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Role of pattern recognition receptors in teleost fish: Recent advances

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Abstract
Pattern recognition receptors (PRRs) are a type of immunological sensor that is responsible for identifying and responding to conserved patterns of microorganisms. Many PRRs, such as TLRs, RLRs, and NLRs, have been found and characterized in teleosts, as well as their downstream molecules. The TLR/RLR/NLR family members, including their expression profiles, signaling pathways, and roles in teleosts. Splicing isoforms from the TLR/RLR/NLR families, which have synergistic and/or antagonistic roles in the response to pathogen infections in teleosts, are extensively demonstrated in this study. This review systematically describes the progress on TLRs, RLRs, and NLRs in teleosts by understanding how TLRs/RLRs/NLRs recognize ligands and transmit signaling in teleosts. Our comprehensive review of PRRs involving involving TLR/RLR/NLR provides a new insight into anitiviral sensing in teleost fishes.

Keywords: Pattern recognition receptors, anitiviral sensing, signaling pathway, teleost

1. Introduction
To diversify the domestic flatfish culture in the northern coast of China, turbot fish were first introduced through Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences [1, 2]. In 1992, for pilot rearing research about 200 turbot juveniles (5-6 cm in length) were acquired from the United Kingdom, which reached sexual maturity in summer of 1995. From the above 200 turbot brood stock eggs, extensive larviculture was accomplished in 1997. After two years of extensive larviculture, commercial-scale juvenile production in the northern coast of Shandong province initiated in 1999.
As the commercial-scale juvenile production was established, the first full-scale turbot grows out systems were built using deep saline well water along the coast of Bohai Bay and the Yellow Sea (Fig.1).

Fig 1: Distribution of Turbot farms and markets in China (red thick arrows represents major markets)

In the last two decades, farming industry of turbot has advanced into one of the major mariculture industries in China. Considering the commercial value and delicacy of turbot, it is widely cultivated in China and have a huge demand overall [3, 4]. Since 2007, the global average annual production of turbot has surpassed 50,000 tons [5].

Shandong, Liaoning and Hebei are the three provinces where most of the turbot farms are been located in China. In addition, one farm is under the municipality of central government, Tianjin which is located along the Bohai bay. Amongst these three farm locations, Shandong province is known to be the origin of turbot farming, thus there are far more turbot farms than any other provinces.

In 2006, according to the statistics provided by the government of China, the greenhouse area in flatfish production mainly in Shandong province was estimated at 5,300 thousand m^2, which included 4,240 m^2 for turbot culture. The annual production in 2006 was estimated at 40 thousand tonnes mainly in the Shandong province contributing to 70% of the total turbot production. China is the leading producer of turbot, where world aquaculture production of turbot rose above 65,000 tons in 2015, mostly due to its rapid development in China [6]. In China, the second largest aquaculture centre for turbot is Liaoning province with an estimated area of 400 thousand m^2 and a total production of 10 thousand tonnes contributing 20 % of the total national aquaculture production. The 2005 fishery statistics of China revealed the total area of flatfish farming consisting three major provincial farming centers of about 8,060 thousand m^2 with a production capacity of 65.3 thousand tonnes, including over 50 thousand tonnes of turbot. According to the fishery statistics (Fishstat plus), the maximum production of flatfish was obtained at 83 thousand tonnes in 2005. Nevertheless, detection of nitrofuran metabolites in the fish samples drawn from Shanghai markets lead to wide distrust in the customers towards the farmed turbot. Such event brought a sudden shrink in the turbot market and product [7, 8].

In recent years, turbot production in China was achieved up to 64,000 tons 2012-13; 67,000 tons 2013; 60,000 tons 2014 and 55,000 tons in 2015 (Fig. 2). According to the data provided by Food and Agriculture Organization (FAO), the farmed turbot production in China is around 60 000 tonnes, while EU aquaculture production is just under 10,000 tonnes and EU fisheries production around 6,000 tonnes per year [9]. According to National Modern Agricultural Industrial Technology System, China, the total production of turbot in 2019 reached approximately 63,000 tons as shown in Fig. 2, respectively [10].

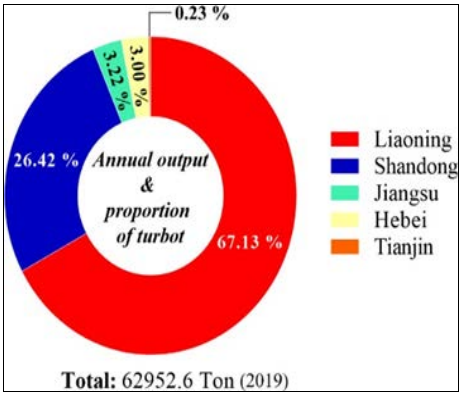

Fig 2: The annual output of turbot in each province in 2019 and its proportion (ton)

1.1 Biological Characteristics of Turbot

Turbot, *Scophthalmus maximus* belongs to the family Bothidae and Suborder Pleuronectoibei. The central body of the turbot is stocky, visceral mass is small and the eyes are located on the left side of head. Turbot's eye side body is snow brown in color, the mouth is big; however the lips are relatively short. Turbot's fins are as follows: dorsal fins are 57-71in number, anal fins 43-52, tail fins 20-22 fins are linked together, and pectoral fins 0-8 [11] (Fig. 3).

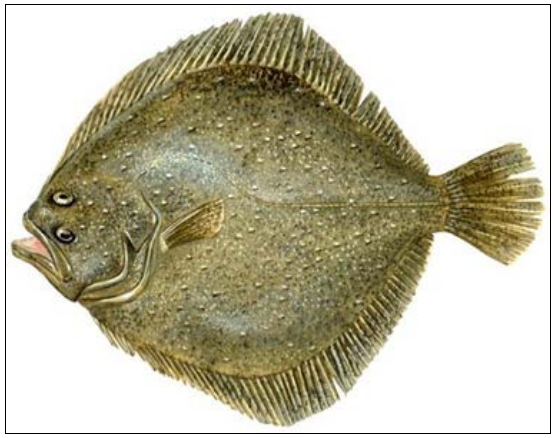

Fig 3: Turbot (*Scophthalmus maximus)* (Adopted from FAO, Fishery report)

Turbot is native to Europe mainly found in the demersal zone. In China, turbot is named as "Duobao" which literally means "peace" and "happiness" or "prosperity". Turbot fish has a salient feature i.e. to adapt life and grow at low temperatures. Turbot can survive and live at extreme temperatures ranging from 0-3 °C. For one year old turbot, the suitable water temperature range is 3-23 °C, however the adaptability to the high temperatures declines with age. Turbot's survival rate is affected with fluctuations in the water temperature, where increase in the temperature above 23-25 °C for long duration affects the survivability. Under proper management conditions, low temperature does not affect or is a threat to the survivability of turbots.

A positive feeding state at 5 °C at the growth size of 10-15 cm in length, has proven that turbot can live at 3-4 °C. Various other culture conditions utilized in the intensive farming consist of clean water with superior transparency have a pH of 7.6-8.2 and illumination of 60-600 lx, oxygen content 3-4 mg/l and salinity at optimal range 20-32. Male turbots reaching sexual maturity at two years and females at three are best for spawning under natural conditions in the duration of May to August. Turbot is spawned in batch/serial wise and can be breed once a year. In addition, age of sexual maturity can be obtained under artificially controlled temperature and light conditions in ~6 months earlier and mature eggs throughout the year.

Turbot can tolerate and adopt to adverse environmental conditions. Turbot cluster together and exhibit high feed utilization and conversion rates. Thus, in northern coastal China, turbot is an ideal fish species for intensive culture.

1.2 Production Cycle

Turbot species is gonochoric in nature with separate sexes, where compared to male individuals' females grow faster and reach maturity after three years and then proceed to lay eggs impulsively in captivity.

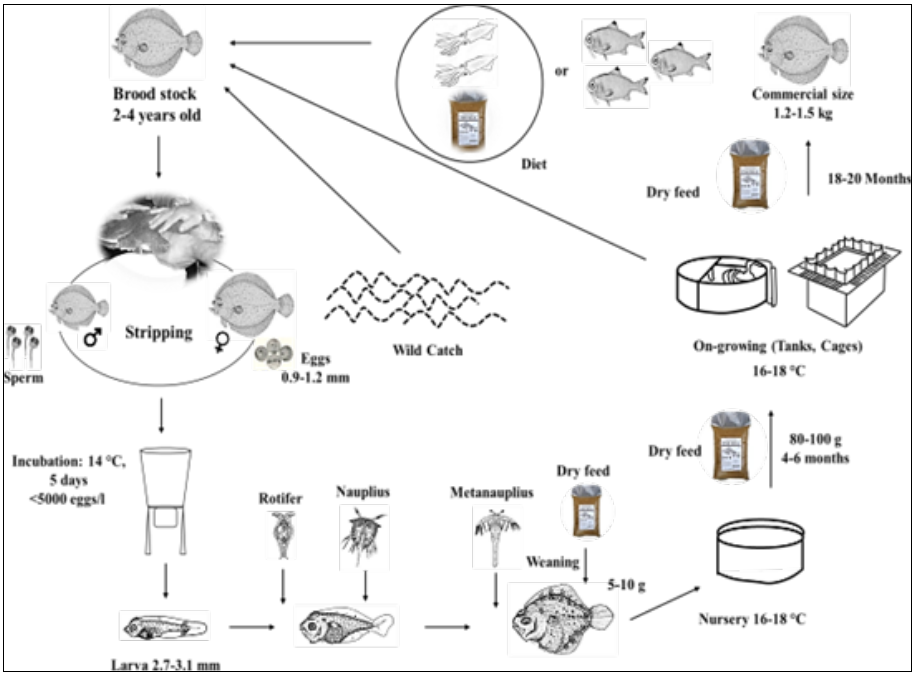

Fig 4: Production cycle of Turbot (*Scophthalmus maximus)* (Cultured Aquatic Species Information Program, FAO)

1.3 Immune System of Teleost

It is reported that fish immune system is equivalent to the vertebrates. Although, there are few differences particularly such as histopathology and location of the vital immune competent cells. In addition, fish immune system functions are similar to the other vertebrates, such as disease resistance and protection against neoplastic cells [12].

Fish comprising the largest vertebrate are known to be very diverse in terms of evolution, which are generally categorized into jawless fish (i.e., Lampreys), and jawed fish. The latter are subdivided into cartilaginous and bony fish i.e., sharks and teleosts, respectively.

Amongst this subdivision, the immune system of teleost is the most intensively studied. In comparison with the vertebrates such as mammals, the immune organs in teleost fish somewhat differ. The immune organs include thymus, kidney, intestinal tract, liver, and spleen [13].

1.3.1 Thymus

Thymus is a white tissue covered by a thin layer of skin that is found under the upper half of the gill operculum [14]. It has a medulla containing immature T lymphocytes and a cortex containing smaller, more evolved lymphocytes, but it is much less developed than in mammals. Allograft rejection, increased macrophage activity, and B-cell activation seem to be linked to these T lymphocytes [15]. The thymus' size is highly dependent on age, season (spawning), and stress: it has been shown to become involute with age or after prolonged stress.

1.3.2 Kidney

The kidney is a dark red organ found along the ventral surface of the vertebrae in teleosts [15]. The anterior portion of the kidney, also known as the anterior or head kidney, is responsible for essential immune functions. In mammals, the anterior kidney acts as the functional counterpart of bone marrow and is involved in hematopoiesis. The anterior kidney contains mitotic and large immature lymphocytes. Immune cells in the kidney, however, perform additional immune functions including phagocytosis and antigen processing. B lymphocytes that produce antibodies are found in the kidney and play an important role in the production of humoral immunity and immunological memory. Furthermore, the reticular endothelial system of circulating and tissue macrophages filters aged blood cells and particulate matter [16].

