The grey water footprint of antimicrobials used for aquaculture production

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June 2022

Master Thesis

University of Twente
Civil Engineering & Management

UNIVERSITY OF TWENTE.

Colophon

Title: The grey water footprint of antimicrobials used for aquaculture production

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June 2022

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Image on cover page: An algal bloom surrounds tilapia pens in Laguna de Bay, Philippines largest lake. PHOTOGRAPHY BY GEORGE STEINMETZ. *Retrieved from* *https://www.nationalgeographic.com/foodfeatures/aquaculture/*

Preface

This thesis is the final part of my master Civil Engineering and Management at the University of Twente. In this thesis I estimate the antimicrobial use in freshwater aquaculture and estimate the resulting grey water footprint. This thesis was completed at the University of Twente's Water Engineering and Management research group, with the supervision of Lara Wöhler and Markus Berger.

My interest in sustainability has led me towards this research project. By doing research related to pharmaceutical pollution I now even better understand the importance of sustainable industries to human health and the gravity of monitoring water quality standards. The results of this study highlight the urgent need of a decrease in antimicrobial consumption, as we are putting not only our human health in jeopardy, but also the environment around us.

I want to thank Lara Wöhler and Markus Berger for their guidance. Their experience in doing research helped me a lot and they pushed me in the right direction multiple times. Besides I want to thank my family and friends who kept supporting me in tough times.

Emiel Dolmans

Nijmegen, June 2022

Summary

Aquaculture has an increasingly important role in feeding a growing world population. However this should be done in a sustainable manner. Currently, intensification of the aquaculture sector results in more animals packed together, increasing the risks of spreading diseases. As a reaction, aquaculture producers use antimicrobials to control disease outbreaks and as treatment for afflicted fish. Unfortunately, the use of these antimicrobials results in water pollution, as effluents of aquaculture farms are discharged into the environment. Besides, with the over consumption of antimicrobials, there is the risk of the emergence of antimicrobial resitsance. The grey water footprint is an indicator to measure freshwater pollution. As the grey water footprint resulting from antimicrobial use in aquaculture has not yet been accounted for in any previous research, the objective of this study is to estimate the antimicrobial consumption in freshwater aquaculture and to determine the resulting grey water footprint. Aquaculture production volumes from the 15 largest freshwater aquaculture producing countries (China, India, Indonesia, Vietnam, Bangladesh, Myanmar, Brazil, Iran, Thailand, Philippines, Egypt, Cambodia, Nigeria, United States of America, Russian Federation) are combined with species specific antimicrobial use coefficients, resulting from a systematic literature review, to estimate the antimicrobial use in each country. Different aquaculture species categories are created, resulting in antimicrobial use data on different species in each country. The grey water footprint is estimated per individual active ingredients for each different species in each country. In conclusion, this study reports that in 2019, the global leading freshwater aquaculture producing countries, which represent 92% of global freshwater aquaculture production, are estimated to consume 12,252 tons of antimicrobials in freshwater aquaculture. Antimicrobial consumption is especially high in the Asia-Pacific region. The most commonly utilized antimicrobial class in global freshwater aquaculture, in terms of frequency of usage, was tetracyclines (70%). Oxytetracycline is the most used antimicrobial in freshwater aquaculture, with a resulting global grey water of 12,860 km^3/y. For comparison, the annual discharge of the Amazon, the worlds largest river, is around half this amount: 6,595 km^3/y. This shows the significance of antimicrobials on the global water pollution. As there is still a large knowledge gap on antimicrobial use, future research should delve into broadening the knowledge about antimicrobial consumption in freshwater aquaculture.

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Glossary

Active pharmaceutical ingredient: "An active pharmaceutical ingredient is any component that provides pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or animals" (FDA, 2017).

Antimicrobial: all agents that work against bacteria (antibacterial), viruses (antiviral), fungus (antifungal), and protozoa (antiprotozoal) are referred to as "antimicrobials". Although the terms "antibiotic" and "antimicrobial" are frequently used interchangeably, "antibiotic" originally referred to a substance produced naturally by microorganisms (fungi and bacteria) that kills or inhibits the growth of another microorganism. So, Antibiotics do not include synthetic (sulfa medicines) or semisynthetic (ampicillin) antimicrobial compounds, as well as those derived from plants (phytochemicals) or animals (lysozyme) (Sutili & Gressler, 2020).

Aquaculture: "the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms that are harvested by an individual or corporate body that has owned them throughout their rearing period contribute to aquaculture while aquatic organisms that are exploitable by the public as a common property resource, with or without appropriate licenses, are the harvest of fisheries" (FAO, 2003).

Aquaculture production: "specifically refers to output from aquaculture activities, which are designated for final harvest for consumption or other purposes, e.g. ornamental purposes. Output is reported in weight (generally in tonnes of live weight equivalent for aquatic animals, in wet weight for aquatic plants)" (FAO, 2003).

Brackishwater Culture: "the cultivation of aquatic organisms where the end product is raised in brackishwater, such as estuaries, coves, bays, lagoons and fjords, in which the salinity may lie or generally fluctuate between 0.5‰ and full strength seawater. If these conditions do not exist or have no effect on cultural practices, production should be recorded under either "Freshwater culture" or "Mariculture". Earlier stages of the life cycle of these aquatic organisms may be spent in fresh or marine waters" (FAO, 2022).

Conversion factor: "In fishery and aquaculture statistics the term conversion factor is used principally when converting the volume or weight of a product at one stage in the production chain to its volume or weight at another stage in the chain. Perhaps the most common use of conversion factors is for the conversion of the landed weight of a product to its live weight equivalent when it was removed from the water" (FAO, 2022).

Freshwater Culture: "the cultivation of aquatic organisms where the end product is raised in freshwater, such as reservoirs, rivers, lakes, canals and groundwater, in which the salinity does not normally exceed 0.5‰. Earlier stages of the life cycle of these aquatic organisms may be spent in brackish or marine waters" (FAO, 2022)

Grey water footprint: **"**the amount of fresh water required to assimilate pollutants to meet specific water quality standards. The grey water footprint considers point-source pollution discharged to a freshwater resource directly through a pipe or indirectly through runoff or leaching from the soil, impervious surfaces, or other diffuse sources" (Water footprint network, 2022).

Landed weight (net weight): **"**Weight of a product at the time of landing, regardless of the state in which the product is landed. That is, the fish may be inter alia whole, gutted or filleted. Consequently, this measure is of limited use for further analysis except where information is available on product type and homogeneity. Where more detailed analysis of the data is required, the landed weight is generally converted to a more meaningful measure often by use of a conversion factor (refer conversion factors)" (FAO, 2022).

Live weight: **"**Total weight of fish when captured or harvested, estimated as if it was alive and prior to processing" (FAO, 2022).

Mariculture: "by mariculture is understood that the cultivation of the end product takes place in seawater, such as fjords, inshore and open waters and inland seas in which the salinity generally exceeds 20‰. Earlier stages in the life cycle of these aquatic organisms may be spent in brackishwater or freshwater" (FAO, 2022).

Abbreviations

AI	Active Ingredient
AMR	Antimicrobial Resistance
AMU	Antimicrobial
APAC	Asia-Pacific region
API	Active Pharmaceutical Ingredient
ASFIS	Aquatic Sciences and Fisheries Information System
BW	Body Weight
EMA	European Medicines Agency
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration
GWF	Grey Water Footprint
OECD	The Organisation for Economic Co-operation and Development
RAS	Recirculating Aquaculture Systems
WHO	World Health Organization

1. Introduction

In this chapter, an introduction to the subject is given. The research gap is presented, resulting in the research aim and the corresponding research questions. The scope of the research is defined, and the used terminology is explained. This chapter ends with the outline of the study.

According to the United Nations (2019), the world population will increase from 7.7 billion in 2019 to 9.7 billion in 2050, and up to 10.9 billion in 2100. This will impact the global food demand. Fukase & Martin (2020) estimate that food demand will double by 2050 compared to 2006 as a result of population growth and rising income per capita, with the latter being the biggest driver. With this increase in food demand, the need for protein rich fish will rise as well (Crona et al., 2020). Aquaculture is playing an increasingly important role in the global fish production, reaching a staggering 46.0% of the total global fish production in 2018, while this was only 25.7% in 2000 (FAO, 2020). These numbers endorse the importance of aquaculture in the next decades to provide animal protein for the global population, as aquaculture "continues to grow faster than other major food production sectors" (FAO, 2018, p.17).

With this increase in demand for fish, the aquaculture sector has seen an transition towards the intensification of aquaculture systems, raising the production output (Henriksson et al., 2018). However, this intensification of the aquaculture systems increases the risk of disease outbreaks among the fish population (Henriksson et al., 2018). Although aquaculture can provide a solution to the growing demand in food, and therefore assure food security, there are concerns about the environmental impacts of aquaculture (Bostock et al., 2010; Naylor et al., 2021). With aquaculture, escaped fish influence wild fish populations by interfering with native fish populations and conveying new diseases to wild fish populations (Bostock et al., 2010). Besides, during aquaculture practices nutrient or chemical waste is released into the environment (Bostock et al., 2010; Naylor et al., 2021). This chemical waste is among other things the result of the use of antimicrobials, which are used to avoid diseases taking hold of the fish populations and treat ill fish (Van Boeckel et al., 2015). Thus, the intensification of the aquaculture sector results in higher risk of disease outbreaks, forcing aquaculture producers to use antimicrobials which pollute the environment.

"Antimicrobials are defined as pharmaceuticals that kill or inhibit the growth of microorganisms and include antibiotics, antivirals, antifungals, and antiprotozoal substances" (Henriksson et al., 2018, p. 1106). They are used as medicines to treat and prevent diseases in humans and animals. Antimicrobials differ in spectrum of activity and kill bacteria in different ways. Therefore, different diseases can be treated with different antimicrobials. Common classes of antimicrobials used in aquaculture are: tetracyclines, penicillins, macrolides, quinolones, sulfonamides, nitrofurans and amphenicols. Lulijwa et al. (2020) observed 67 different antibiotics being used in aquaculture, highlighting the importance of having regulations and supervision over these regulations, so the environment is not overloaded with antimicrobials. This is also acknowledged by Watts et al. (2017), who state that antibiotic use in aquaculture is largely dependent on local regulations, and a lack of regulations often results in overuse of antimicrobials.

As regulations vary widely across countries, antimicrobial use differs as well. In Europe, North America and Japan the use of antimicrobials is strictly regulated, and only a couple of available antimicrobials are authorized for aquaculture usage (Watts et al., 2017). Besides, as stated by Done et al. (2015), the sales data of antimicrobials used in aquaculture are available in the USA and the EU. However in the major global aquaculture producing countries that are located in Asia (FAO, 2020), antibiotic use is not regulated (Henriksson et al., 2018) and data on antimicrobial use in aquaculture is not available (Van Boeckel et al., 2015). In 2019, India and China together constitute 63% of the global aquaculture and even 71% of the global freshwater aquaculture production (FAO, 2021). In India the purchase and application of antimicrobials is entirely unregulated (Done et al., 2015) , while in China prescriptions for veterinary antibiotic use are not required (Maron et al., 2013). This lack of regulation results in the widespread use of different antimicrobials in large quantities (Lulijwa et al., 2020).

As a result of this overuse of antimicrobials, several studies reveal that antimicrobial concentrations are present in water bodies surrounding inland aquaculture farms and therewith are polluting the natural environment (Le & Munekage, 2004; Zou et al., 2011). Antimicrobials have not only been detected in water bodies such as lakes and rivers, but also in sediments (Liu et al., 2021). Because sediment particles have a significant potential to adsorb antibiotics, the degree of antibiotic exposure in sediments is usually even higher than in water (Liu et al., 2021). There are several methods to quantify water pollution by pharmaceuticals. Antimicrobial concentrations in aquatic environments are often measured by using targeted liquid chromatography–mass spectrometry (LC–MS), an analytical technique that combines liquid chromatography's physical separation capabilities with a high sensitivity mass spectrometer (Danner et al., 2019). However, when looking specifically at a certain source of antimicrobial pollution of fresh water, the load of the source can also be used to model the water pollution.

The grey water footprint is a water pollution indicator in volumetric terms, which is based on the pollutant load of a consumed product entering the environment. The grey water footprint (GWF) concept is used in this study to model water pollution by antimicrobials used in freshwater aquaculture. "The grey water footprint shows the 'appropriated waste assimilation capacity'. It is defined as the volume of water required to assimilate waste, quantified as the volume of water needed to dilute pollutants to such an extent that the quality of the ambient water remains above agreed water quality standards" (Hoekstra et al., 2011, p.21). For aquaculture, the grey water footprint of feed has been estimated by Pahlow et al. (2015). However, the grey water footprint resulting from antimicrobial use in aquaculture has not yet been accounted for.

The research objective of this study is to estimate the grey water footprint of antimicrobials related to freshwater aquaculture. The goal is to understand the magnitude of the grey water footprint on a global scale, differentiating between countries and regions and between different fish species. The grey water footprint of antimicrobials in freshwater aquaculture is examined at three spatial levels in this study. A global analysis provides a picture of the severity of water pollution caused by antimicrobial use in freshwater aquaculture. At national level the type of antimicrobials and their amount in the different fish species cultured in the countries of the scope are estimated. At consumer level, the impact of consuming aquaculture products in the Netherlands is estimated.

1.1. Research questions

The problem statement and research objective result in the following research question:

Research question

What is the grey water footprint of antimicrobials used in aquaculture production?

Sub questions

To answer this research question, the following sub questions are defined:

1. *What are the freshwater aquaculture practices in the global leading aquaculture countries?*
2. *What antimicrobials are used in aquaculture in the globally leading aquaculture countries?*
3. *What is the grey water footprint of these antimicrobials?*
4. *What is the impact of consuming aquaculture products (using the example of the Netherlands)?*

1.2. Scope

In 2019, 92% of the global freshwater aquaculture production was supplied by 15 countries: China, India, Indonesia, Vietnam, Bangladesh, Myanmar, Brazil, Iran, Thailand, Philippines, Egypt, Cambodia, Nigeria, United States of America, Russian Federation[1] (FAO, 2021). Global freshwater aquaculture production has an exponential character, the rest of the world's countries only contribute very marginally to the total global freshwater aquaculture production. Therefore this study focusses on the mentioned 15 leading producers which are assumed to be representative for the total global antimicrobial use in freshwater aquaculture.

1.3. Outline

The thesis is outlined as stated in Figure 1. It consists of six chapters: Introduction, background, research methodology, results, discussion and the conclusion. In the introduction, a general introduction to the research is given. The research problem as well as the research questions are stated. In chapter 2, the background of the key concepts is presented. A brief overview of the two key concepts: aquaculture and antimicrobials are described. Chapter 3 consists of the research methodology. The method will be further expanded upon, and the justification for the chosen method is presented. In chapter 4, the results are addressed. The analysis of the data forms the basis to answering question two, three, four and five. In chapter 5, a full discussion of the findings and its implications is presented. Chapter 6 will serve to state the conclusions, as well as limitations of the study and recommendations and/or suggestions for future research.

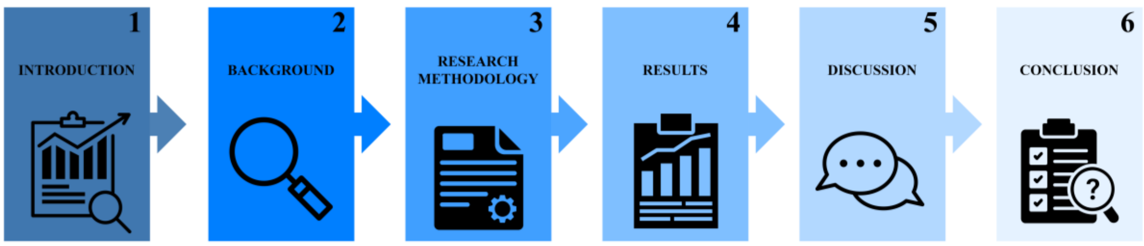


Figure 1, Thesis outline

[1] in order of freshwater aquaculture production

2. Background

In this chapter theoretical background of the research topic is given. Two key themes i.e. aquaculture and antimicrobials are discussed. Based on the discussed literature and findings, research question 1 can be answered.

2.1. Aquaculture systems

There are two ways to make a distinction on aquaculture systems. First, the distinction can be made based on culture operation, i.e. open, semi-closed and closed culture systems that each have different attributes as described below. Secondly aquaculture systems classify from extensive to intensive, depending on the animal density, the extend of inputs (feeding practices, used water, land, capital and labour), and management intensity (FAO, 1989; Lazard & Dabbadie, 2002)

2.1.1. Open, semi-closed and closed aquaculture systems

Open systems use the natural environment as aquaculture farm. Natural water bodies are used for open culture systems. There are different types of open culture systems;

- Cage culture

Stickney (2000) defined cages as "floating structures covered with materials that allow water to freely flow through while retaining confined animals". In cage culture, the fish are held in cages in natural water bodies like rivers and lakes. Depending on the country and the resources available, the cage frame can be made of metal rods, wood, bamboo or PVC pipes. The frame is covered with a mesh made of a wire mesh, mosquito cloth or nylon net.

Figure 2, Left: Cage culture near Akosombo, Ghana. Photo by Curtis Lind, 2009; Right: Tilapia farming in floating cages in Vietnam. Photo by Khaw Hooi Ling, 2007

Figure 3, Left: Adivasi farmers with fish in their small cage, Bangladesh. Photo by Sakil, 2009; Right: small scale cage culture in Magura, Bangladesh. Photo by Khaled Sattar, 2006.

- Raft/Rack culture

Rafts and Racks are used in the culture of molluscs and thus not of relevance for this research.

Figure 4, Left: Molluscs raft in Malaysia. Photo by Astacus, 2008; Right: Oyster racks. Photo by Peter Riou, 2014

- Pen culture

A Pen is defined as "a fixed enclosure in which the bottom is the bed of the water body" (SEAFDEC/IDRC International workshop on Cage and Pen culture, 1979 (Philippines) - Proceedings - Summary Report, P. 20). The difference between cage culture and pen culture is that the cage can be moved, while the pen sits on a permanent location. It is used in shallow regions along river banks and lakes. The pen thus has a benthic fauna on which fish can feed.

Figure 5, Left: A fisherman checking his river fish pen in the Tonlé Sap Rriver, Cambodia. Photo by Ted McGrath, 2019; Right: River pen on the Bassac River bank near Châu Đốc, Vietnam. Photo by Ted McGrath, 2019.

Semi-closed systems are characterized by a frequent water flux going in and out; they are commonly described as *flow-through or once-through systems* (Lawson, 1995). Water is thus seized from a natural source and guided through man-made aquaculture systems.

- Pond culture

Ponds are small land areas filled with water. Earthen ponds are the most common ponds, and are constructed entirely from soil materials. However, ponds can also be created from blocks, bricks or concrete walls. Sometimes even wooden planking is used (FAO, 1992). Ponds can have a different source of water. They can be fed by groundwater, they can be rain fed or they can be fed from a water body as a river, stream, lake, reservoir or irrigation canal (FAO, 1992). Ponds can pollute the surrounding water bodies if their discharge/waste water is not filtered and treated.

Figure 6, Left: Woman feeding fish at her pond in Jessore, Bangladesh. Photo by Yousuf Tushar, 2015; Right: Shrimp ponds near Khulna, Bangladesh. Photo by Mike Lusmore, 2012

- Rice-cum-fish paddies

Rice-cum-fish paddies are paddy fields where rice and aquatic organisms are cultured and reared to marketable sizes in rice paddies (FAO, 2022). Rice-cum-fish culture involves stocking rice fields with fingerlings to produce a fish crop in addition to the main crop of rice. Because it produces both rice and fish, this strategy provides a more efficient use of land. The fish require very little additional labour because during the attending of the rice, the fish can be simultaneously taken care of. Weeding takes less time since fish feed on the growing weeds. Besides, rice yields are also higher due to fewer insects, enhanced mineralization and distribution of nutrients, improved soil aeration and increased organic fertilization. This results in an increased farm output that would not be achieved with separate production systems (Nilsson & Blariaux, 1994).

Figure 7, Left: Feeding fish in rice field ditch, Bangladesh. Photo by WorldFish, 2008; Middle: A farmer showing his prawn capture from his gher (improved pond for combined fish and rice production) in Gabgachia village, Bagerhat district, Bangladesh. Photo by Mélody Braun, 2013; Right: A farmer stands by his integrated rice and fish farm in Laos. Photo by Jharendu Pant, 2013.

- Raceway culture (tank and raceway)

Aquaculture farms which have tanks and/or raceways increase the human control to the aquaculture operation, and conduct highly intensive farming (Pillay & Kutty, 2005). A raceway is commonly a long and narrow concrete canal, designed to provide a flow of water through the system from a supply end to an exit end, enabling dense fish populations (Stickney R. R., 2000; Pillay & Kutty, 2005). The length to width ratio of a raceway is recommended to be about 1:10, with a depth of <1.0 m. The water exchange rate is high with a raceway, e.g., one tank volume exchange every 10 to 15 minutes (Stickney R. R., 2000). The water quality in the raceway is not equally divided along the canal. The water quality is best at the entrance and then deteriorates towards the end of the raceway, as a result of metabolic wastes (Stickney R. R., 2000; Pillay & Kutty, 2005). According to Pillay & Kutty (2005), circular tanks have the advantage that by arranging the water supply and water drainage in a certain way, a whirlpool arises which rids the system of most of the waste material. In raceways and tanks often high value crop is cultured, as a result of the system needing more energy and labour input (Lawson, 1995). Trout is for instance the most common cultivated species in raceways (Pillay & Kutty, 2005).

Figure 8, Left: Cricular tanks. Photo by Todd Marsee, 2014; Right: Raceways in Michagan, USA. Photo by G Bugel, 2009

Closed culture systems are characterized by water that is reconditioned and recirculated to the culture unit(s) Lawson (1995). In closed culture systems, a barrier controls the exchange between the aquaculture farm and the natural environment. In this system, the pollution can be drastically reduced, and the interaction with native species can be avoided, resulting in a restriction on the transfer of parasites and diseases towards freshwater ecosystems

- Water recirculation systems:

Traditional aquaculture practices require lots of fresh water, which is not always available (Lawson T. B., 1995). With recirculating aquaculture systems, also called RAS, water is retained within the system during the fish´s growth stages. The water is filtered and purified within the tanks, and then reused (EUMOFA, 2020). These systems are state of the art, and are currently not used on a big scale (Stickney R. R., 2000)

Figure 9, Left: RAS in Denmark. Photo by Dennis DeLong, 2020; Right: Farmer of Bremnes Seashore at the RAS facility at Trovåg, Norway. Photo by Tommy Olsen, 2018.

All above mentioned aquaculture systems are used in the countries in the scope of this research, although not every system is used in each country. The use of small-scale farming[2] accounts for 70–80% of global aquaculture production (FAO, 2014). Earthen ponds are the most popular freshwater aquaculture system globally. However, raceway tanks, aboveground tanks, pens and cages are widely adapted as well (FAO, 2020). Besides, rice-cum-fish culture is fast spreading, particularly in Asia (FAO, 2020). In the EU, between 1,5 and 2% of total aquaculture production is produced in RAS over the period 2014-2018 (EUMOFA, 2020). In the EU, high variability is seen in the use of RAS. In the Netherlands RAS produce 100% of freshwater aquaculture production, where in large aquaculture producing country Italy it is still in the experimental stage (EUMOFA, 2021). In comparison to traditional aquaculture production systems, production with RAS is technologically complex. Therefore the global percentage of RAS is expected to be even lower as Europe is a technologically advanced region.

[2] small-scale farming is defined as a farm that produces a yearly amount of 50 tonnes of aquaculture in pond-, cage-, pen- or tank- culture

2.1.2. Intensive to extensive aquaculture systems

Aquaculture systems can also be divided according to their intensity of practices. They range from extensive to intensive. In figure 10 the different systems mentioned in chapter 2.1.1 are shown according to their level of intensity.

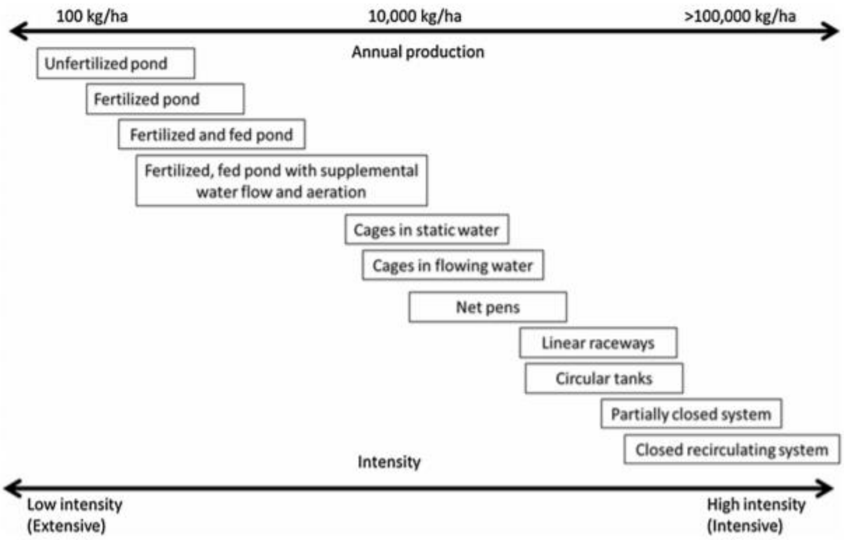


Figure 10. Aquaculture production methods adapted from Stickney (1994)

Extensive aquaculture systems, rely on the natural productivity of the water environment. The fish feed on the naturally present food in the water and thus no supplemental feeding. Nevertheless, according to the FAO (1989), fertilization may be used to stimulate the growth and production of natural food in the water. This system has thus limited inputs, which results in a relatively low productivity[3] of the system . However, because the external inputs of capital and labour are kept low, the return on labour is quite high. A good example of extensive aquaculture is rice field culture, also known as paddy field aquaculture, as the fish feed on the inputs added for the rice cultivation. Extensive aquaculture systems have the advantage of low energy inputs, and thus having a marginal effect on global warming. However, they do affect the water quality in a negative way (Ghamkhar et al., 2021).

Semi-intensive aquaculture systems, depend on the addition of fertilization and supplementary feed. However, still a large chunk of the fish's diet comes from feed naturally present in the water environment. Because the naturally present feed is enhanced, the fish densities are higher than in extensive aquaculture, and thus the production is higher (FAO, 1989). With the higher densities come disadvantages as well, with a higher risk of diseases resulting in higher mortality rates. This results in the semi-intensive aquaculture system being more labour intensive in relation to extensive aquaculture systems

Intensive aquaculture systems lean almost only on supplementary artificially formulated feed. The highest densities of fish are used in this system. According to FAO (1989), the system is managed by the application of lots of inputs (mainly feeds, fertilizers, lime, and pesticides). This system is the most labour intensive, and cost more to be set up, monitor and operate. Production is highest in intensive aquaculture. Recirculating aquaculture systems (RAS) are a good example of intensive aquaculture.

