.

Gathering and collating these various data sources represented an exceptional challenge concerning the consistency of data among each region studied. One limitation of this study that future research could address is the variability in how AMU was reported between regions. Norway and the UK reported AMU according to sales, and prescription data, while Chile reported actual use data according to their government. British Columbia AMU data used prescription data from feed mills – but was considered actual use data due to the general tendency to use all prescribed AMU in BC aquaculture (based on communication with industry veterinarians). The variation in AMU data sources created potential uncertainty that each region's AMU numerator (mg of overall use for AMU indicators) represented an accurate account of all AMU attributable to each region's pooled animal biomass denominator (the PCU). Ultimately, we found that AMU differed so much between regions (apart from the UK and Norway) that

minor details such as AMU type did not alter the big picture concerning relative AMU between regions.

Research question three was answered in Chapter 3 via regional and temporal regression analysis of annual total biomass-adjusted AMU (mg/$PCU_{Slaughter}$) for each region. We performed a variance weighted least squares (VWLS) regression with all regions in the model and determined that Chile was statistically the highest user of antimicrobials in its salmonid aquaculture industry throughout the study period. British Columbia was the second-highest user, followed distantly by the United Kingdom and Norway. The latter two regions salmonid aquaculture AMU were only statistically different throughout the study period when analyzed using VWLS. We could not create a multivariate model containing all regions together with time (year) due to the extreme variability in the AMU data across regions and over time. We analyzed individual regions for temporal trends in AMU and found varying patterns in each. The temporal AMU trend in British Columbia had a significant quadratic relationship with predicted AMU decreasing from the beginning of the study period to 2013, followed by a subsequent increase through 2018. Chilean AMU data over time followed a bimodal distribution. As a result, an OLS model using linear and quadratic splines were used, with the linear spline model having the best fit. This model found significant linear trends for each of the four time periods in the data. Predicted salmonid aquaculture mg/$PCU_{slaughter}$ in Chile increased from 2005 to 2007, decreased to 2010, increased to 2014, and decreased to the end of the study period. The biomass-adjusted AMU plots for Norway suggested a linear relationship between adjusted AMU and year. The coefficient for linear year was significant, while the quadratic was not. Predicted salmonid aquaculture mg/$PCU_{slaughter}$ in Norway decreased from 2004 to 2018. The biomass-adjusted AMU plots for the UK suggested a linear relationship between biomass-adjusted AMU and year. A quadratic relationship between year and adjusted AMU was not significant, and quartiles indicated that the relationship was not linear, and they were significant as a group in the model. Biomass-adjusted AMU in the UK was modeled using year indicator variables defined as quartiles from 2004-2018. Antimicrobial use in the UK was significantly higher in the first quartile (2004-2007), while AMU in all other quartiles did not differ significantly. This suggests that AMU in the UK decreased from 2004-2007 into 2008 and then stayed relatively stable when analyzing biomass-adjusted AMU (mg/$PCU_{Slaughter}$).

The findings in Chapter 3 reinforced the descriptive analyses of Chapter 2. The analyses of biomass-adjusted AMU data for each region presented unique challenges concerning model building due to the extreme variability of AMU between regions and over time. Potential reasons for this extreme variability were discussed and included regional vulnerability, production practices, and species-specific vulnerability. The most important reason among these was likely variable regional vulnerability. When evaluating Chile against Norwegian salmonid aquaculture AMU, the difference is shocking. Without understanding regional disease dynamics and the biological reality of certain pathogens – it would be easy to discount Chilean salmonid aquaculture operations as irresponsible. However, prudent AMU does not always mean achieving the lowest AMU among peers. Chile records such high AMU due to disease pressures absent from Norwegian salmonid aquaculture operations. Norwegian waters are relatively devoid of bacterial pathogens that threaten salmonid aquaculture operations. In addition to regional vulnerability, production practices related to AMD selection also have a large impact on overall unadjusted and adjusted AMU between regions. Chile and BC used a relatively large amount of oxytetracycline (OTC) throughout the study period compared to Norway. Drugs such as OTC are relatively ineffective in aquatic settings; thus, they require large doses to meet recommended dosage levels in salmonids. From an AMU stewardship standpoint, the use of OTC – which is of relatively low importance to human health (As defined by the World Health Organization) is not entirely negative, even when absolute use (by weight) is relatively large in some areas. While Norway records incredibly low levels of AMU in their salmonid aquaculture industry, they have recorded relatively low levels of use of quinolones and fluoroquinolones, which are critically important to human health. I propose that antimicrobial stewardship evaluation must consider the quality (composition) of AMU in addition to the quantity and be flexible when factors outside the control of regions (environment, disease pressure) can elevate the need for AMU.

Overall, the findings from the descriptive and statistical analyses of salmonid production and AMU data from the top four salmonid-producing regions illustrated an extremely large difference in AMU between regions. The first key takeaway from these analyses is that AMU in salmonid aquaculture appears to have declined among many of the top salmonid-producing regions between 2004-2018. This is despite average global salmonid production increasing throughout the study period. This is an exceptional finding as it partially quells fears that increased production would automatically necessitate increased AMU. These findings also

showed the level of variability in AMU between regions for reasons other than production practices and legislation. Variability in environmental factors and species-specific vulnerability have the potential to play extremely important roles in the disease pressures of a region and subsequent AMU required to manage these disease outbreaks. This study has identified suitable AMU indicators for application in salmonid aquaculture AMU monitoring systems/frameworks. Adjusting each region's salmonid aquaculture AMU by their exposed biomass based on annual slaughter weight did not greatly alter apparent trends in regional AMU. However, it further reinforced the disparity in AMU between regions after accounting for relative population sizes. This research characterized unadjusted, as well as biomass-adjusted AMU between the top four salmonid-producing regions. When taken together with existing evidence provided in Chapters 2 and 3, this thesis provided a comparative summary of AMU in the global salmonid aquaculture industry.

Future research that could build upon this thesis includes the integration of AMR and AMU indicators. Analyzing integrated AMU and AMR data could potentially allow for the mapping of any linkages between AMU and AMR in salmonid aquaculture (European Centre for Disease Prevention and Control et al., 2017). Evaluating AMR in salmonid aquaculture is relevant from a One Health perspective owing to the fact that AMR can transfer between aquatic and terrestrial settings (Cabello et al., 2013). Further investigation of potential links between aquaculture AMU/AMR and human AMR is of particular interest in the long-term. If it is found that AMU may not be driving AMR development in salmonid aquaculture as much as previously thought, different stewardship definitions could be developed if the strain of reducing AMU negatively impacts some producers. However, if direct relationships between either AMU quality or quantity are shown to impact the rate of development of AMR in a region, enhanced measures to curb AMU may have to be implemented. Finally, integrating AMU and AMR data could allow for the analysis of other compounding factors that could be driving AMU – whether they be environmental or production-related factors. Further work on developing salmonid ATWs and DDD_{Vets} is required to allow for the use of other types of AMU indicators in salmonid aquaculture AMU surveillance. This work is required for further assessment of antimicrobial stewardship, farm-to-farm comparison, or should it become required, benchmarking. Ultimately, whatever AMU indicator is chosen for AMU surveillance and quantification should be fit-for-purpose. It must satisfy the objective of the surveillance program and motivation for comparison

in a context that is amendable to the salmonid aquaculture industry. The ability to use AMU metrics and indicators such as the $nDDD_{Vet}$ or $nDDD_{Vet}$ per salmonid-days-at-risk will be limited until progress is made to define an acceptable ATW for all regions. This will require industry engagement and buy in, which is crucial if AMU reporting and estimation is to be deemed credible and provide value back to the salmonid aquaculture industry.

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APPENDIX

A.1 LITERATURE REVIEW STRATEGY

A.1.1 TIMELINE AND SCIENTIFIC DATABASES

The search was executed on January 20, 2020, and included literature from January 1, 2016, to present to update a recent review on AMU for general animal surveillance published by Werner, et al., 2018. Communication with the authors indicated that their search was completed in early 2015. The search included five scientific databases:

1. Medline® via Ovid® provides literature (from 1946 – present). It is the world's leading bibliographic source for biomedical scholarly literature and research.
2. CAB Abstracts® via Web of Science™ covers multi-disciplinary literature (from 1910 - present) in the fields of nature, health, and social sciences. CAB Abstracts is the leading English-language bibliographic information service providing access to the world's applied life sciences literature.
3. Embase via Ovid® covers a vast range of biomedical sciences journals, with an added focus on European studies (from 1974 – present) not present in MEDLINE®.
4. AGRICOLA ™ via ProQuest® sources from the United States National Agricultural Library to retrieve global literature (from 1970 – present) on the topic of agriculture.
5. BIOSIS Previews via Web of Science™ covers pre-clinical and experimental research, methods and instrumentation, animal studies, and more (1926 - present).

