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Insights into gene expression responses to infections in teleosts using microarray data: a systematic review

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Abstract

The rapid growth of production in aquaculture in the last decades has brought unwanted consequences affecting fish health and increasing the susceptibility to different infections. This systematic review aimed to analyse and summarize the current knowledge of gene expression responses to infectious diseases in teleosts using viruses, bacteria, fungi and parasites as agents through published microarray data. We conducted searches in electronic databases, including PubMed, Web of Science and SCOPUS until 1 May 2019. We identified 862 citations across databases and manual searches. After removing duplicates, we screened 455 unique references using titles and abstracts, of which 262 proved potentially eligible and evaluated using full text. A total of 79 articles proved eligible for this review. From the articles retrieved, we examined 261 different experiments (or 'studies') and more than a hundred thousand differentially expressed genes (DEGs). This systematic review represents the first catalogue of genes (and their associated processes) that differentially transcribe in different teleost species (13 species) due to infections generated by a large variety of pathogens (38 types). Although the obtained gene expression results are in considerable measure associated with expected immune response, other genes showed surprising significant transcriptional outcomes that may unravel unknown functions related to fish infections. This type of investigations facilitates the visualization of existing gaps in researches that may inspire future analysis in non-traditional but relevant host or pathogen species.

Key words: gene expression, infection, microarray, systematic review, teleost.

Introduction

In the last decades, aquaculture has had rapid growth in production volume and economic yield. This development is due to the increasing demand for seafood that the capture fisheries cannot meet, gaining a significant role as seafood providers and as a source of income and social development in developing countries. This rapid growth, mainly through intensification of production of economically relevant species (e.g. salmonids, carp, tilapia), has brought unwanted consequences affecting fish health and increasing the susceptibility to infections with different types of pathogens (Bostock *et al.* 2010).

Since one of the more critical challenges to aquaculture growth is infectious diseases, which cause multibillion-dollar losses annually (Assefa & Abunna 2018), the study of host–pathogen interactions in infectious diseases in teleosts has grown considerably in the last years. In this context, the use of fish as standard organisms in immunological studies has been useful providing information into the evolution of

the adaptive immune response of vertebrates, including humans (Litman, Rast & Fugmann 2010). At present, however, the primary source of information linked to the response of teleosts to infections comes from fish species of productive interest. Hence, to analyse and interpret existing data becomes a difficult task. Under this scenario, transcriptomic studies are a valuable tool to reach a better understanding of the underlying pathways and mechanisms controlling host response to infection, which has been addressed primarily through microarray hybridization and/or RNA-seq sequencing technologies.

Twenty years ago, complementary DNA microarray (cDNA microarray) emerged as a versatile technology that researchers extensively applied in fish studies. The first analysis of global gene expression in fish employed a cDNA microarray for the goby fish (*Gillichthys mirabilis*), where the authors studied the response of this fish to hypoxia (Gracey, Troll & Somero 2001). To study the response to infection, the first study on fish employed a heterologous human microarray, to detect differentially expressed genes (DEGs) in Atlantic salmon, following *Aeromonas salmonicida* infection (Tsoi *et al.* 2003). However, the divergence of these species limited the results. Since then, more than 30 studies of fish response to infections were published with homologous cDNA microarrays. Then, cDNA microarrays were followed by *in situ* oligonucleotide microarrays, which were used in almost 50 researches to study fish response to infections. Although results showed the highly informative nature of the microarray strategy in fish, new technologies for transcriptomic studies based on high-throughput RNA sequencing (RNA-seq) (Qian *et al.* 2014) are in the way to replace microarray technology; proof of this is that since 2018, only one paper based on microarrays was published to study the transcriptional response of teleosts to infection. Despite this, microarray technique has allowed generating an extensive and exciting set of data on the transcriptional response of fish to pathogens.

This systematic review summarizes the current knowledge of disease challenge studies, including viruses, bacteria, fungi and parasites, as agents in teleosts through published microarray data, and reveals the first integrated catalogue of differentially expressed genes in response to infection in fish.

Methods

Search strategy

This systematic review followed guidelines set by the Preferred Reporting Items for the Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al.* 2009). We performed searches in electronic databases including PubMed, Web of Science and SCOPUS databases from inception to 1 May 2019 using the following keywords: "microarray* AND infectio* AND fish*". In addition, we identified articles through a manual search reviewing cross-references and citations of the included articles, using an iterative protocol as indicated above, which allowed us to retrieve six additional articles for this systematic review. There was no restriction by language, and the publication had to be accepted in the respective journal to enter in this systematic revision.

Study selection process and eligibility criteria

After removing duplicates, two researchers independently assessed the retrieved articles for eligibility excluding those not meeting our selection criteria by evaluating the titles and abstracts. In a second stage, we retrieved the full text of potentially eligible references to define definite inclusion criteria. First criterion: the article had to be original; thus, reviews were excluded. Second criterion: the article had to use wild-type teleosts (*in vivo*, no cell cultures) with no previous vaccination or any treatment and the infections had to be performed in a controlled manner with a wild-type pathogen. Third criterion: the article had to include the comparison by homologous microarray expression of genes that encode proteins of infected (treated) versus non-infected fish (control). Fourth criterion: the article had to include significative change in gene expression data (treated/control) (already analysed by the authors) in the body of the manuscript or Tables S1-S3.

A piloted form, including all four eligibility criteria, helped to document this process. When there was eligibility disagreement, another third reviewer arbitrated to make the final inclusion decision.

Data extraction

A data extraction piloted form was used to extract editorial data from the included articles. This form included the following 11 items: authors' information (corresponding author(s') name(s), year of acceptance of the publication, countries of affiliation and journal where the article was published), study subject (fish species and developmental stage), pathogen information (pathogen species and type) and experimental design (inoculation type, sample time post-infection and organ sampling for gene expression).

A second piloted form was used to extract the information of the microarray data of each article. This form included 13 items that were designed to collect information on the microarray platform information (platform ID, platform name in database, manufacturer, type of probe, number of probes, species, experiment ID, type of assay, dye, pooling, differential expressed genes (DEGs) criteria, statistical test to DEGs and the use of housekeeping gene for qPCR validation).

The entire data extraction process was conducted by two researchers, independently. We solved data discrepancies by discussion, including the participation of a third reviewer as arbiter when agreement was elusive.

Gene name standardization

In order to define a set of commonly DEGs in teleosts in response to infection across all the articles incorporated in this review, we first extracted the gene transcriptional information from the body or Tables S1-S3 (already analysed by the authors) of each of the 79 articles included here. The direction of the transcriptional change (up-regulation or down-regulation of the gene in response to infection) was included in this third piloted form, together with data extracted from the articles that corresponded to each DEG, such as fish species, developmental stage, sample tissue, time post-infection and pathogen species. Again, this data extraction was conducted by two researchers independently, and when there was disagreement, a third person arbitrated to make the final decision. Since direct matching for shared genes among studies was not feasible because authors published their results using different forms of gene identification (e.g. gene name, gene symbol, GenBank accession number, Affymetrix probe set ID or Unigene cluster ID), we had to convert all the different gene identifications to one common description name to compare the sets of genes based on UniProt and/or GeneCards databases. This work was done by three researchers using an iterative strategy, and the quality assessment of the extracted data was done by checking randomly 1% of the gene list of 102,274 entries (Table S3). The analysis for under- or over-enrichment was based on the cumulative distribution function of the hypergeometric distribution of the complete set of up- and down-regulated genes considering significant P-value < 0.05.

Co-authorship network assembly

Authors' names are critical for the accurate links in the network. The same author could be considered with different names on records due to abbreviations, omissions or name changes, while different authors can have the same name. So, manual curation was performed to avoid these problems. The network was constructed using Cytoscape 3.7.1 (Shannon 2003) and the Social Network Cytoscape App version 3, according to Kofia *et al* (2015).

Results and discussion

Systematic review editorial analysis

We initially identified 856 citations across all electronic databases and six through manual searches. We obtained 407 duplicated articles in at least two of the three databases (Figure 1). The remaining 455 articles were screened using titles and abstracts, leaving 262 articles for full-text screening.

After completing all stages of screening of references from electronic databases, 73 articles proved eligible, which were retrieved in different percentages in the three databases: 100% were found in Scopus, 86.3% in PubMed and 76.7% in Web of Science. Then, through the revision of the references and citations lists from the 73 articles previously obtained, we made a manual search for publications meeting our selection criteria that may have been left aside in the search strategy conducted in the electronic databases. Six new articles were recovered, increasing the number of papers used in the systematic review to 79 (Figure 1). This result shows that, when preparing a systematic review, manual curation of the articles retrieved from electronic databases is crucial. Later, all articles were manually evaluated to establish to which database they belonged to, which resulted in 77 of the 79 articles shared by the three databases, except for only two articles that were not present in PubMed. Remarkably, this result indicates that under the focus of this systematic revision, utilizing only one of these databases would have been enough to retrieve between 97% and 100% of the articles. Besides, the sensibility (or recall), defined as the proportion of relevant articles identified by the search strategy, was 92% (73 out of 79), indicating a high sensibility in our search strategy. Also, the precision, defined as the proportion of relevant articles identified as a percentage of all the articles (relevant and irrelevant) detected by the search strategy, was 16% (73 out of 455), indicating a low ability of our search strategy to exclude irrelevant articles. These results are expected since highly sensitive strategies tend to have low levels of precision, which impacts in a higher number needed to read (NNR) to find a relevant article but maximizes the inclusion of most studies of interest (if not all) in the systematic review.

Regarding the number of publications incorporated in this systematic review, mostly ascended from early 2000 until around 2012, after which a prominent drop-down was observed (from approximately 12 articles in 2012 to half in 2014; Figure 2). This decrease tightly synchronized with the general decrease in investigations associated with 'microarray' studies, and interestingly with an increase in the publication amount under 'RNA-seq' technology (Figure 2). This result indicates that for this particular research topic, the methodology implemented is highly influenced by trends and the year of publication of the studies evaluated. In fact, to verify whether any relevant paper regarding this review had been published after May 2019, we repeated the search in January 2020 and found only one new publication since 2018 (Castro *et al*. 2019). Moreover, it suggests that we are currently incorporating most of the microarray studies that will ever be developed on this subject. Thus, we

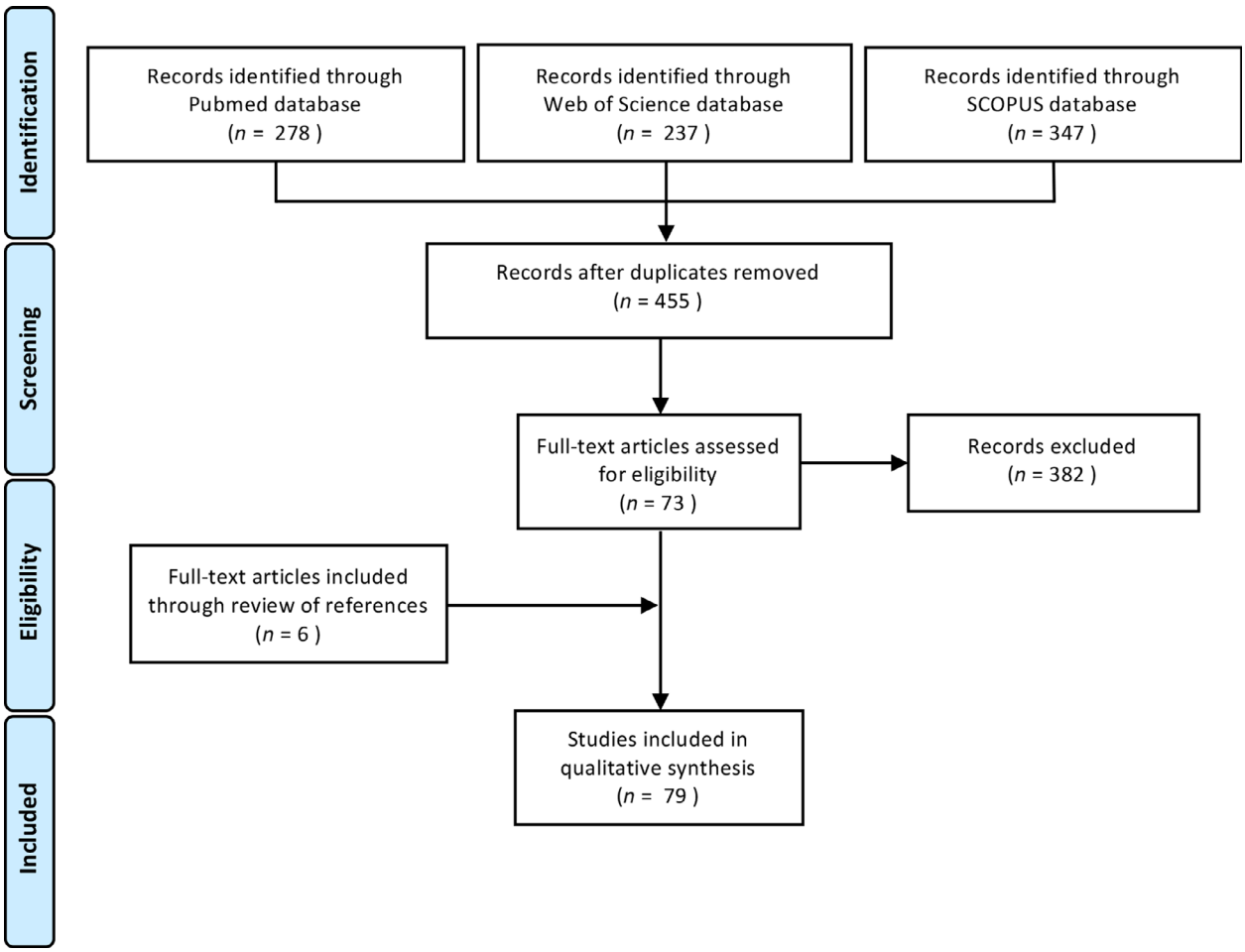

Figure 1 PRISMA flow diagram. PRISMA diagram summarizing the flow throughout the systematic review.

do not expect the need to incorporate many more publications if we would like to conduct an update of this systematic review in the future.

The top three journals in this research area, which published around 50% of the evaluated articles in this systematic review, are Fish and Shellfish Immunology, BMC Genomics and Molecular Immunology (Table S1; Figure 3a). Surprisingly, only one of these three journals is directly associated with fish studies, suggesting that transcriptional response to fish infections, the subject addressed in this systematic review, is of general interest. Also, 15 of the 23 journals that had articles evaluated in this review possessed only one article associated with the theme assessed here, indicating that many journals are open to incorporate studies in this field even though it is not their usual scope. Most of these one-article-in-the-subject journals (11 out of 15) belong to the first journal ranking quartile (Q1) in the SCImago Journal and Country Rank (SJR) (Figure 3a). In this way, despite having few publications on gene expression profiles in teleost infections using microarray data, they are high-ranked journals to publish on this subject.

The country that forms part of the highest number of affiliations in the articles included in this revision is Norway (18 articles), followed by Spain (15 articles), China (13 articles) and Canada (10 articles) (Table S1; Figure 3b), all of which belong to the Northern Hemisphere. In the Southern Hemisphere highlights Australia and Chile with 5 and 3 articles, respectively, showing that many relevant fish farmer countries, such as Norway, China and Chile, develop scientific studies associated with fish response to infections. Nevertheless, it is crucial to consider that the studies involved in this review require relatively expensive methods (hybridization and analysis of microarrays), a reason that could explain the fact that not all outstanding aquaculture countries may afford this kind of approaches.