[3] in kg/ha

2.2. Leading countries in aquaculture production

Using data retrieved from FishstatJ (FAO, 2021), the countries which produce the most total aquaculture and freshwater aquaculture by quantity are identified. There is a noteworthy difference in the ranking of countries with freshwater aquaculture production compared to the country ranking considering total aquaculture production (see Table 1). For instance, Norway is one of the leading producers of aquaculture on global level and the largest aquaculture producing country in Europe. However, it produces mainly marine aquaculture, not freshwater aquaculture. The same counts for the Korean Peninsula. Comparing datasets from different years (e.g. 2016 and 2019 as illustrated in table 1) displays substantial changes in the amount of output per country and year. In the last 3 years, Cambodia has seen an increase of 77% in total aquaculture production. Over the same period, Russia has increased their freshwater aquaculture production by almost 20%.

Table 1, Leading aquaculture countries by quantity in 2019 and 2016

	Aquaculture production 2016 in Tonnes (Thousands)			Aquaculture production 2019 in Tonnes (Thousands)		
Country	**Freshwater production**	**Total production**	**Percentage of freshwater production (%)**	**Freshwater production**	**Total production**	**Percentage of freshwater production (%)**
China	28 845	62 318	46	30 187	68 424	44
India	5 076	5 702	89	6 897	7 800	88
Indonesia	3 217	16 002	20	3 828	15 893	24
Vietnam	2 415	3 582	67	2 984	4 456	67
Bangladesh	1 998	2 204	91	2 271	2 489	91
Myanmar	960	1 018	94	1 029	1 082	95
Brazil	469	543	86	530	600	88
Iran	367	398	92	418	505	83
Thailand	418	963	43	417	964	43
Philippines	300	2 201	14	321	2 358	14
Egypt	276	1 371	20	309	1 642	19
Cambodia	160	173	92	290	307	94
Nigeria	307	307	100	289	290	100
USA	251	445	56	254	490	52
Russia	154	174	89	184	248	74
South Korea	26	1 859	1	34	2 406	1
Japan	35	1 068	3	32	944	3
Ecuador	29	451	6	16	696	2
North Korea	6	620	1	14	680	2
Chile	1	1 050	0	2	1 407	0
Norway	0	1 326	0	0.2	1 453	0

2.3. Antimicrobial agents

In 1928, Alexander Fleming discovered a substance - penicillin - that killed bacteria. In the next decade Fleming with the help of other researchers created a drug that revolutionized the health care and could treat people with bacterial infections. In the next years more antibiotics were discovered, with over 20 new antibiotic classes that have been produced between 1930 and 1962, becoming the corner stone of medical progress in the 20th century (Maffioli, 2013; Review on Antimicrobial Resistance, 2014). Previously serious life threatening illnesses could be treated effectively. However, the discovery of new antibiotics has slowed immensely, with only 4 new classes of antibiotics being discovered since 1962 (Maffioli, 2013; Review on Antimicrobial Resistance, 2014). This is a problem as bacteria have the characteristic to evolve and become resistant to the produced drugs, nullifying all the progress of the past age (Maffioli, 2013; Review on Antimicrobial Resistance, 2014). The current health system greatly depends on antibiotics and should antibiotics fail to work, surgery would become seriously dangerous with higher chances on infections and certain diseases which can currently be treated will return as life threatening. Therefore it is crucial that the global healthcare systems are not inhibited by resistance to antimicrobial drugs (Review on Antimicrobial Resistance, 2014). Antibiotic use results in antimicrobial resistance, and antibiotics should thus be used properly and sparsely (Maffioli, 2013; Review on Antimicrobial Resistance, 2014). Currently antibiotics are used in great quantities in the animal food industry and contribute to the emerging antimicrobial resistance problem (Marshall & Levy, 2011). As humans consume animal protein, antibiotic use in the animal food industry results in antimicrobial resistance in humans as well (O'Neill, 2015). In addition, the overuse of antimicrobials are associated with risks for human health care as antimicrobials used in human health care are similar to antimicrobials used in animal production (FAO, 2007; WHO, 2011). Especially, as antibiotic consumption is expected to rise as a result of increasing animal protein demand (Van Boeckel et al., 2015).

2.3.1. Type of antimicrobial agents

A wide range of antibiotic classes is used within aquaculture, as each class has its specific spectrum of activity. The majority of bacteria that infect fish are either gram-positive or gram-negative (Yanong, 2003). These groups are named after how they react to a gram staining procedure. Gram-positive bacteria are stained blue, while gram-negative bacteria are stained pink. Because each group has a different sort of exterior structure known as the cell wall, they stain differently. Because some antibiotics work better against gram-positive bacteria and others work better against gram-negative bacteria, this distinction is critical for the aqua farmer in the understanding of when to apply which antibiotic. Gram negative bacteria are the most common bacteria in aquaculture e.g. Aeromonas hydrophila, Aeromonas salmonicida, Flavobacterium columnare (which causes columnaris), Vibrio, and Pseudomonas species (Yanong, 2003). Streptococcus is the most common gram-positive bacteria that causes fish disease (Yanong, 2003).The following antibiotic classes are used mostly used in aquaculture:

Tetracyclines are a broad-spectrum antimicrobial drugs and are effective against a wide range of fish pathogens (Noga, 2010). Tetracyclines is the most commonly used drugs for almost all bacterial diseases of fish, as it is cheap and widely available (Park et al., 2012). Tetracyclines are barely metabolised by fish, resulting in almost the entire antibiotic dose being excreted or defecated into the aquatic environment (Park et al., 2012). Besides, the effect of the drug is weaker in freshwater in comparison to marine water (Park et al., 2012). Tetracyclines must be used in a dose 2±5 times higher than the dose for humans and other warm-blooded animals, as fish absorb the drugs on a much lower rate (Burka et al., 1997)

Potentiated Sulfanomides are used for decades (Burka et al., 1997) Since 1935 sulfanomides are used in humans, domestic animals and aquaculture species to treat bacterial infections (Suzuki & Hoa, 2012). Potentiated Sulfanomides combine two antimicrobial drugs, sulfanomides and diaminopyrimidines, such as trimethoprim or ormethoprim (Burka et al., 1997; Park et al., 2012). They have a broad spectrum of activity, and are being used against all major bacterial infections (Burka et al., 1997; Park et al., 2012). Potentiated Sulfanomides are absorbed through the gills, which could be very useful in some situations, as water medication is suitable (Park et al., 2012).

Quinolones have been widely used since the mid 1980s and are considered a broad spectrum antibiotic (Burka et al., 1997). Quinolones are used against all frequently seen diseases, like vibriosis, classical furunculosis, atypical furunculosis and yersiniosis (Burka et al., 1997). As quinolones are effective at low dosage and are relatively cheap, they are used in freshwater aquaculture as well as marine aquaculture(Park et al., 2012; Suzuki & Hoa, 2012).

Macrolides are an important class of antibiotics, active against Gram-positive and Gram-negative cocci (González De La Huebra & Vincent, 2005). Erythromycin is the most important member of the macrolides family, and has been in use in medicines since 1952 (González De La Huebra & Vincent, 2005). Macrolides are the most effective drug against diseases produced by mycoplasma species , and are also effective against mycobacteria, chlamydia and rickettsias (González De La Huebra & Vincent, 2005; Park et al., 2012). This makes it a good substitute and addition to penicillin, as macrolides have a wider spectrum (González De La Huebra & Vincent, 2005; Park et al., 2012).

Amphenicols are extensively used synthetic antibiotics in aquaculture and are active against Gram-positive and Gram-negative bacteria in addition to other groups of micro-organisms (Guo et al., 2021)**.** Chloramphenicol, florfenicol and triamphenicol are the most common antibiotics in this family. Chloramphenicol is used as a very effective drugs against many anaerobic organisms (Hanekamp & Bast, 2015). However, as a result of various harmful and lethal side effects, many countries have banned the use of chloramphenicol in aquaculture and livestock farming including large aquaculture producing countries like the United States, European Union and China (Guo et al., 2021; Li et al., 2019)**.** Hence, florfenicol and triamphenicol are used as substitutes to chloramphenicol (Guo et al., 2021)**.** Being widely available, low of cost and having a broad spectrum, amphenicols are used on a large scale and illegal use is common (Guo et al., 2021)**.**

Penicillins are a commonly used group of antimicrobials in aquaculture, resulting from the fungus Penicillium notatum and belong to the beta-lactam family (Lobanovska & Pilla, 2017; Park et al., 2012). Penicillins work by inhibiting the formation of peptide bridges, preventing new peptidoglycan formation in the bacterial cell wall (Lobanovska & Pilla, 2017; Park et al., 2012). Penicillins are most effective against gram-positive bacteria i.e. Streptococcus (Yanong, 2003).

2.3.2. Administration of drugs

The two main routes of administering antimicrobials in aquaculture are water medication and medicated feed (Noga, 2010).

Water medication (bath treatment) is the most traditional method of drugs administration. This method is mostly applied when the fish suffer from ectoparasites (Park et al., 2012). The drugs are applied and distributed to the water and are absorbed by the fish through the epithelia of the gills, skin and mucosa (Park et al., 2012). This method is however wasteful and relatively costly. Besides, (depending on the aquaculture type) the drugs directly enter the aquatic environment, potentially also affecting organisms the drug is not intended for (Park et al., 2012).

Medicated feed is an alternative method in which the antibiotic is mixed with the feed. The liquid antibiotic is mixed with the feed and within minutes the antibiotic is absorbed into the feed. With this method, waste is reduced which is better for the environment. However, to be effective, fish need to actively feed from the medicated feed. Ill fish tend eat less or eat not at all, while the healthy fish eat more, making this method not always effective (Noga, 2010). Therefore, medicated feed is often used prophylactic (Park et al., 2012). Medicated feed also has its negative effects on the environment as "uneaten food accumulates on the floor, along with medicated faeces containing unmetabolised drugs such as oxytetracycline" (Park et al., 2012, p. 191).

2.3.3. Antibiotic use in the different aquaculture systems

Antibiotics used in different aquaculture systems have different effects on the environment. The most common way of administering antimicrobials in aquaculture is mixing the antimicrobial agent with food (FAO, 2005). In open systems, the food surplus, and fish faeces that are excreted and contain antimicrobials which are not absorbed remain in the natural environment surrounding the fish farms. Antimicrobials remain in the water and the sediment for extensive period of time (FAO, 2005; O'Neill, 2015). Previous studies point out that 70-80 percent of applied antimicrobials end up in the environment (FAO, 2005). As a result, the biodiversity of the surroundings is altered, and antimicrobial resistant bacteria are found in the sediments in several studies (Watts et al., 2017). In closed aquaculture systems, the farming process is isolated from the natural environment. In these systems, process control and water recycling ensure a more sustainable practice, greatly decreasing the amounts of waste and antibiotics reaching the natural environment (Watts et al., 2017). However, these systems are not used often. Integrated aquaculture is also a common way of practicing aquaculture. In this production system aquaculture is combined with another livestock farming operation. Manure and excess feed from livestock like pigs, cattle and poultry, which are located above or adjacent of the fish ponds is directed towards the fish pond, acting as fish feed (Little & Edwards, 2003). As the livestock is generally intensively farmed with application of antimicrobials, these antimicrobials are transferred towards the fish (Hoa et al., 2011; Neela et al., 2015). Hoa et al. (2011) and Neela et al. (2015) indicate that integrated aquaculture systems are sources of antimicrobial resistant genes. Although recovery of waste as aquafeed seems like a sustainable alternative to current fishmeals and fishoils, the transmission of antimicrobial resistant bacteria can pose a danger to human and environmental health (Watts et al., 2017).

2.3.4. Legislation concerning veterinary drug use

Veterinary drug use is greatly controlled and regulated in developed countries, as illustrated by the maximum residue levels set in food by the FDA and EMA and the prohibition of certain antimicrobials (Lulijwa et al., 2020). This is however not the case in developing countries, which together produce most of the aquaculture products, resulting in large differences in antimicrobial use patterns (Watts et al., 2017). Statistics on antimicrobial use within aquaculture are limited and only a few countries register the amount of antimicrobials that are used (Sapkota et al., 2008). Below, the legislation of the countries from the defined scope are discussed.

In China, the Chinese government has approved 13 antibiotics for use in the Chinese aquaculture sector (doxycycline, enrofloxacin, florfenicol, flumequine, neomycin, norfloxacin, oxolinic acid, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, thiamphenicol, and trimethoprim)(Liu et al., 2017). Besides, the Chinese authorities also created a list of 12 antibiotics which are banned: amoxicillin, chloramphenicol, chlortetracycline, ciprofloxacin, erythromycin, furazolidone, gentamycin S, oxytetracycline, penicillin G, streptomycin, sulfamerazine S, and sulfisoxazole (Liu et al., 2017). However, some of these banned antibiotics are still being used (Yuan & Chen, 2012)

In India there is a difference in control on fish produced for domestic use and fish produced for exports. fish produced for exports is mainly marine fish, like frozen shrimps. This is a regulated market as fish exports need to fulfil the European and USA health requirements. The Government of India’s Marine Products Export Development Authority (MPEDA) regulates this aquaculture production. The MPEDA has listed 20 antibiotics and pharmacologically active substances that are banned, which inlcude: Chloramphenicol, Nitrofurans, Neomycin, Sulphamethoxazole, Sulfonamide drugs (except approved Sulfadimethoxine, Sulfabromomethazine and Sulfaethoxypyridazine) and Fluroquinolone. However, fish produced for export is only around 10% of total fish production. The market of fish for domestic consumption is highly unregulated and there is significant use of antibiotics. Antibiotics should be sold on prescriptions, however are seen to be sold by pharmacists over the counter in different parts of India. Besides, antibiotics labelled for use for animals (not fish) or humans are used often in Indian aquaculture (Bhushan, Khurana, & Sinha, 2016).

Indonesia prohibited the use of antibiotics as growth promoter since 1 January 2018, in accordance with the Law No. 41/2014 regarding Farming and Health (ReAct, 2018). The Indonesian government also banned several antibiotic substances; Tetracyclines, Nitrofurans, Chloramphenicol, Dimetridazole/Metronidazole, Nifurpirino and Florfenicol (ASEAN, 2013). The Indonesian government has established a monitoring program to control the antibiotic use in 2004, however this mainly focuses on the shrimp industry. This is the result of shrimps, which are mainly produced for export, being rejected for export towards the USA and EU. Antibiotics are still used for Aquatic products cultivated for the domestic market, as effluent from freshwater aquaculture activities is still seen to have a relatively high level of oxytetracycline, indicating oxytetracycline use during aquaculture practices (Hidayati et al., 2021). Another study by Damayanti et al. (2019) showed that catfish were resistant against ampicillin and the now prohibited tetracycline and chloramphenicol, resulting from antibiotic use (Damayanti et al., 2019). Yet, there are farmers in Indonesia that do use natural compounds as substitutes for antibiotics. According to a study by Caruso et al. (2014), from 379 fish farms in central Java, 80% uses herbal therapy. However, data on antimicrobial use amounts is lacking in Indonesia

Vietnam´s Ministry of Agriculture and Rural Development (MARD) has created a list (Circular No. 10/2016/TT-BNNPTNT) of veterinary drugs permitted or banned for aquaculture use in Vietnam. On this list are 24 banned chemical agents, which include chloramphenicol, florfenicol, nitrofurans, enrofloxacin, fluoroquinolones and ciprofloxacin (Huong et al., 2021; Lulijwa et al., 2020). Antibiotic use in aquaculture officially requires a prescription, however illegal use of banned substances still occurs widely (Lulijwa et al., 2020).

Bangladesh seems not to have a specific legislation in place that controls the use of antibiotics in aquaculture. However, the Aquaculture Medicinal Products' guidelines of the Department of Fisheries state that it is compulsory to acquire a prescription ahead of applying antibiotics (Faruk et al., 2021).

Myanmar banned the following antibiotics; Nitrofurans, Chloramphenicol, Oxolinic acid and Dimetridazole/Metronidazole (ASEAN, 2013). However, the control on illegally sold antibiotics is lacking, resulting in antibiotic misuse. Despite this, a study showed that antibiotics are reported to be used on only 6% of reviewed aquafarms (Belton et al., 2017). Information on use amounts is lacking.

In Brazil, the National Programme for the Control of Residues and Contaminants only authorizes the use of oxytetracycline and florfenicol in the aquaculture sector (Gazal et al., 2020). The preventive use of antibiotics is currently legal in Brazil (Valenti et al., 2021), and it should be expected to occur often. Studies show the use of amoxicillin, tetracycline, sulphonamide and chloramphenicol in tilapia (Ferreira et al., 2021), while another study found residues of oxytetracycline, florfenicol and enrofloxacin in tilapia produced in Brazil (Bortolotte et al., 2021). Quesada et al. (2003) also found erythromycin besides the already mentioned ampicillin and tetracycline in fish samples sold at market in Brazil.

In Iran, antimicrobial resistance has not yet been fully explored, resulting in no legislation. In the past years, the first studies towards residues in fish tissue have been conducted. They conclude that antibiotics are used on a wide scale, as a result of no supervision and monitoring in the aquaculture industry (Rafati, 2017). Adel et al. (2017) showed that Oxytetracycline, Enrofloxacin, and Florfenicol antibiotics are used on a large scale by aquaculture farmers, and concluded that the peak concentrations of Oxytetracycline are equal to studies conducted in other countries. Another study by Barani & Fallah (2015) showed the large use of tetracyclines and florfenicol and low occurrence of fluoroquinolones and sulfonamides in rainbow trout in trout farms. However, there is no data available on dosages or use practices for aqua farms.

In **Thailand**, the Ministry of Agriculture and Cooperatives and the Department of Fisheries banned the use of chloramphenicol, nitrofurans and beta-lactams except amoxicillin in 2002 (Lulijwa et al., 2020), while the Tai food and drug administration has approved the use of oxytetracycline, tetracycline, sulphadimethozine, trimethoprim, sulphadimethoxine-ormethoprim and amoxicillin (Lulijwa et al., 2020)

In the Philippines, different antibiotics are banned, with Nitrofurans, Chloramphenicol and Dimetridazole/Metronidazole the most commonly used antibiotics in aquaculture that are banned in the Philippines (ASEAN, 2013; Cruz-Lacierda et al., 2000; Karunasagar, 2020). Cruz-Lacierda et al. (2008), found in their survey that antibiotics are no longer used in shrimp farming. This contradicts earlier research by Cruz-Lacierda et al. (2000) in which they found that antibiotic use was widespread. According to Sogma et al (2012), most farmers follow regulations on antibiotic use, like minimum withdrawal periods and the maximum residue levels in fish tissue. However, national regulations for aquaculture are relatively new, and more regulations should be added (Somga et al., 2012). Despite the relatively new regulations, aqua-farmers themselves have already embraced environmentally friendly cure methods like probiotics, which are widely available. In shrimp-grow out farms, antibiotics are even not used anymore (Somga et al., 2012).

In Egypt. as in in a lot of the developing countries, antimicrobial use is unregulated. Antibiotics can be bought in pharmacies, but also in general stores and even in some market stalls along the roadside. As a result of insufficient monitoring and assessing of antibiotic residues in fish tissue, the USA and EU have restrictions on Egypt fish imports due to health concerns (Shaalan et al., 2018). Therefore almost all produced aquaculture products are produced for the domestic market.

Cambodia lacks legislation with respect to antibiotic use. With no regulations and limited know how on how to use antibiotics in a responsible way, misuse and overuse is highly likely (Kruijssen et al., 2018). It is reported that antibiotics meant for human use are applied by aquafarmers (Kruijssen et al., 2018).

In Nigeria, legislation on antibiotic use in aquaculture is lacking, and antibiotics are easily available without veterinary prescription and supervision (Kabir et al., 2004).

In the United States of America, the FDA has licensed four antimicrobials that may be applied in aquaculture: florfenicol, oxytetracycline and combinations of sulfadimethoxine and ormethoprim (FDA, 2011).

The Russian Federation seems not to have any legislation for antimicrobial use in aquaculture. There are also no official statistical data and/or research papers available indicating how much antibiotics are used in aquaculture. As there are no regulations on antibiotic use, Russian aquaculture farmers can apply antibiotics in unlimited amounts. Russian aquaculture farmers must meet the Russian veterinary standards on antibiotic residue limits in the final product. However, the lack of a proper control system results in a marginal effect on antibiotic use. Therefore it is expected that antibiotics are used on a large scale in aquaculture.

3. Methodology

In this chapter, the methods that are used to obtain the answers to the stated research questions in chapter 1 are presented.

3.1. General approach

In the following sections the methods used to assess the grey water footprint of antimicrobial pollution from aquaculture are presented. This study adopts the methodology proposed by Schar et al. (2020) to estimate the antimicrobial use, by combining species specific antimicrobial use coefficients (mg/kg) resulting from a systematic literature review with yearly aquaculture production volumes (kg/y). As the resulting data from the literature review is not always presented in the form of species specific antimicrobial use coefficients, formulas to get these species specific antimicrobial use coefficients are presented in chater 3.2.2.. After combining species specific antimicrobial use coefficients (mg/kg) with yearly aquaculture production volumes (kg/y), the resulting species specific antimicrobial use (mg/y) is calculated. The grey water footprint (km^3/y) is estimated based on the water quality standard, as proposed by Hoekstra et al. (2011). The grey water footprint will be defined per species for each country in the scope.

3.2. Antimicrobial quantities used per fish species (species specific antimicrobial use)

To answer research question 2, "*What antimicrobials are used in aquaculture in the global leading aquaculture countries?",* data on antimicrobial use in aquaculture is essential. However this data is scarce and limited research has been done towards antimicrobial use practices, let alone antimicrobial use practices in different countries (Van Boeckel et al., 2015). To make an estimation of the type and quantities of antibiotics used per fish species, this study combines data on aquaculture production per species and country with the estimated average use of antibiotics per kg of body weight per aquaculture species, also called species specific antimicrobial use coefficients.

3.2.1. Aquaculture production volumes

Aquaculture production data is retrieved for the year 2019 from FishstatJ (FAO, 2021) (www.fao.org/fishery/statistics/software/fishstatj/en) for the following 15 countries: China, India, Indonesia, Vietnam, Bangladesh, Myanmar, Brazil, Iran, Thailand, Philippines, Egypt, Cambodia, Nigeria, United States of America, Russian Federation. The FAO database presents production volumes per species, covering high level of details such as presenting data for different trout types. To be able to present results per species, this data is aggregated, following a method proposed by Schar et al. (2020). Aquaculture species categories are defined as follows: carp, catfish, tilapia, trout, pacu and crustaceans. These categories are the most common freshwater aquaculture fish species in the researched countries. There are two other species categories defined, which are then excluded for this study; Molluscs and Algae. They are excluded for this study, because no antimicrobial are used in their production (Rodgers And Furones 2009). Following the approach by Schar et al. (2020), the difference between the total fresh water production and the sum of the eight species categories defined above was assigned to a ninth category, "other", to account for all freshwater aquaculture, for each country. Common aquatic invertebrates are crustaceans and molluscs. In this research it was chosen to add the aquatic invertebrates to the crustaceans category, as choosing the molluscs category would neglect them in this study. In Appendix I and II, all the aquaculture production volumes per country and species category are presented.

3.2.2. Species specific antimicrobial use coefficients

As a next step, the antimicrobial use per aquaculture species category need to be estimated as these are commonly not readily available (Van Boeckel et al., 2015). A systematic literature search identified a number of studies that present different forms of antimicrobial use data.

This literature review was inspired by a search as proposed by Schar et al. (2020). The search terms for "antimicrobial" (antimicrobial; antibiotic; veterinary medicine; VMP; chemicals; drugs; pharmaceuticals; active pharmaceutical ingredient, api; active ingredient, ai); "use" (use; usage; consumption; application; amount; quantity); and "aquaculture" (aquaculture; aquatic; fish; fish farming; aquafarming; freshwater; shellfish; crustaceans), combined with the name of the countries considered in this study were entered into SCOPUS, Web of Science, Google Scholar and PubMed. Peer reviewed literature and grey literature between 2000 and 2020 were considered. From the literature found through the outlined method, further publications were identified by going through the referenced literature. These were additionally considered.

This literature review revealed antimicrobial use data for the different countries, in three different formats: (i) the mean antimicrobial consumption (grams of active ingredient) per tonne of harvested aquaculture animal in all the studied farms[4], (ii) estimates of total antibiotic consumption (pounds) per aquaculture species category, and (iii) surveys on percentages of aquaculture farms using antimicrobials. Data sources used in this study are shown in Table 2. In case of Bangladesh, data from Rico et al. (2013), Hazrat et al. (2016), Jabed Hasan et al. (2020) and Singha et al (2020) is combined into an aggragated species specific antimicrobial use coefficient.

Table 2, Antimicrobial data types

Country	(i) Active ingredient per tonne of harvested aquatic animal	(ii) total antibiotic consumption (pounds) per fish species	(iii) Surveys on percentages of aquaculture farms
China	Rico et al. (2013)	NA	Yuan and Chen (2012)
India	NA	NA	NA
Indonesia	NA	NA	NA
Vietnam	Rico et al. (2013)	NA	NA
Bangladesh	Rico et al. (2013)	NA	Hazrat et al. (2016) Jabed Hasan et al. (2020), Singha et al (2020)
Myanmar	NA	NA	NA
Brazil	NA	NA	NA
Iran (Islamic Rep. of)	NA	NA	NA
Thailand	Rico et al. (2013)	NA	NA
Philippines	NA	NA	Sogma et al (2012)
Egypt	NA	NA	NA
Cambodia	NA	NA	NA
Nigeria	NA	NA	Oyebanji et al. (2018)
United States of America	NA	Benbrook et al. (2002)	NA
Russian Federation	NA	NA	NA

NA: Not Available

[4] Data was converted to the aggregation level of aquaculture species categories defined for this study.

Because of the distinctive data formats, different calculations are needed to determine comparable species specific antimicrobial use coefficients, which are defined as the average use of active pharmaceutical ingredient (API) per kg of aquatic body weight per aquaculture species category (mg/kg). Three methods are presented to calculate the species specific antimicrobial use coefficients: (i) Active ingredient per tonne of harvested aquatic animal, (ii) Total antibiotic consumption (pounds) per fish species, (iii) Surveys on percentage of aquaculture farms. For the countries where no data is available a fourth method is presented (iv).

(i) Active ingredient per tonne of harvested aquatic animal

In their study Rico et al (2013) report the results of a survey on antimicrobial use for different species in four countries. The species match the species categories created in this study. Rico et al (2013) present their data in grams of active ingredient per tonne of harvested product. Which is the same format as the species specific antimicrobial use coefficients. This data can thus directly be used to be multiplied with the production volumes.

