

**From expression profiling to functional validation: evidence hierarchy and
biomarker readiness of circular RNAs in aquatic animals**

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ABSTRACT

Circular RNAs (circRNAs) are covalently closed non-coding RNA molecules that are mainly generated from precursor mRNAs through back-splicing. Owing to the lack of free 5' and 3' ends, circRNAs exhibit high stability and pronounced spatiotemporal expression specificity. In aquatic animals, recent advances in high-throughput sequencing and bioinformatic analysis have greatly expanded the catalog of circRNAs and uncovered their regulatory roles in growth, gonadal development, tissue regeneration, immune defense, and environmental stress responses. This review provides a systematic overview of circRNA biogenesis, representative molecular functions, identification strategies, and bioinformatic resources in aquatic animals, with particular emphasis on circRNA-mediated regulation during bacterial and viral infection, antiparasitic defense, trained immunity, and responses to physicochemical stressors. We further summarize the current evidence associating circRNAs with candidate biomarker potential for economically important traits, infectious disease monitoring, and ecotoxicological assessment. Importantly, we highlight the major bottlenecks that currently limit progress in this field, including incomplete genome annotation, algorithm-dependent identification bias, and the lack of robust *in vivo* functional validation. Overall, aquatic animals provide valuable non-model systems for expanding our understanding of circRNA biology, although many proposed regulatory mechanisms and translational applications remain to be further validated.

Keywords: ceRNA regulation; Micropeptide translation; Host–pathogen interactions; Multi-omics resources; Biomarker discovery; Aquatic immunogenomics



1. Introduction

Aquatic animals provide phylogenetically diverse and environmentally responsive systems for investigating how non-coding RNAs coordinate growth, immune defense, and stress adaptation under complex biotic and abiotic pressures (Su et al., 2023). From an applied perspective, these regulatory processes are also closely linked to disease control, environmental monitoring, and the evaluation of economically important traits in aquaculture (Su et al., 2023).

Circular RNAs (circRNAs) are a class of covalently closed RNA molecules mainly generated from precursor mRNAs through back-splicing, and are characterized by the absence of a 5' cap and a 3' poly(A) tail. Compared with their homologous linear transcripts, circRNAs generally exhibit higher structural stability and stronger resistance to exonuclease-mediated degradation, an attribute heavily dependent on their lack of free terminal ends (Jeck et al., 2013). Furthermore, their biogenesis is tightly regulated by specific trans-acting factors that maintain intron integrity (Stagsted et al., 2021) and govern back-splicing efficiency (Chen & Yang, 2015). Consequently, these molecules not only demonstrate highly pronounced tissue-, developmental stage-, and stress state-specific expression profiles (Kristensen et al., 2019), but also dynamically influence the three-dimensional organization of mRNPs within the cellular microenvironment (Pierron & Weil, 2018). A growing body of evidence has shown that circRNAs are not merely transcriptional by-products. They can participate in the remodeling of post-transcriptional regulatory networks through competing endogenous RNA (ceRNA)-mediated regulation (Hansen et al., 2013; Huang et al., 2017), and can also influence gene expression through interactions with RNA-binding proteins (Kristensen et al., 2019). Meanwhile, the continuous refinement of databases and coding annotation resources for circRNAs with translational potential has further expanded our understanding of their functional boundaries.

In aquatic animals, circRNA research has gradually progressed from early expression profiling toward functional characterization, while also beginning to reveal the value of these systems for studying circRNA regulation in non-model organisms. Available evidence indicates that circRNAs are involved in skeletal muscle growth

(Rbbani et al., 2023), gonadal development and sex determination (Wu et al., 2025; Li et al., 2018), and are also associated with body color variation (Hao et al., 2023; Sun et al., 2024b) and tissue regeneration (Su et al., 2024). With respect to immunity, circRNAs are involved in responses to bacterial infection (Shen et al., 2020; Zhou et al., 2025a), viral infection (Nuanpirom et al., 2025), and parasitic infection (Xia et al., 2024). Under changing environmental conditions, they are also associated with hypoxia response (Huo et al., 2021), salinity variation (Ibrahim et al., 2023), acid–base imbalance (Fan et al., 2025), temperature fluctuation (Rbbani et al., 2024), and pollutant exposure (Özdemir & Arslan, 2022; Wang et al., 2024c). Meanwhile, the development of rRNA-depleted RNA sequencing and back-splicing junction (BSJ) identification algorithms has markedly improved the efficiency of circRNA identification (Drula et al., 2024; Shen et al., 2017; Xu et al., 2017), and long-read sequencing has further enhanced the resolution of full-length circRNAs (Shen et al., 2020). Single-cell RNA sequencing and spatial transcriptomics have provided new tools for investigating their spatiotemporal expression characteristics (Holdener et al., 2025; Liu et al., 2025), while spatial analytical strategies based on in situ detection have further advanced the study of circRNA subcellular localization (Liu et al., 2025). Concurrently, the continuous refinement of bioinformatic infrastructure has laid a robust technical foundation for functional studies in non-model species; this includes the development of comprehensive multi-omics platforms for fish genetics (Liu et al., 2026), dedicated algorithms for constructing cross-species interaction networks (Chiang et al., 2022), species-specific non-coding RNA repositories (Das et al., 2023), curated biomarker knowledgebases (Wang et al., 2024b), and integrative frameworks for aquaculture trait evaluation (Li et al., 2022a).

Despite these advances, research on circRNAs in aquatic animals remains largely in a transitional stage from expression-based correlation analysis to rigorous functional validation. In many species with economically important traits, the fundamental lack of high-quality reference genome annotations inherently exacerbates the susceptibility of back-splicing junction identification to algorithm-derived mapping artifacts (Dodbele et al., 2021; Drula et al., 2024). In addition, in vivo loss-of-function validation and



standardized systems for on-site detection remain insufficiently developed (Chakravarthi & Christenson, 2025; Liu et al., 2025). In view of these limitations, a critical reassessment of the current literature is needed in addition to a descriptive summary of reported circRNAs in aquatic species. In particular, it is important to distinguish circRNA candidates supported only by differential expression or in silico network inference from those validated by junction-specific perturbation, direct interaction assays, and phenotypic rescue experiments (Özdemir & Arslan, 2022; Rbbani et al., 2021; Wang et al., 2024c). This distinction is especially relevant in aquatic animals, where many studies have identified large numbers of differentially expressed circRNAs in response to developmental cues, infection, or environmental stress, yet only a limited subset has been functionally interrogated at the cellular or organismal level. Accordingly, this review is designed to go beyond a general summary of circRNA biogenesis and canonical functions. Compared with previous broad circRNA reviews or fish-centered non-coding RNA summaries, we focus specifically on aquatic animals and organize the available evidence according to biological context, mechanistic support, and translational readiness. In particular, we distinguish expression-based circRNA candidates from molecules supported by direct interaction assays, junction-specific perturbation, or phenotype-linked validation, and we evaluate how far current findings can support functional inference or candidate biomarker development in non-model aquatic systems. By doing so, this review aims to provide an evidence-oriented framework for interpreting circRNA functions in aquatic animals, rather than simply cataloging reported circRNA-associated phenomena.

2. Biological characteristics of circRNAs

Because the biogenesis and canonical functional modes of circRNAs have been extensively reviewed elsewhere, this section provides only a concise conceptual foundation for the subsequent evaluation of circRNA evidence in aquatic animals.

2.1 Basic characteristics of circRNAs

circRNAs are a class of endogenous non-coding RNA molecules with covalently closed circular structures, and their most distinctive feature is the lack of the 5' cap and 3' poly(A) tail that characterize conventional linear RNAs (Chen & Yang, 2015). This



unique closed circular topology confers remarkable stability on circRNAs, enabling them to resist degradation by RNA exonucleases such as RNase R and thereby giving them a longer half-life in vivo than corresponding linear messenger RNAs (mRNAs) (Jeck et al., 2013). Exhibiting broad phylogenetic conservation, circRNAs represent an ancient mode of post-transcriptional regulation pervasive across diverse biological systems, ranging from human transcriptomes (Salzman et al., 2012) and *Drosophila* neural tissues (Westholm et al., 2014), to archaea (Danan et al., 2012), plant viroids (Sanger et al., 1976), and viral genomes (Kos et al., 1986). In terms of expression patterns, circRNAs generally exhibit pronounced tissue specificity, cell type specificity, and developmental stage specificity (Li et al., 2020; Memczak et al., 2013; Westholm et al., 2014). For example, a large number of circRNAs are highly abundant in brain tissue and the nervous system and tend to accumulate with age (Westholm et al., 2014). More recent studies have further shown that some circRNAs in aged brain tissue display exceptionally high stability and may serve as highly stable molecular indicators reflecting age and environmental experience (Kirio et al., 2025). In certain aged tissues or pathological models, circRNAs may even exhibit apparently irreversible accumulation, making them potential high-stability biomarkers of organismal aging and past environmental stress exposure (Kirio et al., 2025).

2.2 Biogenesis of circRNAs

circRNAs are mainly derived from precursor mRNAs (pre-mRNAs) through the process of back-splicing (Figure 1). During this process, the splice donor site (5' splice site) of a downstream exon is joined in reverse order to the splice acceptor site (3' splice site) of an upstream exon, resulting in the head-to-tail circularization of exonic or intronic sequences (Chen & Yang, 2015; Jeck et al., 2013; Kristensen et al., 2019; Stagsted et al., 2021). This process competes with canonical linear splicing, and together the two pathways determine the final fate of the transcript (Ashwal-Fluss et al., 2014).

The biogenesis of circRNAs is precisely regulated and depends on specific cis-acting elements and trans-acting factors. With regard to cis-acting elements, inverted repeat sequences enriched in flanking introns can form hairpin structures through



intramolecular base pairing, thereby bringing back-splicing sites into close spatial proximity and promoting circularization (Aktaş et al., 2017; Jeck et al., 2013). Notably, in the genomes of aquatic teleosts that lack typical Alu repeat elements, this circularization process is usually mediated by other species-specific intronic repeat sequences (Gordiyuk & Mankovska, 2020). With regard to trans-acting factors, multiple RNA-binding proteins (RBPs) have been identified as key regulators, although most foundational evidence comes from mammalian or other model systems rather than aquatic animals. For example, Quaking and Muscleblind can regulate circRNA expression by binding to intronic sequences flanking the back-splicing junction, whereas direct validation of analogous RBP-driven circularization mechanisms in aquatic animals remains limited (Ashwal-Fluss et al., 2014; Errichelli et al., 2017). In addition, the nuclear RNA helicase DEAH-box helicase 9 (DHX9) suppresses the production of aberrant circRNAs and maintains normal RNA processing by binding to and unwinding double-stranded RNA structures formed by inverted repeat sequences such as Alu elements (Aktaş et al., 2017). Collectively, these findings indicate that circRNA biogenesis is a tightly regulated process controlled by both cis-acting elements and trans-acting factors. Figure 1 schematically illustrates an intron pairing-driven model of circRNA biogenesis and representative nuclear–cytoplasmic export routes. The figure highlights how complementary intronic sequences promote back-splicing, how this process competes with canonical linear splicing, how RBPs and DHX9 modulate circularization efficiency, and how circRNAs may be exported through length-associated, m⁶A-dependent, and XPO4-dependent routes. Because several export mechanisms remain incompletely validated in aquatic animals, these routes are presented as representative models rather than fully resolved pathways across all aquatic taxa.

2.3 Biological functions of circRNAs

To support the subsequent discussion of aquatic animal studies, the major functional modes of circRNAs are briefly summarized as follows (Figure 2):

ceRNA-mediated “molecular sponge” function: This is currently the most extensively studied functional model in aquatic functional genomics. Through miRNA



response elements (MREs) within their sequences, circRNAs can sequester specific miRNAs, thereby relieving the post-transcriptional repression exerted by miRNAs on downstream target mRNAs and participating in the regulation of gene expression networks (Hansen et al., 2013; Huang et al., 2017). Representative examples include ciRS-7 in the human brain (Hansen et al., 2013; Memczak et al., 2013) and Sry circRNA in mice (Hansen et al., 2013). However, the regulatory efficacy of the ceRNA mechanism is strongly constrained by molecular stoichiometry. For a circRNA to function as an effective miRNA sponge in vivo, its abundance should be comparable to, or higher than, that of the corresponding target miRNA within the same cellular compartment. Therefore, ceRNA networks inferred only from co-expression patterns or predicted binding sites should be interpreted with caution, especially in aquatic animals where absolute copy-number measurements are still uncommon. Future studies should combine relative expression analysis with absolute quantification of circRNAs, target miRNAs, and downstream mRNAs to determine whether the proposed ceRNA interactions are stoichiometrically plausible.

Participation in protein interactions and translational potential: In addition to interactions at the nucleic acid level, circRNAs can bind RNA-binding proteins (RBPs) through specific sequence motifs and act as scaffolds, decoys, or transport carriers for protein interactions, thereby regulating protein function or nucleocytoplasmic shuttling (Kristensen et al., 2019). Moreover, a small subset of circRNAs containing an internal ribosome entry site (IRES) and an open reading frame (ORF) exhibits cap-independent translational potential (Jeck et al., 2013; Kristensen et al., 2019). The genome of hepatitis delta virus (HDV), discovered as early as 1986, had already suggested the protein-coding capacity of single-stranded circular RNAs (Kos et al., 1986). In recent years, with the establishment of databases and coding annotation resources such as circRNADb and riboCIRC (Chen et al., 2016; Li et al., 2021a), an increasing number of endogenous or pathogen-derived circRNAs in aquatic animals have been reported to possess translational potential and, in some cases, to encode micropeptides, with some candidate molecules receiving support from ribosome association data or proteomic evidence. Nevertheless, predicted ORFs, IRES elements, or ribosome association



signals alone do not necessarily prove productive translation, and candidate circRNA-derived micropeptides should be further validated by peptide-level evidence and loss-of or gain-of-function assays under native biological conditions (Li et al., 2021a; Chakravarthi & Christenson, 2025).

Potential roles in transcriptional and splicing regulation: In addition to ceRNA activity, protein interactions, and translational potential, some circRNAs may also influence the transcriptional output of their parental genes and the splicing of precursor mRNAs within the nucleus, thereby participating in broader gene expression regulatory networks. This view is mainly based on studies in model animals and cell systems (Aktaş et al., 2017; Ashwal-Fluss et al., 2014); in aquatic animals, related discussions still rely largely on inferences from circularization mechanisms and cross-species analogy (Errichelli et al., 2017; Gordiyuk & Mankovska, 2020). Accordingly, current circRNA research in aquatic animals still focuses primarily on ceRNA network inference and the validation of a limited number of circRNAs with translational potential (Huang et al., 2017; Kristensen et al., 2019).

Taken together, the currently proposed functions of circRNAs in aquatic animals are not equally supported by experimental evidence. At present, the strongest body of evidence is concentrated in two categories: ceRNA-like regulation inferred from circRNA-miRNA-mRNA relationships and a limited number of circRNAs with translational potential supported by ribosome association or peptide-level evidence. By contrast, protein-scaffolding functions, transcriptional modulation, and splicing-related effects remain much less explored in aquatic species and are often extrapolated from mammalian systems rather than directly demonstrated. This imbalance has two important implications. First, the apparent predominance of ceRNA mechanisms in aquatic animals may reflect methodological accessibility rather than true biological dominance. Second, mechanistic claims should be interpreted with caution unless they are supported by junction-specific perturbation, direct binding validation, appropriate subcellular localization evidence, and phenotypic rescue. Therefore, a major challenge for the field is not simply to expand the catalog of functional hypotheses, but to establish a more rigorous hierarchy of evidence for assigning circRNA function in non-



model organisms. Figure 2 summarizes the representative functional modes of circRNAs, including miRNA sponge activity (Hansen et al., 2013; Huang et al., 2017), interactions with RNA-binding proteins (RBPs) (Errichelli et al., 2017; Kristensen et al., 2019), cap-independent translation into micropeptides (Kristensen et al., 2019; Chen et al., 2016; Li et al., 2021a), and potential roles in transcriptional and splicing regulation within the nucleus (Aktaş et al., 2017; Ashwal-Fluss et al., 2014).

3. Identification strategies and bioinformatic analysis

With the development of high-throughput sequencing technologies, circRNA identification in aquatic animals, which are typically non-model organisms, has gradually evolved into a relatively mature technical workflow. Given the high heterozygosity of aquatic animal genomes, the abundance of repetitive sequences, and the frequent occurrence of whole-genome duplication events that generate paralog-rich genomic backgrounds, accurate identification of back-splicing junctions (BSJs) is a prerequisite for subsequent functional studies.

Although circRNAs are widely present in genomes and exhibit considerable regulatory potential at the post-transcriptional level, their unique molecular features have long posed a major technical challenge. Specifically, most circRNAs lack a 3' poly(A) tail, and their exonic sequences extensively overlap with those of homologous linear precursor mRNAs. As a result, conventional transcriptome library construction methods based on oligo (dT) enrichment cannot efficiently capture this large class of molecules. This limitation constituted a long-standing technical blind spot in early non-coding RNA research. Only in recent years has the widespread adoption of specific enrichment protocols, such as rRNA-depleted RNA sequencing, successfully navigated the topological detection challenges inherent to these transcripts (Drula et al., 2024). When these enrichment strategies are systematically coupled with advances in BSJ-oriented identification algorithms across diverse taxa (Shen et al., 2017), researchers have markedly improved analytical sensitivity, and have even developed computational pipelines capable of extracting preliminary circRNA catalogs from historically archived, conventional linear transcriptome libraries (Xu et al., 2017). In this section, we systematically summarize identification strategies ranging from conventional bulk

tissue sequencing to single-cell and spatially resolved approaches, and evaluate recent progress in the construction of relevant multi-omics databases, thereby clarifying how these methodological advances have laid an indispensable technical foundation for dissecting the multidimensional functions of circRNAs.

3.1 Sequencing strategies and identification algorithms

Studies have shown that even conventional linear transcriptome RNA-seq datasets can be used for preliminary circRNA identification through dedicated computational pipelines, such as CIRC, find_circ, and segemehl (Xu et al., 2017). In large yellow croaker (*Larimichthys crocea*), 975 circRNA molecules with potential miRNA sponge activity were successfully identified from routine transcriptomic data, of which 65% were derived from exonic regions and displayed marked alternative splicing characteristics (Xu et al., 2017).