In order to examine the relations among groups of investigators working on gene expression profiles in teleost infection using microarray data, we used the Cytoscape platform to generate a co-authorship network that

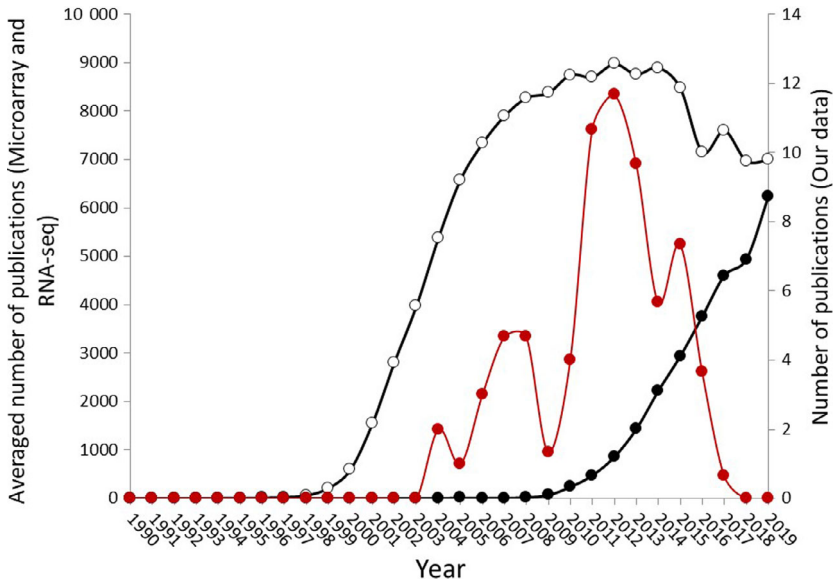

Figure 2 Articles by year of distribution. Microarray (white dots) information and RNA-seq (black dots) information were obtained searching the keywords 'microarray' or 'RNAseq, RNA seq, RNA-seq', in PubMed, Web of Science and Scopus databases from 1990 to 2019. The graph shows the averaged information from the three databases. Our data (red dots, secondary axis) correspond to the average of the manuscripts selected and evaluated in this systematic review from the three databases for all the years evaluated. –o–, Microarray; –●–, RNA-seq; –●–, Our data.

incorporated all the authors that participated in the 79 articles included in this revision (Figure 3c). We can observe that many of the nodes or authors were associated with one large network module, which was composed of 165 out of the 382 total authors of this review and which congregated the 45.6% (36 out of 79) of the articles included here. Nine of the eleven authors who participated in 5 or more of the articles (black nodes) were encompassed in this module, highlighting A. Krasnov, who contributed in 16 of the publications (Figure 3c, square node). Only two more modules contained a black node, which was also the networks with the highest number of articles following the large module (6 articles each). Also, these three same modules contained the authors that were keystone components for network structure by having the 50% superior values of betweenness centrality of the whole network (Figure 3c, five nodes with orange border). Together with Krasnov, H. Takle was one of the central authors in the most significant module, which outstandingly only participated in two of the 79 articles analysed (Figure 3c, yellow node with orange border in the most extensive module). Also, the other three of these keystone authors (Y. Wang, B. Koop and B. Nowak) were present in smaller network modules. Wang was present in one module with five publications, while Koop and Nowak were part of another module with 5 and 4 articles each (Figure 3c, nodes with orange border in medium-size modules). These results indicate that despite the smaller contribution in the number of publications, some authors, and especially their strategic connections, have been fundamental in maintaining network assembly.

The smallest network modules (Figure 3c, modules formed by eleven or fewer nodes) were mostly shaped by authors who had participated in only one of the articles included in this systematic review: research groups with fewer connections are new in this subject. Alternatively, this fact could be explained by the recent shift to RNA sequencing regarding gene expression global techniques, which have resulted in a reduction in transcriptomic analyses using microarrays (Figure 2). We can conclude that the set of more than 20 different author association networks generated in this analysis indicates that many of the authors did not develop collaborative strategies, even though they were all interested in studying teleost infection using microarray data.

Other aspects obtained from the 79 articles that entered this systematic review were the species of fish and types of pathogens that caused the infections, which are summarized in Figure 4. Thirteen different teleost species and 38 pathogens were investigated. A neighbour-joining cladogram of the fish species showed that they were grouped in five clusters and that the one formed by the salmonids (*Oncorhynchus mykiss* and *Salmo salar*) contained the highest number of articles reviewed. As for the pathogen species, *Piscine novirhabdovirus* (VHSV) was the most represented in the articles.

In most cases, the infections studied were generated by a bacterium (n = 35) or a virus (n = 31). At the same time,

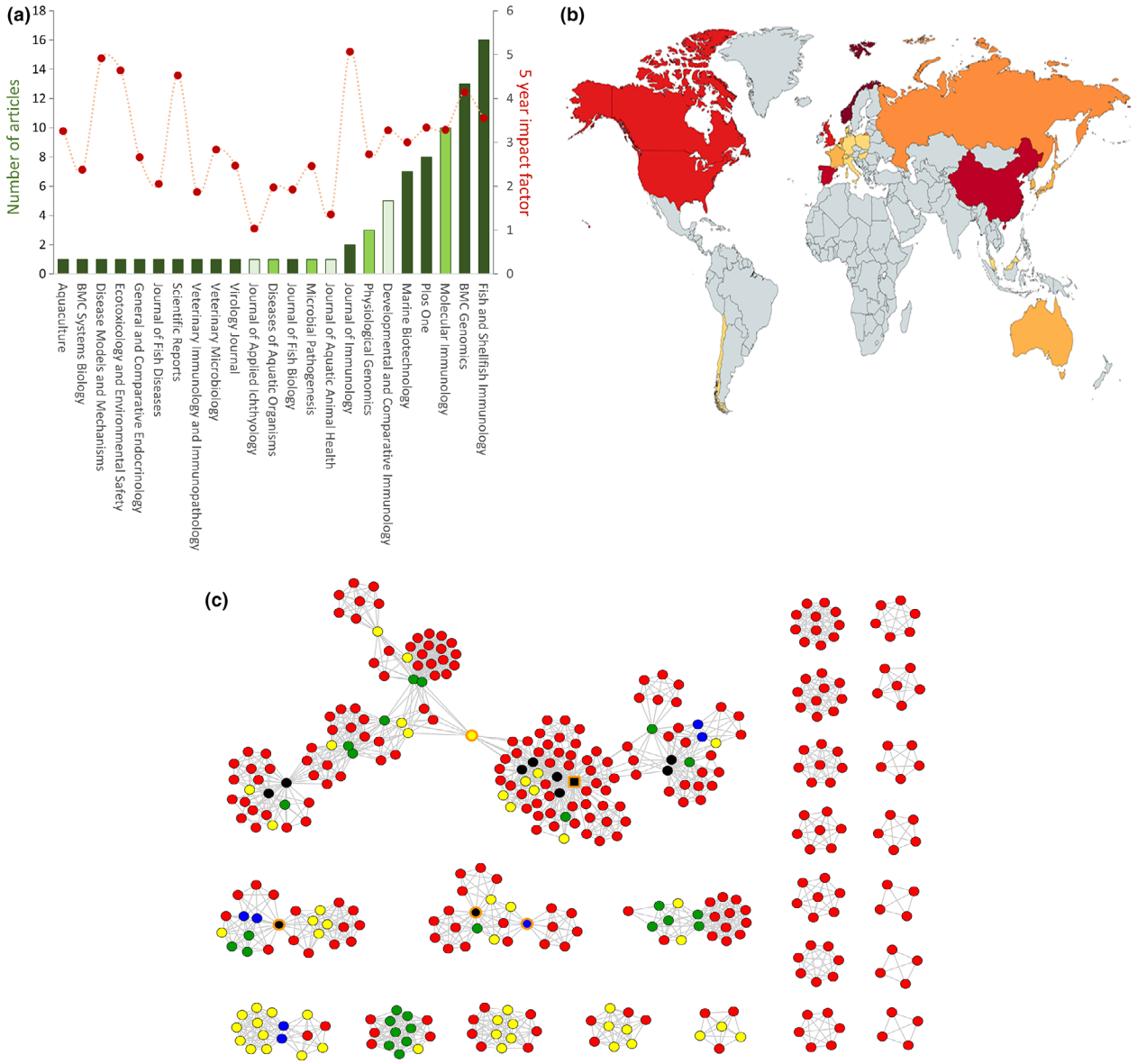

Figure 3 Editorial Panel. (a) Study distribution per journal published. Red line: 5- year impact factor (until 2018) per journal (obtained from Journal Citation Reports database). Dark green, first-quartile journal (i.e. Q1); light green, second-quartile journal (i.e. Q2); very light green, third-quartile journal (i.e. Q3). (b) Distribution of countries participating in the articles evaluated. Number of coauthored works: ■, 1; ■, 2; ■, 3; ■, 4; ■, ≥5. (c) Co-authorship network. Square node corresponds to the author that participated in the highest number of articles included in this review (A. Krasnov). Nodes with orange border correspond to keystone components for network structure by having the 50% superior values of betweenness centrality of the whole network (A. Krasnov, H. Takle, Y. Wang, B. Koop and B. Nowak). Number of publications included: ■, 1–3; ■, 4–6; ■, 7–9; ■, 10–12; ■, 13–15; ■, +16.

only 15 articles were associated with a eukaryotic pathogen (fungi or parasites), indicating that the first two pathogens are of more general interest at least for this kind of investigation purposes. Nonetheless, when looking at the combination of the developmental stage of the fish and the pathogen involved in the infection, juvenile *Salmo salar* and the eukaryotic ectoparasite *Lepeophtheirus salmonis* had the highest value ($n = 4$) (Table S1; Figure 4). This result is most probably attributable to the need to increase the knowledge of this devastating disease in this economically significant specimen.

Most articles studied in this systematic review were related to infections in juvenile fish ($n = 55$) (Figure 4), defined as fish from 30 to 89 days of development,

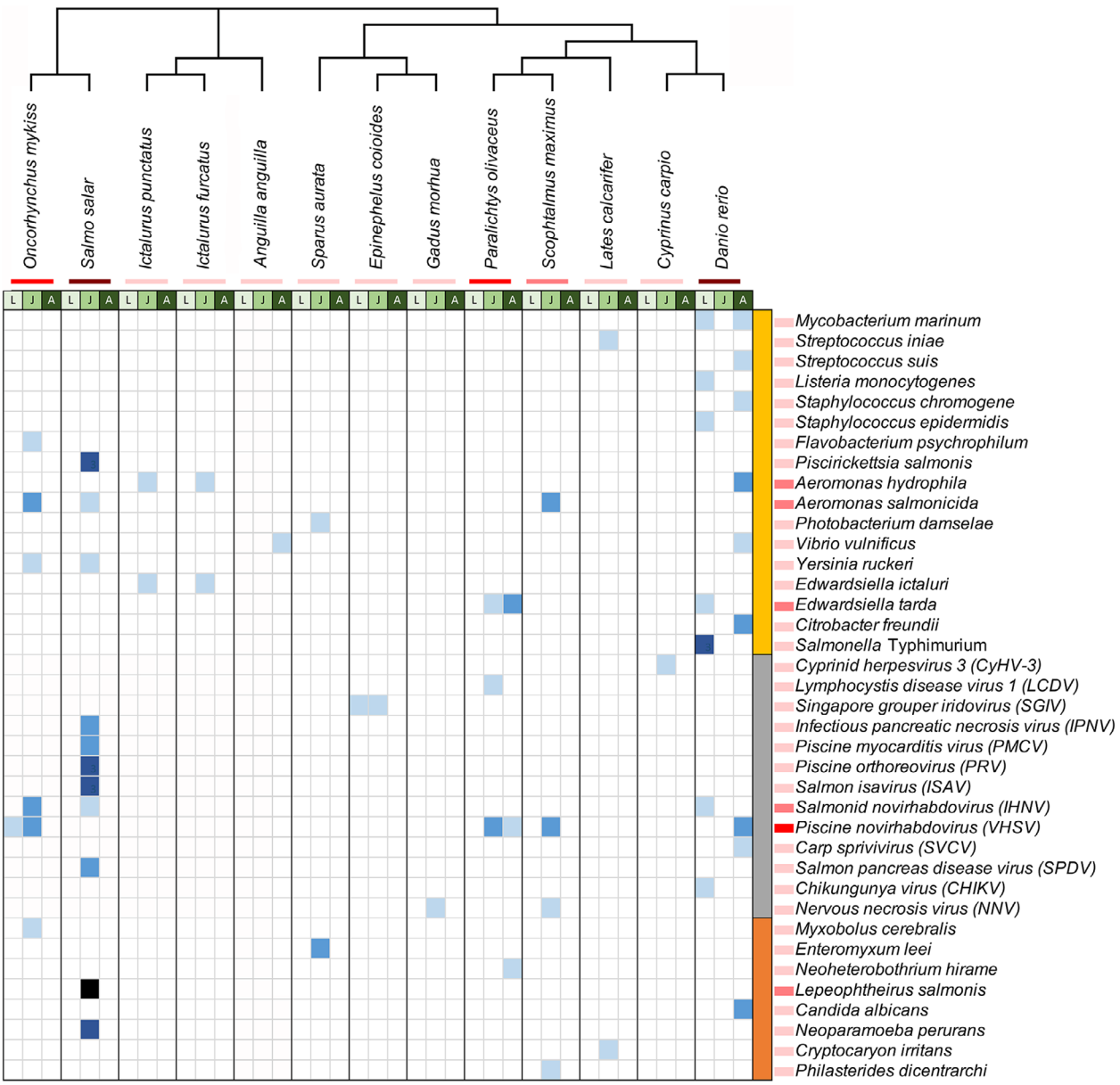

Figure 4 Diagram of fish and pathogen information of the articles incorporated in this systematic review. Top, phylogeny of the fish species in different developmental stages (L, larvae; J, juvenile; A, adult). Right, bacteria (yellow), virus (grey) and fungi and parasites (orange). Phylogeny of fish was performed using the neighbour-joining method (Saitou & Nei 1987). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl & Pauling 1965) and are in the units of the number of amino acid substitutions per site. The analysis involved 14 amino acid sequences from the cytochrome oxidase subunit I of all the fish species used in this systematic review obtained from NCBI database (Meynard *et al*. 2012; Chen *et al*. 2014). There was a total of 516 positions in the final data set. Evolutionary analyses were conducted in MEGA7 (Kumar, Stecher & Tamura 2016). Number of studies involving fish/pathogen species: , 1 to 3; , 4 to 6; , 7 to 9; , >10. Number of studies: , 0; , 1; , 2; , 3; , 4.

according to Kimmel *et al*. (1995), while larvae (3 to 29 days) and adults (90 days to 2 years old) were present in only 9 and 17 articles, respectively (Figure 4). In fact, in all the articles that studied *Salmo salar*, which was the species with the highest number of studies considered in this review, were juvenile fish (Table S1; Figure 4). This fact

may be because, in this teleost species, this stage of development is more comfortable to handle experimentally than bigger fish. On the other hand, *Danio rerio* was the second most reported fish species in the articles considered in this review, presented no studies involving juvenile specimens (Table S1; Figure 4). This can be due to the fact that most of the advantages of zebrafish as a model species occur in embryonic/larvae stages (transparency of the larvae, rapid development, low cost, genetic engineering, high throughput, etc), making its use more frequent in these stages (Torraca & Mostowy 2018). Also, this species is almost the only one from this systematic review that was used to study human bacterial, viral and fungal pathogens (except for the work on *Anguilla anguilla* infected with *Vibrio vulnificus*). Interestingly, this species is the second most widely used organism as a research model in human biomedicine (Aleström & Winther-Larsen 2016).