(ii) Total antibiotic consumption (pounds) per fish species

Benbrook et al. (2002) present their data in total applied antibiotic mass in pounds per fish species. The total applied antibiotic mass is the total weight of the drug, so this consists of the active ingredient(s) in the product, as well as the rest of the substances that make out the antibiotic. The used antibiotics (Romet 30, Terramycin 200 and aquaflor) each consist thus of 1 or 2 active ingredients and some filler materials to make a consumable drug. The presented data of Benbrook et al. (2002) has to be recalculated to the species specific antimicrobial use coefficients. First the active pharmaceutical ingredient (API) per mg of antibiotic is determined using formula 1 and the information presented in table 3, converting pounds to mg in the process.

$$API = total\ applied\ antibiotic\ mass\ \times \frac{\%\ API}{100} \quad (1)$$

Where API is the active pharmaceutical ingredient in mg, total applied antibiotic mass in mg, and $\%\ API$ is the percentage of the active pharmaceutical ingredient on the total applied mass

Table 3, active pharmaceutical ingredients in antimicrobials allowed for aquaculture in the USA

Antibiotic	% of Active pharmaceutical ingredient
Romet 30©	25% Sulfamethoxine
Romet 30©	5% Ormetoprim
Terramycin 200©	44.09% Oxytetracyline
Aquaflor©	50 % florfenicol

Now that the API (mg) per fish species is determined, the species specific antimicrobial use coefficients can be determined by dividing the total amount of active pharmaceutical ingredient per fish species by the aquaculture production volume in the corresponding year, also called the population correction unit of each fish species (PCU)

$$\alpha_{s;1} = \frac{API}{PCU} \quad (2)$$

Where α_s is the antimicrobial use coefficients for species (s), API is the active pharmaceutical ingredient in mg and PCU is the population correction unit in kg of body weight.

(iii) Surveys on percentage of aquaculture farms

The third method uses surveys on percentage of aquaculture farms that use antimicrobials to calculate the species specific antimicrobial use coefficients. The average use amount of antibiotic in 2019 is defined by combining the recommended dose of applied mass with the amount of adjacent days the antimicrobial is applied and the number of times the antimicrobial is applied in a year. The amount of times an antimicrobial is applied depends on the amount of applications per culture cycle and the amount of culture cycles in a year. By combining the amount of antibiotic with the average percentage of active pharmaceutical ingredient, the average active pharmaceutical ingredient is determined. However not all farms apply antimicrobials. The percentage of aquaculture farms that use antimicrobials is combined with the average antimicrobial use on the farms that use antimicrobials to estimate the average antimicrobial use on the total aquaculture farm population of the countries. As the antimicrobials are applied during the culture cycle of the fish, and not at their final harvest weight, a correction factor should be used in order to not overestimate the antimicrobial use, called the weight ratio of harvest.

$$\alpha_{s;2} = D \cdot d \cdot n \cdot c \cdot \frac{\% F}{100} \cdot WR \tag{3}$$

Where, D is the recommended daily dose (mg/kg BW); d is the amount of days an antimicrobial is applied; n is the number of applications per culture cycle; c is the number of culture cycles in a year; $\% F$ is the percentage of farms applying antimicrobials; and WR is the weight ratio of harvest;

The following assumptions have been made:

- According to Rico et al (2014), aquaculture farmers applied antibiotics 1-3 times per production cycle. For this study an average of 2 antibiotic rounds per culture cycle has been taken.
- It is estimated by the researcher that on average the application of antibiotics happens at 30% of their final body weight. This is based on research by Phan et al. (2009), which reported more antibiotic application in the early to mid-months of the production cycle, as there is a higher disease occurrence in the first months of the culture cycle. The weight ratio is therefore set at 0.3
- The number of farming cycles differs per cultured species and the environment where the fish species are cultivated. The average farming cycles are displayed in table 4. (Rico, 2014)

Table 4, farming cycles of different fish species

Fish Species	Average farming cycles a year
Carp	1
Catfish	2
Tilapia	2
Crustaceans	2

The recommended doses and their accompanying days of appliance are taken from literature and displayed in table 5.

Table 5, recommended antimicrobial doses for aquaculture

Drug class	Compound	Recommended daily dose active ingredient	Recommended number of days	Data Source
Aminoglycoside	Neomycin	10 mg/kg BW	5-7 days	Rico et al. (2013)
Aminoglycosides	Kanamycin	50 mg/kg BW	5-7 days	Rico et al. (2013)
Aminoglycoside	Gentamicin	10 mg/kg BW	5-7 days	Rico et al. (2013)
Aminoglycoside	Streptomycin	10 mg/kg BW	5-7 days	Rico et al. (2013)
Amphenicols	Florfenicol	10-20 mg/kg BW	3-5 days	Rico et al. (2013)
Amphenicols	Chloramphenicol	10-20 mg/kg BW	3-5 days	Rico et al. (2013)
Diaminopyrimidine	Ormetoprim	10 mg/kg BW	5-7 day	Rico et al. (2013)
Diaminopyrimidine	Trimethoprim	10 mg/kg BW	5-7 day	Rico et al. (2013)
Macrolide	Erythromycin	40-75 mg/kg BW	5-10 days	Boonyaratpalin (1990); Liao et al. (1996)
Nitrofuran	Furazolidone	10 mg/kg BW	3-6 days	Kou et al. (1988); Liao et al. (1992)
Nitroimidazole	Metronidazole	50 mg/kg BW	5 days	Janet Whaley & Ruth Francis-Floyd (1991)
Penicillin	Amoxicillin	40-80 mg/kg BW	5-7 days	Arthur et al. (2000)
Penicillins	Ampicillin	50 mg/kg BW	4-5 days	Rico et al. (2013)
Penicillin	Benzypenicillin	50 mg/kg BW	4-5 days	Rico et al. (2013)
Quinolones	Enrofloxacin	10-20 mg/kg BW	5-7 days	Rico et al. (2013)
Quinolones	Oxolinic acid	10-20 mg/kg BW	4-7 days	Yuan and Chen (2012)
Quinolones	Tyrosine	10-20 mg/kg BW	5-7 days	Rico et al. (2013)
Quinolones	Norfloxacin	10 mg/kg BW	5-7 days	Arthur et al. (2000)
Sulfonamides	Sulfadimethoxine	50 mg/kg BW	5-7 days	Rico et al. (2013)
Sulfonamides	Sulfamethoxazole	50 mg/kg BW	5-7 days	Rico et al. (2013)
Sulfonamides	Sulfadiazine	25 mg/kg BW	5 days	EMEA(EMEA, 1995b)
Sulfonamides	Sulfamethazine/ Sulfadimidine	25 mg/kg BW	5 days	EMEA/MRL/(EMEA, 1995b)
Sulfonamides	sulfaquinoxaline	50 mg/kg BW	5-7 days	Rico et al. (2013)
Tetracycline	Oxytetracycline	60-80 mg/kg BW	7-10 days	Rico et al. (2013)
Tetracycline	Chlortetracycline	75 mg/kg	10 days	EMEA/MRL/023/95 (EMEA, 1995a)
Tetracycline	Doxycycline	20 mg/kg BW	3-5 days	Xu et al. (2020)

(iv) Countries lacking data

Due to lacking data, not all species specific antimicrobial use coefficients can be calculated. In this case, the mean species specific antimicrobial use coefficients of all the calculated countries has been taken. For each country, the drugs legislation has been considered. Non-authorized APIs have been removed for each country from the mean species specific antimicrobial use coefficients. There is one exception in Egypt, where the species specific antimicrobial use coefficients of Nigeria has been taken, as these countries share a lot of characteristics. In both countries antimicrobial use is highly unregulated, antibiotics can be bought in market stalls along the roadside and products are produced for the domestic market. Besides, semi-intensive aquaculture production in earthen ponds is dominating the aquaculture sector in both countries.

3.2.3. Total antimicrobial use

Combining aquaculture production volumes with species specific antimicrobial use coefficients results in the total antimicrobial use per country:

$$L_{s,c} = P_{s,c} \cdot 1000 \cdot \alpha_{s,c} \tag{4}$$

Where $L_{s,c}$ are the antimicrobial use volumes for species (s) in country (c); $P_{s,c}$ are the production volumes per species (s) and country (c); and α_s are the antimicrobial use coefficients for species (s) in country (c).

It should be mentioned that the production volumes are from 2019, while the antimicrobial use coefficients are the result of a literature study over the years 2000-2020. Because there is a lack of data, the data of these different years is combined in this study.

To get better insight in the different antimicrobials used, and their importance to human health, the WHO list of medically important antimicrobials is used to categorize the used antimicrobials within global aquaculture.

3.3. Grey water footprint of antimicrobials used in aquaculture per country

The grey water footprint is estimated per individual active ingredients for each different species in each country. To estimate the total grey water footprint of each fish species in each country, the critical load is taken, which is defined as “the load of pollutants that will fully consume the assimilation capacity of the receiving water body” (Hoekstra et al., 2011, p. 188). To calculate the grey water footprint of a country per kg of produced aquaculture product, the total grey water footprint is devided by the total production in concerned country. The grey water footprint is defined from the following formula (Hoekstra et al., 2011):

$$GWF = \frac{L}{c_{max} - c_{nat}} \tag{5}$$

Where GWF is the grey water footprint in $m^3\ y^{-1}$; L is the pollutant load in kg y^{-1}; c_{max} is the maximum acceptable concentration in kg m^{-3}; and c_{nat} is the natural concentration in the receiving water body in kg m^{-3}.

pollutant load L

To derive the pollutant load, the annual antimicrobial use of each country defined in chapter 3.2 is used. However, not all the antibiotics end up in the environment. It is estimated in a previous research that 70-80% of the antibiotics used end up in the environment (FAO, 2005). Therefore, the determined total antimicrobials for each country are multiplied by the factor 0.75.

Maximum acceptable concentration

The maximum acceptable concentration is defined with the predicted no-effect concentration (PNEC), which indicates a maximum concentration of a chemical at which the chemical is expected not to harm the environment. A variety of PNEC values for antimicrobials can be found in literature. For this study, the PNEC values defined by the members of the AMR Industry Alliance have been chosen, as this list includes most of the antibiotics used within aquaculture. The PNEC values are based on published scientific methodologies. The AMR Industry Alliance has defined two different values for each antibiotic; The PNEC-Environment (PNEC-ENV) represents the maximum value at which ecological species are protected, “and incorporate assessment factors consistent with standard environmental risk methodologies” based on the methodology of Brandt et al. (2015) (AMR Industry Alliance, 2018, p. 1). The PNEC-Minimum Inhibitory Concentration (PNEC-MIC) represents the maximum concentration at which the antibiotic is expected to be protective against antibiotic resistance promotion and the values are the result of following the methodology of Bengtsson-Palme and Larsson (2016). The lowest value of the two is chosen during this study. Not all antibiotics used in aquaculture are defined by the AMR Industry Alliance, and missing values are completed with studies by Bergmann et al. (2011) and De Liguoro et al. (2010).

Table 6, PNECs of antimicrobials used in aquaculture

Antibiotic class WHO (2011)	Active Pharmaceutical Ingredient (API)	PNEC-ENV (µg/L)	PNEC -MIC (µg/L)	Lowest Value (µg/L)	Source
Aminoglycoside	Neomycin	0.03	2.0	0.03	AMR Industry Alliance (2018)
Aminoglycosides	Kanamycin	1.1	2.0	1.1	AMR Industry Alliance (2018)
Aminoglycoside	Gentamicin	0.20	1.0	0.2	AMR Industry Alliance (2018)
Aminoglycoside	Streptomycin	N/A	16	16	AMR Industry Alliance (2018)
Aminoglycosides	Apramycin sulfate	N/A	N/A	1000	Bergmann et al. (2011)
Amphenicols	Florfenicol	N/A	2.0	2.0	AMR Industry Alliance (2018)
Amphenicol	Chloramphenicol	N/A	8.0	8.0	AMR Industry Alliance (2018)
Cephalosporins	Cephalexin	0.08	4.0	0.08	AMR Industry Alliance (2018)
Diaminopyrimidines	Ormetoprim	N/A	N/A	0.50	Bergmann et al. (2011)
Diaminopyrimidines	Trimethoprim	100	0.50	0.50	AMR Industry Alliance (2018)
Macrolides	Erythromycin	0.50	1.0	0.50	AMR Industry Alliance (2018)
Nitrofuran	Furazolidone	N/A	N/A	1.3	Bergmann et al. (2011)
Nitroimidazole	Metronidazole	N/A	0.13	0.13	AMR Industry Alliance (2018)
Penicillins	Amoxicillin	Testing On-Going	0.25	0.25	AMR Industry Alliance (2018)
Penicillins	Ampicillin	0.87	0.25	0.25	AMR Industry Alliance (2018)
Penicillin	Benzypenicillin	N/A	0.25	0.25	AMR Industry Alliance (2018)
Polymyxin	Colistin	N/A	2.0	2.0	AMR Industry Alliance (2018)
Quinolones	Enrofloxacin	1.9	0.06	0.06	AMR Industry Alliance (2018)
Quinolones	Oxolinic acid	N/A	N/A	0.23	Bergmann et al. (2011)
Quinolones	Norfloxacin	120	0.50	0.50	AMR Industry Alliance (2018)
Quinolones	Ciprofloxacin	0.45	0.06	0.06	AMR Industry Alliance (2018)
Quinolones	Levofloxacin hydrate	Testing On-Going	0.25	0.25	AMR Industry Alliance (2018)
Rifamycins	Rifampicin	N/A	0.06	0.06	AMR Industry Alliance (2018)
Sulfonamides	Sulfadimethoxine	50	N/A	50	AMR Industry Alliance (2018)
Sulfonamides	Sulfamethoxazole	0.60	16	0.60	AMR Industry Alliance (2018)
Sulfonamides	Sulfadiazine	720	N/A	720	AMR Industry Alliance (2018)
Sulfonamides	Sulfamethazine/sulfadimidine	N/A	N/A	0.0152	Bergmann et al. (2011)
Sulfonamides	Sulfaquinoxaline	N/A	N/A	2.0	De Liguoro et al. (2010)
Tetracyclines	Oxytetracycline	18	0.50	0.50	AMR Industry Alliance (2018)
Tetracyclines	Chlortetracycline	N/A	N/A	0.03	Bergmann et al. (2011)
Tetracyclines	Doxycycline	Testing On-Going	2.0	2.0	AMR Industry Alliance (2018)

Natural concentration in the receiving water body

The natural concentration of antimicrobials in the environment is 0.

Grey water footprint

Therefore, the grey water footprint is estimated with the following formula:

$$GWF = \frac{L \cdot 0.75}{PNEC} \tag{6}$$

3.4. Grey water footprint of consuming farmed fish in the Netherlands

To understand the impact of consuming imported farmed fish in the Netherlands, the grey water footprint per kg of fish species are used. With the use of trade data, the impact of consuming farmed fish in the Netherlands is captured. Fish trade data for the Netherlands is obtained from The European Market Observatory for Fisheries and Aquaculture (EUMOFA)(EUMOFA Statistics). The previously determined freshwater species categories were created here as well, so the trade data can be used in combination with the calculated grey water footprint to calculate the impact of consuming farmed fish in the Netherlands.

The import data is used to determine the percentage of in which country an consumed aquaculture product is produced. As the Netherlands is historically a country of trade, the fish species a consumer buys in the supermarket can be produced in different countries, depending on the supermarket or the moment a consumer buys the product. Each aquaculture species consumed in the Netherlands is thus divided over the different countries it might originate from, with its corresponding percentage of occurance and the accompanying water footprint in that country. This method results in an average grey water footprint per fish species, in which the different grey water footprint of each country of origin has been taken into account.

With the use of consumption data, the impact of an average Dutch consumer is also captured. Consumer data of the Netherlands is obtained from the Bureau Risicobeoordeling & Onderzoek (BuRO), who requested the data of the Dutch population from the most recent food consumption survey executed by the Front Office Voedsel- en Productveiligheid of the Rijksinstituut voor Volksgezondheid en Milieu (RIVM). The data consists of a recent food consumption survey from 2012-2016 among people aged 1 to 79 years. The average Dutch consumer consumes 16.2 grams of fish, crustaceans and shellfish a day (RIVM, 2020). The results are shown in table 56 in the Appendix. In table 7, the relevant freshwater species groups, with their average consumption are displayed.

Table 7, Consumption of freshwater fish products in the Netherlands by 1-79 year olds (VCP 2012-2016; n=4,313)

Fish species	Average consumption of the Dutch (g/day)	Average yearly consumption of the Dutch (g)
Carp	Not eaten in the Netherlands	0
Catfish	0.9	328.5
Crustaceans	1.1	401.5
Pacu	Not eaten in the Netherlands	0
Tilapia	0.1	36.5
Trout	0.2	73

The average consumption is shown in landed weight, so clean for consumption. To calculate the grey water footprint, the fish consumption in live weight is needed, as the antibiotic use is also presented in live weight. To convert landed weight to live weight, conversion factors are used. Conversion factors can be very specific, often defined for a specific species caught or grown in a particular area or country and processed by a specific method (FAO, 2022). As it is unclear in what state (whole fish; headed and gutted; fillets) the aquatic products arrive in the Netherlands, an overarching conversion factor has been chosen based on figure 11.

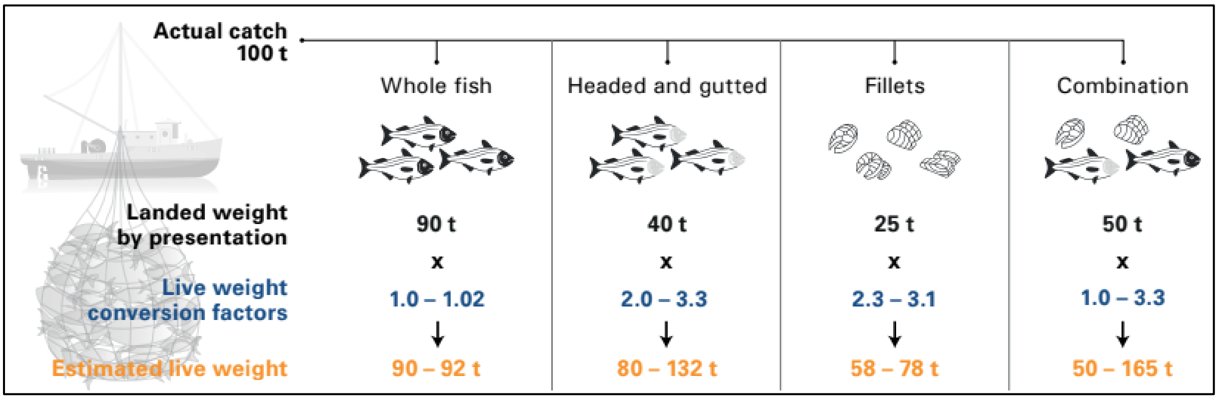


Figure 11, conversion factors based on unknown fish species and unknown processing (EJF, 2021)

The average of 1.0-3.3 is taken. This results in a conversion factor of 2.15. This is almost exactly the same conversion factor as in the report of the Dutch fish board where they state that Dutch people consumed 9.3 kilos of fish, shellfish and shellfish per person, which they convert with conversion tables to 20 kilos of live weight (Nederlands Visbureau, 2021). Therefore, the chosen average conversion factor of 2.15 seems plausible. In table 8, the average live weight consumption of the Dutch is presented.

Table 8, Consumption of freshwater fish products in the Netherlands by 1-79 year olds

Fish species	Average daily consumption of the Dutch (grams of landed weight)	Average yearly consumption of the Dutch (grams of landed weight)	Average yearly consumption of the Dutch (grams of live weight)
Carp	Not eaten in the Netherlands	0	0
Catfish	0.9	328.5	706
Crustaceans	1.1	401.5	863
Pacu	Not eaten in the Netherlands	0	0
Tilapia	0.1	36.5	78
Trout	0.2	73	157

By combining the average yearly consumption with the trade data, the grey water footprint of consuming freshwater aquaculture products of an average Dutch consumer is captured.

4. Results

In this chapter, the results of the proposed methods are presented.

4.1. Antimicrobial use

In this chapter the antimicrobial use of the investigated countries is presented. The antimicrobials are categorized based on their antibiotic class as mentioned on the WHO's critically important antimicrobial list (WHO, 2011). This provides a clear overview as many different antibiotics were found in this study, as well as gives an insight in the antibiotics used that are important for human health care.

4.1.1. Total antimicrobial use of each country

In 2019, the global leading freshwater aquaculture producing countries, which represent 92% of global freshwater aquaculture production, are estimated to consume 12,252 tons of antimicrobials in freshwater aquaculture (Table 50). Antimicrobial consumption mostly occurs in the Asia-Pacific (APAC) region, with China and India being the largest antimicrobial consumers. This can be contributed to the fact that most of the aquaculture production is situated in Asia. The five countries with the biggest share of antimicrobial consumption all reside in the Asia-Pacific region: China (78.3%), India (9.3%), Indonesia (2.1%), Vietnam (2.0%) and Myanmar (1.3%). The first four countries also produce the most freshwater aquatic animal products in 2019: China (59.9%), India (13.8%), Indonesia (7.7%) and Vietnam (6.0%). Bangladesh (4.6%) produces more freshwater aquatic animal products in 2019 compared to Myanmar (2.1%). However, due to its relative low antimicrobial use, Bangladesh is surpassed by Myanmar for total antimicrobial consumption. In figure 12, total antimicrobial use in freshwater aquaculture is illustrated. Tetracyclines are the most used antimicrobials in most of the researched countries. Indonesia is an exception, as the Indonesian government banned tetracyclines. However it should be noted that illegal use of oxytetracycline is still common.

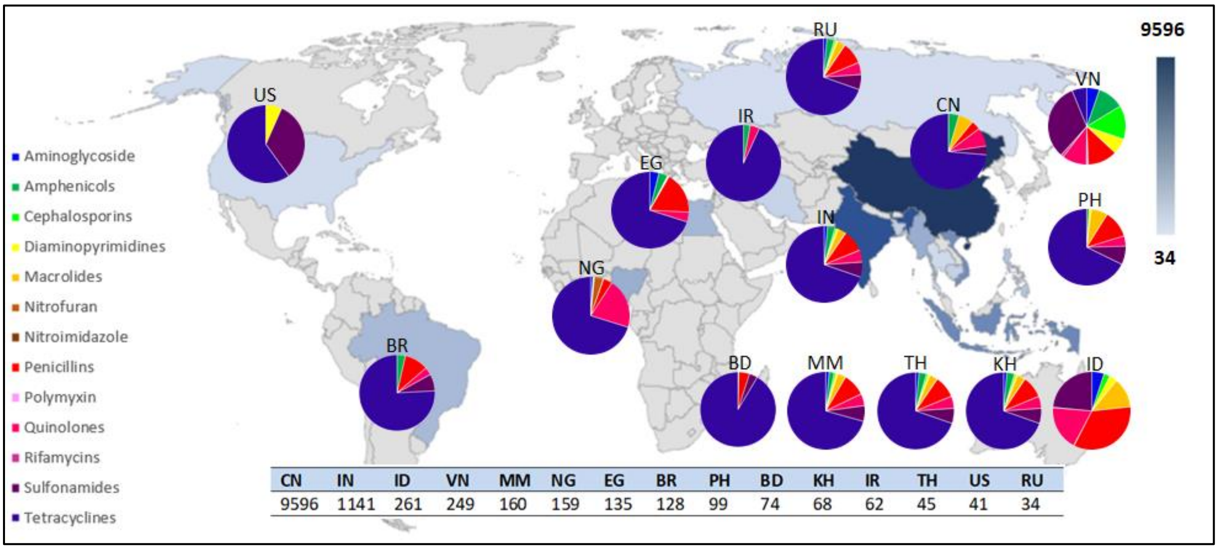


Figure 12, Total antimicrobial use in aquaculture in tonnes per year. Country abbreviations: CN: China; IN: India; ID: Indonesia; VN: Vietnam; MM: Myanmar; NG: Nigeria; EG: Egypt; BR: Brazil; PH: Philippines; BD: Bangladesh; KH: Cambodia; IR: Iran; TH: Thailand; US: United States; RU: Russia.

What becomes clear from figure 12 is that there is a large difference in total antimicrobial use per country. However, also the antimicrobial use per kg produced aquaculture product differs significantly. In Nigeria for instance, aquaculture farmers use on average 16 times the amount of antibiotics per kg of fish (552 mg/kg) that Bangladeshi aquaculture farmers use (33 mg/kg). This can be partly explained by the lack of cultivation of crustaceans in Nigeria, which use relatively small amounts of antimicrobials. Besides, the lack of legislation on antimicrobial use in Nigeria results in a high average antimicrobial consumption.

Egypt (437 mg/kg), China (320 mg/kg) and Philippines (307 mg/kg) are also at the high end of the antimicrobial consumption spectrum. For the Philippines this can be explained by the fact that the Philippines aquaculture production system is focussed on marine crustaceans, in which antimicrobials are no longer used (Somga et al., 2012). Freshwater aquaculture focuses on finfish, especially tilapia (81%), and freshwater crustaceans are not produced. The culture of finfish in combination with relatively new regulations results in high antimicrobial use. In Egypt aquaculture production also focuses on finfish, with carp (47%) and tilapia (40%) as the most produced aquaculture species, automatically resulting in a higher average antimicrobial use. Besides, the last two decades have shown a transition from traditional extensive aquaculture systems to semi-intensive and intense aquaculture system (Adeleke et al., 2021; Ali et al., 2020). Also, Antimicrobial use is highly uregulated and antimicrobials can be bought in market stalls along the roadside. As a result, overuse of antimicrobials has resulted in restrictions on fish imports by the USA and EU health departments (Shaalan et al., 2018). The backbone of Chinese aquaculture historically consists of the polyculture of different species of carp. These cyprinids (carps) are mostly cultivated in ponds, which is often semi intensive or intensive cultivation (Hu et al., 2021; Wang et al., 2015). The combination of intensive cultivation of finfish and the widespread use of banned substances results in high antimicrobial use.