A.1.2 SEARCH STRATEGIES AND RESULTS

Medline® via Ovid®

Component	Search Terms	# Results
1. Surveillance	(surveillance* or inspect* or control* or metric* or measure* or observ* or scrutin* or examin*or monitor* or track* or evaluat*).ti,ab,kw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	10,320,274
2. Use	("Use*" or Usage* or Treat* or Appli* or Prescribe* or admin* or distribut* or sell* or sale* or metric or metrics or distribut*).ti,ab,kw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	12,242,908
3. Antimicrobial	(antibiotic* or antimicrobial* or anti-microbial* or anti-biotic* or anti-bacterial* or antibacterial* OR multidrug or medication* or drug* or antiinfective or anti-infective or anti-infective agent*).ti,ab,kw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	2,230,687
4. Metric	(PDD or prescribed daily dose or ACD or animal course dose or DCD or defined course dose or DCDA or defined course dose animal or ADDD or animal defined daily dose or DDDA or defined daily dose animal or DDD or UDD or UCD or PCU or daily defined dose or daily course dose or population correct* unit or used daily dose or APCU or adjusted population correct* unit or DOT or DPD or Daily Product Dose or treatment incidence or Treatment frequency or treatment incidence rate or treatment frequency or sale* data or product related daily dose or animal daily dose).ti,ab,kw.	55,775

5. Animal	(Cattle or cow or bull or bulls or steer or calf or calves or bos taurus or beef or veal or pig or piglet or swine or hog or sow or pork or sus scrofa domesticus or chick or chicken or chickens or rooster or hen or broiler or gallus gallus domesticus or turkeys or meleagris gallopavo or turkey or gobbler or poultr*) or ((farm* or domestic or aquaculture or livestock) and (fish* or finfish or fin-fish or atlantic salmon or pacific salmon or arctic char or black cod or chinook salmon or coho or tilapia)).ti,ab,kw.	956,412
6. 1 AND 2 AND 3 AND 4 AND 5		129
7. limit 6 to yr="2016 - Current"		60

Embase® via Ovid®

Component	Search Terms	# Results
1. Surveillance	(surveillance* or inspect* or control* or metric* or measure* or observ* or scrutin* or examin*or monitor* or track* or evaluat*).ti,ab,kw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	13,233,562
2. Use	("Use*" or Usage* or Treat* or Appli* or Prescribe* or admin* or distribut* or sell* or sale* or metric or metrics or distribut*).ti,ab,kw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	15,503,048

3. Antimicrobial	(antibiotic* or antimicrobial* or anti-microbial* or anti-biotic* or anti-bacterial* or antibacterial* or multidrug or medication* or drug* or antiinfective or anti-infective or anti-infective agent*).ti,ab,kw. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	3,101,711
4. Metric	(PDD or prescribed daily dose or ACD or animal course dose or DCD or defined course dose or DCDA or defined course dose animal or ADDD or animal defined daily dose or DDDA or defined daily dose animal or DDD or UDD or UCD or PCU or daily defined dose or daily course dose or population correct* unit or used daily dose or APCU or adjusted population correct* unit or DOT or DPD or Daily Product Dose or treatment incidence or Treatment frequency or treatment incidence rate or treatment frequency or sale* data or product related daily dose or animal daily dose).ti,ab,kw.	72,770
5. Animal	(Cattle or cow or bull or bulls or steer or calf or calves or bos taurus or beef or veal or pig or piglet or swine or hog or sow or pork or sus scrofa domesticus or chick or chicken or chickens or rooster or hen or broiler or gallus gallus domesticus or turkeys or meleagris gallopavo or turkey or gobbler or poultr*) or ((farm* or domestic or aquaculture or livestock) and (fish* or finfish or fin-fish or atlantic salmon or pacific salmon or arctic char or black cod or chinook salmon or coho or tilapia)).ti,ab,kw.	897,833
6. 1 AND 2 AND 3 AND 4 AND 5		148
7. limit 6 to yr="2016 - Current		62

AGRICOLA ™ via ProQuest

Component	Search Terms	# Results
1. Surveillance	noft(surveillance* or inspect* or control* or metric* or measure* or observ* or scrutin* or examin*or monitor* or track* or evaluat*)	1,555,184
2. Use	noft("Use*" or Usage* or Treat* or Appli* or Prescribe* or admin* or distribut* or sell* or sale* or metric or metrics or distribut*)	2,184,459
3. Antimicrobial	noft(antibiotic* or antimicrobial* or anti-microbial* or anti-biotic* or anti-bacterial* or antibacterial* or multidrug or medication* or drug* or antiinfective or anti-infective or anti-infective agent*)	262,309
4. Metric	noft(PDD or prescribed daily dose or ACD or animal course dose or DCD or defined course dose or DCDA or defined course dose animal or ADDD or animal defined daily dose or DDDA or defined daily dose animal or DDD or UDD or UCD or PCU or daily defined dose or daily course dose or population correct* unit or used daily dose or APCU or adjusted population correct* unit or DOT or DPD or Daily Product Dose or treatment incidence or Treatment frequency or treatment incidence rate or treatment frequency or sale* data or product related daily dose or animal daily dose)	47,031
5. Animal	noft(Cattle or cow or bull or bulls or steer or calf or calves or bos taurus or beef or veal or pig or piglet or swine or hog or sow or pork or sus scrofa domesticus or chick or chicken or chickens or rooster or hen or broiler or gallus gallus domesticus or turkeys or meleagris gallopavo or turkey or gobbler or poultr*) or ((farm* or domestic or aquaculture or livestock) and (fish* or finfish or fin-fish or atlantic salmon or pacific salmon or arctic char or black cod or chinook salmon or coho or tilapia))	668,750
6. 1 AND 2 AND 3 AND 4 AND 5		914
7. Filter 2016-01-01 – 2020-01-01		198

CAB Abstracts® via Web of Science™

Component	Search Terms	# Results
1. Surveillance	Topic: ((surveillance* or inspect* or control* or metric* or measure* or observ* or scrutin* or examin*or monitor* or track* or evaluat*))	4,962,453
2. Use	Topic: (("Use*" or Usage* or Treat* or Appli* or Prescribe* or admin* or distribut* or sell* or sale* or metric or metrics or distribut*))	5,715,468
3. Antimicrobial	Topic: ((antibiotic* or antimicrobial* or anti-microbial* or anti-biotic* or anti-bacterial* or antibacterial* or multidrug or medication* or drug* or antiinfective or anti-infective or anti-infective agent*))	1,587,251
4. Metric	Topic: ((PDD or prescribed daily dose or ACD or animal course dose or DCD or defined course dose or DCDA or defined course dose animal or ADDD or animal defined daily dose or DDDA or defined daily dose animal or DDD or UDD or UCD or PCU or daily defined dose or daily course dose or population correct* unit or used daily dose or APCU or adjusted population correct* unit or DOT or DPD or Daily Product Dose or treatment incidence or Treatment frequency or treatment incidence rate or treatment frequency or sale* data or product related daily dose or animal daily dose))	163,423
5. Animal	Topic: ((Cattle or cow or bull or bulls or steer or calf or calves or bos taurus or beef or veal or pig or piglet or swine or hog or sow or pork or sus scrofa domesticus or chick or chicken or chickens or rooster or hen or broiler or gallus gallus domesticus or turkeys or meleagris gallopavo or turkey or gobbler or poultr*) or ((farm* or domestic or aquaculture or livestock) and (fish* or finfish or fin-fish or atlantic salmon or pacific salmon or arctic char or black cod or chinook salmon or coho or tilapia)))	1,774,047
6. 1 AND 2 AND 3 AND 4 AND 5		7,658
7. 6 limited to 2016-2020		1,055

Biosis® via Web of Science™

Component	Search Terms	# Results
1. Surveillance	Topic: ((surveillance* or inspect* or control* or metric* or measure* or observ* or scrutin* or examin*or monitor* or track* or evaluat*))	10,101,482
2. Use	Topic: (("Use*" or Usage* or Treat* or Appli* or Prescribe* or admin* or distribut* or sell* or sale* or metric or metrics or distribut*))	12,24,1802
3. Antimicrobial	Topic: ((antibiotic* or antimicrobial* or anti-microbial* or anti-biotic* or anti-bacterial* or antibacterial* or multidrug or medication* or drug* or antiinfective or anti-infective or anti-infective agent*))	5,126,564
4. Metric	Topic: ((PDD or prescribed daily dose or ACD or animal course dose or DCD or defined course dose or DCDA or defined course dose animal or ADDD or animal defined daily dose or DDDA or defined daily dose animal or DDD or UDD or UCD or PCU or daily defined dose or daily course dose or population correct* unit or used daily dose or APCU or adjusted population correct* unit or DOT or DPD or Daily Product Dose or treatment incidence or Treatment frequency or treatment incidence rate or treatment frequency or sale* data or product related daily dose or animal daily dose))	457,235
5. Animal	Topic: ((Cattle or cow or bull or bulls or steer or calf or calves or bos taurus or beef or veal or pig or piglet or swine or hog or sow or pork or sus scrofa domesticus or chick or chicken or chickens or rooster or hen or broiler or gallus gallus domesticus or turkeys or meleagris gallopavo or turkey or gobbler or poultr*) or ((farm* or domestic or aquaculture or livestock) and (fish* or finfish or fin-fish or atlantic salmon or pacific salmon or arctic char or black cod or chinook salmon or coho or tilapia))	1,391,050
6. 1 AND 2 AND 3 AND 4 AND 5		3,909
7. 6 limited to 2016-2020		562

A.2 SUPPLEMENTARY TABLES

Table A.2.1. Average treatment weights used to calculate Population Correction Units for various terrestrial animal species (adapted from the European Medicines Agency (2019) and Radke (2017).

Animal Category	Average treatment weights	
	ESVAC weights (kg)[a, b]	**Adjusted weights (kg)[b]**
Pigs		
Suckling piglets	4	4
Weaner pigs	12	12
Sows/boars	240	240
Slaughter pigs	65 (25-105)*	65
Finisher	65	65
Imported/exported pigs for slaughter	65	65
Imported/exported pigs for fattening	25	-
Cattle		
Slaughter cows	500	627
Slaughter heifers	200	269 (45-493)*
Slaughter bullocks and bulls	500	329 (45-612)*
Slaughter calves and young cattle	140	169 (45-293)*
Imported/exported cattle for slaughter	500	299
Imported/exported cattle for fattening	140	169 (45-293)*
Livestock dairy cows	500	627
Veal calves	80	80
Poultry		
Slaughter broilers	1	1
Slaughter turkeys	6.5	6.5
Imported/exported broilers for slaughter	1	1
Finfish		
Slaughter fish	***Total slaughter weight (kg)***	

[a] (European Medicines Agency, 2019)

[b] (Radke, 2017)

* Weight used (weight range for category in brackets), where applicable.