Moreover, we could observe that most articles interested in Gram-positive bacteria studied the infections in *Danio rerio* (except for one publication of juvenile *Lates calcarifer* infected with *Streptococcus iniae*), showing a remarkable bias in gene expression investigations using microarrays involving this group of bacteria. For instance, we noticed the lack of investigations regarding infections by the Gram-positive bacterium *Renibacterium salmoninarum,* the agent causing bacterial kidney disease (BKD). This disease generates morbidity and mortality in both farmed and wild fish in most regions of the world where salmonids are found; it is also the most significant cause of infectious disease-related mortality in restoration programmes for several endangered species (Wiens *et al.* 2008). Moreover, to the best of our knowledge, no general transcriptomic studies (including RNA-seq) have been performed in infection studies involving *Renibacterium salmoninarum*. Hence, this could be an exciting niche for future disease studies on fish of productive and conservation interest (*i.e.* not *Danio rerio*) produced by a Gram-positive bacterium.

Microarray platform information

From the total publications utilized in this systematic review, 65.8% (52 out of 79) explicitly indicated the ID access code of the microarray platform to the Gene Expression Omnibus (GEO) database: https://www.ncbi.nlm.nih.gov/geo/ (The National Center for Biotechnology Information, USA) or to the ArrayExpress database: https://www.ebi.ac.uk/arrayexpress/ (The European Bioinformatics Institute_EMBL-EBI) (Table S2). Among the publications that indicated their ID code, we identified 37 different microarray platforms used. From the publications that did not indicate their ID code, we estimated at least other 15, based on their descriptions. These platforms ranged between 0.4 and 60 K probes each, being most of them based on *in situ* oligonucleotides (60.7%). These chips were mainly manufactured by Agilent Technologies (48.1%), while the others are based on spotted DNA/cDNA, which were mainly manufactured in university core facilities. Remarkably, almost three-quarters of the platforms used were developed for salmonids (40 out of 79) and *Danio rerio* (19 out of 79), indicating that most of the current knowledge of the transcriptional response of fish to infections is centred on these two species, which is explained by their commercial relevance and their impact as a teleost model, respectively.

In the field of study of this review, most of the platforms have been used to carry out two-colour competitive assays (65.8%), being the Cy3 and Cy5 the most popular fluorophores utilized (in over 80% of the cases). Interestingly, in most of the studies (70%), the RNAs extracted from the same conditions were mixed (pooling) in competitive hybridization assays. Whereas this is mainly explained because this experimental approach is cheaper than the hybridization of individual fish, the knowledge of the associated biological variability is compromised with this strategy. Although we know this is the trend, we recommend for future investigations to maximize biological replicates of individual fish, while the technical replicas may be minimized.

Finally, it is relevant to highlight that only in 58.2% of the publications explicitly reported the ID access code to the whole gene expression data; hence, more than 40% of the articles cannot be incorporated into future meta-analysis studies. For instance, although seven of the publications directed their study in the transcriptional response of *Paralichthys olivaceus* to different pathogens, none of these studies is available as raw data, precluding the generation of integrated knowledge of the transcriptional response to the infection of this species. For this reason, we suggest that for future researches based on microarray and/or RNA-seq, the raw expression data must be available in the appropriate databases.

Gene expression analysis

From the 79 articles retrieved in this systematic review, we examined most of the conditions evaluated in all the articles, including the different fish species, stages of development of the fish, tissue samples, times post-infection and pathogen species. From this analysis, we obtained 261 different experiments (from now on called 'studies') that contemplated: 13 different fish species, three stages of development of the fish, 18 different tissue samples, several post-infection times and 38 types of pathogens (17 species of bacteria, 13 viruses and eight eukaryotic pathogens) (Table S3). Overall, more than 100,000 DEGs were found, and those present in more than 50% of the studies

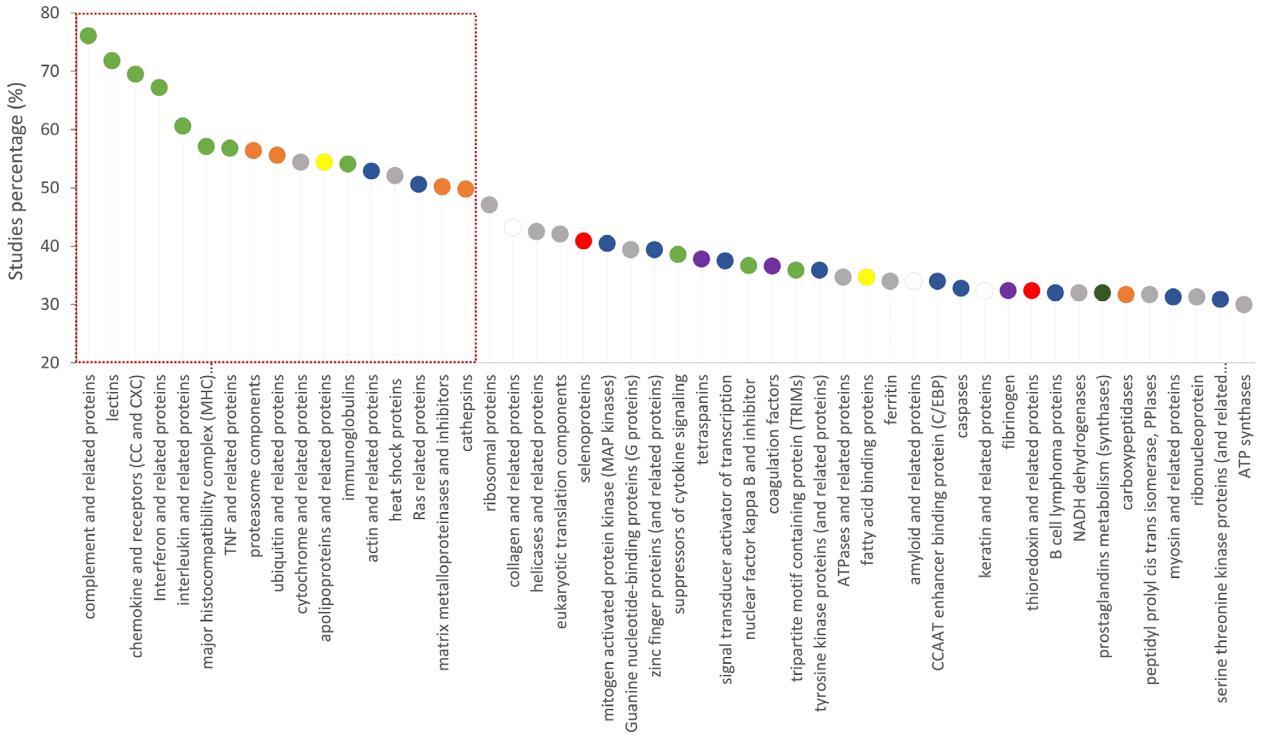

Figure 5 Ranking of the proteins functions from the 261 studies evaluated. Proteins with more than 30% of representation in the studies are shown. Red box, proteins that accounted for over 50% of representation in the studies and that were profoundly investigated. ○, Housekeeping cellular functiona and/or response to stress ●, Antioxidant metabolism; ●, Regulations and dynamics of cellular processes; ●, Classical components of immune response to infection; ●, Coagulation system component; ●, Transport and metabolism of lipids; ●, Protein degradation; ○, Other functions.

(Figure 5) were grouped according to their general functions and classified as 'classical components of immune response to infection', 'protein degradation processes', 'transport and metabolism of lipids', 'housekeeping cellular functions and/or response to stress' and 'regulation and dynamics of cellular processes'. These processes are explored and described in detail below.

Classical components of immune response to infection

The complement system

The complement system (CS) is part of the innate immune system of vertebrates. It consists of a series of proteins that are synthesized in many fish tissues and exist in the plasma and on cell surfaces as inactive precursors (Boshra, Li & Sunyer 2006; Nakao *et al.* 2011). Three distinct pathways can activate complement, and all of them produce the same protective effects: opsonization, recruitment of phagocytic cells and formation of membrane-attack complexes. In the present work, 76.1% of all the studies reviewed showed DEGs encoding proteins of the complement system. In contrast, 84.6% species of fish and 84.2% of the pathogens analysed are related to some DEG associated with CS. Also, the proportion of up- and down-regulated genes related to CS were significantly enriched with respect to the complete proportion of up-and down-regulated DEGs of this review, correspondingly.

In teleosts, the CS exerts essential functions, from alerting hosts of the presence of potential pathogens and their clearing, to the development of adaptive immune response (Holland & Lambris 2002; Nakao *et al.* 2011). In fish, this system is active against bacteria, viruses and eukaryotic pathogens even at low temperatures and the serum titres of alternative pathway components are up to ten times higher compared with mammals (Zarkadis, Mastellos & Lambris 2001). Interestingly, it has been described that CS of fish has a broader recognition capacity of microorganisms than mammals, making the CS a pivotal point in systemic and local fish defence system (Sunyer & Lambris 1998; Sunyer, Zarkadis & Lambris 1998; Nonaka & Smith 2000; Boshra, Li & Sunyer 2006). Mechanisms underlying protection are not fully elucidated and, in some cases, seem to be species-specific. Also, transcripts encoding complement components show a broad tissue distribution, being present in head kidney, intestine, skin and gills, among others (Løvoll *et al.* 2007; Encinas *et al.* 2010). This fact suggests that

teleost complement system is not active only in circulating blood or body fluids, but also at the interface to the outer environment, where pathogens enter the host.

Protective activity against bacteria by the CS has been recognized as one of the main bactericidal mechanisms in teleosts (Ellis 2001; Holland & Lambris 2002). In fact, of all the bacterial species analysed in the present work, 16 out of 17 (94.1%) showed transcriptional regulation of CS components in the host, higher than with viruses (10 out of 13, 76.9%) and fungi and parasites (6 out of 8, 75%). However, for viral diseases, it has been described that the neutralizing activity of antibodies against rhabdoviruses, such as viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV), is dependent on the complement activity (Iwama *et al.* 1996). For parasites instead, an important role has been described for complement C3 protein in binding to the surface of parasites (Harris, Soleng & Bakke 1998). Remarkably, the gene that encodes to complement C3 protein was the most represented among the CS differentially expressed genes ($n = 333$). The CS stands out as the most represented group across the studies and agents of the articles included in this systematic review, reaffirming the importance of this pathway in infection and teleost–pathogen interaction.

Lectins

Lectins are a superfamily of carbohydrate-binding proteins with a significant number of members throughout almost all living species and viruses. These proteins have been recognized as critical components of innate immunity, showing functions as regulators of adaptive immune responses by recognizing bacterial or viral components on dendritic cells (van Vliet *et al.* 2008), promoting signals that initiate or modulate cytokine responses, and inducing lymphocyte maturation and polarization to the invading pathogens (den Dunnen, Gringhuis & Geijtenbeek 2010). Also, as pattern-recognition receptors, lectins bind to a wide range of carbohydrates expressed on microorganism surfaces, including mannose and fucose, leading to their phagocytosis and lysis by the complement system (Møller-Kristensen *et al.* 2007). In teleosts, lectin repertoire is ample and complex, with representatives from most lectin families described so far and with tissue-specific expression and localization consistent with their distinct biological roles in innate and adaptive immunity (Vasta *et al.* 2011).

In the present work, 71.8% of all the studies reviewed showed DEGs encoding lectins. All fish species and 89.5% of the pathogens analysed in this review are associated with some DEG encoding for lectins. The proportions of up- and down-regulated genes related to lectins were significantly enriched with respect to the complete proportion of up- and down-regulated DEGs of this review, respectively. The two most represented lectins observed in this systematic review were galectins (41%; $n = 540$) and C-type lectins (25.3%; $n = 333$), both with functions as recognition factors of microbial glycans (Vasta 2009; Stowell *et al.* 2010) or as regulators of multiple adaptive immune functions by modulating B-cell development, inducing or preventing T-cell apoptosis and modulating T-cell responses (Vasta *et al.* 1999; Jayaraman *et al.* 2010; Liu & Rabinovich 2010; Sehrawat *et al.* 2010). Also, lectins have shown to play an essential role in host defence against infections caused by different types of pathogens (Jack & Turner 2003). All the bacterial species used to challenge fish analysed in this review showed transcriptional regulation of host lectins, followed by viral (11 out of 13 = 84.6%) and eukaryotic pathogen (6 out of 8 = 75%) types. The present work reaffirms the undeniable importance of lectins in innate immune responses against infections, standing out as the second group with more DEGs in all the studies evaluated.

Cytokines

Chemokine and receptors (CC and CXC). Chemokines or chemoattractant cytokines constitute a protein family regulating immune cell migration under both inflammatory and normal physiological conditions (Bird & Tafalla 2015). During infections, chemokines possess a decisive role in promoting leucocyte mobilization and regulating the first steps of both innate and acquired immune responses (Esche, Stellato & Beck 2005). The chemokine superfamily consists of many chemokine ligands and receptors, with the ligands being classified into four groups based on the position of the first two cysteine residues (CC, CXC, CX3C and XC). On the other hand, chemokine receptors are formed by seven transmembrane helixes connected by intra- and extracellular loops (Zlotnik & Yoshie 2000).

Since 1998, when the first teleost fish chemokine gene was reported (Dixon *et al.* 1998), many fish chemokine sequences have been identified. The repertoire of chemokines is more extensive in fish than in mammals, being known to evolve faster than other immune genes (Peatman & Liu 2007), which makes these molecules highly divergent in the different teleost species. Thus, the function and regulation of many fish chemokines are still unknown.

In the present work, 69.5% of all the studies reviewed showed DEGs encoding proteins of the chemokine system. In contrast, 92.3% of the fish species fish and 86.8% of the pathogens analysed in this review are linked with some DEG encoding for chemokines. Additionally, the proportion of up- and down-regulated genes related to chemokines were significantly enriched concerning the complete proportion of up- and down-regulated DEGs of this review.

From the 971 chemokine DEGs, most corresponded to chemokine ligands (68.4%), from which 488 matched up

to CC chemokines and only 176 to CXC. This differential regulation bias could be because CC is the most abundant chemokine family ligand in fish genomes (Nomiyama, Osada & Yoshie 2013). Finally, when investigating the gene expression profiles of chemokine ligands and receptors, most studies were over-represented in all the types of pathogens analysed in this systematic review (94.1% of the bacterial species, 84.6% of the types of viruses and 75% of the fungi and parasites), highlighting the relevance of this family of proteins during the infection in teleosts.

Interferon and related proteins. Interferons (IFNs) constitute a family of cytokines secreted by host cells that signal in response to the presence of pathogens to activate and regulate innate and acquired immune mechanisms around and in the infected cell (Zhang 2017; Ferreira *et al.* 2018). In teleosts, IFN types I and II are present. While fish type I IFN has a remarkable effect over bacterial and viral infections either positively or negatively, fish type II IFN prompts the response from the immune system against pathogens by inducing gene expression of IFN-stimulated genes (Zou & Secombes 2011; Boudinot *et al.* 2016). Functional studies of IFNs in teleosts are abundant. For instance, Chang and co-workers injected pre-smolt salmons with an antigen of infectious salmon anaemia virus and with a plasmid encoding Atlantic salmon type I IFN, resulting in a decrease in mortality in the infected fish (Chang, Sun & Robertsen 2015).