1.3.3 Intestinal Tract

Antigen uptake and processing have been shown to occur primarily in the intestine. It runs around the bottom portion of the trout's posterior peritoneal cavity. In comparison to humans, the intestine of fish does not contain lymphocyte aggregates. The stratum granulosum's granular cells, on the other hand, have been linked to mucosal immunity. Macrophages, B lymphocytes, immunoglobulin-negative T lymphocytes, and normal cytotoxic cells are all found in this gut- associated lymphoid tissue [15].

1.3.4 Liver

The liver is a large organ situated in the anterior peritoneal cavity that performs many of the functions that are recognized in mammals. The presence of phagocytic mononuclear cells in the liver sinusoids suggests that the liver is also involved in the presentation of particulate antigens. They are thought to be the same as mammalian Kupffer cells. The liver of rainbow trout can also produce C-reactive protein (CRP) [14-16].

1.3.5 Spleen

The spleen, like the spleens of other vertebrates, is the primary filter of blood borne antigens and also serves as an immunopoietic organ. It is involved in hematopoiesis in teleost fish and may have immune functions similar to lymph nodes in mammals (fish do not have lymph nodes). It has a smooth texture and a dark red color and is located in the lower posterior abdominal cavity. The outer capsule of the spleen is made up of connective tissue and a pulp matrix. Both hematopoietic red pulp and lymphopoietic white pulp are contained in the pulp. The spleen can store a large number of mature erythrocytes that can be released into the bloodstream

when required, and it is also where thrombocytes are produced [15, 17, 18].

1.4 Nonspecific Immune System

Fish live in a marine ecosystem that is conducive to the transmission of disease-causing species. Fish have a well-developed nonspecific immune system as a result. The first line of defense against pathogens is the skin, lateral line, and gills. Mucus, which is continually secreted by goblet cells and contains antibodies as well as lysozyme, coats the skin of fish. The epidermis cells are the next line of protection, followed by the scales. Fish are more susceptible in areas that aren't covered by mucus or scales, such as their gills, and macrophages can be found on the surfaces of their gills. Antigens from pathogens such as *Yersinia ruckeri* bacteria, provided as a suspension in water, have also been shown to be very successful in triggering strong immune responses in rainbow trout. *Aeromonas salmonicida*, for example, does not provoke such a strong response. Rainbow trout's nonspecific immune response is focused on the activation of mononuclear phagocytes (macrophages) and polymorphonuclear granulocytes (PMN), both of which can be activated by opsonic antibodies and complement. Complement activation can occur in fish through both the classical and alternative pathways. Both pathways result in pathogen lysis, but teleosts also have other lytic processes in their serum that have yet to be identified. Inflammatory reactions in fish include the movement of neutrophils, eosinophils, basophils (not all species), macrophages, and lymphocytes to the infection site; also small temperature changes have been observed. Soluble factors including cytokines, eicosanoids, and complement components induce chemotaxis to the site of inflammation. In teleost fish, only a few cytokines have been definitively described. Fish exhibit biological activity for interleukin-1 (IL-1), transforming growth factor (TGF)-1, and tumor necrosis factor (TNF)-1, indicating that these cytokines have receptors. Furthermore, molecular cloning and sequencing have revealed the existence of an IL-2-like, IFN-like, IL-1, and TGF-gene. Carp macrophages and neutrophilic granulocytes have also been discovered to secrete an IL-1-like factor, and supernatants from carp leukocyte cultures stimulated with mitogen or alloantigen have been found to contain IL-2-like lymphocyte growth-promoting activity [15, 17].

1.5 Specific Immune System

As previously stated, fish can absorb antigens in the water not only through their skin, gills, and lateral line, but also orally [17]. T and B cells are activated, produce cytokines, and thus induce plasma cells to produce antibodies after antigen is presented via the major histocompatibility complex (MHC) I or II. Only IgM antibodies have been discovered in fish up to this point [19]. In addition, unlike human IgM, which is pentameric, fish IgM is a tetrameric molecule. Fish produce specific antibodies after being immunized, which have properties like agglutination, precipitation, complement fixation, Opsonization, and skin sensitization [18]. In fish species, isotype switching, rapid titer increases, affinity, or maturation have not been described. Because antibody production in fish is largely temperature dependent, immunizations and leukocyte cultivation should be performed at the optimum temperature for the species, such as 15–20 °C for trout [19]. Fish have been shown to express MHC class I and II molecules, as previously mentioned. Full-length MHC class I -chain cDNA and full-length MHC class II -chain cDNA have been described in Atlantic salmon (*Salmo salar*), carp (*C. carpio*), zebrafish (*Danio rerio*), and rainbow trout (*Oncorhynchus mykiss*) using molecular cloning (*O. mykiss*). Both carp and Atlantic salmon have MHC class II- chain expression in their thymus, head kidney, spleen, and peripheral blood, though at different levels [18, 19].

2. Fish Immune Response to Viral Pathogens

Since 2002 to 2021 (till date), there have been several researches on the antiviral immune response in turbot (*Scophthalmus maximus*). The maximum number of research published concerning antiviral immune response in turbot were in “Fish and Shellfish Immunology contributing >50 % of the total published researches. The second largest was in “Developmental and Comparative Immunology” contributing >15 %, followed by “Aquaculture” >7 %, respectively. In addition, various other scientific journals contributed >2 %, >1 % and <1 % in the total published research on antiviral immune response in turbot (Fig. 5), respectively.

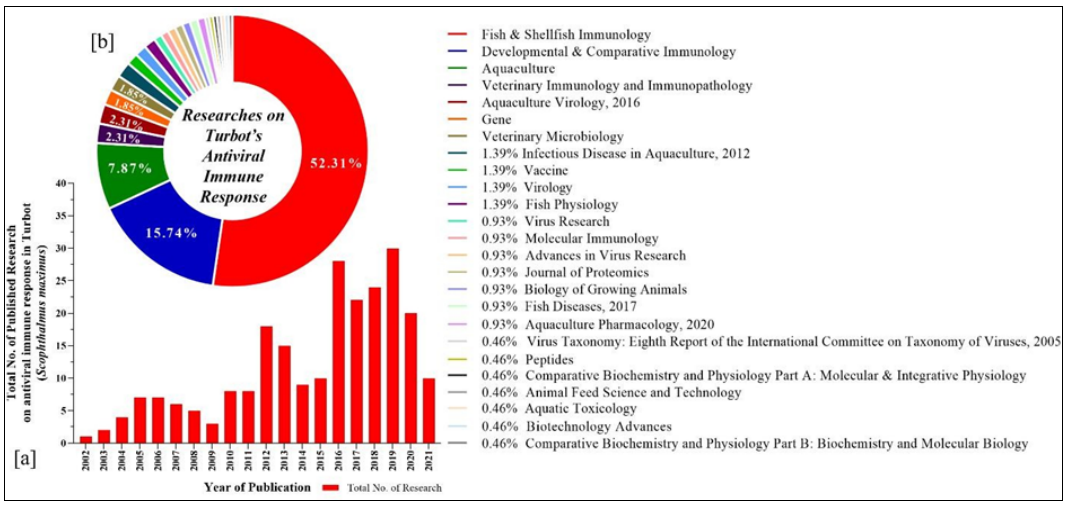

Fig 5: (a) Total publication on antiviral immune response in turbot (2002-2021); and (b) Share of publications in international scientific research journals (Data acquired from various journals)

In aquaculture, viral diseases cause major losses. Some viruses, such as Infectious Pancreatic Necrosis Virus (IPNV) in salmonids, damage only young fish, while others, such as Viral Hemorrhagic Septicemia virus (VHSV) and Infectious

Salmon Anemia virus, destroy fish during their lives (ISAV). Many fish viral pathogens cause the host to remain in a persistent carrier state. These fish have the potential to shed virus into the water and infect other fish. Low levels of virus can often be isolated from the kidneys of carrier fish, and evidence suggests that certain viruses, such as IPNV, can replicate in and release from kidney macrophages without being cytolytic. The processes involved in viral carrier states in fish and how viruses escape host defenses are poorly understood. Fish have two types of innate defenses against viruses: constitutive and receptive. The activity of non-specific cytotoxic cells against virus-infected cells is a constitutive innate antiviral response in fish, and interferon production, which is non-specifically inducible by virus infection is a receptive mechanism. Complement appears to play an important role in virus neutralization mediated by the particular antibody response in certain cases, such as protection against rhabdoviruses, VHSV, and IHNV. Other constitutive defenses could include lectins (which can attach to glycosylated residues on virus surfaces) and non-specific lysins (which can lyse the envelopes of enveloped viruses), but evidence for these in fish is missing. Viruses must first bind to the cell surface, penetrate the cell membrane, and engage the cell's biochemistry for nucleic acid and protein production in order to replicate in fish cells. All of these stages will necessitate molecular recognition, and if this isn't present, the cell will be immune to viral replication. Although some progress is being made with VHS and ISA, very little is known about virus/host identification for viral diseases of fish [20]. IFN is generated in response to viral double-stranded RNA (dsRNA), and the synthetic dsRNA polyinosinic polycytidylic acid (poly I:C) is a powerful inducer of IFN. Most viruses generate dsRNA at some point during their replication, and it appears that animals have evolved an innate ability to recognize these molecules and react to them [20]. IFN operates by inducing the expression of a variety of proteins in host cells that prevent viral mRNA from being translated. 2',5'-oligoadenylate synthetase, protein kinase P1, and Mx proteins are among the proteins that have been identified [21]. In a variety of fish species, IFN-like activity has been observed, but neither the protein nor the genes have been isolated [22]. Rainbow trout, Atlantic salmon, and Atlantic halibut have all had their Mx protein genes cloned [23-25]. A sensitive approach for detecting INF responses in fish has been to use RT-PCR to detect Mx gene mRNA expression or labeled antibodies to detect Mx protein expression [26]. Noteworthy, in 2006, the value of farmed turbot production in China reached US$400 million. Because of high density stocking and poor management, more and more epizootic diseases of farmed turbot have recently emerged in China. In 2004, China announced a fish disease that caused high mortality and severe damage to turbot cultures. The spleen, kidney, cranial connective tissue, and endocardium all showed cell hypertrophy as a result of the viral infection. Based on microscopic analysis and transmission electron microscopy, the causative agent was determined to be an iridovirus-like virus (TEM). Further, this virus was identified as the iridovirus and termed as Turbot reddish body iridovirus (TRBIV) [27].

3. Fish Immune Response to Bacterial Pathogens

Since 1996 to 2021 (till date), there have been several researches on the antibacterial immune response in turbot (*Scophthalmus maximus*). The maximum number of research published concerning antibacterial immune response in turbot were in "Fish and Shellfish Immunology contributing >50 % of the total published researches. The second largest was in "Developmental and Comparative Immunology" contributing >15 %, followed by "Aquaculture" >17 % and "Developmental and Comparative Immunology" >10 %, respectively. In addition, various other scientific journals contributed >1 %, and <1 % in the total published research on antibacterial immune response in turbot (Fig. 6), respectively. Development of broad-spectrum antimicrobial substances and acute phase proteins, non- classical complement activation, release of cytokines, inflammation, and phagocytosis are among fish's innate defense mechanisms against bacteria. Many of these defenses' natures and mechanisms are discussed in other articles in this volume. Although these mechanisms provide effective defenses against invasion by saprophytic bacteria in the environment, pathogenic bacteria have developed ways to escape many of them. In certain cases, non-immunized fish tend to coexist in a carrier state with highly virulent bacterial pathogens without displaying any signs of morbidity, implying that the innate defense mechanisms provide some security. Disease outbreaks and mortalities, on the other hand, are often the product of stressed fish, with their defense mechanisms probably weakened [28].