The identification process generally involves several key steps. First, total RNA sequencing following rRNA depletion is used to capture non-polyadenylated transcripts (Shen et al., 2017; Xu et al., 2017). Subsequently, algorithms such as CIRC2 are employed to identify reads spanning back-splicing junctions. By analyzing paired information within CIGAR strings, CIRC2 can effectively exclude interference from linear splicing and is currently regarded as one of the more sensitive algorithms (Shen et al., 2017). However, in aquatic species with WGD-derived paralog-rich genomes, high allelic heterozygosity, or abundant repetitive regions, short reads may map ambiguously to homologous loci and generate spurious BSJ signals or inaccurate parental gene assignments. Therefore, candidate BSJs in these taxa should be interpreted more cautiously and filtered using stricter criteria, including unique junction-spanning read support, consistency across multiple circRNA callers, exclusion of repetitive-region artifacts, and experimental validation by divergent primer amplification and Sanger sequencing across the BSJ. In addition, for non-model organisms, the integration of long-read sequencing technologies such as PacBio SMRT enables full-length circRNA identification and helps avoid errors introduced by short-read assembly (Shen et al., 2020). In zebrafish, the combined use of find_circ and segemehl led to the identification of 3,868 circRNAs, revealing relatively strong cross-



species evolutionary conservation (Shen et al., 2017).

Notably, with the decreasing cost of high-throughput sequencing and the continuous optimization of identification algorithms, these approaches have been widely applied in transcriptomic studies of aquatic animals under pathogen infection, environmental stress, and vaccine immunization. Through sequencing analyses of different tissues, such as the spleen, liver, intestine, and skin as immune barrier tissues, researchers have successfully screened large numbers of differentially expressed circRNAs (DE-circRNAs) involved in host immune responses and predicted their potential immunoregulatory pathways (see Table 1). These data not only validate the effectiveness of current identification strategies, but also provide a rich pool of candidate molecules for subsequent functional validation and biomarker development.

Furthermore, although conventional bulk RNA-seq provides a panoramic view of expression profiles, it inevitably masks single-cell heterogeneity and spatial distribution patterns within complex tissue microenvironments (Fan et al., 2019; Wang et al., 2024a). In recent years, single-cell RNA sequencing and spatial transcriptomics have begun to be introduced into studies of development and immune microenvironments in aquatic animals (Holdener et al., 2025). However, spatial analysis of circRNAs has long faced a technical bottleneck: most commercial spatial transcriptomic arrays rely heavily on probe capture targeting the 3' poly(A) tails of transcripts, making it difficult to effectively detect circRNAs, which naturally lack poly(A) tails, using routine spatial transcriptomic methods (Holdener et al., 2025). To overcome this limitation, the recently developed rolling circle amplification-based single-molecule fluorescence in situ hybridization technique, abcFISH, has provided a new strategy for the in situ quantification of circRNAs. By using probes specifically targeting BSJ sites in combination with exponential signal amplification, this approach effectively excludes interference from homologous linear mRNA sequences (Liu et al., 2025). In the future, such high-resolution in situ detection methods may promote circRNA functional validation toward single-cell and subcellular spatial localization (Liu et al., 2025).

3.2 Commonly used analytical tools and databases

To accurately identify circRNAs from massive sequencing datasets and dissect

their complex regulatory networks, a variety of standalone software tools and databases have been developed, greatly improving the efficiency of non-coding RNA research in aquatic animals.

CircMiMi is a software tool specifically designed for constructing circRNA-miRNA-mRNA interaction networks. It supports 18 species, including fishes, and can automatically retrieve miRNA binding sites and predict target genes based on given BSJ coordinates, making it a core tool for dissecting ceRNA mechanisms (Chiang et al., 2022). During experimental validation, the design of divergent primers targeting BSJ sites is a major challenge. The web-based platform CircPrime addresses this issue by automatically extracting flanking genomic sequences and generating highly specific PCR primers, thereby ensuring that amplified products originate only from circular molecules rather than linear precursors (Sharko et al., 2023). In addition, with respect to translational potential, the riboCIRC database provides information on circRNAs with ribosome-association evidence across multiple species, offering an important resource for mining protein-coding circRNAs in aquatic animals (Li et al., 2021a). This database includes large amounts of mass spectrometry-validated peptide-coding information, thereby providing new clues for identifying antimicrobial peptide-like molecules in aquatic animals (Li et al., 2021a). Another relevant database is CCncRNAdb, which was developed for common carp, an important aquatic model species. It integrates lncRNA and circRNA information and provides tissue-specific expression profiles across 28 tissues, thereby supporting the precise screening of candidate biomarkers (Das et al., 2023). In addition, the BioKA database, which covers biomarkers for diseases and traits across multiple species, also provides substantial integrative support for homologous comparison and diagnostic target development of circRNAs in animal pathological models (Wang et al., 2024b).

With the explosive growth of whole-genome and transcriptome sequencing data from aquatic animals, related analytical tools, dedicated databases, and integrative multi-omics platforms have continued to improve (Li et al., 2022a; Chiang et al., 2022; Das et al., 2023; Liu et al., 2026; Wang et al., 2024b). Among them, the recently released comprehensive fish multi-omics database iFish represents a systematic



advance in this field (Liu et al., 2026). This platform highly integrates multi-omics data from 88 fish species. Within its transcriptome module, 197,554 circRNA molecules have been systematically identified and quantified, together with more than 9,000 transcriptomic and single-cell sequencing samples (Liu et al., 2026). Researchers can use this platform to query dynamic circRNA expression profiles across developmental stages, tissue dimensions, and infection states (Liu et al., 2026), and can combine these data with existing animal circRNA resources for cross-species conservation analysis (Liu et al., 2026; Memczak et al., 2013). Such multi-omics data engines have markedly shortened the time from high-throughput circRNA discovery to downstream mechanistic validation in aquatic animals, while also providing data support for molecular breeding target selection (Li et al., 2022a; Rbbani et al., 2021). To facilitate the rapid selection of appropriate analytical tools, [Table 2](#) summarizes the bioinformatic tools and specialized database resources commonly used in aquatic animal circRNA research.

Although methodological advances have markedly increased circRNA discovery in aquatic animals, improved detection does not necessarily translate into improved biological certainty. In practice, circRNA annotation in non-model species remains highly sensitive to genome completeness, read depth, sequencing strategy, and algorithm choice, and different pipelines may recover overlapping but non-identical circRNA sets. This issue is especially relevant in aquatic taxa with high genomic heterogeneity, lineage-specific repeats, or incomplete annotation of exon-intron structures, because false-positive BSJ calls and inaccurate parental transcript assignment can directly distort downstream ceRNA network construction and functional interpretation. Moreover, the current analytical workflow still tends to favor transcript abundance profiling over isoform-resolved and cell-resolved validation. Thus, future methodological progress should be evaluated not only by the number of newly detected circRNAs, but also by improvements in reproducibility across pipelines, precision of BSJ annotation, full-length isoform resolution, and compatibility with functional perturbation assays. From this perspective, the key methodological transition for the field is from high-throughput circRNA detection to high-confidence circRNA



attribution.

Another important issue is the normalization of circRNA abundance across species, tissues, and experimental conditions. Because validated reference circRNAs are still lacking for most aquatic animals, future studies should avoid relying on a single unverified internal control. A more reliable strategy is to combine multiple levels of normalization, including library-size-normalized BSJ read counts for sequencing datasets, divergent primer-based qRT-PCR validation, assessment of circRNA-to-linear host transcript ratios, and the use of experimentally validated reference genes or exogenous spike-in controls when appropriate (Bustin et al., 2009; Dodbele et al., 2021; Drula et al., 2024). Such strategies would improve comparability across studies and help distinguish true circRNA regulation from changes caused by sequencing depth, RNA quality, host gene transcription, or sample composition.

4. Regulation of basic physiological processes and economically important traits in aquatic animals by circRNAs

With the aid of the high-throughput sequencing and multi-omics approaches described above, researchers have been able to construct circRNA landscapes of aquatic animals at the whole-transcriptome level in a relatively systematic manner (Shen et al., 2017; Xu et al., 2017). Before moving on to pathogen infection and environmental stress, it is first necessary to define the physiological regulatory baseline under homeostatic conditions. The industrial and economic value of aquatic animals depends heavily on rapid skeletal muscle accretion (Bai et al., 2022; Huang et al., 2025; Liu et al., 2021; Rbbani et al., 2023), precise gonadal differentiation (Gong et al., 2022; Hou et al., 2023; Li et al., 2025; Zhang et al., 2025), and, in some economically important species, the capacity for tissue repair and regeneration (Su et al., 2024). Owing to their high stability and pronounced spatiotemporal expression specificity (Li et al., 2022a; Jeck et al., 2013; Rbbani et al., 2021), circRNAs may not only reflect changes in these complex developmental programs but may also participate in their regulation. Existing studies indicate that circRNAs can regulate skeletal muscle growth and flesh quality traits through ceRNA networks (Rbbani et al., 2023; Liu et al., 2021; Bai et al., 2022), and are also closely associated with economically important traits such as sex

determination, germ cell differentiation, and body color variation (Gong et al., 2022; Zhang et al., 2025; Hao et al., 2023).

Across studies on growth, gonadal differentiation, body coloration, and tissue regeneration, one recurrent feature is that circRNAs are frequently positioned at regulatory convergence points linking developmental signaling pathways to economically important phenotypes. However, the evidentiary strength of these associations varies substantially among traits. In most cases, circRNAs are first identified as trait-associated molecules through differential expression profiling and are then placed into putative ceRNA networks; only a smaller number of studies proceed to direct validation of circRNA-miRNA interaction or functional perturbation. Consequently, the current literature supports the view that circRNAs are informative molecular correlates of physiological state, but provides more limited evidence that they are primary causal regulators of complex production traits. This distinction is important because economically important traits in aquatic animals are typically polygenic, environmentally modulated, and developmentally dynamic. circRNAs may therefore act less as isolated master regulators and more as context-dependent integrators of transcriptional, post-transcriptional, and physiological variation.

4.1 Regulatory networks of skeletal muscle development and flesh quality traits

Growth rate is a core economically important trait in aquatic animals and depends primarily on skeletal muscle hyperplasia and hypertrophy. In this process, circRNAs not only regulate gene expression levels, but also participate deeply in the control of myogenic differentiation and muscle structural adaptation.

In skeletal muscle development and the shaping of economically important traits, circRNAs are frequently proposed as regulatory nodes in growth-related signaling networks. In many studies, they are predicted or partially validated to regulate metabolic and differentiation pathways through ceRNA-like interactions with specific miRNAs, thereby potentially contributing to muscle hyperplasia and hypertrophy. In teleosts, circRNAs exhibit species-specific regulatory strategies. In Nile tilapia (*Oreochromis niloticus*), one key molecule positively associated with muscle growth, circMef2c, has been identified. Mechanistic analysis showed that circMef2c can



competitively sequester miR-34, miR-130b-5p, and miR-202, and form a candidate ceRNA regulatory network together with growth-related genes such as *igfbp2*, *myod1*, and *fgf14*, thereby participating in the regulation of skeletal muscle growth and myogenic differentiation in Nile tilapia (Rbbani et al., 2023).

The regulatory targets of circRNAs differ markedly among fish with different feeding habits. In blunt snout bream (*Megalobrama amblycephala*), the differentially expressed novel_circ_0001608 was predicted to regulate *pik3r1* (the regulatory subunit of PI3K) by sequestering dre-miR-153b-5p, thereby affecting the metabolic sensitivity of the insulin/IGF-1 pathway (Liu et al., 2021). In the carnivorous largemouth bass (*Micropterus salmoides*), by contrast, circRNA389 and circRNA399 occupy central regulatory positions. By sequestering members of the let-7 family and miR-133, these circRNAs may delay cellular differentiation and maintain the proliferative pool of precursor cells (Bai et al., 2022).

In addition, the long-term plasticity of fish growth in response to environmental temperature, namely the lasting effects of early thermal experience, is also subject to epigenetic regulation by circRNAs. In embryonic temperature experiments in Nile tilapia, low temperature induced a significant downregulation of circTTN in adult skeletal muscle. circTTN is derived from the *Titin* gene, and Titin is a key structural protein that determines sarcomere elasticity. Changes in circTTN may reflect a biophysical adaptation in which fish alter splicing patterns to regulate passive muscle tension in response to the viscosity of cold water. Meanwhile, circNxen was significantly upregulated in groups exposed to temperatures deviating from the optimum (24°C and 32°C), suggesting that it may serve as a candidate biomarker for assessing early thermal stress experience in juvenile fish (Rbbani et al., 2024).

In mollusks, studies in Pacific abalone (*Haliotis discus hannai*) have further linked circRNAs to the control of organ size. Differentially expressed circRNAs were enriched in the Hippo signaling pathway and lipid transport processes, suggesting that shellfish may use circRNAs to coordinate the allocation between muscle growth and energy (lipid) storage. This provides new molecular targets for improving flesh quality traits in shellfish (Huang et al., 2025).



Taken together, studies on skeletal muscle development suggest that circRNA regulation of growth in aquatic animals is more likely to converge at the level of shared biological processes than at the level of conserved individual molecules. Different species often recruit distinct circRNAs, yet these molecules repeatedly map to a relatively limited set of pathways related to myogenic differentiation, insulin/IGF signaling, structural remodeling, and energy allocation. This pattern implies that circRNAs may function as modular regulatory nodes that tune pathway activity in a species- and context-dependent manner, rather than as universally conserved master regulators of growth.

4.2 Sex determination, gonadal development, and germ cell differentiation

Sex control and reproductive health are fundamental to the sustainable development of aquaculture. circRNAs display pronounced spatiotemporal specificity during gonadal differentiation and gametogenesis, and some molecules are also associated with toxicological responses and immune-related regulation.

Sexual size dimorphism (SSD) is widespread in aquatic animals. In Chinese tongue sole (*Cynoglossus semilaevis*), researchers identified a conserved circRNA derived from the W chromosome-linked gene *CNOT6L*. Because this molecule is present only in female (ZW) individuals, it may serve as a highly specific candidate marker for genetic sex identification and may help address the problem of pseudo-males interfering with breeding programs. More importantly, this circRNA shows high abundance in the spleen and head kidney, the major immune organs of females (Li et al., 2018). This finding provides a clue to the genetic basis of sex-related differences in the immune system and suggests that sex chromosomes may influence female immune surveillance networks through specific circRNAs in association with ovarian development. In addition to sex chromosome-specific molecules, autosomal circRNAs also contribute to the establishment of SSD. Recent studies constructed ceRNA networks in male and female Chinese tongue sole and found that molecules such as novel_circ_004374 may regulate key genes including *HIPK2* (homeodomain-interacting protein kinase 2) and *SETD2* by sequestering miR-130, thereby influencing the female-biased larger body size phenotype at the epigenetic level (Gong et al., 2022).



In addition, within the brain-pituitary-gonadal axis of Japanese flounder (*Paralichthys olivaceus*), circRNAs participate in the establishment of SSD by regulating genes such as *frizzled3* and *apelin*. Among these, *apelin* is involved in energy balance and hormone secretion and may help explain the faster growth rate of female individuals (Zhang et al., 2025). In the Chinese soft-shelled turtle (*Pelodiscus sinensis*), an important special aquaculture species, and in common carp (*Cyprinus carpio*), circRNAs finely regulate the expression of key sex-determination genes such as *foxl3*, *fgf9*, and the steroidogenic enzyme *hsd17 β 3* through the Wnt/FoxO pathway or through competitive binding to novel_313 (Hou et al., 2023; Ma et al., 2021; Zhu et al., 2022a). This circRNA-mediated mode of sex regulation also appears to be conserved in lower aquatic animals. In Pacific oyster (*Crassostrea gigas*), integrated transcriptomic analyses revealed that genes such as *Fem1c* and *Spefl* are precisely regulated by circRNA-miRNA networks. These networks are deeply involved in oocyte meiosis and energy metabolism and ultimately determine the direction of sex differentiation in shellfish (Guo et al., 2025). In the echinoderm sea cucumber (*Apostichopus japonicus*), differentially expressed circRNAs have been shown to affect sex hormone synthesis through the TGF- β and AMPK signaling pathways, thereby regulating sex differentiation and filling a knowledge gap in marine invertebrates (Li et al., 2025).

Hormone-induced gonadal maturation likewise depends on circRNA-mediated signal transduction. In spotted scat (*Scatophagus argus*), during IGF3-induced ovarian maturation, the circRNA Lachesis_group5 sequesters novel_miR_622, thereby relieving repression of genes involved in extracellular matrix (ECM) remodeling and oocyte maturation. This not only promotes germ cell development but also provides a physical basis for follicular wall remodeling before ovulation (Li et al., 2022b). In the aquatic model fish medaka (*Oryzias latipes*), circ880 has been confirmed to act as a sponge for miR-375-3p and is specifically highly expressed in spermatogonia, suggesting a role in maintaining male germ cell stemness (Zhang et al., 2021). Similar regulatory networks have also been demonstrated in Nile tilapia (*Oreochromis niloticus*), where gonadal circRNAs are enriched in binding sites for miR-212 and miR-200b and directly target key reproductive genes such as *ZP3* (zona pellucida

glycoprotein 3) and *HSD17b3*, thereby maintaining the homeostasis of spermatogenesis and oogenesis (Zhong et al., 2022). During ovarian development in Chinese tongue sole, circRNA expression displays strict temporal specificity, and most of these molecules exhibit resistance to RNase R degradation. By participating in signaling pathways such as MAPK, they are involved throughout the entire process from oocyte growth to maturation (Li et al., 2021b).

In invertebrates, circRNA regulation of the reproductive cycle is often tightly coupled with environmental or behavioral stimuli. In ark shell (*Scapharca broughtonii*), dynamic changes in circRNA expression across different stages of ovarian development were found to be mainly enriched in the steroid hormone biosynthesis pathway, suggesting that these molecules may act as molecular switches initiating reproduction in shellfish (Wang et al., 2023b). In mud crab (*Scylla paramamosain*), mating behavior has been shown to markedly accelerate ovarian maturation, a process accompanied by pronounced remodeling of circRNA expression profiles. Studies indicate that mating-induced circRNAs promote rapid oocyte proliferation and volume increase by targeting the renin-angiotensin system (RAS) and the TGF- β signaling pathway (Yu et al., 2024).

4.3 Body color variation and tissue regeneration

Body color is not only an economically important trait in aquatic animals, but is also closely linked to immune status. In leopard coral grouper (*Plectropomus leopardus*), comparative skin transcriptomic analyses between red-bodied and black-bodied individuals revealed that ceRNA networks constructed from differentially expressed circRNAs were mainly enriched in pathways related to melanin metabolism and immune responses (Hao et al., 2023). Given the homology and functional coordination between pigment cells and immune defense systems in lower vertebrates, such as melanomacrophage centers, these body color-related circRNAs may simultaneously regulate immune surveillance capacity. This suggests that body color variation may provide a visual clue for evaluating potential disease resistance in fish. In the clam *Cyclina sinensis*, molecules such as novel_circ_0004787 regulate shell pigmentation and biomineralization by sequestering hru-miR-1985, further supporting a general role for non-coding RNAs in the formation of structural coloration (Sun et al.,



2024b).