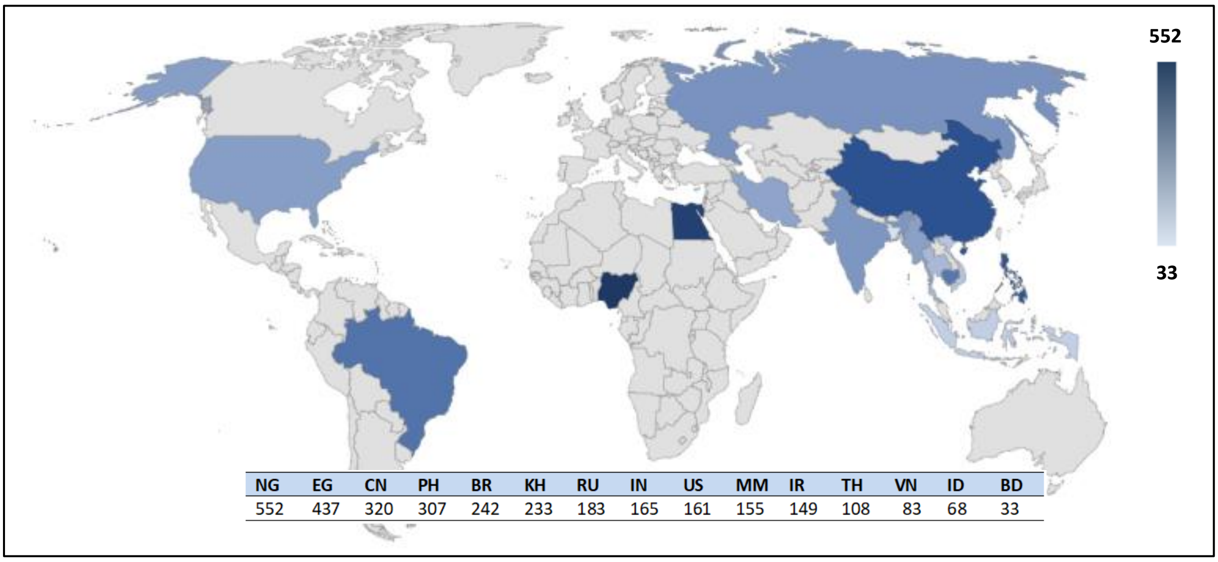


Figure 13, Antimicrobial use in mg antimicrobials per kg of fish (mg/kg)[5]. Country abbreviations: NG: Nigeria; EG: Egypt; CN: China; PH: Philippines; BR: Brazil; KH: Cambodia; RU: Russia; IN: India; US: United States; MM: Myanmar; IR: Iran; TH: Thailand; VN: Vietnam; ID: Indonesia; BD Bangladesh.

It is also remarkable that the United States have a relatively high average antimicrobial consumption for a developed country. This can be explained by the fact that the data that have been used for the United States comes from a study out of 2002. Since then, more attention has been given towards limiting antimicrobial use. It should be noted that data from Rico et al. (2013)[6] is relatively low

[5] It is important to note that this antimicrobial use data shows no information on selection of drugs/drug categories, variations in drug potencies, resistance selection pressures, or importance of antimicrobials for human health. However, it does provide an important overview of the use practices in different countries. With the calculated grey water footprint from the next chapter, pollution differences resulting from the use of distinct antimicrobials are taken into account with the use of PNECs.

[6] The paper from Rico et al. (2013) is used to get antimicrobial use data of the following countries: Bangladesh, Vietnam, Thailand and China.

compared to other data gathered. This might be the result of Rico et al. researching aquaculture farms producing for foreign markets, which often have having more stringent regulations and thus lower antimicrobial use. On average, 245 mg/kg of antimicrobials are used globally in freshwater aquaculture.

4.1.2. Antimicrobial classes

The World Health Organization (WHO) has created a list of antimicrobials which are used in human health care, and categorized the medicines into three groups: critically important, highly important and important (WHO, 2011). Besides, antimicrobials that are used for veterinary use only are stated as well. In the conducted literature study, 31 different types of antibiotics have been found to have been used in aquaculture. Figure 14 shows the different antimicrobial classes, most commonly used in freshwater aquaculture.

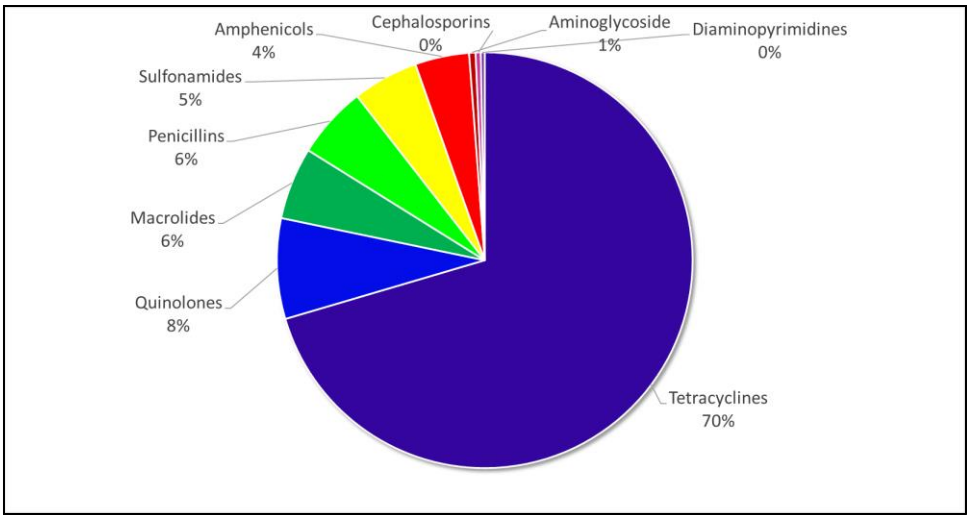


Figure 14, Antimicrobial classes used in freshwater aquaculture

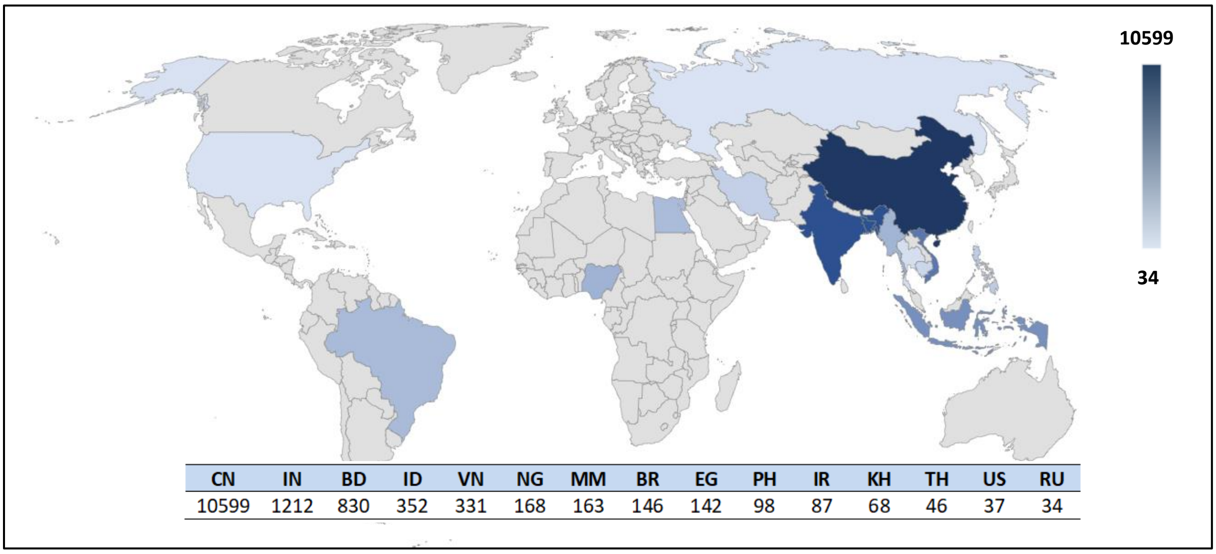
The most commonly utilized antimicrobial classes in freshwater aquaculture, in terms of frequency of usage, were; tetracyclines (70%), quinolones (8%), macrolides (6%), penicilins (6%), sulfanomides (5%) and amphenicols (4%). Together, highly important and critically important antimicrobials for human medicine embody around 95% of all antimicrobial use in aquaculture, while antimicrobials supposed to be for veterinary use only constitute a mere 5% of all antimicrobials used in aquaculture. Antimicrobials classified as critically important and very important for human medicine by the WHO accounted for respectively 18 percent and 77 percent of antimicrobial consumption (Table 50 in the Appendix).

Table 9, the WHO´s Critically Important Antimicrobials for Human Medicine list

Antibiotic class	Antibiotics	On the WHO's critically important antimicrobials list (2011)
Aminoglycosides	Neomycin	Critically important
Aminoglycosides	Kanamycin	Critically important
Aminoglycosides	Gentamicin	Critically important
Aminoglycosides	Streptomycin	Critically important
Aminoglycosides	Apramycin	Veterinary use only
Amphenicols	Florfenicol	Veterinary use only
Amphenicol	Chloramphenicol	Highly important
Cephalosporins	Cefalexin	Highly important
Diaminopyrimidine	Ormetoprim	Highly important
Diaminopyrimidine	Trimethoprim	Highly important
Macrolides	Erythromycin	Critically important
Nitrofuran	Furazolidone	Important
Nitroimidazole	Metronidazole	Important
Penicillins	Amoxicillin	Critically important
Penicillins	Ampicillin	Critically important
Penicillins	Benzylpenicillin	Critically important
Polymyxins	Colistin	Critically important
Quinolones	Enrofloxacin	Veterinary use only
Quinolones	Norfloxacin	Critically important
Quinolones	Ciprofloxacin	Critically important
Quinolones	Oxoinic acid	Critically important
Quinolones	Levofloxacin	Critically important
Rifamycin	Rifampicin	Critically important
Sulfonamides	Sulfamethoxazole	Highly important
Sulfonamides	Sulfadiazine	Highly important
Sulfonamides	Sulfadimidine	Highly important
Sulfonamides	Sulfadimethoxine	Highly important
Sulfonamides	Sulfaquinoxaline	Veterinary use only
Tetracyclines	Doxycycline	Highly important
Tetracyclines	Oxytetracycline	Highly important
Tetracyclines	Chlortetracycline	Highly important

4.1.3. Total GWFs per country

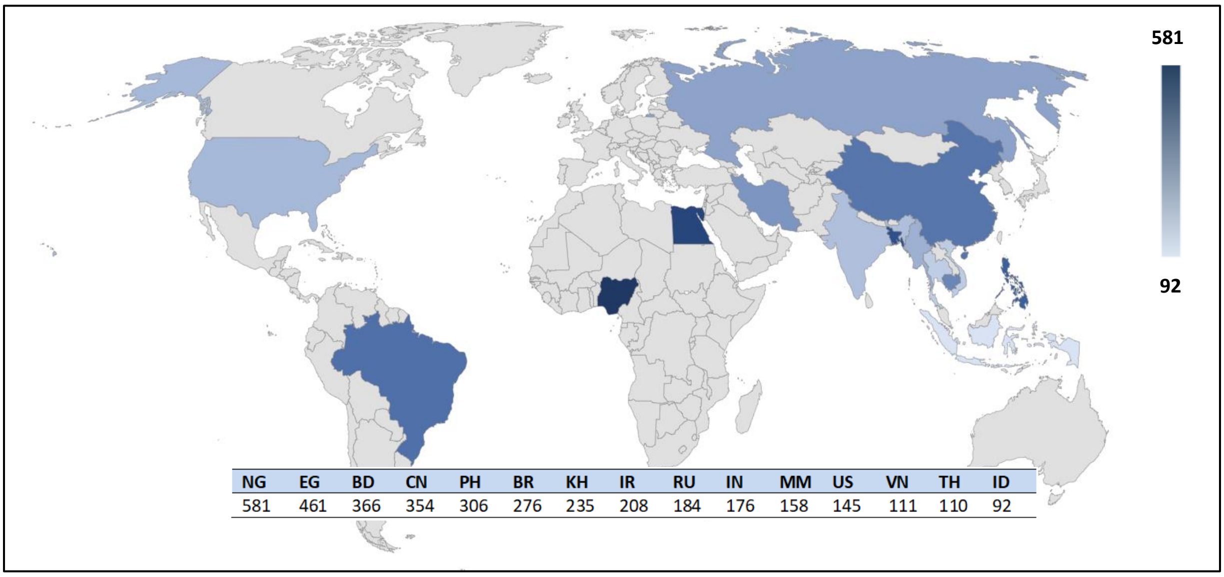
In 2019, the total grey water footprint as a result of antimicrobial consumption in freshwater aquaculture in the 15 countries of the scope is 14,314 km^3/y. This is the accumulation of national grey water footprints resulting from the critical loads in the different countries. Oxytetracycline is by far the most used substance, although in some countries like Indonesia, oxytetracycline is a banned substance. The global grey water footprint as a result of the use of oxytetracycline in freshwater aquaculture is 12,860 km^3/y. The five countries with the highest grey water footprint are all part of the APAC region. China is responsible for three quarters of the global GWF and has by far the largest total grey water footprint among the investigated countries (10,599 km^3/y). Most of China's grey water footprint is the result of carp production (67%).



CN	IN	BD	ID	VN	NG	MM	BR	EG	PH	IR	KH	TH	US	RU
10599	1212	830	352	331	168	163	146	142	98	87	68	46	37	34

Figure 15, total grey water footprint in km^3/y

The average global grey water footprint of freshwater fish produced in aquaculture as a result of antimicrobial use is 286 m^3/kg. The grey water footprint differs greatly among different countries, as seen in figure 16. Indonesia has a relative low grey water footprint per produced kilogram (92 m^3/kg) while Nigeria has a large grey water footprint per produced kilogram (581 m^3/kg). The countries that have a relative high grey water footprint generally have a lack of regulations on antibiotic use.



NG	EG	BD	CN	PH	BR	KH	IR	RU	IN	MM	US	VN	TH	ID
581	461	366	354	306	276	235	208	184	176	158	145	111	110	92

Figure 16, grey water footprint in m3/kg of aquatic body weight

4.2. Information per fish species (antimicrobial consumption per species group)

In this research, seven species categories are created: carp, tilapia, catfish, trout, pacu, crustaceans and "other". Globally, carp is the largest freshwater aquaculture species group (57.6%), followed by catfish (12.3%) and the multi-species group "other" (11.6%). The antimicrobial consumption shows a somewhat similar view. However, it becomes clear that antimicrobial consumption in the farming of crustaceans is relatively low (14 mg/kg), while the antimicrobial consumption in the farming of the multi-species group "other" is relatively high (474 mg/kg)

Table 10, global average of antimicrobial consumption and grey water footprint of different fish species

	Freshwater aquaculture production		Antimicrobial consumption			Grey water footprint		
	tons (thousands)	%	Use in tons	%	Consumption in mg/kg fish	WF in m^3/y	%	WF in m^3/kg
Carp	28758	57.6	7387	60.3	257	8.28182E+12	57.9	288
Tilapia	4453	8.9	396	3.2	89	4.54978E+11	3.2	102
Catfish	6128	12.3	1496	12.2	244	2.14527E+12	15.0	350
Trout	301	0.6	84	0.7	278	1.04315E+11	0.7	347
Pacu	332	0.7	94	0.8	283	1.0718E+11	0.7	323
Crustaceans	4220	8.4	59	0.5	14	2.27073E+11	1.6	54
Other	5773	11.6	2737	22.3	474	3.1793E+12	22.2	551
Total	**49963**	**100**	**12252**	**100**	**245**	**1.43141E+13**	**100**	**286**

With the use of the presented PNECs in table 6 and the presented antimicrobial consumption, the grey water footprint is calculated. The total grey water footprint is highest for carp production (57.9%). However, the grey water footprint relative to the production is actually relatively low (288 m^3/kg). This is in contrast to catfish, where the grey water footprint relative to the production is actually relatively high (350 m^3/kg). The success of the intensification of pangasius (catfish) is partly the result of the species physiological characteristics. The pangasius can directly extract oxygen from the air, thus tolerating low amounts of oxygen in the water production (World Bank, 2010). This means that fish farmers do not have to worry too much about the oxygen content of the water, and can breed a lot of fish in a small space. A floating cage of one cubic meter in the Mekong Delta can contain up to 150 fish. As a result, bacterial infections are more common and more antibiotics are used.

4.3. Grey water footprint of consuming farmed freshwater fish in the Netherlands

In this chapter the Dutch freshwater fish imports are presented. The grey water footprint of the average Dutch consumer is defined.

4.3.1. Fish imports

Dutch consumers can buy an array of different types of fish at the supermarkets and fish sellers. They can buy; locally caught fish like codfish, pollock and herring; imported caught or farmed marine fish; and imported caught or farmed freshwater fish. Total fish imports in 2019 are 1,135,988 tonnes of fish. Almost all farmed freshwater fish consumed in the Netherlands is imported. In 2019, the Netherlands produces 46,350 tonne farmed fish. However, freshwater fish only contributes around 11% of the total produced aquaculture, with a production of 2,700 tonnes of North African catfish and 2,200 tonnes of European eel. Most farmed fish consumed in the Netherlands is imported. Farmed freshwater fish are imported from 12 out of the 15 countries from this studies scope. Only Brazil, Cambodia and Iran do not export their freshwater aquaculture products to the Netherlands. The Netherlands also imports freshwater aquaculture products from countries outside this studies scope. These imports are combined in the group "Other". In figure 17 and table 11, the freshwater imports are displayed. Pacu is not consumed in the Netherlands and therefore not imported. Trout is not imported from any country out of the scope. Trout mainly is imported from European countries (EUMOFA, 2022). A total of 3,381 tonnes of Trout is imported by the Netherlands in 2019.

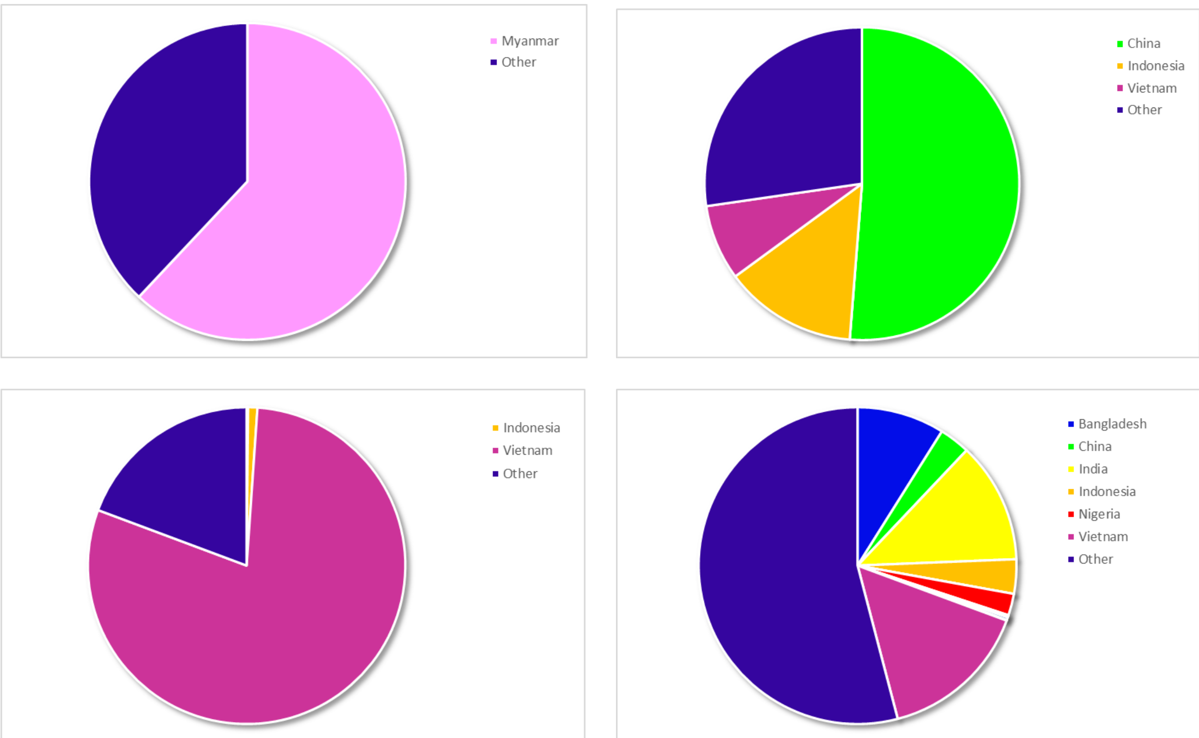


Figure 17, Fish imports in the Netherlands, clockwise: carp, tilapia, catfish and crustaceans

The Netherlands imported 219 tonnes of carp in 2019 from Myanmar, making it the main source of imported carp of the Netherlands (62%). Tilapia is supplied from different countries. A total of 7,730 tonnes of tilapia is imported in 2019. China (51.2%), Indonesia (13.6%) and Vietnam (7.8%) are the main suppliers of tilapia, with respectively 3,961, 1,055 and 603 tonnes. Catfish is imported to the

Netherlands on a large scale. 17,722 tonnes of catfish are imported from different exporters. The bulk of the catfish products are imported from Vietnam (79.6%), which is the world's biggest pangasius (catfish) producer. With 89,937 tonnes, crustaceans are imported to the Netherlands on a large scale, far exceeding the other specie groups. Most crustaceans are imported from countries outside the scope (54.1 %). From the researched countries, Vietnam (15.3%) is the country with most crustacean exports to the Netherlands followed by India (12.3%) and Bangladesh (8.9%), with respectively 13,753; 11,086 and 8,008 tonnes of aquatic products.

Table 11, Fish imports in the Netherlands

	Trout		Carp		Tilapia		Catfish		Crustaceans	
	Quantity (tonnes)	%	Quantity (tonnes)	%	Quantity (tonnes)	%	Quantity (tonnes)	%	Quantity (tonnes)	%
Bangladesh							25	0.1	8,008	8.9
Brazil										
Cambodia										
China					3,961	51.2			2,815	3.1
Egypt									0,360	0
India					0,350	0			11,086	12.3
Indonesia					1,055	13.6	168	0.9	3,152	3.5
Iran										
Myanmar			219	62.0					0,018	0
Nigeria									1,987	2.2
Philippines									80	0.1
Russia									126	0.1
Thailand									267	0.3
USA									39	0
Vietnam					603	7.8	14,108	79.6	13,753	15.3
Other	3,381	100	134	38.0	2,110	27.3	3,420	19.3	48,623	54.1
Total	**3,381**	**100**	**353**	**100**	**7,730**	**100**	**17,722**	**100**	**89,937**	**100**

4.3.2. Yearly grey water footprint as a result of consuming freshwater aquaculture products by an average Dutch consumer

The average Dutch consumer has a yearly grey water footprint of 280 m^3 as a result of consuming freshwater aquaculture products. The largest external water footprint of Dutch aquaculture consumption lies in the Mekong delta in Vietnam (71 m^3/y). This grey water footprint is mainly the result of catfish consumption. Dutch consumers also have a large external water footprint in Bangladesh and India with 43 m^3/y and 30 m^3/y respectively. These water footprints are the result of crustaceans consumption.

Table 12, Grey water footprint of average Dutch consumer in m^3/y

	Fish Species				
Country	**Crustaceans**	**Trout**	**Tilapia**	**Catfish**	**Total**
Bangladesh	41.83			0.75	42.58
China	1.12		0.31		1.43
India	29.78				29.78
Indonesia	3.06		1.08	0.64	4.79
Thailand	0.02				0.02
Vietnam	0.29		0.76	69.57	70.61
Other	26.29	54.39	2.19	47.72	130.59
Total	**102.39**	**54.39**	**4.34**	**118.68**	**279.81**

Table 13, GWF of average Dutch consumer

Other	Vietnam	Bangladesh	India	Indonesia	China	Thailand
131	71	43	30	4.8	1.4	0.02

5. Discussion

In this chapter the results of the previous chapter are compared to the existing state of the art literature. Subsequent methods to reduce antimicrobial use in aquaculture are discussed. In the end, limitations of this study are presented

5.1. Results in context

According to O'Neill (2015), antibiotic dosages in aquaculture might be larger proportionally to the dosages used in livestock. A study by Van Boeckel et al. (2015) estimated that the global consumption of antimicrobials in food animal production was 63,151 tons in 2010, and was expected to rise to 105,596 tons, by 2030. This study excluded aquaculture. Comparing antibiotic use per kilogram of produced animal shows interesting results. Van Boeckel et al. (2015) estimate that the antimicrobial consumption per kilogram of animal produced in the world was 45 mg/kg, 148 mg/kg, 172 mg/kg for cattle, chicken, and pigs, respectively. A more recent study of Tiseo et al. (2020) estimated that in 2017, globally, 93,309 tonnes of antimicrobials are used in food animals[7]. While on average cattle, chicken and pigs used 42 mg/kg, 68 mg/kg and 193 mg/kg respectively. The present study shows the significant share of antimicrobials used in freshwater aquaculture, as with an estimated consumption of 12,252 tonnes freshwater aquaculture has a share of over 11% of total global antimicrobials used in food animal production[8]. The total annual global antimicrobial consumption is estimated at 100,000–200,000 tonnes (Wise, 2002). Antimicrobials used in freshwater aquaculture therefore consist of between 6%-12% of total gloabal antimicrobial use.

Comparing the antimicrobial consumption per kilogram of animal produced shows that the average antimicrobial consumption in freshwater aquaculture (245 mg/kg) has extremely high consumption rates compared to livestock consumption[9].

Table 14, antimicrobial consumption in food animals

Antimicrobial consumption in year (Study)	Antimicrobials per kilogram of produced animal (mg/kg)				Total global antimicrobial consumption in animal production (tonnes)	
	Cattle	Chicken	Pigs	Freshwater Aquaculture	Livestock	Freshwater Aquaculture
2010; (Van Boeckel et al., 2015)	45	148	172	-	63,151	-
2017; (Tiseo et al., 2020)	42	68	193	-	93,309	-
Present Study (2022)	-	-	-	245		12,252

The results of this study have shown large differences in antimicrobial conumption between the different researched countries. On average, Nigeria consumes 552 mg/kg, over 10 times as much as Bangladesh, that only consumes 33 mg/kg. However, the existing literature on antimicrobial use in marine aquaculture also shows how different antimicrobial use practices are depending on their geographical location. Notoriously large antimicrobial consumer Chile uses over 1400 times the amount of antimicrobials Norway uses (Burridge et al., 2010). The results of this study seem to align with studies on antimicrobial use in marine aquaculture.

[7] cattle, chicken and pigs, so excluding aquaculture

[8] This is a combination of Tiseo et al (2020) and the present study. It should be noted that this excludes marine and brackish aquaculture.

[9] van Boeckel et al. (2015) and Tiseo et al. (2020) do not state which antimicrobials are used. Therefore, the total antimicrobial use data in livestock is not one on one comparable with antimicrobial use data in aquaculture as there is no information on selection of drugs/drug categories, variations in drug potencies, resistance selection pressures, or importance of antimicrobials for human health

Table 15, Antimicrobials used in Atlantic salmon aquaculture, quantities used in 2007 and quantities applied relative to production (Burridge et al., 2010)

Country	Salmon production (metric ton)	kg (active ingredient) used	kg therapeutant/ metric ton produced	mg therapeutant/ kg produced (mg/kg)
Norway	821,997	649	0.0008	0.8
Chile	330,791	385,600	1.17	1170
UK	132,528	1553	0.0117	11.7
Canada	121,370	21,330	0.175	175

A more recent study by Miranda et al. (2018) estimated that between 2013 and 2016, Chilean farmers used on average 580 mg of antimicrobials per kg of harvested salmon annually. This falls in the same range as the results of this study, where Nigeria is the top antimicrobial user with 552 mg/kg.