Table A.2.2. Annual British Columbian species-specific salmonid aquaculture total slaughter biomass (kg) 2004-2018.

Year	Atlantic Salmon	Pacific Salmon	Trout
2004	46,100,000	15,700,000	115,000
2005	53,800,000	16,600,000	171,000
2006	71,000,000	7,000,000	274,000
2007	73,300,000	5,600,000	505,000
2008	77,200,000	4,200,000	635,000
2009	72,700,000	3,600,000	523,000
2010	74,500,000	4,200,000	600,000
2011	79,400,000	3,800,000	758,000
2012	72,900,000	5,500,000	593,000
2013	58,300,000	4,100,000	62,000
2014	64,100,000	3,600,000	44,000
2015	89,800,000	3,200,000	-
2016	90,500,000	2,300,000	1,136,000
2017	83,100,000	2,600,000	33,000
2018	84,500,000	2,600,000	1,190,000

Table A.2.3. Annual drug-specific antimicrobial use (kg) (AMU) of antimicrobial drugs used in British Columbian salmonid aquaculture. AMU in species other than Atlantic Salmon, Pacific Salmon trout were excluded. Other AMU includes the erythromycin and lincomycin. TMS - Trimethoprim + Sulfadiazine, SMOR - Sulfadimethoxine + Ormetoprim.

Year	Florfenicol	Oxytetracycline	TMS	SMOR	Other
2004	63.06	18,846.48	249.76	183.52	1,026.06
2005	93.53	14,584.87	436.22	715.46	25.14
2006	26.25	7,061.09	680.02	311.76	-
2007	18.25	7,792.40	582.31	78.34	-
2008	57.66	4,775.38	657.07	-	-
2009	95.61	4,103.29	814.56	-	-
2010	588.27	4,550.84	503.20	21.75	-
2011	624.13	2,422.86	376.46	2.63	-
2012	693.68	4,176.88	106.28	-	-
2013	345.21	3,568.28	124.98	-	-
2014	1,197.99	4,243.07	64.38	42.50	-
2015	2,535.46	12,044.36	0.18	152.40	-
2016	2,627.18	2,371.90	-	95.36	-
2017	2,421.02	3,060.05	-	-	-
2018	4,139.89	7,625.17	-	-	-

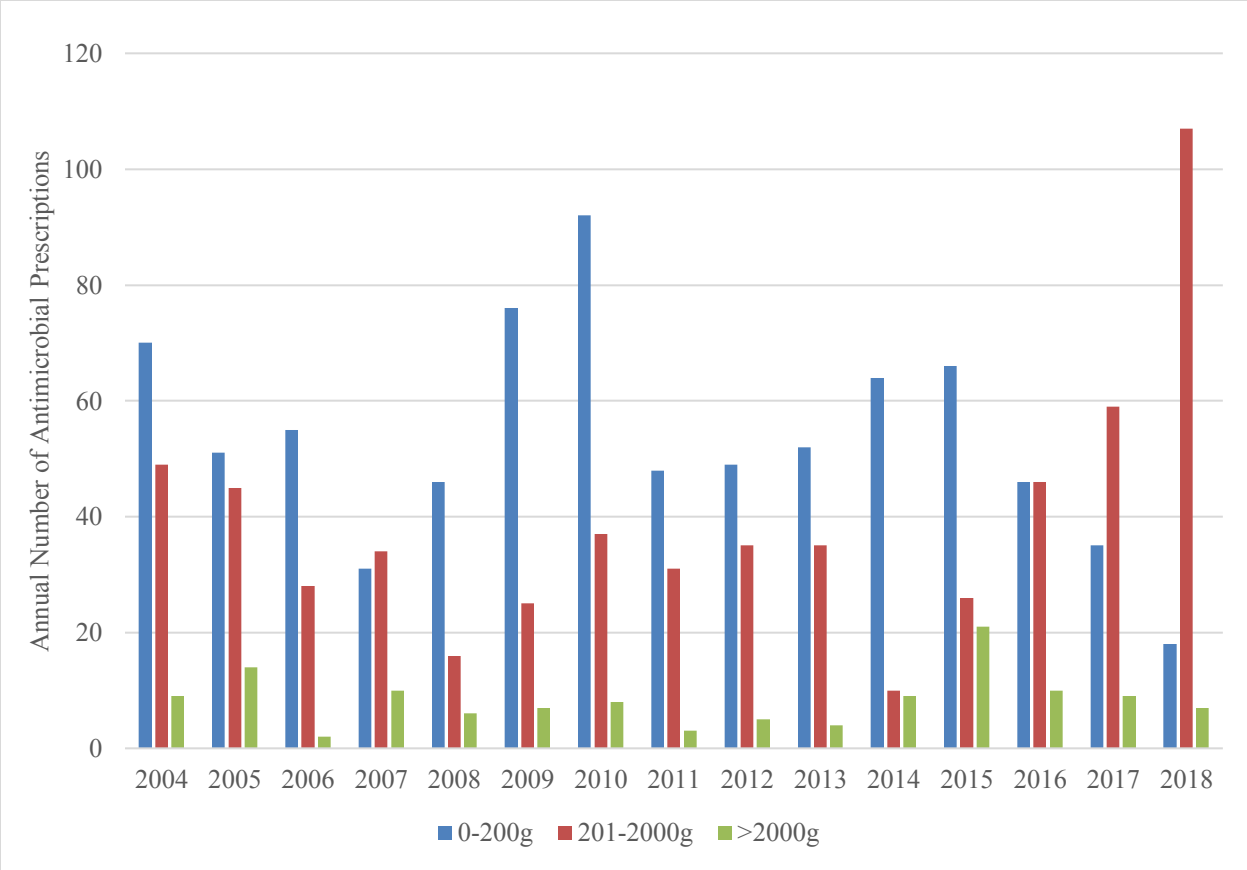

Figure A.2.1. Annual number of prescriptions by production class (size of salmon) for Atlantic/Pacific salmon, and trout produced in British Columbia. Antimicrobials included are: oxytetracycline, florfenicol, sulfadiazine + trimethoprim, sulfadimethoxine + ormetoprim, erythromycin, and lincomycin.

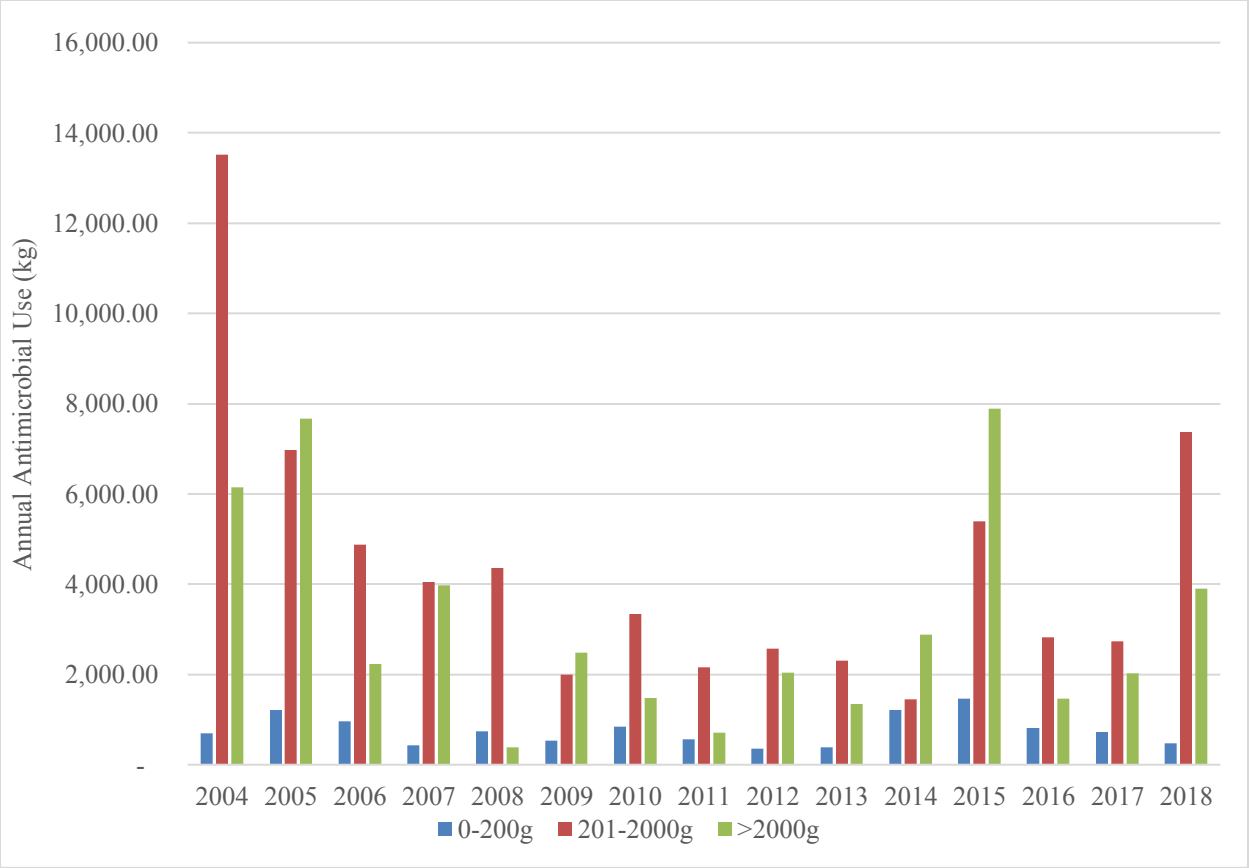

Figure A.2.2. Annual overall antimicrobial use (kg) (AMU) by production class for Atlantic/Pacific salmon, and trout produced in British Columbia. Antimicrobial drugs included are: oxytetracycline, florfenicol, sulfadiazine + trimethoprim, sulfadimethoxine + ormetoprim, erythromycin, and lincomycin.