At present, studies on tissue regeneration-related circRNAs in aquatic animals remain relatively limited. Existing direct evidence indicates that in the echinoderm sea cucumber (*Apostichopus japonicus*), circRNA8388 can participate in ECM remodeling by regulating miR-2392, thereby promoting intestinal regeneration after evisceration. This finding is important for elucidating the molecular regulatory mechanisms underlying injury repair and recovery during aquaculture in sea cucumbers (Su et al., 2024). Overall, circRNAs may participate in regeneration in aquatic animals by regulating tissue remodeling, extracellular matrix turnover, and local homeostatic recovery. However, current mechanistic evidence is still concentrated in only a few species and remains to be further validated in a broader range of aquaculture organisms.

To systematically present the diverse functions of circRNAs in the formation of economically important traits in aquatic animals, [Table 3](#) summarizes their expression characteristics and potential roles in growth, development, reproduction, and regeneration, thereby providing a basis for subsequent screening of candidate molecular biomarkers.

Taken together, existing studies indicate that circRNAs are widely associated with the regulation of growth, sex differentiation, reproduction, pigmentation, and tissue regeneration in aquatic animals, and thus represent an important post-transcriptional layer contributing to the formation of economically important traits. At the same time, the current evidence base remains heterogeneous in both taxonomic coverage and mechanistic depth. For most traits, the literature is still dominated by differential expression analysis and ceRNA network inference, whereas direct causal validation remains limited to a relatively small number of circRNAs and species. This imbalance suggests that circRNAs should presently be regarded as informative regulatory candidates and sensitive indicators of developmental state, rather than as broadly established master determinants of production traits. Importantly, the functions of circRNAs are not confined to homeostatic physiology. Once aquatic animals are exposed to invading pathogens, the same regulatory features that make circRNAs responsive to developmental transitions may also position them as dynamic modulators



of host defense. On this basis, the following section turns to infection-related immunity and examines how circRNAs participate in bacterial and viral infections through pathogen-specific immune regulatory networks.

5. circRNA-mediated immune regulation during bacterial and viral infection

5.1 circRNA-mediated immune regulation in bacterial infection

Bacterial infection studies provide a useful framework for assessing how circRNAs modulate innate immune signaling and downstream effector responses in aquatic animals. Compared with viral infection, bacterial challenge more prominently involves Toll/NF- κ B-centered inflammatory regulation, phagocytosis, autophagy, apoptosis, and pathogen clearance. This subsection therefore focuses on representative circRNA axes involved in these processes, while noting that the strength of mechanistic evidence varies among studies and remains constrained by BSJ annotation accuracy, model availability, and junction-specific functional validation.

The NF- κ B pathway is one of the most intensively studied signaling axes of circRNA regulation during bacterial infection. After infection of blunt snout bream with *Aeromonas hydrophila*, circARHGEF15 is significantly upregulated and relieves the repression of *hepcidin* by sequestering miR-214, thereby promoting the expression of inflammatory factors such as *il-1 β* , *il-6*, *il-8*, and *tnf- α* and enhancing host antibacterial immunity (Lin et al., 2024). In contrast, circRXRBB participates in the negative regulation of inflammation through the miR-155/*socs1a* axis; its downregulation after infection helps release SOCS1a-mediated inhibition and promotes the timely initiation of inflammatory responses (Zhai et al., 2025). A similar regulatory pattern has also been observed in black rockfish. After *Edwardsiella tarda* infection, novel_circ_0004195, novel-530, and I κ B form a regulatory network that participates in NF- κ B pathway activation by affecting I κ B expression (Cao et al., 2021). In addition, the miR-286/I κ B α 1 and miR-786/I κ B α 2 axes are regulated by circRNA-4254 and circRNA-273, respectively, and jointly influence NF- κ B signal transduction (Wang et al., 2025). In invertebrates, circRNAs likewise participate in Toll receptor-related antibacterial responses. After infection of sea cucumber with *Vibrio splendidus*, circMETTL7A regulates Toll receptor expression through miR-137 and activates the NF- κ B pathway

(Guo et al., 2024b). These findings suggest that circRNA-mediated Toll/NF- κ B regulation may represent a recurrent regulatory pattern across different aquatic species, although the strength of evidence varies among individual circRNA axes. Its core role lies in determining the activation threshold and persistence of inflammatory responses, and it also indicates that circRNAs in bacterial infection do not merely amplify inflammatory signaling in a unidirectional manner, but instead exert fine regulation between inflammatory initiation and negative feedback balance.

Beyond inflammatory signaling, circRNAs are also widely involved in the effector processes of bacterial clearance. In sea cucumber, circRNA432 enhances coelomocyte phagocytosis through the miR-2008/ELMO1 axis (Fu et al., 2023). The m6A-modified circRNA388 is exported to the cytoplasm under the mediation of YTHDC1/CRM1 and promotes autophagy through the miR-2008/AjULK1 axis, thereby enhancing the clearance of intracellular bacteria (Liu et al., 2024). These studies indicate that, in invertebrates, circRNAs not only participate in the regulation of pathogen recognition-related signaling, but can also directly act on innate immune effector processes such as phagocytosis and autophagy to promote pathogen uptake and intracellular degradation. In fishes, transcriptomic studies likewise show that bacterial infection-related circRNAs are broadly enriched in pathways associated with endocytosis, lysosomes, and immunometabolism. After infection of black rockfish with *Aeromonas salmonicida*, differentially expressed circRNAs are mainly associated with endocytosis, JAK-STAT, and FoxO pathways (Gao et al., 2023); after infection of Nile tilapia with *Streptococcus agalactiae*, differentially expressed circRNAs are mainly enriched in pathways related to cytokine responses, metabolic regulation, and lysosome formation (Shen et al., 2020). In addition, during *Vibrio* or *Aeromonas* infection in Japanese flounder and turbot, differentially expressed circRNAs are also significantly involved in chemokines, cell adhesion molecules, and humoral immune networks (Xue et al., 2021a; Ning & Sun, 2021; Cai et al., 2023a). These findings indicate that the regulatory scope of circRNAs in bacterial infection has expanded from a single inflammatory pathway to multiple levels, including pathogen uptake, intracellular clearance, cell migration, and tissue immune remodeling.



At later stages of infection or under combined stress conditions, circRNAs further participate in the regulation of the balance between apoptosis and autophagy. After infection of yellow catfish with *Aeromonas veronii*, circPIK3C2A shows a pronounced stage-dependent regulatory pattern: it promotes autophagy and inhibits apoptosis at the early stage of infection, which is conducive to pathogen clearance; at the later stage, it promotes apoptosis and inhibits autophagy, thereby limiting persistent infection and secondary tissue damage (Zhou et al., 2025b). Under combined hypoxia and bacterial infection stress, downregulation of circArid4b aggravates hepatocyte apoptosis and inflammatory responses, suggesting that circRNAs also participate in the interactive regulation of environmental stress and infection responses (Zhou et al., 2025a). Notably, some circRNAs can directly influence the stability of signaling proteins by translating micropeptides. In miiuy croaker, circRnf103 and circMIB2 encode Rnf103-177aa and MIB2-134aa, respectively, both of which competitively inhibit TRAF6 ubiquitin-mediated degradation and maintain NF- κ B pathway activity, thereby enhancing host antibacterial capacity (Zheng et al., 2023a; Zheng et al., 2023b). However, because circRNA-encoded micropeptides may be prone to false-positive annotation when inferred only from ORF prediction, IRES prediction, ribosome association, or heterologous overexpression, such claims should ideally be supported by orthogonal evidence, including polysome profiling, Ribo-seq, high-resolution mass spectrometry of endogenous tissues, and functional validation in species-matched or physiologically relevant cellular contexts (Li et al., 2021a; Chakravarthi & Christenson, 2025). In addition, circRNA254, circRNA1149, and circ-FGL1 in sea cucumber participate in apoptosis regulation through molecules such as BAG2, Bax, and AjMyc/AjOTUB1, respectively (Zeng et al., 2023; Guo et al., 2023; Guo et al., 2024a). Therefore, circRNAs in bacterial infection are not only important components of ceRNA networks, but are also gradually emerging as key nodes linking signal transduction, maintenance of protein homeostasis, and the regulation of cell fate outcomes.

Overall, circRNA regulation in bacterial infection is centered on Toll/NF- κ B signaling (Lin et al., 2024; Guo et al., 2024b), and extends to pathogen clearance processes such as phagocytosis and autophagy (Fu et al., 2023; Liu et al., 2024) as well

as to effector pathways including endocytosis/lysosomes, JAK-STAT, FoxO, and apoptosis (Shen et al., 2020; Gao et al., 2023; Zhou et al., 2025b). Their major functions can be summarized in three aspects: first, participation in the initiation of inflammatory responses and the balance of negative feedback; second, promotion of pathogen clearance processes such as phagocytosis, autophagy, and intracellular degradation; and third, influence on cell fate outcomes through the regulation of apoptosis, autophagy, and ubiquitination-related mechanisms. Taken together, circRNAs in bacterial infection have formed a multilayered regulatory network spanning signal recognition, effector execution, and maintenance of tissue homeostasis. [Figure 3](#) schematically summarizes the major regulatory modules and signaling pathways involved in circRNA-mediated antibacterial immunity in aquatic animals, highlighting how circRNAs coordinate inflammatory regulation, pathogen clearance, and cell fate/protein homeostasis during bacterial infection.

These antibacterial regulatory modules provide a basis for comparison with viral infection, where circRNAs are more closely linked to nucleic acid sensing and interferon-related effector outputs. In addition to the schematic overview of antibacterial immune pathways above, [Table 4](#) further summarizes representative circRNAs that have been functionally validated in bacterial infection models of aquatic animals, together with their major regulatory pathways and immunological significance.

5.2 circRNA-mediated immune regulation in viral infection

Compared with bacterial infection, viral infection more clearly highlights the breadth of functional modes potentially adopted by circRNAs, including ceRNA regulation, protein interaction, and, in some cases, protein-coding potential. Across existing studies, circRNAs are repeatedly connected to core antiviral axes involving MITA/STING, MAVS, TRIF, interferon-related signaling, complement-associated responses, and endocytosis-linked processes. This pattern suggests that circRNAs may act less as isolated antiviral switches than as regulatory amplifiers or tuners embedded within pre-existing innate immune circuits. At the same time, antiviral circRNA function appears to be highly context-dependent. The same pathway may contribute to resistance in one host-pathogen system yet be co-opted for immune evasion or



dissemination in another. Thus, the most important unresolved question is not simply which circRNAs are differentially expressed during viral infection, but which of them directly alter viral replication dynamics, host tissue damage, or transmission-related phenotypes under physiologically relevant conditions.

After viral infection, the host first relies on pattern recognition receptors and their adaptor proteins to establish antiviral signaling. In miiuy croaker, circRasGEF1B enhances host anti-rhabdovirus immunity through the miR-21-3p/MITA axis (Chu et al., 2021), whereas circCBL exerts antiviral effects by promoting MITA-related signaling activation (Lv et al., 2023b). In addition, circPIKfyve upregulates MAVS through miR-21-3p, and its circularization is also regulated by the RNA-binding protein QKI (Su et al., 2021); circDtx1 promotes downstream signal transduction through the miR-15a-5p/TRIF axis (Zheng et al., 2021a). These studies indicate that one important role of circRNAs in viral infection is to regulate the establishment and amplification of antiviral signaling around upstream recognition nodes such as MITA/STING, MAVS, and TRIF. Functionally, such circRNAs largely determine whether, after viral nucleic acids are recognized, the relevant signals can be effectively transmitted upward and converted into subsequent interferon and antiviral gene expression. Thus, in viral infection, circRNAs first regulate the key step of whether antiviral immunity can be established in a timely manner.

Once upstream recognition signaling is activated, circRNAs further participate in the regulation of inflammatory and interferon pathways. In miiuy croaker, circPlce1 and circRara are both significantly upregulated after poly (I:C) stimulation and can synergistically activate NF- κ B and IRF3 signaling, thereby promoting the expression of inflammatory factors and antiviral genes (Lv et al., 2023a; Xin et al., 2022). circBCL2L1, in turn, maintains TRAF6 stability by sequestering miR-30c-3-3p, thereby enhancing innate antiviral responses (Zheng et al., 2021b). These findings indicate that circRNAs in viral infection not only participate in the regulation of the interferon system, but also play a coupled amplification role between inflammatory signaling and antiviral effector outputs. However, circRNAs in viral infection do not always act as positive regulators. The host circRNA circVPS13D can be translated into



VPS13D-170aa, which promotes MAVS ubiquitin-mediated degradation and inhibits IFN production, thereby weakening host antiviral capacity (Wang et al., 2023a). This indicates that circRNAs also participate in the negative regulation of antiviral immunity, and that their effects depend on the specific molecule involved, the stage of infection, and the context of host-pathogen interactions. Thus, the circRNA network in viral infection also exhibits bidirectional regulatory characteristics in which activation and inhibition coexist.

Beyond the models that have undergone in-depth functional validation, transcriptomic studies in different species and tissues also indicate that circRNAs broadly participate in antiviral processes such as TLR, NLR, endocytosis, complement, and antigen processing and presentation. After grass carp infection with grass carp reovirus, differentially expressed circRNAs in the spleen are mainly associated with complement and coagulation cascades, whereas those in kidney cells are more enriched in endocytosis- and infection-related pathways (He et al., 2017; Liu et al., 2019a). After infectious hematopoietic necrosis virus infection in rainbow trout, differentially expressed circRNAs in the skin are significantly involved in TLR, NLR, and cytokine receptor interaction pathways (Sun et al., 2024a). After iridovirus infection in orange-spotted grouper, differentially expressed circRNAs in the spleen are mainly associated with immune factor activation and regulation of cellular activity (Cai et al., 2023b); after megalocytivirus infection in Japanese flounder, differentially expressed circRNAs are related to antigen processing and presentation and T-cell receptor signaling pathways (Wu et al., 2021). These studies suggest that circRNA-mediated antiviral regulation is not confined to classical interferon pathways, but also covers mucosal barriers, humoral immunity, and downstream adaptive immune processes. In crustaceans, the antiviral role of circRNAs is also closely linked to the regulation of their biogenesis. After *Litopenaeus vannamei* is infected with WSSV, QKI is activated and promotes circRNA generation; silencing QKI leads to a decrease in circRNA levels and aggravates viral replication (Phiwthong et al., 2025). Among these circRNAs, circAGO3 has been shown to play an important antiviral role, and its knockdown significantly accelerates shrimp mortality (Nuanpirom et al., 2025). In addition, under

yellow head virus and WSSV infection, differentially expressed circRNAs in the hemolymph of *L. vannamei* involve Toll-like receptor and multiple other immune-related pathways and form complex circRNA-miRNA-mRNA regulatory networks (Massu et al., 2024; Limkul et al., 2023). Overall, circRNA regulation in viral infection exhibits marked species specificity and tissue specificity and has gradually expanded from single-molecule functional analyses to studies of systemic immune networks.

Overall, circRNAs in viral infection mainly act around upstream recognition nodes such as MITA/STING, MAVS, and TRIF (Chu et al., 2021; Su et al., 2021; Zheng et al., 2021a), and further cooperate with pathways such as NF- κ B, IRF3, and IFN to amplify antiviral effects (Lv et al., 2023a; Wang et al., 2023a; Xin et al., 2022). At the receptor level, viral nucleic acid sensing in teleosts involves both endolysosomal/lysosomal Toll-like receptors (TLRs) and cytosolic RIG-I-like receptors (RLRs) (Su, 2025). In particular, TLR22 has been experimentally shown to localize to lysosomes and to participate in dsRNA sensing under acidic conditions, rather than being simply represented as a plasma membrane receptor (Ji et al., 2020). Because teleost TLR signaling is characterized by species-specific variation, incompletely defined receptor-adaptor relationships, and negative regulatory mechanisms, the corresponding connections in the revised Figure 4 are shown in a generalized manner rather than as fully resolved linear pathways (Su, 2025). In addition, the QKI-circRNA axis and circAGO3 studies in shrimp suggest that circRNA biogenesis itself can also affect antiviral capacity (Nuanpirom et al., 2025; Phiwthong et al., 2025). Their major roles lie in promoting viral recognition, amplifying antiviral signaling, and regulating downstream effector execution. Compared with bacterial infection, circRNAs in viral infection more prominently serve as crucial links between pathogen nucleic acid recognition and antiviral effector output. Figure 4 schematically illustrates representative circRNA-regulated antiviral signaling axes in aquatic animals. In the revised schematic, viral RNA recognition is summarized more broadly as TLR- and RLR-associated sensing, TLR22 is placed within the endolysosomal/lysosomal compartment, and dashed arrows are used to indicate generalized or incompletely resolved signaling connections that converge on IRF3/7- and NF- κ B-mediated



interferon and inflammatory responses.

5.3 Immune evasion and regulation of pathogen spread in studies of bacterial and viral infection

During host-pathogen interactions, circRNAs not only participate in immune activation, but may also become important molecules mediating immune evasion or influencing pathogen spread. Compared with bacterial infection, the related evidence is more substantial in viral infection, but both contexts suggest that circRNAs have dual biological significance in host defense and pathogen adaptation.

In viral studies, circRNA-mediated negative regulation of immunity is particularly typical. In miiuy croaker, VPS13D-170aa translated from circVPS13D can promote MAVS degradation and inhibit IFN production, thereby weakening host antiviral immunity (Wang et al., 2023a). This suggests that, while host-derived circRNAs help maintain immune homeostasis, they may also be exploited by pathogens under specific conditions to create a molecular environment favorable for persistent infection. In bacterial infection, although direct evidence for immune evasion mediated by pathogen-derived circRNAs is still limited, existing studies have already revealed that host anti-inflammatory networks may become potential windows of benefit for pathogens. For example, the inflammation-braking mechanism reflected by the circRXRBB-*socs1a* axis indicates that the negative regulatory circuitry retained by the host to avoid uncontrolled inflammation may also reduce pathogen clearance efficiency at the early stage of infection (Zhai et al., 2025). Therefore, from a functional perspective, circRNA-mediated negative regulation of immunity is not only a mechanism required for the host to maintain tissue homeostasis, but may also constitute a molecular basis for pathogen immune evasion.