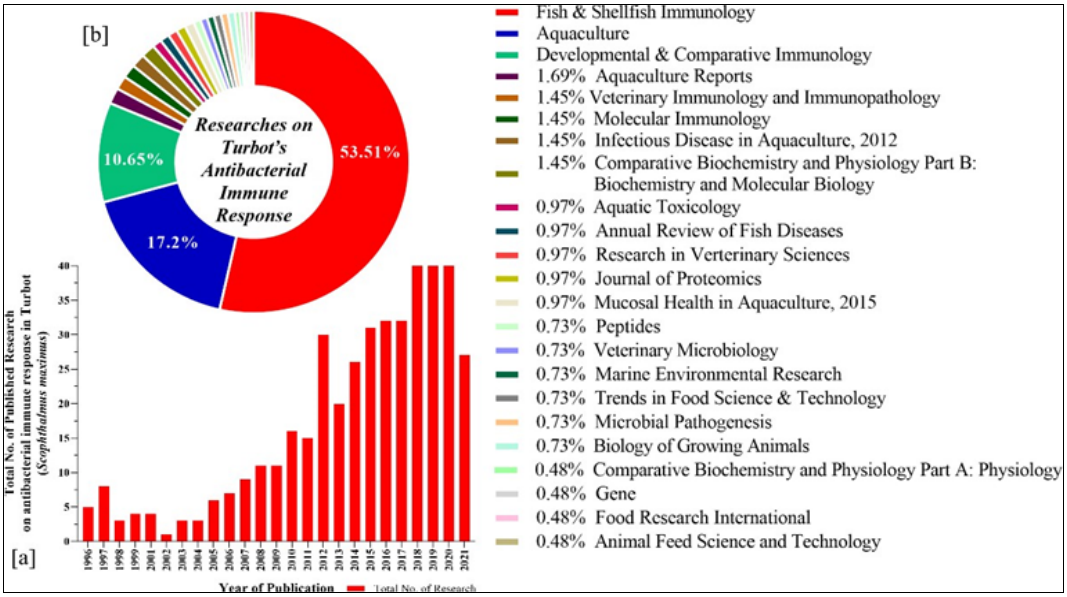

Fig 6: (a) Total publication on antibacterial immune response in turbot (1996-2021); and (b) Share of publications in international scientific research journals (Data acquired from various journals)

Amongst several diseases in fish, Vibriosis is known to cause by a variety of Vibrio species. Vibriosis is a common fish disease in both marine and brackish waters. It has been found in over 50 different species of marine fish, and it is a significant hindrance to the culture of marine salmonids [29]. Disease outbreaks in intense culture are normal in late summer, when water temperatures rise. Vibrio (*Listonella*) *anguillarum* is a Gram-negative curved rod with polar flagella that is halophilic. This bacterial species has been found to cause vibriosis in a variety of finfish species, including turbot (*Scophthalmus* maximus), eels (*Anguilla Anguilla*), and salmonids (*Oncorhynchus nerka*) [30, 31]. In cultured cobia, Rachycentron canadum, high mortality is normal, with 100 percent morbidity and mortality rates generally exceeding 80 % [32]. Fish less than 4 months old (500g) tend to be the most vulnerable to this bacterial pathogen, with the highest mortality rates. Hemorrhagic septicemia, skin discoloration, red necrotic lesions in the abdominal muscle, abdominal distension, exophthalmia, and erythema at the base of the fins, vent, and in the mouth are some of the symptoms that may occur [33].

4. Pattern Recognition Receptors (PRRs) and progress in teleost

Innate host defense relies heavily on pattern recognition receptors (PRRs). However, PRR signaling can be detrimental to the host in a number of diseases. Excessive pathogen-induced PRR signaling can cause tissue damage. However, over the last decade, many diseases have been reported in which endogenous factors induce excessive PRR signaling. In living organisms, a number of sensors are detected by non-self-viral components. Restriction enzymes are used by bacteria as a protective mechanism to destroy invading nucleotides [34]. Plants and invertebrates have developed a technique called post-transcriptional gene silencing (PTGS) that can degrade viral mRNA and thus suppress viral gene expression [35, 36]. To direct unique viral RNA degradation, the PTGS uses RNA induced silencing complexes (RISC) containing small RNAs cleaved from double-stranded viral RNAs by DICER family members and Argonaute proteins [37-40]. In vertebrates, a more sophisticated surveillance network has developed to detect viral lipids, proteins, and nucleotides and activate an antiviral response, which is known to be coordinated by interferons in jawed vertebrates (IFNs) [41-47]. These sensors, along with those that detect other pathogens (such as bacteria, fungi, and viruses), are known as pattern recognition receptors (PRRs), since they can recognize unique structures that are conserved across pathogen types, known as pathogen associated molecular patterns (PAMPs), in any part of the cell. Membrane- bound receptors such as Toll-like receptors (TLRs), which are anchored on the cell surface or in subcellular organelles such as endosomes, and cytosolic proteins such as RIG-I-like receptors (RLRs), DNA binding proteins, and nucleotide-binding domain leucine-rich repeat-containingmolecules (NLRs), which are dispersed in the cytosol, are characterized PRRs in the sense of viral sensing [48-54]. To fight viral infection, teleost fish, like all jawed vertebrates, have both an innate and adaptive immune system. Mammalian study has made significant progress in elucidating the molecular mechanisms of host antiviral defense in recent years, and although fish immunologists are still catching up, several primary components of the antiviral system have been discovered (Fig. 7) [55].

These findings indicate that much of the endogenous antiviral mechanism in vertebrates is well conserved. IFNs, for example, are critical in mounting an efficient antiviral response in fish, just as they are in mammals. However, the lineage that gave rise to teleost fish diverged from that that gave rise to tetrapods early in vertebrate evolution, and fish may have extended some viral sensing gene families, lost others, or preserved genes that were later lost in mammals [56-65].

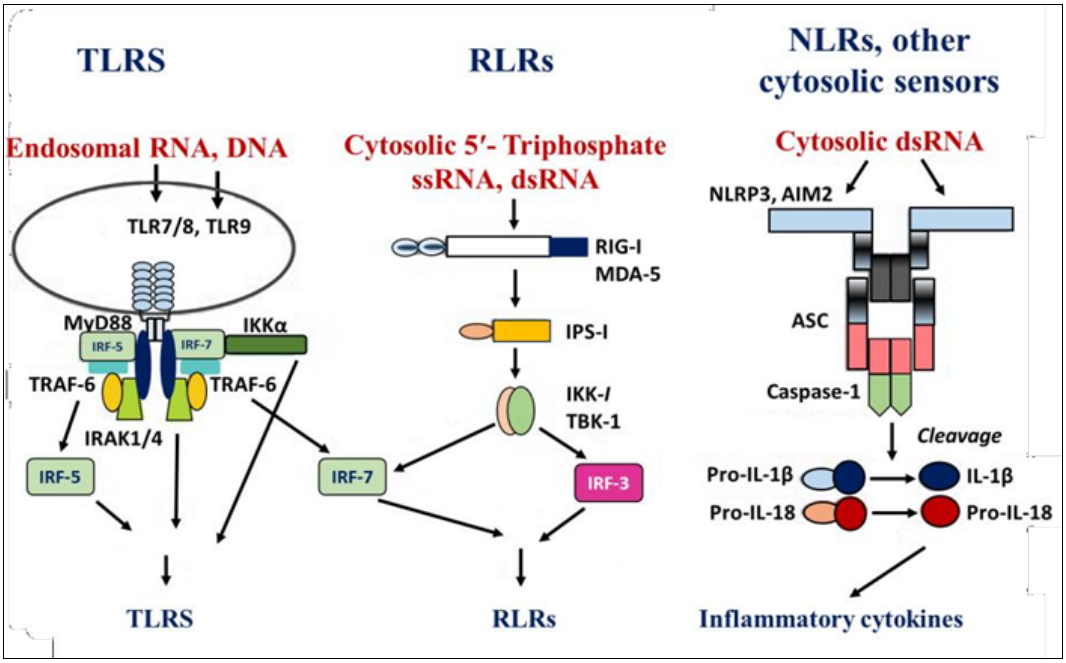

Fig 7: Three classes of pattern recognition receptors implicated in viral nucleic acids recognition in mammalian cells. NLR, nucleotide oligomerization domain–like receptor; RLR, retinoid acid- inducible gene-I-like receptor; TLR, Toll-like receptor

4.1.1 Rig-I like Receptors (RLRs)

In teleost fish, retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are found in low numbers. RIG-I, MDA5, and LGP2, as well as downstream molecules such as MITA, TRAF3, and TBK1, have been discovered in a variety of fish organisms. However, the absence of RIG-I in

Acanthopterygian fish is astonishing, and it would be necessary to determine the existence and function of the RIG-I gene in a wide range of Teleostei taxa. *In vivo* and *in vitro*, viral pathogens, as well as synthetic dsRNA, poly (I:C), can induce RLRs in fish, resulting in the development oftype I interferons (IFNs) and the expression of IFN-stimulated genes (ISGs). Bacterial pathogens, such as Edwardsiella tarda, and their components, such as lipopolysaccharide, have found to induce RLR expression, but it is unclear if this is mediated by direct recognition by RLRs or by crosstalk with other pattern recognition receptors that recognize specifically bacterial pathogen- associated molecular patterns. RLR-activated type I IFN production, on the other hand, can be inhibited in fish by molecules like TBK-1-like protein and IRF10, which have been found to inhibit RIG-I and MAVS-activated type I IFN production, as well as block MITA and bind ISRE motifs, respectively (Fig. 8) [66, 67]. The evolutionary occurrence of RLRs in fish, as well as their recognized ligands, especially those from their fish pathogens, and the mechanisms involved in RLR signaling pathways, are thought to be of great interest for further research [66, 67].