In the present work, 67.2% of all the studies reviewed showed DEGs encoding IFNs. All thirteen fish species and 86.8% of the pathogens analysed in this review are related to some DEG that encodes for IFNs. Also, the proportion of up- and down-regulated genes related to IFNs were significantly enriched concerning the complete proportion of up-and down-regulated DEGs of this review. Protective activity against viruses by the IFNs has been recognized as one of the main antiviral mechanisms in teleosts (Valero *et al.* 2015). All the viral infections analysed in the present work showed transcriptional regulation of IFNs in the host, higher than with bacteria (14 out of 17, 82.4%) and fungi and parasites (6 out of 8, 75%). However, for bacterial infections it has been described that *Paralichthys olivaceus* injected with *Edwardsiella tarda* had an increase of 60% in the mortality when type II IFN was added to the injection (Pereiro, Figueras & Novoa 2019), showing that interferons have a behaviour that depends on the pathogen that infects. It has also been described that IFN-inducible Mx proteins, together with their antiviral function, support antiparasitic defence against *Ichthyophthirius multifiliis* (Saleh *et al.* 2019). Remarkably, the gene that encodes for one IFN-inducible protein (IFN-inducible protein 44) was the most represented among the IFN differentially expressed genes of this review (n = 1396). According to the role of IFNs in all vertebrate immune responses to infections, this systematic review endorses the importance of this family of proteins in infective processes in teleosts.

Interleukin and related proteins. Interleukins (ILs) are a family of secreted proteins that belong to cytokines, involved in the modulation of immune systems in an autocrine or paracrine manner (Wang *et al.* 2011). They are synthesized by leucocytes, monocytes, macrophages and endothelial cells, and bind to their specific receptor to perform an essential role in the communication between leucocytes (Abbas, Lichtman & Pillai 2014). Interleukins can induce an inflammatory and anti-inflammatory response, activation and differentiation of cells from the immune system; besides, they influence proliferation, maturation, migration and adhesion, modulating responses to infection (Choy Kok *et al.* 2003).

Recently, Zou et al. (Zou & Secombes 2016) described ILs in teleosts. They confirmed that virtually all the ILs found in fish have their orthologous in mammals except for the novel IL-1 family member (nIL-1Fm), who would act as an antagonist of IL-1β (Zou & Secombes 2016). In trout, it has been demonstrated that IL-22 enhances fish survival after infection with *Yersinia ruckeri* by up-regulating the expression of antimicrobial peptides (Monte *et al.* 2011), while IL-1β influences the migration of phagocytes into the site of infection (Hong *et al.* 2003).

In the present work, 60.6% of all the studies reviewed showed DEGs encoding proteins of ILs. Eleven out of thirteen species of fish (84.6%) and 68.4% (26 out of 38) of the pathogens analysed in this review are associated with some DEG that encodes for proteins of ILs. Also, the proportions of up- and down-regulated genes related to ILs were significantly enriched for the complete proportion of up-and down-regulated DEGs of this review.

Defensive activity against viruses and bacteria by the ILs has been recognized as one of the main antivirals and bactericidal mechanisms in teleosts (Huising *et al.* 2005; Ribeiro *et al.* 2010). In fact, of all the viral and bacterial infections analysed in the present work, 10 out of 13 (76.9%) species of viruses and 12 out of 17 (70.6%) species of bacteria showed transcriptional regulation of ILs in the host. In contrast, studies in fungi and parasites infections showed only 50% of transcriptional regulation (4 out of 8). Regarding fish viral infections, crucian carp infected with Cyprinid herpesvirus 2 enhances the expression of IL-11 (Podok *et al.* 2014). Also, rainbow trout increased significantly the expression of nIL-1Fm against infection with *Yersinia ruckeri* (Wang *et al.* 2009), the same as in infection of parasites, when gilthead sea bream infected with *Enteromyxum* spp. increased the expression of IL-1 ten days after the challenge began (Gómez, Bartholomew & Sunyer 2014). Parasites and virus pathogens, IL-1 (or fish IL-1,

nIL-1Fm), seem to be important in the response of the immune system. Remarkably, the gene that encodes to IL-1 protein was the most represented among the IL DEGs ($n = 131$).

The ILs family represents a critical modulator of the immune system on teleost fish that helps to combat all kinds of infections, a response that is also visible in this systematic review where a large percentage of the studies display gene expression changes in genes related to ILs.

Tumour necrosis factor and related proteins. Tumour necrosis factors (TNFs) are pleiotropic cytokines involved in both early inflammatory and acquired immune responses, which modulate processes such as haematopoiesis and antibody production and are expressed by activated macrophages and lymphocytes (MacEwan 2002; Rahman & McFadden 2006). There are specific cell surface receptors for these cytokines, named TNF receptors (TNFr), a superfamily of transmembrane proteins characterized by their repeated cysteine-rich extracellular sequence homology and by their binding capacity to more than one ligand with specific high affinity (Locksley, Killeen & Lenardo 2001; MacEwan 2002). Signalling of the TNFr can result in two different pathways, the promotion of inflammation and/or the induction of apoptosis (Benedict 2003). In the present work, 56.8% of all the studies reviewed showed DEGs encoding proteins of TNF and related proteins. Eleven out of thirteen species of fish (84.6%) and 71.1% (27 out of 38) of the pathogens analysed are linked with some DEG that encodes for a TNF or related protein.

In teleosts, two genes have been found to encode TNF-α in a variety of species. Like in other species, in fish TNF/TNFr have essential roles in inflammation, antiviral immunity and the immune response against general pathogens (Zou *et al.* 2003; Kono *et al.* 2006; Glenney & Wiens 2007). There are studies where the knockdown of a TNFr in zebrafish increases the susceptibility to *Mycobacterium marinum* infection (Clay, Volkman & Ramakrishnan 2008; Zou & Secombes 2016). In contrast, the knockdown of TNF-α and TNF-β leads to a reduction in the liver size, together with the association of TNF-α with the inflammatory bowel disease onset (Qi *et al.* 2010; Marjoram *et al.* 2015; Zou & Secombes 2016).

Nevertheless, most function-related information about TNF and related proteins comes from transcriptomic approaches and *in vitro* assays with recombinant proteins (Buhrmann *et al.* 2017; Hong *et al.* 2019). In this aspect, transcripts encoding TNF are highly expressed in lymphoid and haematopoietic tissues such as head kidney, spleen, and also in the epithelium of the intestine (Kono *et al.* 2006). Upon cell–pathogen interaction, the TNF genes tend to get up-regulated (Li & Zhang 2016; Morick & Saragovi 2017). The proportion of up-regulated genes related to TNF were significantly enriched for the complete proportions of up-regulated and down-regulated DEGs of this review.

Of all the bacterial infections analysed in the present work, 15 out of 17 (88.2%) species showed transcriptional regulation of TNF in the host, higher than the types of viruses (9 out of 13; 69.2%) and fungi and parasites (3 out of 8; 37.5%) engaged in infections. The genes that encode to TNFrs were the most represented among the TNF DEGs ($n = 408$), probably because the activation of TNFr induces all the immune responses to infections.

Major histocompatibility complex

Major histocompatibility complex (MHC) proteins are fundamental components of the vertebrate adaptive immune system in the recognition and presentation of invading pathogens. MHC *loci* are subdivided into classical and non-classical types. The first ones have a function in the presentation of antigens to T-cell receptors, showing high polymorphism along with broad expression domains; and the latter ones have a function not been fully characterized with low variability and tissue-specific expression (Wilson 2017).

In teleosts, MHC knowledge is primarily obtained from analyses done in species with sequenced genomes (Grimholt 2016). MHC-I is subdivided into five significant lineages (U, Z, S, L and P) based on sequence divergence and tissue expression patterns, and MHC-II is subdivided into three significant lineages (DA, DB and DE) (Wilson 2017). While MHC-II is considered a critical component of vertebrate adaptive immunity and memory, some fish species lack the MHC-II/CD4 pathway. For instance, Atlantic cod (*Gadus morhua*) has lost the genes associated with MHC-II, compensating the loss by expanding their MHC-I genes (Star *et al.* 2011). The peptide-binding ability of MHC molecules varies due to the composition of the peptide-binding residues, having high or low affinity to increase or lower their susceptibility against pathogens (Potts & Slev 1995). For these reasons, MHC molecules are frequently used as candidate genes to evaluate disease resistance traits in fish (Ozaki *et al.* 2001; Kjøglum *et al.* 2006; Munang'andu, Galindo-Villegas & David 2018).

Studies on MHC and disease resistance in teleosts are related to a quantitative trait *locus* with significant allelic variation in classical MHC-I, which do not indicate differences in disease resistance, but possibly influence the function of MHC-II and non-classical MHC-I (Yamaguchi & Dijkstra 2019). For example, MHC-II polymorphisms have shown disease resistance/susceptibility in Nile tilapia (*Oreochromis niloticus*) against *Streptococcus agalactiae* and *Aeromonas hydrophilla* (Gao *et al.* 2019; El-Magd *et al.* 2019). We could also observe that all agents analysed in this study induce transcriptional regulation of MHC genes in

high proportions: 100% (8 out of 8) of the species of fungi and parasites, 76.5% (13 out of 17) of the bacterial species and 84.6% (11 out of 13) of the viruses.

More than half (57.1%) of all the studies reviewed in this work showed DEGs associated with MHC genes with a significant representation of MHC-I genes (54.7%) over MHC-II genes (45.3%), probably due to the ubiquitous expression of MHC-I molecules (Dijkstra et al. 2003). Eleven out of thirteen species of fish (84.6%) and 84.2% (32 out of 38) of the pathogens are related to some DEGs that encode for MHC proteins. Moreover, the proportions of up- and down-regulated genes related to MHC were significantly enriched regarding the complete proportion of up- and down-regulated DEGs analysed in this review, respectively.

In sum, MHC proteins are essential proteins in establishing the adaptive immune response and the resulting disease resistance in natural infections or vaccine prophylaxis. The high percentage of pathogenic agents that induce MHC DEGs in this study shows their important role in host–pathogen interactions.

Immunoglobulins

Immunoglobulins (Ig) are the key effector molecules of the adaptive humoral immune response. Ig binds to microorganisms to facilitate their killing and clearance by phagocytes, a process called opsonization, and by this preventing pathogen entry. Also, they can interact with the innate and adaptive immune system driving the clearance of bacteria, virus, fungi and parasites, can form immune complexes, clear toxins, activate the complement system, eliminate infected cells, increase antigen presentation and regulate inflammation (Lu *et al*. 2018). Nevertheless, many bacteria elude the effect of Ig by interfering with opsonization through the expression of surface proteins that bind Ig outside the antigen recognition region (Mintz & Fives-Taylor 1994; Sandt & Hill 2001; Nordenfelt *et al*. 2012; Chi *et al*. 2018), neutralizing this host protective mechanism.

In the present work, 54.4% of all the studies reviewed showed DEGs encoding Ig or Ig receptors. Eleven out of thirteen species of fish (84.6%) and 68.4% (26 out of 38) of the pathogens analysed in this review are associated with some DEG. Besides, the proportions of up- and down-regulated genes related to Ig were significantly enriched concerning the complete proportion of up- and down-regulated DEGs of this review.

In the last two decades, many teleost Ig genes have been identified by *in silico* data mining from the enormous gene and EST databases of many fish species (Hikima, Jung & Aoki 2011). In teleosts, three different Ig heavy-chain isotypes have been identified: IgM, IgD and IgT/IgZ (teleosts/zebrafish). IgM is the most prevalent Ig in plasma (Flajnik 2002; Flajnik & Kasahara 2010) and can be expressed on the surface of B cells or secreted as a tetrameric antibody in serum or mucus (Mashoof & Criscitiello 2016). Their effector functions are complement system activation, agglutination for phagocytosis and cellular cytotoxicity (Kaattari *et al*. 1998). The role of IgD is not thoroughly described, but it is proposed to be the induction of antimicrobial, opsonizing, pro-inflammatory and B-cell-activating factors on basophils (Chen *et al*. 2009). IgT/IgZ are Ig exclusive of teleosts (Flajnik & Kasahara 2010). They are recognized as dedicated mucosal Ig isotypes presented in mucous as tetramers (Zhang *et al*. 2010; Mashoof *et al*. 2014). In this review, IgT/IgZ were differentially expressed in *Salmo salar* and *Danio rerio*, respectively, and IgM was differentially expressed in 61.5% of fish species studied (8 out of 13). These Ig expressions occurred in different organs and were induced by viral, bacterial and parasite agents.

In the present work, 10 out of 17 bacterial species (58.7%) showed transcriptional regulation of Ig in the host, lower than fungi and parasites (7 out of 8, 87.5%) and viruses (9 out of 13, 69.2%). Ig has a vital role in response to infection, and this review supports it. Nevertheless, Ig DEGs were not strongly represented given the percentages of studies or pathogens in this review when compared with other proteins, probably because since they are elements of the adaptive immune response, their transcriptional regulation will depend on later sampling or antigen re-exposition.

Protein degradation processes

Protein turnover (degradation of intracellular proteins)

The ubiquitin–proteasome system. The ubiquitin–proteasome system (UPS) is the main intracellular pathway for degradation of proteins that are misfolded or damaged, having a critical role in the regulation of the cell cycle, apoptosis and immune responses through the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ƙB) and antigens of major histocompatibility complex I (Thibaudeau & Smith 2019). Proteins that are selected for degradation are marked with a small protein called ubiquitin by a covalent union mediated by three enzymes. The first one is E1 (ubiquitin-activating enzyme) that uses ATP to bind the ubiquitin protein that then passes to E2 (ubiquitin carrier) to make a complex with E3 (ubiquitin–protein ligase) that selects the target protein for degradation. This process repeats until a polyubiquitin chain is made. Then, the proteins with a polyubiquitin chain are recognized by the proteasome, a multiprotein complex whose primary role is the degradation of proteins. Some viruses can manipulate the UPS to improve their life cycle by recruiting the cellular E3 and by this selecting antiviral proteins for degradation. In the present work, 55.6% of all the studies reviewed showed DEGs encoding

proteins of the UPS. All thirteen species of fish (100%) and 78.9% (30 out of 38) of the pathogens analysed in this review are related to DEGs that encode for proteins of the UPS. Also, the proportions of up- and down-regulated genes related to UPS were significantly enriched for the complete proportion of up- and down-regulated DEGs of this review.

In teleosts, the UPS is present by the expression of ubiquitin- and proteasome-related proteins that are highly conserved (Tokumoto 1998; Tacchi *et al.* 2010). Interestingly, the UPS in mammals is responsible for muscle protein degradation during starvation, while in fish, UPS is down-regulated under this same condition, indicating the existence of another pathway for this process in teleosts (Martin *et al.* 2002).

In this review, of all the bacterial infections analysed, 14 out of 17 (82.4%) species of bacteria showed transcriptional regulation of the UPS components, a number that was higher than viral infections (10 out of 13, 76.9%) and fungi and parasites (6 out of 9, 75%). Viral infections of *Salmo salar* with salmonid alphavirus (SAV) have shown that E3 is highly expressed when the peak of the infection is in course (Heidari *et al.* 2015). Also, in bacterial infections, the UPS is a candidate regulator of fish innate immune signalling pathways; for instance, *Danio* rerio infected with *Aeromonas hydrophila* highly expressed btr20, an E3 ubiquitin ligase involved in the regulation of the expression of the NF-ƙB for the activation of the innate immune response (Zhang *et al.* 2015). In this work, the gene that encodes the proteasome subunit beta protein was the most represented among the UPS DEGs (n = 439) and is part of the catalytic activity of the proteasome.

