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Antimicrobial resistance genes in the aquaculture sector: global reports and research gaps.

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Abstract

Aquaculture has been one of the fastest-growing food production systems over the last decade and increased intensification of production has created conditions that favour disease outbreaks. Antibiotics are commonly applied in the animal food sector to fight against antibacterial infections, however their inappropriate use contributes to the emergence of antibiotic-resistant bacteria. Investment in research and capacity-strengthening, in parallel to enforcing existing regulations around antimicrobial use, are potentially powerful tools in tackling the threat of antimicrobial resistance (AMR) emanating from animal producing systems such as aquaculture. However, directing investment effectively is challenging due to the limited data available that hinders the identification of risk areas for current and future AMR emergence. Here, we aim to partially fulfill this gap by analyzing the current peer-reviewed literature reporting AMR genes in aquaculture food production systems and combining the data in a systematic map.

Systematic searches of three bibliographic databases, a search engine, and 120 reviews returned 10 699 articles which were screened at title and abstract and then by full text (n = 1100). 218 articles, spanning 39 countries and 6 continents, met all inclusion criteria and were coded to retrieve bibliographic, methodology and study outcome data. AMR gene detections were associated with 44 families of fish and crustaceans and 75 genera of bacteria, with most studies employing primer-based methods to detect ARGs. A narrative synthesis explores implications for future research and policy as well as limitations of the systematic mapping methodology.

Keywords: aquaculture, antimicrobial resistance, genes, fish, crustacean, one health

Introduction

Aquaculture is currently responsible for producing close to half of all aquatic animals consumed globally (FAO 2019). Driven by dwindling stocks in wild capture fisheries and increased demand for fish and seafood products globally, aquaculture has been one of the fastest growing food production sectors since the turn of the century, with an annual growth rate of 5.8% during the period 2001-2016 (FAO 2018; Lulijwa et al. 2019). This growth has been supported in part by the intensification of production methods, much of which has occurred in low and middle-income countries, particularly in Asia (Brunton et al. 2019). Intensification increases the proximity of animals to each other and can negatively impact water quality, creating crowded and environmentally challenging conditions that lead to physiological stress and impaired immune function favouring disease emergence (Cabello et al. 2013; Santos and Ramos 2018; Lulijwa et al. 2019). Antibiotics are commonly applied to treat pathogen outbreaks and mitigate associated economic losses (Santos and Ramos 2018; Brunton et al. 2019). Although prophylactic use of antibiotics is prohibited in most countries, inappropriate antibiotic use (including for growth promotion), which is partly supported by limited regulations and controls, creates selective pressures that favour the emergence of antibiotic-resistant bacteria (Watts et al. 2017; Henriksson et al. 2018; Brunton et al. 2019; Reverter et al. 2020)

Aquaculture differs from other food production sectors in terms of its biodiversity and socio-economic context, presenting unique opportunities for AMR emergence and distinct challenges to addressing this emergence. For example, aquaculture is an evolving food-production system

cultivating close to 600 species in a variety of culture systems over a broad geographical area (194 producing countries) (FAO 2018; Henriksson et al. 2018). Furthermore, the majority of global aquaculture production is centred in sub-tropical and tropical regions, which are prone to more rapid and severe disease outbreaks (Leung and Bates 2013; Reverter et al. 2020). As no antibiotics have been specifically developed for aquaculture, those designed for livestock and humans are used, some of which are extremely important in human medicine (e.g. kanamycin) (Henriksson et al. 2018). These are generally incorporated into feed and applied metaphylactically at the population level. Unfortunately, as fish do not efficiently metabolize antibiotics and monitoring feed intake is difficult in the aquatic environment, a large proportion can be lost to the environment as uneaten feed, undigested feed, and secreted antimicrobial metabolites, with some studies indicating retention as low as 20 - 30 % (Watts et al. 2017; Santos and Ramos 2018; Lulijwa et al. 2019). These antibiotics then interact with an aquatic microbiome that harbours a large variety of mobile genetic elements where significant genetic exchange and recombination can occur (Watts et al. 2017; Santos and Ramos 2018; Thornber et al. 2019). In addition, the regulatory framework governing the use of antibiotics in aquaculture varies greatly among countries, with limited capacity for monitoring and enforcement in many of the developing countries that are major aquaculture producers (Santos and Ramos 2018; Brunton et al. 2019).

Research and capacity-strengthening (both in the technical and institutional sense) are potentially powerful tools in tackling the threat of AMR emanating from aquaculture as they directly address many previously identified mechanisms for controlling antimicrobial use around biosecurity, diagnostics, education, vaccines, alternative treatments and legislation (Henriksson

et al. 2018). However, gaining maximum impact from programs addressing AMR requires ways of identifying areas of greatest risk for current and future AMR emergence to effectively direct resources. Accessing this information through current global AMR surveillance systems is difficult as they are generally disconnected and underdeveloped, with a strong focus on humans (IACG 2018). The World Health Organisation Global Antimicrobial Surveillance System (GLASS) has only enrolled 71 countries, with fewer than 50 countries reporting AMR rates in the latest report (WHO 2018). In terms of the food and agriculture sector, surveillance systems are even less developed and coordinated. While some high-income regions and countries, particularly Europe, the United States, Canada, Japan, and Australia have established some form of veterinary surveillance program (Schrijver et al. 2018; Sharma et al. 2018), there has been less activity in low and middle-income countries (LMIC) around this issue. Current initiatives, such as the Food and Agriculture Organization Assessment Tool for Laboratories and AMR Surveillance Systems (ATLASS) (FAO 2020) are at the level of mapping AMR surveillance capacity in LMIC's with the aim of strengthening technical capacity, coordination, and harmonization among actors, both internally and regionally/globally.

Fundamentally, AMR surveillance systems track (either directly or indirectly) the genetic determinants of resistance. These are the genes that code for the protective mechanisms that microorganisms have developed, through Darwinian selection, to counter naturally occurring toxic substances produced by themselves or other microorganisms, including environmental fungi and saprophytic bacteria (Holmes et al. 2016). The majority of antimicrobial drugs are these naturally produced substances or synthetic derivatives thereof, with only a few fully synthetic types (Holmes et al. 2016). Culture-based AMR assessment methods, such as the disc-

106 diffusion test, test for the phenotypic expression of resistance by exposing microorganisms to
107 antimicrobials and observing susceptibility (Reller et al. 2009). More recently, advances in
108 molecular biology have facilitated the direct identification of resistance genes in
109 microorganisms, either through targeted primers or secondary analysis of whole genome
110 sequences. Genes conferring resistance to antimicrobials can emerge in a microbial population
111 either through mutation and dissemination via normal vertical inheritance or acquired from other
112 strains or species through horizontal gene transfer mechanisms. These include conjugation by
113 plasmids, transduction by bacteriophages, or natural transformation by extracellular DNA
114 (Lerminiaux and Cameron 2019).

115

116 Despite the risks for AMR emergence and dissemination associated with the rapidly expanding
117 aquaculture sector, there are limited data sources from which to extract information on the
118 incidence and geographic distribution of AMR, and particularly the genetic determinants of
119 resistance, in the context of global aquaculture. Recently, Reverter et al. (2020) conducted a
120 meta-analysis to explore the impact of global warming and AMR on aquaculture, including using
121 data from antimicrobial susceptibility studies to calculate a Multi-Antibiotic Resistance index
122 (MAR) of aquaculture-related bacteria for 40 countries. Data from research studies targeting
123 resistance genes could provide complementary insight into the nature of AMR in aquaculture,
124 with research microbiologists potentially functioning as a loose proxy for a global observation
125 network. Here we set out to test this proposition. The objective of this synthesis was to identify,
126 collate, and describe the peer-reviewed literature that has reported antibiotic-resistant genes in
127 bacteria sampled from aquaculture food productions systems. The goal was to provide
128 preliminary insights into the distribution and nature of AMR in aquaculture in the absence of an

integrated global AMR surveillance system in these food production systems. Specifically, we asked: What is the global incidence, composition, and geographic distribution of genetic determinants of antibiotic resistance in bacteria associated with aquaculture food production systems?

Approach

This systematic map followed the protocol published at the inception of this project on the Open Science Forum (https://osf.io/wsj5n/) informed by the Collaboration for Environmental Evidence Guidelines (CEE 2019) and complies with Reporting Standards for Systematic Evidence Syntheses (ROSES) (Haddaway et al. 2018). Our methods deviated from the protocol through the adjustment of the search string to fit requirements for the ProQuest database, the incorporation of additional terms in the coding sheet and the method of data extraction, which was shifted from a Google form to an excel spreadsheet.

Searching for articles

The search strategy aimed to capture relevant studies in the peer-reviewed literature using three databases focused on peer-reviewed publications and a single web-based search engine. In addition, the reference sections of relevant review articles were searched to identify articles not previously found.

150 ***Definition of the question components***

151 *Population*

152 Aquaculture food-production systems are defined as those that involve cultivating an organism in
153 an aquatic environment with direct human involvement in the form of seed addition, feed
154 addition, habitat engineering, water quality manipulation, or a combination thereof. This
155 synthesis aimed to target intensive aquaculture food-producing systems where the application of
156 antibiotics is focused on the finfish and crustacean sectors of global production and excluded the
157 extensively farmed plant and mollusk sectors.

158 *Measure of antibiotic resistance*

159 A genetic indicator of resistance was selected (i.e. the presence / absence of antibiotic resistance
160 genes as defined by the Comprehensive Antimicrobial Resistance Database (CARD -
161 https://card.mcmaster.ca) (Alcock et al. 2020). This methodology was adopted as it provides a
162 standardized method for AMR detection that partially mitigates operational, reagent quality, and
163 interpretational issues associated with culture-based methods and potentially provides
164 information on non-culturable components of the microbiome.