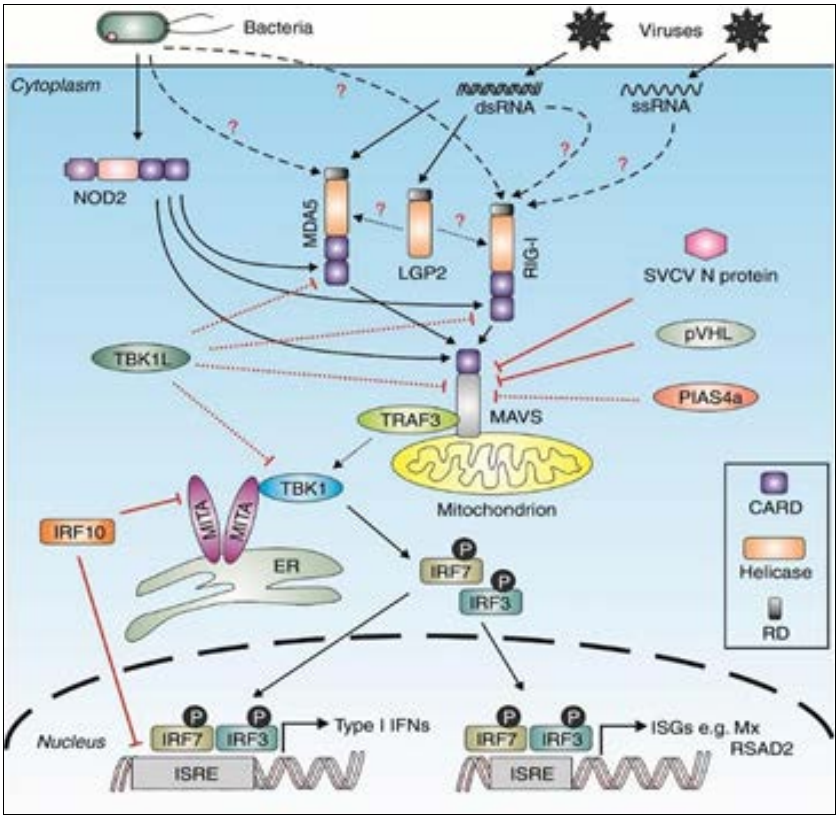

Fig 8: RIG-like receptors (RLRs) in teleost fish

RIG-like receptors (RLRs), which belong to DExD/H box RNA helicases, are the most important cytosolic receptors for viral RNA recognition. RLR stands for retinoic acid-inducible gene I (RIG-I, also known as DEAD box polypeptide 58, DDX58), melanoma differentiation- associated gene 5 (MDA5, also known as interferon induced with helicase C domain 1, IFIH1), and laboratory of genetics and physiology 2 (LGP2, or DExH box polypeptide 58, DHX58) [68] (Fig. 8). Over the last few decades, significant progress has been made in the field of fish immunology, with orthologous genes of mammalian RIG-I, MDA5, and LGP2 being discovered in teleost fish. In addition their functions also been examined in a variety of fish species, including the model fish zebrafish (Danio rerio) [69, 70] and some of the economically important fish species for instance, turbot (*Scophthalmus maximus*), rainbow trout (*Oncorhynchus mykiss*) and Japanese flounder (Paralichthys olivaceus) [67, 71, 72].

When RLRs are transcripted in fish, they may be spliced at the RNA level, resulting in sequence deletion or insertion in some functional domains. After searching the zebrafish genome database, researchers discovered two spliced transcripts of MDA5, MDA5a and MDA5b, that are derived from the same gene. MDA5a comprises all 16 exons, while MDA5b has a frameshift due to the deletion of the HELICc and RD domains from the predictive protein due to a missing partial sequence in the eleventh exon. The RIG-I gene in zebrafish has two transcripts, RIG-Ia and RIG- Ib, with a 114-nt sequence inserted in the second CARD domain of RIG-Ia that has no homology with those in other fish species or mammals. In reality, the RIG-I genomic DNA sequence in the current zebrafish genome (GRCz10) hasn't been fully assembled, so it's still uncertain if the two isoforms are encoded by a single gene or two duplicated genes. In rainbow trout, the LGP2 gene has a splicing variant, LGP2b, which is 54 amino acids (aa) shorter than LGP2a, due to premature termination due to an unspliced intron at the 3' end region of the LGP2b open reading frame (ORF) [69-71].

RIG-I is currently only present in Cypriniformes, Siluriformes, and Salmoniformes, while MDA5 and LGP2 genes are found in Cypriniformes, Siluriformes, Salmoniformes, and other Acanthopterygii fish species [69, 73-76]. It's unclear if RIG-I genes have been lost in Acanthopterygii fish, which necessitates further research into this community of fish. Indeed, attempts to find orthologues of RIG-I in Japanese pufferfish (*Takifugu rubripes*), tetraodon (*Tetraodon nigroviridis*), medaka (*Oryzias latipes*), three-spined stickleback (*Gasterosteus aculeatus*), gilt-head sea bream (*Sparus aurata*), and European sea bass (*Sparus*

aurata) have been unsuccessful (*Dicentrarchus labrax*). RIG-I has not been discovered in mandarin fish or "Chinese perch" (*Siniperca chuatsi*). RIG-I genes have been found in a wide variety of Teleostei taxa, but more advanced research is needed to confirm their presence [77-79]. RLRs in fish have protein domains that are identical to those found in mammalian RLRs. A DExD/H box helicase domain (DEXDc), a helicase C-terminal domain (HELICc), a regulatory domain (RD), and two caspase activation and recruitment domains (CARDs) at the N-terminal region of RIG-I and MDA5, but not LGP2, are present in all RLR molecules, with CARD playing an important role in signaling transduction [69-71, 80, 81].

Pathogenic bacteria or their components have been shown in experiments to significantly increase the expression of RLRs in teleost fish. The infection of zebrafish ZF4 cells with Edwardsiella tarda, an intracellular Gram-negative bacterial pathogen, resulted in a significant increase in RIG-I and MDA5 mRNA levels [70]. In the liver of channel catfish, E. ictaluri induced the expression of RIG-I, MDA5, and LGP2. Similarly, MDA5 and LGP2 were up-regulated in grass carp RIG-I, MDA5, and LGP2 in primary trunk kidney cells following LPS exposure *in vitro* in LPS stimulated peripheral blood leucocytes and leucocytes from the kidney of Japanese flounder. 38 However, in LPS-stimulated brain and fry cells of sea perch, MDA5 expression was decreased, and it was believed that signal pathways mediated by TLRs or NLRs were likely to be inhibited as a result of MDA5 expression. While TLR4 orthologues have been found in some teleost fish (not all teleosts), such as zebrafish, the mechanisms involved in LPS recognition and signaling transduction remain unknown in fish. TLR4 orthologues in fish, on the other hand, aren't interested in LPS identification. Fish can withstand higher levels of LPS than mammals, according to studies, and toll like receptor adaptor molecule 2 (TICAM2, also known as TRAM and TIRP), a vital adaptor for signal transduction triggered by LPS in mammals, is missing in fish. As a result, the pathways involved in LPS recognition and RLR expression in response to LPS may be a promising area for future research. Furthermore, RLRs are thought to play a role in antibacterial immunity in fish, as well as their antiviral effect [53, 69, 72, 82-87].

4.1.2 Toll-like Receptors (TLRs)

The Toll gene, which encodes a type I transmembrane receptor, was discovered in 1985 to be involved in Drosophila embryogenesis [88]. The cytoplasmic signaling domain of the Drosophila Toll receptor is conserved in the mammalian receptor of interleukin 1, one of the most important cytokines that regulates inflammatory responses during the early stages of bacterial and viral infection. Later, in Toll gene mutated flies that are highly susceptible to fungal infection, the function of the Toll receptor in innate immunity was confirmed [53]. To date, there are more than 13 members of the Toll-like receptor (TLR) family in vertebrates that recognize a variety of compounds derived from bacteria, fungi, protozoa, and viruses. TLRs all have several leucine rich repeats in the extracellular region that help them recognize PAMPs from bacteria, fungi, and viruses, as well as a conserved intracellular interleukin-1 receptor domain that helps them elicit a particular immune response. TLR3 and TLR7/8 are known to sense viral RNA, while TLR9 recognizes unmethylated CpG viral DNA, allowing the interferon system to be activated. Non- mammals have recently discovered a new TLR subfamily (named TLR21/22) that also plays a role in the identification of viral nucleotide PAMPs [89] (Fig. 9).

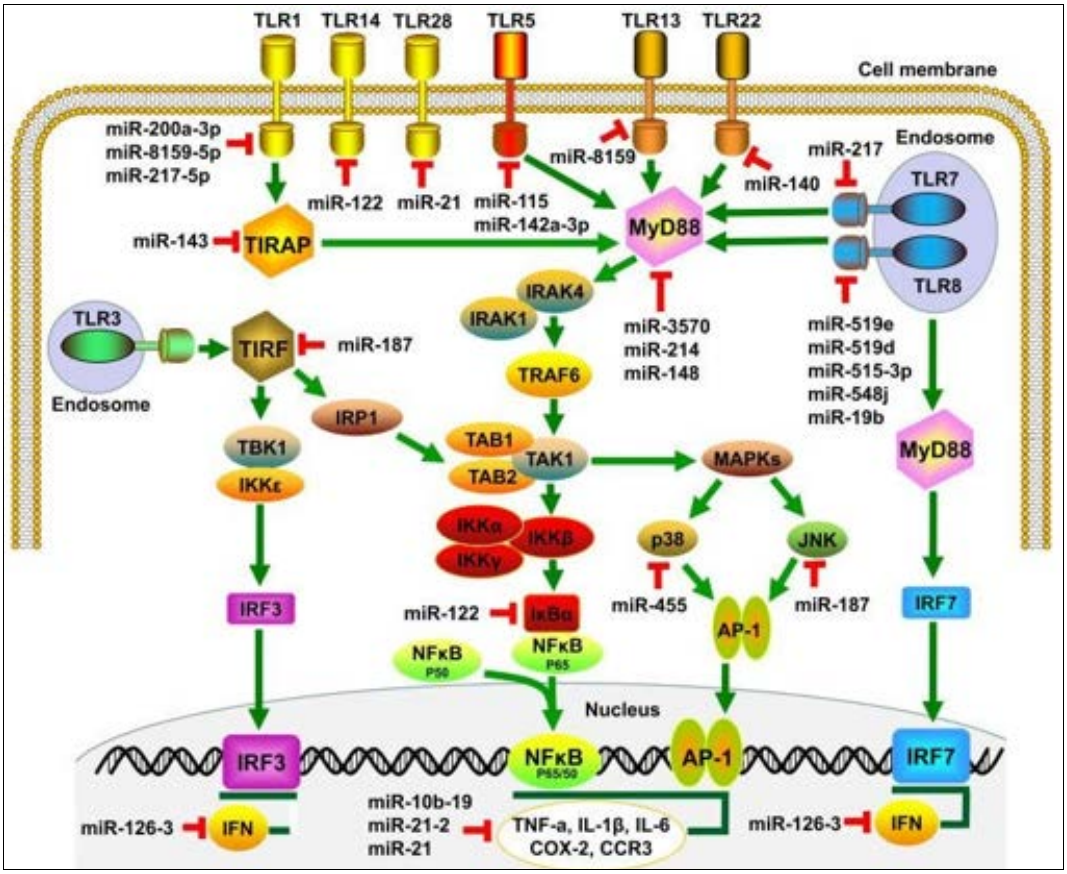

Fig 9: Toll-like receptors (TLRs) in teleost fish [90]

TLR3 recognizes synthetic polyI: C and double-stranded RNA. It is primarily found on the membrane of endosomes in dendritic cells, where the viral genome is uncoated for replication and transcription after entry into host cells [91]. TLR3 is found on the surface of human fibroblasts and epithelial cells. TLR3 dimerizes after activation and then recruits adapter proteins via the TIR domain to initiate a cascade of events that results in the induction of IFN expression [92, 93]. The crystal structure of the human TLR3 ectodomain has been determined, revealing a horseshoe form with 23 leucine-rich repeats (LRRs) with a typical signature of LxxLx LxxNx Lxx Lxxxx FxxL. The N and C terminal LRR domains are specifically involved in dsRNA molecule binding, which is thought to be at least 40-45 bp long [94].

TLR7 and TLR8 are two other TLRs that detect viral RNA PAMPs in the endosome. TLR7/8, unlike TLR3, bind to single-stranded viral RNA and enhance antiviral responses through various pathways depending on cell type. TLR7/8 can activate MyD88 in dendritic cells (DCs), the main professional antigen-presenting cells for viral pathogens, allowing phosphorylation of IRF1 and IRF7 and thus promoting IFN development. Other cell types, on the other hand, use NF- kB and MAPK signaling to boost the production of proinflammatory cytokines and chemokines including IL-1β, IL-6, IL-8, and TNF [95, 96]. TLR9 is a PRR that responds to DNA PAMPs. It is essential for sensing microbial nucleotide PAMPs exposed in the endosome in conjunction with TLR3, 7 and 8. TLR9 binds to unmethylated CpG DNA from bacteria or viruses and activates NF- kB and IRF-7 to produce proinflammatory cytokines and type I IFNs through MyD88 [97].