Pahlow et al. (2015) conducted the first attempt to systematically estimate the water footprint of aquaculture. This research focussed on the water footprint of aquafeed and neglected the impact of water refreshment rates and the use of antimicrobials. According to this research, the average water footprint of commercially fed fish was 1,974 m^3/tonne (83% green, 9% blue, and 8% grey). Although this study identified that during aquafeed production a considerable amount of water is consumed and the water system is polluted, the total impact of the aquaculture system is not captured. A more recent study by Guzmán-Luna et al. (2021) estimates the water footprint[10] of extensive, semi-intensive and intensive freshwater tilapia aquaculture in Mexico over the five production phases along the chain[11] (GWFs displayed in Table 16). This study incorporated aquafeed, fertilizer and hormones as well as water refreshment rates, but excluded antimicrobial use. The water footprint estimated by Guzmán-Luna et al. is significantly higher than the water footprint estimated by Pahlow et al. (2015), as this water footprint included water footprints related to the fish ponds i.e. water evaporation etc.

Table 16, Water footprint of Tilapia (Guzmán-Luna et al., 2021)

	Production system		
	Extensive	Semi-intensive	Intensive
Blue water footprint (m^3/tonne)	927	2909	13,027
Green water footprint (m^3/tonne)	5	7827	7831
Grey water footprint (m^3/tonne)	398	1873	1873
Total	**1,330**	**12,609**	**22,731**

The study by Guzmán-Luna et al. (2021) has similar results to a study by Gephart et al. (2017), in which the freshwater footprint of aquaculture, barring antimicrobials, in China was estimated. The water footprint in this study was estimated between 3,349-21,215 m^3/tonne, depending on the species produced, the feed composition, the province in China and the culture system. Comparing the total water footprint of Tilapia over the five production stages to the grey water footprint of antimicrobial use in freshwater aquaculture, shows that the grey water footprint of antimicrobial use

[10] Blue, green and grey water footprint
[11] i.e. broodstock, breeding, fattening, processing, and transportation phases

is up to 30 times as high as the total water footprint estimated by Guzmán-Luna et al. (2021), which excluded antimicrobial use (581,378 and 22,731 m^3/tonne). This only emphasizes the significance of antimicrobial use on the grey water footprint of the aquaculture production cycle and therefore the importance of reducing antimicrobial use in aquaculture.

Table 17, Grey water footprint of antimicrobial use in freshwater aquaculture

	Average	Min (Indonesia)	Max (Nigeria)
Grey water footprint	286,495 m^3/tonne	91,911 m^3/tonne	581,378 m^3/tonne

A study by Wöhler et al. (2020) estimated the grey water footprint of veterinary pharmaceuticals applied to livestock in Germany and the Netherlands. Beef was estimated to have a grey water footprint related to pharmaceutical use of 654 m^3/kg in Germany, while the GWF in the Netherlands was estimated to be 148 m^3/kg. Pork has the largest grey water footprint in the Netherlands (212 m^3/kg). Chicken has a relatively small grey water footprint with 15 m^3/kg and 0.14 m^3/kg in Germany and Netherlands respectively.

Table 18, Grey water footprint related to veterinary pharmaceutical use (Wöhler et al., 2020)

	Grey water footprint related to veterinary pharmaceutical use (m^3/kg)	
	Germany	Netherlands
Beef	654	148
Pig	51	212
Chicken	15	0.14

With an average GWF of 286 m^3/kg, aquaculture products have a relatively large grey water footprint compared to the grey water footprint of other livestock.

The global average water footprint over the period 1996-2005 is estimated by Mekonnen and Hoekstra (2011) to be 1,385 m^3/y per capita. This value includes all water consumption from domestic use (3.8%), industrial products (4.7%) and agriculture products (92%). The grey water footprint estimates by Mekonnen and Hoekstra only include nitrogen fertilisers related water pollution and do not include other fertiliser components and pesticides. Besides it excludes pharmaceutical-related water pollution. The global grey water footprint as a result of the use of oxytetracycline in freshwater aquaculture is 12,860 km^3/y. This results in a global average grey water footprint from the antimicrobial oxytetracycline of 1,600 m^3/y per capita, thus exceeding the global average water footprint calculated by Mekonnen and Hoekstra (2011). Besides, with a global grey water of 12,860 km^3/y, the global grey water footprint as a result of antimicrobial use in freshwater aquaculture is twice the the annual discharge of the Amazon, the worlds largest river: 6,595 km^3/y.

The water footprint of Dutch consumers, excluding pharmaceutical-related water pollution, is about 2,300 m^3/y over this same time period (1996-2005) (Van Oel et al., 2008). This study shows that the average Dutch consumer has a grey water footprint related to antimicrobial use in freshwater aquaculture products of 280 m^3/y. Dutch consumers thus have a relatively low grey water footprint as a result of eating low amounts of freshwater aquaculture products. However, the water footprint is an external water footprint, and consumers often are not aware that the pollution affects people in other parts of the world.

The above indicates the scale of antimicrobial use in aquaculture. Recent studies have shown antimicrobial resistance to tetracycline, penicillin and ampicillin in China, India, Vietnam, Brazil, Iran, Thailand, the Phillipines and the USA (Elmahdi et al., 2016; Huys et al., 2007). Tetracycline is by far the most used antimicrobial infreshwater aquaculture (70%). Tamminen et al. (2011) conclude that "tetracycline resistance genes are highly persistent and do not disappear from aquaculture sites, even after several years without antibiotic use" (p. 390). Antibiotics that persist in the aquatic environment and build up in animal tissue of aquaculture products may pose a health risk to humans by entering the food chain (Lulijwa et al., 2020). Besides, antibiotic residues can also be consumed by wild fish and as a result, the safety of captured aquatic products is jeopardized (Boxall et al., 2004). Antibiotic residues in human food can cause adverse drug reactions (ADR) and the development of antibiotic resistance for significant bacterial infections (Liu et al., 2017). For example, commonly used antimicrobials in aquaculture like penicillin, tetracycline, and sulphonamides, are antigenic and may trigger allergic reactions in consumers (Li, 2008). Besides, damage to organs can occur in humans as a result of bioaccumulation of antimicrobial residues (Zheng & Su, 2010). Reducing the antimicrobial consumption is thus from vital concern.

5.2. Reducing antimicrobial consumption

The above shows that antimicrobial use is very high in aquaculture, even exceeding antimicrobial rates in other animal food production. To reduce environmental burden as well as to impede development and spreading of antibiotic resistances, it is essential to decrease the antimicrobial use in the aquaculture industry. By looking at the success Norway has had with reducing antimicrobial use, several mechanisms which can be used to reduce antimicrobial use are identified.

In Norway, strict regulations and the increased use of vaccinations have decreased antimicrobial use by 99% between 1987 and 2013, despite the production output of aquaculture growing more than 20 fold over this time period (Norwegian Ministries, 2015; O'Neill, 2015). Over the period 2007-2014, the consumption of antimicrobials in Norway salmon aquaculture has been around 1 mg/kg produced fish, while 2014 set a record low of 0.36 mg of active ingredient per kilogram of produced salmon (The Norwegian Veterinary Institute, 2016). This is significantly lower than the average antimicrobial consumption of 245 mg/kg found in this study. In the early stages of industrialized aquaculture, Norway experienced numerous severe bacterial infection outbreaks, putting pressure on the farmed fish population. This resulted in the widespread use of antimicrobials (figure 19). The government recognized this problem, and started regulating antimicrobial use by law. Antimicrobials can only be prescribed by veterinarians and aquamedicine biologists to treat clinically confirmed infectious illnesses (The Norwegian Veterinary Institute, 2016). Besides, antimicrobials are exclusively available through pharmacies, and the government monitors the antimicrobial sales. By focussing on creating effective vaccines, Norway could increase its productivity without the use of antimicrobials, creating a unique sustainable aquaculture sector (The Norwegian Veterinary Institute, 2016). Bernhard & Hannisdal (2021) report that in 2020, a total of 48 veterinary antimicrobial treatment prescriptions were issued by Norwegian aquaculture farms, the lowest amount ever. As a result of the stringent standards in Norway, 99 percent of the produced salmon is farmed without the use of antimicrobials (Bernhard & Hannisdal, 2021)

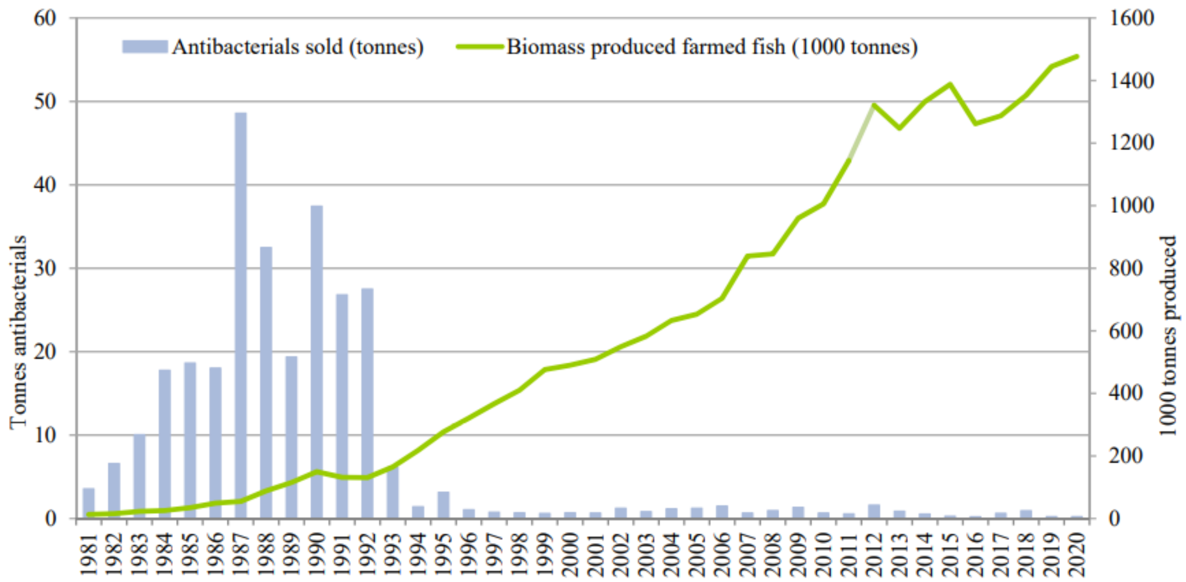


Figure 18, Sales, in tonnes of active substance, of antibacterial veterinary medicinal products for therapeutic use in farmed fish (including cleaner fish) in Norway in 1981-2020 versus tonnes produced (slaughtered) farmed fish (NORM/NORM-VET 2020, 2021).

As shown by Norway, vaccines can be very efficient against bacterial disease outbreaks in fish. However, not everywhere the implementation of vaccines is plausible, as developing vaccines and administrating them is relatively costly (Secombes, 2008). This view is strengthened by Phu et al. (2016), who found in their survey that amongst Vietnamese aquaculture farmers there is scepticism about the vaccines' economic sustainability in the low profit pangasius market due to the high vaccine costs and the intensive labour required to inject individual fish. Besides, vaccines are not yet available for crustaceans and mollusks, as they have an alternative adaptive immune system compared to fish (Amatul-Samahah et al., 2020; Johnson et al., 2008). Therefore, other mechanisms to decrease antimicrobial use are discussed. The government can influence the use of antimicrobials with regulations on the direct use of antimicrobials or regulations on antimicrobial levels in the final products. As seen in Norway, regulations on how antimicrobials can be applied and under which circumstances can have a significant impact on total antimicrobial consumption (Henriksson et al., 2018). Setting maximum residue levels can result in lower antimicrobial use. However, only a small sample size can be tested as screening is time consuming and resource intensive. Besides, a change in the moment of application within the production cycle can influence the residue levels in the final product while antimicrobial consumption remains equal (Henriksson et al., 2018). Furthermore, import regulations, as applied by the EU and the US, can also result in aquaculture farmers using different antimicrobial quantities in products meant for domestic use and products for export to highly regulated markets. Moreover, an export switch towards countries with less stringent regulations can also be the result, having no impact on the antimicrobial consumption. Despite, on account of the US repeatedly rejecting shipments of Vietnamese pangasius because of exceeding enrofloxacin limits, the Vietnamese government banned the use of enrofloxacin in 2012 (Henriksson et al., 2018). Banning or approving certain antimicrobials is also a way to regulate antimicrobial use. Even though, Liu et al (2017) and Yuan & Chen (2012) still found evidence of the use of banned substances in Chinese aquaculture. Besides, approving certain antimicrobials may result in the overuse of those approved antimicrobials.

Most western countries have veterinarians to accurately diagnose diseases. However, famers in developing countries, where most aquaculture is produced, often lack proper diagnoses capacity, resulting in unsuitable use of antimicrobials (Henriksson et al., 2018; Phu et al., 2015). Educating farmers could solve this. Besides, probiotics can play an important role. A relatively recent term, probiotics refer to microorganisms that have been linked to positive effects on the host. Aquatic animal growth rates, feed utility, and survival rates have all increased as a result of the use of probiotics (Hai et al., 2015). Probiotics also improve the water quality (Hai et al., 2015). They are seen as environment friendly and safe growth promoters and pathogen-controlling agents, which can be a good alternative to antimicrobial use in aquaculture (El-Saadony et al., 2022; Hoseinifar et al., 2018). However, the current state of knowledge on probiotics' impact on the immune system is quite limited, and further study is needed (Hoseinifar et al., 2018).

Eco-certification of aquaculture products can enhance a move away from antimicrobial consumption, and is often regarded as a strategy for reducing environmental impacts of the aquaculture industry (Jonell et al., 2013). Especially as consumers are increasingly well informed about the advantages of buying antimicrobial-free products (Tiseo et al., 2020). As a result of consumer preferences, a number of big corporations, including McDonald's, have demanded that direct suppliers of meat products end the use of antimicrobials for growth promotion (MacDonald & Wang, 2011).

Consequently, nations who reduce antimicrobial use first obtain an economic advantage in securing export markets (Tiseo et al., 2020).

Reporting of antimicrobial sales meant for aquaculture is essential, and countries should document their use and help promote antimicrobial stewardship globally. Preferably, surveillance systems should be put into place in collaboration with drug-manufacturers and wholesalers to guarantee that the monitored data is accurate (Vander Stichele et al., 2004). Although systematic surveillance systems might not be implemented in low- and middle-income countries (LMICs) in the near future, point-prevalence surveys can serve as a starting point for tracking antimicrobial sales in certain geographical areas (Cuong et al., 2018).

Recirculating aquaculture systems (RAS) is a relatively new aquaculture technique in which negative environmental impacts are reduced by having a close to zero discharge (Watts et al., 2017). In RAS, waste created during the aquaculture process is collected and treated with mechanical and biological filtration processes after which the water can be reused again (Almeida et al., 2019; Watts et al., 2017). However, antimicrobials used during the aquaculture process tend to impede the microbiome of the biofilters, and getting rid of these chemical residues from the aquaculture tanks is hard, resulting in the spread of antimicrobial resistant pathogens in the aquaculture tanks (Almeida et al., 2019). The combination of phage therapy, in which viruses (phages) exclusively target and kill bacteria, with RAS is an interesting technique that is currently used on experimental scale (Almeida et al., 2019). Aquaculture producers can attain long-term security from typical aquaculture bacterial infections by supplementing RAS tanks with the proper type of phage, safeguarding their productivity while avoiding the expense and hazards of antibiotic treatment (Almeida et al., 2019). Although RAS in combination with phase therapy seems to be a solution which could reduce antimicrobial use and its effect on the environment, due to the high costs it is not expected to be a viable solution for low- and middle-income countries (LMICs) in the near future.

Whether the above mentioned intervention methods are a success or not to decrease antimicrobial consumption depends on different factors. For example, time intensive implementation and high costs influence the effectiveness of the intervention methods in different countries, as resources are varying in each receiving system (Léger et al., 2021). As the successful implementation of the interventions depends a lot on the context i.e. cultural, political and ecological differences, it can be concluded that no one method is the right method, and a combination of all the above must be applied.

5.3. Limitations of this study

This study shows the antimicrobial consumption of freshwater aquaculture and the resulting grey water footprint. Although this study gives insight in the magnitude of the global antimicrobial consumption, a couple of limitations within this study should be taken into account.

First, as a result of a lack of data on antimicrobial consumption in freshwater aquaculture, this study relies ondata of only a few studies, and specifically heavy on the research by Rico et al. (2013). Having a small sample results in a limited generalizability, as the sample does not fully represent the population. However, the gathered data was sufficient to form a broad picture and overall impression of antimicrobial use in freshwater aquaculture.

Second, the data used might not always be representable for all production systems. For example, Rico et al investigated the use of antimicrobials in 252 aquaculture farms in 4 different countries[12], but the majority of the aquaculture farms included in this research were producing for foreign markets (Rico et al., 2013). As regulations are more stringent for foreign markets, the actual total antimicrobial use is possibly higher, and as a result total antimicrobial use might be underestimated in this study.

Third, not enough information is available on the antimicrobial use per aquaculture species. For instance, information on antimicrobial use in China, globally the largest aquaculture producer, is very limited. Hardly any regions/provinces have information on antimicrobial use, and the regions that have datasets available often do not have information on different aquaculture species. In this study, due to the lack of data, results of certain aquaculture species are now also used for completely different aquaculture species. However, this might give an incomplete picture, especially as there are notorious fish species in which the average antimicrobial use is shown to be very high in comparison to other fish species.

Besides, in the methodology of this study, if there is a lack of data, the mean species specific antimicrobial use coefficients of all the calculated countries has been taken. The assumption has been made that antimicrobials that are illegal in a country are not used in the concerned country, and they are thus removed from the list of mean species specific antimicrobial use coefficients. However, in fact, the illegal use of banned substances still occurs widely (Lulijwa et al., 2020). Besides, the lack of use of illegal antimicrobials will probably be substituted by more use of legal antimicrobials. This is not taken into account in this study, and therefore the total antimicrobial use might be underestimated.

Because the lack of accurate data on the percentages of different aquaculture systems in different countries, there is no difference made between water pollution as a result of the different aquaculture systems. Combining occurrence rates of different aquaculture systems in each country and the antimicrobial use in the different aquaculture systems would give a more complete image. Especially as open aquaculture systems are thought to affect surrounding water bodies more then closed aquaculture systems. It is currently assumed that 75% of the pollutant load ends up in the environment. However, in closed systems this value would be much lower. Taking this into account would give a more complete picture. Since no clear data is available this could not be integrated into this study. However, as between 1,5 and 2% of total aquaculture production is produced in

[12] Bangladesh, China, Thailand and Vietnam

recirculating aquaculture systems in the EU over the period 2014-2018 (EUMOFA, 2020), and the global average is expected to be even lower, the estimation of 75% ending up in the environment still holds up.

Sixth, the results of this study rely heavily on the dosages used by the aquaculture farmers. However, the quality of antimicrobials used in developing countries differs a lot. Phu et al (2015) found that most antimicrobial products used in Vietnamese aquaculture contained active compounds over ±10% of the concentration as stated on the product label. Besides, recommended doses on product labels very greatly between different brands. For the active ingredient doxycycline, recommended antimicrobial doses in Vietnam differentiate between 5 to 40 mg per kg fish (Phu et al., 2015). It should thus be expected that the doses of used antimicrobial products differentiate a lot, especially in developing countries where there is a lack of approval procedures and quality monitoring programs of antimicrobial products. This study has used recommended doses as stated on product labels found on site in farms, to calculate antimicrobial use. A variation of ±10% in antimicrobial doses is assumed across global production, resulting in a global antimicrobial consumption between 11,138-13,477 and a global grey water footprint as a result of oxytetracycline use between 11,691-14,146.

Seventh, it should be noted that the effect of integrated aquaculture is not taken into account. With integrated farming, manure and excess food from livestock containing residues of medication are spilled over to fish. The fish are usually not additionally fed (Gibson et al., 2020; Petersen et al., 2002). Petersen et al. (2002) found a clear increase in antimicrobial resistance in the water-sediment samples as a result of integrated aquaculture. It is therefore important to note that the total amount of antimicrobials in the aquaculture production chain is underestimated.

Eight, the grey water footprint depends a lot on the chosen set of PNECs. The selection of water quality thresholds is required for the calculation of the grey water footprint. As there is no consensus on which thresholds should be utilized, this action is a value choice (Mikosch et al., 2021). Water quality thresholds are generally derived from national or international water quality standards. Because the thresholds can differ greatly, the grey water footprint is strongly dependent on the standard used to calculate it. For this study the precautionary PNECs stated in the Methodology are chosen. Using a different water quality standard impacts the grey water footprint outcome.

Ninth, In the method of this study, the average farming cycles per fish species are stated. In reality these farming cycles differ a lot depending on the climate in which the aquaculture is taking place. For instance, Egypt has a dry climate with very little precipitation while Indonesia has a humid climate with lots of precipitation. This results in different farming cycles. For instance, in the arid climate of Egypt the grow-out cycle of tilapia is around 9 months compared to 3-4 months in Indonesia (Gephart et al., 2017). In future studies these differences should be taken into account to create a more complete picture.

Laslty, in the estimation of the grey water footprint of consuming farmed fish in the Netherlands, conversion factors are used. These conversion factors depend on the aquaculture species as well as the country where the aquaculture products are from. The state of the products (whole fish; headed and gutted; fillets) affect the conversion factors in addition to whether gutting, heading and filleting is processed with hands or with machinery. An overall average conversion factor of 2.15 has been chosen for all aquaculture products, as the state of the aquaculture products was unkown.

6. Conclusion & recommendations

In this chapter the final conclusions are stated and recommendations are proposed

6.1. Conclusions

This study is the first attempt to estimate antimicrobial use in freshwater aquaculture, and estimate the resulting grey water footprint. In conclusion, this study reports that in 2019, the global leading freshwater aquaculture producing countries, which represent 92% of global freshwater aquaculture production, are estimated to consume 12,252 tons of antimicrobials in freshwater aquaculture. Antimicrobials used in freshwater aquaculture therefore consist of over 13% of total global antimicrobials in food animal production and between 6%-12% of total gloabal antimicrobial use. Worrying levels of medically important antimicrobials to humans are found to be used in aquaculture in this study. Oxytetracycline is the most used antimicrobial in freshwater aquaculture, and is classified as highly important to humans. Antimicrobial consumption is especially high in the APAC region. The resulting grey water footprint overshadows the total water footprint of the rest of the aquaculture production chain, with a total grey water footprint as a result of the use of oxytetracycline in freshwater aquaculture of 12,860 km^3/y. Besides, the present study might still underestimate the use of antimicrobials as the effect of integrated aquaculture is not taken into account. Better recording of antimicrobial use in different aquaculture systems and locations should lead to a better overview of hotspots. Frontrunners such as Norway have shown that it is possible to reduce antimicrobial consumption while increasing aquaculture production. Countries that have high antimicrobial consumption, like Nigeria, Egypt, China and The Philippines, may want to consider Norwegian practices to reduce their antimicrobial consumption as antimicrobial resistance poses a big risk for global society. Alternatives to antimicrobial use are the use of vaccines and probiotics. Also, different mechanisms can be used to reduce antimicrobial use in freshwater aquaculture including government regulations on maximum residue levels and banning antimicrobial substances. Besides, educating farmers on proper antimicrobial use avoids antimicrobial overuse, as farmers in developing countries currently often lack proper diagnoses capacity. Furthermore, as a result of consumer preferences, eco-certification of aquaculture products can boost a move away from antimicrobial consumption. Finally, recirculating aquaculture systems in combination with phage therapy is an alternative, although the high costs will make this not a viable solution for low- and middle-income countries in the near future.

6.2. Outlook

There is still a large knowledge gap on antimicrobial use in a lot of countries. Especially data on antimicrobial use in freshsater aquaculture is scarce. As freshwater aquaculture systems differ from marine aquaculture systems, they should be evaluated and assessed separately, resulting in an even smaller pool of literature. For this research, only data from a couple of countries could be used.Thus, future research should delve into broadening the knowledge about antimicrobial consumption in freshwater aquaculture. This should start with setting up standardized methods to collect antimicrobial use data. Antimicrobial consumption surveillance according to these standardized methods should result in reliable and compareable antimicrobial use data from different parts of the world, for different fish species, different aquaculture systems and different water environments. Besides, a differentiation must be made between products produced for domestic markets or products produced for export, as products produced for export are often subject to more stringent rules than those products produced for domestic markets. Collecting this comparable data will facilitate comprehending antimicrobial use in aquaculture systems and results in finding the best solutions to decrease antimicrobial use in aquaculture.

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Photography credits

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Channel catfish is harvested at an aquaculture farm in Itta Bena, Mississippi. PHOTOGRAPHY BY BRIAN SKERRY. Retrieved from https://www.nationalgeographic.com/foodfeatures/aquaculture/

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Fisherman feeding at an aquaculture farm in the Mekong river near Nong Khai, Thailand. PHOTOGRAPHY BY THIRAWATANA PHAISALRATANA. Retrieved from Getty images

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Japanese scallops at an aquaculture farm off Canada's Vancouver Island. PHOTOGRAPHY BY BRIAN SKERRY. Retrieved from https://www.nationalgeographic.com/foodfeatures/aquaculture/

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Freshwater prawn aquaculture in a small pond near Khulna, Bangladesh. PHOTOGRAPHY BY JIM RICHARDSON. Retrieved from https://www.nationalgeographic.com/foodfeatures/aquaculture/

Appendices

Appendix I: Country background

China

Practices used

Chinese freshwater aquaculture has historically been a polyculture. This is the result of a historical event during the reign of the Tang dynasty (618-906 A.D.). The Tang emperor in China had the family name of Li, which is the same as the name of the Chinese word for common carp. The emperor banned the culture of the common carp, which was then widely cultivated by the Chinese as a food source (Rabanal, 1988). As a result, the Chinese people started looking for other fish species for their pond culture, discovering new fish species in the mud carp, silver carp, the big-head carp and the grass carp. An additional advantage was that the Chinese found out that these different fish species feed on different types of food and stay in different environmental niches in the ponds. The mud carp are bottom feeders, the silver carp and big-head carp are midwater feeders and the grass car are top feeders. This led to the polyculture of the four Chinese family carps, thereby maximizing the productivity of freshwater pond culture. Monoculture of the common carp was since replaced by polyculture of family carps, which is still practiced today (FAO, 1983).

Freshwater aquaculture methods in China consists mainly of pond culture, pen culture, cage culture and rice-fish culture (Hu et al., 2021). Pond culture is the most common way of aquaculture as it has an output of 74% of the total freshwater aquaculture (Hu et al., 2021). Pond culture mostly consists of carp farming systems, which are almost always set up as polyculture systems (Wang et al., 2015). Pond aquaculture is often intensive cultivation or semi intensive cultivation. In natural water bodies (rivers and lakes), the most common aquaculture practice is net cage culture (Wang et al., 2015). Species cultured in cage systems are generally higher value species which are intended for export (Tilapia). Pen culture is after cage culture the most common aquaculture practice in natural water bodies. As well as with cage culture, there is a shift from the low end carps towards higher value species e.g. crustaceans. Rice field culture, also known as paddy field aquaculture, is an aquaculture practice which dates long back in Chinese history. Here we see a trend as well towards high valued species like Chinese soft/shelled turtles, crayfish and mitten crabs (Hu et al., 2021). Most of Chinese freshwater aquaculture practices are conducted in the provinces in the Yangtze River Basin, which lies in the tropical and subtropical belt.