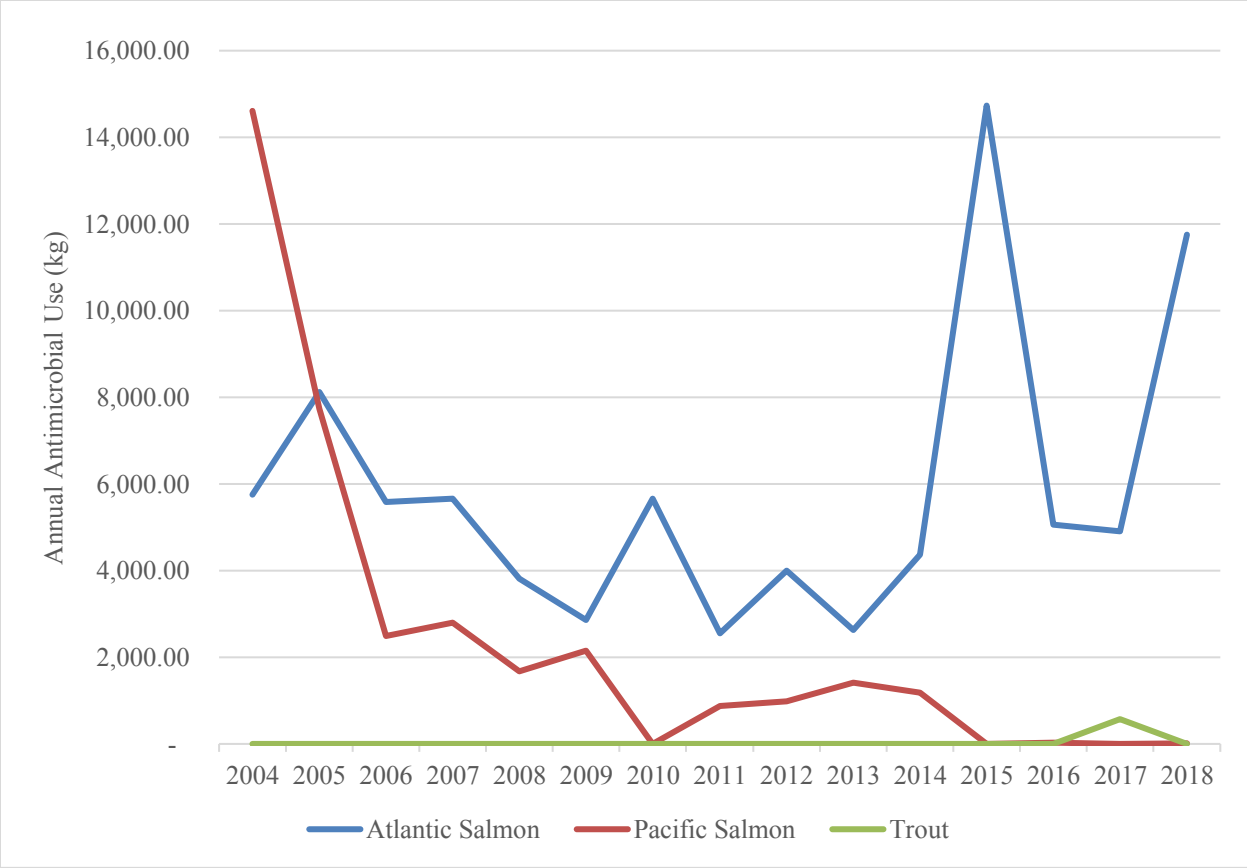

Figure A.2.3. Annual overall antimicrobial use (kg) (AMU) by species produced in British Columbia. Includes data for Atlantic/Pacific salmon, and trout. Antimicrobial drugs included are: oxytetracycline, florfenicol, sulfadiazine + trimethoprim, sulfadimethoxine + ormetoprim, erythromycin, and lincomycin.

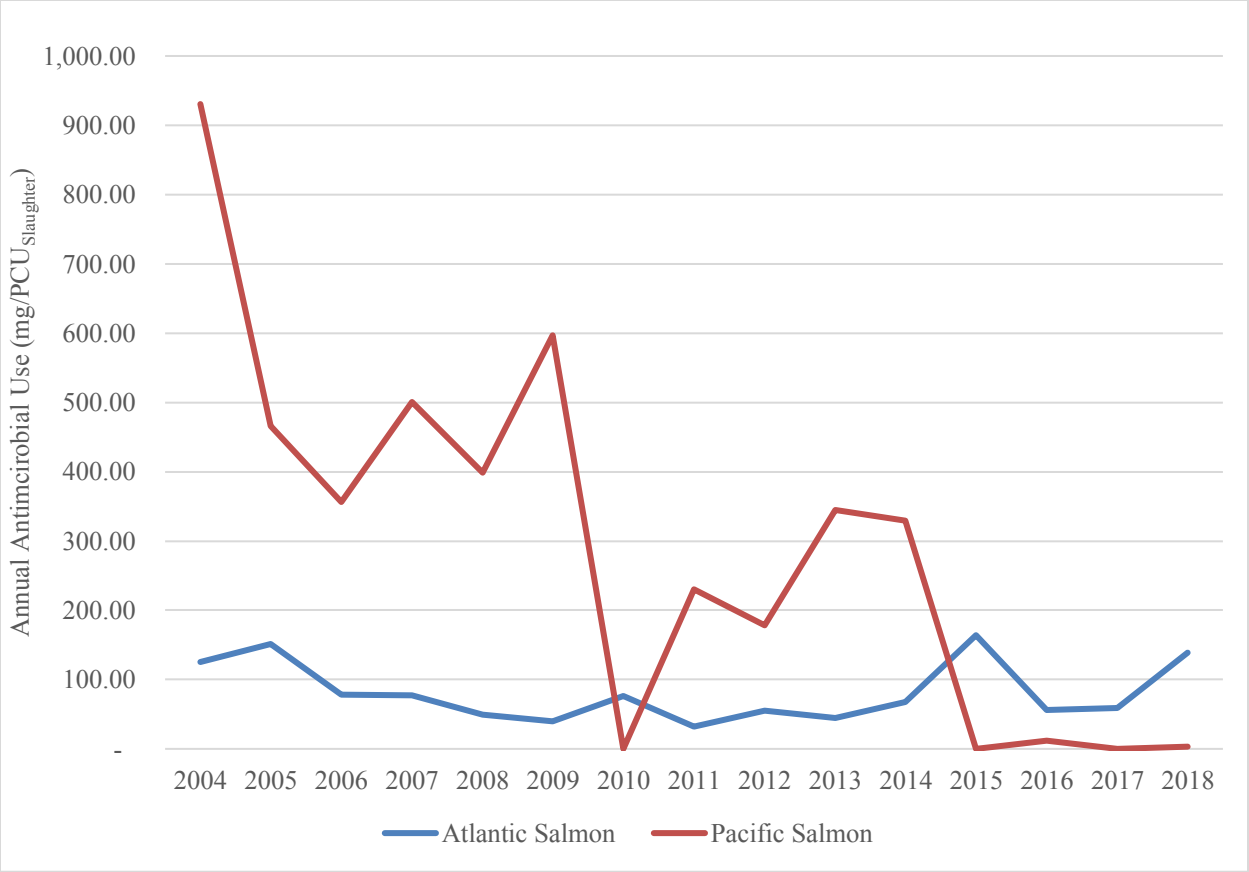

Figure A.2.4. Annual species-specific biomass-adjusted antimicrobial use (AMU) (mg/$PCU_{Slaughter}$) by species produced in British Columbia from 2004-2018. Data excludes trout for illustrative purposes.