In addition to immune evasion, the roles of pathogen-derived circRNAs in viral infection are not unidirectionally pro-viral, but can exhibit bidirectional effects in which virus-promoting and virus-limiting activities coexist. The circ-udg derived from cyprinid herpesvirus 2 can be translated into circ-udg-P147 and promotes viral replication by increasing UDG protein levels (Zhu et al., 2022b). In contrast, in grass carp reovirus, some virus-derived circRNAs can induce apoptosis and inhibit viral gene

expression (Pan et al., 2021). More representatively, the WSSV-derived circVP28 is translated into ceVP28, which competitively binds the host Rab7 receptor and thereby blocks subsequent viral attachment and invasion (Limkul et al., 2025). These findings indicate that pathogen-derived circRNAs do not necessarily always serve pathogen amplification, but may play different roles in promoting or restricting infection at different stages of the viral replication cycle, with functions closely related to the viral replication cycle, infection stage, and host selective pressure. By comparison, studies on pathogen-derived circRNAs participating in the restriction of pathogen spread during bacterial infection remain scarce, and the relevant mechanisms remain to be further clarified. Nevertheless, existing viral studies have clearly shown that circRNAs are not only host immune regulatory molecules, but may also act as important mediators of pathogen self-regulation during replication and spread.

Research on immune evasion and regulation of pathogen spread has further expanded the potential translational relevance of circRNAs in disease prevention and control. On the one hand, negatively regulatory circRNAs and their encoded products may serve as important targets for identifying pathogen immune evasion strategies; for example, MAVS degradation-related circRNAs may hold promise for antiviral intervention (Wang et al., 2023a). On the other hand, pathogen-derived circRNAs and their functional fragments that inhibit viral invasion or spread may provide references for the design of novel anti-infective molecules; for example, circVP28 can block viral entry by competing for the host receptor, thereby offering a new strategy for non-antibody-dependent antiviral intervention (Limkul et al., 2025). In addition, vaccine immunization studies have shown that, after turbot is inoculated with inactivated *A. salmonicida* vaccine, some circRNAs participate in the regulation of cell adhesion molecules and immunoglobulin superfamily-related molecules, suggesting that circRNAs may also be involved in the formation of immune memory-like responses (Xue et al., 2021b). Therefore, whether in pathogen recognition, immune enhancement, or vaccine adjuvant development, circRNAs show translational potential, although this remains to be further validated. To more systematically summarize the representative functional circRNAs and their modes of action during viral infection in aquatic animals,



Table 5 summarizes the major modes by which host-derived circRNAs and pathogen-derived circRNAs participate in antiviral immunity, immune evasion, and the regulation of pathogen spread.

6. circRNA-mediated antiparasitic defense, trained immunity, and environmental stress responses

In addition to bacterial and viral infections, parasitic infection and environmental stressors such as salinity, pH, hypoxia, and pollutant exposure are also major factors affecting the health and farming performance of aquatic animals. Existing studies indicate that circRNAs are not passive expression products under these biotic and abiotic stress conditions, but instead participate in host defense, stress adaptation, and toxicological responses through multiple mechanisms, including ceRNA regulation, metabolic reprogramming, maintenance of organelle homeostasis, and epitranscriptomic modifications. Compared with the regulatory patterns observed in bacterial and viral infections, which are centered on pathogen recognition, amplification of inflammatory responses, and effector execution, the functions of circRNAs discussed in this chapter more strongly reflect their integrative roles in host-environment interactions. Specifically, circRNAs form composite regulatory networks that connect immunity, metabolism, and physiological homeostasis. These networks are involved in host-pathogen interactions during parasitic infection (Xia et al., 2024; Zhou et al., 2025d), the establishment of trained immunity (Báez-Magaña et al., 2025; Guzman et al., 2025), maintenance of osmotic and acid-base homeostasis (Cao et al., 2023; Ibrahim et al., 2023; Fan et al., 2025), hypoxia adaptation (Huo et al., 2021; Zhou et al., 2025a; Zhou et al., 2025c), and pollutant toxicity transmission (Wang et al., 2024c; Özdemir & Arslan, 2022).

6.1 circRNA regulation in parasitic infection and trained immunity

Compared with bacterial and viral infections, the number of studies on parasitic infection-related circRNAs remains relatively limited. Nevertheless, available findings have already demonstrated their important roles in host-pathogen interactions and secondary immune enhancement. On the one hand, circRNA changes following parasitic infection are associated with tissue repair, metabolic remodeling, and the

907 maintenance of local immune homeostasis (Xia et al., 2024; Zhou et al., 2025d). On the
908 other hand, studies on trained immunity suggest that circRNAs may participate in the
909 establishment and maintenance of secondary defensive states in invertebrates (Guzman
910 et al., 2025). This phenomenon is consistent both with the general theoretical
911 framework of trained immunity (Báez-Magaña et al., 2025) and with studies on
912 vaccine-induced memory-like immune responses (Xue et al., 2021b).

913 Parasitic infection often causes host tissue damage, nutrient depletion, and
914 persistent immune pressure. Therefore, the defensive process depends not only on
915 inflammatory activation, but also on damage repair and metabolic regulation. After
916 infection of tiger puffer with *Cryptocaryon irritans*, researchers identified multiple
917 differentially expressed circRNAs and constructed ceRNA regulatory axes including
918 LOC105418663-circ_0000361-fru-miR-204a-fzd3a. Among these, *fzd3a* is a key
919 component of the Wnt signaling pathway, suggesting that circRNAs under parasitic
920 infection may participate in gill tissue repair and the maintenance of local immune
921 balance by regulating Wnt- and mTOR-related networks (Xia et al., 2024). This
922 indicates that circRNA regulation in parasitic infection is not limited to the
923 inflammatory response itself, but also involves post-infection tissue reconstruction and
924 homeostatic recovery. In crustaceans, parasitic infection-related regulation mediated by
925 circRNAs more prominently reflects the bidirectional interaction between host and
926 pathogen. After infection of swimming crab with the microsporidian *Ameson portunus*,
927 transcriptomic remodeling was observed not only in the host but also in the pathogen
928 itself, in which circRNAs and other non-coding RNA molecules involved in metabolic
929 and proliferative regulation were detected (Zhou et al., 2025d). The results showed that
930 some basal metabolic pathways in the host were suppressed during infection, whereas
931 genes associated with DNA replication and stress responses were upregulated,
932 suggesting that the host may restrict intracellular substrate availability to inhibit the
933 proliferation of obligate intracellular parasites. Meanwhile, non-coding molecules such
934 as circRNAs and lncRNAs cooperatively participated in infection-induced metabolic
935 reprogramming and regulation of cellular responses (Zhou et al., 2025d). Therefore, the
936 key role of circRNA regulation in parasitic infection lies not only in immune signaling



937 activation, but also in the deeper competition between host and pathogen over material
938 utilization and cellular resource allocation.

939 In recent years, trained immunity has gradually become an important topic in
940 immune studies of aquatic invertebrates. Although these organisms lack a typical
941 adaptive immune system, current evidence indicates that invertebrates can still generate
942 broad secondary enhancement of defense through innate trained immunity (Báez-
943 Magaña et al., 2025). In an immune stimulation model induced by supplementation
944 with freeze-dried *Lactobacillus plantarum* in Pacific white shrimp, host survival was
945 significantly improved upon secondary challenge with *Vibrio parahaemolyticus* and
946 white spot syndrome virus (WSSV). Transcriptomic analysis further showed that this
947 secondary protective effect was accompanied by extensive remodeling of circRNA
948 expression profiles, with pronounced changes in multiple candidate molecules
949 associated with pattern recognition and stress defense, such as circRNAs related to
950 DSCAM, Ficolin-1, and HSP70 (Guzman et al., 2025). These findings suggest that
951 circRNAs are involved not only in post-transcriptional regulation during the acute
952 response stage, but may also serve as important molecular nodes for maintaining the
953 trained immunity state. From a broader perspective, the significance of circRNAs in
954 trained immunity may lie in their ability to convert prior stimulation into a sustained
955 molecular state, thereby influencing the magnitude and efficiency of subsequent
956 defensive responses. Similar phenomena are also indirectly supported by vaccine
957 immunization studies. After inoculation of turbot with an inactivated *Aeromonas*
958 *salmonicida* vaccine, some circRNAs participated in the formation of early memory-
959 like immune responses by regulating networks related to cell adhesion molecules and
960 the immunoglobulin superfamily (Xue et al., 2021b). Therefore, although trained
961 immunity and memory-like immune responses are not completely equivalent, existing
962 evidence indicates that circRNAs may play a bridging role in the continuous process of
963 “primary stimulation-state maintenance-secondary enhancement.”

964 Overall, although studies on circRNAs related to parasitic infection and trained
965 immunity are still in their infancy, two relatively clear functional directions have
966 emerged. First, circRNAs in parasitic infection mainly act around host-pathogen



interactions, tissue repair, and metabolic reprogramming (Xia et al., 2024; Zhou et al., 2025d). Second, circRNAs in trained immunity and memory-like immune remodeling may participate in the establishment and maintenance of broad secondary defensive states (Xue et al., 2021b; Báez-Magaña et al., 2025; Guzman et al., 2025). Taken together, the role of circRNAs in this context is more strongly reflected in their function as important regulatory molecules linking immediate defense, restoration of tissue homeostasis, and sustained immune remodeling.

6.2 circRNA regulation in environmental stress and toxicology

Compared with pathogen infection, studies on circRNAs related to environmental stress place greater emphasis on their roles in immune-metabolic trade-offs and the maintenance of internal homeostasis. Existing research indicates that circRNAs are involved in salinity and osmotic adaptation (Cao et al., 2023; Ibrahim et al., 2023), regulation of acid-base homeostasis (Fan et al., 2025), responses to hypoxia and combined stress (Zhou et al., 2025a; Huo et al., 2021; Zhou et al., 2025c), and toxicity transmission following pollutant exposure (Wang et al., 2024c; Özdemir & Arslan, 2022). In addition, some ceRNA networks are also associated with glycogen reserves and energy metabolism (Wang et al., 2021). Thus, under environmental stress conditions, circRNAs display pronounced tissue specificity and species specificity, and may influence stress adaptation by regulating metabolic enzymes, ion homeostasis-related molecules, and organelle transport processes.

With respect to osmotic and salinity adaptation, aquatic animals often remodel metabolic networks through circRNAs to maintain cellular water content and ion balance. Under acute high-salinity stress in razor clam, TAT- and HYAL4-related circRNAs participate in the regulation of amino acid synthesis and membrane transport, and the downregulation of HYAL4 helps reduce hyaluronan hydrolysis and maintain the water-retention capacity of the extracellular matrix, thereby enhancing tissue osmotic buffering capacity (Cao et al., 2023). In Hong Kong oyster, six core differentially expressed circRNAs were detected under salinity stress, and these molecules were mainly associated with taurine, hypotaurine, and phenylalanine metabolism, suggesting that shellfish may maintain cellular homeostasis through the



metabolism of small-molecule osmolytes (Ibrahim et al., 2023). In addition, studies in Pacific oyster further indicate that non-coding RNA networks can participate in environmental adaptation by regulating insulin signaling and glycogen reserves, suggesting that circRNA-mediated osmotic regulation is fundamentally linked to energy allocation and metabolic storage (Wang et al., 2021). Under combined pH and alkalinity stress, the role of circRNAs is further reflected in their dual regulation of antioxidant responses and acid-base homeostasis. In Pacific white shrimp exposed to high pH and high alkalinity, novel_circ_004981 and novel_circ_021024 participate in the regulation of glutathione peroxidase and carbonic anhydrase-3 expression, respectively (Fan et al., 2025). The former mainly functions in reactive oxygen species scavenging, whereas the latter is a key enzyme for maintaining hemolymph acid-base balance. These findings indicate that the important functions of circRNAs under acid-base stress lie not only in coping with environmentally induced oxidative damage, but also in maintaining ionic and acid-base microenvironmental stability so as to ensure the normal operation of basic cellular metabolic processes (Fan et al., 2025).

Hypoxia stress is one of the most common stress types in aquaculture environments, and its biological consequences often involve impaired energy metabolism, organelle dysfunction, and tissue damage. Studies of hypoxia response in Chinese mitten crab showed that the ceRNA network associated with circRNA12 is deeply involved in Golgi-endoplasmic reticulum transport (Zhou et al., 2025c). Because hypoxia readily induces endoplasmic reticulum stress and the accumulation of unfolded proteins, circRNAs may play key roles in relieving ER stress and determining whether cells shift toward adaptive survival or programmed cell death by regulating material transport between organelles (Zhou et al., 2025c). At the same time, hypoxia exposure is accompanied by abnormalities in HIF-1 signaling and phospholipid metabolism, suggesting that circRNAs occupy a relatively upstream regulatory position in hypoxia-induced tissue injury and metabolic remodeling. Compared with a single environmental factor, combined stress more closely resembles real aquaculture settings and more effectively reflects the integrative regulatory capacity of circRNAs under multiple pressures. Under the combined effects of high temperature and hypoxia in sea



cucumber, five common differentially expressed circRNAs were identified, and their functional enrichment mainly involved metabolic processes, signal transduction, and ion responses. This suggests that invertebrates may possess a “core environmental stress response system” composed of conserved circRNAs to maintain basal metabolic homeostasis under multiple adverse conditions (Huo et al., 2021). Because most aquatic animals are ectothermic, temperature may affect circRNA regulation not only by altering stress-responsive gene expression (Huo et al., 2021; Rbbani et al., 2024), but also by influencing RNA secondary structure, RBP binding, splice-site accessibility, and back-splicing efficiency, all of which are closely related to circRNA biogenesis (Chen & Yang, 2015; Aktaş et al., 2017; Kristensen et al., 2019). In this sense, thermal stress may reshape circRNA landscapes at both the biogenesis and stability levels. However, direct evidence linking temperature-dependent RNA folding or splice-site accessibility to altered circRNA biogenesis in aquatic species remains limited. Future studies combining temperature-gradient experiments, time-course sampling, host linear transcript analysis, and BSJ-specific validation will be important for determining whether thermally responsive circRNAs reflect changes in transcription, RNA stability, or back-splicing efficiency. In addition, under the interaction between hypoxia and bacterial infection, downregulation of circArid4b in yellow catfish aggravates apoptosis and inflammatory responses, indicating that environmental factors and infection stimuli are not independent of one another, but can jointly reshape circRNA networks and thereby affect defensive strategies and tissue injury outcomes (Zhou et al., 2025a). Therefore, studies of hypoxia and combined stress suggest that the function of circRNAs in aquatic animals has expanded from simple stress responses to important integrative nodes that coordinate multiple external signals and allocate metabolic and defensive resources.

Exposure to environmental pollutants has further pushed circRNA research into the field of ecotoxicology. Compared with general physicochemical stressors, pollutant-induced circRNA abnormalities not only reflect stress states, but also often directly participate in toxicity transmission, thereby providing greater insight into the molecular basis of adverse outcomes. Current studies mainly focus on exposure



scenarios involving endocrine disruptors, antimicrobial agents, and pesticides. A common feature among these contexts is that circRNA changes are often accompanied by phenotypes such as reproductive injury, neurotoxicity, oxidative stress, and cell death, and are associated with epigenetic modifications and imbalances in complex non-coding RNA networks. In studies of antimicrobial exposure, circRNAs have already been incorporated into the adverse outcome pathway (AOP) framework. In a zebrafish model of triclosan toxicity, circRNA dysregulation was shown to serve as a key event in the AOP, linking molecular initiating events to final toxic outcomes (Wang et al., 2024c). Among these molecules, circRNA48 was markedly dysregulated after triclosan exposure and could mediate multiple adverse outcomes, including neurotoxicity, cardiotoxicity, oxidative stress, and inflammatory responses, through subsequent imbalance of miRNA/mRNA networks. More importantly, knockdown of circRNA48 significantly alleviated the relevant toxic phenotypes, suggesting that it occupies a functional position in the toxicity transmission cascade (Wang et al., 2024c). In addition to antimicrobial agents, pesticide exposure can likewise induce stable and specific circRNA responses. After zebrafish exposure to cypermethrin or chlorpyrifos, 62 significantly downregulated circRNAs were detected. These molecules were mainly associated with classical toxicity-related pathways such as MAPK, p53, and apoptosis, suggesting that they may serve as early indicators of neurotoxicity and cellular injury (Özdemir & Arslan, 2022). Compared with conventional protein markers, circRNAs exhibit higher stability both in vivo and in vitro and retain a certain degree of spatiotemporal specificity. Therefore, they may serve as early warning indicators of pollutant exposure and sublethal toxic effects (Wang et al., 2024c; Özdemir & Arslan, 2022).

Together, these studies link environmentally responsive circRNAs to osmotic and acid-base regulation (Cao et al., 2023; Ibrahim et al., 2023; Fan et al., 2025), hypoxia-related immune-metabolic trade-offs and combined stress responses (Zhou et al., 2025a; Huo et al., 2021; Zhou et al., 2025c), and pollutant-triggered adverse outcome pathways (Wang et al., 2024c; Özdemir & Arslan, 2022). Thus, circRNAs in environmental stress models should be viewed not only as stress-responsive indicators, but also as candidate



regulatory nodes connecting physiological homeostasis with toxicological mechanisms. Table 6 further summarizes representative circRNAs involved in parasitic infection, environmental stress, and toxicological exposure across different aquatic species.

Across the physiological, immune, and environmental contexts discussed above, circRNA-associated functions show clear taxon- and evidence-dependent patterns. To provide an integrated overview, we constructed a functional landscape map of circRNAs across major aquatic animal taxa, including fish, mollusks/shellfish, crustaceans, echinoderms, and aquaculture-associated reptiles (Figure 5). This figure summarizes representative functional categories reported in these groups and distinguishes relatively well-represented or better-supported categories from more limited, preliminary, or expression-associated evidence by label weight.

7. Candidate biomarker potential of circRNAs in aquatic animals

The biomarker potential of circRNAs in aquatic animals should be evaluated against criteria that extend beyond differential expression alone. This is particularly important in non-model aquatic species, where BSJ annotation, normalization strategy, and validation systems remain insufficiently standardized. Similarly, ceRNA-based biomarker interpretation should be supported not only by predicted circRNA-miRNA-mRNA relationships, but also by quantitative evidence showing that the candidate circRNA is sufficiently abundant and reproducibly regulated under the target condition.