165 *Geographical scope*

166 Global, no limits on geographical scope.

167

168 ***Search terms and language***

169 An initial set of English search terms relevant to the different components of the research
170 question were compiled. A list of common names of cultured fish and crustacean species was
171 extracted from the FAO Fishery Statistical Collection: Global Aquaculture Production accessed

through the FAO FishStatJ software (http://www.fao.org/fishery/statistics/global-aquaculture-production/en) (FAO 2016). Lists of antibiotic names and antibiotic resistance genes (ARG) were extracted from CARD, a curated collection of characterized, peer-reviewed resistance determinants and associated antibiotics (Alcock et al. 2020). Initial attempts to develop search strings using specific gene names extracted from the CARD database were abandoned due to the non-specificity of wildcards when using this approach.

A set of search strings was developed and modified through a scoping exercise using Web of Science Core Collections and Scopus to evaluate the sensitivity associated with alternate terms and wildcards. The terms were broken into four components (aquaculture descriptors, cultured species / habitat descriptors, resistance descriptors, and resistance units) and combined using Boolean operators "AND" and/or "OR" (see Supplementary Material A). The comprehensiveness of the search was assessed using a collection of benchmark papers (n = 25) to ensure that these articles identified as relevant were represented in search results. (see Supplementary Material A).

Searches

Three bibliographic databases (ISI Web of Science Core Collection, Scopus and ProQuest Dissertations & Theses Global) were searched in July 2019 using the primary search string as described in Supplementary Material A. The search string for ProQuest was condensed by the removal of antibiotic names to meet the limitations of the search function of this database (Supplementary Material A). The Carleton University institutional subscription was used to conduct the searches (Supplementary Material A). A further search was also performed using a condensed search string (256-character limit for searches) on the web-based search engine

Google Scholar. The top 200 most relevant results were exported. In addition, the reference sections of 120 review articles identified as potentially relevant (113 at title and abstract screening and 7 from full text screening) were screened manually for articles that were within the scope of this systematic map and not captured by the previous searches. No updates to the search were performed during the systematic mapping process.

Article screening and study eligibility criteria

Screening process

Results from the bibliographic database were exported as either an .RIS file (Scopus, ProQuest) or as a coded .txt files (ISI Web of Science, Google Scholar) and then imported into CADIMA (Kohl et al. 2018), an open access online tool for systematic review management, where duplicates were removed. Numeric outcomes of the search strategy are described in the ROSES report (see Supplementary Material B).

All articles were screened at two distinct stages. Initial screening at title and abstract was followed by a second round of screening at full text using a pre-established set of eligibility criteria (Table 1). Prior to each stage of screening, a consistency check was conducted between the reviewers using a subset of articles. At title and abstract, 1070/10699 articles (10%) were screened by two reviewers (JK and LK) with a Kappa score of 0.61 (SE = 0.042, 95% confidence interval 0.528 – 0.693) indicating good agreement. All discrepancies were discussed between the two reviewers and reconciled before proceeding with screening. Any articles that were unclear were flagged for a second opinion and eligibility discussed between reviewers to reach a

decision. At full text, 120/1150 articles (10%) were again screened by two reviewers with a Kappa score of 0.817 (SE = 0.058, 95% confidence interval 0.703 - 0.931) indicating very good agreement between reviewers.

Study validity assessment

We did not appraise the validity of individual studies.

Data Extraction

Following screening, articles selected as eligible for data extraction were processed by one of two reviewers (JK and LK) using a standard template (Table 2). The template was established in an Excel spreadsheet and captured key information in the broad categories of (1) bibliographic information, (2) culture system descriptors, and (3) bacteria and resistance using a combination of pre-populated drop-down menus and open-ended input as required.

Meta-data extraction was conducted down to the level of unique bacterial species or sample. Therefore, within each article, reports of the same gene in multiple strains/cultures of the same species were recorded as a single detection. However, reports of the same gene in multiple strains/cultures of the same species, but with differing aquaculture system, locality or sample origin, were counted as separate detections.

Following extraction, each potential gene was referenced against the CARD database for a match to a gene and standardized to a single term based on the CARD database nomenclature if required (for example, tet(a), tet(A), tet-A *etc*. were standardised to tetA). Ancillary data relating

238 to each matched gene, including the drug classes it is associated with, the resistance mechanism,
239 and gene family were extracted as per the CARD ontology (Supplementary Material C)

240

241 **Findings**

242

243 **Number and types of articles**

244 A search of three bibliographic databases and Google Scholar returned 14 000 individual
245 records. After duplicate removal, 10 699 articles were screened at abstract and title according to
246 the eligibility criteria, of which 1150 records passed through to screening at full text. The
247 majority of these articles (n = 1100) were retrieved through Carleton University institutional
248 subscriptions or inter-library loans, with 50 articles unobtainable given available resources (e.g.
249 not accessible via inter-library loan system) or did not meet inclusion criteria (e.g. conference
250 abstracts, non-English language publications). Following screening at full text, 890 articles were
251 excluded for the following reasons: (1) study population (n = 176), (2) study outcome (n = 173),
252 (3) study methodology (culture-based; n = 478), duplicates (n = 52), article type (review article;
253 n = 7) and article type (conference abstract; n = 4). A total of 210 articles were selected for
254 inclusion in the systematic map. In addition, 8 articles were included from searches of the
255 bibliographic sections of relevant reviews. Accordingly, 218 articles were included in the
256 systematic map database and synthesis (see Supplementary Material B – ROSES form and
257 Supplementary Material D – Full text screening outcomes).

258 The included articles varied across several metrics. There was a marked increase in the number
259 of articles published annually since the first article in 1987 until 2019. Most articles (> 80 %)

were published in the last 10 years, with more than 50 % being published in the last 5 years (Figure 1A). All articles, barring two PhD theses, came from the commercially published literature (Figure 1B). Articles came from 83 journals, with the top 5 contributors being *Science of the Total Environment* (n = 13), *Antimicrobial Agents and Chemotherapy* (n = 10), *Aquaculture* (n = 10), *Microbial Drug Resistance* (n = 8) and *Frontiers in Microbiology* (n = 8) (Figure 1C). The residence of the primary authors aligned with the country of sampling or the location of the experiments in most articles (190 of 218 articles).

Systematic Map

The systematic map is composed of two key components, namely (1) a database containing meta-data and coding for all studies selected for inclusion (see Supplementary Material E - Data) and (2) a series of heat-maps to visualize patterns in the data extracted from these studies. Due to space limitations some heatmaps are truncated, however, the full datasets used to generate the heatmaps are provided (see Supplementary Material F - Heatmaps).

Geographic distribution of studies

The 218 articles included in the systematic map reported on 226 studies. A study was defined by the location of sample collection at the country level, as such, some articles reported on samples collected in more than one country. More than half of the studies were conducted in just 5 countries, namely China (n = 47), Japan (n = 23), Thailand (n = 17), Republic of Korea (n = 17) and the United States (n = 14). At a continental scale, Asia accounted for over half of the studies

(n = 129), followed by Europe (n = 47), North America (n = 20), South America (n = 17), Africa (n = 9) and Australia (n = 4) (Figure 2, Supplementary Material E - Data).

Study characteristics

To detect antibiotic resistance genes, 85% of studies employed primer-based polymerase chain reaction (PCR) techniques. A further 13 % adopted whole genome sequencing of bacteria (or plasmids) combined with gene databases to identify sequences that matched known ARGs, while 2% employed alternative methods such as DNA probes (Figure 3A).

Primer-based studies had a higher mean number of samples (5.0 ± 6.5) per study compared to those using genome-based methods (1.8 ± 1.7) (Figure 3B). By contrast, primer-based studies reported less ARGs per study (20.0 ± 28.4 *vs* 41.7 ± 79.5) and per sample (5.0 ± 7.1 *vs* 17.8 ± 24.6) compared to genome-based methods (Figure 3C,D).

Sample characteristics

Fish aquaculture systems accounted for 90% of the 1023 separate detections extracted from the 226 studies. Within fish aquaculture systems, samples taken directly from aquaculture organisms accounted for 50% of the samples, followed by water samples, sediment samples and feed samples, which accounted for a further 32%, 13%, 3%, and 2% of samples respectively (Figure 4). Data detailing the culture system where samples were taken from were not available in 39% of studies. Where such data were available, pond and cage culture were the most prevalent sources of samples, accounting for 61% of samples (Figure 4). In terms of the bacterial genus

associated with samples, Aeromonas, Vibrio, Pseudomonas and Enterococcus were the most commonly reported, accounting for 20%, 9%, 6%, and 4% of samples, respectively. No bacterial genus was associated with 16% of samples, reflecting studies where the bacterial cultures were not identified or where DNA was sampled directly from the environment or aquaculture organisms (Figure 5).

Antibiotic resistance genes

Cross-referencing all extracted potential ARGs against the CARD database resulted in 201 studies with a match, resulting in a total of 4467 potential gene detections. Of these, 375 were discarded as the match related to a gene family, enzyme, bacteria, integron, or plasmid rather than a specific gene. Ultimately 4092 individual gene detections were considered for further analysis (Figure 6).