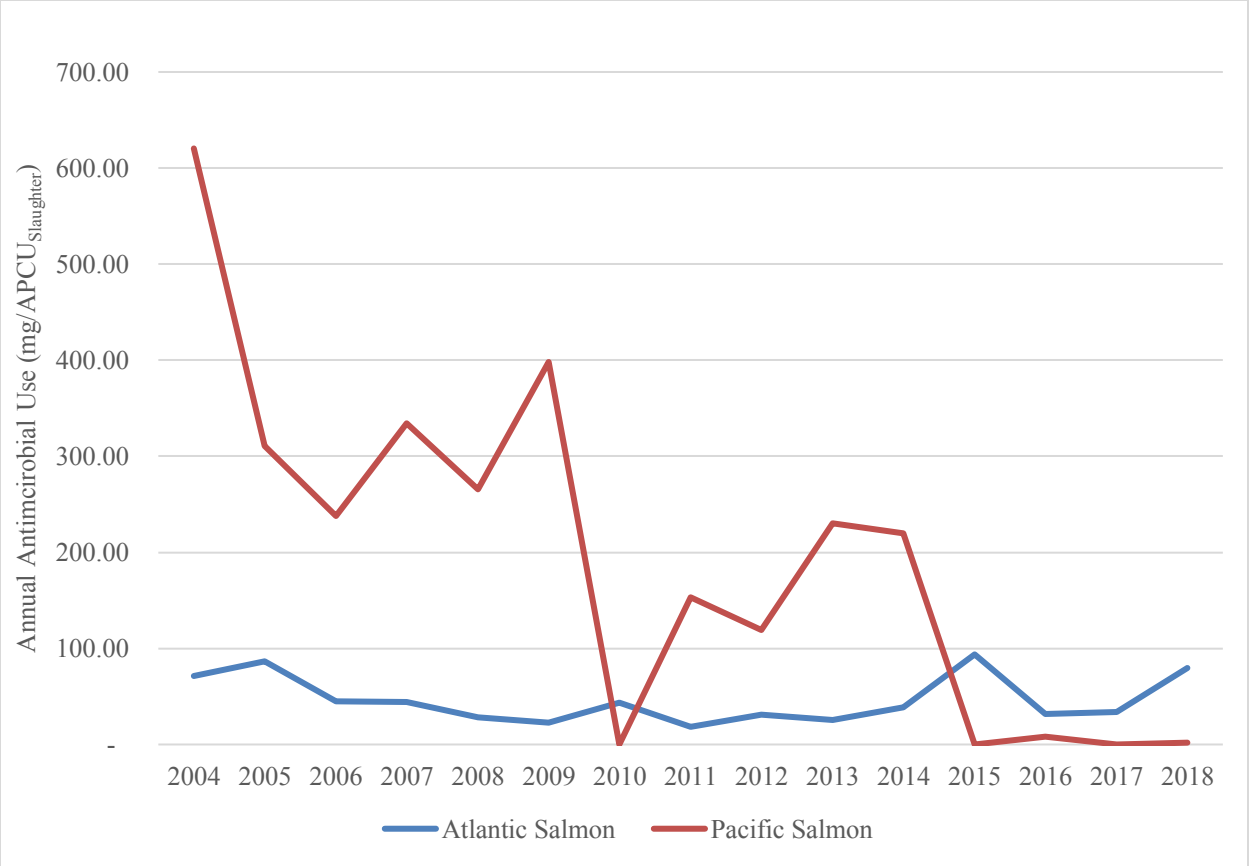

Figure A.2.5. Annual species-specific biomass-adjusted antimicrobial use (AMU) (mg/APCU$_{Slaughter}$) by species produced in British Columbia from 2004-2018. Data excludes trout for illustrative purposes.

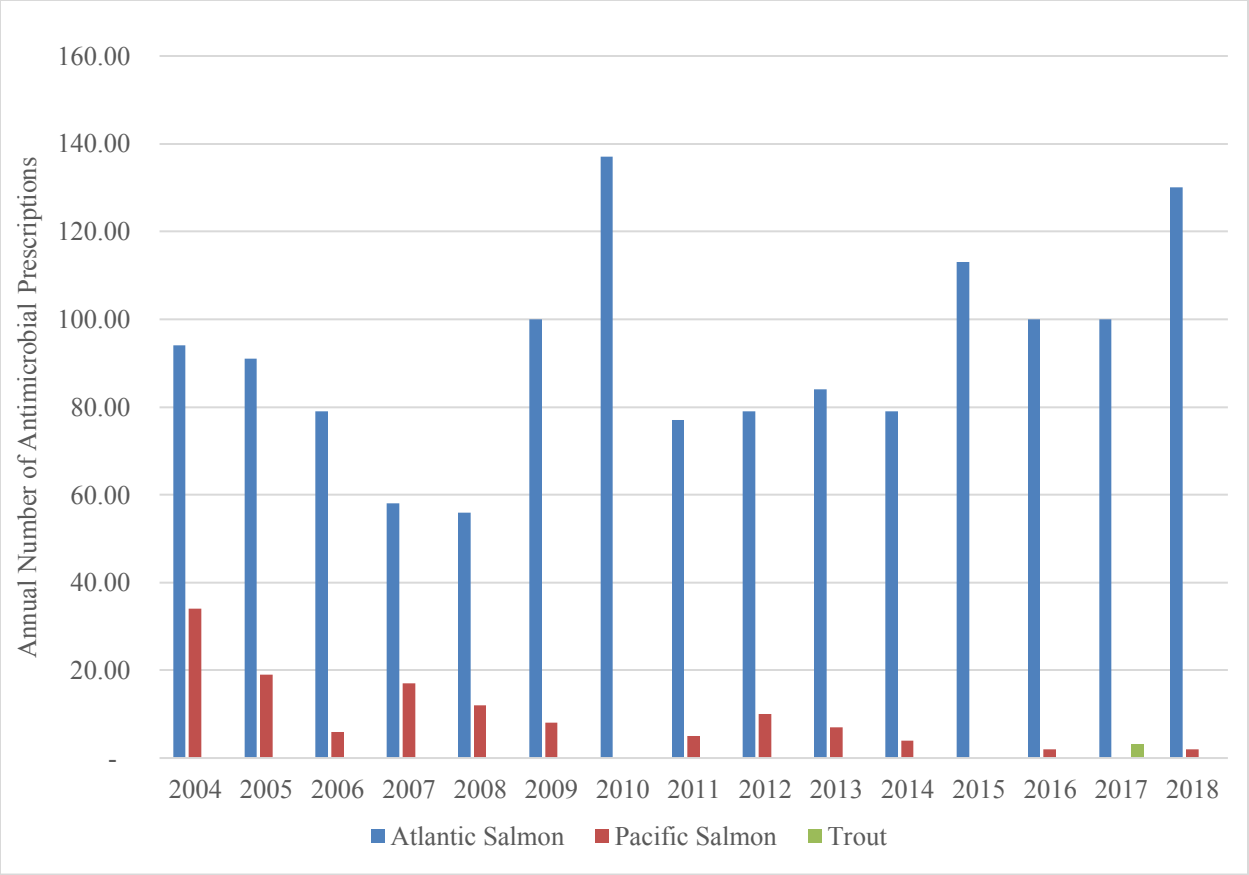

Figure A.2.6. Annual number of antimicrobial prescriptions by salmonid species produced in British Columbia. Antimicrobial drugs included are: oxytetracycline, florfenicol, trimethoprim + sulfadiazine, sulfadimethoxine + ormetoprim, erythromycin, and lincomycin.

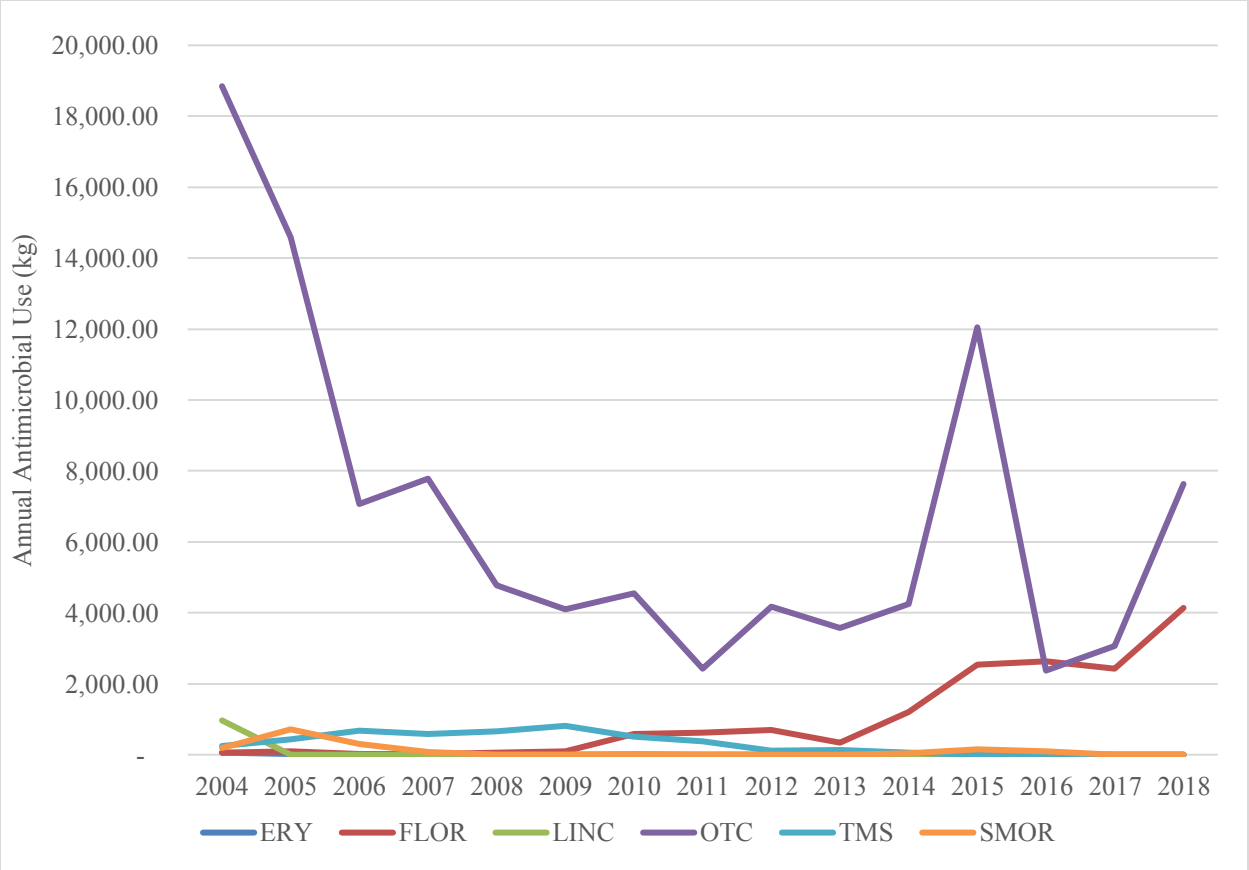

Figure A.2.7. Annual drug-specific antimicrobial use (AMU) (kg) in British Columbia. FLOR – Florfenicol, OTC – Oxytetracycline, ERY – Erythromycin, LINC – Lincomycin, TMS – Trimethoprim + Sulfadiazine, SMOR – Sulfadimethoxine + Ormetoprim.