An informative circRNA biomarker should ideally satisfy at least four conditions: robust detectability across biological replicates and populations, clear association with a defined physiological or pathological state, sufficient specificity to distinguish the target condition from unrelated stressors, and practical compatibility with field-applicable sampling and detection workflows. Based on the evidence summarized above, the dynamic expression patterns of circRNAs in aquatic animals suggest that these molecules may provide informative molecular readouts of physiological and pathological state transitions (Rbbani et al., 2021). As aquatic veterinary medicine and ecological monitoring move toward greater precision, there is an increasing need for indicators that are sensitive, non-invasive, and stable under ex vivo conditions (Zoccali et al., 2024). Conventional detection strategies based on linear mRNAs or proteins are

often constrained by sample quality and limited detection windows. By contrast, owing to their covalently closed circular topology, circRNAs show high resistance to exonuclease-mediated degradation, which supports their consideration as stable candidate indicators (Li et al., 2022a; Ren et al., 2020). Similar stability-related biomarker discussions have also been reported in broader disease contexts (Shafaghat et al., 2025; Zhao et al., 2024). In addition, the pronounced spatiotemporal expression specificity of circRNAs suggests preliminary value as candidate auxiliary indicators for the early warning of aquatic diseases (Shen et al., 2020), stage-specific monitoring of infection progression (Cai et al., 2023a), and ecotoxicological assessment under pollutant exposure scenarios (Özdemir & Arslan, 2022; Wang et al., 2024c). Crucially, topological advantages such as exonuclease resistance do not automatically confer field-deployable diagnostic utility. In complex aquatic environments, where physiological baselines are profoundly shaped by ontogeny, fluctuating abiotic factors, and highly variable husbandry conditions, any candidate circRNA biomarker must demonstrate rigorous stability across diverse genetic populations and consecutive generations. Furthermore, it must empirically outperform, or significantly complement, existing conventional transcriptomic and biochemical indicators in real-world aquaculture settings. Therefore, this chapter treats circRNAs as preliminary, proof-of-concept candidate indicators. Although the following sections summarize their association with economically important trait evaluation, infectious disease monitoring, and ecotoxicological assessment, mature industrial or veterinary application will require substantial longitudinal and cross-cohort validation.

7.1 Candidate molecular biomarkers for growth-, sex-, and reproduction-related economically important traits

In the field of selective breeding in aquaculture, traditional breeding has long relied heavily on phenotypic measurements, which are time-consuming and readily influenced by environmental factors. Within the framework of marker-assisted breeding (Martini et al., 2021), tilapia circMef2c has shown promise as a candidate marker for evaluating growth potential (Rbbani et al., 2023), whereas the W chromosome-specific circRNA identified in Chinese tongue sole may serve as a



candidate marker for sex identification (Li et al., 2018). In addition, some circRNAs associated with immune responses and reproductive timing also suggest that they may serve as candidate molecular indicators for evaluating health status or reproductive progression (Chu et al., 2021; Li et al., 2021b).

7.2 Early warning and stage-specific monitoring of infectious diseases

Host-derived circRNAs can show infection-associated expression specificity, thereby providing a potential molecular basis for the early warning and stage-specific monitoring of aquatic diseases. Unlike conventional PCR, which mainly relies on the direct detection of pathogen-derived nucleic acids, host circRNAs essentially reflect endogenous molecular responses triggered by infectious stimuli. Therefore, even at stages when pathogen load is still low and obvious clinical symptoms have not yet appeared, detectable changes in circRNA expression may already be present. For example, in the early asymptomatic stage of *Aeromonas hydrophila* infection in blunt snout bream, circARHGEF15 is already significantly upregulated and participates in the initiation of inflammatory responses through the miR-214/Hepcidin axis, suggesting that it may serve as a candidate molecular indicator for early warning of infection (Lin et al., 2024). The potential significance of such candidate molecular indicators lies in their possible ability to shift the diagnostic window earlier and to provide auxiliary information for timely management decisions before obvious clinical symptoms appear.

In addition to early warning, circRNAs may also be used to track infection progression and pathological stage transitions. Existing studies indicate that different circRNAs often exhibit stage-dependent and dynamic expression patterns at different phases of infection. For example, in the model of *Streptococcus agalactiae* infection in Nile tilapia, circRNA expression profiles can distinguish different pathological stages, including activation of pro-inflammatory responses at the early stage, metabolic remodeling at the middle stage, and enhanced lysosomal function together with tissue injury at the later stage (Shen et al., 2020). During *Vibrio anguillarum* infection in turbot, some differentially expressed circRNAs may also reflect key transition points at which the host response shifts from early innate immunity to subsequent adaptive

immunity (Cai et al., 2023a). Therefore, compared with single indicators that only indicate whether infection is present, circRNAs may have exploratory value as auxiliary candidate markers for stratifying infection progression and assessing disease severity, provided that their performance is confirmed across independent cohorts and field-relevant conditions.

In the context of viral infection, circRNAs may also provide supplementary information for evaluating the status of active viral replication. For pathogens such as GCRV and WSSV that can establish persistent or latent infections, the mere detection of viral genomic DNA/RNA may not always accurately reflect the current activity state of infection. Existing studies have shown that some virus-derived circRNAs or host-derived antiviral circRNAs are closely associated with the infection process, such as the WSSV-derived circVP28 and the host-derived antiviral circRNA circAGO3 (Limkul et al., 2025; Nuanpirom et al., 2025). These molecules may be regarded as candidate indicators for evaluating viral infection activity, although current evidence is still insufficient to support stable stratification of latency or replication activity. In addition, some virus-derived circRNAs may also reflect viral replication activity to a certain extent. For example, circ-udg encoded by CyHV-2 has been shown to promote viral replication (Zhu et al., 2022b). Therefore, from a translational perspective, incorporating both host-derived circRNAs and virus-derived circRNAs into a combined detection system may help improve the overall assessment of viral infection activity and provide supplementary information for outbreak risk evaluation.

7.3 Assessment of aquatic environmental stress and ecotoxicological exposure

Marked fluctuations in aquatic environmental factors, including hypoxia, temperature variation, salinity and pH imbalance, as well as exposure to pesticides, antimicrobial agents, and other pollutants, can substantially affect growth, immunity, and tissue homeostasis in aquatic animals. Compared with linear mRNAs, circRNAs possess relatively high molecular stability and stress-state specificity, and therefore may be explored as candidate molecular indicators for environmental quality assessment and animal welfare monitoring. Functionally, environmentally responsive circRNAs can reflect hypoxia and combined stress states (Zhou et al., 2025a; Huo et



al., 2021), and are involved in the regulation of acid-base homeostasis and oxidative stress (Fan et al., 2025), suggesting that they may serve as candidate molecular indicators for environmental risk assessment in aquaculture waters. Under pollutant exposure scenarios, circRNAs are also associated with adverse outcomes such as neurotoxicity and inflammatory responses (Özdemir & Arslan, 2022; Wang et al., 2024c). Heat stress studies further suggest that they may serve as candidate indicators of variation in thermotolerance (Quan et al., 2021).

Under general environmental stress conditions, changes in circRNA expression often reflect the transition of the organism from homeostasis to a state of stress compensation. For example, in the hypoxia stress model of yellow catfish, downregulation of circArid4b is associated with enhanced inflammation and aggravated apoptosis, suggesting that it may be used to assess the degree of hypoxia stress and the risk of tissue injury (Zhou et al., 2025a). In the heat stress model of rainbow trout, the ceRNA network associated with novel_circ_003889 participates in the regulation of heat shock proteins such as HSP90 and HSP70, and may serve as a candidate molecular indicator of heat stress intensity and differences in thermotolerance (Quan et al., 2021). These findings suggest that, in aquaculture management settings, dynamic monitoring of specific circRNAs may help identify fish that are in an early stress state or a mildly compromised health state, thereby providing a reference for aeration, water quality regulation, and stocking density management.

In ecotoxicological studies, circRNAs also show relatively high sensitivity to pollutant exposure type and toxicity mechanism. In zebrafish, for example, both pyrethroid insecticides (cypermethrin) and organophosphorus pesticides (chlorpyrifos) can induce marked alterations in circRNA expression profiles in brain tissue, mainly affecting classical toxicity-related pathways such as MAPK, p53, and apoptosis (Özdemir & Arslan, 2022). Importantly, the circRNA response patterns induced by different pollutants are not completely identical, suggesting that they may be useful for distinguishing different mechanisms of toxic action. Another study showed that circRNA48 is aberrantly expressed after triclosan exposure and participates in adverse outcome pathways involving neurotoxicity, oxidative stress, and inflammatory



responses (Wang et al., 2024c). Therefore, from a translational perspective, circRNAs may provide candidate early-warning indicators of waterborne pollutant exposure and may help clarify toxicity transmission mechanisms, although their incorporation into ecotoxicological assessment systems will require further cross-exposure, cross-stage, and field-based validation.

A critical issue is that biomarker candidacy and biomarker readiness are not equivalent. Many circRNAs reported in aquatic animals should currently be regarded as candidate indicators rather than validated diagnostic or prognostic markers. In most cases, the available evidence is still derived from single-study transcriptomic comparisons, often under controlled experimental settings and with limited validation across populations, developmental stages, or co-occurring environmental stressors. As a result, the translational gap remains substantial. For example, a circRNA that tracks infection severity under laboratory challenge may not retain the same discriminatory power in field conditions where temperature fluctuation, hypoxia, salinity change, or nutritional stress occur simultaneously. Therefore, future biomarker studies should prioritize multi-cohort validation, longitudinal sampling, receiver operating characteristic-based performance evaluation, and direct comparison with existing clinical or aquaculture-relevant indicators. Table 7 summarizes the candidate circRNA biomarkers currently identified in aquatic animals that are associated with disease diagnosis, environmental monitoring, and the evaluation of economically important traits, and that show preliminary translational relevance, thereby providing a reference for subsequent functional validation and translational application studies.

8. Summary and future challenges

In recent years, research on circRNAs in aquatic animals has expanded from descriptive expression profiling to a broader framework encompassing basic physiological regulation, pathogen-associated immunity, and environmental adaptation. Available evidence indicates that circRNAs are involved in muscle growth, gonadal development, tissue regeneration, infection-related immune regulation, and environmental stress responses, and that some molecules may hold promise as candidate biomarkers. However, the current body of evidence still relies predominantly



on expression profiling, ceRNA network inference, and a limited number of functional studies. Accordingly, circRNAs in aquatic animals are more appropriately viewed at present as a candidate molecular system with substantial research value, rather than as a mature route for industrial application.

In aquaculture, because circRNAs exhibit relatively high stability and pronounced spatiotemporal expression specificity, they may serve as candidate auxiliary indicators for health assessment, disease monitoring, and the screening of economically important traits, but their practical utility still requires standardized validation. Figure 6 provides an integrative overview of the current evidence for circRNAs in aquatic animals, their candidate biomarker potential, the major bottlenecks restricting translational progress, and the future directions needed to advance this field. The following sections therefore focus on the key bottlenecks restricting deeper investigation and translational application, particularly from the perspectives of methodology and validation systems (Dodbele et al., 2021).

8.1 Bottlenecks in genome annotation and circRNA identification

Accurate identification of circRNAs depends heavily on the quality of the reference genome. At present, apart from a few model fishes such as zebrafish, carp, and tilapia, many aquatic species of major economic importance, particularly mollusks, crustaceans, and echinoderms, still lack high-quality chromosome-level genomes, or have highly incomplete genome annotations with poorly defined intron-exon boundaries. As a result, when RNA-seq data are used to identify back-splicing junctions (BSJs), agreement among different prediction algorithms is often limited, and the false-positive rate remains relatively high. Therefore, multi-algorithm cross-validation is usually required, together with further experimental confirmation by RNase R treatment, RT-PCR, or Sanger sequencing (Dodbele et al., 2021; Drula et al., 2024).

Another major challenge in deeply dissecting circRNA functions in aquatic animals lies in their highly complex genomic background. For example, cyprinid fishes such as common carp and salmonids have undergone lineage-specific whole-genome duplication (WGD) events during evolution, resulting in large numbers of highly similar homologous genes and paralogs in their genomes (Wang et al., 2024a). During



the alignment of high-throughput RNA sequencing reads, this extensive sequence similarity can readily lead to misalignment, causing traditional short-read assembly-based algorithms to generate a high false-positive rate in BSJ identification (Dodbele et al., 2021; Drula et al., 2024). In addition, many marine invertebrates, such as shrimp and oysters, possess highly complex genomes and a heavy burden of repetitive sequences, which further interferes with high-quality reference genome assembly and precise intron-exon boundary annotation (Dodbele et al., 2021; Drula et al., 2024). Therefore, mining aquatic animal circRNAs using only short-read transcriptomic data has inherent limitations. To overcome these problems, future studies should adopt a tiered validation strategy rather than relying on a single prediction pipeline. At the computational level, candidate BSJs should be supported by multiple algorithms, sufficient unique junction-spanning reads, careful filtering of repetitive or paralogous genomic regions, and comparison with the expression of corresponding linear host transcripts. At the experimental level, high-confidence candidates should be validated by RNase R treatment, divergent primer amplification, Sanger sequencing across the BSJ, and, where feasible, full-length isoform confirmation using PacBio Iso-Seq or Nanopore sequencing. Such a combined strategy would help reduce false-positive BSJ calls caused by WGD-derived paralogs, polyploidy, or high heterozygosity and improve the reliability of circRNA annotation in non-model aquatic species (Shen et al., 2020; Dodbele et al., 2021; Drula et al., 2024).

8.2 Limitations of mechanistic dissection and bottlenecks in *in vivo* functional validation

Current approaches for functional dissection and validation of circRNAs in aquatic animals are still subject to severe methodological constraints, which constitute one of the core bottlenecks limiting deeper progress in this field. Firstly, basic mechanistic research shows a pronounced tendency toward homogenization. Existing studies rely excessively on bioinformatically predicted circRNA-miRNA-mRNA co-expression networks, and most conclusions remain confined to the traditional ceRNA hypothesis centered on miRNA sponge activity. However, co-expression, sequence complementarity, and pathway enrichment alone are insufficient to demonstrate



functional miRNA sequestration. To strengthen ceRNA-related claims, future studies should include absolute or near-absolute quantification of circRNAs and target miRNAs, direct validation of circRNA-miRNA binding, subcellular co-localization analysis, and rescue experiments showing that the downstream phenotype depends on the proposed circRNA-miRNA-mRNA axis. By contrast, more complex molecular events involving circRNA-mediated transcriptional regulation, protein scaffold formation, phase separation, and the translation of specific micropeptides have not yet been explored in a sufficiently systematic manner (Dodbele et al., 2021; Kristensen et al., 2019).

Secondly, the lack of *in vitro* cell models severely restricts the physiological relevance of mechanistic validation. Many aquatic species with major economic value, especially marine crustaceans and shellfish, still lack immortalized cell lines that can be stably passaged. This forces researchers to rely on heterologous mammalian expression systems, such as human embryonic kidney cells (HEK293T), when validating homologous protein interactions or RNA interactions. Such heterologous systems cannot faithfully simulate the species-specific intracellular microenvironment or physicochemical stressors of aquatic animals, nor can they adequately reflect differences in circRNA expression, RNA structure, RBP binding, and back-splicing efficiency that may occur in poikilothermic animals under different water temperatures (Dodbele et al., 2021; Rbbani et al., 2024). Therefore, primary cells, short-term tissue explants, and organoid-like systems may serve as intermediate validation platforms when stable species-matched cell lines are unavailable. For circRNA-derived micropeptides, a minimal validation workflow should also prioritize native biological context whenever possible. Candidate coding circRNAs should first be screened by ORF/IRES prediction and ribosome-associated evidence, then validated by BSJ-specific amplification, polysome profiling or Ribo-seq, and peptide-level detection using high-resolution mass spectrometry of endogenous tissues. Functional relevance should further be tested by junction-specific perturbation or circRNA recoding/rescue experiments in species-matched cell lines, primary cells, tissue explants, or *in vivo* models, rather than relying solely on heterologous mammalian overexpression systems

(Dodbele et al., 2021; Li et al., 2021a; Chakravarthi & Christenson, 2025).

Finally, *in vivo* loss-of-function validation faces extremely high technical barriers. Because most circRNA sequences overlap extensively with their homologous linear mRNAs and differ only slightly at the back-splicing junction (BSJ), conventional RNA interference (RNAi) approaches, such as siRNA injection, are highly prone to severe off-target effects, leading to nonspecific silencing of homologous host linear transcripts and thereby confounding phenotypic interpretation (Chakravarthi & Christenson, 2025; Dodbele et al., 2021). Targeted RNA perturbation systems such as CRISPR-Cas13 may provide useful tools for BSJ-directed circRNA knockdown, but their specificity should not be overestimated. Even when sgRNAs are designed across the BSJ, collateral RNA cleavage, local sequence similarity, spatial accessibility of the circular junction, or partial complementarity to homologous linear transcripts may still cause unintended degradation of linear host mRNAs. Therefore, Cas13-based circRNA perturbation should be accompanied by careful sgRNA screening, measurement of both circular and linear host transcripts, transcriptome-wide off-target assessment, and rescue experiments whenever possible (Chakravarthi & Christenson, 2025). At the same time, delivery remains a major practical bottleneck in non-model aquatic animals, especially crustaceans, mollusks, and echinoderms, where stable cell lines and efficient *in vivo* delivery systems are often unavailable. Thus, before direct *in vivo* application, Cas13- or RNAi-based perturbation strategies should ideally be optimized in species-matched cell lines, primary cells, tissue explants, or organoid-like systems, followed by validation in physiologically relevant *in vivo* contexts. In parallel, with the aid of more advanced single-molecule spatial transcriptomic techniques, such as abcFISH, resolving the *in situ* distribution patterns of circRNAs within complex tissue microenvironments under stress conditions will be crucial for deciphering their spatiotemporally specific biological functions (Liu et al., 2025).

In summary, circRNAs have emerged as an important regulatory layer linking transcriptome plasticity to development, immunity, and environmental adaptation in aquatic animals. The current literature clearly demonstrates that circRNAs are widespread, dynamically regulated, and biologically informative across a broad range

of aquatic taxa. At the same time, the field remains characterized by a marked asymmetry between discovery and validation: large numbers of circRNAs have been cataloged, but relatively few have been assigned functions through rigorous mechanistic analysis. As a consequence, the strongest conclusions currently supported are that circRNAs constitute sensitive indicators of physiological and pathological state and that a subset of these molecules participates in post-transcriptional regulatory circuits, particularly ceRNA-like networks and, in rarer cases, translation-related processes. By contrast, claims regarding broad causal control, conserved master regulators, or immediate biomarker deployability should still be regarded as provisional. Looking forward, the most important advances will come from integrating improved genome annotation, isoform-resolved circRNA identification, spatially and temporally resolved expression analysis, and junction-specific perturbation strategies *in vivo* (Chakravarthi & Christenson, 2025; Dodbele et al., 2021; Pedrosa-Gerasmio et al., 2025). Such progress will be essential for transforming aquatic circRNA research from an increasingly rich descriptive field into a mechanistically grounded and translationally credible discipline.