ARGs associated with resistance to a single antibiotic class accounted for 75 % of all detections. Within this group of ARGs associated with a single antibiotic class, five antibiotic classes accounted for over 85 % of the detections. The classes were tetracycline antibiotics (39 %), sulfonamide antibiotics (22 %), aminoglycoside antibiotics (13%), phenicol antibiotics (6%), and diaminopyrimidine antibiotics (6%) (Figure 7). In terms of organism type, 76% of detected ARGs were associated with finfish aquaculture, 22% with crustacean aquaculture, and the remainder either combined fish/crustacean aquaculture or no data were provided. Within fish aquaculture, data relating to specifics of the culture system were not available in 38% of detections. Where data were available, many detections were associated with freshwater pond aquaculture (17 %), marine cage culture (11 %), and ornamental culture (6 %). Within

crustacean aquaculture, pond culture was dominant, associated with 64 % of detections (Figure 7, Figure 10).

In terms of specific ARGs, 418 unique genes were reported, with just 60 of these responsible for over 75% of all reported detections. Within this frequently reported group, those associated with resistance to tetracycline antibiotics accounted for 46 % of the detections, followed by ARGs associated with resistance to sulphonamides (21 %), aminoglycosides (10 %), and multiple antibiotics (8%). The 10 most commonly detected ARGs were sul1 (n = 339), tet(A) (n = 248), sul2 (n = 252), tetM (n = 219), tet(B) (n = 184), floR (n = 105), tetE (n = 103), tet(D) (n = 103), tet(C) (n = 79), and tetW (n = 70) (Figure 8, Supplementary Material F - Heatmaps).

The Salmonidae were the family most commonly associated with reported ARGs, accounting for 22 % of all detections. Other prominent families included the Cyprinidae (7 %) and the Cichlidae (6 %). The Penaeidae accounted for 15 % of detections. A family name could not be assigned to 18 % of the detections (Figure 8).

It was no possible to associate ARGs with a bacterial genus in just under half the detections (46%). Where data on the bacterial genus of ARGs reported, ARGs were most commonly associated with the genus Aeromonas (27 %), Vibrio (10%), Escherichia (8 %), Pseudomonas (7 %), and Enterococcus (5 %).

Limitations of the map

Limitations related to the search strategy

345 The search strategy was wide-ranging given the use of a broad search-string that included both
346 generic terminology and specific aquaculture organism and antibiotic names. However, scientific
347 names were not included in the search-string component related to the targeted aquaculture
348 organisms, and this may have influenced the number of results obtained. Furthermore, the finite
349 time and resources available for this synthesis meant that the search was confined to the
350 commercially published peer-reviewed literature. It is possible that valuable complementary
351 information can be found in the grey literature, particularly databases and reports emanating
352 from country and regional surveillance programs and networks, however searching these sources
353 was beyond the resources of this synthesis.

354 The use of English as the search language could have biased the search results. While the search
355 engines used were locating non-English language articles that provided English abstracts, we
356 acknowledge that a section of the relevant literature published entirely in non-English languages
357 was excluded. The inclusion of non-English language literature sources and the exploration of
358 the grey literature, particularly as it relates to government and producer commissioned studies,
359 should be considered to improve the robustness of future syntheses on this subject.

360

361 ***Limitations in coding and synthesis***

362 Interpretation of the information presented in this systematic map should consider the following
363 caveats regarding the data extraction, synthesis, and presentation process. First, no critical
364 appraisal of the quality of the studies included in the systematic map was conducted. This is
365 likely less of an issue given the use of a present/absent genetic indicator of resistance, compared

366 to cultured-based methods (e.g. diffusion disks) where both study design parameters and the
367 interpretation of results are more variable.

368 Second, interpretation of heatmaps that include the variables of either "culture system
369 descriptor" or "bacterial sample origin descriptor" should be undertaken with the knowledge that
370 in some cases multiple values were assigned to these parameters. For example, multiple samples
371 collected from an aquaculture organism, water, and sediment were pooled before analysis.
372 However, only the first of these was used for the heatmaps. Two or more bacterial sample
373 descriptors or two or more culture systems were present in 16% and 3 % of total samples,
374 respectively (see Supplementary Material E – Data).

375 Third, while the CARD database served as a useful reference to identify and categorize potential
376 ARGs, it is likely that some potential ARGs excluded using CARD were in fact valid and could
377 be identified using other means. These data points (n = 597) have been retained and are available
378 (see Supplementary Material F – Heatmaps, sheet "DATA_Expanded", column "AA", value =
379 "2") for future analysis.

380

381 ***Limitations of the evidence base***

382 This systematic map specifically selected studies that used a genetic indication of antibiotic
383 resistance. This approach is advantageous in that it standardized to some degree the method for
384 AMR detection and partially mitigated some of the limitations associated with culture-based
385 methods. However, it also potentially introduces its own set of biases. First, the presence of an
386 ARG does not necessarily imply expression of the gene and associated antibiotic resistance in the

phenotype. Simultaneous application of standardized culture-based, antibiotic-exposure tests and genetic sequencing would be required to confirm an association.

Second, the detection of ARGs indicates their presence in a sample, but also directly reflects the study methodology employed. This is particularly true in primer-based studies, where the choice of primers directly influences the boundaries of the results that can be obtained. Studies that reference sequenced genomes against gene databases are less prescriptive, however, selection criteria, such as the percentage similarity to confirm a match, can influence outcomes.

Third, the current synthesis did not consider the temperature of aquaculture systems when extracting reports of AMR genes. Recent research indicates that antimicrobial use is accompanied by a parallel factor, in the form of higher temperature, in driving the selection and emergence of AMR (MacFadden et al. 2018; Reverter et al. 2020). As such, the presence of AMR genes reflects complexity beyond the outcomes of a simple linear process resulting from antimicrobial use.

Fourth, studies selected for inclusion in this systematic map did not necessarily form part of a systematic surveillance program and were in some cases conducted in response to disease outbreaks in aquaculture facilities. Reference to disease, either in terms of the health of individual culture organisms sampled or general outbreak conditions, was associated with 32 % of the included articles (see Supplementary Material E). The remaining 68% either explicitly mentioned healthy culture organisms or no disease-specific information were provided. As such, both the location of the studies and particularly the species of bacteria associated with ARGs would be biased by the interest of the investigators and common pathogens, respectively. The situation prevailing the studies cannot be assumed to be similar amongst all studies.

Given the previous four points, any attempt to interpret the heatmaps presented as directly indicative of the distribution and prevalence of ARGs in global aquaculture should be undertaken with caution. Clearly, the use of literature derived ARG distribution and prevalence is insufficient to provide a clear picture of the nature of AMR in global aquaculture. While this synthesis provides some insights into research gaps made apparent by the characteristics of the scientific literature on the subject, more robust data is needed to direct effective measures to address AMR in the sector. This could at least partly be achieved by combining ARG data with other measures of AMR, such as those derived from culture-dependent techniques.

Discussion and conclusions

The systematic map presented here provides a comprehensive synthesis of available information related to the distribution and composition of genetic resistance determinants in fish and crustacean aquaculture food-production systems. This synthesis identified a total of 218 articles (226 unique sampling studies at country level) reporting potential ARGs in bacteria sampled from aquaculture systems, spanning 39 countries across 6 continents. These ARG detections were associated with 44 families of fish and crustaceans and 75 genera of bacteria, with most studies (85 %) employing primer-based methods to isolate and amplify specific sequences associated with known ARGs. This map not only depicts general patterns in the available evidence, but also highlights knowledge gaps and biases in the existing evidence base, particularly imbalances between research output and total aquaculture production at the country level.

Approximately 95% of the global production of finfish and crustacean aquaculture can be attributed to 21 countries (FAO 2016). Country aquaculture production (CAP; as a proportion of total global finfish and crustacean aquaculture production) can be compared to the number of studies from each of these 21 countries (NS; as a proportion of all studies included (n = 226)) in an index (NS/CAP) (Figure 11). Values below 1 indicate a proportionally lower research output reporting ARGs relative to total aquaculture production in a country. This reduced reporting could theoretically either result from a reduced prevalence of AMR genes in these countries (i.e. studies are being conducted but are not finding and reporting ARG's and were therefore not captured in the current synthesis). Alternatively, the lack of reporting could reflect low relative research effort and/or capacity, despite high ARG prevalence on the ground. Interestingly, 8 of the top 10 producing countries globally show index values below 1, highlighting potential knowledge gaps in the prevalence and composition of ARGs in aquaculture systems in these countries. A recent review of culture-based studies reporting antimicrobial resistance (AMR) in aquaculture (Reverter et al. 2020) found that the levels of AMR, calculated using a multi-antibiotic resistance (MAR) index, were reasonably high (> 0.3) in many of these countries, suggesting that reduced prevalence of AMR is unlikely to be the cause of the lack of representation in the literature.

Implications for policy / management

One of the key strategic objectives of the Global Action Plan on AMR (GAPAMR) (WHO, 2016) is to strengthen the knowledge and evidence base through surveillance and research. This strategy envisions both (1) generating knowledge on the incidence, prevalence, pathogen range, and geographical patterns of AMR and (2) developing an understanding of how resistance

453 develops and spreads, including how resistance circulates within and between humans, animals,
454 and the environment. While large scale susceptibility testing would go a long way in addressing
455 the first point, genetic approaches would offer considerable insight into the second. The outputs
456 of this systematic map (i.e. the map database and heatmaps) provide a current collection of the
457 existing peer-reviewed evidence regarding the incidence and global distribution of AMR genes
458 in aquaculture food production systems.