Products produced/fish types

China has a long history with aquaculture, as the earliest records of aquaculture date date back 2500 years. It is thus no surprise that China is the world's largest aquaculture producer in the world. With a volume of 68.424 million metric tons in 2019, the country contributed over two thirds of the global total aquaculture production. Almost halve of the total aquaculture production comes from freshwater aquaculture.

Table 19, Freshwater aquaculure in China in 2019

Freshwater Aquaculture China 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	19 528
Tilapia	1 642
Catfish	1 212
Trout	39
Pacu	69
Crustaceans	3 983
Molluscs	190
Algae	55
Other	3 469
Total	**30 187**

As seen in table 19, China has the largest number of fish species cultured in freshwater. Traditionally, different type of cyprinids (carps) are cultured, including: Grass carp, silver carp, bighead carp, common carp, crucian carp, wuchang bream and the black carp. The carp production contributes 65% of China's freshwater aquaculture production in 2019 (figure 20). The introduction of non-indigenous species has seen an increase in the culture of tilapia and catfishes. The Nile Tilapia is the most dominant fish after the cyprinids. Crustaceans also play an important role in Chinese freshwater aquaculture, as seen in Figure 20. In China, the growing middle class will result in a change in demand towards more carnivorous species, as those fish species are seen as tastier and more exclusive. However, the backbone of Chinese freshwater aquaculture will still be the polyculture of carp.

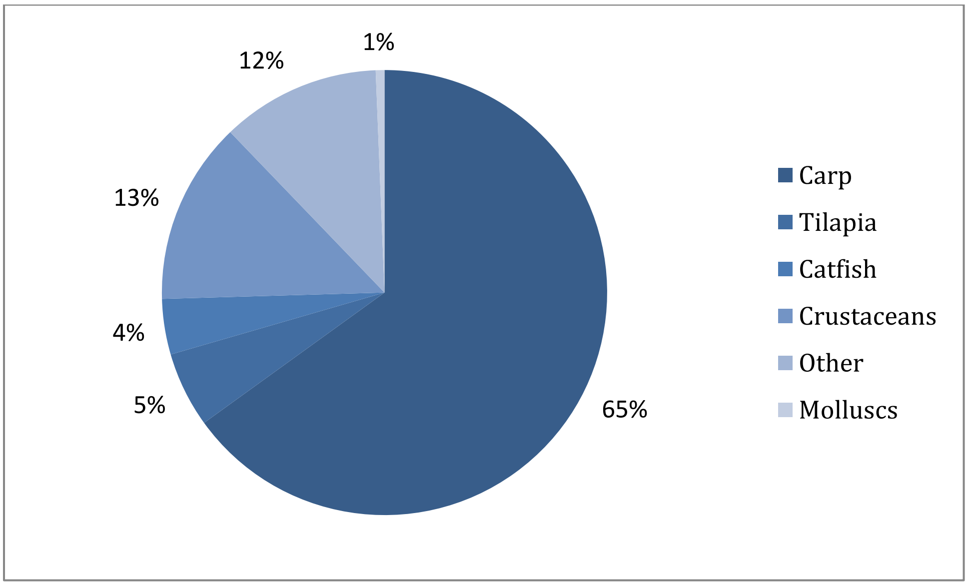

Figure 19, Fish specie categories in freshwater aquaculture in China in 2019

India

Practices used

Indian aquaculture has grown a lot, increasing six and half fold over the last two decades, as a result of greater cost-efficiency in culture technology (Jayasankar, 2018). Most aquaculture practices in India are focussed on freshwater aquaculture, with almost 90 percent of the total production being freshwater aquaculture (FAO, 2021). This is a result of India's geographical position in the monsoon belt, which results in lots of rainfall. Consequently, freshwater aquaculture bodies like ponds and tanks are scattered all over India, however the three states, Andhra Pradesh, Karnataka and West Bengal constitute 50 percent of the total area of freshwater aquaculture bodies in India (Katiha et al., 2005). Historically, carp polyculture in ponds is the common aquaculture practice in India. Instead of combining 10 or more species like they do in China, the Indian culture system stands on the use of 3 to 6 carp species (Jayasankar, 2018). Carp farming in rice fields and ponds is traditionally a family business, with small farms (Swiss Re, 2015). Lately, aquaculture in tanks and reservoirs is practiced (Swiss Re, 2015), as well as cage culture as a result of floating extruded fish feeds being available in India since 2008. Catfishes are produced as well of late (Jayasankar, 2018). Almost all the produced freshwater aquaculture is meant for the domestic market, with only the giant freshwater prawn produced for export (Jayasankar, 2018).

Products produced/fish types

With 75 percent, carp production dominates Indian freshwater aquaculture (FAO, 2021). Catfish has become the 2nd most produced fish and is produced on large scales in the North-eastern regions of India, especially in the state of Assam (FAO, 2021)

Table 20, Freshwater aquaculure in India in 2019

Freshwater Aquaculture India 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	5151
Tilapia	0
Catfish	724
Trout	1
Pacu	0
Crustaceans	9
Molluscs	0
Other	1013
Total	**6897**

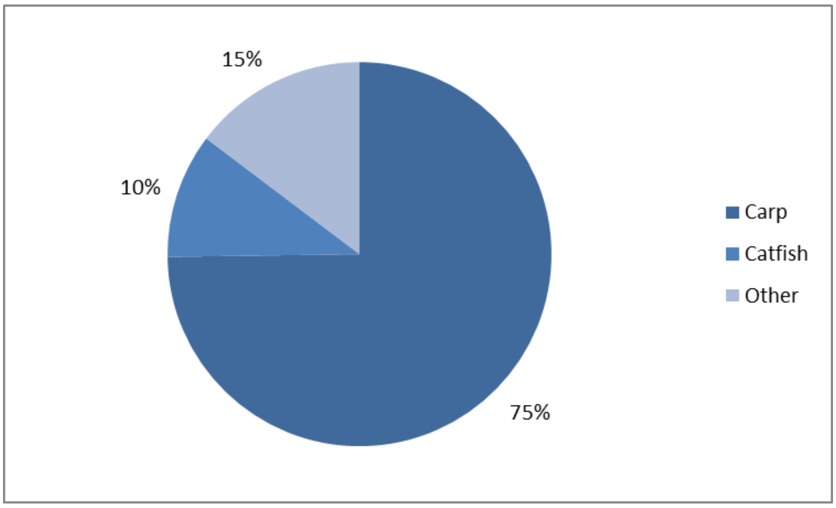

Figure 20, Fish specie categories in freshwater aquaculture in India in 2019

Indonesia

Practices used

After China, Indonesia is the world's second largest fishery producer. The circumstances of Indonesia are superb for fish production, as Indonesia is known for being the world's biggest archipelago country, consisting of 17.508 islands. Besides long stretches of coastal area, Indonesia resides in the Coral Triangle, resulting in lots of aquatic life. However overfishing has resulted in a diminishing industry of capture fisheries, and a more prominent role for aquaculture (Henriksson et al., 2019). A study by Phillips et al. (2015) indicates that by 2030, capture fisheries will be overtaken by aquaculture as dominant method of fish production. Indonesia's aquaculture sector mainly focuses on freshwater aquaculture, with the cultivation of freshwater finfish species, and the production of seaweed (Henriksson et al., 2019). Mariculture remains very small. Freshwater aquaculture is mainly practiced in backyard ponds. In 2009, 56% of all freshwater aquaculture activities took place in ponds (Sari, 2010). The other three freshwater culture systems that are used are; floating net cage (24%), cage (10%) and paddy field (9%) (Sari, 2010). Cage culture is practiced on a more commercial scale. Floating net cage culture is practiced in lakes and reservoirs, while cage culture is practiced in rivers and canals (FAO, 2005). Cage culture is practiced as an intensive aquaculture system. Aquaculture practiced in paddy fields is often a side activity of local farmers and not practiced on a commercial scale. Freshwater aquaculture is mainly practiced in the following five provinces; West Java (34 percent), East Java (13 percent), West Sumatra (8 percent), Central Java (7 percent) and South Sumatra (5 percent) (FAO, 2005).

Products produced/fish types

Indonesian aquaculture is based on three pillars, carp, tilapia and catfish culture.

Table 21, Freshwater aquaculure in Indonesia in 2019

Freshwater Aquaculture Indonesia 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	716
Tilapia	1167
Catfish	1480
Trout	0
Pacu	73
Crustaceans	34
Molluscs	0
Other	358
Total	3828

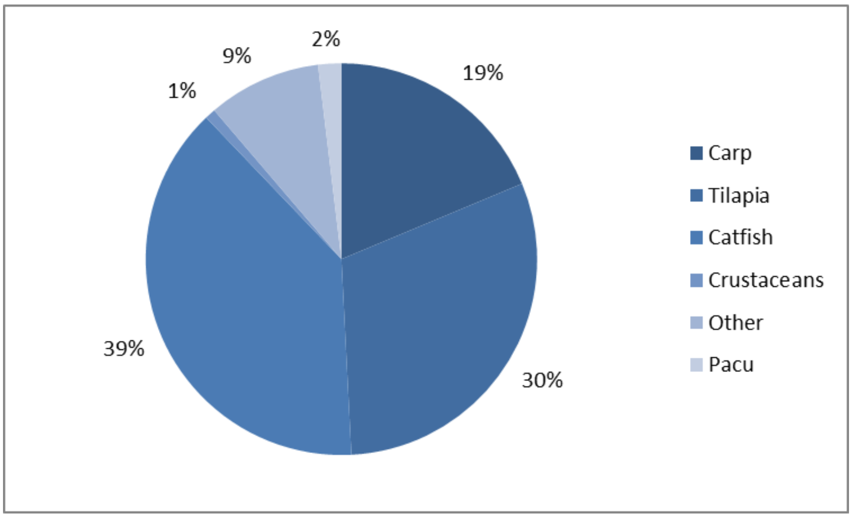

Figure 21, Fish specie categories in freshwater aquaculture in Indonesia in 2019

Vietnam

Practices used

The aquaculture sector in Vietnam consists mainly of brackish and freshwater production systems (World Bank, 2010). With 98%, shrimp dominates the brackish aquaculture as fish dominates the freshwater production with 99% (World Bank, 2010; FAO, 2021). Inland aquaculture in Vietnam consists of pond and cage culture. Pond culture and rice culture are mainly done in the deltas of North and Middle Vietnam, while cage culture is practiced in reservoirs created in the mountains and in the Mekong river in the South of Vietnam. Pond culture is growing over the last years, as at the same time the share of cage culture is diminishing. Pen culture is a new system and the use of this system is growing in the Mekong Delta. According to the World Bank (2010), the Mekong Delta accounts for 75% of Vietnam's cultured fish production, while being dominated by freshwater catfish cultivation. The Red River Delta, residing in the North of Vietnam, constitutes 15% of Vietnam's fish production (World Bank, 2010). Intensive catfish culture in the Mekong delta is the biggest part of Vietnam´s aquaculture sector, as they are grown in earthen ponds, adjoining rivers, allowing the water transfer between rivers and ponds. The success of the intensification of pangasius is partly the result of the species physiological characteristics. The pangasius can directly extract oxygen from the air, thus tolerating low amounts of oxygen in the water production (World Bank, 2010). This means that fish farmers do not have to worry too much about the oxygen content of the water, and can breed a lot of fish in a small space. A floating cage of one cubic meter in the Mekong Delta can contain 150 fish.

Products produced/fish types

Vietnam is the world's biggest pangasius (catfish) producer. Pangasius is mainly cultivated in the Mekong delta. Tilapia and carps are cultivated in ponds.

Table 22, Freshwater aquaculure in Vietnam in 2019

Freshwater Aquaculture Vietnam 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	574
Tilapia	263
Catfish	1613
Trout	0
Pacu	24
Crustaceans	20
Molluscs	0
Other	489
Total	**2983**

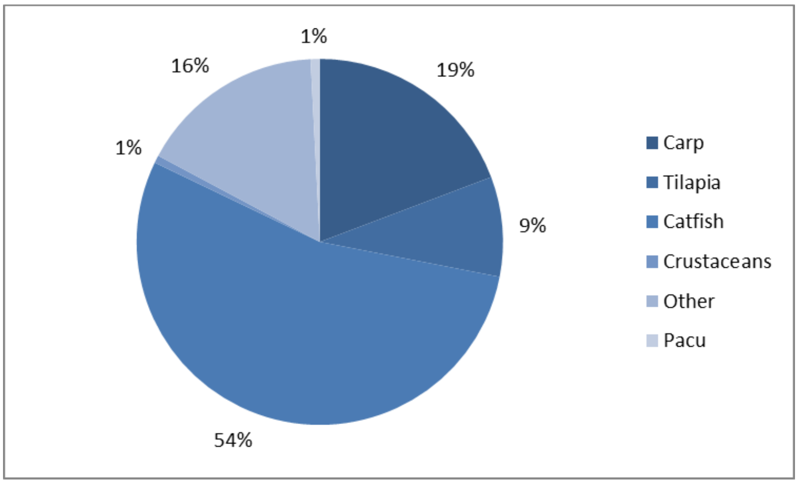

Figure 22, Fish specie categories in freshwater aquaculture in Vietnam in 2019

Bangladesh

Practices used

The Ganges Delta, the world's largest river delta, resides in Bangladesh. As a result, rivers and inland water bodies constitute 7% of the total surface of Bangladesh, and 80% of the country consists of floodplains (Larive International & LightCastle Partners, 2021). Historically, fish plays an important role in Bangladeshi life and is the preferred origin of animal protein. 58% of consumed animal protein comes from fish in Bangladesh (Shamsuzzaman et al., 2020), and fish consumption will only rise as the growing GDP (8%) will result in an increase in purchasing power (Larive International & LightCastle Partners, 2021). 57% of Bangaledshi fish production comes from aquaculture (Shamsuzzaman et al., 2020), of which 91% is freshwater aquaculture (FAO, 2021). Bangladeshi aquaculture is characterized by smallholders. Bangladesh culture systems are predominantly semi-intensive farming systems. However, production output is largest in intensive farming systems (42% vs 35%) as a result of higher average production output of this system (Larive International & LightCastle Partners, 2021). Pond farming is dominant in Bangladesh, as 52% of the farms are pond farms, contributing 79% of total aquaculture production. 9% of the production is produced on farms using floodplains (Larive International & LightCastle Partners, 2021). Farming in lakes or rivers with cage or pen culture is only a small percentage (Shamsuzzaman et al., 2017), as the construction costs and high costs for supplementary feed hamper wide adoption among the poor farmers.

Products produced/fish types

The aquaculture sector of Bangladesh is mainly focussed on polyculture of carp. This will only increase, as a result of increasing purchasing power shifting the focus of farmers towards culture of high value species like carp at the expense of pangasius and tilapia (Parven & Ahmed, 2010; Larive International & LightCastle Partners, 2021). Tilapia (21%) and pangasius (15%, catfishes) are seen as inferior fish, with lower prices and impressions of the use of low-quality feed (Larive International & LightCastle Partners, 2021).

Table 23, Freshwater aquaculure in Bangladesh in 2019

Freshwater Aquaculture Bangladesh 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	1229
Tilapia	350
Catfish	479
Trout	0
Pacu	0
Crustaceans	61
Molluscs	0
Other	152
Total	2271

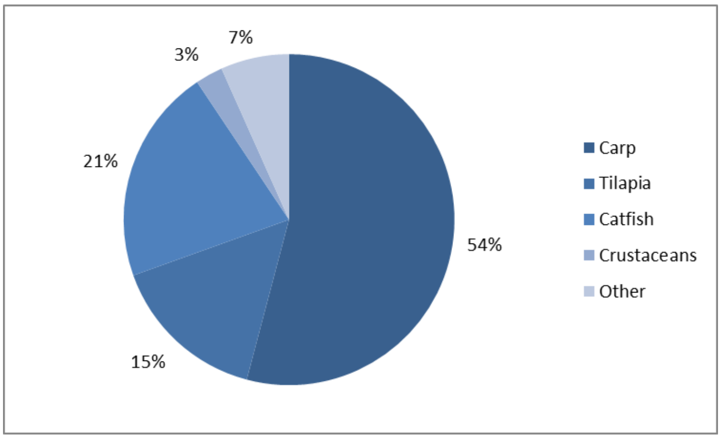

Figure 23, Fish specie categories in freshwater aquaculture in Bangladesh in 2019

Myanmar

Practices used

Myanmar has seen an economical rise in the past years, which has resulted in a large fishing industry. Most of this industry is focussed on capture fisheries. Aquaculture only consists of 1/3th of total production (Tezzo et al., 2018). With 95%, freshwater aquaculture dominates the aquaculture sector in Myanmar (Belton et al., 2015; The World Bank, 2019). Aquaculture in Myanmar is fixated on one region, the Ayeyarwady Delta. The Ayeyarwady Delta is a large delta in the South of Myanmar, covering 3.2% of Myanmar's land area (Soe et al., 2020) . Here, the Irrawaddy River meets the sea, resulting in lots of fresh- and brackish water bodies. 90% of aquaculture cultivation takes place in the Ayeyarwady Delta region, as there is an abundance of water and the close proximity to Yangon, Myanmar's largest city, result in a large distribution area with adequate transport infrastructure (Belton et al., 2015). In this region, pond culture is practiced. Most of the aqua farms are small and medium sized, however large farms contribute most to the total production, as 70% of total pond area is cultivated by large farms (Karim et al., 2020). These large farms often use additional feeding practices (Tezzo et al., 2018), and are semi-intensive (Karim et al., 2020). Cage culture is exclusively practiced in Inle Lake, and contributes only marginally to the total freshwater aquaculture production (FAO, 2016).

Products produced/fish types

Myanmar's freshwater aquaculture sector is strongly dominated by carp production. This overdependence can be a problem in the future, as relying on one species carries risks. Rohu (Labeo rohita) is the carp species of choice, as Rohu contributes to 70% of the total production volume (Tezzo et al., 2018) . In larger ponds where polyculture is practiced, tilapia can also be found. Most of the production is produced for domestic markets (Tezzo et al., 2018).

Table 24, Freshwater aquaculure in Myamar in 2019

Freshwater Aquaculture Myanmar 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	897
Tilapia	69
Catfish	40
Trout	0
Pacu	5
Crustaceans	10
Molluscs	0
Other	9
Total	**1030**

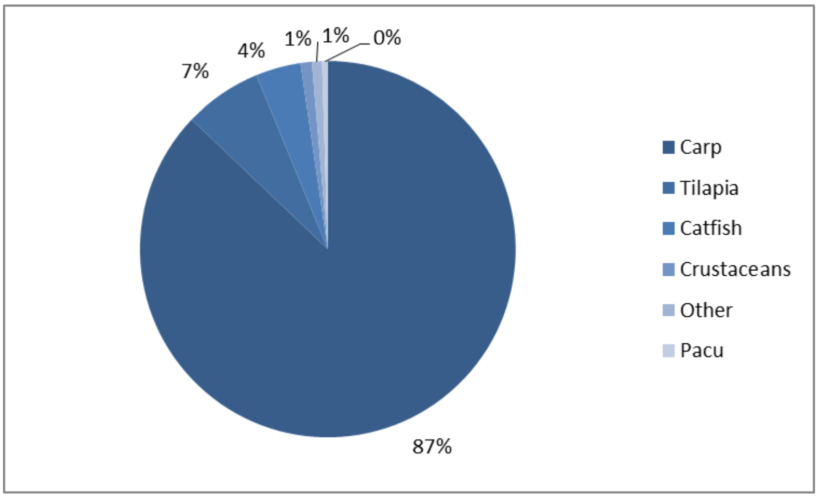

Figure 24, Fish specie categories in freshwater aquaculture in Myanmar in 2019

Brazil

Practices used

Brazil as a country has massive potential for freshwater aquaculture, as it has certain advantageous characteristics as a country; 12 percent of the available global freshwater is located in Brazil, there is enough space with 5.5 million hectares of lake and reservoir areas, the weather is favourable and native fish species suitable for aquaculture (Bueno et al., 2015; Nobile et al., 2020; Valenti et al., 2021). This was recognized by the Dutch, which started aquaculture in Brazil during their occupation in the 17th century by the West India Company (WIC) (Valenti et al., 2021). However, only since the 1970's did aquaculture become somewhat professionally organised (Valenti et al., 2021). Industrial aquaculture is hence a new enterprise. Currently, most of the aquaculture production is produced in lots of small scale farms, with Brazil having over 200 thousand freshwater fish farms and 80 percent of the aquaculture farms having less than 2ha (Valenti et al., 2021). The southern regions contribute the most to the country´s aquaculture production, with an output of almost 50 percent of Brazil´s aquaculture production (Valenti et al., 2021). Aquaculture in Brazil is focussed on freshwater, as freshwater aquaculture produces 90 percent of the total aquaculture production, and 95 percent of the total amount of aquaculture farms are freshwater farms. Currently, Brazil produces 1 percent of the global freshwater aquaculture (FAO, 2021). However, the aquaculture sector has seen a significant growth in the past years, as the freshwater aquaculture production has increased with 25 percent in the last 5 years (Valenti et al., 2021). Pond production systems are the most common fresh water aquaculture systems in Brazil, with 80 percent of the production produced in earthen ponds (Valenti et al., 2021). These are mostly small local farmers, as 95 percent of the pond aquaculture farms are smaller than 2 ha, and only 0.1 percent are greater than 50 ha (Valenti et al., 2021). Aquaculture in these small farms is generally a secondary activity, where the farmers "buy seeds and feed from other companies and selling to processing plants, wholesalers, retailers or even directly to consumers" (Valenti et al., 2021, p. 3). Aquaculture parks, meant to produce fish with net cages in reservoirs are being promoted by the Brazilian government in the past years (Bueno et al., 2015), resulting in an increased significance in the aquaculture sector. With the amount of available fresh water resources, Brazil can become the worlds largest net-cage producer (Bueno et al., 2015). Raceways are not used much in Brazil, and is almost only confined to the production of trout (Valenti et al., 2021). The modernization of aquaculture practices is not widely used yet, as recirculating aquaculture systems (RAS) are also rare. Integrated aquaculture is done in earthen ponds following the Chinese model, adding tilapias and catfishes to the common carp and Chinese carps (Valenti et al., 2021).

Products produced/fish types

Table 25, Freshwater aquaculure in Brazil in 2019

Freshwater Aquaculture Brazil 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	18
Tilapia	324
Catfish	11
Trout	2
Pacu	161
Crustaceans	0
Molluscs	0
Other	14
Total	**530**

Brazil produced 324 thousand tonnes of tilapia in 2019, making it the 4th largest tilapia producer in the world, surpassing the Philippines and Thailand in recent years (Nobile et al., 2020; FAO, 2021). As seen in figure 26, the non-native tilapia consists of 61 percent of Brazil's freshwater aquaculture production. Tilapia is traditionally produced in earthen ponds, however Brazil has seen a switch towards production in net-cages in large reservoirs which have higher productivity (Valenti et al., 2021). The native family of pacu's has the second highest output, with 30 percent. Pacu's are almost only produced in South America. The category of pacu consists of the following native South American round shaped fishes; pacu, patinga, tambaqui, tambacu and tambatinga. These officially do not belong to the same family, however have such a close resemblance, that for this study they are put in the same category. There are worries in Brazil that the introduction of non-native species results in biological diversity loss, as non-native parasites will be passed onto native species resulting in new diseases in Brazil, while also invading genotypes (Nobile et al., 2020). Carp polyculture used to be significant, however the production of carp has seen a decline with the rise of tilapia production (Valenti et al., 2021). In recent times, the production of the catfish Pangasius has seen an increase of interest.

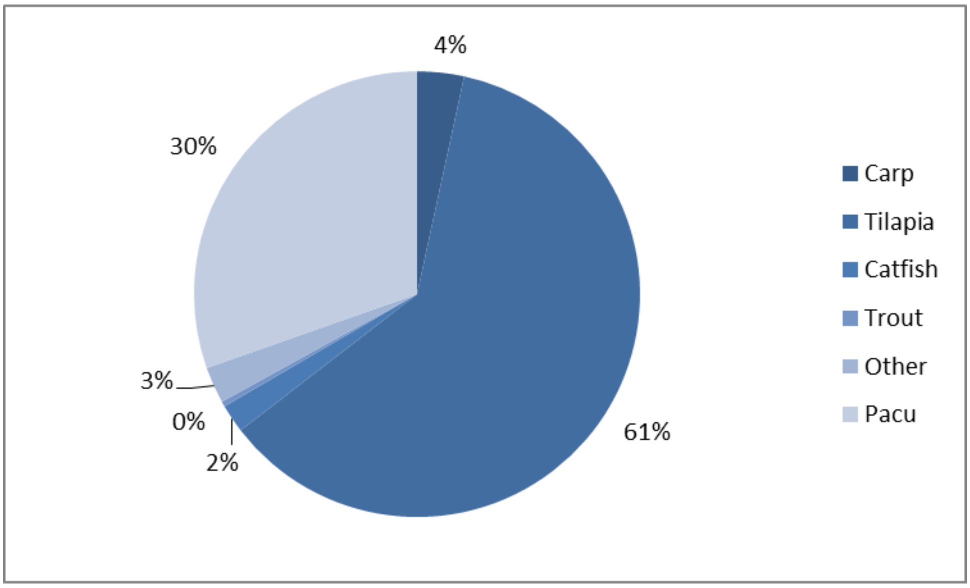

Figure 25, Fish specie categories in freshwater aquaculture in Brazil in 2019

Iran

Practices used

Aquaculture is a relatively new practice in Iran, as it has only emerged in the last four decades as a result of investments in aquaculture development in the early 1980s. As weather conditions vary significantly across Iran there are various types of culture systems used (Kalbassi et al., 2013). Extensive aquaculture is carried out in inland lakes and reservoirs all over Iran (Kalbassi et al., 2013). The numerous dams that are built to provide Iran from electricity and manage the growing water scarcity have created many reservoirs with outstanding conditions for aquaculture. Most of the freshwater aquaculture is produced in earthen ponds, where carps are produced in semi-intensive aquaculture(Kalbassi et al., 2013). Rainbow trout is cultured in concrete raceways in provinces in the centre, north western and western parts of the country, where mountains provide cool summers and cold winters (Kalbassi et al., 2013). Recirculating aquaculture systems are rare in Iran, as the productivity levels are lower as projected and the production costs are bigger in comparison to the more commonly used raceway systems (Kalbassi et al., 2013). In the Northern part of Iran, fish culture in rice paddies has benefited rural families. This is however a small share of the total production (Kalbassi et al., 2013). Cage culture is practiced in reservoirs on a small scale.