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Footnotes

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Figure legends

Figure 1 Schematic model of intron pairing-driven back-splicing, its competition with linear splicing, and regulation by RBPs and DHX9

Precursor mRNA (pre-mRNA) can undergo back-splicing when complementary intronic sequences bring an upstream 3' splice acceptor and a downstream 5' splice donor into close spatial proximity, generating a circular RNA (circRNA) with a characteristic back-splicing junction (BSJ). This process competes with canonical linear splicing and is regulated by both cis-acting intronic elements and trans-acting factors. RNA-binding proteins (RBPs), represented here by Quaking (QKI) and Muscleblind (MBL), can facilitate circRNA biogenesis, whereas DHX9 suppresses back-splicing by unwinding double-stranded RNA structures formed by complementary intronic sequences. Representative nuclear export routes are also shown, including length-associated export mediated by DExD/H-box RNA helicases, m⁶A-dependent export through the YTHDC1/CRM1 axis, and XPO4-dependent export associated with sequence or structural features of circRNAs. The aquatic animal example illustrates that, in *Apostichopus japonicus* challenged with *Vibrio splendidus*, YTHDC1/CRM1-mediated export of m⁶A-modified circRNA388 regulates the miR-2008/AjULK1 axis and induces autophagy. Some export routes and regulatory mechanisms shown in this schematic remain putative or incompletely validated in aquatic species and should therefore be interpreted as representative models rather than universally established pathways.

Abbreviations: circRNA, circular RNA; pre-mRNA, precursor mRNA; mRNA, messenger RNA; BSJ, back-splicing junction; RBP, RNA-binding protein; QKI, Quaking; MBL, Muscleblind; DHX9, DEAH-box helicase 9; NPC, nuclear pore complex; DExD/H-box, DEAD-box/DEAH-box; UAP56/DDX39B, ATP-dependent RNA helicase UAP56/DEAD-box helicase 39B; URH49/DDX39A, UAP56-related helicase 49/DEAD-box helicase 39A; m⁶A, N⁶-methyladenosine; YTHDC1, YTH domain-containing protein 1; CRM1/XPO1, chromosome region maintenance 1/exportin 1; XPO4, exportin 4; miR, microRNA; AjULK1, *Apostichopus japonicus* unc-51-like autophagy activating kinase 1.

Figure 2 Representative molecular mechanisms and functional modes of circRNAs

CircRNAs exert regulatory functions through several representative molecular mechanisms, including ceRNA-mediated regulation, protein interaction, micropeptide translation, and potential nuclear regulation. (A) In ceRNA-mediated regulation, circRNAs sequester specific miRNAs through miRNA response elements (MREs), thereby relieving miRNA-mediated repression of downstream target mRNAs and increasing target protein expression. (B) In protein interaction, circRNAs bind RNA-binding proteins (RBPs) and may function as scaffolds, decoys, or carriers to modulate protein activity, localization, or stability. (C) A subset of circRNAs containing an internal ribosome entry site (IRES), an open reading frame (ORF), or other translation-promoting elements can recruit ribosomes and be translated into micropeptides in a cap-independent manner. (D) Some circRNAs may also participate in nuclear regulation by influencing parental gene transcription and pre-mRNA splicing; however, these nuclear functions remain less directly validated, particularly in aquatic animals. Solid green arrows indicate commonly reported or better-supported mechanisms, dashed green arrows indicate potential or less directly validated mechanisms, red blunt-ended lines indicate inhibition/repression, and gray dashed arrows indicate schematic links to representative regulatory modes.

Abbreviations: circRNA, circular RNA; ceRNA, competing endogenous RNA; miRNA, microRNA; MRE, miRNA response element; mRNA, messenger RNA; RBP, RNA-binding protein; IRES, internal ribosome entry site; ORF, open reading frame; pre-mRNA, precursor mRNA.

Figure 3 Major regulatory modules and signaling pathways involved in circRNA-mediated antibacterial immunity in aquatic animals

Upon bacterial infection, pathogen-derived cues are sensed by Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs), leading to the establishment of a multilayered circRNA regulatory network centered on Toll/NF- κ B signaling. This network can be functionally divided into three major modules. In the pathogen clearance module, circRNA432 and circRNA388 promote bacterial elimination through the miR-2008/ELMO1 axis and the miR-2008/AjULK1 axis, thereby

enhancing phagocytosis and autophagy, respectively. In the inflammatory regulation module, circARHGEF15, circRXRBB, and circMETTL7A modulate inflammatory activation and homeostatic balance through the miR-214/Hepcidin, miR-155/SOCS1a, and miR-137/Toll receptor pathways, respectively, converging on Toll/NF- κ B signaling. In the cell fate/protein homeostasis module, circRnf103 and circMIB2 encode micropeptides that stabilize TRAF6 and support antibacterial signaling, whereas circPIK3C2A dynamically coordinates the balance between autophagy and apoptosis in a stage-dependent manner, thereby contributing to tissue homeostasis during infection.

Abbreviations: circRNA, circular RNA; miR, microRNA; TLR, Toll-like receptor; PRR, pattern recognition receptor; NF- κ B, nuclear factor kappa B; ELMO1, engulfment and cell motility 1; AjULK1, *Apostichopus japonicus* unc-51-like autophagy activating kinase 1; SOCS1a, suppressor of cytokine signaling 1a; TRAF6, tumor necrosis factor receptor-associated factor 6.

Figure 4 Representative circRNA-regulated antiviral signaling axes in aquatic animals

Upon viral infection, viral RNA can be sensed by endolysosomal/lysosomal Toll-like receptors (TLRs) and cytosolic RIG-I-like receptors (RLRs), leading to adaptor-centered antiviral signaling pathways involving TRIF, MITA/STING, MAVS, and an auxiliary MyD88-related branch. circRasGEF1B relieves miR-21-3p-mediated repression of MITA/STING, circCBL promotes MITA/STING-associated antiviral signaling, circPIKfyve enhances MAVS signaling by antagonizing miR-21-3p, and circDtx1 blocks miR-15a-5p-dependent repression of TRIF. These circRNA-regulated upstream signaling axes converge on IRF3/7 and NF- κ B, thereby promoting the expression of interferons (IFNs), interferon-stimulated genes (ISGs), and inflammatory cytokines. Dashed arrows indicate generalized, auxiliary, or incompletely resolved signaling connections. This schematic highlights the role of circRNAs as regulatory nodes linking viral recognition to downstream antiviral effector responses.

Abbreviations: circRNA, circular RNA; miR, microRNA; TLR, Toll-like receptor; RLR, RIG-I-like receptor; RIG-I, retinoic acid-inducible gene I; TRIF, TIR-domain-



containing adaptor-inducing interferon- β ; MyD88, myeloid differentiation primary response 88; MITA, mediator of IRF3 activation; STING, stimulator of interferon genes; MAVS, mitochondrial antiviral-signaling protein; IRF3/7, interferon regulatory factors 3 and 7; NF- κ B, nuclear factor kappa B; IFN, interferon; ISG, interferon-stimulated gene.

Figure 5 Functional landscape of circRNAs across aquatic animal taxa.

This schematic summarizes representative functional categories associated with circular RNAs (circRNAs) across major aquatic animal taxa. Fish-related studies include growth/muscle development, sex determination/reproduction, body color variation, immune defense, environmental stress/toxicology, and antiparasitic defense. Mollusks/shellfish, used here mainly to denote molluscan shellfish, include growth/energy allocation, shell pigmentation/biomineralization, sex differentiation/reproduction, and osmotic/salinity stress. Crustaceans include antiviral immunity, reproduction/ovarian maturation, trained immunity/immune priming, and environmental stress adaptation. Echinoderms mainly represent sea cucumber studies involving antibacterial innate immunity, intestinal regeneration, and sex differentiation/gonadal development. Reptiles denote aquaculture-associated aquatic or semi-aquatic reptiles discussed in the review, currently represented mainly by sex-determination-related evidence. Bold labels indicate functional categories that are relatively well represented or better supported in the current literature, whereas regular labels indicate limited, preliminary, expression-associated, or mainly inferential evidence. The figure presents representative functional categories rather than an exhaustive catalog and does not imply that all listed roles have been causally validated. Abbreviation: circRNAs, circular RNAs.

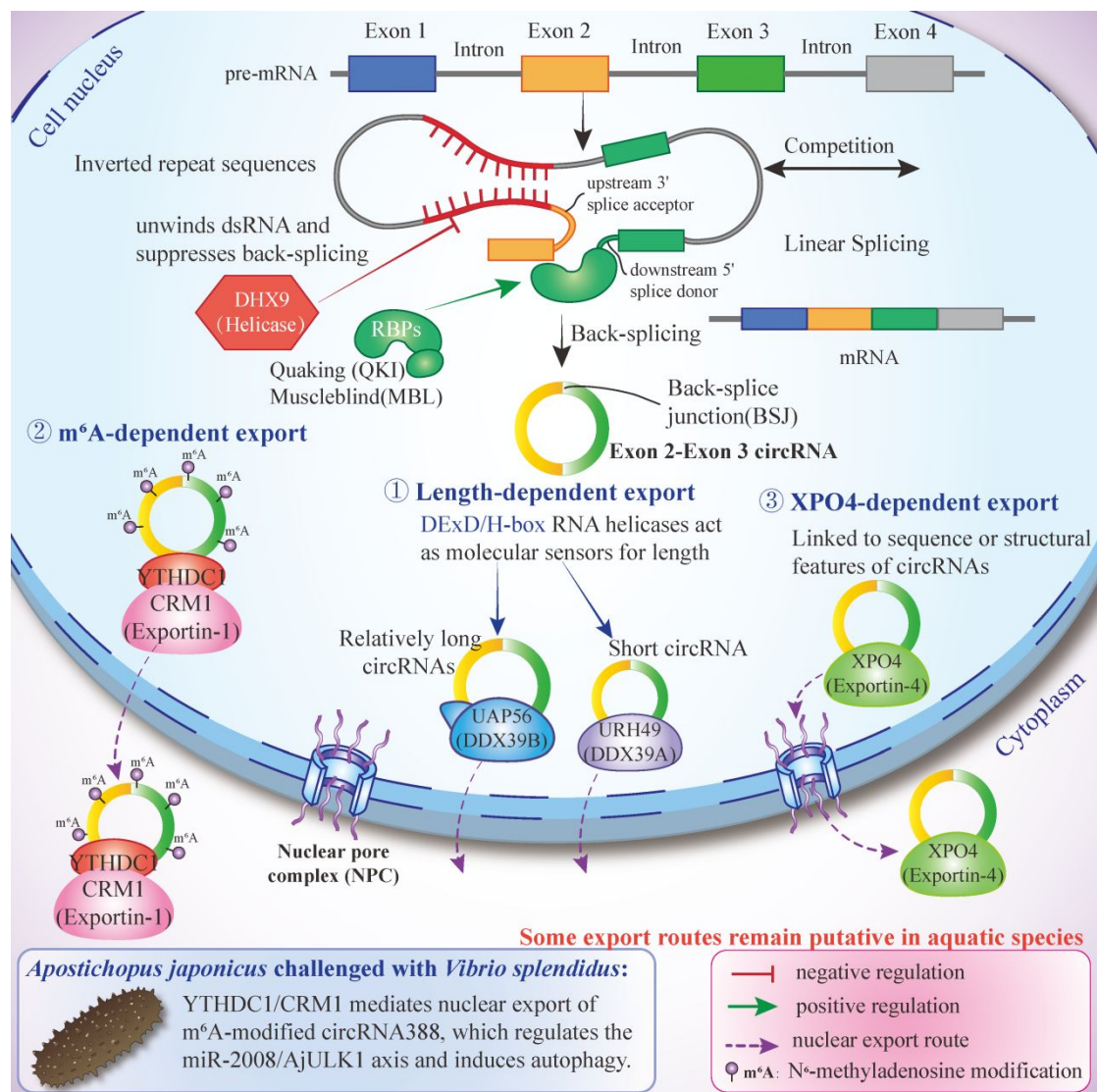
Figure 6 Current evidence, candidate biomarker potential, major bottlenecks, and future directions of circRNAs in aquatic animals

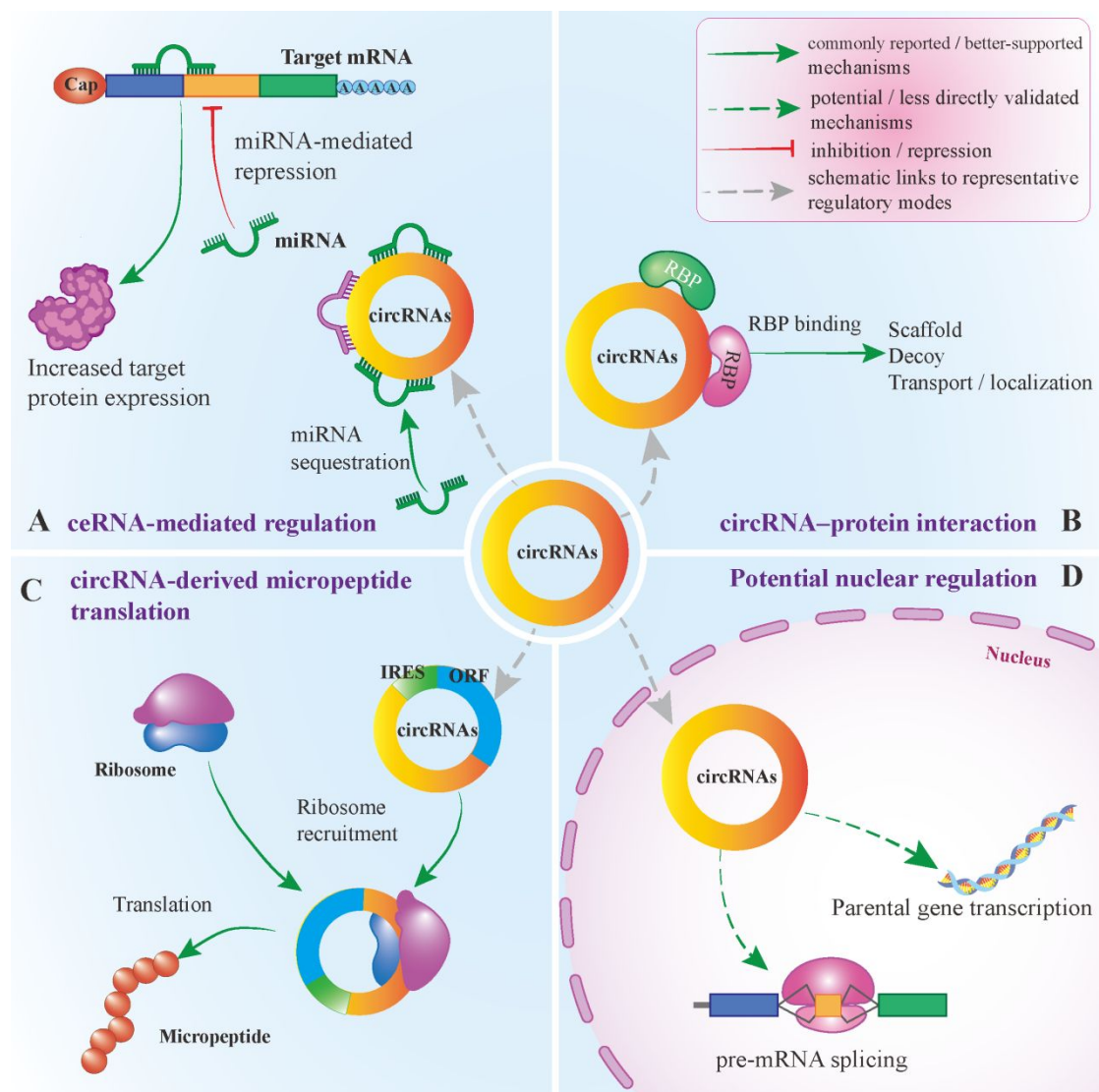
Current studies indicate that circRNAs in aquatic animals are involved in basic physiological processes and economically important traits, pathogen-associated responses, and environmental stress responses. Their potential application as candidate biomarkers is supported by several advantageous properties, including high stability,

