

CRISPR 3.0: Beyond Genome Editing - The Emerging Future of Aquatic Animal Health

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ABSTRACT

Aquaculture is expanding rapidly, but infectious diseases continue to threaten production, profitability, and animal welfare. At the same time, CRISPR technology is evolving beyond its original role as a programmable DNA-cutting system. Base editing and prime editing enable increasingly precise genetic modification. Meanwhile, Cas12- and Cas13-based systems offer new possibilities for pathogen detection and RNA targeting. This broader technological landscape is sometimes described as “CRISPR 3.0”. However, the term is conceptual rather than a formally standardized scientific classification. For aquatic animal health, its importance may lie not only in genome editing, but also in rapid pathogen diagnosis, functional investigation of host-pathogen interactions, precision breeding and future pathogen-targeted interventions. CRISPR-based assays have already been demonstrated for important shrimp pathogens, and recent work has extended multiplex detection of white spot syndrome virus (WSSV) and *Ecytonucleospora hepatopenaei* (EHP) to rapid, field-oriented formats. Researchers are also investigating genome editing for disease-related traits in fish, while more advanced approaches such as prime editing and RNA targeting are emerging. However, most applications beyond diagnostics remain experimental and face challenges involving delivery, editing efficiency, ecological safety, animal welfare and regulation. CRISPR is therefore unlikely to replace conventional biosecurity and disease-management practices. Instead, its greatest potential may be as one component of an integrated precision-health framework for aquaculture.

INTRODUCTION:

Aquaculture has become an essential part of the global food supply. However, increasing production intensity also increases the need to maintain animal health. Viral, bacterial and parasitic diseases can spread rapidly through cultured populations, causing mortality, poor growth and substantial economic losses. For shrimp, pathogens such as white spot syndrome virus (WSSV), *Ecytonucleospora hepatopenaei* (EHP) and the bacteria associated with acute hepatopancreatic necrosis disease (AHPND) are major concerns. Fish farming faces a similarly diverse range of viral, bacterial and parasitic diseases. Conventional approaches, including biosecurity, pathogen surveillance, selective breeding, vaccination, water-quality management, and appropriate therapeutics, remain the foundation of aquatic animal health. However, advances in molecular biology are opening another possibility: can we detect disease earlier, understand it more precisely, and manage it at the level of genes and nucleic acids?

Here, CRISPR becomes particularly interesting. The CRISPR-Cas9 system transformed molecular biology by enabling researchers to direct a nuclease to a selected DNA sequence using guide RNA (Jinek *et al.*, 2012). What began as a programmable molecular “scissor” has since developed into a much broader toolkit. Researchers can now modify individual DNA bases, introduce programmed sequence changes, regulate gene activity, target RNA, and detect pathogen nucleic acids. This broader evolution is sometimes called “CRISPR 3.0.” The term should be used carefully. It is not an officially defined third generation of CRISPR technology. Rather, it is a useful conceptual term for describing the movement from conventional genome cutting toward increasingly programmable manipulation and detection of biological information.

For aquaculture, the important question is therefore not simply: Can CRISPR edit an aquatic animal? The more useful question is: Could CRISPR become part of the aquatic animal health toolbox?

1. From Molecular Scissors to a Molecular Toolkit

The first CRISPR revolution was based on targeted DNA cleavage. Cas9 could be programmed to recognize a particular DNA sequence and generate a double-strand break, allowing researchers to disrupt or modify genes (Jinek *et al.*, 2012). The technology subsequently became more precise. Base editing allows particular nucleotide conversions without conventional double-strand DNA cleavage. Prime editing, introduced by Anzalone *et al.* (2019), extended this concept by combining a Cas9 nickase with reverse transcriptase and a specialized guide RNA to introduce programmed substitutions, insertions, and deletions.

The progression can be broken down as follows:

- I. CRISPR-Cas9 → Cut DNA
- II. Base editing → Change selected DNA letters
- III. Prime editing → Programmed sequence changes by writing.
- IV. Cas13 → Target RNA
- V. Cas12/Cas13 → Detect nucleic acids

This distinction matters for aquatic health because different problems require different tools. A disease outbreak may require rapid pathogen detection, not genome editing. A host-pathogen study may require functional gene manipulation. A breeding programme may eventually require precise modification of

a favourable genetic variant. CRISPR is increasingly capable of addressing all three.

2. CRISPR Diagnostics: The Most Immediate Opportunity?

Perhaps the strongest near-term connection between CRISPR and aquaculture is diagnostics. Some CRISPR proteins, particularly Cas12 and Cas13, can recognize specific nucleic-acid sequences and subsequently generate a detectable signal. When coupled with rapid amplification methods such as recombinase polymerase amplification (RPA), these systems can operate under relatively simple, constant-temperature conditions. This is important for aquaculture because disease diagnosis is often most valuable before widespread mortality occurs.