Products produced/fish types

Iran is the worlds biggest trout producer in the world, producing 198800 tons of rainbow trout in 2019, contributing 21 percent of the total global trout production (FAO, 2021). Rainbow trout is a salmonid fish which is also cultured in fresh water in Europe and North America, and represents over 97 percent of the trout category. Besides rainbow trout, carp polyculture in earthen ponds is also practiced in Iran.

Table 26, Freshwater aquaculure in Iran in 2019

Freshwater Aquaculture Iran 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	215
Tilapia	0
Catfish	0
Trout	199
Pacu	0
Crustaceans	0
Molluscs	0
Other	4
Total	**418**

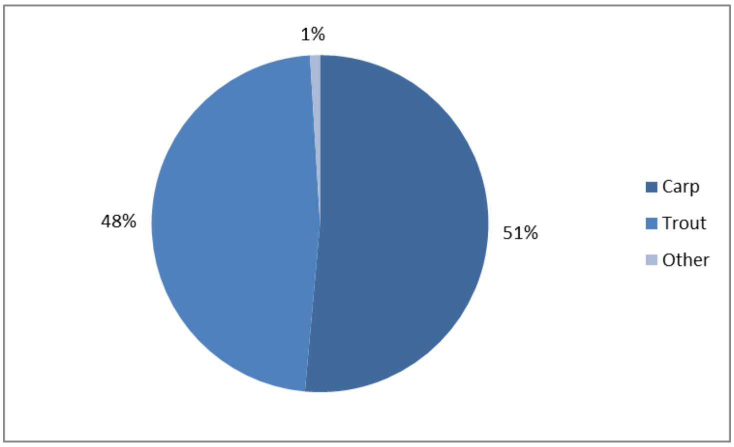

Figure 26, Fish specie categories in freshwater aquaculture in Iran in 2019

Thailand

Practices used

Aquaculture in Thailand follows the same trend as the rest of South East Asia, showing a big increase over the past 20 years (Sampantamit et al., 2020). Marine aquaculture produces the main bulk, with a production of 57% of total aquaculture production. Freshwater aquaculture constitutes for 43% of total aquaculture production (DoF, 2018). While coastal aquaculture consists mainly of shellfish culture (crustaceans) and the culture of molluscs, freshwater aquaculture is dominated by finfish production (DoF, 2018). Freshwater fish is cultivated in ponds, cages, paddy rice fields and ditches (DoF, 2018; Wongbusarakum et al., 2019), and is primarily produced for domestic consumption. Marine aquaculture generally produces products with high value which are moslty exported (Wongbusarakum et al., 2019). Thailand's department of fisheries distinguishes four freshwater aquaculture systems; pond culture, which constitutes almost 92% of the total freshwater aquaculture production; cage culture (7%), paddy rice field culture (<1%) and ditch culture (<1%). With over 97%, the percentage of pond farms in relation to the total amount of freshwater aquaculture farms is even bigger. Cage farms have a relative high production output. Most inland aquaculture is produced in Central Thailand (Sampantamit et al., 2020).

Products produced/fish types

Thailand has quite a diverse freshwater aquaculture sector. Over half of the production is tilapia fish. However, there is also a substantial cultivation of catfish, carps, crustaceans and other not classified fish species.

Table 27, Freshwater aquaculure in Thailand in 2019

Freshwater Aquaculture Thailand 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	25
Tilapia	214
Catfish	116
Trout	0
Pacu	0
Crustaceans	31
Molluscs	0
Other	31
Total	**417**

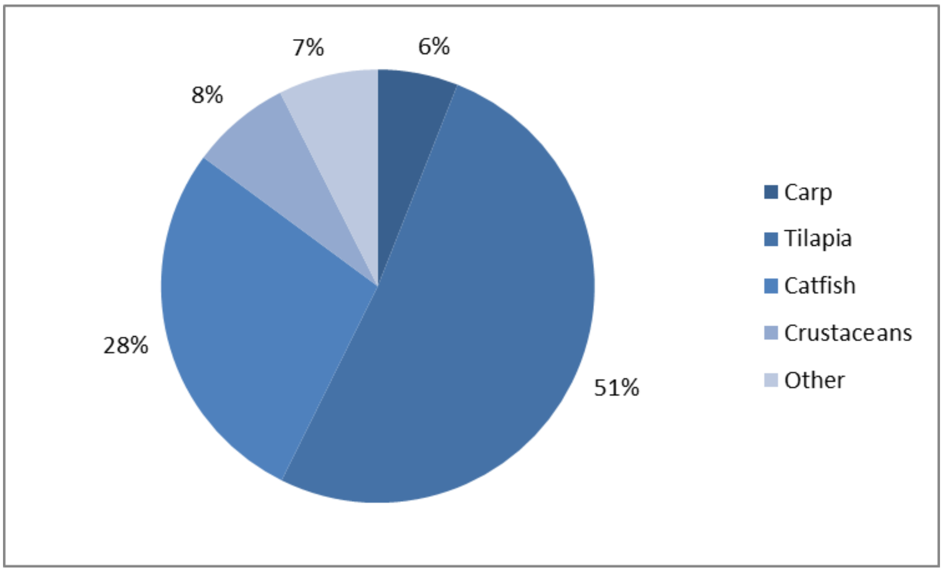

Figure 27, Fish specie categories in freshwater aquaculture in Thailand in 2019

Philippines

Practices used

Philippines aquaculture is largely based around the culture of seaweeds, making the Philippines the third largest producer of seaweeds globally (PSA, 2018). The Philippines produced 321 thousand tonnes of freshwater aquaculture in 2019, 14% of the total aquaculture production (BFAR, 2020). Most of the aquaculture is produced in fishponds (52%). Cages (32%) and fish pens (17%) are used as well (BFAR, 2020). Shallow earthen fishponds are cultivated as semi- intensive systems by small farmers (FAO, 2005). Around ¾ of the fishponds are smaller than 1 hectare, averaging an extent of 0.13 hectares per farm (PSA, 2018). These small farms are named “backyard fishponds”, as the farmer lives in the surrounding area of the aquafarm (PSA, 2018).

Products produced/fish types

Freshwater aquaculture in the Philippines is based on tilapia cultivation (81%). For the largest part, tilapia is cultivated in fishponds (54%), followed by fish cages (38%)(FAO, 2005). Carp is produced in small amounts as well, mostly in freshwater fish pens in Laguna Lake, Philippines biggest lake (FAO, 2005).

Table 28, Freshwater aquaculure in Philipines in 2019

Freshwater Aquaculture Philippines 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	13
Tilapia	261
Catfish	5
Trout	0
Pacu	0
Crustaceans	0
Molluscs	0
Other	42
Total	**321**

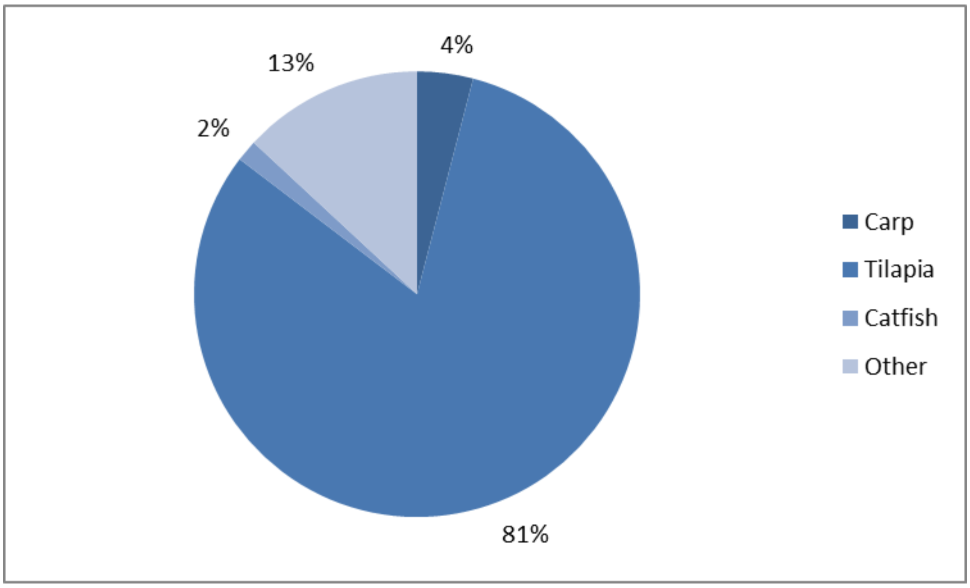

Figure 28, Fish specie categories in freshwater aquaculture in Philippines in 2019

Egypt

Practices used

Egypt's economy and food supply is dependent on the fishing industry, which consists of fisheries and aquaculture (Rothuis et al., 2013; Soliman & Yacout, 2016). Aquaculture dominates the national fisheries production, contributing 77% (Adeleke et al., 2021; Ali et al., 2020). Besides, Egypt produces the most aquatic products in all of Africa, and is the 8th largest aquaculture producer globally (Eltholth et al., 2015). Most of the aquaculture production is centered around the Nile delta north of Cairo (Rothuis et al., 2013). Historically aquaculture was done in a traditional extensive aquaculture system, named a "hosha" (Kaleem & Bio Singou Sabi, 2020; Rothuis et al., 2013; Soliman & Yacout, 2016). In this system, a pond is constructed on the lake/river shore, directing water and fish from the water body towards the hosha, trapping fish in the hosha. The fish then relied on natural food. As the hosha system resulted in environmental damage, and resulted in competition with lake fishing, the system was prohibited by the government (Kaleem & Bio Singou Sabi, 2020; Rothuis et al., 2013; Soliman & Yacout, 2016). Rice farming used to be used as aquaculture system, but with the decrease in rice cultivation as a result of water scarcity, this system is waning as well (Soliman & Yacout, 2016). The last two decades have seen a switch from traditional extensive aquaculture systems to semi-intensive and intensive aquaculture systems (Adeleke et al., 2021; Ali et al., 2020). Currently, semi-intensive aquaculture production in earthen ponds is dominating the aquaculture sector, contributing 86% of the total production (Adeleke et al., 2021; Kaleem & Bio Singou Sabi, 2020). These farms are often quite large. However, there is a shift taking place towards more intensive pond aquaculture, with earthen ponds that are smaller but deeper then semi-intensive earthen ponds (Rothuis et al., 2013). Besides intensive pond culture, intensive tank and cage farming is also fast developing (Kaleem & Bio Singou Sabi, 2020).

Products produced/fish types

The freshwater aquaculture sector is dominated by tilapia and carp production. Tilapia is only produced for domestic consumption, as the industry is not able to meet the food safety standards of the EU and USA (Adeleke et al., 2021).

Table 29, Freshwater aquaculure in Egypt in 2019

Freshwater Aquaculture Egypt 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	147
Tilapia	123
Catfish	6
Trout	0
Pacu	0
Crustaceans	0
Molluscs	0
Other	33
Total	**309**

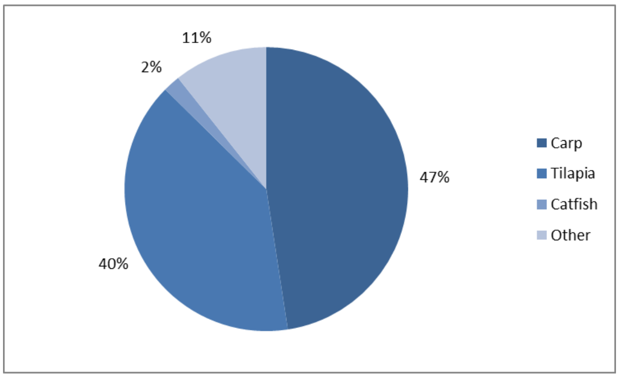

Figure 29, Fish specie categories in freshwater aquaculture in Egypt in 2019

Cambodia

Practices used

Fish has always played an important role in Cambodian life. Over 80% of consumed animal protein stems from fish in Cambodia (Richardson & Suvedi, 2018).The lion's share of fish produced in Cambodia comes from inland water bodies (Richardson & Suvedi, 2018). The Mekong River and Tonlé Sap, the largest freshwater lake in Southeast Asia which covers about 7.5% of Cambodia's surface, reside in Cambodia. Cambodia's fishing industry is mainly focussed on (inland) fisheries (Joffre et al., 2017), while aquaculture only represents 10% of Cambodia's total fish production (Joffre et al., 2010). Although it is widely accepted that cage and pen culture originated from Cambodia, the impact of aquaculture is still marginal. However, wild fish resources are now decreasing, as a result of overfishing leading to an increasing important role for aquaculture. Freshwater aquaculture dominates the aquaculture industry. There are 5 freshwater aquaculture systems used in Cambodia which contribute to the total aquaculture production in Cambodia; smallholder low-input pond culture (5%); smallholder high-input pond culture (18%); small and medium-sized enterprise (SME) intensive pond culture (22%); freshwater cage culture (53%) and rice-fish systems (0.2%) (Joffre et al., 2010, 2016). Although most farms are smallholder low-input pond systems (>50%), they have a low production output as a result of these farms having only 1 to 2 small ponds. Freshwater cages are for the major part located in the Tonlé Sap lake and the Mekong River, while the pond culture is located in lower flood plains (Joffre et al., 2016). Rice-fish culture is a relatively new culture system, and is not widely adopted in Cambodia. Overall, intensive aquaculture contributes around 75% of the total production output, while 25% stems from extensive/semi-intensive systems (Lang, 2015)

Products produced/fish types

Cambodian freshwater aquaculture is dominated by carps and catfishes. snakeheads are also a common freshwater specie, which is however in this study categorized within the "other" category. Tilapia cultivation is quickly growing in the last years, as tilapia is becoming more popular with the Cambodians (Joffre et al., 2019)

Table 30, Freshwater aquaculure in Cambodia in 2019

Freshwater Aquaculture Cambodia 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	94
Tilapia	11
Catfish	97
Trout	0
Pacu	0
Crustaceans	0
Molluscs	0
Other	88
Total	**290**

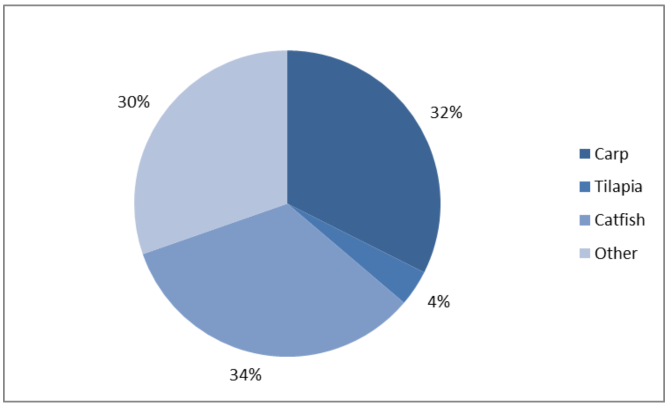

Figure 30, Fish specie categories in freshwater aquaculture in Cambodia in 2019

Nigeria

Practices used

With a population of over 200 million, Nigeria has the largest population of Africa, as well as the highest fish demand in Africa (Adeleke et al., 2021). As a result, an immense growth of aquaculture has made Nigeria Africa's second largest freshwater aquaculture producer, with a production output of almost 300.000 tonnes (FAO, 2021). Aquaculture is stimulated by the Federal government of Nigeria as well, in a way to manage the increasing demand for fish and to broaden its oil based economy (Adewumi, 2015). Aquaculture in Nigeria is based on small scale freshwater aquaculture, with 80% of production coming from small scale farmers (Kaleem & Bio Singou Sabi, 2020).

The use of semi intensive freshwater ponds is the most common way of aquaculture in Nigeria(Kaleem & Bio Singou Sabi, 2020). Earthen ponds are a regular system used in locations with a high water table (Adeleke et al., 2021). Historically , flow through systems like tanks and raceways are the first inland aquaculture systems used in Nigeria, and are still common (Adeleke et al., 2021). Ibemere & Ezeano (2014) show in their study that 31.1% of the interviewed fish farmers (n=90) cultivated in concrete fish tanks, while 23.3% used earthen ponds. Cage and pen culture contributed 14.4% and 8.9% respectively. Recirculating aquaculture systems are rare in Nigeria. Cage culture is a growing method for aquaculture production in Nigeria, and currently already has an important share of the total production output (Adeleke et al., 2021).

Products produced/fish types

Aquaculture in Nigeria is dominated by the cultivation of African catfishes, as seen in figure 32. Alongside catfishes, tilapia and carp are produced as well in large quantities.

Table 31, Freshwater aquaculure in Nigeria in 2019

Freshwater Aquaculture Nigeria 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	24
Tilapia	22
Catfish	190
Trout	0
Pacu	0
Crustaceans	0
Molluscs	0
Other	54
Total	**290**

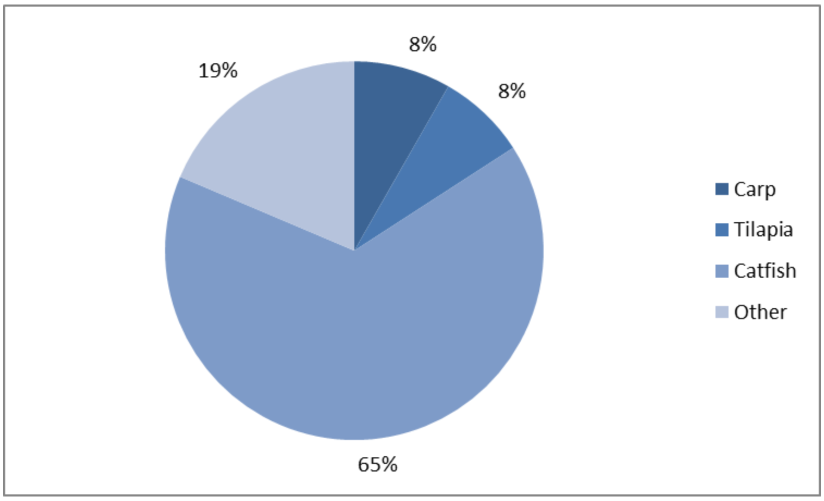

Figure 31, Fish specie categories in freshwater aquaculture in Nigeria in 2019

United States of America

Practices used

Fish production is an important part of the American economy. America is ranked 5th of the world in tonnes fish production, of which 17% comes from aquaculture and 83% from fisheries (OECD, 2021). The aquaculture sector of the United States consists of 56 % freshwater aquaculture, overshadowing marine aquaculture production (FAO, 2021). However, the increasing global demand for fish products has not resulted in a swift expansion of the aquaculture sector as seen in other countries discussed in this chapter. This is the result of campaigns of environmental activism in the last two decades, influencing public perception and governmental perspectives of the aquaculture sector (FAO, 2021). Historically, the cultivation of channel catfish began in the 1960s in the southern states of Louisiana, Arkansas, Alabama, and Mississippi, alongside the Mississippi river and its tributaries (Olin, 2012). According to Olin (2012), these states account for over 90% of the catfish production of the United States, primarily using earthen ponds. Most of the produced trout origins from Idaho, as Idaho accounts for 50% of the total farmed trout in the United States (Olin, 2012). The rainbow trout is mainly cultivated in raceways. Crustaceans are often cultivated in a rotational system of rice or soybeans. This is done in shallow earthen ponds (FAO, 2021). According to the FAO (2021), the aquaculture sector is considered as intensive, while using more and more technology, accentuated by the increasing interest in recirculation aquaculture systems.

Produced fish species

The freshwater aquaculture sector is dominated by channel catfish, accounting for 60% of the total freshwater aquaculture production. Channel fish is a relatively cheap fish species, produced for domestic markets (FAO, 2021). Besides channel catfishes, with 6%, rainbow trout has a marginal role in the total production output of the United States. However, besides finfish, crustaceans also play a very significant role in the freshwater aquaculture in the United States. Crustaceans include two species: the giant freshwater prawn and the red swamp crayfish, with the latter dominating heavily. In response to climate change, product diversification is being stimulated, and research into new species is being orchestrated.

Table 32, Freshwater aquaculure in United States of America in 2019

Freshwater Aquaculture United States of America 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	1
Tilapia	7
Catfish	153
Trout	15
Pacu	0
Crustaceans	72
Molluscs	0
Other	6
Total	**254**

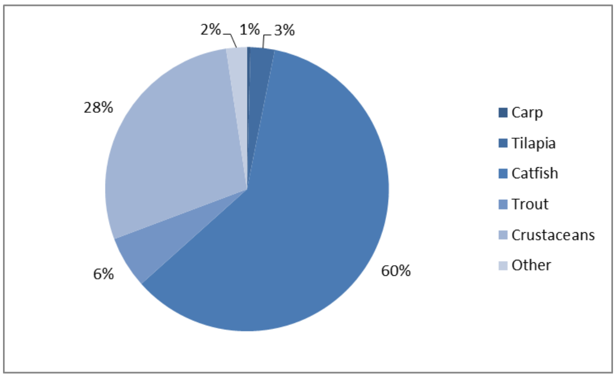

Figure 32, Fish specie categories in freshwater aquaculture in USA in 2019

Russian Federation

Practices used

Russia is the 6th global fish producing country. However, with under 5%, the share of aquaculture on the total fish production is very small (Gurkovskaya et al., 2019). In its history, Russia has always focussed on capture fisheries. Fish farming was not the first priority, and has been neglected ever since, resulting in a lagged development to this day (Kalinina et al., 2019). Fresh water aquaculture contributes 56% of the total aquaculture production in 2017 (Gurkovskaya et al., 2019). The bulk of the freshwater aquaculture production is produced in the Southern district and the North Western district which together produced 58% of aquaculture production in 2016 (Kalinina et al., 2019). Of the 3000 aquaculture farms in Russia, most of them operate with pond systems, as carps are mostly produced in ponds. Pond aquaculture is practiced on an extensive and semi-intensive level. There is a lack of technology use in Russian aquaculture, with a lot of small farms with low production output. Trout is produced in cages in the North Western district, as water temperatures in lakes are excellent. Trout cultivation in tanks is practiced in the Southern district.

Products produced/fish types

Russian freshwater aquaculture is based on carp and trout cultivation.

Table 33, Freshwater aquaculure in Russian Federation in 2019

Freshwater Aquaculture Russian Federation 2019	
Category	**Quantity in Tonnes (Thousands)**
Carp	126
Tilapia	0
Catfish	2
Trout	45
Pacu	0
Crustaceans	0
Molluscs	0
Other	11
Total	**184**

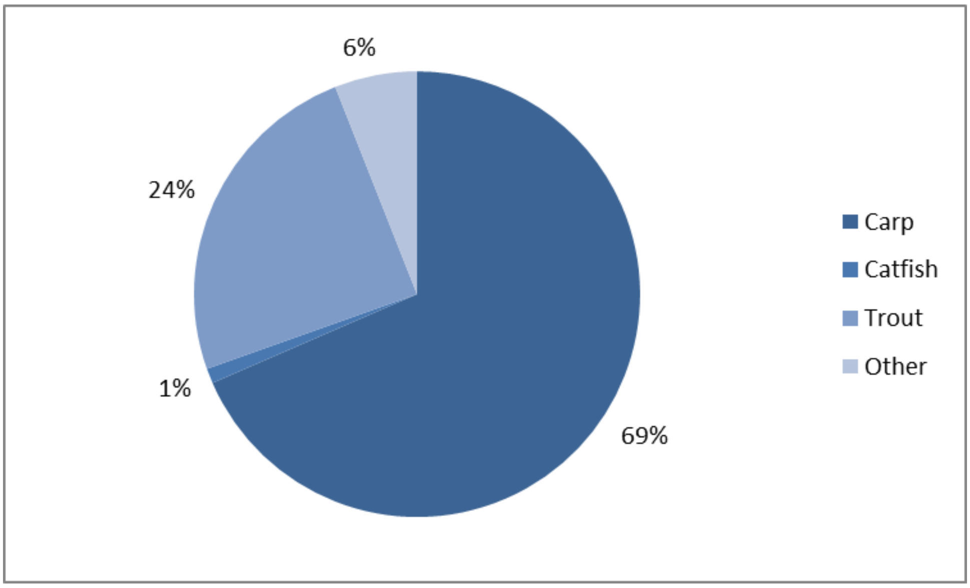

Figure 33, Fish specie categories in freshwater aquaculture in Russia in 2019

Appendix II: Production data

Table 34, Freshwater aquaculture production of China in 2019

Freshwater Aquaculture China 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Grass carp(=White amur)	Carp	5 533
Silver carp	Carp	3 810
Bighead carp	Carp	3 102
Common carp	Carp	2 885
[Carassius spp]	Carp	2 756
Red swamp crawfish	Crustaceans	2 090
Nile tilapia	Tilapia	1 231
Chinese mitten crab	Crustaceans	779
Wuchang bream	Carp	763
Black carp	Carp	680
Whiteleg shrimp	Crustaceans	671
Freshwater fishes nei	Other	670
Yellow catfish	Catfish	537
Largemouth black bass	Other	478
Snakehead	Other	462
Blue-Nile tilapia, hybrid	Tilapia	411
Pond loach	Other	357
Amur catfish	Catfish	355
Mandarin fish	Other	337
Chinese softshell turtle	Other	325
Asian swamp eel	Other	314
Channel catfish	Catfish	298
Japanese eel	Other	234
Oriental river prawn	Crustaceans	225
Giant river prawn	Crustaceans	140
Frogs	Other	107
Sturgeons nei	Other	102
Chinese mystery snail	Molluscs	93
Pirapatinga	Pacu	69
Chinese pond mussel	Molluscs	58
Spirulina nei	Algae	55
Aquatic invertebrates nei	Crustaceans	52
River and lake turtles nei	Other	46
Rainbow trout	Trout	39
Freshwater prawns, shrimps nei	Crustaceans	26
Chinese longsnout catfish	Catfish	22
Freshwater molluscs nei	Molluscs	21
Asian clam	Molluscs	18
Clearhead icefish	Other	14
Pond smelt	Other	11
Obscure pufferfish	Other	10
Salmonoids nei	Other	2
Freshwater mussel shells	Molluscs	1
[Haematococcus pluvialis]	Algae	0

Swimming crabs, etc. nei	Crustaceans	0
Mud carp	Carp	0
Total		**30 187**

Table 35, Freshwater aquaculture production of India in 2019

Freshwater Aquaculture India 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Catla	Carp	3055
Roho labeo	Carp	1256
Freshwater fishes nei	Other	1013
Striped catfish	Catfish	594
Silver carp	Carp	504
Mrigal carp	Carp	266
Torpedo-shaped catfishes nei	Catfish	130
Grass carp(=White amur)	Carp	27
Common carp	Carp	25
Orangefin labeo	Carp	14
Giant river prawn	Crustaceans	9
Manipur osteobrama	Carp	5
Rainbow trout	Trout	1
Kelee shad	Other	0
Climbing perch	Other	0
Giant tiger prawn	Trout	0
Monsoon river prawn	Crustaceans	0
River prawns nei	Crustaceans	0
Aquatic plants nei	Algae	0
Snakeheads(=Murrels) nei	Other	0
Total		**6897**