459 Furthermore, where data were available, the association between reported AMR genes and
460 bacterial genus offers mixed insights. The two most commonly reported genera, namely
461 *Aeromonas spp*. and *Vibrio spp*., are considered major bacterial pathogens in aquaculture (Figure
462 9) (Reverter et al. 2020). However, other major pathogens such as *Edwardsiella spp.*, *Yersinia*
463 *spp.*, *Lactococcus spp*. and *Streptococcus spp*. were less commonly associated with AMR genes.
464 Moreover, the wide diversity of bacterial genera with AMR genes reported from aquaculture
465 settings would support the indication that these systems, and the larger aquatic environments
466 they exist in, are active reservoirs of AMR (Marti et al. 2014). It is likely that AMR is already
467 influencing production by limiting antimicrobial treatment options for at least some of the major
468 bacterial disease-causing agents, with potential consequences for antimicrobial use as farmers
469 seek out alternative antimicrobials or adjust dosage in response.

470 From an international policy perspective, this systematic map potentially highlights regions
471 where support, either in the form of direct research funding or capacity-strengthening, can be
472 directed to develop locally generated data on the genetic determinants of AMR in local
473 aquaculture systems (Figure 11). In addition, high costs associated with establishing genetic
474 analyses capacity could be partially circumvented through the establishment of regional or
475 international partnerships to facilitate knowledge and capacity sharing. Further to this, patterns

476 emerging from this systematic map may allow targeting of research effort to aquaculture systems
477 (i.e. marine fish cage, freshwater fish pond, freshwater ornamental fish, crustacean pond, and
478 some polyculture systems) that show high incidences of AMR genes (Figure 7). However, this
479 approach should be undertaken with the consideration that increased reporting of AMR genes in
480 these systems may reflect increased research effort rather than increased prevalence.

481

482 ***Implications for research***

483 Several opportunities and considerations for future evidence synthesis or primary research are
484 highlighted by the current systematic map.

485 1. The reported incidence of ARGs in ornamental fish would benefit from further
486 investigation given the AMR dissemination risks associated with the high mobility of live
487 animals on a global scale.

488 2. Gaps in geographic coverage from many of the large producers of aquaculture products,
489 particularly in Asia. It is possible that this is an artefact of language bias in the
490 systematic map methodology used here.

491 3. Further synthesis to explore the incidence in aquaculture of ARGs considered important
492 to human medicine. The World Health Organization list of Critically Important
493 Antimicrobials for Human Consumption (WHO 2017) provides a useful reference in this
494 regard.

495 4. While relatively few studies employed whole-genome approaches to detecting ARGs
496 (Figure 3), those studies that did generally reported a higher diversity of ARGs, likely an

497 outcome of bypassing primer selection issues and/or their ability to capture non-
498 culturable or accessory components (i.e. the phageome) of the microbiome. Given the
499 ability of ARGs to move between components of the microbiome, complementing
500 targeted investigations of specific pathogens with ecosystem-level environmental
501 sampling of microbiome DNA would provide a more nuanced understanding of ARG
502 incidence and potential risk.

503

504 **Acknowledgements**

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507 the IDRC or its Board of Governors

508 **References**

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629 **Table captions**

630 Table 1: Eligibility criteria

631 Table 2: Data extraction template

632

633 **Tables**

634 Table 1

Title and Abstract
Population
1. Articles that report on a relevant food production system (i.e. aquaculture involving fish or crustacean species).
Study design / outcome
2. Articles report the sampling of bacteria from the water, sediment and other surfaces, infrastructure and resident biological organisms directly associated with an aquaculture farm, including the direct outflow.
Full text
Study design / outcome
2.1 Articles that extract DNA from bacterial or environmental samples and report the occurrence of genetic resistance determinants following PCR using suitable primers, or through secondary analysis of whole genomes.
(Articles that reported resistance to antimicrobials using culture-based methods were excluded but flagged for future investigation.)

635

636 Table 2

Category	Open input	Pre-populated categories
Bibliographic Information		
Citation	x	
Journal	x	
Publication year		1900 - 2019
Publication title	x	
Primary author name	x	
Primary author country	x	
Corresponding author name	x	
Corresponding author contact	x	
Abstract	x	
Keywords	x	
Culture System Descriptors		
Country of study		List – 246 countries
Region / province	x	
Latitude	x	
Longitude	x	
Year of study		1900 – 2019 *or* range
Water salinity	x	Freshwater | Brackish | Marine | Other | no data
Cultured animal(s) common name	x	
Cultured animal(s) scientific name	x	
Cultured animal(s) family name	x	
Cultured animal(s) type		Fish | Crustacean | Combined | no data
Culture system descriptor #1		Broodstock | Hatchery | Pond | Raceway | Tank | Cage | RAS | Ornamental | Basket | Well | Fish/Duck polyculture | Fish/Chicken polyculture | Fish/Goose polyculture | Fish/Swine polyculture | Outflow | no data
Culture system descriptor #2		*as above*
Culture system descriptor #3		*as above*
Culture system descriptor: Other	x	
Bacteria and Resistance		
Bacteria sample origin descriptors #1		Sediment | Aquaculture organism | Accessory organism | Feed | Waste | Soil
Bacteria sample origin descriptors #2		*as above*
Genetic analyses method		Primer | Genome
Genetic analyses method – other	x	
Bacterial species	x	
Genetic resistance determinant	x	

637

638 **Figure captions**

639 Figure 1: Number of articles included in the systematic map by publication type (A), publication
640 year (B), and journal name for journals contributing five or more articles (C).

641

642 Figure 2: Geographic distribution of studies selected for inclusion in the systematic map.
643 Numbers correspond to the number of studies from each country or regions. Map created with
644 Microsoft Excel using data available in Supplementary File E.

645

646 Figure 3: Methods employed to detect genetic resistance determinants (ARGs) (A), the mean ±
647 SD number of unique samples analyzed per study by methodology (B), the mean ± SD number
648 of ARGs detected per study by methodology (C) and the mean ± SD number of ARGs reported
649 per sample by study.

650

651 Figure 4: Heatmap depicting the number of unique samples extracted from 226 studies for each
652 organism type, focused by the origin of the sample and the primary aquaculture system
653 descriptor.

654

655 Figure 5: A heatmap depicting the number of unique samples extracted from 226 studies by
656 bacterial genus and the origin of the sample (categorized by organism type).

657

Figure 6: Flowchart of outcomes resulting from the cross-referencing of potential genetic resistance determinants, extracted from 226 studies, against the Comprehensive Antibiotic Resistance Database (CARD).

Figure 7: Heatmap depicting the number of gene detections for each organism type, focused by culture salinity, primary aquaculture system descriptor, and the antibiotic class associated with the ARG (as per CARD).

Figure 8: Heatmap depicting the number of gene detections of the most commonly reported ARGs, accounting for 75% of total detections, focused by family of the culture organism.

Figure 9: Heatmap depicting the number of gene detections of the most commonly reported ARGs (75% of total detections), focused by genus of the bacteria associated with the originating sample.

Figure 10: Heatmap depicting the number of gene detections of the most commonly reported ARGs (75% of total detections), focused by culture organism, water salinity, and primary culture system.

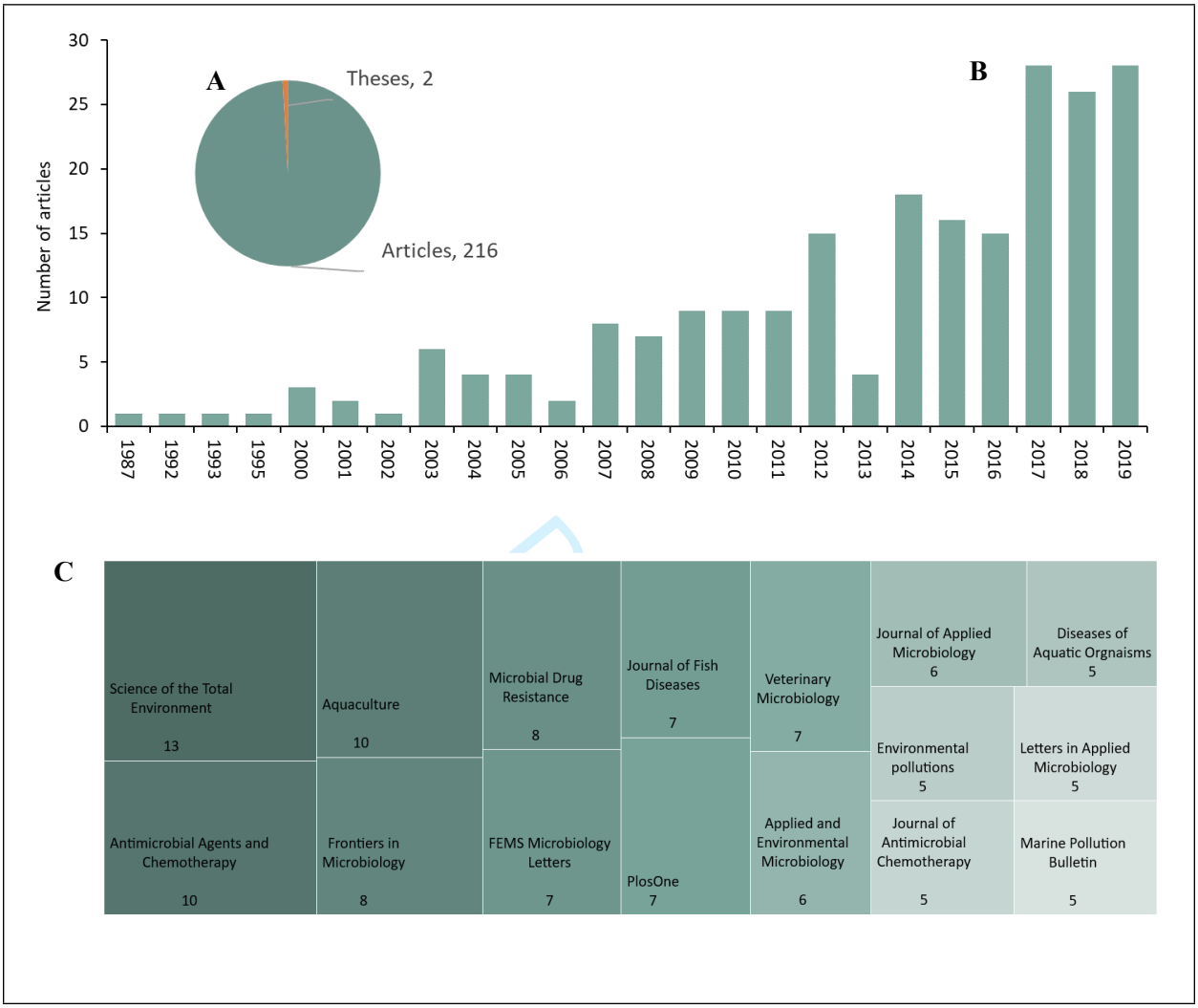
Figure 11: Comparison of country aquaculture production (CAP; proportion of total global finfish and crustacean production) against the number of studies (NS; as a proportion of all