Cathepsins. Cathepsins (CTS), initially described as lysosomal proteolytic enzymes, include cysteine proteases, serine proteases and aspartic proteases (Chwieralski, Welte & Bühling 2006; Turk *et al.* 2012). The release of CTS from the lysosomes into the cytoplasm is a precondition for their participation in apoptosis regulation, a crucial element of immunoregulation and differentiation (Chwieralski, Welte & Bühling 2006). In teleosts, CTS have been described as relevant factors in immune activity, such as their hydrolytic properties and increased expression during bacterial and viral infections (Kim *et al.* 2017). For instance, an up-regulated expression of CTS B was observed in Nile tilapia during infection with *Streptococcus agalactiae*, which was validated through the analysis of the hydrolytic activity of the purified CTS B recombinant protein (Liang *et al.* 2018). Similarly, CTS B had an increase in tongue sole (*Cynoglossus semilaevis*) expression when exposed to infection with *Vibrio anguillarum*, and proteolytic activity was also demonstrated in the recombinant protein CTS B (Chen & Sun 2012). Moreover, in crayfish, an effect on the Toll pathway (immune response) was observed through RNA interference of CTS L (Dai *et al.* 2017).

In the present work, 49.8% of all the studies reviewed showed DEGs encoding proteins of CTS. Ten out of thirteen species of fish (76.9%) and 76.3% of the pathogens analysed in this review are linked with some DEGs that encode for CTS. Besides, the proportions of up- and down-regulated genes related to CTS were significantly enriched concerning the complete proportion of up- and down-regulated DEGs of this review. Also, for all the bacterial infections analysed in the present work, 14 out of 17 (82.4%) showed transcriptional regulation of CTS components in the host, higher than with viruses (10 out of 13, 76.9%) and fungi and parasites (5 out of 8, 62.50%). The gene that encodes for cathepsin L (CTS L) protein was the most represented among the CTS differentially expressed genes (n = 110). Robust changes in the expression of CTS in response to pathogens affirm the importance of this group of proteolytic enzymes during infections in teleost.

Degradation and signalling of extracellular proteins

Matrix metalloproteinases and inhibitors. Matrix metalloproteinases (MMPs) are a family of zinc endopeptidases that collectively can cleave most of the extracellular matrix constituents (Birkedal-Hansen *et al.* 1993). The tissue inhibitors of metalloproteinases (TIMPs) are naturally occurring proteins that specifically inhibit MMPs to maintain the balance between matrix destruction and formation (Wojtowicz-Praga, Dickson & Hawkins 1997). MMPs play an essential role in the host's immune response through the recruitment of effector cells through the promotion of inflammatory activity; however, excessive inflammation following infection may cause tissue damage that favours the spread of the pathogen. It has been reported that infections with bacterial and viral pathogens have been associated with increases in MMPs expression in affected tissues (Elkington, O'Kane & Friedland 2005; Chaves-Pozo *et al.* 2008). In the present work, 50.2% of all the studies reviewed showed DEGs encoding proteins of MMPs. Nine out of thirteen species of fish (69.2%) and 76.3% (29 out of 38) of the pathogens analysed in this review are related to some DEGs that encode for MMPs. Moreover, the proportions of up- and down-regulated MMP-related genes were significantly enriched with respect to the complete proportion of up- and down-regulated DEGs considered in this review.

In teleost's infections, the up-regulation of genes that express MMPs and TIMPs has been observed with diverse types of pathogenic agents (Zhang *et al.* 2003; Chadzinska *et al.* 2008; Shan *et al.* 2016). For instance, TIMP 2b in gilthead seabream (*Sparus aurata* L.) infected with *Vibrio anguillarum* was induced in competent immune organs in inflammatory stages. At the same time, TIMP 2a presented

a modest increase in liver and blood (Chadzinska *et al.* 2008). In MMP-9 knocked-down cells of *Danio rerio*, an effect on macrophage migration during infection with Listeria monocytogenes has been observed, showing the importance of MMP-9 for protection against bacterial pathogens (Zhang *et al.* 2003). Remarkably, MMP-9 was the most represented MMP in this work (n = 253). Also, of all the infections accomplished by bacterial species analysed in the present work, 15 out of 17 (88.2%) showed transcriptional regulation of MMP components in the host. In viruses and the group of fungi and parasites, the representation was lower (61.5% and 75%, respectively).

In this systematic review, we could observe that MMPs and their inhibitor genes were highly represented in the analysed studies, which is not surprising since many reports have shown that these genes are differentially expressed during fish infections (Zhang *et al.* 2003; Manicone & McGuire 2008; Xu *et al.* 2012; Shan *et al.* 2016).

Transport and metabolism of lipids

Apolipoproteins and related proteins

Apolipoproteins (APOs) are proteins that bind lipids to form lipoprotein particles. They transport lipids and hydrophobic vitamins in the blood, cerebrospinal fluid and lymph (Ren *et al.* 2019). APOs act as ligands for cell membrane receptors and enzyme cofactors (e.g. apolipoprotein CII and apolipoprotein AI) (Kastelein *et al.* 2008). Different APOs bind to lipids to form lipoprotein particles of different densities, which can be divided into several types: chylomicrons (CM), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL) and high-density lipoproteins (HDL) (Di Angelantonio *et al.* 2009).

APOs play a vital role in the transport of lipids in fish, and it has been demonstrated in teleosts that APOs possess antimicrobial activity and have crucial roles in innate immunity (Tian *et al.* 2019). Proteins and peptides such as APOs are essential in the primary defence of epithelial barriers in fish (Magor & Magor 2001). APOs A1 and A2 from the epidermis and epidermal mucus of the carp (*Cyprinus carpio* L.) have been characterized by immunohistochemistry and Western blot analysis. They have shown to be part of the innate immune system of teleost fish through bactericidal and/or bacteriostatic tests (Concha *et al.* 2004). Similar functions of APO A1 against bacteria have also been evidenced in rainbow trout (*Oncorhynchus mykiss*) (Concha *et al.* 2004) and orange-stained grouper (*Epinephelus coioides*) (Concha *et al.* 2004). It was also found that the protein profile of APO A1, APO A2 and APO-14kDa in mucus changed significantly in Atlantic salmon (*Salmo salar*) after infection with *Lepeophtheirus salmonis* and *Vibrio anguillarum* (Wei *et al.* 2015). In the channel catfish (*Ictalurus punctatus*), it was found that a series of APO genes, including APO A, were differentially expressed after infection by *Edwardsiella ictaluri* and *Flavobacterium columnare* (Easy & Ross 2009). These studies suggest that APO genes are potential immune modulators of fish, fulfilling a fundamental role in the innate immune response and the epithelial barrier. In the present work, 54.4% of all the studies reviewed showed DEGs encoding apolipoproteins. Ten out of thirteen species of fish (76.9%) and 71.1% (27 out of 38) of the pathogens analysed in this review are linked with some DEGs that encode for APOs. Also, we could observe similar representations of the bacterial and parasitic species used for the infections (76.5 and 75%, respectively), while viruses had a lower representation (8 out of 13 = 61.5%). Finally, we can highlight that genes that encode for the APO A family were the most prominent among the APO DEGs (n = 184), with the APO AI gene being the most prominent within this family (n = 75), suggesting a relevant role of this protein in the fish–pathogen interaction during the infection.

Housekeeping cellular functions and/or response to stress

Cytochromes and related proteins

Cytochromes are haem-containing proteins whose characteristic mode of action involves the transfer of a single electron between the Fe(II) and Fe(III) of the haem group. In vertebrates, they are classified based on the nature and mode of binding to the said group as cytochromes a, b and c, among others (Ichiye 2013; Chertkova *et al.* 2017; Ortega Ugalde *et al.* 2019). Cytochromes b and c are involved in the electron transport chain in the mitochondria, mainly the unidirectional electron flow along the chain, which is possible given the differential reduction potential (Chertkova *et al.* 2017). On the other hand, cytochromes P450, which are a group of monooxygenases, could be included in the cytochrome b class, according to Nomenclature Committee of the International Union of Biochemistry (NC-IUB), since both cytochromes are enzymes in which proto-haem is non-covalently attached to the protein (Nomenclature Committee of the International Union of Biochemistry (NC-IUB) 1991; Ortega Ugalde *et al.* 2019). Although the group of cytochromes a, b and c and the group of cytochromes P450 have different biological functions, both share the ability to binding haem and seem to be relevant in the response to pathogens, and hence were analysed together in this section. There are also related proteins such as the cytochrome reductases and oxidases that allow cytochrome REDOX functions (Ichiye 2013). In the present work, 54.4% of all the studies reviewed showed DEGs encoding cytochromes and related proteins. Twelve out of thirteen species of fish (92.3%) and 78.9% (30 out of 38) of the pathogens analysed in this review are associated

with some DEG that encodes for cytochromes and related proteins. Also, the proportions of up- and down-regulated genes related to cytochromes were significantly enriched with respect to the total up- and down-regulated DEGs of this review.

The function and structure of cytochromes a, b and c are highly conserved among species (Datta *et al.* 2018; Little *et al.* 2018). Since reactive oxygen species response is vital for host defence, the knockdown of cytochrome b-245 reduces the resistance of *Danio rerio* against *Salmonella* infections (Masud *et al.* 2019). In the case of cytochrome c, it is released to function as a pro-apoptotic factor by triggering the caspase cascade as a response to pathogen infection (Datta *et al.* 2018). Moreover, for viral diseases, it has been described that heat-shock protein 27 can bind to cytochrome c and induce apoptosis after red-spotted grouper nervous necrosis virus infection (Le *et al.* 2017). Interestingly, regarding this protein, the genes that encode cytochrome c were the most represented among the cytochromes DEGs ($n = 531$) in the analysed studies. On the other hand, cytochrome P450s (CYPs) encode one of the most diverse enzyme superfamilies in nature. They catalyse oxidative reactions of endogenous molecules and exogenous chemicals and have shown association with the transcriptional response of catfish following bacterial infections (Zhang *et al.* 2014). Interestingly, of all the infections analysed in the present work, 14 out of 17 species of bacteria (82.4%) showed transcriptional regulation of cytochromes and related proteins in the host, higher than viruses (9 out of 13, 69.2%) and lower than fungi and parasites (7 out of 8, 87.5%). Functional analyses of these proteins are still needed in teleosts to get better insights into their involvement during infectious processes.

Heat-shock proteins

Heat-shock proteins (HSPs) are among the most immunogenic antigens found in nature and have been shown to play roles in inflammation, acting as damage-associated molecular patterns (DAMPs), in antigen presentation and also in intercellular signalling (Kaul & Thippeswamy 2011; Land 2015). HSPs are expressed constitutively, and their synthesis is increased in the heat-shock response, a fundamental cellular defence mechanism that protects prokaryotic or eukaryotic cells from various insults during periods of stress caused by infection, inflammation, extreme temperatures, radiation or similar events (Zügel & Kaufmann 1999).

HSPs from hosts and pathogens can induce proinflammatory molecules as a danger or pathogen signal respectively, and also exert roles over adaptive immunity through the transport of antigenic peptides into antigen-presenting cells (Stewart & Young 2004). Also, HSP expression in intracellular pathogens is essential for their survival in host cells such as macrophages (Fields *et al.* 1986; Buchmeier & Heffron 1990; Hombach *et al.* 2014).

Studies regarding HSPs in fish are scarce, including slight information of the functional significance of the multiple somewhat divergent copies of HSPs in fish genomes (Demeke & Tassew 2016). For most fish species, HSP studies have been limited to *in vitro* examination with more emphasis on abiotic than biotic stresses.

In the present work, 52.1% of all the studies reviewed showed DEGs encoding HSPs. Eleven out of thirteen species of fish (84.6%) and 71.1% (27 out of 38) of pathogens analysed in this review are related to some DEGs that encode for HSPs. Additionally, the proportion of up-regulated and down-regulated genes related to HSPs were significantly enriched with respect to the complete proportion of up-regulated and down-regulated DEGs of this review.

Most studied HSPs can be grouped in three main categories based on their molecular weight: members of HSP70 (68–73 kDa), which are the most widely studied HSPs in aquatic organisms and are principally involved in the processing and presentation of antigens and maturation of dendritic cells in mammals (DeNagel & Pierce 1992; Srivastava 2002); HSP90 (85–90 kDa), whose members are active in supporting various components of the cytoskeleton and steroid hormone receptors (Csermely *et al.* 1998); and low molecular weight HSPs that have diverse species-specific functions and are induced only during stress (Ciocca *et al.* 1993). Among HSP DEGs, the genes that encode for HSP70 were the most represented ($n = 390$, 32.2%), followed by HSP90 ($n = 214$, 17.7%) and only 75 DEGs (6.2%) encoded for low molecular weight HSPs. Thus, there is about 40% of less explored HSP-related proteins that are yet highly differentially expressed, indicating that bigger attention needs to be focused on them at least when studying fish infections. Also, among the bacterial infections, DEGs associated with HSPs contemplated 82.4% of the species analysed in this work; at the same time, the group formed by fungi and parasites and viruses enclosed fewer types, only 62.5 and 61.5%, respectively. Thus, despite the scarce information regarding studies of fish HSPs, this systematic review showed that these proteins seem to be quite relevant during teleost's infections; hence, more studies regarding HSPs in teleost–pathogen interactions are needed to fill up the knowledge gap.

Regulation and dynamics of cellular processes

Actin and related proteins

The cytoskeleton is a fundamental and complex matrix of protein fibres that mainly gives shape and mechanical resistance to the cells (Fuchs & Cleveland 1998). In eukaryotes, the cytoskeleton is composed of three types of protein filaments: microtubules, intermediate filaments and

microfilaments. Microtubules are dynamic structures made of tubulin proteins that exert essential functions maintaining cellular structure, providing intracellular transport and helping in the formation of cilia and flagella, among others (Desai & Mitchison 1997). Intermediate filaments can be formed of different types of proteins (mainly keratin) and have the role of organizing the internal three-dimensional structure of the cell (Markl & Schechter 1998; Infante *et al.* 2007), while microfilaments are filaments formed by linked monomers of actin. Microfilaments have many vital roles in the cell, including giving the cell shape and structure and participating in organelle movement, cell signalling, cell motility, cell division and serving as tracks for the movement of the motor protein myosin, among others (Gunning *et al.* 2015). Additionally, reconfigurations and reorganizations of the host actin have been observed during cellular infections by pathogens that are unable to survive and replicate outside host cells, such as viruses and strictly intracellular bacteria or parasites (Elliott *et al.* 2001; Radtke *et al.* 2006; Bhavsar, Guttman & Finlay 2007; Frénal & Soldati-Favre 2009; Taylor, Koyuncu & Enquist 2011). These pathogens are capable of extensively manipulating the host actin in order to facilitate host invasion, maintain their replicative niche and egress from host epithelial cells (Caven & Carabeo 2020).

As for teleosts, these changes in actin dynamics have been detected in different cell lines upon infection with infectious pancreatic necrosis virus (Levican *et al.* 2017; Levicán-Asenjo *et al.* 2019) and also during the infection process of the intracellular bacterium *Piscirickettsia salmonis* in fish macrophages (Ramírez, Ǵomez & Marshall 2015). This fact could explain why in the present work, at least 52.9% of all the studies reviewed showed DEGs encoding for actin or actin-related proteins. Ten out of thirteen species of fish (76.9%) and 65.8% (25 out of 38) of the pathogens analysed in this review are linked with some DEGs encoding for this group of proteins.