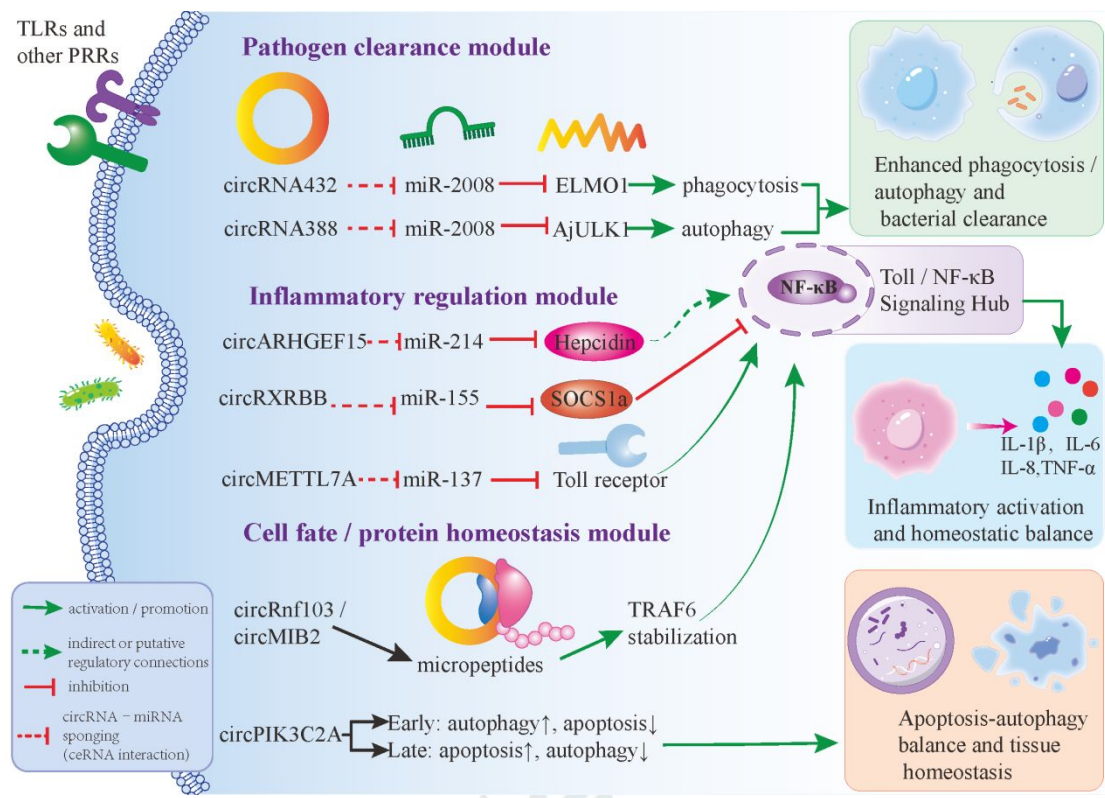


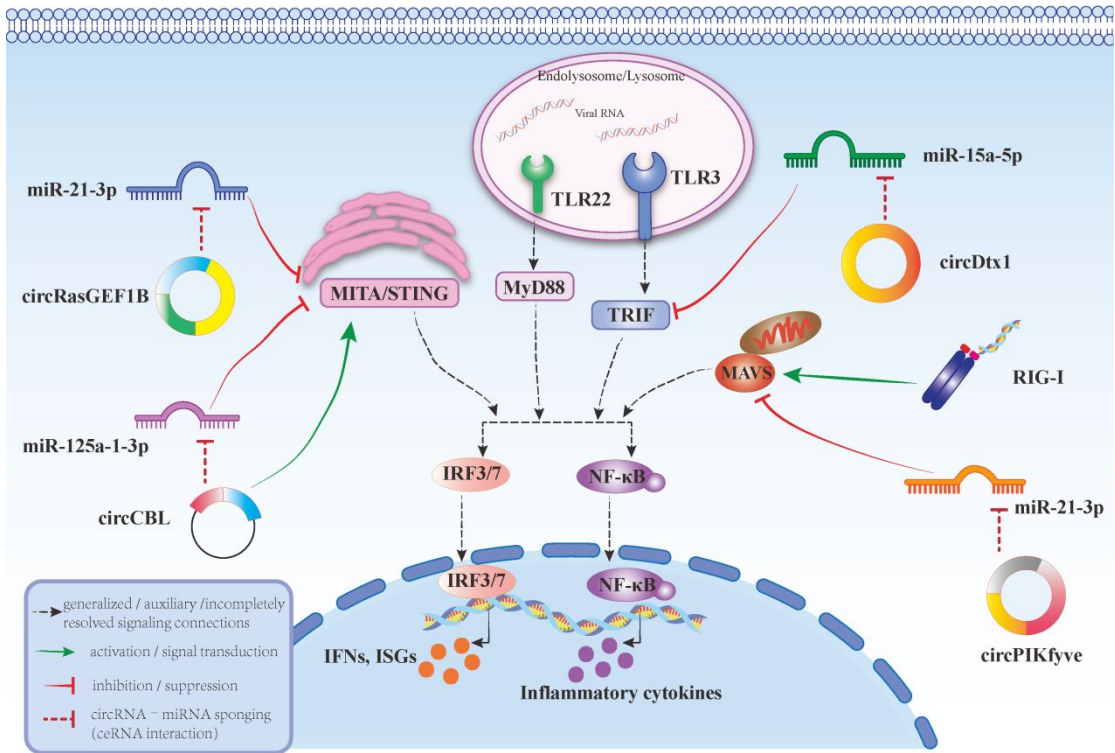
back-splicing junction (BSJ) specificity, spatiotemporal expression patterns, and detectability in tissues and body fluids. On this basis, circRNAs may hold promise for applications in trait evaluation, disease monitoring, and environmental assessment. However, several major bottlenecks still limit further progress, including incomplete BSJ annotation, mechanistic gaps, and insufficient experimental validation. Future advances in long-read sequencing, cross-validation using multiple algorithms, spatial and single-cell tools, CRISPR-Cas13-based functional interrogation, and non-invasive detection strategies may help promote the transition of circRNAs from candidate molecular markers to more robust application-oriented systems in aquatic animals.

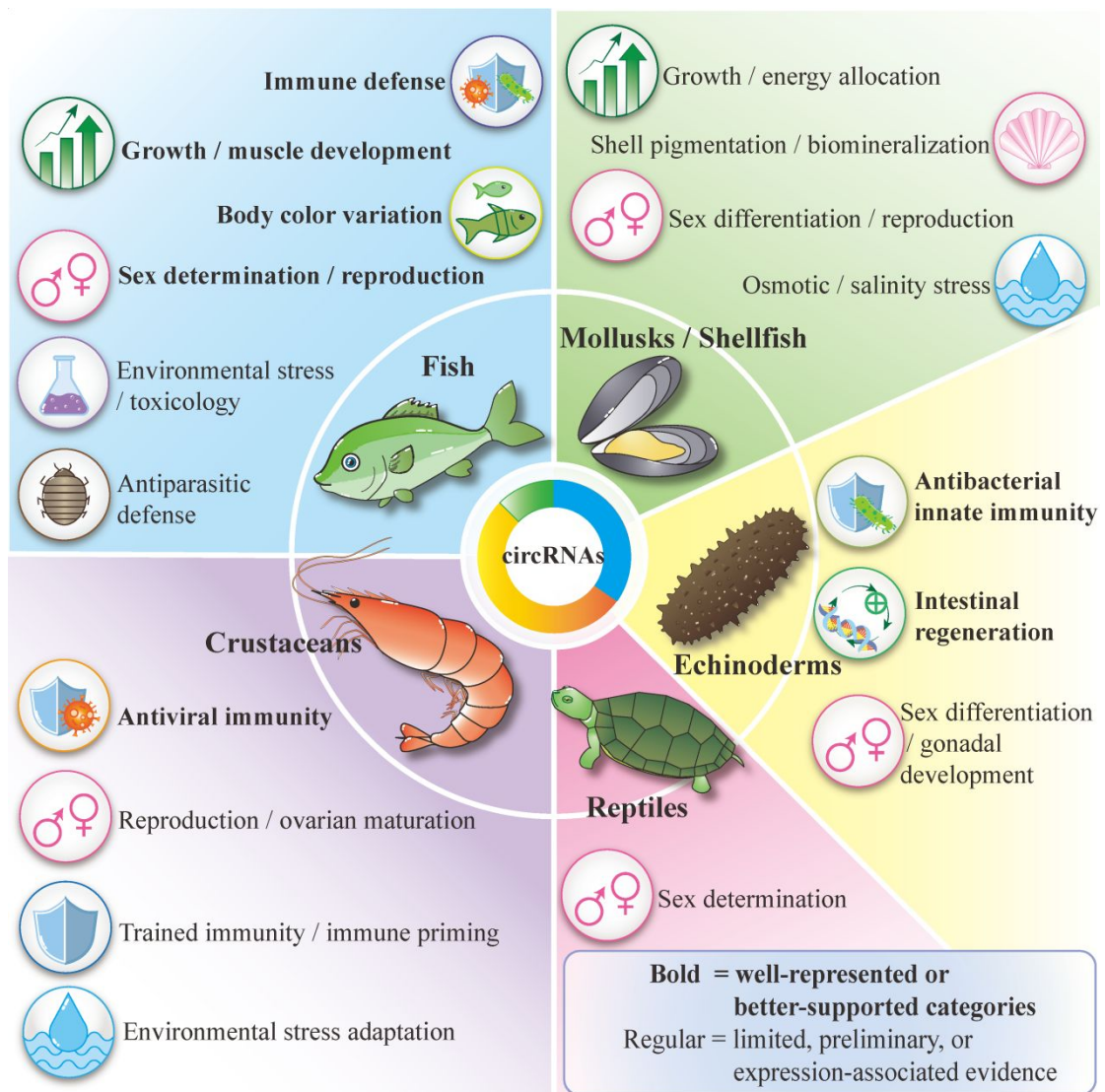
Abbreviations: circRNA, circular RNA; BSJ, back-splicing junction; CRISPR-Cas13, clustered regularly interspaced short palindromic repeats-associated protein 13.

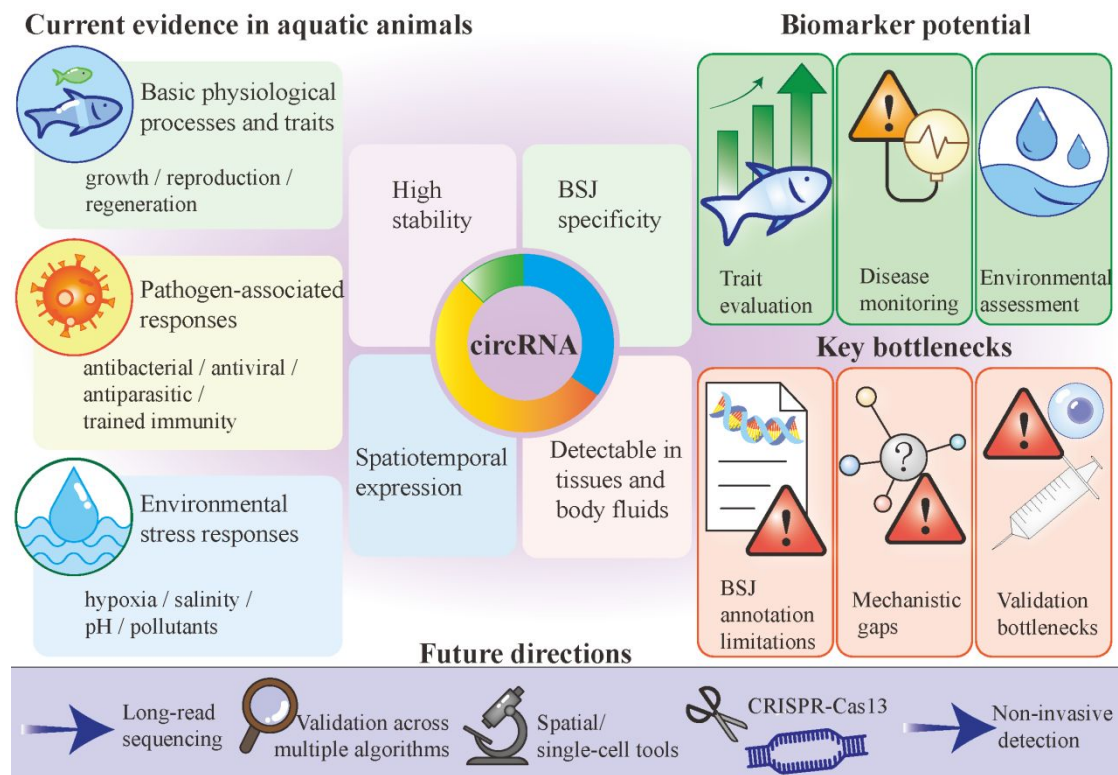












1957 **Table 1 High-throughput sequencing-based expression profiling of infection-related circRNAs in aquatic**
1958 **animals**

Species	Pathogen/Stimulus	Tissue	No. of DE-circRNAs	Major Enriched Pathways/Functions	Ref.
Bacterial infection					
Japanese flounder (<i>P. olivaceus</i>)	<i>Edwardsiella tarda</i>	Intestine	62	Intestinal immune-related pathways	(Xiu et al., 2019)
Black rockfish (<i>S. schlegelii</i>)	<i>Edwardsiella tarda</i>	Intestine	87	NF-κB signaling pathway; chemokine signaling pathway	(Cao et al., 2021)
Turbot (<i>S. maximus</i>)	<i>Aeromonas salmonicida</i>	Spleen	119	Cell adhesion molecules; chemokines and other immune-related genes	(Xue et al., 2021a)
Nile tilapia (<i>O. niloticus</i>)	<i>Streptococcus agalactiae</i>	Spleen/head kidney	61	Cytokine responses; energy metabolism; lysosome formation	(Shen et al., 2020)
Black rockfish (<i>S. schlegelii</i>)	<i>Aeromonas salmonicida</i>	Spleen	39	Endocytosis; JAK-STAT and FoxO signaling pathways	(Gao et al., 2023)
Japanese flounder (<i>P. olivaceus</i>)	<i>Vibrio anguillarum</i>	Spleen	148	Immune-related pathways (a complex ceRNA network was constructed)	(Ning & Sun, 2021)
Yellow catfish (<i>P. fulvidraco</i>)	<i>Aeromonas veronii</i>	Spleen	47	Platelet activation; humoral immunity; Ral signaling transduction	(He et al., 2024)
Turbot (<i>S. maximus</i>)	<i>Vibrio anguillarum</i>	Liver	31	Activation of innate immunity at the early stage and adaptive immunity at the later stage	(Cai et al., 2023a)
Turbot (<i>S. maximus</i>)	<i>A. salmonicida</i> vaccine	Spleen	111	Cell adhesion molecules; immunoglobulin superfamily (immune memory-like responses)	(Xue et al., 2021b)
Viral infection					
Grass carp (<i>C. idella</i>)	Grass carp reovirus (GCRV)	Spleen	41	Metal ion binding; coagulation and complement cascades	(He et al., 2017)
Rainbow trout (<i>O. mykiss</i>)	Infectious hematopoietic necrosis virus (IHNV)	Skin	34	Toll-like receptor (TLR) and NOD-like receptor (NLR) signaling pathways; cytokine-receptor interactions	(Sun et al., 2024a)

Orange-spotted grouper (<i>E. coioides</i>)	Singapore grouper iridovirus (SGIV)	Spleen	123	Regulation of cellular activity; activation of immune factors	(Cai et al., 2023b)
Japanese flounder (<i>P. olivaceus</i>)	Megalocytivirus	Spleen	169	Antigen processing and presentation (MHC); T-cell receptor (TCR) signaling	(Wu et al., 2021)
Pacific white shrimp (<i>L. vannamei</i>)	Yellow head virus (YHV)	Hemolymph	358	Toll-like receptor signaling pathway and other immune-related pathways	(Mass u et al., 2024)
Grass carp (<i>C. idella</i>)	Grass carp reovirus (GCRV)	Kidney cells (CIK)	76	Endocytosis; immune- and infection-related pathways	(Liu et al., 2019a)

Abbreviations: DE-circRNAs, differentially expressed circRNAs; TLR, Toll-like receptor; NLR, NOD-like receptor; MHC, major histocompatibility complex; TCR, T-cell receptor; CIK, *Ctenopharyngodon idellus* kidney; GCRV, grass carp reovirus; IHNV, infectious hematopoietic necrosis virus; SGIV, Singapore grouper iridovirus; YHV, yellow head virus; JAK-STAT, Janus kinase-signal transducer and activator of transcription.



Table 2 Commonly used bioinformatic tools and databases for circRNA research in aquatic animals

Category	Tool/Database	Main Function and Application Description	Ref.
Identification algorithm	CIRI/CIRI2	Efficient BSJ-based identification algorithms used for the de novo prediction of circRNAs in aquatic model species such as zebrafish.	(Shen et al., 2017; Xu et al., 2017)
Identification algorithm	find_circ	A classic circRNA identification tool, often used together with CIRI to improve prediction accuracy; it has been applied in zebrafish studies.	(Shen et al., 2017)
Identification algorithm	segemehl	An algorithm for sequence alignment and detection of circular RNA splicing events, with specific applications reported in zebrafish studies.	(Shen et al., 2017)
Functional/network prediction	CircMiMi	A standalone Python package specifically designed for constructing cross-species circRNA-miRNA-mRNA regulatory networks, including those in aquatic animals.	(Chiang et al., 2022)
Functional/network prediction	miRanda	An industry-standard miRNA target prediction algorithm widely used to predict the miRNA sponge activity of circRNAs within the ceRNA mechanism.	(Cao et al., 2021; Liu et al., 2019b; Ning & Sun, 2021; Wang et al., 2021; Xiu et al., 2019)
Functional/network prediction	TargetScan	Another widely used miRNA target prediction tool, often used together with miRanda to screen high-confidence binding sites.	(Liu et al., 2019b)
Experimental design tool	CircPrime	A web-based platform specifically developed for designing divergent primers to validate circRNA junction sequences.	(Sharko et al., 2023)
Specialized database	CCncRNAdb	A common carp-specific non-coding RNA database that integrates lncRNA and circRNA information, together with associated ceRNA network data.	(Das et al., 2023)
Specialized database	riboCIRC	A comprehensive database dedicated to circRNAs with translational potential, providing Ribo-seq evidence to facilitate studies on the functions of circRNA-encoded micropeptides; it includes data from aquatic model species such as zebrafish.	(Li et al., 2021a)
Database	iFish	The most comprehensive fish multi-omics database currently available, integrating data from 88 fish species. Within its transcriptome module, 197,554 circRNAs have been systematically identified and quantified, supporting cross-tissue expression profile queries, visualization, and data download.	(Liu et al., 2026)
Identification algorithm	find_circ	In the iFish transcriptome module, circRNA identification follows a workflow based on back-splicing junction recognition; the anchor realignment strategy represented by find_circ provides a classic reference for its algorithm design.	(Liu et al., 2026; Memczak et al., 2013)
Functional/network prediction	iFish – Function Module	Supports cross-species conservation analysis of ncRNA sequences based on BLASTN (identity $\geq 50\%$, E-value $\leq 1e-5$). In addition, a co-expression network was constructed from 9,797 RNA-seq	(Liu et al., 2026)

samples (Pearson correlation coefficient ≥ 0.8 , FDR < 0.05), which can be used to predict potential regulatory relationships between circRNAs and protein-coding genes.

Visualization tool	JBrowse	Integrated into iFish as a genome browser covering 88 fish species, allowing users to intuitively inspect the genomic location and structure of circRNAs, as well as their relationships with neighboring genes.	(Liu et al., 2026)
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1964 **Abbreviations:** circRNA, circular RNA; miRNA, microRNA; mRNA, messenger RNA; ceRNA, competing endogenous RNA;
1965 ncRNA, non-coding RNA; BSJ, back-splicing junction; CIRI/CIRI2, circRNA identifier/circRNA identifier 2; Ribo-seq, ribosome
1966 profiling sequencing; BLASTN, Basic Local Alignment Search Tool for nucleotides; FDR, false discovery rate; RNA-seq, RNA
1967 sequencing; E-value, expect value.