679 studies) using an index (NS/CAP) from each of these 21 countries which cumulatively account
680 for 95% of total finfish and crustacean aquaculture production.

681

682 **Figures**

683 Figure 1



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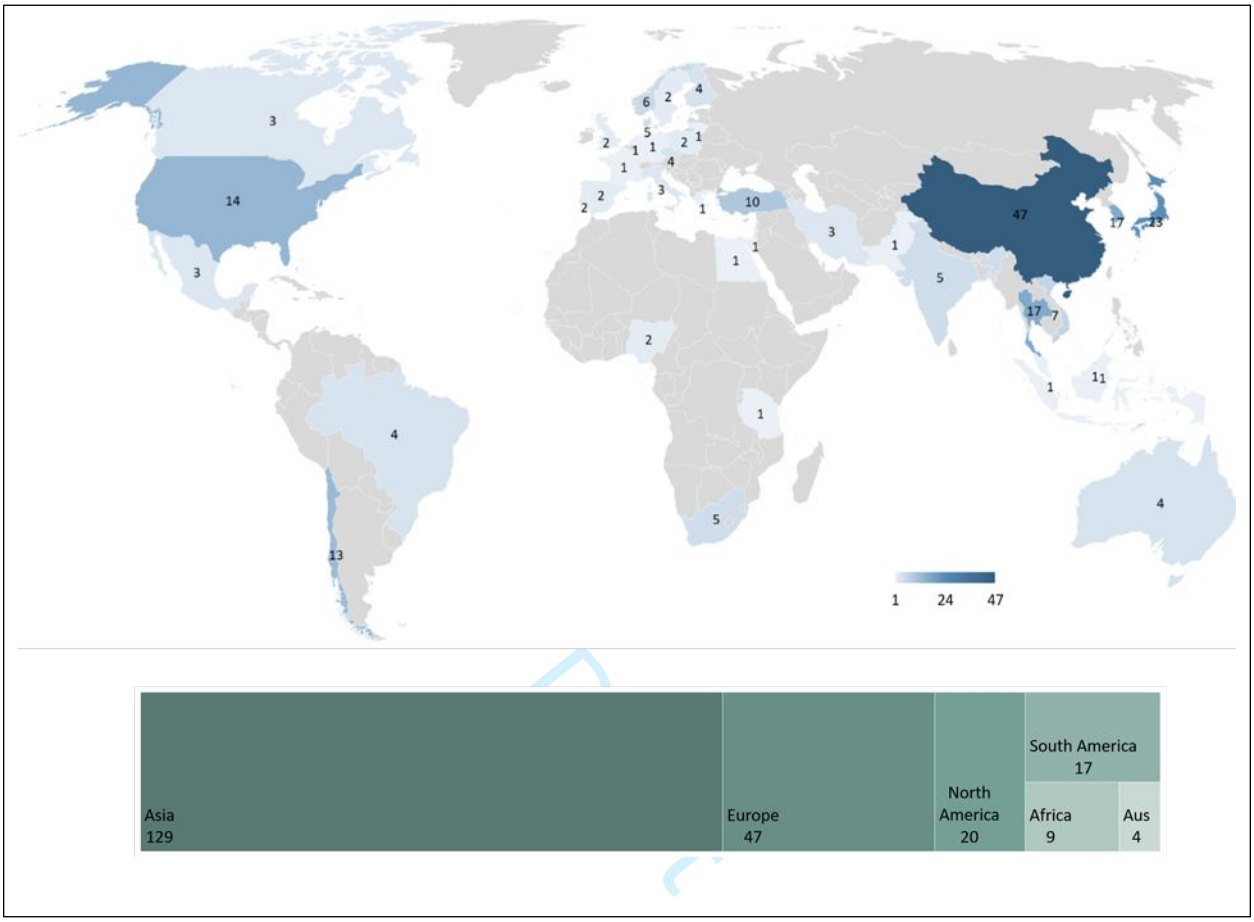
686 Figure 2



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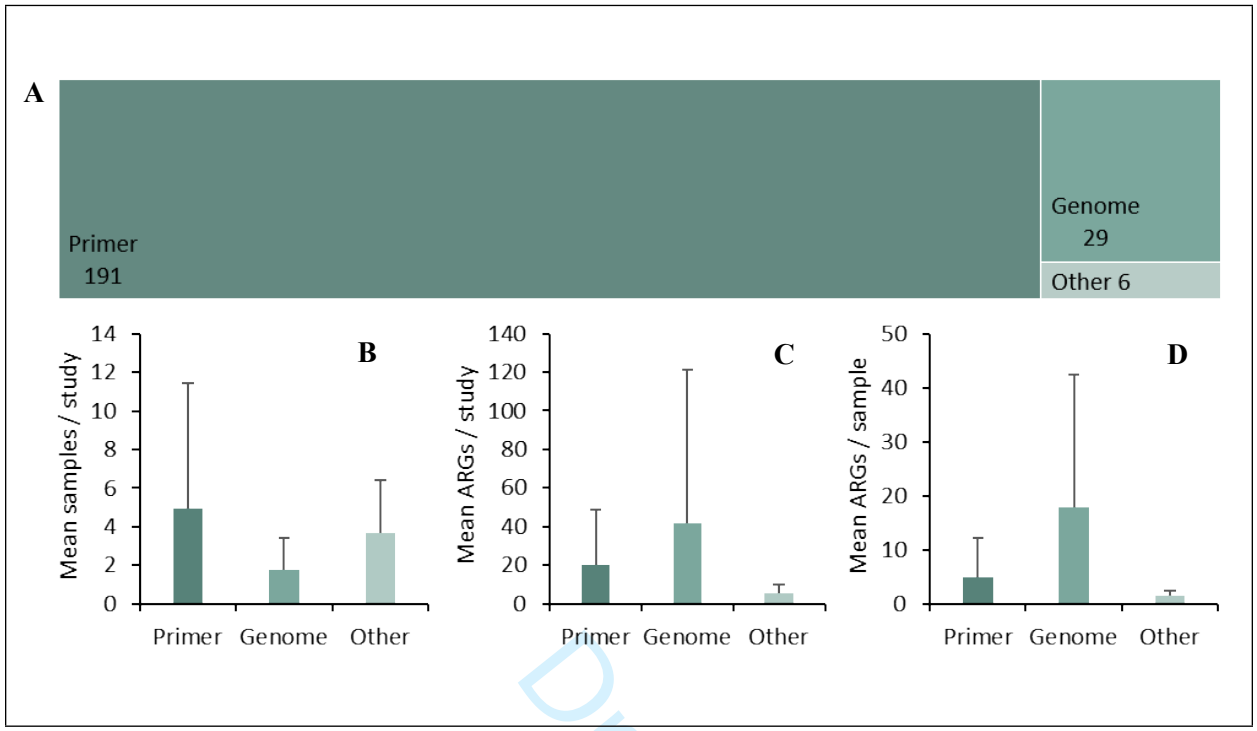
688 Figure 3