A subfamily of TLRs found in fish is not found in humans. TLR21/22 were discovered by bioinformatics research of the Fugu genome and are now known as TLR21/22. Three copies of TLR21 and a single copy of TLR22 are found in lamprey. TLR22 homologues are found in amphibians and birds but not in fish, suggesting that TLR22 was lost in tetrapods and is now a fish-specific TLR. This TLR community (TLR21) is thought to have been lost in mammals during evolution [98-100].

4.1.3 Nod-like Receptors (NLRs)

The intracellular pathogen recognition receptors (PRRs) known as nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) play a key role in pathogen recognition and subsequent activation of innate immune signaling pathways. Many different teleost fish species have been found to express many NLR subfamily members, including NOD1, NOD2, NLR-C3, NLR-C5, and NLR-X1. Lipopolysaccharides (LPS), peptidoglycans (PGN), and polyinosinic- polycytidylic acid [Poly (I:C)] are among the ligands that trigger these receptors. *In vitro* and *in vivo*, synthetic dsRNA and bacterial or viral infections have been shown to stimulate these receptors.

NLRs have an unusual structure in that they lack a signal peptide and a transmembrane domain, instead consisting of three domains: (a) A variable number of leucine-rich repeats (LRRs) in the C-terminal region, (b) an ATPase-active central nucleotide oligomerization (NACHT) domain, and (c) an N-terminal effector-binding domain (EBD). The C-terminal region is involved in ligand recognition and NLR activation, while the NACHT domain is involved in NLR activation and inflammatory response control, and the EBD is involved in interactions with adaptor molecules and downstream effector proteins.

The EBDs in mammals are structurally diverse, with four distinct domain types described. CARD (caspase-activation and recruitment domain), PYD (pyrin domain), BIR (baculovirus inhibitor of apoptosis protein repeat), and AD are the acronyms for these proteins (acidic activation domain). The NLRs in mammals are classified into five subfamilies based on the identities of these different N-terminal EBDs: NLR-A (AD containing), NLR-B (BIR containing), NLR-C (CARD containing), NLR-P (PYD containing), and NLR-X (X containing) (X stands for domain with no strong homology to the N-terminal domain of any other NLR subfamily member) [101]. The NLRs and their functions in the teleost fish are shown in the figure given below (Fig. 10).

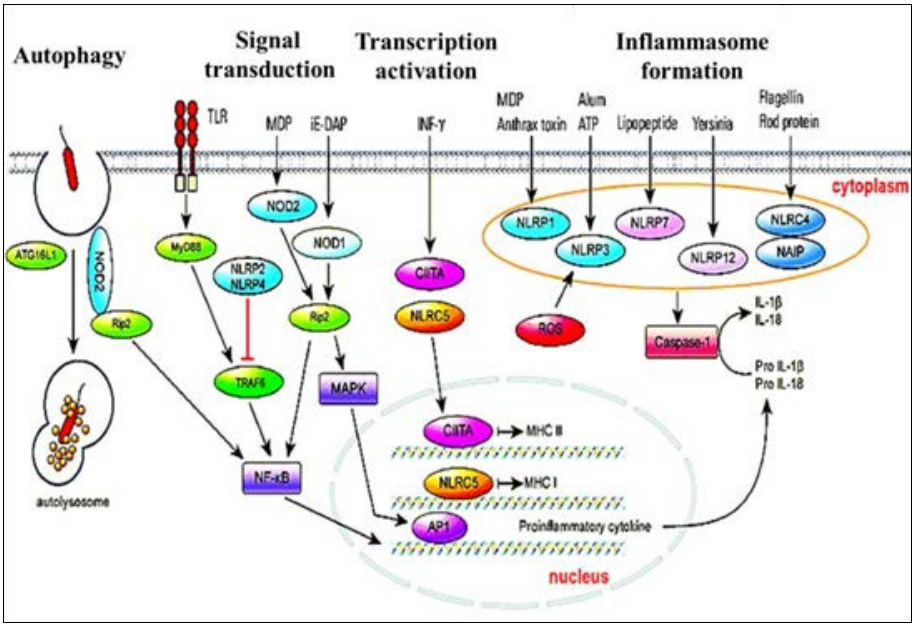

Fig 10: NOD-like receptors (NLRs) and functions of NOD-like receptors [102]

NLR expression in mammalian cells can be caused by bacteria, viruses, and synthetic dsRNA. NOD1, NOD2, NLR-C3, NLR-C5, and NLR-X1 are also expressed in a number of fish species in response to *in vitro* and *in vivo* stimulation

with lipopolysaccharides (LPS), peptidoglycans (PGN), or polyinosinic-polycytidylic acid [Poly(I:C)], as well as viral or bacterial infection. The expression of each NLR, on the other hand, varies depending on the fish species and the pathogenic stimulus. NOD1, NOD2, NLR-C3, NLR-C5, and NLR-X1 are widely expressed in the brain, gill, head kidney, trunk kidney, heart, intestine, liver, muscle, skin, spleen, stomach, ovary, blood, and leukocytes of healthy adult channel catfish (Ictalurus punctatus). Only NOD1 expression was increased in the intestine after stimulation with Edwardsiella ictaluri, a Gram-negative intracellular bacterium [103]. However, the findings of another study were very different. NOD1, NOD2, NLR-C3, and NLR-C5 expression was significantly increased in the intestine, liver, and head kidney after infection with either two Gram-negative bacteria, Edwardsiella tarda or Aeromonas hydrophila, a Gram-positive bacteria, Streptococcus in iae, or channel catfish haemorrhage reovirus (CCRV). In the spleen, however, expression was clearly reduced after infection with Aeromonas hydrophila or CCRV. NLR-X1 expression was significantly increased in the intestine, liver, and head kidney after exposure to the four pathogens described above, while expression in the spleen was clearly decreased. Streptococcus iniae caused the greatest increase in NOD mRNA levels among these four pathogens, while CCRV only slightly increased NOD gene expression [104].

It was evidently detected that after infection with the Gram-negative bacterium *Vibrio anguillarum*, turbot NLR-C3a and NLR-C3b expression was strongly induced in the intestine, but not in the skin or gills [105]. Similarly, infection with Gramnegative bacteria such as Vibrio alginolyticus or Staphylococcus aureus substantially increased the expression of intestinal NLR- C3 in Asian seabass (*Lates calcarifer*) [106]. The expression of turbot NLR-C3a and NLR-C3b increased more in the intestine after infection with Streptococcus iniae than in the skin or gill tissues [105]. The expression of the NLR-C3 gene in flounders was also significantly increased after stimulation with Edwardsiella tarda [107]; however, expression of the NLR-C3 gene in Channel catfish (Ictalurus punctatus) was significantly decreased after infection with Edwardsiella ictaluri [108, 109]. Similarly, after stimulation with LPS or infection with either Streptococcus iniae or Edwardsiella tarda, expression of a Japanese flounder (Paralichthys olivaceus) NLR-C transcript that clustered with the NLR-C3 group was significantly increased. Furthermore, NLR- C3 mRNA levels were clearly increased in the liver, trunk kidney, head kidney, and intestinal tissues 12 hours after infection with Aeromonas hydrophila [110]. In goldfish (*Carassius auratus*), the spleen has the highest basal mRNA expression levels, followed by the intestine, gills, brain, and kidney, with lower expression levels in the heart, liver, and muscle. Macrophages had a higher level of transcript expression than other immune cell populations. NLRC-3L expression in macrophages was significantly increased after infection with heat-killed Aeromonas salmonicida at the protein level [111]. The role of NOD1 and NOD2 in bacterial and viral immune responses has been investigated in mammals [112-115]. NOD1 and NOD2 can interact directly with IPS-1 (interferon-b promoter stimulator protein 1) in mammals, causing NF-jB (nuclear factor kappa B) and IRF-3 to be activated (interferon regulatory factor 3). These proteins then trigger the formation of pro- inflammatory cytokines and type I interferons (interferon). These processes are thought to serve as a link between innate and adaptive immunity, and their activation triggers a powerful immune response that helps to control viral and bacterial infections [116] (Fig. 11).

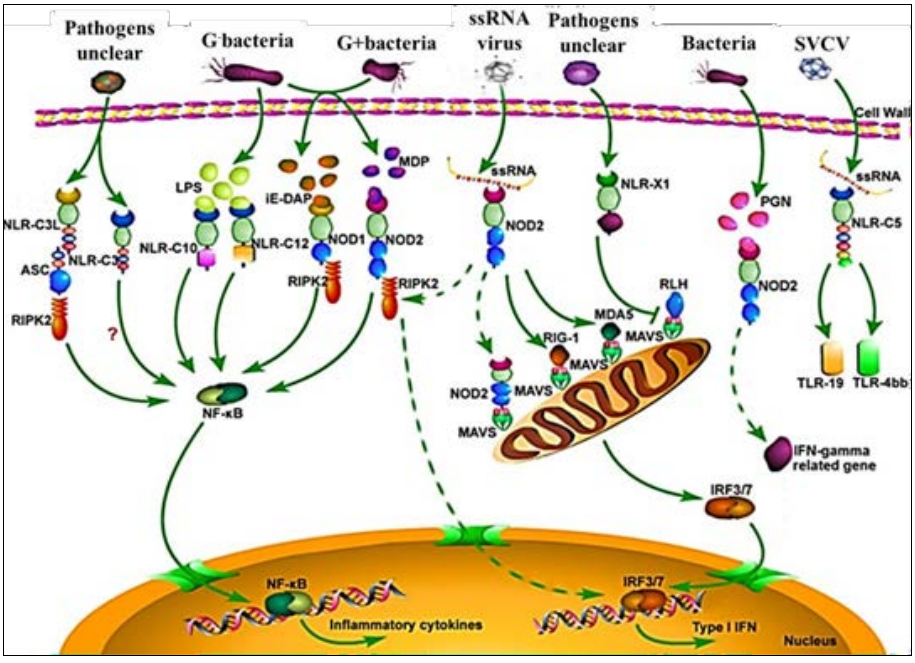

Fig 11: NOD-mediated signaling pathway in teleost fish [117]

In contrast to other fish species, the roles of NOD2 in response to viral and bacterial infections have been studied extensively in zebrafish. Overexpression of NOD2 in zebrafish (*Danio rerio*) embryos, for example, can result in increased expression of immune-related genes. The expression of genes encoding PRRs and cytokines involved in the antiviral response, such as RIPK2, MDA5, RIG-I, type I IFN, and MAVS, was increased in particular; these findings suggest a link between NOD2 expression and antiviral activity of these molecules. The expression of NOD2, MAVS, and RIPK2 was also significantly increased after infection with SVCV or Edwardsiella tarda. The NF-jB and type I IFN promoters were also activated when zebrafish NOD2 was overexpressed. NOD2 and RIPK2 co-expression resulted in

substantially increased NF-jB activity as well as increased resistance to viral and bacterial infections. Furthermore, MAVS may enhance NOD2-induced NF-jB and IFN promoter activation, implying that, as in mammals, NOD2 can interact with MAVS to induce a viral immune response in fish [118].