Researchers have already investigated CRISPR-based detection for WSSV, AHPND, and EHP. A recent multiplex assay combined RPA with Cas12a and Cas13a to simultaneously detect WSSV and EHP in shrimp. The reported detection limits were 20 copies of target DNA for EHP and 200 copies for WSSV, respectively. The assay operated at 37°C in less than one hour, produced distinct visual signals and showed no false positives with DNA from other shrimp pathogens tested (Kanitchinda *et al.*, 2025). The technology is moving further toward field-oriented testing. This matters because conventional PCR and qPCR remain highly useful but generally depend on laboratory infrastructure, thermal cycling equipment, and trained personnel. CRISPR platforms that combine isothermal amplification with fluorescence or lateral-flow readouts could potentially simplify testing closer to the farm. However, CRISPR diagnostics should not yet be considered a complete replacement for PCR or qPCR. Sample preparation, target selection, analytical validation, quantitative interpretation and

regulatory acceptance remain important considerations. Nevertheless, the direction is clear: CRISPR diagnostics could potentially shift aquatic disease surveillance from laboratory-dependent testing toward faster, more portable molecular detection.

3. Could CRISPR Help Produce Disease-Resilient Animals?

Diagnostics tell us what pathogen is present. Genome editing may eventually help us understand why some animals respond differently to infection. Host genetics influence disease resistance, and selective breeding already exploits naturally occurring variation in disease-related traits. CRISPR provides researchers with a way to test candidate genes and investigate their functional roles experimentally (Lin *et al.*, 2025). This could eventually contribute to precision breeding. For example, suppose genomic studies identify a particular genetic variant associated with improved disease resistance. Researchers could experimentally test whether changing that sequence actually alters susceptibility. This distinction between association and causation is important.

CRISPR-based genome editing has already been used in several aquatic species to target aquaculture-relevant traits, including disease-related phenotypes. However, most applications remain experimental, and a single gene rarely controls complex traits such as disease resistance. Genetic background and environmental conditions can strongly influence the resulting phenotype (Lin *et al.*, 2025). Another important point is that CRISPR does not automatically mean non-transgenic. The technology is simply a molecular tool. It can disrupt an existing gene, introduce a precise mutation, or insert an external gene. These outcomes have different biological and regulatory implications.

4. Prime Editing: Towards Genetic Fine-Tuning

One of the most interesting developments for future aquaculture breeding is prime editing. Researchers often use traditional CRISPR-Cas9 to disrupt genes. Prime editing offers the potential for more precise changes. Imagine that researchers identify a naturally occurring DNA variant associated with resistance to a particular pathogen. Rather than eliminating the entire gene, a future breeding strategy could theoretically reproduce that specific variant. Prime editing was developed to make programmed sequence changes without requiring a conventional double-strand DNA break (Anzalone *et al.*, 2019). This is scientifically attractive, but its application in aquaculture remains emerging rather than established. Recent reviews indicate that base and prime editing are still much less developed in aquatic species than conventional CRISPR-Cas9 editing.

Nevertheless, these technologies could eventually expand the precision with which aquaculture traits are investigated and modified (Lin *et al.*, 2025). Therefore, prime editing in an aquatic research model shows technological feasibility, not that precision-edited, disease-resistant shrimp are ready for commercial farming. That distinction is essential when discussing the future of CRISPR in aquaculture.

5. Beyond DNA: The RNA Dimension

CRISPR technology is also moving beyond DNA. Cas13 systems can target RNA, creating new possibilities for studying gene expression and host-pathogen interactions. This is particularly relevant to aquatic health because many important aquatic viruses have RNA genomes. Researchers have already investigated RNA-targeting CRISPR systems in aquatic organisms. Such studies demonstrate that programmable RNA

manipulation can function in fish and provide a foundation for future investigations of host-pathogen interactions. Could the same principle eventually be used against RNA viruses? Possibly, but this remains a research question.

A programmable antiviral system would have to reach the correct tissues, remain active long enough to influence infection, and avoid unintended effects on host RNA. Viruses can also mutate, potentially escaping sequence-specific interventions. Thus, RNA-targeting CRISPR is a promising research direction, not yet a farm-ready antiviral treatment.

6. Could CRISPR Directly Fight Aquatic Viruses?

This is perhaps the most exciting possibility. Instead of simply detecting a pathogen, could CRISPR be designed to interfere directly with the pathogen inside the animal? Experimental studies have begun exploring this concept for WSSV in shrimp. This research shows that pathogen-targeted CRISPR systems can be designed and tested in vivo. However, the gap between **proof of concept and practical treatment** is substantial.

A farm-scale antiviral CRISPR system would need to overcome several problems:

- efficient delivery into infected tissues;
- sufficient duration of activity;
- viral sequence variation and escape;
- possible effects on host nucleic acids;
- scalability; and
- economic feasibility.