Table 36, Freshwater aquaculture production of Indonesia in 2019

Freshwater Aquaculture Indonesia 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Nile tilapia	Tilapia	1130
Torpedo-shaped catfishes nei	Catfish	1034
Common carp	Carp	631
Pangas catfishes nei	Catfish	441
Giant gourami	Other	205
Milkfish	Other	79
Pirapatinga	Pacu	73
Nilem carp	Carp	44
Mozambique tilapia	Tilapia	37
Silver barb	Carp	36
Indonesian snakehead	Other	29
Whiteleg shrimp	Crustaceans	27
Freshwater fishes nei	Other	21
Snakeheads(=Murrels) nei	Other	9

Kissing gourami	Other	7
Asian redtail catfish	Catfish	6
Hoven's carp	Carp	6
Giant river prawn	Crustaceans	5
Climbing perch	Other	4
Snakeskin gourami	Other	4
Giant tiger prawn	Crustaceans	2
Frogs	Other	0
River eels nei	Other	0
Marble goby	Other	0
Penguin wing oyster	Molluscs	0
Gudgeons, sleepers nei	Other	0
Red claw crayfish	Crustaceans	0
Total		**3828**

Table 37, Freshwater aquaculture production of Vietnam in 2019

Freshwater Aquaculture Vietnam 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Striped catfish	Catfish	1600
Freshwater fishes nei	Other	476
Cyprinids nei	Carp	440
Tilapias nei	Tilapia	263
Common carp	Carp	134
Pirapatinga	Pacu	24
Giant river prawn	Crustaceans	20
Torpedo-shaped catfishes nei	Catfish	13
Frogs	Other	11
Sturgeons nei	Other	2
River and lake turtles nei	Other	1
Freshwater molluscs nei	Molluscs	0
Total		**2984**

Table 38, Freshwater aquaculture production of Bangladesh in 2019

Freshwater Aquaculture Bangladesh 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Striped catfish	Catfish	447
Tilapias nei	Tilapia	350
Roho labeo	Carp	304
Silver carp	Carp	232
Mrigal carp	Carp	204
Catla	Carp	190
Common carp	Carp	99
Freshwater fishes nei	Other	96
Grass carp(=White amur)	Carp	60
Giant river prawn	Crustaceans	52
Climbing perch	Other	51

Cyprinids nei	Carp	50
Silver barb	Carp	44
Orangefin labeo	Carp	34
Philippine catfish	Catfish	16
Stinging catfish	Catfish	15
Freshwater prawns, shrimps nei	Crustaceans	9
Asian barbs nei	Carp	7
Olive barb	Carp	6
Striped snakehead	Other	1
Spotted snakehead	Other	1
Wallago	Other	1
Bronze featherback	Other	1
Great snakehead	Other	1
Clown knifefish	Other	0
Total		**2271**

Table 39, Freshwater aquaculture production of Myanmar in 2019

Freshwater Aquaculture Myanmar 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Roho labeo	Carp	361
Silver barb	Carp	260
Common carp	Carp	256
Tilapias nei	Tilapia	69
Striped catfish	Catfish	25
Torpedo-shaped catfishes nei	Catfish	15
Giant river prawn	Crustaceans	10
Silver carp	Carp	9
Freshwater fishes nei	Other	8
Catla	Carp	5
Pirapatinga	Pacu	5
Bighead carp	Carp	4
Grass carp(=White amur)	Carp	1
Streaked prochilod	Other	1
Stinging catfish	Catfish	0
Mrigal carp	Carp	0
Giant gourami	Other	0
Vatani rohtee	Carp	0
Total		**1029**

Table 40, Freshwater aquaculture production of Brazil in 2019

Freshwater Aquaculture Brazil 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Nile tilapia	Tilapia	324
Cachama	Pacu	101
Tambacu, hybrid	Pacu	32
Cyprinids nei	Carp	18

Pacu	Pacu	12
Sorubims nei	Catfish	11
Tambatinga, hybrid	Pacu	8
Freshwater fishes nei	Other	4
[Brycon spp]	Pacu	4
[Brycon amazonicus]	Pacu	3
Streaked prochilod	Other	3
[Leporinus spp]	Other	3
Rainbow trout	Trout	2
Arapaima	Other	2
Pirapatinga	Pacu	2
Trahira	Other	1
Banded astyanax	Other	1
American bull frog	Other	0
Giant river prawn	Crustaceans	0
[Cichla spp]	Other	0
Dorado	Other	0
River and lake turtles nei	Other	0
Total		**530**

Table 41, Freshwater aquaculture production of Iran in 2019

Freshwater Aquaculture Iran 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Rainbow trout	Trout	199
Silver carp	Carp	118
Common carp	Carp	54
Grass carp(=White amur)	Carp	32
Bighead carp	Carp	11
Sturgeons nei	Other	3
Freshwater fishes nei	Other	1
Danube crayfish	Crustaceans	0
Giant river prawn	Crustaceans	0
Total		**418**

Table 42, Freshwater aquaculture production of Thailand in 2019

Freshwater Aquaculture Thailand 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Nile tilapia	Tilapia	214
Africa-bighead catfish, hybrid	Catfish	102
Giant river prawn	Crustaceans	31
Silver barb	Carp	21
Freshwater fishes nei	Other	17
Striped catfish	Catfish	13
Snakeskin gourami	Other	7
East Asian bullfrog	Other	2
Roho labeo	Carp	2
Striped snakehead	Other	1

Giant gourami	Other	1
Climbing perch	Other	1
Common carp	Carp	1
Silver carp	Carp	1
Indonesian snakehead	Other	1
Mrigal carp	Carp	0
Chinese softshell turtle	Other	0
Mozambique tilapia	Tilapia	0
Knifefishes	Other	0
Marble goby	Other	0
Gouramis nei	Other	0
Asian swamp eel	Other	0
Isok barb	Carp	0
Total		**417**

Table 43, Freshwater aquaculture production of Philippines in 2019

Freshwater Aquaculture Philippines 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Nile tilapia	Tilapia	170
Tilapias nei	Tilapia	91
Milkfish	Other	41
Cyprinids nei	Carp	13
Torpedo-shaped catfishes nei	Catfish	5
Striped snakehead	Other	1
Freshwater fishes nei	Other	0
Giant gourami	Other	0
Giant river prawn	Crustaceans	0
Total		**321**

Table 44, Freshwater aquaculture production of Egypt in 2019

Freshwater Aquaculture Egypt 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Nile tilapia	Tilapia	123
Common carp	Carp	80
Silver, bighead carps nei	Carp	67
Mullets nei	Other	33
North African catfish	Catfish	6
Bayad	Catfish	0
River eels nei	Other	0
European seabass	Other	0
Gilthead seabream	Other	0
Grass carp(=White amur)	Carp	0
Total		**309**

Table 45, Freshwater aquaculture production of Cambodia in 2019

Freshwater Aquaculture Cambodia 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Pangas catfishes nei	Catfish	90
Striped snakehead	Other	66
Silver barb	Carp	50
Cyprinids nei	Carp	32
Snakeskin gourami	Other	19
Nile tilapia	Tilapia	11
Torpedo-shaped catfishes nei	Catfish	7
Hoven's carp	Carp	6
Common carp	Carp	4
Climbing perch	Other	3
Silver carp	Carp	0
Grass carp(=White amur)	Carp	0
Giant river prawn	Crustaceans	0
Frogs	Other	0
Bighead carp	Carp	0
Asian swamp eel	Other	0
Red claw crayfish	Crustaceans	0
Freshwater siluroids nei	Catfish	0
Philippine catfish	Catfish	0
Mozambique tilapia	Tilapia	0
Total		**290**

Table 46, Freshwater aquaculture production of Nigeria in 2019

Freshwater Aquaculture Nigeria 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
North African catfish	Catfish	157
Torpedo-shaped catfishes nei	Catfish	29
Cyprinids nei	Carp	24
Tilapias nei	Tilapia	22
Nile perch	Other	16
Aba	Other	7
Reticulate knifefish	Other	6
African bonytongue	Other	5
Characins nei	Other	5
Upsidedown catfishes	Catfish	5
Grass-eaters nei	Other	4
Citharinus nei	Other	4
Kafue pike	Other	4
Parachanna snakeheads nei	Other	3
Freshwater fishes nei	Other	1
Mullets nei	Other	0
Bagrid catfish	Catfish	0
Naked catfishes	Catfish	0
Total		289

Table 47, Freshwater aquaculture production of United States of America in 2019

Freshwater Aquaculture United States of America 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Channel catfish	Catfish	153
Red swamp crawfish	Crustaceans	72
Rainbow trout	Trout	15
Tilapias nei	Tilapia	7
Striped bass, hybrid	Other	4
Sturgeons nei	Other	1
Freshwater fishes nei	Other	1
Grass carp(=White amur)	Carp	0
Cyprinids nei	Carp	0
Barramundi(=Giant seaperch)	Other	0
American yellow perch	Other	0
Sea trout	Trout	0
Arctic char	Trout	0
Giant river prawn	Crustaceans	0
Common carp	Carp	0
Bighead carp	Carp	0
Total		**254**

Table 48, Freshwater aquaculture production of Russian Federation in 2019

Freshwater Aquaculture Russian Federation 2019		
ASFIS species (Name)	**Category**	**Quantity in Tonnes (Thousands)**
Common carp	Carp	70
Rainbow trout	Trout	45
Silver carp	Carp	39
Grass carp(=White amur)	Carp	9
Cyprinids nei	Carp	7
Whitefishes nei	Other	5
Sturgeons nei	Other	4
Channel catfish	Catfish	2
Northern pike	Other	1
Freshwater fishes nei	Other	1
Roaches nei	Carp	0
European perch	Other	0
Aquatic invertebrates nei	Crustaceans	0
Freshwater crustaceans nei	Crustaceans	0
Pike-perch	Other	0
Freshwater bream	Other	0
Salmonoids nei	Trout	0
Chum(=Keta=Dog) salmon	Trout	0
Tench	Carp	0
Burbot	Other	0
Tilapias nei	Tilapia	0
Mullets nei	Other	0
Black carp	Carp	0

Atlantic salmon	Trout	0
Three-spined stickleback	Other	0
Total		**184**

Appendix III: Results

Table 49, Total freshwater production per year (tonnes x 1000)

Country	Total freshwater production per year (tonnes x thousand)	Percentage
China	29942	59.93
India	6898	13.81
Indonesia	3828	7.66
Vietnam	2983	5.97
Bangladesh	2271	4.55
Myanmar	1029	2.06
Brazil	530	1.06
Iran	418	0.84
Thailand	417	0.83
Philippines	321	0.64
Egypt	309	0.62
Cambodia	290	0.58
Nigeria	289	0.58
United States	254	0.51
Russia	184	0.37
Total	**49963**	**100**

Table 50, Total Antimicrobial use (tonnes/year)

Country	Total antimicrobial use (t/y)	Percentage
China	9596	78.32
India	1141	9.32
Indonesia	261	2.13
Vietnam	249	2.03
Myanmar	160	1.30
Nigeria	159	1.30
Egypt	135	1.10
Brazil	128	1.05
Philippines	99	0.81
Bangladesh	74	0.61
Cambodia	68	0.55
Iran	62	0.51
Thailand	45	0.37
United States	41	0.33
Russia	34	0.28
Total	**12252**	**100**

Table 51, Total antibiotic use (tonnes)

Country	Total antibiotic use (tonnes)	Percentage
Tetracyclines	8658	70.30
Quinolones	962	7.81
Penicillins	695	5.65
Macrolides	693	5.63
Sulfonamides	629	5.10
Amphenicols	512	4.16
Aminoglycoside	57	0.46
Cephalosporins	52	0.42
Diaminopyrimidines	43	0.35
Nitrofuran	7	0.05
Rifamycins	4	0.03
Polymyxin	3	0.03
Nitroimidazole	1	0.01
Total	**12252**	**100**

Table 52, WHO antibiotics importance categories (tonnes of antibiotics)

Country	Veterinary	Critically Important	Highly Important	Important	Total
China	426	1746	7424	0	9596
India	70	154	979	1.32	1204
Indonesia	36	124	101	0	261
Vietnam	53	48	148	0	249
Bangladesh	0	3	71	0	162
Myanmar	9	21	132	0.18	160
Brazil	8	13	107	0	136
Iran	4	0	58	0	128
Thailand	3	6	36	0.03	99
Philippines	7	21	71	0	74
Egypt	5	30	100	0.50	68
Cambodia	4	9	55	0.07	62
Nigeria	6	35	118	0.60	45
United States	0	0	41	0	41
Russia	2	4	27	0.03	34
Total	**634**	**2213**	**9468**	**2.7**	**12252**
Percentage	**5.15**	**17.97**	**76.86**	**0.02**	**100**

Table 53, Average mg antimicrobials per kg of fish (mg/kg)

Country	Average mg antibiotics per kg of fish (mg/kg)
Nigeria	552
Egypt	437
China	320
Philippines	307
Brazil	242
Cambodia	233
Russia	183
India	165
United States	161
Myanmar	155
Iran	149
Thailand	108
Vietnam	83
Indonesia	68
Bangladesh	33
Total	**245**

Table 54, Total grey water footprint (km^3/y)

Country	Total grey water footprint (km^3/y)	Percentage
China	10599	74.04
India	1212	8.47
Bangladesh	830	5.80
Indonesia	352	2.46
Vietnam	331	2.31
Nigeria	168	1.17
Myanmar	163	1.14
Brazil	146	1.02
Egypt	142	0.99
Philippines	98	0.69
Iran	87	0.61
Cambodia	68	0.48
Thailand	46	0.32
United States	37	0.26
Russia	34	0.24
Total	**14314**	**100**

Table 55, GWF per kg of fish (m^3/kg)

Country	GWF per kg of fish (m^3/kg)
Nigeria	581
Egypt	461
Bangladesh	366
China	354
Philippines	306
Brazil	276
Cambodia	235
Iran	208
Russia	184
India	176
Myanmar	158
United States	145
Vietnam	111
Thailand	110
Indonesia	92
Total	**286**

Table 56, Consumption of fish products in the Netherlands by 1-79 year olds (VCP 2012-2016; n=4,313)

Fish product	Made of	Species group	Average (g/day)	Average (g/y)
Salmon		Marine	3,6	1314
Tuna		Marine	1,3	474.5
Codfish		Marine	1,0	365
Salt Herring		Marine	0,9	328.5
White fish	N.S.	N.S.	0,9	328.5
Pangasius		Catfish	0,7	255.5
Fried Fish	Codfish	Marine	0,6	219
Gamba		Crustaceans	0,6	219
Pollock		Marine	0,5	182.5
Mackerel		Marine	0,5	182.5
Fried Fish	N.S.	N.S.	0,4	146
Fried Fish	N.S.	N.S.	0,4	146
Fish Sticks	Pollock	Marine	0,4	146
Sole		Marine	0,3	109.5
Fish Sticks	N.S.	N.S.	0,3	109.5
Mussels		Molluscs	0,3	109.5
Shrimps (N.S.)		Crustaceans	0,3	109.5
Eel		Other	0,2	73
Sour Herring		Marine	0,2	73
Fried Fish	Codfish	Marine	0,2	73
Calamari		Molluscs	0,2	73
Fish Sticks	Codfish	Marine	0,2	73
Plaice		Marine	0,2	73
Trout		Trout	0,2	73
Fish (N.S.)		N.S.	0,1	36.5
Zander/pikeperch		Other	0,1	36.5
Dutch Shrimp		Crustaceans	0,1	36.5
Tilapia		Tilapia	0,1	36.5
Sardines		Marine	0,1	36.5
Freshwater fish (N.S.)		Other	0,1	36.5
Marine fish (N.S.)		Marine	0,1	36.5
Herring (N.S.)		Marine	0,1	36.5
Fried Fish	Pangasius	Catfish	0,1	36.5
Fried Fish	Pollock	Marine	0,1	36.5
Fried Fish	Pollock	Marine	0,1	36.5
Fish burger	Codfish	Marine	0,1	36.5
Fish fillet	Pangasius	Catfish	0,1	36.5
Fish fillet	Pollock	Marine	0,1	36.5
Crab sticks	Crab	Crustaceans	0,1	36.5
Anchovies		Marine	0,1	36.5
Dab(limanda limanda)		Marine	0,1	36.5
Perch		Other	0,1	36.5

N.S. (not specified)

Table 57, China

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	19528	6408	328	Oxytetracycline	7.11093E+12	364.14
Tilapia	1642	12	7	Gentamycin sulfate	12622875000	7.6875
Catfish	1212	795	656	Oxytetracycline	8.82675E+11	728.28
Trout	39	26	656	Oxytetracycline	28402920000	728.28
Pacu	69	45	656	Oxytetracycline	50251320000	728.28
Crustaceans	3983	33	8	Enrofloxacin	1.66788E+11	41.875
Other	3469	2277	656	Oxytetracycline	2.5264E+12	728.28
Total	**29942**	**9596**	**320**	**Oxytetracycline**	**1.05987E+13**	**354**

Table 58, India

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	5151	680.0352513	132.0200449	Oxytetracycline	7.22337E+11	140.2323
Tilapia						
Catfish	724	191.165025	264.0400898	Oxytetracycline	2.03056E+11	280.4647
Trout	1	0.26404009	264.0400898	Oxytetracycline	280464677.5	280.4647
Pacu						
Crustaceans	9	2.376360808	264.0400898	Oxytetracycline	2524182098	280.4647
Other	1013	267.472611	264.0400898	Oxytetracycline	2.84111E+11	280.4647
Total	**6898**	**1141.313288**	**165.4556811**	**Oxytetracycline**	**1.21231E+12**	**175.7478**

Table 59, Indonesia

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	716	26.92036584	37.59828	Enrofloxacin	36298881595	50.69676
Tilapia	1167	87.75437693	75.19655	Enrofloxacin	1.18326E+11	101.3935
Catfish	1480	111.2908979	75.19655	Enrofloxacin	1.50062E+11	101.3935
Trout						
Pacu	73	5.489348343	75.19655	Enrofloxacin	7401727253	101.3935
Crustaceans	34	2.55668279	75.19655	Enrofloxacin	3447379816	101.3935
Other	358	26.92036584	75.19655	Enrofloxacin	36298881595	101.3935
Total	**3828**	**260.9320377**	**68.16406**	**Enrofloxacin**	**3.51836E+11**	**91.91106**

Table 60, Vietnam

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	574	26.67665	46.475	Cephalexin	35516250000	61.875
Tilapia	263	24.44585	92.95	Cephalexin	32546250000	123.75
Catfish	1613	149.92835	92.95	Cephalexin	1.99609E+11	123.75
Trout						
Pacu	24	2.2308	92.95	Cephalexin	2970000000	123.75
Crustaceans	20	0.0288	1.44	Oxytetracycline	43200000	2.16
Other	489	45.45255	92.95	Cephalexin	60513750000	123.75
Total	**2983**	**248.763**	**83.39356353**	**Cephalexin**	**3.31155E+11**	**111.0140798**

Table 61, Myanmar

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	897	123.1987578	137.3453265	Oxytetracycline	1.25788E+11	140.2323388
Tilapia	69	18.95365505	274.6906529	Oxytetracycline	19352062750	280.4646775
Catfish	40	10.98762612	274.6906529	Oxytetracycline	11218587101	280.4646775
Trout						
Pacu	5	1.373453265	274.6906529	Oxytetracycline	1402323388	280.4646775
Crustaceans	10	2.746906529	274.6906529	Oxytetracycline	2804646775	280.4646775
Other	9	2.472215876	274.6906529	Oxytetracycline	2524182098	280.4646775
Total	**1030**	**159.7326147**	**155.0802084**	**Oxytetracycline**	**1.6309E+11**	**158.3400097**

Table 62, Bangladesh

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	1229	13.534977	11.013	Chlortetracycline	1.29967E+11	105.75
Tilapia	350	1.6632	4.752	amoxicillin	4989600000	14.256
Catfish	479	43.200531	90.189	Chlortetracycline	5.06543E+11	1057.5
Trout						
Pacu						
Crustaceans	61	2.0602872	33.7752	Chlortetracycline	33214500000	544.5
Other	152	13.708728	90.189	Chlortetracycline	1.6074E+11	1057.5
Total	**2271**	**74.1677232**	**32.65861876**	**Chlortetracycline**	**8.30464E+11**	**365.6819683**

Table 63, Brazil

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	18	2.219280729	123.2933739	Oxytetracycline	2524182098	140.2323388
Tilapia	324	79.89410626	246.5867477	Oxytetracycline	90870555521	280.4646775
Catfish	11	2.712454225	246.5867477	Oxytetracycline	3085111453	280.4646775
Trout	2	0.493173495	246.5867477	Oxytetracycline	560929355.1	280.4646775
Pacu	161	39.70046638	246.5867477	Oxytetracycline	45154813083	280.4646775
Crustaceans						
Other	14	3.452214468	246.5867477	Oxytetracycline	3926505485	280.4646775
Total	**530**	**128.4716956**	**242.3994256**	**Oxytetracycline**	**1.46122E+11**	**275.7020698**

Table 64, Iran

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	215	21.59483858	100.4411	Oxytetracycline	30149952835	140.2323388
Tilapia						
Catfish						
Trout	199	39.97556165	200.8822	Oxytetracycline	55812470829	280.4646775
Pacu						
Crustaceans						
Other	4	0.803528877	200.8822	Oxytetracycline	1121858710	280.4646775
Total	**418**	**62.3739291**	**149.2199**	**Oxytetracycline**	**87084282374**	**208.3356038**

Table 65, Thailand

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	25	3.409907243	136.3962897	Oxytetracycline	3505808469	140.2323388
Tilapia	214	1.5622	7.3	Amoxicillin	2272680000	10.62
Catfish	116	31.64393921	272.7925794	Oxytetracycline	32533902594	280.4646775
Trout						
Pacu						
Crustaceans	31	0.14012	4.52	Amoxicillin	274350000	8.85
Other	31	8.456569962	272.7925794	Oxytetracycline	8694405004	280.4646775
Total	**417**	**45.21273642**	**108.4238283**	**Oxytetracycline**	**45941076067**	**110.1704462**

Table 66, Philippines

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	13	2.03896875	156.84375	Oxytetracycline	2030437500	156.1875
Tilapia	261	81.8724375	313.6875	Oxytetracycline	81529875000	312.375
Catfish	5	1.5684375	313.6875	Oxytetracycline	1561875000	312.375
Trout						
Pacu						
Crustaceans						
Other	42	13.174875	313.6875	Oxytetracycline	13119750000	312.375
Total	**321**	**98.65471875**	**307.3355724**	**Oxytetracycline**	**98241937500**	**306.0496495**

Table 67, Egypt

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	147	42.16810892	286.8578838	Oxytetracycline	44422058091	302.1908714
Tilapia	123	70.56703942	573.7157676	Oxytetracycline	74338954357	604.3817427
Catfish	6	3.442294606	573.7157676	Oxytetracycline	3626290456	604.3817427
Trout						
Pacu						
Crustaceans						
Other	33	18.93262033	573.7157676	Oxytetracycline	19944597510	604.3817427
Total	309	135.1100633	437.2493957	**Oxytetracycline**	**1.42332E+11**	**460.6210369**

Table 68, Cambodia

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	94	13.09384594	139.2962	Oxytetracycline	13181839844	140.2323388
Tilapia	11	3.064517135	278.5925	Oxytetracycline	3085111453	280.4646775
Catfish	97	27.02346928	278.5925	Oxytetracycline	27205073721	280.4646775
Trout						
Pacu						
Crustaceans						
Other	88	24.51613708	278.5925	Oxytetracycline	24680891623	280.4646775
Total	**290**	**67.69796943**	**233.4413**	**Oxytetracycline**	**68152916641**	**235.0100574**

Table 69, Nigeria

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	24	6.884589212	286.8578838	Oxytetracycline	7252580913	302.1908714
Tilapia	22	12.62174689	573.7157676	Oxytetracycline	13296398340	604.3817427
Catfish	190	109.0059959	573.7157676	Oxytetracycline	1.14833E+11	604.3817427
Trout						
Pacu						
Crustaceans						
Other	54	30.98065145	573.7157676	Oxytetracycline	32636614108	604.3817427
Total	**290**	**159.4929834**	**549.9758048**	**Oxytetracycline**	**1.68018E+11**	**579.372843**

Table 70, USA

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	1	0.21475	214.75	Oxytetracycline	249675000	249.675
Tilapia	7	1.50325	214.75	Oxytetracycline	1747725000	249.675
Catfish	153	17.7327	115.9	Oxytetracycline	8698050000	56.85
Trout	15	4.704	313.6	Oxytetracycline	6637500000	442.5
Pacu						
Crustaceans	72	15.462	214.75	Oxytetracycline	17976600000	249.675
Other	6	1.2885	214.75	Oxytetracycline	1498050000	249.675
Total	**254**	**40.9052**	**161.0441**	**Oxytetracycline**	**36807600000**	**144.911811**

Table 71, Russia

Fish Species	Production (Tonnes x1000)	Total antibiotic use (tons)	Average mg antibiotic per kg of fish (mg/kg)	Critical load	WF (m^3/y)	WF per kg of fish (m^3/kg)
Carp	126	17.55132541	139.2962	Oxytetracycline	17669274685	140.2323388
Tilapia						
Catfish	2	0.557184934	278.5925	Oxytetracycline	560929355.1	280.4646775
Trout	45	12.53666101	278.5925	Oxytetracycline	12620910489	280.4646775
Pacu						
Crustaceans						
Other	11	3.064517135	278.5925	Oxytetracycline	3085111453	280.4646775
Total	**184**	**33.70968848**	**183.2048**	**Oxytetracycline**	**33936225982**	**184.4360108**

Table 72, Grey water footprint per kg of fish of trout imports of the Netherlands

	Trout	
Country of origin	Percentage of availability on Dutch markets	GWF (m^3/kg)
Other	100	347

Table 73, Grey water footprint per kg of fish of tilapia imports of the Netherlands

	Tilapia	
Country of origin	Percentage of availability on Dutch markets	GWF (m^3/kg)
China	51.2	8
Indonesia	13.6	101
Vietnam	7.8	124
Other	27.3	102
Average	100	55

Table 74, Grey water footprint per kg of fish of crustaceans imports of the Netherlands

	Crustaceans	
Country of origin	Percentage of availability on Dutch markets	GWF (m^3/kg)
Bangladesh	8.9	545
China	3.1	42
India	12.3	280
Indonesia	3.5	101
Thailand	0.3	9
Vietnam	15.3	2
Other	56.6	54
Average	100	119

Table 75, Grey water footprint per kg of fish of catfish imports of the Netherlands

	Catfish	
Country of origin	Percentage of availability on Dutch markets	GWF (m^3/kg)
Bangladesh	0.1	1058
Indonesia	0.9	101
Vietnam	79.6	124
Other	19.3	280
Average	100	155