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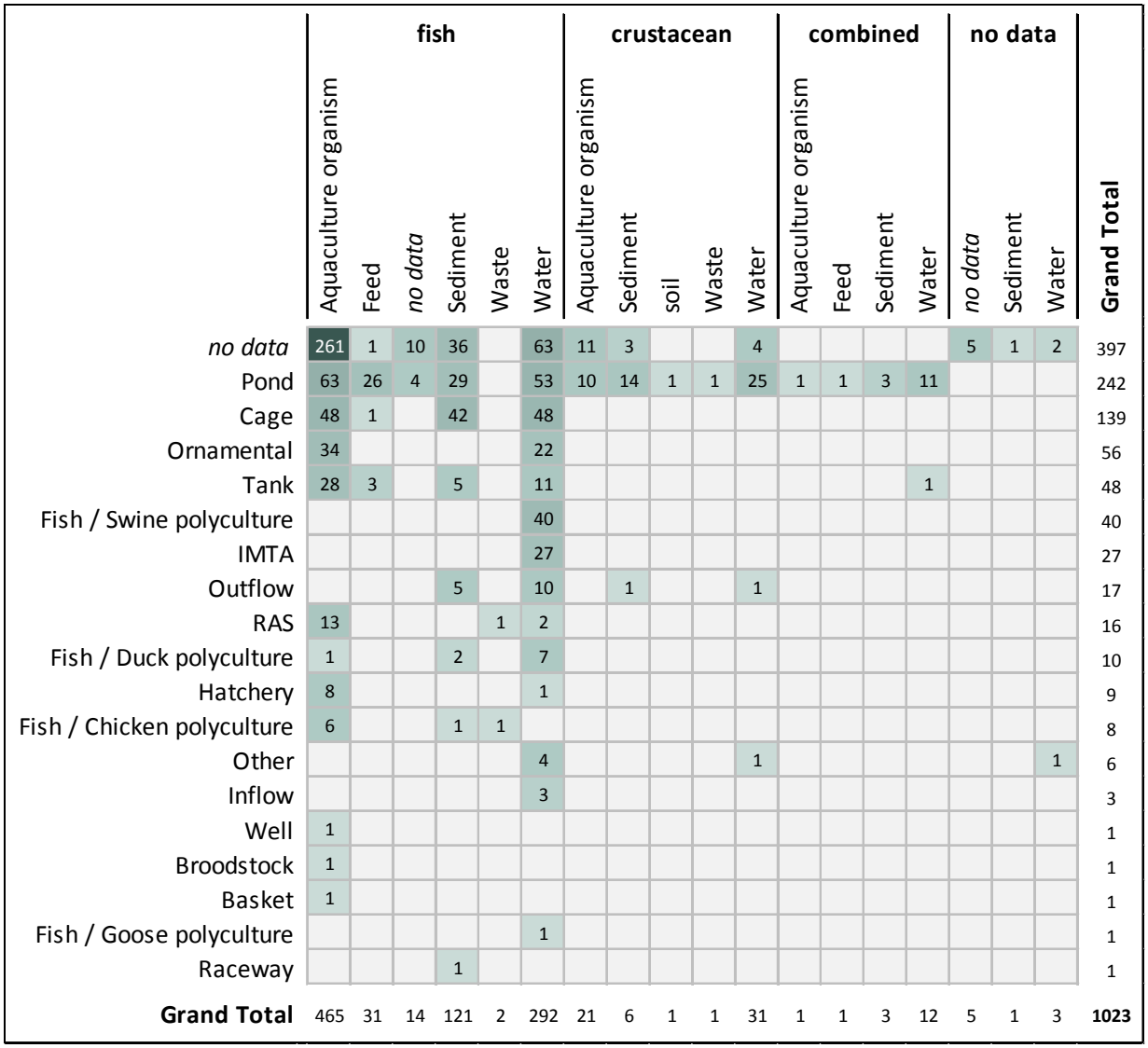
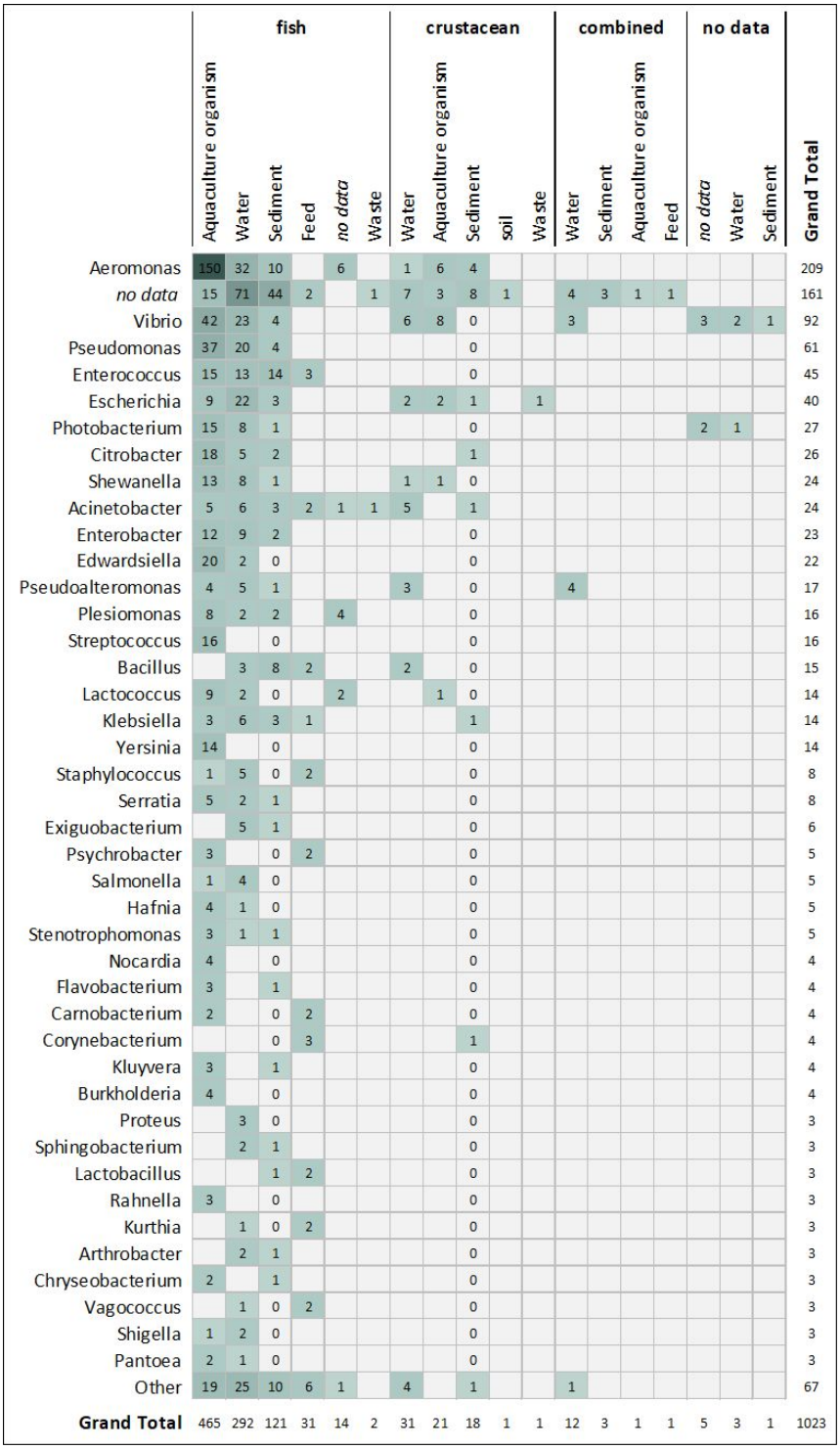


	fish						crustacean					combined				no data			
	Aquaculture organism	Feed	*no data*	Sediment	Waste	Water	Aquaculture organism	Sediment	soil	Waste	Water	Aquaculture organism	Feed	Sediment	Water	*no data*	Sediment	Water	**Grand Total**
no data	261	1	10	36		63	11	3			4					5	1	2	397
Pond	63	26	4	29		53	10	14	1	1	25	1	1	3	11				242
Cage	48	1		42		48													139
Ornamental	34					22													56
Tank	28	3		5		11									1				48
Fish / Swine polyculture						40													40
IMTA						27													27
Outflow				5		10		1			1								17
RAS	13				1	2													16
Fish / Duck polyculture	1			2		7													10
Hatchery	8					1													9
Fish / Chicken polyculture	6			1	1														8
Other						4					1							1	6
Inflow						3													3
Well	1																		1
Broodstock	1																		1
Basket	1																		1
Fish / Goose polyculture						1													1
Raceway				1															1
Grand Total	465	31	14	121	2	292	21	6	1	1	31	1	1	3	12	5	1	3	**1023**

691

692 Figure 5



	fish						crustacean					combined				no data			
	Aquaculture organism	Water	Sediment	Feed	*no data*	Waste	Water	Aquaculture organism	Sediment	soil	Waste	Water	Sediment	Aquaculture organism	Feed	*no data*	Water	Sediment	**Grand Total**
Aeromonas	150	32	10		6		1	6	4										209
no data	15	71	44	2		1	7	3	8	1		4	3	1	1				161
Vibrio	42	23	4				6	8	0			3				3	2	1	92
Pseudomonas	37	20	4						0										61
Enterococcus	15	13	14	3					0										45
Escherichia	9	22	3				2	2	1		1								40
Photobacterium	15	8	1						0							2	1		27
Citrobacter	18	5	2						1										26
Shewanella	13	8	1				1	1	0										24
Acinetobacter	5	6	3	2	1	1	5		1										24
Enterobacter	12	9	2						0										23
Edwardsiella	20	2	0						0										22
Pseudoalteromonas	4	5	1				3		0			4							17
Plesiomonas	8	2	2		4				0										16
Streptococcus	16		0						0										16
Bacillus		3	8	2			2		0										15
Lactococcus	9	2	0		2			1	0										14
Klebsiella	3	6	3	1					1										14
Yersinia	14		0						0										14
Staphylococcus	1	5	0	2					0										8
Serratia	5	2	1						0										8
Exiguobacterium		5	1						0										6
Psychrobacter	3		0	2					0										5
Salmonella	1	4	0						0										5
Hafnia	4	1	0						0										5
Stenotrophomonas	3	1	1						0										5
Nocardia	4		0						0										4
Flavobacterium	3		1						0										4
Carnobacterium	2		0	2					0										4
Corynebacterium			0	3					1										4
Kluyvera	3		1						0										4
Burkholderia	4		0						0										4
Proteus		3	0						0										3
Sphingobacterium		2	1						0										3
Lactobacillus			1	2					0										3
Rahnella	3		0						0										3
Kurthia		1	0	2					0										3
Arthrobacter		2	1						0										3
Chryseobacterium	2		1						0										3
Vagococcus		1	0	2					0										3
Shigella	1	2	0						0										3
Pantoea	2	1	0						0										3
Other	19	25	10	6	1		4		1			1							67
Grand Total	465	292	121	31	14	2	31	21	18	1	1	12	3	1	1	5	3	1	1023