NLRs are intracellular PRRs in teleost fish that are responsible for the identification of different PAMPs. NLRs, such as NOD1, NOD2, NLR-C3, NLR-C5, and NLR-X1, are critical components of the immune response. NOD1 recruits RIPK2 in response to pathogen stimulation, and NOD2 recruits downstream adaptors such as RIPK2, RIG-I, and MDA5, resulting in the activation of NF-jB or IRF-3/7 and the formation of pro-inflammatory cytokines and type I IFN. Some NLRs, such as NLR-C3 and NLR-C5, can, on the other hand, suppress the development of pro-inflammatory cytokines and type I interferons. Despite the discovery of numerous NLRs and downstream effectors in some teleost fish species, the origin and evolution of NLRs, NLR ligand specificity, and mechanisms controlling NLR signaling pathways in teleost fish remain poorly understood. Importantly, to gain a complete understanding of the role of NLRs in fish, further research is required in these areas [107].

4.1.4 C-type Lectin Receptors (CLR)

CLR is a large receptor family with over 1,000 members that perform a variety of functions such as cell adhesion, complement activation, tissue remodeling, platelet activation, endocytosis, phagocytosis, and innate immunity activation [119]. TLR15, which belongs to the TLR2 subfamily phylogenetically, has been found to be unique to avian species [120]. RLRs, which are involved in the antiviral signaling pathway, have been extensively studied in a variety of vertebrate organisms [67]. Since the discovery of a novel NLR subfamily (NLRC) in zebrafish with a PRY/SPRY (B30.2) domain [121, 122], reports about NLRs in bony fish have gradually increased, especially about NOD1, NOD2, and NOD3 genes [109, 123].

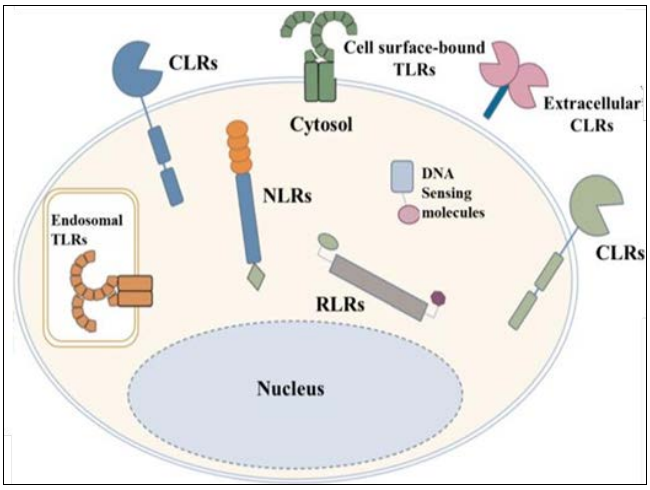

Fig 12: C-type Lectin Receptors (CLR)

In comparison to TLRs and RLRs, there have been few systematic studies of the CLRs family in bony fish (Fig. 12). Though many researchers have worked on the evolution of fish innate immunity systems in the last two decades, most studies have primarily focused on teleost fish especially zebrafish (Danio rerio) [67, especially zebrafish (Danio rerio) {Jault, 2004 #395, 124, 125] especially zebrafish (Danio rerio).

CLRs are proteins that can identify a wide range of PAMPs and/or DAMPs, as well as endogenous ligands. They can be found as soluble or transmembrane proteins. Traditionally, they were thought to only bind carbohydrates, but they can also bind lipid and protein ligands. Based on motifs present in the CTLD, traditional carbohydrate-binding CLRs can be classified into two groups: those that bind mannose-type carbohydrates through their EPN motif and those that bind galactose-type carbohydrates through their QPD motif. CLRs have been classified into 17 classes for clustering purposes based on domain organization and phylogeny. In CLRs, the groups II, III and V have developed roles in antimicrobial innate immunity and are beginning to emerge in adaptive immunity [126-128].

4.2 Biological insights gained in fish studies using RNA-seq

The discovery and subsequent characterization of immune-related genes and unique pathways involved in immune responses from fish transcriptomes before and after immune challenges aids in the development of immune-based therapy for fish diseases, the selection of disease-resistant fish brood stocks, and the understanding of the origin and evolution of the immune system. This type of research has used RNA-seq on live fish, fish embryos, and fish primary cells.

Fish immune responses to pathogens are studied using RNA-seq primarily in economically important species. For example, using Illumina sequencing, the transcriptomic response of channel catfish gills to *Flavobacterium columnare*, a Gram-negative bacterium implicated in fish disease outbreaks around the world, was investigated [129]. Using a 1.5-fold shift cut-off, 2605 uniquely annotated genes were found to have important differential expression patterns in pathogen identification, cytoskeletal dynamics, cell junction integrity, oxidative stress responses, apoptosis, lysosomal processes, and pro- and anti-inflammatory pathways. RNA-seq identified fifteen differentially expressed genes, which were confirmed by quantitative PCR (qPCR). A rhamnose- binding lectin (RBL) gene was highlighted in this study, with a 105-fold increase in expression. RBL ligands, L-rhamnose and D-galactose, strongly protected channel catfish against columnaris disease in a dose-dependent manner, according to a subsequent study by the same group [130]. The same team looked into the transcriptomic changes in the intestinal epithelium of channel catfish after being exposed to *Edwardsiella ictaluri* [131]. In this study, 1633 differentially expressed genes were discovered, many of which are involved in actin cytoskeletal polymerization/remodeling and junctional control in pathogen entry and subsequent inflammatory responses. *Vibrio harveyi* was challenged in Asian seabass (*Lates calcarifer*) [132] and Japanese sea bass (*Lateolabrax japonicus*) [133].

RNA-seq was used to examine the immunization-related gene expression patterns of zebrafish and European sea bass immunized with vaccines against *Edwardsiella tarda* and *Vibrio anguillarum*, respectively, to explain the host immune mechanisms underlying the protective effects of vaccines and enhance their immunogenicity in future efforts. 4565 genes were found to be differentially expressed in zebrafish liver transcriptome samples before and after immunization (2186 up-regulated and 2379 down-regulated) [134]. Further qPCR analysis revealed that genes encoding factors involved in the MHC-I processing pathway were upregulated, while genes

involved in the MHC-II processing pathway were downregulated. Differential expression was found for 496 transcripts in the head kidney and 336 transcripts in the gut in an RNA-seq analysis with European sea bass [135].

Aside from *in vivo* research, RNA-seq was used to study the immune response of fish embryos and primary cells. RNA-seq and tag-based sequencing were used to investigate the innate host immune response to inflammatory bacterial infection in zebrafish embryos infected with *Salmonella typhimurium* [136]. Two sequencing methods were used in this analysis, which reported a clear correlation of sequence read counts per transcript and an overlap of 241 transcripts that were differentially expressed in response to infection. Transcripts encoding transcription factors, signal transduction proteins, cytokines and chemokines, complement factors, proteins involved in apoptosis and proteolysis, antimicrobial proteins, and many other recognized and novel proteins not previously linked to the immune response were discovered. RNA-Seq study of Poly (I:C) (Polyinosinic: polycytidylic acid)-challenged rainbow trout erythrocytes also revealed a diverse collection of differentially expressed mRNA transcripts linked to a variety of physiological systems, including the endocrine, reproductive, and immune systems.

4.3 Mechanism of Pathogen Infection in Teleost

RNA-Seq technology has been used in studies to better understand fish pathogens and pathogen evasion strategies against the immune system, often during bacterial infection. The most widely used fish species, the zebrafish (*Danio rerio*), is an outstanding research model. The role of Traf6 function in the innate immune response {Stockhammer, 2010 #428} and transcriptome changes during infection [136] were studied using zebrafish embryos infected with *Salmonella typhimurium*. Similarly, during *Mycobacterium marinum* (*M. marinum*) infection, immune-responsive behavior of muscle tissue were discovered in zebrafish [137]. *M. marinum* infection showed 39 TIR domains with transcript isoforms and genes in common carps (*Cyprinus carpio*) [138]. Infections with *Mycobacterium marinum* in fish are used as a model to study tuberculosis in humans caused by *Mycobacterium tuberculosis. Edwardsiella ictaluri* (*E. ictaluri*) causes enteric septicemia in catfish, which is a financially significant disease that affects the catfish aquaculture industry. The value of actin cytoskeletal polymerization and remodeling, as well as junctional control of *E. ictaluri* entry by intestinal barrier destruction, was discovered during research on pathogen entry mechanisms and the mucosal immune response in channel catfish (*Ictalurus punctatus*) [131]. The immune evasion mechanisms of *Flavobacterium columnare* (*F. columnare*), which targets mucosal organs such as the gill and the skin as entry points, were studied using RNA-Seq. The pathogen was evaded by a strain of channel catfish susceptible to *F. columnare* infection that had a basal polarization of gill mucosal compartments with a putative mucosecretory and tolerogenic phenotype [139]. The European sea bass (Dicentrarchus labrax) was given an oral vaccine against *V. anguillarum*, which resulted in increased expression of unusual transcripts including leukocyte immune-type receptors and cullin or supervillain [135].

Viruses are a major threat to the fish farming industry, resulting in significant economic losses. If a virus has spread beyond a limited number of host cells, a variety of nonspecific and unique host defense mechanisms are enabled in an effort to contain the virus and mitigate host cell damage [140]. The role of MDA5 and Janus kinase (JAK)-mediated signaling pathways in preventing viral invasion was discovered using RNA-Seq [141]. The Singapore grouper iridovirus (SGIV) infected orange-spotted grouper had a major impact on the mitogen-activated protein kinase, chemokines, TLRs, and RIG-I signaling pathways [142]. Megalocytivirus, another iridovirus, is known to cause disease outbreaks during the summer months when the temperatures are high. The temperature has a major role in the global transcription of turbot (*Scophthalmus maximus*) during megalocytivirus infection in low and high temperatures, which affects the immune system, according to comparative transcriptomics of turbot (*Scophthalmus maximus*) during megalocytivirus infection in low and high temperatures [143].

RNA-Seq-based transcriptomics study is an invaluable method not only for revealing host– viral interactions in aquatic species, but also for characterizing viral mechanisms for evading host defense mechanisms.

5. Conclusion

The key members of the TLR/RLR/NLR families were summarized in teleost in this review. Despite the significant similarity of PRRs and their downstream components between teleost and mammal, teleost PRR signaling pathways have certain unique characteristics. As a result, more extensive investigations are needed to increase our understanding of PRR networks in teleosts. Notably, more research is needed in these areas to acquire a complete understanding of TLRs, RLRs, and NLRs in teleosts. Understanding the evolution of TLRs/RLRs/NLRs in teleosts is essential to thoroughly investigate the vital function and illuminate the mechanism of TLRs/RLRs/NLRs after pathogen infection. While TLRs/RLRs/NLRs expression patterns in teleosts rise following stimulation, the ligand specificities of individual TLRs/RLRs/NLRs are poorly understood and require further research. The precise immunoregulatory mechanisms involved in the immunological response of teleost TLRs/RLRs/NLRs are rarely disclosed. As a result, molecules participating in signaling pathways mediated by TLRs, RLRs, and NLRs must yet be discovered and functionally defined. Thus, these areas are systematically explored in this review which will shed new light on the diversity and function of PRRs in teleost.