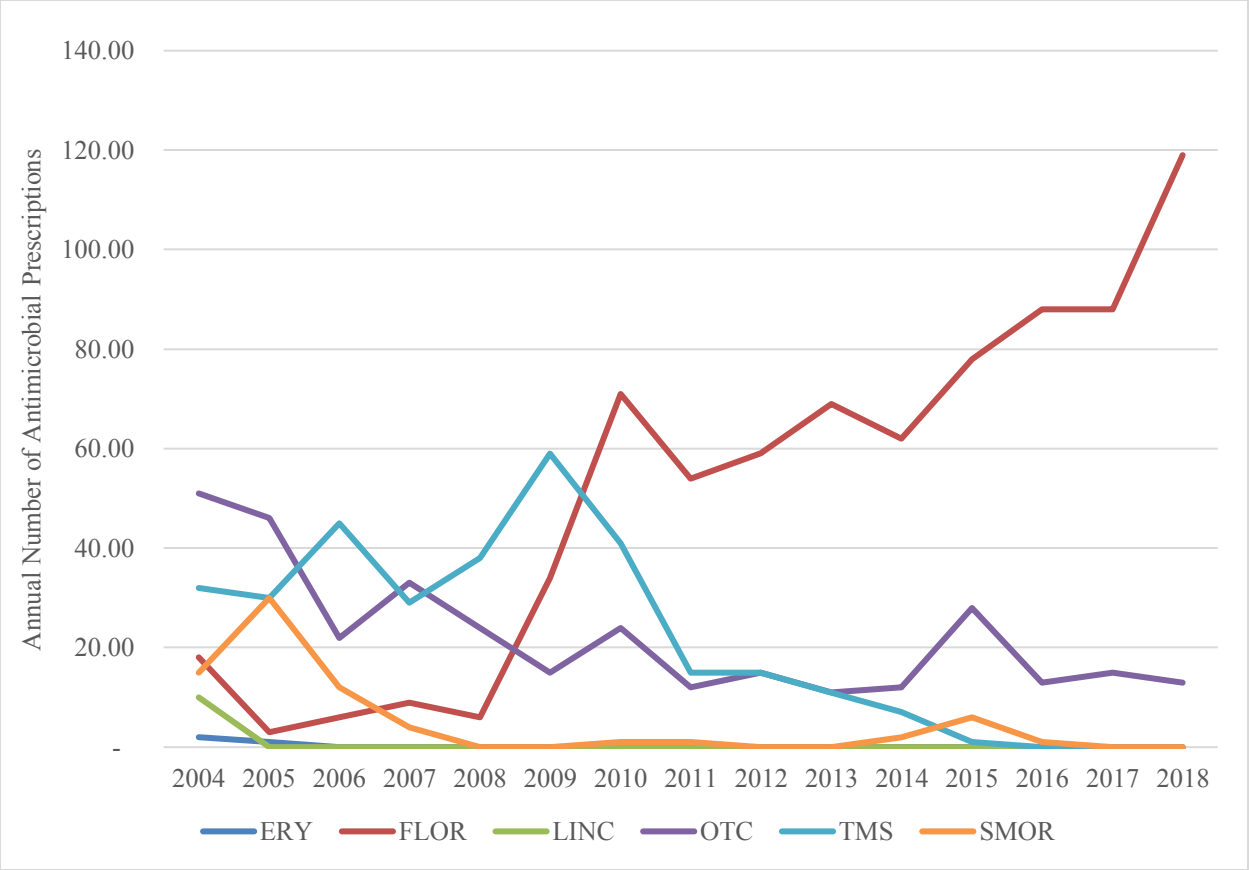

Figure A.2.8. Annual number of antimicrobial prescriptions by antimicrobial type for British Columbia salmonid aquaculture. ERY – Erythromycin, FLOR – Florfenicol, LINC – Lincomycin, OTC – Oxytetracycline, TMS - Trimethoprim + Sulfadiazine, SMOR - Sulfadimethoxine + Ormetoprim.

Table A.2.4. Norwegian annual species-specific salmonid aquaculture total slaughter biomass (kg) 2004-2018.

Year	Atlantic Salmon	Trout
2004	563,850,862	63,277,616
2005	586,356,589	58,723,746
2006	629,766,059	62,580,339
2007	744,124,942	77,673,694
2008	737,254,367	85,565,877
2009	862,304,940	74,304,353
2010	939,536,408	54,674,870
2011	1,064,868,228	58,554,081
2012	1,232,094,919	74,677,795
2013	1,168,323,615	71,552,270
2014	1,258,355,858	68,986,189
2015	1,303,345,775	73,007,387
2016	1,233,619,240	87,851,475
2017	1,236,352,762	66,999,332
2018	1,282,003,214	68,344,798

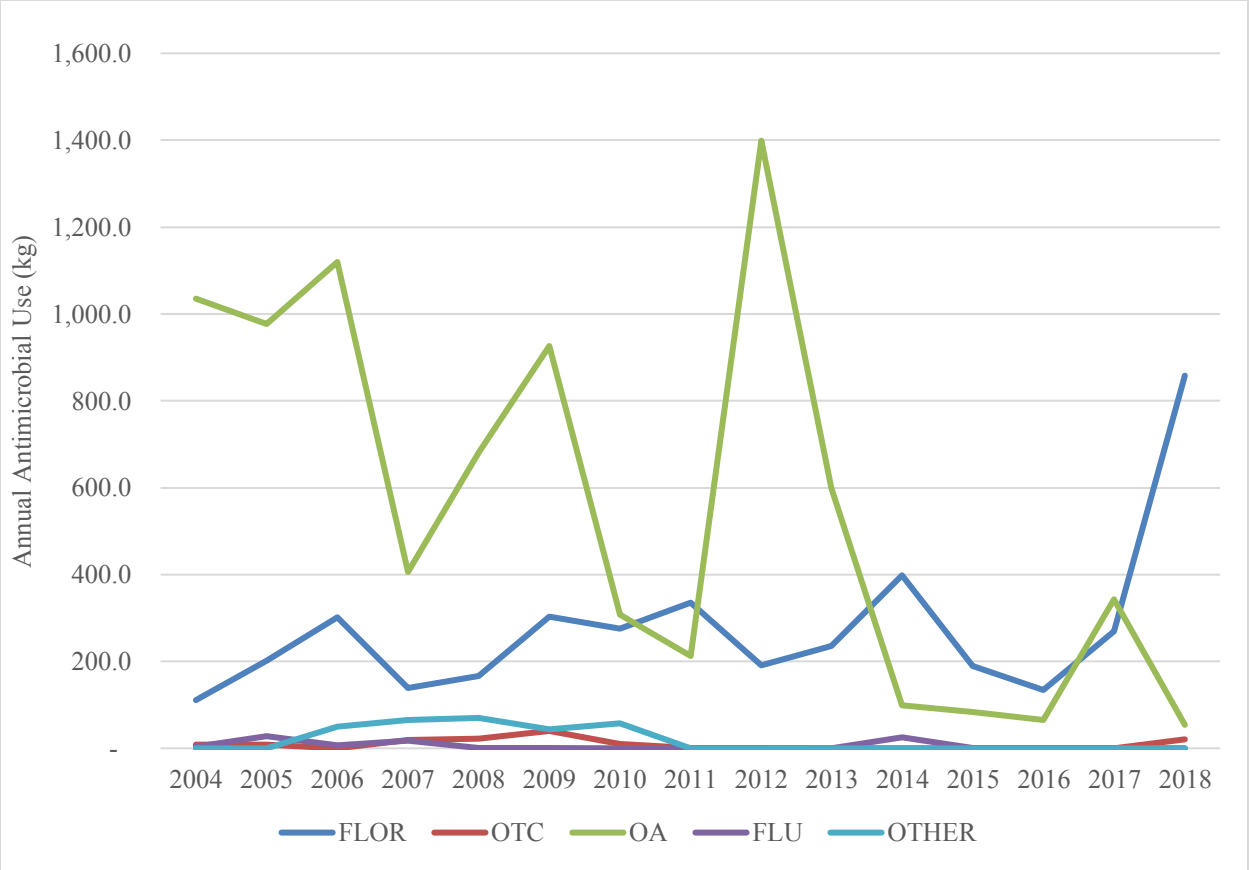

Figure A.2.9. Norwegian annual total unadjusted salmonid aquaculture antimicrobial use (AMU) (kg) 2004-2018. FLOR – Florfenicol, OTC – Oxytetracycline, OA – Oxolinic Acid, FLU – Flumequine. Other antimicrobials include spectinomycin + lincomycin.