Regarding the different pathogens that transcriptionally are associated with actin regulation, 11 species involved bacterial infections out of the total 17 species evaluated in this work (64.7%), together with 8 out of the 13 types of viruses (61.5%) and, interestingly, 6 out of the total eight types of fungi and parasites (75%), achieving the former the highest percentage. Remarkably, differentially expressed actin-related genes have been detected in studies involving the ectoparasites *Lepeophtheirus salmonis*, *Cryptocaryon irritans*, *Neoparamoeba perurans* and *Neoheterobothrium hirame*. Likewise, differential expression of actin-related teleost genes as a result of bacterial infections was associated with extracellular and/or intracellular pathogens, suggesting that this regulation could be part of the response of fish to infection or the manipulation of the pathogen by secretion of effectors.

Intriguingly, and despite demonstrating that infections induce dramatic cytoskeletal reorganizations, actin transcripts are usually used as housekeeping in fish gene expression analyses (*e.g.* qPCR assays) (Verrier *et al.* 2012; Kumar, Abd-Elfattah & El-Matbouli 2015) (Table S2). Hence, according to the findings obtained in this systematic review, we recommend avoiding actin and cytoskeleton components as gene expression normalizers in studies of fish response to infections, independently whether the pathogen is intracellular or free-living. Collectively, these results indicate a crucial role of the cytoskeleton and its components in the fish–pathogen interaction during the infection.

Ras-related proteins

The Ras superfamily proteins, which are expressed in all animal cell lineages and organs, share a primary biochemical activity: binding to guanosine triphosphate (GTP) and hydrolysis (Walker *et al.* 1982). Members of the Ras superfamily are grouped into subfamilies depending on their function, among which the following five subfamilies stand out: subfamily of Ras proteins that are functional signal transducers (Chiu *et al.* 2002; Roy, Wyse & Hancock 2002); subfamily of Rho proteins that are collaborators and initiators of cell transformation and have participation in cell proliferation and cancer (Sahai & Marshall 2002); the subfamily of Rab and Ran proteins, which regulate vesicle formation, movement and fusion in proteins and traffic pathways (Pereira-Leal & Seabra 2001; Segev 2001); subfamily the protein ARF (and SARA) that are regulators of intracellular membrane and protein traffic, besides being in charge of the remodelling of the cytoskeleton (Randazzo *et al.* 2000); and the subfamily of the Gα proteins linked to the G proteins (Guanine nucleotide fixative) and the transduction of signals from the receptor.

Ras-related proteins in teleosts have been attributed importance in cell growth, proliferation, differentiation and organ development, as well as in pathogen–host relationship (Kumar, Abd-Elfattah & El-Matbouli 2015; Zhao 2017; Wei *et al.* 2019). For instance, H-Ras protein of the teleost Nile tilapia (*Oreochromis niloticus*) regulates its adaptive immune responses, since a decrease in this protein is associated with significant changes in the number of lymphocytes (Wei *et al.* 2019). In contrast, Rab1a3 protein of *Cyprinus carpio* induces the immune response against exogenous bacterial infections (Zhao 2017). It has also been shown that the expression of Rab-11b gene was significantly up-regulated in rainbow trout exposed with the parasite *Tetracapsuloides bryosalmonae* (Kumar, Abd-Elfattah & El-Matbouli 2015).

In the present work, at least 50.6% of all the studies reviewed showed DEGs encoding Ras-related proteins.

Eleven out of thirteen species of fish (84.6%) and 73.7% (28 out of 38) of the pathogens analysed in this review are associated with DEGs encoding for these proteins. In addition, the proportions of up-regulated genes of the Ras proteins are significantly enriched with respect to the complete proportion of up-regulated DEGs of this review, a condition that also happens for down-regulated DEGs.

As described above, Ras proteins are relevant in the fish–pathogen relationship. In fact, of all the bacterial infections analysed in the present work, 14 species out of 17 (82.4%) showed transcriptional regulation of Ras genes, higher than the types of viruses (10 out of 13; 76.9%) and eukaryotic pathogens (4 out of 8; 50%). In this study, the gene family encoding for Rab was the most represented among the differentially expressed Ras-related proteins ($n = 332$), suggesting that membrane trafficking, vesicle formation and membrane fusion are relevant processes for the response to infection in teleosts.

This systematic review allowed the generation of the first catalogue of differentially expressed genes in response to infection in teleost fish based on information obtained from microarrays. With this method, it is possible to estimate expression changes in miles of genes simultaneously. Hence, this catalogue allows the 'rescue' of genes whose transcriptional behaviour was not considered or discussed in their studies, but still form groups of shared genes among the different studies that allow considering their possible impact on the response of fish to infections. Additionally, this catalogue permits to know whether a gene of interest shows transcriptional changes in response to a specific pathogen, species of fish, development state, tissue and/or time of infection (*e.g.* early or late). Therefore, we consider that this catalogue can be widely used as a database for the compression of the impact of individual genes and biological processes in response to infection.

Remarkably, the biological processes described were not chosen based on our knowledge a priori, but rather emerged from the classification of genes present in more than 50% of the studies evaluated. Since microarrays allow to characterize only the transcriptional layer, it is relevant that the most abundantly represented process was 'classical components of immune response to infection', because it allowed validating the efficacy of microarray technology and the clustering strategy to address the question. Furthermore, this approach permitted us to identify other genes and processes that showed surprising significant transcriptional outcomes that, with posterior analyses, may unravel unknown (and probably relevant) functions related to fish infections. For instance, in the regulation and dynamics of cellular processes, we could observe that actin and related proteins displayed differential expression patterns during infections with most of the agents evaluated in the studies. This finding is intriguing, considering that the transcript of actin is usually used as a housekeeping gene for quantitative PCR validations in many fish microarrays (Table S2).

Since different types of pathogens have evolved several strategies to evade antimicrobial through the reprogramming of host gene expression (Connolly & Fearnhead 2017; Villares, Berthelet & Weitzman 2020), we did not emphasize discussing the sense of DEG transcriptional changes (up- or down-regulation). This decision was made because the sense of change could be due to the active transcriptional response of the host or to the manipulation that the pathogen exerts on host regulatory machinery. For instance, Castro and co-workers (Castro *et al.* 2019) studied the transcriptional response of *Oncorhynchus mykiss* to the bacterium *Lactococcus garvieae* in spleen and head kidney. Interestingly, the gene encoding for the CK11 chemokine was consistently down-regulated in both tissues, even though CK11 was recently described as a teleost chemokine with a potent antimicrobial activity (Muñoz-Atienza *et al.* 2019). Because of this, we considered all DEGs only as transcriptionally regulated genes as other types of studies are necessary to define more finely the reasons for their transcriptional change.

One of the limitations of our approach to generate the catalogue of DEGs was that it only considered the genes that had an annotation (unknown DEGs were not considered). Also, we did not contemplate the value of fold change as a factor to evaluate since the different authors do not use the same statistical criteria, so we consider them incomparable. These aspects could be addressed by a statistical meta-analysis approach based on gene expression raw data. However, since the purpose of this study was to show an overview of this topic as vast as possible, we decided to include all studies even when the raw expression data were not reported. This strategy allowed us, for example, to include data of gene expression of *Paralichthys olivaceus* infected with lymphocystis disease virus 1 (LCDV) and *Neoheterobothrium hirame*, *Epinephelus coioides* infected with Singapore grouper iridovirus (SGIV) and *Gadus morhua* infected with Nervous necrosis virus (NNV), which could not be part of a meta-analysis since no raw data of these studies are available.

This systematic review summarizes the current knowledge of disease challenge studies, including viruses, bacteria, fungi and parasites, as agents in teleosts through published microarray data. This kind of studies is indispensable to better understand the fish response to infections, highlighting the components and mechanisms linked to immune response, as well as other not so expected mechanisms that only show a significant response when taking the data from all the articles together. Also, this type of analysis helps visualize gaps in the studies that may inspire future enlarged analysis in non-studied host or pathogen species.

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References

Abbas AK, Lichtman AHH, Pillai S (2014) *Cellular and Molecular Immunology*, 8th edn. Available at: https://www-clinicalkey-com.ep.fjernadgang.kb.dk/#!/browse/book/3-s2.0-C20130013230 Accessed: 8 February 2020.

Aleström P, Winther-Larsen HC (2016) Zebrafish offer aquaculture research their services. In: Simon, M (ed) *Genomics in Aquaculture*, pp. 165–194. Elsevier Inc. https://doi.org/10.1016/B978-0-12-801418-9.00007-X

Assefa A, Abunna F (2018) Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary Medicine International* **2018**: 1–10.

Benedict CA (2003) Viruses and the TNF-related cytokines, an evolving battle. *Cytokine & Growth Factor Reviews* **14**(3-4): 349–357.

Bhavsar AP, Guttman JA, Finlay BB (2007) Manipulation of host-cell pathways by bacterial pathogens. *Nature* **449**(7164): 827–834.

Bird S, Tafalla C (2015) Teleost chemokines and their receptors. *Biology* **4**(4): 756–784.

Birkedal-Hansen H, Moore WGI, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A *et al.* (1993) Matrix metalloproteinases: A review. *Critical Reviews in Oral Biology & Medicine* **4**(2): 197–250.

Boshra H, Li J, Sunyer JO (2006) Recent advances on the complement system of teleost fish. *Fish & Shellfish Immunology* **20**(2): 239–262.

Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K *et al.* (2010) Aquaculture: global status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**(1554): 2897–2912.

Boudinot P, Langevin C, Secombes C, Levraud J-P (2016) The peculiar characteristics of fish Type I interferons. *Viruses* **8**(11): 298.

Buchmeier NA, Heffron F (1990) Induction of Salmonella stress proteins upon infection of macrophages. *Science* **248**(4956): 730–732.

Buhrmann C, Popper B, Aggarwal BB, Shakibaei M (2017) Resveratrol downregulates inflammatory pathway activated by lymphotoxin α (TNF-β) in articular chondrocytes: Comparison with TNF-α. *PLoS One* **12**(11): e0186993.

Castro R, Coll J, Blanco MDM, Rodriguez-Bertos A, Jouneau L, Fernández-Garayzábal JF *et al.* (2019) Spleen and head kidney differential gene expression patterns in trout infected with Lactococcus garvieae correlate with spleen granulomas. *Veterinary Research* **50**(1): 32.

Caven L, Carabeo RA (2020) Pathogenic puppetry: Manipulation of the host actin cytoskeleton by Chlamydia trachomatis. *International Journal of Molecular Sciences* **21**(1): 90.

Chadzinska M, Baginski P, Kolaczkowska E, Savelkoul HFJ, Lidy Verburg-van Kemenade BM (2008) Expression profiles of matrix metalloproteinase 9 in teleost fish provide evidence for its active role in initiation and resolution of inflammation. *Immunology* **125**(4): 601–610.

Chang CJ, Sun B, Robertsen B (2015) Adjuvant activity of fish type I interferon shown in a virus DNA vaccination model. *Vaccine* **33**(21): 2442–2448.

Chaves-Pozo E, Castillo-Briceño P, García-Alcázar A, Meseguer J, Mulero V, García-Ayala A (2008) A role for matrix metalloproteinases in granulocyte infiltration and testicular remodelation in a seasonal breeding teleost. *Molecular Immunology* **45**(10): 2820–2830.

Chen L, Sun L (2012) Cathepsin B of Cynoglossus semilaevis: Identification, expression, and activity analysis. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* **161**(1): 54–59.

Chen K, Xu W, Wilson M, He B, Miller NW, Bengtén E *et al.* (2009) Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell–stimulating programs in basophils. *Nature Immunology* **10**(8): 889–898.

Chen J-N, López JA, Lavoué S, Miya M, Chen W-J (2014) Phylogeny of the Elopomorpha (Teleostei): Evidence from six nuclear and mitochondrial markers. *Molecular Phylogenetics and Evolution* **70**(1): 152–161.

Chertkova RV, Brazhe NA, Bryantseva TV, Nekrasov AN, Dolgikh DA, Yusipovich AI *et al.* (2017) New insight into the mechanism of mitochondrial cytochrome c function. *PLoS One* **12**(5): e0178280.

Chi Y, Huang Z, Chen Q, Xiong X, Chen K, Jin X *et al.* (2018) Loss of RUNX1 function results in B cell immunodeficiency but not T cell in adult Zebrafish. *Royal Society Open Science* **8**(7): 180043.

Chiu VK, Bivona T, Hach A, Sajous JB, Silletti J, Wiener H *et al.* (2002) Ras signalling on the endoplasmic reticulum and the Golgi. *Nature Cell Biology* **4**(5): 343–350.

Choy Kok C, Schwenger GT, Osmond RIW, Urwin DL, Sanderson CJ (2003) Interleukin-5. In: Angus, WT & Lotze, MT (eds) *The Cytokine Handbook*, pp. 263–279. Elsevier Inc. https://books.google.cl/books?id=orVuGH_nrSMC&lpg=PA263&ots=Qjbmhd9uy-&dq=The%20Cytokine%20Handbook%20Choy%202003&hl=es&pg=PA260#v=onepage&q=The%20Cytokine%20Handbook%20Choy%202003&f=false.

Chwieralski CE, Welte T, Bühling F (2006) Cathepsin-regulated apoptosis. *Apoptosis* **11**(2): 143–149.

Ciocca DR, Oesterreich S, Chamness GC, MCGuire Wl, Fuqua SAW (1993) Biological and Clinical Implications of Heat Shock Protein 27000 (Hsp27): a Review. *JNCI Journal of the National Cancer Institute* **85**(19): 1558–1570.

Clay H, Volkman HE, Ramakrishnan L (2008) Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity* **29**(2): 283–294.

Concha MI, Smith VJ, Castro K, Bastías A, Romero A, Amthauer RJ (2004) Apolipoproteins A-I and A-II are potentially

important effectors of innate immunity in the teleost fish Cyprinus carpio. *European Journal of Biochemistry* **271**(14): 2984–2990.

Connolly PF, Fearnhead HO (2017) Viral hijacking of host caspases: an emerging category of pathogen–host interactions. *Cell Death & Differentiation* **24**(8): 1401–1410.

Csermely P, Schnaider T, So″ti C, Prohászka Z, Nardai G (1998) The 90-kDa molecular chaperone family. *Pharmacology & Therapeutics* **79**(2): 129–168.

Dai L-S, Chu S-H, Yu X-M, Li Y-Y (2017) A role of cathepsin L gene in innate immune response of crayfish (*Procambarus clarkii*). *Fish & Shellfish Immunology* **71**: 246–254.

Datta D, Khatri P, Singh A, Saha DR, Verma G, Raman R *et al.* (2018) Mycobacterium fortuitum-induced ER-Mitochondrial calcium dynamics promotes calpain/caspase-12/caspase-9 mediated apoptosis in fish macrophages. *Cell Death Discovery* **4**(1): 1–13.

Demeke A, Tassew A (2016) Heat shock protein and their significance in fish health. *Research & Reviews: Journal of Veterinary Sciences* **2**(1), 1–10.

DeNagel DC, Pierce SK (1992) A case for chaperones in antigen processing. *Immunology Today* **13**(3): 86–89.

Desai A, Mitchison TJ (1997) Microtubule polymerization dynamics. *Annual Review of Cell and Developmental Biology* **13**(1): 83–117.

Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A *et al.* (2009) Major lipids, apolipoproteins, and risk of vascular disease. *JAMA - Journal of the American Medical Association* **302**(18): 1993–2000.

Dijkstra JM, Köllner B, Aoyagi K, Sawamoto Y, Kuroda A, Ototake M *et al.* (2003) The rainbow trout classical MHC class I molecule Onmy-UBA*501 is expressed in similar cell types as mammalian classical MHC class I molecules. *Fish & Shellfish Immunology* **14**(1): 1–23. https://doi.org/10.1006/fsim.2001.0407

Dixon B, Shum B, Adams EJ, Magor K, Hedrick RP, Muir DG *et al.* (1998) CK-1, a putative chemokine of rainbow trout (Oncorhynchus mykiss). *Immunological Reviews* **166**(1): 341–348.

den Dunnen J, Gringhuis SI, Geijtenbeek TBH (2010) Dusting the sugar fingerprint: C-type lectin signaling in adaptive immunity. *Immunology Letters* **128**(1): 12–16.

Easy RH, Ross NW (2009) Changes in Atlantic salmon (Salmo salar) epidermal mucus protein composition profiles following infection with sea lice (Lepeophtheirus salmonis). *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* **4**(3): 159–167.

Elkington PTG, O'Kane CM, Friedland JS (2005) The paradox of matrix metalloproteinases in infectious disease. *Clinical and Experimental Immunology. Wiley-Blackwell* **142**(1): 12–20.

Elliott DA, Coleman DJ, Lane MA, May RC, Machesky LM, Clark DP (2001) Cryptosporidium parvum infection requires host cell actin polymerization. *Infection and immunity* **69**(9): 5940–5942.

Ellis AE (2001) Innate host defense mechanisms of fish against viruses and bacteria. *Developmental and Comparative Immunology* **25**(8–9): 827–839.

El-Magd MA, El-Said KS, El-Semlawy AA, Tanekhy M, Afifi M, Mohamed TM (2019) Association of MHC IIA polymorphisms with disease resistance in Aeromonas hydrophila-challenged Nile tilapia. *Developmental & Comparative Immunology* **96**: 126–134.

Encinas P, Rodriguez-Milla MA, Novoa B, Estepa A, Figueras A, Coll J (2010) Zebrafish fin immune responses during high mortality infections with viral haemorrhagic septicemia rhabdovirus. A proteomic and transcriptomic approach. *BMC Genomics* **11**(1): 518.

Esche C, Stellato C, Beck LA (2005) Chemokines: Key Players in Innate and Adaptive Immunity. *Journal of Investigative Dermatology* **125**(4): 615–628.

Ferreira VL, Borba HHL, Bonetti AF, Leonart LP, Pontarolo R (2018) Cytokines and Interferons: Types and Functions. *Autoantibodies and Cytokines IntechOpen*. https://doi.org/10.5772/intechopen.74550.

Fields PI, Swanson RV, Haidaris CG, Heffron F (1986) Mutants of Salmonella typhimurium that cannot survive within the macrophage are avirulent. *Proceedings of the National Academy of Sciences* **83**(14): 5189–5193.

Flajnik MF (2002) Comparative analyses of immunoglobulin genes: surprises and portents. *Nature Reviews Immunology* **2**(9): 688–698.

Flajnik MF, Kasahara M (2010) Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nature Reviews Genetics* **11**(1): 47–59.

Frénal K, Soldati-Favre D (2009) Role of the Parasite and Host Cytoskeleton in Apicomplexa Parasitism. *Cell Host & Microbe* **5**(6): 602–611.

Fuchs E, Cleveland DW (1998) A structural scaffolding of intermediate filaments in health and disease. *Science* **279**(5350): 514–519.

Gao FY, Zhang D, Lu MX, Cao JM, Liu ZG, Ke Xl *et al.* (2019) MHC Class IIB gene polymorphisms associated with resistance/susceptibility to Streptococcus agalactiae in Nile tilapia Oreochromis niloticus. *Diseases of Aquatic Organisms* **133**(3): 253–261.

Glenney GW, Wiens GD (2007) Early diversification of the TNF superfamily in Teleosts: Genomic characterization and expression analysis. *The Journal of Immunology* **178**(12): 7955–7973.

Gómez D, Bartholomew J, Sunyer JO (2014) Biology and mucosal immunity to myxozoans. *Developmental & Comparative Immunology* **43**(2): 243–256.

Gracey AY, Troll JV, Somero GN (2001) Hypoxia-induced gene expression profiling in the euryoxic fish Gillichthys mirabilis. *Proceedings of the National Academy of Sciences of the United States of America* **98**(4): 1993–1998.

Grimholt U (2016) MHC and evolution in teleosts. *Biology* **5**(1): 6.

Gunning PW, Ghoshdastider U, Whitaker S, Popp D, Robinson RC (2015) The evolution of compositionally and functionally distinct actin filaments. *Journal of Cell Science* **128**(11): 2009–2019.

Harris PD, Soleng A, Bakke TA (1998) Killing of Gyrodactylus salaris (Platyhelminthes, Monogenea) mediated by host complement. *Parasitology* **117**(2): 137–143.

Heidari Z, Tinsley J, Bickerdike R, McLoughlin MF, Zou J, Martin SAM (2015) Antiviral and metabolic gene expression responses to viral infection in Atlantic salmon (Salmo salar). *Fish & Shellfish Immunology* **42**(2): 297–305.

Hikima J, Jung T-S, Aoki T (2011) Immunoglobulin genes and their transcriptional control in teleosts. *Developmental & Comparative Immunology* **35**(9): 924–936.

Holland MCH, Lambris JD (2002) The complement system in teleosts. *Fish & Shellfish Immunology* **12**(5): 399–420.

Hombach A, Ommen G, MacDonald A, Clos J (2014) A small heat shock protein is essential for thermotolerance and intracellular survival of Leishmania donovani. *Journal of Cell Science* **127**(21): 4762–4773.

Hong S, Peddie S, Campos-Pérez JJ, Zou J, Secombes CJ (2003) The effect of intraperitoneally administered recombinant IL-1beta on immune parameters and resistance to *Aeromonas salmonicida* in the rainbow trout (*Oncorhynchus mykiss*). *Developmental and Comparative Immunology* **27**(9): 801–12.

Hong S, Wang TY, Secombes CJ, Wang T (2019) Different origins of paralogues of salmonid TNR1 and TNFR2: Characterisation and expression analysis of four TNF receptor genes in rainbow trout *Oncorhynchus mykiss*. *Developmental & Comparative Immunology* **99**: 103403.

Huising MO, Kruiswijk CP, van Schijndel JE, Savelkoul HFJ, Flik G, Verburg-van Kemenade BML (2005) Multiple and highly divergent IL-11 genes in teleost fish. *Immunogenetics* **57**(6): 432–443.

Ichiye T (2013) In: Electron transfer proteins: Overview. Roberts, GCK (ed) *Encyclopedia of Biophysics*, pp. 614–621. Springer, Berlin, Heidelberg.

Infante C, Manchado M, Asensio E, Cañavate J (2007) Molecular characterization, gene expression and dependence on thyroid hormones of two type I keratin genes (sseKer1 and sseKer2) in the flatfish Senegalese sole (Solea senegalensis Kaup). *BMC Developmental Biology* **7**(1): 118.

Iwama GK, George K, Nakanishi T (1996) *Fish Physiology. Volume 15, Fish Immune System: Organism, Pathogen, and Environment*. Academic Press, Cambridge.

Jack DL, Turner MW (2003) Anti-microbial activities of mannose-binding lectin. *Biochemical Society Transactions* **31**(Pt 4): 753–757.

Jayaraman P, Sada-Ovalle I, Beladi S, Anderson AC, Dardalhon V, Hotta C *et al*. (2010) Tim3 binding to galectin-9 stimulates antimicrobial immunity. *Journal of Experimental Medicine* **207**(11): 2343–2354.

Kaattari S, Evans D, Klemer J, Kaattari S, Evans D, Kieraer J (1998) Varied redox forms of teleost IgM: an alternative to isotypic diversity? *Immunological Reviews* **166**(1): 133–142.

Kastelein JJP, van der Steeg WA, Holme I, Gaffney M, Cater NB, Barter P *et al*. (2008) Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. *Circulation* **117**(23): 3002–3009.

Kaul G, Thippeswamy H (2011) Role of heat shock proteins in diseases and their therapeutic potential. *Indian Journal of Microbiology* **51**(2): 124–31.

Kim J-W, Kong HJ, Nam B-H, Park JY, Kim D-H, Jeong J-M *et al*. (2017) The first report of cathepsin A gene in a teleost (rock bream, *Oplegnathus fasciatus*): An investigation of immune functions upon infection with several pathogens. *Developmental & Comparative Immunology* **76**: 262–267.

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the zebrafish. *Developmental Dynamics* **203**(3): 253–310.

Kjøglum S, Larsen S, Bakke HG, Grimholt U (2006) How specific MHC class I and class II combinations affect disease resistance against infectious salmon anaemia in Atlantic salmon (Salmo salar). *Fish & Shellfish Immunology* **21**(4): 431–441.

Kofia V, Isserlin R, Buchan AMJ, Bader GD (2015) Social Network: a Cytoscape app for visualizing co-authorship networks. *F1000Research* **4**: 481.

Kono T, Zou J, Bird S, Savan R, Sakai M, Secombes CJ (2006) Identification and expression analysis of lymphotoxin-beta like homologues in rainbow trout Oncorhynchus mykiss. *Molecular Immunology* **43**(9): 1390–1401.

Kumar G, Abd-Elfattah A, El-Matbouli M (2015) Identification of differentially expressed genes of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in response to Tetracapsuloides bryosalmonae (Myxozoa). *Parasitology Research* **114**(3): 929–939.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**(7): 1870–1874.

Land WG (2015) The role of damage-associated molecular patterns (DAMPs) in human diseases: Part II: DAMPs as diagnostics, prognostics and therapeutics in clinical medicine. *Sultan Qaboos University Medical Journal* **15**(2), 157–70.

Le Y, Jia P, Jin Y, Liu W, Jia K, Yi M (2017) The antiviral role of heat shock protein 27 against red spotted grouper nervous necrosis virus infection in sea perch. *Fish & Shellfish Immunology* **70**: 185–194.

Levican J, Miranda-Cárdenas C, Soto-Rifo R, Aguayo F, Gaggero A, León O (2017) Infectious pancreatic necrosis virus enters CHSE-214 cells via macropinocytosis. *Scientific Reports* **7**(1): 3068.

Levicán-Asenjo J, Soto-Rifo R, Aguayo F, Gaggero A, Leon O (2019) Salmon cells SHK-1 internalize infectious pancreatic necrosis virus by macropinocytosis. *Journal of Fish Diseases* **42**(7): 1035–1046.

Li MF, Zhang J (2016) CsTNF1, a teleost tumor necrosis factor that promotes antibacterial and antiviral immune defense in a manner that depends on the conserved receptor binding site. *Developmental & Comparative Immunology* **55**: 65–75.

Liang F-R, He H-S, Zhang C-W, Xu X-M, Zeng Z-P, Yuan J-P *et al*. (2018) Molecular cloning and functional characterization of cathepsin B from Nile tilapia (*Oreochromis niloticus*). *International Journal of Biological Macromolecules* **116**: 71–83.

Litman GW, Rast JP, Fugmann SD (2010) The origins of vertebrate adaptive immunity. *Nature Reviews Immunology* **10**(8): 543–553.

Little AG, Lau G, Mathers KE, Leary SC, Moyes CD (2018) Comparative biochemistry of cytochrome c oxidase in animals. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **224**: 170–184.

Liu F-T, Rabinovich GA (2010) Galectins: regulators of acute and chronic inflammation. *Annals of the New York Academy of Sciences* **1183**(1): 158–182.

Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF Receptor Superfamilies. *Cell* **104**(4): 487–501.

Løvoll M, Fischer U, Mathisen GS, Bøgwald J, Ototake M, Dalmo RA (2007) The C3 subtypes are differentially regulated after immunostimulation in rainbow trout, but head kidney macrophages do not contribute to C3 transcription. *Veterinary Immunology and Immunopathology* **117**(3-4): 284–295.

Lu LL, Suscovich TJ, Fortune SM, Alter G (2018) Beyond binding: antibody effector functions in infectious diseases. *Nature Reviews Immunology* **18**(1): 46–61.

MacEwan DJ (2002) TNF ligands and receptors - A matter of life and death. *British Journal of Pharmacology* **135**(4), 855–875.

Magor BG, Magor KE (2001) Evolution of effectors and receptors of innate immunity. *Developmental & Comparative Immunology* **25**(8–9): 651–682.

Manicone A, Mcguire J (2008) Matrix metalloproteinases as modulators of inflammation. *Seminars in Cell & Developmental Biology* **19**(1): 34–41.

Marjoram L, Alvers A, Deerhake ME, Bagwell J, Mankiewicz J, Cocchiaro JL *et al.* (2015) Epigenetic control of intestinal barrier function and inflammation in zebrafish. *Proceedings of the National Academy of Sciences* **112**(9): 2770–2775.

Markl J, Schechter N (1998) Fish intermediate filament proteins in structure, evolution, and function. *Sub-Cellular Biochemistry* **31**, 1–33.

Martin S, Blaney S, Bowman A, Houlihan D (2002) Ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Oncorhynchus mykiss*): Effect of food deprivation. *Pflugers Archiv European Journal of Physiology* **445**(2): 257–266.

Mashoof S, Criscitiello M (2016) Fish Immunoglobulins. *Biology* **5**(4): 45.

Mashoof S, Pohlenz C, Chen PL, Deiss TC, Gatlin D, Buentello A *et al.* (2014) Expressed IgH μ and τ transcripts share diversity segment in ranched *Thunnus orientalis*. *Developmental & Comparative Immunology* **3**(1): 76–86.

Masud S, Prajsnar TK, Torraca V, Lamers GEM, Benning M, Van Der Vaart M *et al.* (2019) Macrophages target Salmonella by Lc3-associated phagocytosis in a systemic infection model. *Autophagy* **15**(5), 796–812.

Meynard CN, Mouillot D, Mouquet N, Douzery EJ P (2012) A Phylogenetic Perspective on the Evolution of Mediterranean Teleost Fishes. *PLoS One* **7**(5): e36443.

Mintz KP, Fives-Taylor PM (1994) Identification of an immunoglobulin Fc receptor of Actinobacillus actinomycetemcomitans. *Infection and Immunity* **62**(10): 4500–4505.

Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med* **6**(7): e1000097.

Møller-Kristensen M, Hamblin MR, Thiel S, Jensenius JC, Takahashi K (2007) Burn injury reveals altered phenotype in Mannan-binding Lectin-deficient mice. *Journal of Investigative Dermatology* **127**(6): 1524–1531.

Monte MM, Zou J, Wang T, Carrington A, Secombes CJ (2011) Cloning, expression analysis and bioactivity studies of rainbow trout (*Oncorhynchus mykiss*) interleukin-22. *Cytokine* **55** (1): 62–73.

Morick D, Saragovi A (2017) Inhibition of nervous necrosis virus by ribavirin in a zebrafish larvae model. *Fish & Shellfish Immunology* **60**: 537–544.

Munang'andu H, Galindo-Villegas J, David L (2018) Teleosts genomics: progress and prospects in disease prevention and control. *International Journal of Molecular Sciences* **19**(4): 1083.

Muñoz-Atienza E, Aquilino C, Syahputra K, Al-Jubury A, Araújo C, Skov J *et al.* (2019) CK11, a Teleost Chemokine with a Potent Antimicrobial Activity. *The Journal of Immunology* **202** (3): 857–870.