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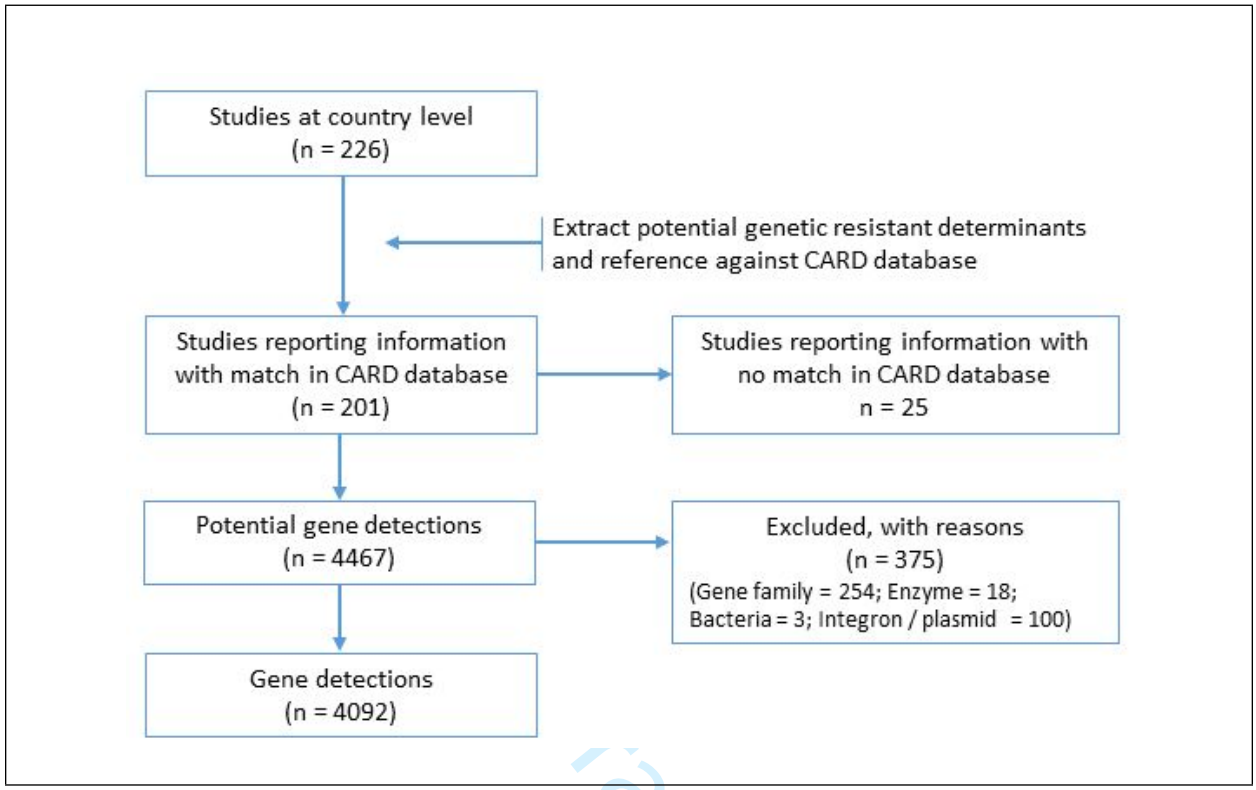
694 Figure 6



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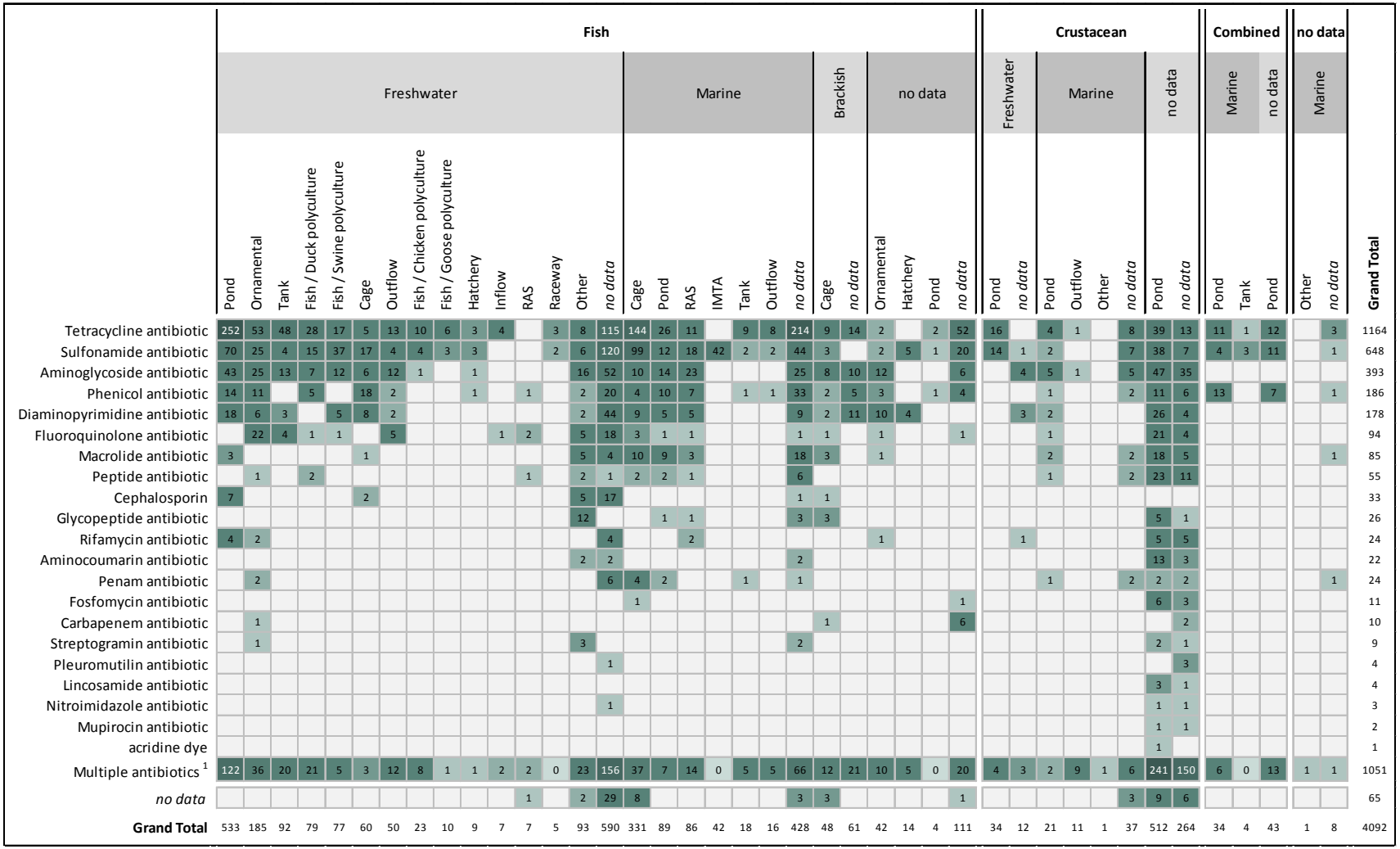
696 Figure 7



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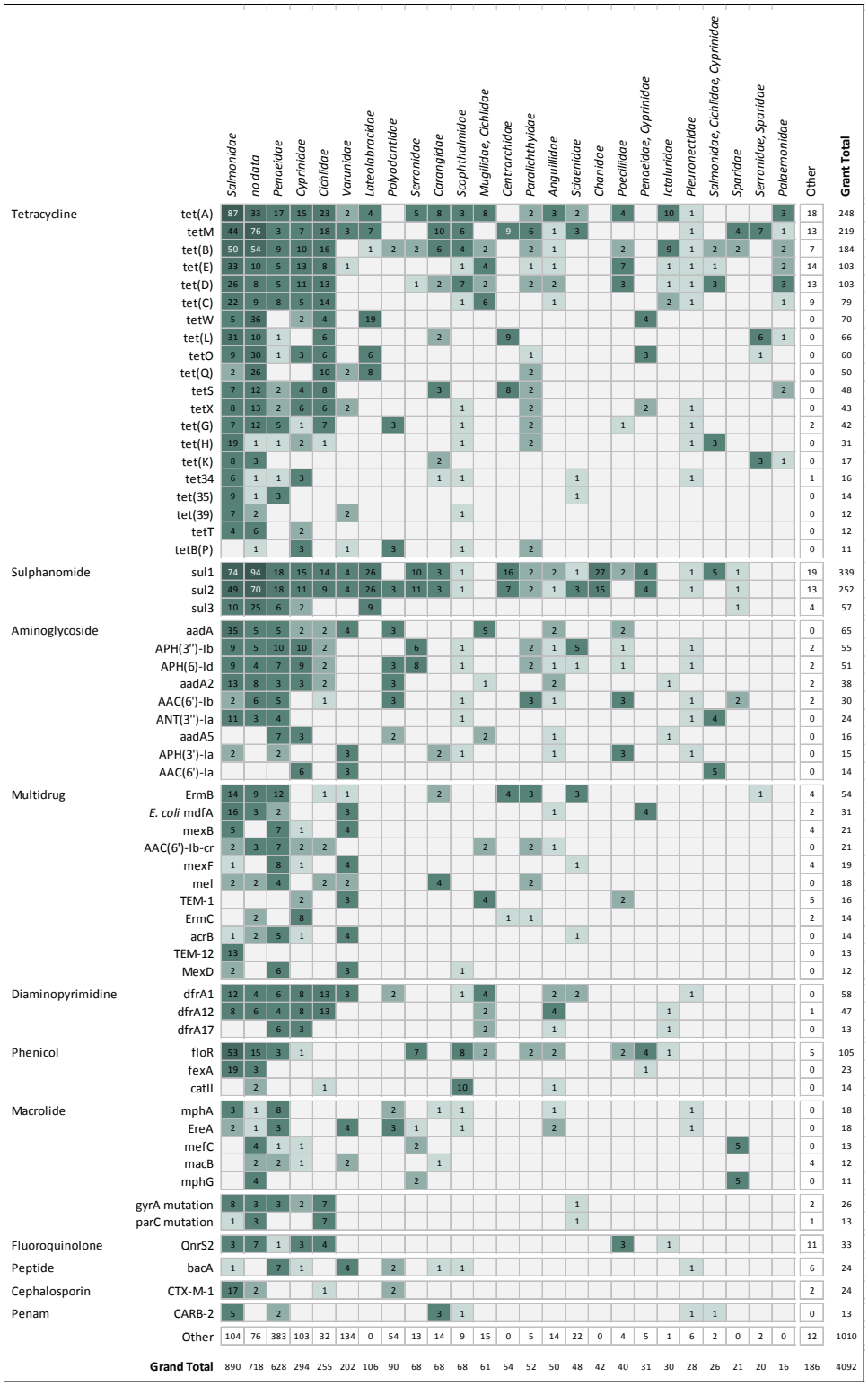
698 Figure 8