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References

1. Lei J *et al*. Review of “green house + deep well seawater” industrialized culture pattern of turbot (*Scophthalmus maximus*). Marine Fisheries Research 2002;23(4):1-7.
2. Xinfu LQL. An Primary Study on Culture of Turbot, Scophthalmus Maeoticus L. [J]. Modern Fisheries Information 1995, 11.
3. Li X *et al*. Effect of flow velocity on the growth, stress and immune responses of turbot (*Scophthalmus maximus*) in recirculating aquaculture systems. Fish & shellfish immunology 2019;86:1169-1176.
4. Jia R *et al*. The physiological performance and immune response of juvenile turbot (*Scophthalmus maximus*) to nitrite exposure. Comparative Biochemistry and

Physiology Part C: Toxicology & Pharmacology 2016;181:40-46.
5. FAO. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome 2020.
6. Martínez P *et al*. Turbot (*Scophthalmus maximus*) genomic resources: application for boosting aquaculture production, in Genomics in aquaculture, Elsevier 2016, 131-163.
7. Huang S-P, Yang Z-Y. Economical analysis on the turbot industrial cultivation in China. Guangdong Agricultural Sciences 2011;16:113-116.
8. Cang P, Yang Z, Duan Y, The economies of scale of turbot industrial running water aquaculture system in China: a case from Shandong province. Turkish Journal of Fisheries and Aquatic Sciences 2018;18(1):167-173.
9. FAO. The state of World Fisheries and Aquaculture 2020. Sustainability in action. Rome 2020.
10. Fisheries C. National Modern Agricultural Industrial Technology System Turbot industry development report 2021, (1).
11. Aydın İ *et al*. Turbot and flounder aquaculture. Deniz Çoban 106.
12. Burchiel SW *et al*. Assessment of immunotoxicity by multiparameter flow cytometry. Toxicological Sciences, 1997;38(1):38-54.
13. Ostrander GK, Hopkins J. The laboratory fish 2000.
14. Rand GM. Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment. CRC press 1995.
15. Powell DB. Immune system, in the laboratory fish. Elsevier 2000, 441-449.
16. Press CM, Evensen Ø. The morphology of the immune system in teleost fishes. Fish & shellfish immunology 1999;9(4):309-318.
17. Anderson D, Zeeman M. Immunotoxicology in fish 1995.
18. Pastoret P-P *et al*. Handbook of vertebrate immunology. 1998: Academic Press.
19. Köllner B *et al*. Evaluation of immune functions of rainbow trout (*Oncorhynchus mykiss*)—how can environmental influences be detected? Toxicology letters 2002;131(1-2):83-95.
20. Johansen LH, Sommer AI. Multiplication of infectious pancreatic necrosis virus (IPNV) in head kidney and blood leucocytes isolated from Atlantic salmon, Salmo salar L. Journal of Fish Diseases 1995;18(2):147-156.
21. Stark GR *et al*. How cells respond to interferons. Annual review of biochemistry 1998;67(1):227-264.
22. Robertsen B. Modulation of the non-specific defence of fish by structurally conserved microbial polymers. Fish & Shellfish Immunology 1999;9(4):269-290.
23. Trobridge GD, J-A, Leong C. Characterization of a rainbow trout Mx gene. Journal of Interferon & Cytokine Research 1995;15(8):691-702.
24. Trobridge GD, Chiou PP, Leong J. Cloning of the rainbow trout (*Oncorhynchus mykiss*) Mx2 and Mx3 cDNAs and characterization of trout Mx protein expression in salmon cells. Journal of virology 1997;71(7):5304-5311.
25. Trobridge G *et al*. Induction of the Mx protein of rainbow trout *Oncorhynchus mykiss in vitro* and *in vivo* with poly I: C dsRNA and infectious hematopoietic necrosis virus. Diseases of Aquatic Organisms 1997;30(2):91-98.
26. Nygaard R *et al*. Induction of Mx protein by interferon and double-stranded RNA in salmonid cells. Fish & shellfish immunology 2000;10(5):435-450.
27. Shi C-Y *et al*. The first report of an iridovirus-like agent infection in farmed turbot, *Scophthalmus maximus*, in China. Aquaculture 2004;236(1-4):11-25.
28. Hiney M, Smith P, Bernoth E-M. Covert Aeromonas salmonicida infections, in *Furunculosis*. Elsevier 1997, 54-97.
29. Smail D, Woo P, Bruno D. Fish Diseases and Disorders: Viral, Bacterial and Fungal Infections 1999.
30. Pizer SM *et al*. Adaptive histogram equalization and its variations. Computer vision, graphics, and image processing 1987;39(3):355-368.
31. Antipa R, Gould R, Amend D. *Vibrio anguillarum* vaccination of sockeye salmon *Oncorhynchus nerka (Walbaum)* by direct and hyperosmotic immersion. Journal of Fish Diseases 1980;3(2):161-165.
32. Reed P, Francis-Floyd R, Klinger R. Monogenean parasites of fish. University of Florida IFAS Extension 2002.
33. Lin YK, Liu DC. Comparison of physical–chemical properties of type I collagen from different species. Food Chemistry 2006;99(2):244-251.
34. Sabin LR, Hanna SL, Cherry S. Innate antiviral immunity in Drosophila. Current opinion in immunology 2010;22(1):4-9.
35. Zipfel C. Pattern-recognition receptors in plant innate immunity. Current opinion in immunology 2008;20(1):10-16.
36. Ding S-W, Voinnet O. Antiviral immunity directed by small RNAs. Cell 2007;130(3):413-426.
37. Deddouche S *et al*. The DExD/H-box helicase Dicer-2 mediates the induction of antiviral activity in drosophila. Nature immunology 2008;9(12):1425.
38. Garcia-Ruiz H *et al*. Arabidopsis RNA-dependent RNA polymerases and dicer-like proteins in antiviral defense and small interfering RNA biogenesis during Turnip Mosaic Virus infection. The Plant Cell 2010;22(2):481-496.
39. Tolia NH, Joshua-Tor L. Slicer and the argonautes. Nature chemical biology 2007;3(1):36-43.
40. Aliyari R, Ding SW. RNA-based viral immunity initiated by the Dicer family of host immune receptors. Immunological reviews 2009;227(1):176-188.
41. Li M *et al*. Interferon-λs: the modulators of antivirus, antitumor, and immune responses. Journal of leukocyte biology 2009;86(1):23-32.
42. Robertsen B. The interferon system of teleost fish. Fish & shellfish immunology 2006;20(2):172-191.
43. Billiau A, Matthys P. Interferon-γ: a historical perspective. Cytokine & growth factor reviews 2009;20(2):97-113.
44. Samuel CE. Antiviral actions of interferons. Clinical microbiology reviews 2001;14(4):778-809.
45. Samuel CE. Mechanisms of the antiviral action of interferons. Progress in nucleic acid research and molecular biology 1988;35:27-72.
46. Pestka S *et al*. Interferons and their actions. Annual review of biochemistry 1987;56(1):727-777.
47. Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. Journal of general virology 2008;89(1):1-47.
48. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010;140(6):805-820.
49. Brennan K, Bowie AG. Activation of host pattern

recognition receptors by viruses. Current opinion in microbiology 2010;13(4):503-507.
50. Pichlmair A, e Sousa CR. Innate recognition of viruses. Immunity 2007;27(3):370-383.
51. Sabbah A *et al*. Activation of innate immune antiviral responses by Nod2. Nature immunology 2009;10(10):1073.
52. Wilkins C, Gale Jr M. Recognition of viruses by cytoplasmic sensors. Current opinion in immunology 2010;22(1):41-47.
53. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nature immunology 2010;11(5):373.
54. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. International immunology 2009;21(4):317-337.
55. Shayakhmetov DM, Di Paolo NC, Mossman KLJMT. Recognition of virus infection and innate host responses to viral gene therapy vectors 2010;18(8):1422-1429.
56. Zou J *et al*. Identification and bioactivities of IFN-γ in rainbow trout *Oncorhynchus mykiss*: the first Th1-type cytokine characterized functionally in fish. The Journal of Immunology 2005;175(4):2484-2494.
57. Zou J *et al*. Identification of a second group of type I IFNs in fish sheds light on IFN evolution in vertebrates. The Journal of Immunology 2007;179(6):3859-3871.
58. Xu C *et al*. Alpha interferon and not gamma interferon inhibits salmonid alphavirus subtype 3 replication *in vitro*. Journal of virology 2010;84(17):8903-8912.
59. Savan R *et al*. Structural conservation of interferon gamma among vertebrates. Cytokine & growth factor reviews 2009;20(2):115-124.
60. Furnes C, Seppola M, Robertsen B. Molecular characterisation and expression analysis of interferon gamma in Atlantic cod (Gadus morhua). Fish & shellfish immunology 2009;26(2):285-292.
61. Chang M *et al*. Identification of an additional two-cysteine containing type I interferon in rainbow trout *Oncorhynchus mykiss* provides evidence of a major gene duplication event within this gene family in teleosts. Immunogenetics 2009;61(4):315-325.
62. Casani D *et al*. Molecular characterisation and structural analysis of an interferon homologue in sea bass (*Dicentrarchus labrax* L.). Molecular immunology 2009;46(5):943-952.
63. Robertsen B *et al*. Atlantic salmon interferon genes: cloning, sequence analysis, expression, and biological activity. Journal of Interferon & Cytokine Research 2003;23(10):601-612.
64. Long S *et al*. Identification of a cDNA encoding channel catfish interferon. Developmental & Comparative Immunology 2004;28(2):97-111.
65. Altmann SM *et al*. Molecular and functional analysis of an interferon gene from the zebrafish, Danio rerio. Journal of virology 2003;77(3):1992-2002.
66. Onoguchi K, Yoneyama M, Fujita T. Retinoic acid-inducible gene-I-like receptors. Journal of Interferon & Cytokine Research 2011;31(1):27-31.
67. Chen SN, Zou PF, Nie P. Retinoic acid-inducible gene I (RIG-I)-like receptors (RLR s) in fish: current knowledge and future perspectives. Immunology 2017;151(1):16-25.
68. Kumar H, Kawai T, Akira S. Pathogen recognition in the innate immune response. Biochemical Journal 2009;420(1):1-16.
69. Zou PF *et al*. Higher antiviral response of RIG-I through enhancing RIG-I/MAVS- mediated signaling by its long insertion variant in zebrafish. Fish & shellfish immunology 2015;43(1):13-24.
70. Zou PF *et al*. Melanoma differentiation-associated gene 5 in zebrafish provoking higher interferon-promoter activity through signalling enhancing of its shorter splicing variant. Immunology 2014;141(2):192-202.
71. Chang M *et al*. Expression and functional characterization of the RIG-I-like receptors MDA5 and LGP2 in Rainbow trout (*Oncorhynchus mykiss*). Journal of virology 2011;85(16):8403-8412.
72. Ohtani M *et al*. Characterization and antiviral function of a cytosolic sensor gene, MDA5, in Japanese flounder, Paralichthys olivaceus. Developmental & Comparative Immunology 2011;35(5):554-562.
73. Yang C *et al*. Identification of a retinoic acid-inducible gene I from grass carp (Ctenopharyngodon idella) and expression analysis *in vivo* and *in vitro*. Fish & shellfish immunology 2011;30(3):936-943.
74. Sun F *et al*. Fish MITA serves as a mediator for distinct fish IFN gene activation dependent on IRF3 or IRF7. The Journal of Immunology 2011;187(5):2531-2539.
75. Feng H *et al*. Expression profiles of carp IRF-3/-7 correlate with the up-regulation of RIG-I/MAVS/TRAF3/TBK1, four pivotal molecules in RIG-I signaling pathway. Fish & shellfish immunology 2011;30(4-5):1159-1169.
76. Rajendran K *et al*. Pathogen recognition receptors in channel catfish: II. Identification, phylogeny and expression of retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). Developmental & Comparative Immunology 2012;37(3-4):381-389.
77. Sarkar D, DeSalle R, Fisher PB. Evolution of MDA-5/RIG-I-dependent innate immunity: independent evolution by domain grafting. Proceedings of the National Academy of Sciences 2008;105(44):17040-17045.
78. Zou J *et al*. Origin and evolution of the RIG-I like RNA helicase gene family. BMC evolutionary biology 2009;9(1):1-14.
79. Chen H-Y *et al*. RIG-I specifically mediates group II type I IFN activation in nervous necrosis virus infected zebrafish cells. Fish & shellfish immunology 2015;43(2):427-435.
80. Jia P *et al*. Identification and characterization of the melanoma differentiation– associated gene 5 in sea perch, *Lateolabrax japonicus*. Developmental & Comparative Immunology 2016;61:161-168.
81. Huang T *et al*. Identification and expression profiling analysis of grass carp Ctenopharyngodon idella LGP2 cDNA. Fish & shellfish immunology 2010;29(2):349-355.
82. Ohtani M *et al*. Evolutional conservation of molecular structure and antiviral function of a viral RNA receptor, LGP2, in Japanese flounder, Paralichthys olivaceus. The Journal of Immunology 2010;185(12):7507-7517.
83. Chen L *et al*. Trunk kidney of grass carp (Ctenopharyngodon idella) mediates immune responses against GCRV and viral/bacterial PAMPs *in vivo* and *in vitro*. Fish & shellfish immunology 2013;34(3):909-919.
84. Sullivan C *et al*. The gene history of zebrafish tlr4a and tlr4b is predictive of their divergent functions. The Journal of Immunology 2009;183(9):5896-5908.