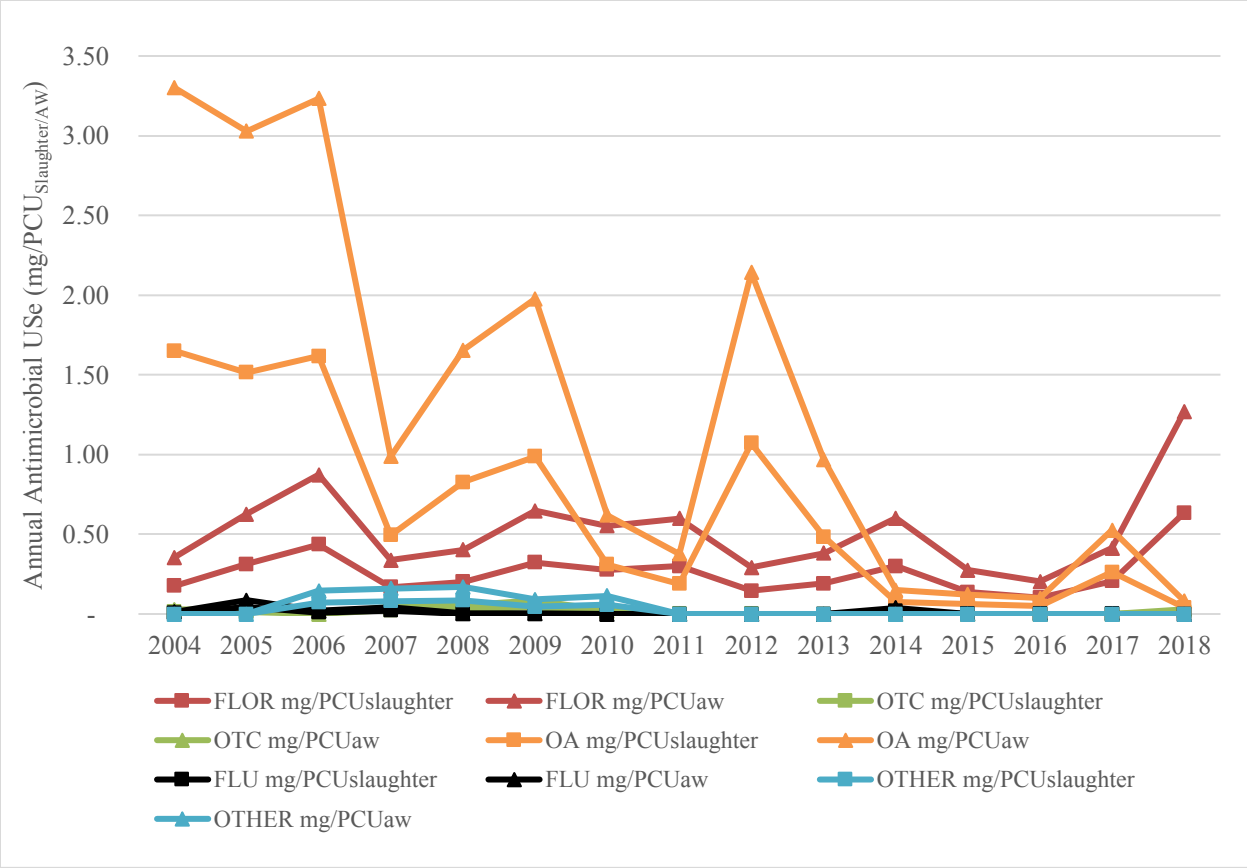

Figure A.2.10. Drug-specific annual biomass-adjusted salmonid aquaculture antimicrobial use (AMU) levels for Norway (mg/PCU$_{Slaughter, AW}$). PCU – Adjusted Population Correction Unit, AW – Average weight, FLOR – Florfenicol, OTC – Oxytetracycline, FLU – Flumequine, OA – Oxolinic Acid. Other includes spectinomycin + lincomycin.

Table A.2.5. Chilean annual species-specific salmonid aquaculture total slaughter biomass (kg) 2004-2018.

Year	Atlantic Salmon	Pacific Salmon	Trout
2004	357,547,640	91,781,000	125,841,000
2005	380,360,910	102,148,000	119,368,000
2006	346,600,000	72,900,000	138,000,000
2007	325,880,520	115,572,070	161,317,000
2008	388,847,000	92,389,000	149,411,000
2009	204,013,000	120,605,000	149,558,000
2010	123,233,000	123,380,000	220,244,000
2011	264,354,000	160,679,000	224,459,000
2012	399,678,000	164,504,000	262,767,000
2013	493,463,000	147,003,000	145,625,000
2014	644,459,000	158,949,000	151,773,000
2015	621,884,000	154,109,000	107,109,000
2016	532,225,000	110,980,000	84,607,000
2017	614,173,000	164,193,000	76,960,000
2018	669,237,000	174,595,000	80,069,000

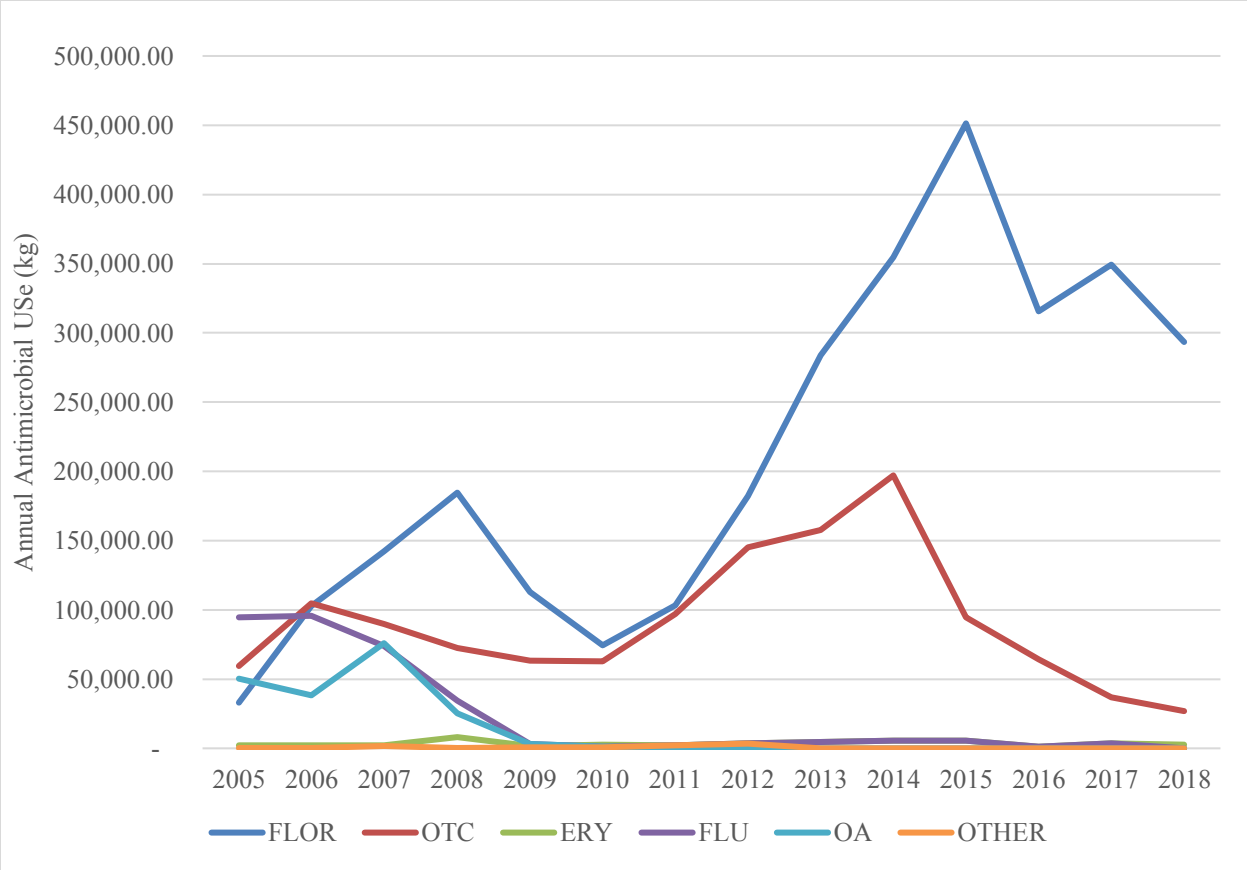

Figure A.2.11. Chilean annual drug-specific salmonid aquaculture antimicrobial use (AMU) (kg) 2005-2018. FLOR – Florfenicol, OTC – Oxytetracycline, OA – Oxolinic Acid, FLU – Flumequine, ERY – Erythromycin. Other antimicrobial drugs include Trimethoprim + Sulfadiazine and amoxicillin. Chilean AMU data were missing for 2004.

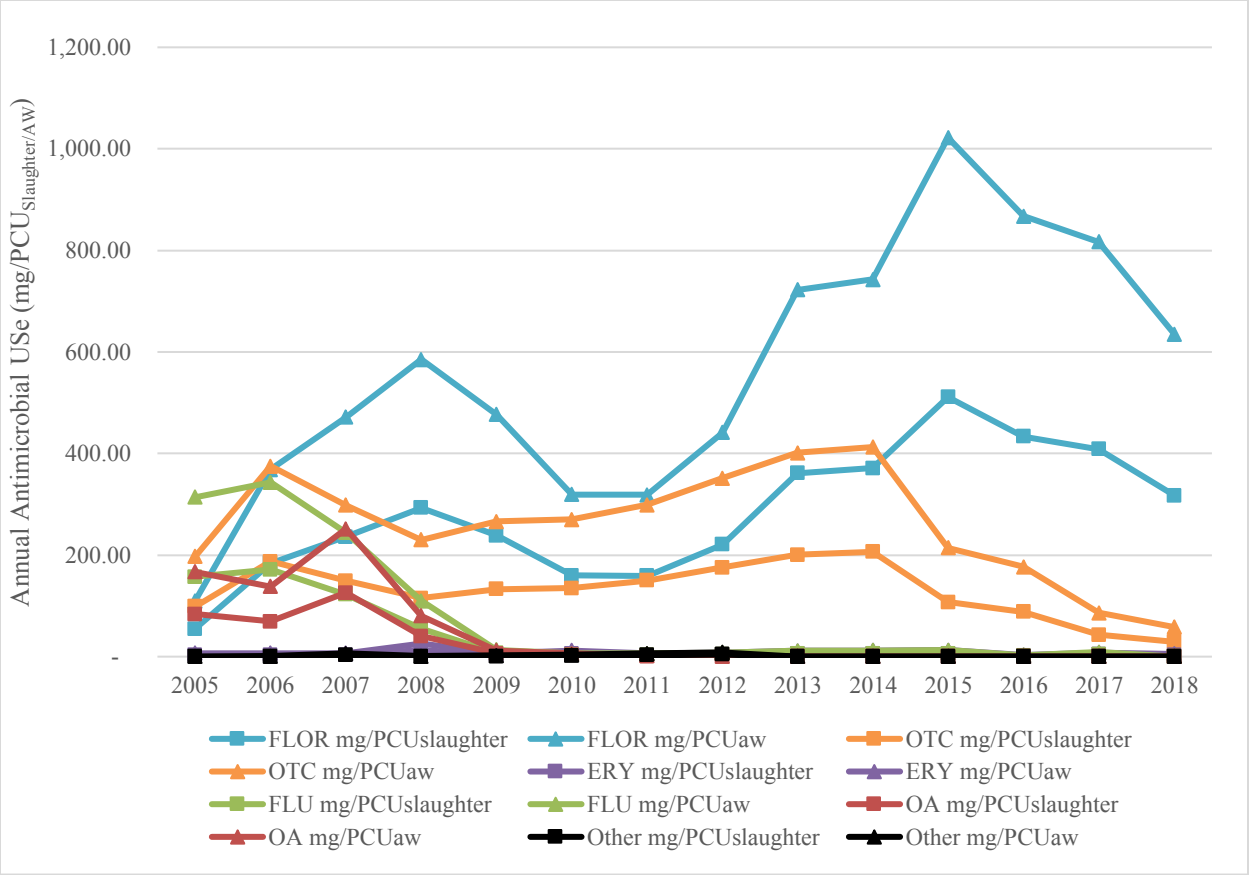

Figure A.2.12. Drug-specific annual biomass-adjusted salmonid aquaculture antimicrobial use (AMU) levels for Chile (mg/PCU$_{Slaughter, AW}$). PCU – Population Correction Unit, AW – Average weight, FLOR – Florfenicol, OTC – Oxytetracycline, ERY – Erythromycin, FLU – Flumequine, OA – Oxolinic Acid. Other includes trimethoprim + sulfadiazine and amoxicillin.