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700 Figure 9

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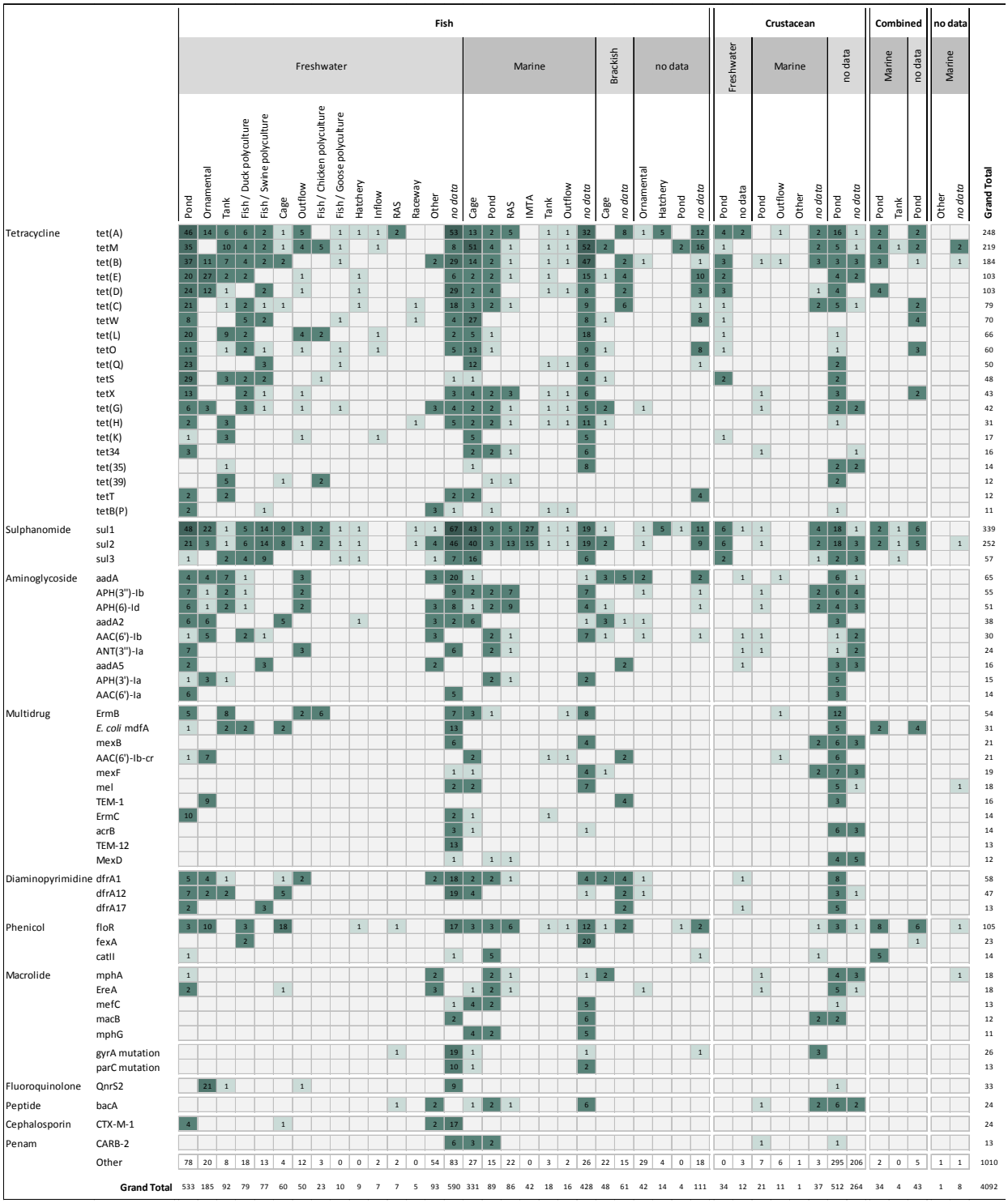
702 Figure 10



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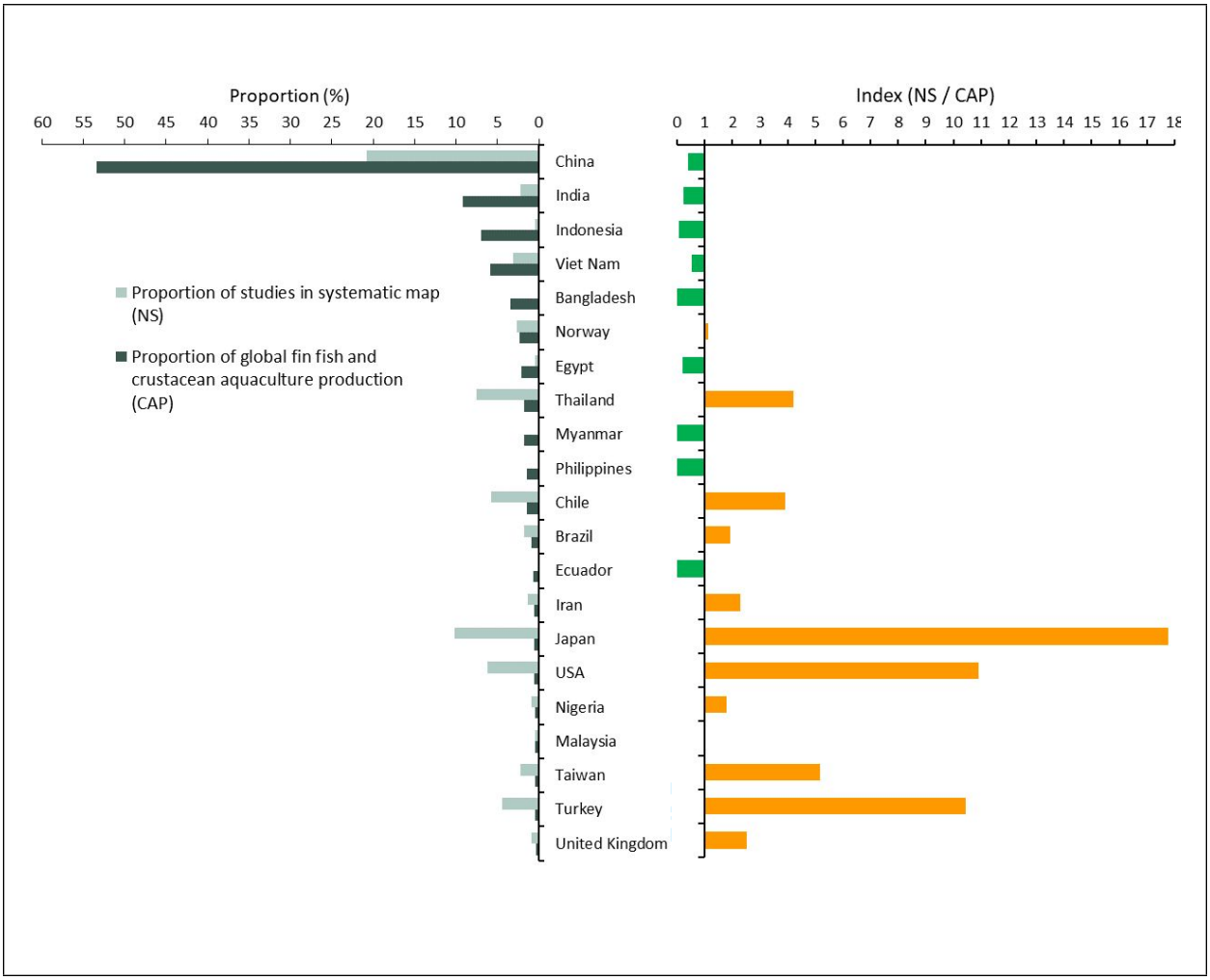
704 Figure 11



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