85. Bi D *et al*. Recognition of lipopolysaccharide and activation of NF-κB by cytosolic sensor NOD1 in teleost fish. Frontiers in immunology 2018;9:1413.
86. Berczi I, Bertok L, Bereznai T. Comparative studies on the toxicity of Escherichia coli lipopolysaccharide endotoxin in various animal species. Canadian journal of microbiology 1966;12(5):1070-1071.
87. Sullivan C *et al*. Evidence for evolving Toll-IL-1 receptor-containing adaptor molecule function in vertebrates. The Journal of Immunology 2007;178(7):4517-4527.
88. Anderson KV, Jürgens G, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. Cell 1985;42(3):779-789.
89. Oshiumi H *et al*. Prediction of the prototype of the human Toll-like receptor gene family from the pufferfish, Fugu rubripes, genome. Immunogenetics 2003;54(11):791-800.
90. Zhou Z *et al*. MicroRNA regulation of Toll-like receptor signaling pathways in teleost fish 2018;75:32-40.
91. Matsumoto M *et al*. Subcellular localization of Toll-like receptor 3 in human dendritic cells. The Journal of Immunology 2003;171(6):3154-3162.
92. Botos I *et al*. The toll-like receptor 3: dsRNA signaling complex. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms 2009;1789(9-10):667-674.
93. Choe J, Kelker MS, Wilson IA. Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science 2005;309(5734):581-585.
94. Liu L *et al*. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science 2008;320(5874):379-381.
95. Diebold SS. Recognition of viral single-stranded RNA by Toll-like receptors. Advanced drug delivery reviews 2008;60(7):813-823.
96. Lee HK *et al*. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 2007;315(5817):1398-1401.
97. Kumagai Y, Takeuchi O, Akira S. TLR9 as a key receptor for the recognition of DNA. Advanced drug delivery reviews 2008;60(7):795-804.
98. Kasamatsu J *et al*. Phylogenetic and expression analysis of lamprey toll-like receptors. Developmental & Comparative Immunology 2010;34(8):855-865.
99. Oshiumi H *et al*. Pan-vertebrate toll-like receptors during evolution. Current genomics 2008;9(7):488-493.
100. Temperley ND *et al*. Evolution of the chicken Toll-like receptor gene family: a story of gene gain and gene loss. BMC genomics 2008;9(1):1-12.
101. Pontillo A, Crovella S. NOD-like receptors: a tail from plants to mammals through invertebrates. Current Protein and Peptide Science 2017;18(4):311-322.
102. Kim YK, Shin J-S, Nahm MHJYMJ. NOD-like receptors in infection, immunity, and diseases 2016;57(1):5.
103. Sha Z *et al*. NOD-like subfamily of the nucleotide-binding domain and leucine-rich repeat containing family receptors and their expression in channel catfish. Developmental & Comparative Immunology 2009;33(9):991-999.
104. Li M *et al*. Expression profiles of NODs in channel catfish (*Ictalurus punctatus*) after infection with Edwardsiella tarda, Aeromonas hydrophila, Streptococcus iniae and *channel catfish hemorrhage reovirus*. Fish & shellfish immunology 2012;33(4):1033-1041.
105. Hou Z *et al*. Characterization and expression profiling of NOD-like receptor C3 (NLRC3) in mucosal tissues of turbot (*Scophthalmus maximus* L.) following bacterial challenge. Fish & shellfish immunology 2017;66:231-239.
106. Paria A *et al*. Identification of Nod like receptor C3 (NLRC3) in Asian seabass, *Lates calcarifer*: characterisation, ontogeny and expression analysis after experimental infection and ligand stimulation. Fish & shellfish immunology 2016;55:602-612.
107. Zhang L *et al*. Nucleotide-binding and oligomerization domain (NOD)-like receptors in teleost fish: Current knowledge and future perspectives. Journal of fish diseases 2018;41(9):1317-1330.
108. Chang MX *et al*. The expanding and function of NLRC3 or NLRC3-like in teleost fish: recent advances and novel insights. Developmental & Comparative Immunology 2020, 103859.
109. Rajendran K *et al*. Pathogen recognition receptors in channel catfish: I. Identification, phylogeny and expression of NOD-like receptors. Developmental & Comparative Immunology 2012;37(1):77-86.
110. Unajak S *et al*. Molecular characterization, expression and functional analysis of a nuclear oligomerization domain proteins subfamily C (NLRC) in Japanese flounder (Paralichthys olivaceus). Fish & shellfish immunology 2011;31(2):202-211.
111. Xie J, Belosevic M. Characterization and functional assessment of the NLRC3-like molecule of the goldfish (*Carassius auratus* L.). Developmental & Comparative Immunology 2018;79:1-10.
112. Barnich N *et al*. Membrane recruitment of NOD2 in intestinal epithelial cells is essential for nuclear factor–κB activation in muramyl dipeptide recognition. The Journal of cell biology 2005;170(1):21-26.
113. Kufer T *et al*. Role for erbin in bacterial activation of Nod2. Infection and immunity 2006;74(6):3115-3124.
114. Tattoli I *et al*. The Nodosome: Nod1 and Nod2 control bacterial infections and inflammation. in Seminars in immunopathology. Springer 2007.
115. Uehara A *et al*. Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. Molecular immunology 2007;44(12):3100-3111.
116. Ting JP, Duncan JA, Lei Y. How the noninflammasome NLRs function in the innate immune system. Science 2010;327(5963):286-290.
117. Zhang L *et al*. Nucleotide-binding and oligomerization domain (NOD)-like receptors in teleost fish: Current knowledge and future perspectives 2018;41(9):1317-1330.
118. Zou PF *et al*. NOD2 in zebrafish functions in antibacterial and also antiviral responses via NF-κB, and also MDA5, RIG-I and MAVS. Fish & shellfish immunology 2016;55:173-185.
119. Dambuza IM, Brown GD. C-type lectins in immunity: recent developments. Current opinion in immunology 2015;32:21-27.
120. Brownlie R, Allan B. Avian toll-like receptors. Cell and tissue research 2011;343(1):121-130.
121. Laing KJ *et al*. A genomic view of the NOD-like receptor family in teleost fish: identification of a novel NLR

subfamily in zebrafish. BMC evolutionary biology 2008;8(1):1-15.
122. Howe K *et al*. Structure and evolutionary history of a large family of NLR proteins in the zebrafish. Open biology 2016;6(4):160009.
123. Li J, Chu Q, Xu T. A genome-wide survey of expansive NLR-C subfamily in miiuy croaker and characterization of the NLR-B30. 2 genes. Developmental & Comparative Immunology 2016;61:116-125.
124. Drickamer KT, Maureen E. Recent insights into structures and functions of C-type lectins in the immune system. Current opinion in structural biology 2015;34:26-34.
125. Meijer AH *et al*. Expression analysis of the Toll-like receptor and TIR domain adaptor families of zebrafish. Molecular immunology 2004;40(11):773-783.
126. Weis WI, Taylor ME, Drickamer K. The C-type lectin superfamily in the immune system. Immunological reviews 1998;163(1):19-34.
127. Drickamer K, Dell A, Fadden AJ. Genomic analysis of C-type lectins. In Biochemical Society Symposia Portland Press 2002.
128. Zelensky AN, Gready JE. The C-type lectin-like domain superfamily. The FEBS journal 2005;272(24):6179-6217.
129. Sun F *et al*. Transcriptomic signatures of attachment, NF-κB suppression and IFN stimulation in the catfish gill following columnaris bacterial infection. Developmental & Comparative Immunology 2012;38(1):169-180.
130. Beck BH *et al*. Putative roles for a rhamnose binding lectin in Flavobacterium columnare pathogenesis in channel catfish Ictalurus punctatus. Fish & shellfish immunology 2012;33(4):1008-1015.
131. Li C *et al*. RNA-seq analysis of mucosal immune responses reveals signatures of intestinal barrier disruption and pathogen entry following Edwardsiella ictaluri infection in channel catfish, Ictalurus punctatus. Fish & shellfish immunology 2012;32(5):816- 827.
132. Xia JH *et al*. Analysis of stress-responsive transcriptome in the intestine of Asian seabass (*Lates calcarifer*) using RNA-Seq. DNA research 2013;20(5):449-460.
133. Xiang L-X *et al*. Deep sequencing-based transcriptome profiling analysis of bacteria- challenged *Lateolabrax japonicus* reveals insight into the immune-relevant genes in marine fish. BMC genomics 2010;11(1):1-21.
134. Yang D *et al*. RNA-seq liver transcriptome analysis reveals an activated MHC-I pathway and an inhibited MHC-II pathway at the early stage of vaccine immunization in zebrafish. BMC genomics 2012;13(1):1-16.
135. Sarropoulou E *et al*. Characterization of European sea bass transcripts by RNA SEQ after oral vaccine against *V. anguillarum*. Marine biotechnology 2012;14(5):634-642.
136. Ordas A *et al*. Deep sequencing of the innate immune transcriptomic response of zebrafish embryos to Salmonella infection. Fish & shellfish immunology 2011;31(5):716-724.
137. Chen L *et al*. Characterization of Mycobacterium marinum infections in zebrafish wounds and sinus tracts. Wound Repair and Regeneration 2017;25(3):536-540.
138. Zhang Y *et al*. Identification of common carp innate immune genes with whole-genome sequencing and RNA-Seq data. Journal of integrative bioinformatics 2011;8(2):165-175.
139. Peatman E *et al*. Basal polarization of the mucosal compartment in Flavobacterium columnare susceptible and resistant channel catfish (Ictalurus punctatus). Molecular immunology 2013;56(4):317-327.
140. Smail D, Munro E. The virology of teleosts. Fish pathology 2012, 186-291.
141. Du X *et al*. Transcriptome profiling of spleen provides insights into the antiviral mechanism in Schizothorax prenanti after poly (I: C) challenge. Fish & shellfish immunology 2017;62:13-23.
142. Huang Y *et al*. Transcriptome analysis of orange-spotted grouper (Epinephelus coioides) spleen in response to Singapore grouper iridovirus. BMC genomics 2011;12(1):1-12.
143. Zhang J, Sun L. Transcriptome analysis reveals temperature-regulated antiviral response in turbot *Scophthalmus maximus*. Fish & shellfish immunology 2017;68:359-367.