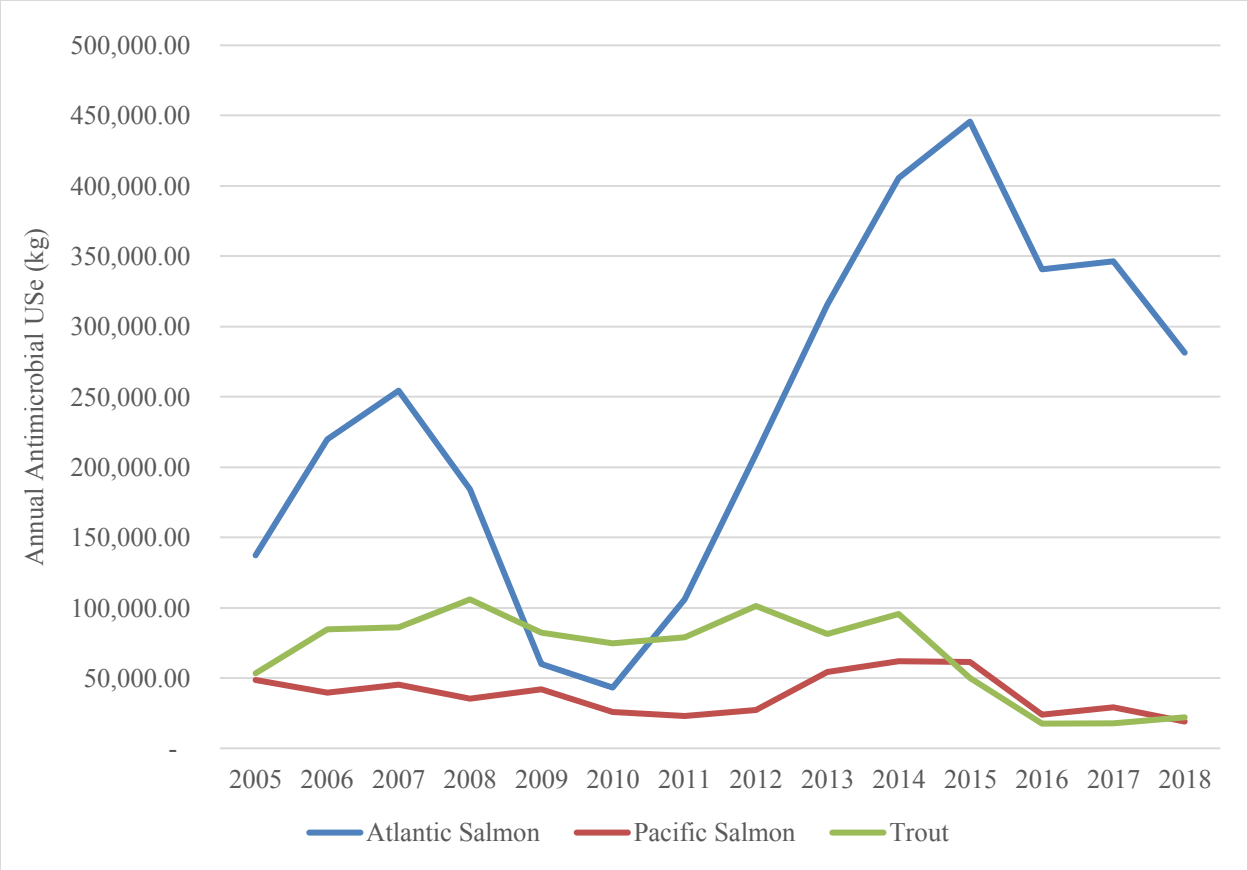

Figure A.2.13. Chilean annual species-specific salmonid aquaculture antimicrobial use (kg) (AMU) 2005-2018. Chilean antimicrobial use data missing for 2004.

Table A.2.6. United Kingdom annual species-specific salmonid aquaculture total slaughter biomass (kg) 2004-2018.

Year	Atlantic Salmon	Trout
2004	158,099,000	15,285,000
2005	129,823,000	12,458,000
2006	131,973,000	12,981,000
2007	130,104,000	15,128,000
2008	128,744,000	13,089,500
2009	144,663,000	14,929,000
2010	154,633,100	13,593,500
2011	158,309,000	12,152,000
2012	162,548,000	14,591,000
2013	163,518,000	12,466,000
2014	179,397,040	12,706,780
2015	172,146,260	14,838,610
2016	163,134,520	13,851,060
2017	189,707,000	13,041,358
2018	156,025,000	11,859,170

Table A.2.7. British Columbian biomass-adjusted drug-specific salmonid aquaculture antimicrobial use using the indicators milligrams of active ingredient per population correction unit average weight and slaughter ($mg/PCU_{AW/Slaughter}$) 2004-2018. Other includes erythromycin and lincomycin.

	Florfenicol		**Oxytetracycline**		**TMS**		**SMOR**		**Other**	
Year	mg/PCU_{AW}	mg/PCU Slaughter	mg/PCU_{AW}	mg/PCU Slaughter	mg/PCU_{AW}	mg/PCU Slaughter	mg/PCU_{AW}	mg/PCU Slaughter	mg/PCU_{AW}	mg/PCU Slaughter
2004	2.04	1.02	608.79	304.39	8.36	4.18	5.93	2.96	33.14	16.57
2005	2.65	1.33	413.34	206.67	12.36	6.18	20.28	10.14	0.71	0.36
2006	0.67	0.34	180.42	90.21	17.38	8.69	7.97	3.98	-	-
2007	0.46	0.23	196.27	98.13	14.67	7.33	1.97	0.99	-	-
2008	1.52	0.76	115.81	57.90	15.93	7.97	0.05	0.03	-	-
2009	2.78	1.39	105.67	52.84	20.98	10.49	-	-	-	-
2010	14.67	7.34	113.51	56.75	12.55	6.28	0.54	0.27	-	-
2011	14.92	7.46	57.93	28.96	9.00	4.50	0.06	0.03	-	-
2012	17.55	8.78	105.68	52.84	2.69	1.34	-	-	-	-
2013	8.36	4.18	86.43	43.21	3.03	1.51	-	-	-	-
2014	34.74	17.37	123.71	61.85	1.87	0.93	1.23	0.62	-	-
2015	53.99	26.99	256.95	128.47	0.00	0.00	3.25	1.62	-	-
2016	57.00	28.50	50.98	25.49	-	-	2.05	1.02	-	-
2017	55.51	27.76	71.09	35.55	-	-	-	-	-	-
2018	94.38	47.19	187.39	93.70	-	-	-	-	-	-

Table A.2.8. British Columbian biomass-adjusted drug-specific salmonid aquaculture antimicrobial use using the indicators milligrams of active ingredient per adjusted population correction unit average weight and slaughter (mg/APCU$_{AW/Slaughter}$) 2004-2018. Other includes erythromycin and lincomycin.

	Florfenicol		**Oxytetracycline**		**TMS**		**SMOR**		**Other**	
Year	mg/APCU$_{AW}$	mg/APCU Slaughter	mg/APCU$_{AW}$	mg/APCU Slaughter	mg/APCU$_{AW}$	mg/APCU Slaughter	mg/APCU$_{AW}$	mg/APCU Slaughter	mg/APCU$_{AW}$	mg/APCU Slaughter
2004	1.21	0.60	361.02	180.51	4.96	2.48	3.52	1.76	19.66	9.83
2005	1.57	0.78	244.47	122.23	7.31	3.66	11.99	6.00	0.42	0.21
2006	0.39	0.19	104.47	52.23	10.06	5.03	4.61	2.31	-	-
2007	0.27	0.13	113.37	56.68	8.47	4.24	1.14	0.57	-	-
2008	0.88	0.44	66.74	33.37	9.18	4.59	0.03	0.02	-	-
2009	1.60	0.80	60.89	30.45	12.09	6.04	-	-	-	-
2010	8.46	4.23	65.47	32.74	7.24	3.62	0.31	0.16	-	-
2011	8.59	4.29	33.34	16.67	5.18	2.59	0.04	0.02	-	-
2012	10.14	5.07	61.05	30.52	1.55	0.78	-	-	-	-
2013	4.85	2.43	50.16	25.08	1.76	0.88	-	-	-	-
2014	20.04	10.02	71.35	35.68	1.08	0.54	0.71	0.36	-	-
2015	31.03	15.52	147.69	73.84	0.00	0.00	1.87	0.93	-	-
2016	32.70	16.35	29.24	14.62	-	-	1.18	0.59	-	-
2017	31.91	15.96	40.87	20.43	-	-	-	-	-	-
2018	54.20	27.10	107.61	53.81	-	-	-	-	-	-