Nakao M, Tsujikura M, Ichiki S, Vo TK, Somamoto T (2011) The complement system in teleost fish: Progress of post-homolog-hunting researches. *Developmental & Comparative Immunology* **35**(12): 1296–1308.

Nomenclature Committee of the International Union of Biochemistry (NC-IUB), 1998 (1991) Nomenclature Committee of the International Union of Biochemistry (NC-IUB). Nomenclature of electron-transfer proteins. Recommendations. *European Journal of Biochemistry* **200**(3), 599–611.

Nomiyama H, Osada N, Yoshie O (2013) Systematic classification of vertebrate chemokines based on conserved synteny and evolutionary history. *Genes to Cells* **18**(1): 1–16.

Nonaka M, Smith SL (2000) Complement system of bony and cartilaginous fish. *Fish and Shellfish Immunology* **10**(3): 215–228.

Nordenfelt P, Waldemarson S, Linder A, Mörgelin M, Karlsson C, Malmström J *et al.* (2012) Antibody orientation at bacterial surfaces is related to invasive infection. *The Journal of Experimental Medicine* **209**(13): 2367–2381.

Ortega Ugalde S, Boot M, Commandeur JNM, Jennings P, Bitter W, Vos JC (2019) Function, essentiality, and expression of cytochrome P450 enzymes and their cognate redox partners in Mycobacterium tuberculosis: are they drug targets? *Applied Microbiology and Biotechnology* **103**(9): 3597–3614.

Ozaki A, Sakamoto T, Khoo S, Nakamura K, Coimbra MR, Akutsu T *et al.* (2001) Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*). *Molecular Genetics and Genomics* **265**(1), 23–31.

Peatman E, Liu Z (2007) Evolution of CC chemokines in teleost fish: a case study in gene duplication and implications for immune diversity. *Immunogenetics* **59**(8): 613–623.

Pereira-Leal JB, Seabra MC (2001) Evolution of the Rab family of small GTP-binding proteins. *Journal of Molecular Biology* **313**(4): 889–901.

Pereiro P, Figueras A, Novoa B (2019) Insights into teleost interferon-gamma biology: An update. *Fish & Shellfish Immunology* **90**: 150–164.

Podok P, Xu L, Xu D, Lu L (2014) Different expression profiles of Interleukin 11 (IL-11), Intelectin (ITLN) and Purine nucleoside phosphorylase 5a (PNP 5a) in crucian carp (Carassius auratus gibelio) in response to Cyprinid herpesvirus 2 and Aeromonas hydrophila. *Fish & Shellfish Immunology* **38**(1): 65–73.

Potts WK, Slev PR (1995) Pathogen-based models favoring MHC genetic diversity. *Immunological Reviews* **143**(1): 181–197.

Qi F, Song J, Yang H, Gao W, Liu N-A, Zhang B *et al.* (2010) Mmp23b promotes liver development and hepatocyte proliferation through the tumor necrosis factor pathway in zebrafish. *Hepatology* **52**(6): 2158–2166.

Qian X, Ba Y, Zhuang Q, Zhong G (2014) RNA-Seq technology and its application in fish transcriptomics. *OMICS: A Journal of Integrative Biology* **18**(2): 98–110.

Radtke K, Dohner K, Sodeik B (2006) Viral interactions with the cytoskeleton: a hitchhiker's guide to the cell. *Cellular Microbiology* **8**(3): 387–400.

Rahman MM, McFadden G (2006) Modulation of tumor necrosis factor by microbial pathogens. *PLoS Path* **2**(2): 66–77.

Ramírez R, Ǵomez FA, Marshall SH (2015) The infection process of Piscirickettsia salmonis in fish macrophages is dependent upon interaction with host-cell clathrin and actin. *FEMS Microbiology Letters* **362**(1): 1–8.

Randazzo PA, Nie Z, Miura K, Hsu VW (2000) Molecular aspects of the cellular activities of ADP-ribosylation factors. *Science Signaling* **2000**(59): re1.

Ren L, Yi J, Li W, Zheng X, Liu J, Wang J *et al.* (2019) Apolipoproteins and cancer. *Cancer Medicine* **8**(16): 7032–7043.

Ribeiro CMS, Hermsen T, Taverne-Thiele AJ, Savelkoul HFJ, Wiegertjes GF (2010) Evolution of recognition of ligands from gram-positive bacteria: similarities and differences in the TLR2-mediated response between mammalian vertebrates and teleost fish. *The Journal of Immunology* **184**(5): 2355–2368.

Roy S, Wyse B, Hancock JF (2002) H-Ras signaling and K-ras signaling are differentially dependent on endocytosis. *Molecular and Cellular Biology* **22**(14): 5128–5140.

Sahai E, Marshall CJ (2002) RHO–GTPases and cancer. *Nature Reviews Cancer* **2**(2): 133–142.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 4406-425.

Saleh M, Kumar G, Abdel-Baki A-AS, Dkhil MA, El-Matbouli M, Al-Quraishy S (2019) Quantitative proteomic profiling of immune responses to Ichthyophthirius multifiliis in common carp skin mucus. *Fish & Shellfish Immunology* **84**: 834–842.

Sandt CH, Hill CW (2001) Nonimmune binding of human immunoglobulin A (IgA) and IgG Fc by distinct sequence segments of the EibF cell surface protein of Escherichia coli. *Infection and Immunity* **69**(12): 7293–7303.

Segev N (2001) Ypt/Rab GTPases: Regulators of Protein Trafficking. *Science Signaling* **2001**(100): re11.

Sehrawat S, Reddy PBJ, Rajasagi N, Suryawanshi A, Hirashima M, Rouse BT (2010) Galectin-9/TIM-3 Interaction Regulates Virus-Specific Primary and Memory CD8+ T Cell Response. *PLoS Path* **6**(5): e1000882.

Shan Y, Zhang Y, Zhuo X, Li X, Peng J, Fang W (2016) Matrix metalloproteinase-9 plays a role in protecting zebrafish from lethal infection with Listeria monocytogenes by enhancing macrophage migration. *Fish & Shellfish Immunology* **54**: 179–187.

Shannon P (2003) Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research* **13**(11): 2498–2504.

Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. *Nature Reviews Immunology* **2**(3): 185–194.

Star B, Nederbragt AJ, Jentoft S, Grimholt U, Malmstrøm M, Gregers TF *et al.* (2011) The genome sequence of Atlantic cod reveals a unique immune system. *Nature. Nature Publishing Group* **477**(7363): 207–210.

Stewart GR, Young DB (2004) Heat-shock proteins and the host–pathogen interaction during bacterial infection. *Current Opinion in Immunology* **16**: 506–510.

Stowell SR, Arthur CM, Dias-Baruffi M, Rodrigues LC, Gourdine J-P, Heimburg-Molinaro J *et al.* (2010) Innate immune lectins kill bacteria expressing blood group antigen. *Nature Medicine* **16**(3): 295–301.

Sunyer JO, Lambris JD (1998) Evolution and diversity of the complement system of poikilothermic vertebrates. *Immunological Reviews* **166**(1): 39–57.

Sunyer J, Zarkadis IK, Lambris JD (1998) Complement diversity: A mechanism for generating immune diversity? *Immunology Today* **19**(11): 519–523.

Tacchi L, Bickerdike R, Secombes CJ, Pooley NJ, Urquhart KL, Collet B *et al.* (2010) Ubiquitin E3 ligase atrogin-1 (Fbox-32) in Atlantic salmon (Salmo salar): Sequence analysis, genomic structure and modulation of expression. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **157**(4): 364–373.

Taylor MP, Koyuncu OO, Enquist LW (2011) Subversion of the actin cytoskeleton during viral infection. *Nature Reviews Microbiology* **9**(6): 427–439.

Thibaudeau TA, Smith DM (2019) A Practical review of proteasome pharmacology. *Pharmacological Reviews* **71**(2): 170–197.

Tian Y, Wen H, Qi X, Mao X, Shi Z, Li J *et al.* (2019) Analysis of apolipoprotein multigene family in spotted sea bass (*Lateolabrax maculatus*) and their expression profiles in response to Vibrio harveyi infection. *Fish & Shellfish Immunology* **92**: 111–118.

Tokumoto T (1998) Nature and role of proteasomes in maturation of fish oocytes. *International Review of Cytology* **186**: 261–294.

Torraca V, Mostowy S (2018) Zebrafish infection: From pathogenesis to cell biology. *Trends in Cell Biology* **28**(2): 143–156.

Tsoi SC M, Cale JM, Bird IM, Ewart V, Brown LL, Douglas S (2003) Use of human cDNA microarrays for identification of differentially expressed genes in atlantic salmon liver during aeromonas salmonicida infection. *Marine Biotechnology* **5**(6): 545–554.

Turk V, Stoka V, Vasiljeva O, Renko M, Sun T, Turk B *et al.* (2012) Cysteine cathepsins: From structure, function and regulation to new frontiers. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1824**(1): 68–88.

Valero Y, Morcillo P, Meseguer J, Buonocore F, Esteban MA, Chaves-Pozo E *et al.* (2015) Characterization of the IFN pathway in the teleost fish gonad against vertically transmitted viral nervous necrosis virus. *Journal of General Virology* **96**(8): 2176–2187.

van Vliet SJ, Paessens LC, Broks-van den Berg VCM, Geijtenbeek TBH, van Kooyk Y (2008) The C-Type Lectin Macrophage Galactose-Type Lectin Impedes Migration of Immature APCs. *The Journal of Immunology* **181**(5): 3148–3155.

Vasta GR (2009) Roles of galectins in infection. *Nature Reviews Microbiology* **7**(6): 424–438.

Vasta GR, Quesenberry M, Ahmed H, O'Leary N (1999) C-type lectins and galectins mediate innate and adaptive immune functions: their roles in the complement activation pathway. *Developmental & Comparative Immunology* **23**(4-5): 401–420.

Vasta GR, Nita-Lazar M, Giomarelli B, Ahmed H, Du S, Cammarata M *et al.* (2011) Structural and functional diversity of the lectin repertoire in teleost fish: Relevance to innate and adaptive immunity. *Developmental & Comparative Immunology* **35**(12): 1388–1399.

Verrier ER, Langevin C, Tohry C, Houel A, Ducrocq V, Benmansour Ab *et al.* (2012) Genetic Resistance to Rhabdovirus Infection in Teleost Fish Is Paralleled to the Derived Cell Resistance Status. *PLoS One* **7**(4): e33935.

Villares M, Berthelet J, Weitzman JB (2020) The clever strategies used by intracellular parasites to hijack host gene expression. *Seminars in Immunopathology* **42**(2): 215–226.

Walker J, Saraste M, Runswick MJ, Gay NJ (1982) Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *The EMBO Journal* **1**(8): 945–951.

Wang T, Bird S, Koussounadis A, Holland JW, Carrington A, Zou J *et al.* (2009) Identification of a Novel IL-1 Cytokine Family Member in Teleost Fish. *The Journal of Immunology* **183**(2): 962–974.

Wang T, Huang W, Costa MM, Secombes CJ (2011) The gamma-chain cytokine/receptor system in fish: More ligands and receptors. *Fish & Shellfish Immunology* **31**(5): 673–687.

Wei J, Gao P, Zhang P, Guo M, Xu M, Wei S *et al.* (2015) Isolation and function analysis of apolipoprotein A-I gene response to virus infection in grouper. *Fish & Shellfish Immunology* **43**(2): 396–404.

Wei X, Zhao T, Zhang Y, Ai K, Li H, Yang J (2019) Involvement of H-Ras in the adaptive immunity of Nile tilapia by regulating lymphocyte activation. *Fish & Shellfish Immunology* **89**: 281–289.

Wiens GD, Rockey DD, Wu Z, Chang J, Levy R, Crane S *et al.* (2008) Genome sequence of the fish pathogen renibacterium salmoninarum suggests reductive evolution away from an environmental arthrobacter ancestor. *Journal of Bacteriology* **190**(21): 6970–6982.

Wilson AB (2017) MHC and adaptive immunity in teleost fishes. *Immunogenetics* **69**(8–9): 521–528.

Wojtowicz-Praga SM, Dickson RB, Hawkins MJ (1997) Matrix metalloproteinase inhibitors. *Investigational new drugs* **15**(1): 61–75.

Xu X-Y, Shen Y-B, Fu J-J, Liu F, Guo S-Z, Yang XM *et al.* (2012) Matrix metalloproteinase 2 of grass carp Ctenopharyngodon idella (CiMMP2) is involved in the immune response against bacterial infection. *Fish & Shellfish Immunology* **33**(2): 251–257.

Yamaguchi and Dijkstra (2019) Major Histocompatibility Complex (MHC) Genes and Disease Resistance in Fish. *Cells* **8**(4): 378.

Zarkadis IK, Mastellos D, Lambris JD (2001) Phylogenetic aspects of the complement system. *Developmental and Comparative Immunology* **25**(8–9): 745–762.

Zhang K (2017) Overview of Interferon: Characteristics, signaling and anti-cancer effect. *Archives of Biotechnology and Biomedicine* **1**(1): 1–16.

Zhang J, Bai S, Zhang X, Nagase H, Sarras MP (2003) The expression of gelatinase A (MMP-2) is required for normal development of zebrafish embryos. *Development Genes and Evolution* **213**(9): 456–463.

Zhang Y-A, Salinas I, Li J, Parra D, Bjork S, Xu Z *et al.* (2010) IgT, a primitive immunoglobulin class specialized in mucosal immunity. *Nature Immunology* **11**(9): 827–835.

Zhang J, Yao J, Wang R, Zhang Y, Liu S, Sun L *et al.* (2014) The cytochrome P450 genes of channel catfish: Their involvement in disease defense responses as revealed by meta-analysis of RNA-Seq data sets. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1840**(9): 2813–2828.

Zhang X, Zhao H, Chen Y, Luo H, Yang P, Yao B (2015) A zebrafish (Danio rerio) bloodthirsty member 20 with E3 ubiquitin ligase activity involved in immune response against bacterial infection. *Biochemical and Biophysical Research Communications* **457**(1): 83–89.

Zhao Z (2017) Cloning and functional analysis of an immune-induced promoter in common carp(Cyprinus carpio). *Journal of Fisheries of China* **41**(12): 1829–1837.

Zlotnik A, Yoshie O (2000) Chemokines: A new classification system and their role in immunity. *Journal of Cultural Heritage* **12**: 121–127.

Zou J, Secombes CJ (2011) Teleost fish interferons and their role in immunity. *Developmental and Comparative Immunology* **35** (12): 1376–1387.

Zou J, Secombes CJ (2016) The function of fish cytokines. *Biology* **5**(2): 23.

Zou J, Secombes CJ, Long S, Miller N, Clem LW, Chinchar VG (2003) Molecular identification and expression analysis of tumor necrosis factor in channel catfish (Ictalurus punctatus). *Developmental & Comparative Immunology* **27**(10): 845–858.

Zuckerkandl E, Pauling L (1965) Evolutionary divergence and convergence in proteins. *Evolving Genes and Proteins* 97–166.

Zügel U, Kaufmann SHE (1999) Role of heat shock proteins in protection from and pathogenesis of infectious diseases. *Clinical Microbiology Reviews* **12**(1): 19–39.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Editorial information of the articles incorporated in the systematic review.

Table S2. Microarray technical information of the articles incorporated in the systematic review.

Table S3. Differentially expressed genes (DEGs) of the articles incorporated in the systematic review.