As such, CRISPR-based antiviral intervention should currently be viewed as an experimental approach, not a substitute for WSSV biosecurity or other traditional disease-

management measures. What happens to Biosafety? The responsible use of genome-edited aquatic animals in future aquaculture should be subject to environmental risk assessments. One major issue is what may happen if these animals escape into natural water bodies. Even if they fail to reproduce, they can still mingle with the wild. One strategy is reproductive containment, which researchers have investigated using genetic methods that target germ-cell development in fish to prevent reproduction and genetic mixing with wild populations. Sterility alone does not guarantee environmental safety. Experiments with sterile animals may affect predation, competition, and transmission of zoonotic pathogens. Because of this, future CRISPR-based aquaculture will require closing gaps in biological and physical containment, environmental risk assessment, regulatory approval, and long-term monitoring.

7. CRISPR 3.0: From Gene Editing to Precision Aquatic Health

The greatest potential may not come from one CRISPR technology. It may come from integration. Imagine a future health-management pipeline:

1. Pathogen surveillance
2. Rapid CRISPR detection
3. Sequencing and pathogen characterization
4. Genomic and AI-assisted analysis
5. Host-pathogen target identification
6. Functional CRISPR studies
7. Precision breeding or experimental intervention
8. Health and environmental monitoring

In this framework, CRISPR would work alongside sequencing, genomics, artificial intelligence, selective breeding, microbiome research, biosecurity and environmental monitoring (Fig. 1). This is a more realistic vision of CRISPR 3.0 than the idea that genome editing alone will “solve” aquaculture diseases.

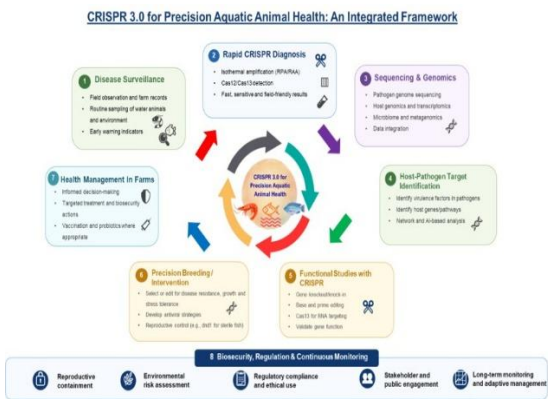


Fig1. CRISPR 3.0 for precision aquatic animal health: an integrated approach

8. What Is Real Today and What Is Still Future?

Application	Current position
CRISPR-based pathogen diagnostics	Demonstrated
CRISPR genome editing in aquatic animals	Demonstrated experimentally
Disease-resistance gene studies	Established research area
Prime/base editing in aquatic species	Emerging
RNA targeting in aquatic organisms	Experimental
CRISPR-based antiviral intervention	Proof-of-concept/research stage
Commercial CRISPR-edited disease-resistant shrimp	Not established
Farm-scale CRISPR therapeutics	Prospect

The distinction is critical. A technology can be scientifically possible without being technologically mature.

9. The Road Ahead

The most realistic near-term contribution of CRISPR to aquatic animal health is likely to be diagnosis and functional research, rather than direct genetic or antiviral intervention. The evidence for diagnostics is already advancing rapidly. Recent work is moving toward multiplex detection, isothermal amplification, visual readouts and increasingly field-oriented systems. The 2025 WSSV/EHP multiplex assay, for example, combined Cas12a and Cas13a with RPA and produced results in less than one hour at 37°C (Kanitchinda *et al.*, 2025).

Genome editing, meanwhile, provides researchers with powerful tools to understand host-pathogen biology and investigate traits relevant to aquaculture. More advanced technologies such as base and prime editing may eventually allow finer genetic modification. However, their practical deployment will require considerably more research (Lin *et al.*, 2025). The key challenge, therefore, is not simply demonstrating that CRISPR works. It is determining where CRISPR provides a genuine advantage over existing technologies.

CONCLUSION

CRISPR began as a molecular pair of scissors. It is becoming a much larger biological toolkit. It can now cut DNA, modify genetic letters, make programmed sequence changes, target RNA and detect pathogen nucleic acids. For aquatic animal health, the most immediate opportunity is probably rapid pathogen detection. More ambitious opportunities include functional investigation of disease-related genes, precision breeding, reproductive containment, and potentially pathogen-targeted interventions. However, CRISPR 3.0 should not be presented as a magic solution.

Aquaculture will still need biosecurity, good husbandry, surveillance, selective breeding and responsible disease management. The real promise lies in combining these established approaches with increasingly precise molecular technologies. The future of CRISPR in aquaculture may therefore not be about editing animals simply because we can. It may be about detecting disease earlier, understanding host-pathogen interactions more precisely, and making carefully justified interventions when the biology, safety, and economics support them. That is where CRISPR 3.0 could move from an exciting molecular concept toward a meaningful component of precision aquatic animal health management.

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