1968 **Table 3 Expression characteristics and functions of circRNAs involved in growth, development, reproduction,**
1969 **and regeneration in aquatic animals**

Physiological Process	Species	Key Molecule/Expression Profile	Target/Regulatory Network	Biological Function	Immune/Metabolic Link and Biomarker Potential	Ref.
Muscle growth and flesh quality	Nile tilapia (<i>O. niloticus</i>)	circMef2c	Sequesters miR-34, miR-130b-5p, and miR-202; forms a candidate ceRNA network associated with <i>igfbp2</i> , <i>myod1</i> , and <i>fgf14</i>	Promotes muscle hyperplasia and hypertrophy; regulates myogenic differentiation	Involves the mTOR pathway, suggesting a resource trade-off between growth and immunometabolism	(Rbbani et al., 2023)
	Blunt snout bream (<i>M. amblycephala</i>)	novel_circ_0001608	Sequesters dre-miR-153b-5p; targets <i>pik3r1</i>	Regulates insulin/IGF-1 pathway sensitivity	Affects metabolic homeostasis	(Liu et al., 2021)
	Largemouth bass (<i>M. salmoides</i>)	circRNA389, circRNA399	Sequesters the let-7 family and miR-133	Delays cellular differentiation and maintains the proliferative pool of precursor cells	The let-7 family comprises classic immunoregulatory factors linking growth and defense	(Bai et al., 2022)
	Pacific abalone (<i>H. discus hannai</i>)	250 DE-circRNAs (large vs. small individuals)	Hippo signaling pathway; lipid transport network	Coordinates muscle growth and energy (lipid) storage	Potential molecular targets for improving flesh quality in shellfish	(Huang et al., 2025)
Body color variation and development	Leopard coral grouper (<i>P. leopardus</i>)	Body color-related circRNA clusters	Constructs ceRNA networks; involves melanin metabolism and participates in the regulation of red/black body color variation	Regulates red/black body color variation	Differentially expressed genes are enriched in immune pathways, suggesting an association between body color variation and potential immune responses	(Hao et al., 2023)
	Clam (<i>C. sinensis</i>)	novel_circ_0004787	Sequesters hru-miR-1985	Regulates shell pigmentation and biomineralization	Reveals the role of non-coding RNAs in structural coloration	(Sun et al., 2024b)
	Zebrafish (model fish, <i>D. rerio</i>)	1,028 temporally expressed circRNAs (throughout embryonic development)	NOTCH signaling pathway; co-expression with host genes	Participates in fine temporal regulation during embryonic development	Reference molecular biomarkers for temporal regulation during embryonic development in aquatic model fish	(Liu et al., 2019b)
Sex determination and dimorphism	Chinese tongue sole (<i>C. semilaevis</i>)	W chromosome-specific circRNA showing female-specific expression through chromosome-linked inheritance	Chromosome-linked inheritance; highly expressed in the spleen and other tissues	Female-specific expression; a highly specific molecular marker for sex identification	May serve as a potential molecular marker for genetic sex identification	(Li et al., 2018)
	Chinese tongue sole (<i>C. semilaevis</i>)	novel_circ_004374	Sequesters miR-130; targets HIPK2	Regulates body-size traits associated with sexual size dimorphism (SSD)	Explains the epigenetic basis of the female-biased larger body size phenotype	(Gong et al., 2022)
	Japanese flounder (<i>P. olivaceus</i>)	Brain/pituitary/muscle SSD-related networks	Regulates <i>frizzled3</i> and <i>apelin</i> pathways	Regulates SSD through the brain-pituitary-gonadal axis	Associated with energy balance and hormone secretion	(Zhang et al., 2025)



	Chinese soft-shelled turtle (special aquaculture species, <i>P. sinensis</i>)	710 DE-circRNAs (testis vs. ovary)	Wnt/FoxO pathway; targets <i>foxl3</i> and <i>fgf9</i>	Finely regulates key genes involved in sex determination	Potential targets for sex-control breeding	(Ma et al., 2021; Zhu et al., 2022a)
	Pacific oyster (<i>C. gigas</i>)	644 sex-biased circRNAs	Regulates <i>Fem1c</i> and <i>Spef1</i>	Determines the direction of sex differentiation in shellfish	A molecular hub linking energy metabolism and reproductive allocation	(Guo et al., 2025)
	Sea cucumber (<i>A. japonicus</i>)	584 DE-circRNAs	TGF- β and AMPK pathways	Affects sex hormone synthesis	Fills the knowledge gap in sex differentiation in marine invertebrates	(Li et al., 2025)
	Common carp (<i>C. carpio</i>)	Gonadal differential expression profiles	Competitively binds novel_313; targets <i>hsd17β3</i>	Regulates steroidogenic enzymes	Potential molecular targets for monosex breeding	(Hou et al., 2023)
	Spotted scat (<i>S. argus</i>)	Lachesis_group5	Sequesters novel_miR_622	Relieves suppression of ECM remodeling	Ovarian maturation and ovulation mechanisms under IGF3 induction	(Li et al., 2022b)
	Nile tilapia (<i>O. niloticus</i>)	141 DE-circRNAs	Sequesters miR-212 and miR-200b; targets <i>ZP3</i>	Maintains gonadal developmental homeostasis	Indicators for sex identification and gonadal maturity	(Zhong et al., 2022)
Reproduction and gametogenesis	Medaka (model fish, <i>O. latipes</i>)	circ880	Sequesters miR-375-3p	Maintains the stemness of male germ cells	Reference molecular marker for studies of male germ cells	(Zhang et al., 2021)
	Mud crab (<i>S. paramamosain</i>)	Mating-induced circRNA clusters	Targets the RAS and TGF- β pathways	Promotes rapid oocyte proliferation	Reveals the molecular regulatory mechanism by which behavioral stimuli such as mating drive reproductive remodeling	(Yu et al., 2024)
Tissue regeneration	Sea cucumber (<i>A. japonicus</i>)	circRNA8388	Sequesters miR-2392	Extracellular matrix (ECM) remodeling	Promotes intestinal regeneration and recovery after evisceration	(Su et al., 2024)

- 1970 **Abbreviations:** DE-circRNAs, differentially expressed circRNAs; SSD, sexual size dimorphism; ECM, extracellular matrix; RAS,
- 1971 renin–angiotensin system; mTOR, mechanistic target of rapamycin; IGF-1, insulin-like growth factor 1; IGF3, insulin-like growth
- 1972 factor 3; HIPK2, homeodomain-interacting protein kinase 2; TGF- β , transforming growth factor beta; AMPK, AMP-activated
- 1973 protein kinase; *ZP3*, zona pellucida glycoprotein 3.



1974 **Table 4 Key functional circRNAs involved in bacterial infection and their immunoregulatory mechanisms in**
1975 **aquatic animals**

circRNA Name	Host Species	Pathogen/Stimulus	Mechanism	Target	Immune Function/Biological Outcome	R e f.
circARHGEF15	Blunt snout bream (<i>M. amblycephala</i>)	<i>Aeromonas hydrophila</i>	ceRNA	miR-214	Relieves the repression of hepcidin and promotes the expression of inflammatory factors (il-1 β , il-6)	(Lin et al., 2024)
circRXRBB	Blunt snout bream (<i>M. amblycephala</i>)	<i>Aeromonas hydrophila</i>	ceRNA	miR-155	Negative feedback regulation; maintains SOCS1a levels under homeostatic conditions to prevent excessive inflammation	(Zhai et al., 2025)
novel_circ_0004195	Black rockfish (<i>S. schlegelii</i>)	<i>Edwardsiella tarda</i>	ceRNA	novel-530	Activates the NF- κ B pathway and initiates immune responses	(Cao et al., 2021)
circRNA-4254	Black rockfish (<i>S. schlegelii</i>)	<i>Edwardsiella tarda</i>	ceRNA	miR-286	Regulates I κ B α 1 and activates the NF- κ B pathway	(Wang et al., 2025)
circPIK3C2A	Yellow catfish (<i>P. fulvidraco</i>)	<i>Aeromonas veronii</i>	Regulation of parental gene	pik3c2a	Biphasic regulation: promotes autophagy and inhibits apoptosis at the early stage of infection; promotes apoptosis and inhibits autophagy at the later stage	(Zhou et al., 2025b)
circArid4b	Yellow catfish (<i>P. fulvidraco</i>)	<i>Aeromonas veronii</i> + hypoxia	Regulation of parental gene	Arid4b	Downregulation aggravates hepatocyte apoptosis, but reduces host susceptibility to bacterial infection	(Zhou et al., 2025a)
circRNA432	Sea cucumber (<i>A. japonicus</i>)	<i>Vibrio splendidus</i>	ceRNA	miR-2008	Relieves the repression of ELMO1 and enhances phagocytosis	(Fu et al., 2023)
circRNA388	Sea cucumber (<i>A. japonicus</i>)	<i>Vibrio splendidus</i>	m6A modification/ ceRNA	miR-2008	Upregulates AjULK1 and induces autophagic bacterial clearance	(Liu et al., 2024)
circMETTL7A	Sea cucumber (<i>A. japonicus</i>)	<i>Vibrio splendidus</i>	ceRNA	miR-137	Maintains Toll receptor expression and activates the NF- κ B pathway	(Guo et al., 2024b)
circRNA254	Sea cucumber (<i>A. japonicus</i>)	<i>Vibrio splendidus</i>	ceRNA	miR-375	Downregulated after infection, thereby relieving BAG2-associated regulation and promoting apoptosis	(Zeng et al., 2023)



circ-FGL1	Sea cucumber (<i>A. japonicus</i>)	<i>Vibrio splendidus</i>	Protein interaction	AjMyc	Downregulated after infection, releasing AjMyc into the nucleus to initiate Cyt c/Bax transcription and induce apoptosis	(Gu o et al., 202 4a)
circRnf103	Miiuy croaker (<i>M. miiuy</i>)	<i>Vibrio anguillarum</i>	Protein- coding	TRAF6 (binding)	Translates Rnf103-177aa as a decoy to prevent TRAF6 degradation and restore immune signaling	(Zh eng et al., 202 3a)
circMIB2	Miiuy croaker (<i>M. miiuy</i>)	<i>Vibrio anguillarum</i>	Protein- coding	TRAF6 (binding)	Translates MIB2-134aa, attenuates MIB2-mediated TRAF6 degradation, and inhibits bacterial colonization	(Zh eng et al., 202 3b)

- 1976 **Abbreviations:** ceRNA, competing endogenous RNA; miR, microRNA; m6A, N6-methyladenosine; NF-κB, nuclear factor kappa
- 1977 B; ELMO1, engulfment and cell motility 1; AjULK1, *Apostichopus japonicus* unc-51-like autophagy activating kinase 1; SOCS1a,
- 1978 suppressor of cytokine signaling 1a; IκB, inhibitor of κB; BAG2, Bcl-2-associated athanogene 2; Bax, Bcl-2-associated X protein;
- 1979 Cyt c, cytochrome c; TRAF6, tumor necrosis factor receptor-associated factor 6.



1980 **Table 5 Functional circRNAs involved in antiviral immunity in aquatic animals**

Source	circRNA Name	Host Species	Virus/Stimulus	Mechanism	Target	Function/Outcome	Ref.
Host-derived	circRasGEF1B	Miiuy croaker (<i>M. miiuy</i>)	<i>Siniperca chuatsi</i> rhabdovirus (SCRV)	ceRNA	miR-21-3p	Relieves the suppression of MITA (STING) and enhances antiviral immunity	(Chu et al., 2021)
Host-derived	circCBL	Miiuy croaker (<i>M. miiuy</i>)	SCRV	ceRNA/cooperative regulation	MITA	Promotes MITA expression and enhances antiviral responses	(Lv et al., 2023b)
Host-derived	circBCL2L1	Miiuy croaker (<i>M. miiuy</i>)	Viral infection	ceRNA	miR-30c-3-3p	Protects TRAF6 from degradation and enhances innate immunity	(Zheng et al., 2021b)
Host-derived	circPIKfyve	Miiuy croaker (<i>M. miiuy</i>)	Viral infection	ceRNA	miR-21-3p	Increases MAVS levels (under QKI regulation)	(Su et al., 2021)
Host-derived	circDtx1	Miiuy croaker (<i>M. miiuy</i>)	Viral infection	ceRNA	miR-15a-5p	Blocks TRIF downregulation and activates the NF-κB/IRF3 pathway	(Zheng et al., 2021a)
Host-derived	circVPS13D	Miiuy croaker (<i>M. miiuy</i>)	Viral infection	Protein-coding	MAVS (binding)	Translates VPS13D-170aa, promotes MAVS degradation, and negatively regulates IFN production (immune evasion)	(Wang et al., 2023a)
Host-derived	circAGO3	Pacific white shrimp (<i>L. vannamei</i>)	White spot syndrome virus (WSSV)	ceRNA	circAGO3/miRNA/mRNA	A key antiviral molecule; silencing accelerates mortality	(Nuanpirom et al., 2025)
Virus-derived	circ-udg	Gibel carp (<i>C. gibelio</i>)	Cyprinid herpesvirus 2 (CyHV-2)	Protein-coding	Viral replication complex	Translates circ-udg-P147 and facilitates viral replication (promotes infection)	(Zhu et al., 2022b)
Virus-derived	v-circRNAs	Grass carp (<i>C. idella</i>)	Grass carp reovirus (GCRV)	ceRNA	viral/host miRNAs	Some exhibit activities that induce apoptosis and inhibit viral gene expression	(Pan et al., 2021)
Virus-derived	circVP28	Pacific white shrimp (<i>L. vannamei</i>)	WSSV	Protein-coding	Host Rab7 receptor	Translates the truncated protein ceVP28, which competitively binds the host receptor and partially blocks viral particle invasion	(Limkul et al., 2025)

1981 **Abbreviations:** ceRNA, competing endogenous RNA; SCR, *Siniperca chuatsi* rhabdovirus; WSSV, white spot syndrome virus;

1982 GCRV, grass carp reovirus; CyHV-2, cyprinid herpesvirus 2; IFN, interferon; MITA, mediator of IRF3 activation; STING,

1983 stimulator of interferon genes; MAVS, mitochondrial antiviral-signaling protein; TRIF, TIR-domain-containing adaptor-inducing

1984 interferon-β; IRF3, interferon regulatory factor 3; NF-κB, nuclear factor kappa B; QKI, Quaking; TRAF6, tumor necrosis factor

1985 receptor-associated factor 6; Rab7, Ras-related protein Rab-7.



1986 **Table 6 Regulatory mechanisms and functional roles of circRNAs associated with parasitic infections and**
1987 **environmental stress in aquatic animals**

Species	Stress Type	Core circRNA (or Key Finding)	Main Biological Function and Mechanism	Ref.
Tiger puffer (<i>Takifugu rubripes</i>)	<i>Cryptocaryon irritans</i> infection (protozoan)	circ_0000361	Regulates the Wnt and mTOR signaling pathways and participates in antiparasitic immune responses	(Xia et al., 2024)
Swimming crab (<i>Portunus trituberculatus</i>)	<i>Ameson portunus</i> infection (microsporidian)	Microsporidian-derived circRNAs	Regulate pathogen metabolism; induce host metabolic reprogramming and upregulation of DNA replication-related genes	(Zhou et al., 2025d)
Chinese razor clam (<i>Sinonovacula constricta</i>)	Acute hypersaline challenge	TAT/HYAL4-associated circRNAs	Regulate amino acid synthesis and membrane transport; downregulation of HYAL4 reduces hyaluronan hydrolysis and helps maintain cellular water content	(Cao et al., 2023)
Hong Kong oyster (<i>Crassostrea hongkongensis</i>)	Salinity stress	Six core differentially expressed circRNAs	Participate in taurine, hypotaurine, and phenylalanine metabolism and regulate cellular osmotic balance	(Ibrahim et al., 2023)
Pacific white shrimp (<i>Litopenaeus vannamei</i>)	Combined pH and alkalinity stress	novel_circ_004981 and novel_circ_021024	Regulate glutathione peroxidase (GPx) and carbonic anhydrase-3 (CA-3), thereby modulating the antioxidant system	(Fan et al., 2025)
Chinese mitten crab (<i>Eriocheir sinensis</i>)	Hypoxia stress	circRNA12	Affects Golgi-endoplasmic reticulum transport and enhances programmed cell death and immune effects	(Zhou et al., 2025c)
Yellow catfish (<i>Pelteobagrus fulvidraco</i>)	Hypoxia + bacterial infection (combined stress)	circArid4b	Downregulation relieves inhibition of the parental gene <i>Arid4b</i> , enhances apoptosis and inflammatory responses, and improves antibacterial resistance	(Zhou et al., 2025a)
Sea cucumber (<i>Apostichopus japonicus</i>)	High temperature + hypoxia (combined stress)	Five shared circRNAs, including novel_circ_0003311	Mediates the “regulation of response to stimulus,” involving metabolic processes, signal transduction, and ion responses	(Huo et al., 2021)
Zebrafish (model fish, <i>Danio rerio</i>)	Triclosan exposure (antimicrobial agent)	circRNA48	Contributes to the adverse outcome pathway (AOP) framework and mediates neurotoxicity, cardiotoxicity, oxidative stress, and inflammatory responses	(Wang et al., 2024c)



Zebrafish (model fish, <i>Danio rerio</i>)	Cypermethrin/chlorpyrifos exposure (pesticides)	62 significantly downregulated circRNAs	Specifically regulate the MAPK, p53, and apoptosis pathways and may serve as candidate biomarkers of neurotoxicity	(Özdemir & Arslan, 2022)
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1988 **Abbreviations:** GPx, glutathione peroxidase; CA-3, carbonic anhydrase-3; AOP, adverse outcome pathway; mTOR, mechanistic

1989 target of rapamycin; DNA, deoxyribonucleic acid; HYAL4, hyaluronidase 4; TAT, tyrosine aminotransferase; MAPK, mitogen-

1990 activated protein kinase; p53, tumor protein p53.

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Table 7 Promising candidate circRNA biomarkers in aquatic animals

Application Domain	Candidate Biomarker (Host/Virus)	Target/Mechanism	Indicative Function/Trait	Ref.
Disease diagnosis	circARHGEF15 (blunt snout bream)	miR-214/Hepcidin	Early warning of bacterial infection; initiation of inflammatory responses	(Lin et al., 2024)
Disease diagnosis	circVP28 (WSSV/shrimp)	Viral structural protein	Candidate indicator of viral infection activity	(Limkul et al., 2025)
Disease diagnosis	circAGO3 (shrimp)	Immune pathway	Candidate indicator of host antiviral responses	(Nuanpirom et al., 2025)
Environmental monitoring	circArid4b (yellow catfish)	Apoptosis/inflammation	Severity of hypoxia stress; risk of tissue injury	(Zhou et al., 2025a)
Environmental monitoring	novel_circ_003889 (rainbow trout)	HSP90/HSP70	Heat stress intensity; evaluation of thermotolerance	(Quan et al., 2021)
Evaluation of economically important traits	circMef2c (Nile tilapia)	miR-34, miR-130b-5p, and miR-202/ <i>igfbp2</i> -, <i>myod1</i> -, and <i>fgf14</i> -related network	Candidate indicator associated with growth rate and skeletal muscle development	(Rbbani et al., 2023)
Evaluation of economically important traits	W-linked circRNA (Chinese tongue sole)	CNOT6L	Auxiliary indicator for female genetic sex identification and pseudo-male discrimination	(Li et al., 2018)

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Abbreviations: miR, microRNA; WSSV, white spot syndrome virus; HSP90, heat shock protein 90; HSP70, heat shock protein

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70; CNOT6L, CCR4-NOT transcription complex subunit 6 like; W-linked, W chromosome-linked.

