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Review Article

CRISPR-Cas Applications in Fish Genomics: Implications for Selective Breeding and Fisheries Sustainability

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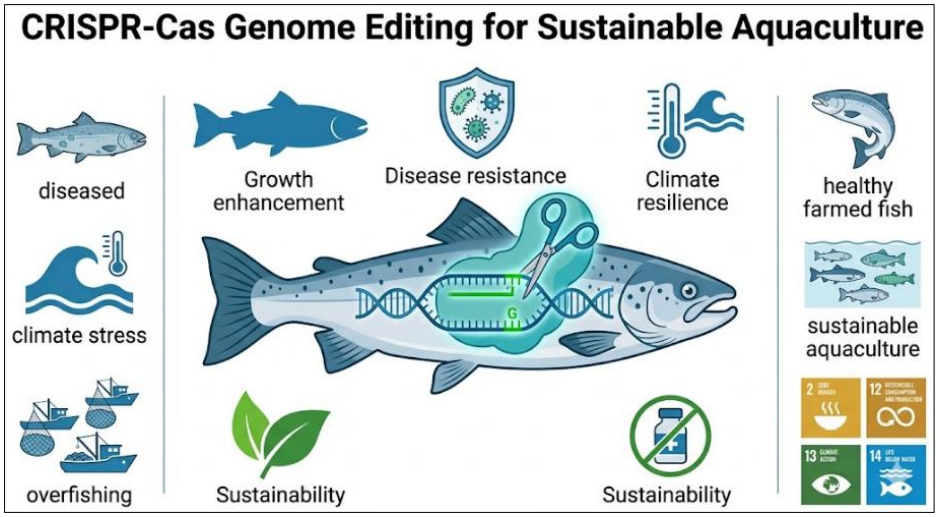
Abstract



Population growth, habitat destruction, climate change, and new diseases are all threats to the world's fisheries and aquaculture. Traditional selective breeding has achieved success, but has some drawbacks such as long breeding cycles, polygenetic nature of traits, and limited genetic diversity in closed populations. The potential of CRISPR-Cas genome editing is a recent technology that has been used in zebrafish since 2013, and is now revolutionizing the genetic improvement of Atlantic salmon, Nile tilapia, rainbow trout, channel catfish, grass carp, and others. This precision has been further improved by advanced technologies such as base editors, prime editors, CRISPRa, and CRISPRi. Applications include growth enhancement through myostatin disruption; increased disease resistance, reproductive efficiency, flesh quality and thermal/osmotic stress tolerance. These tackle key production bottlenecks, while contributing to lower use of antibiotics, lower environmental footprint and climate-resilient systems based on the UN SDGs. Even with these challenges, there are significant issues off-target effects, mosaicism, regulatory issues, public acceptance and polygenetic traits. Now, new technologies like artificial intelligence-driven design, multi-omics, multiplex editing, and epigenome editing provide answers. To be fully commercialised, regulatory harmonisation and transparent communication are paramount.

Keywords: CRISPR-Cas9, Fish Genomics, Aquaculture, Selective Breeding, Genome Editing, Myostatin, Disease Resistance, Fisheries Sustainability, Precision Breeding, Climate Resilience.

1. INTRODUCTION

Fisheries and aquaculture are an important component of global food security, providing nearly 20% of animal protein for more than 3.3 billion people and contributing to the lives of some 600 million people (Dongyu, (2024). Global fish production in 2020 reached a record 214 million tonnes, with aquaculture accounting for almost 49% of this, as capture fisheries approach sustainable limits, the proportion of aquaculture production is expected to exceed 55% by 2030. The sector accounts for more than USD 400 billion per year and plays a key role in food policies in Asia, Sub-Saharan Africa, Latin America and Pacific Island countries (PIC) (Naylor *et al.,* 2021). Advancement in aquaculture is directly connected to the United Nations Sustainable Development Goals 2 (Zero Hunger) and 14 (Life Below Water) and is a geopolitical and humanitarian priority. As demand for protein is projected to increase by 70% by 2050, when the global population will have grown to 9.7 billion, aquaculture will need to play a major role in meeting this demand with very limited potential of land use for terrestrial livestock and capture fisheries (Naylor *et al.,* 2021; Froehlich *et al.,* 2018).

Although the industry has grown by leaps and bounds, it is still plagued by considerable biological, environmental and socioeconomic issues. Pathogens include infectious salmon anaemia virus (ISAV), Aeromonas hydrophila, white spot syndrome virus (WSSV), Flavobacterium columnare and grass carp reovirus (GCRV) that are responsible for losses of over USD 6 billion annually (Maldonado *et al.,* 2022; Ijaz *et al.,* 2024). These outbreaks cause high dependency on antibiotics and chemicals, and thus increase the danger of antimicrobial resistance. The changing climate also contributes to production in multiple ways, by modifying water temperature, oxygen levels, salinity, and disease dynamics. Atlantic salmon, for example, thrives in a temperature range between 12-14°C and projected sea surface temperature changes of 1.5-3.0°C by mid-century could lead to reduced growth by 10-25% and increased susceptibility to diseases such as amoebic gill disease (Benedicenti *et al.,* 2019; Qasim *et al.,* 2025). Unsustainable feed ingredients, such as fishmeal and fish oil sourced from wild-fish, can also compromise marine ecosystem integrity (Naylor *et al.,* 2021). Despite of significant improvements in traditional selective breeding (10-15% improvement per generation in salmonids growth rates), such breeding has been constrained by long generation times (5-10 years), narrow genetic variation within closed populations and the inefficiency in addressing polygenetic traits (Gjedrem *et al.,* 2012). These constraints underscore the critical need for innovative biotechnological solutions.

In the early 2000s, the zinc finger nuclease (ZFNs) technology was developed and shown to be able to induce targeted modification in fish; this technology was more complicated and expensive (Bogdanove & Voytas, 2011). TALEN technology was developed in about 2010, and was also demonstrated to be able to induce targeted modification in fish, but was also more complicated and expensive (Wood *et al.,* 2011). The introduction of CRISPR-Cas9, inspired by its immune function in bacteria and its ability to be program, simplified, made efficient and affordable the editing of the genome (Cong *et al.,* 2013). For the applications in fish genomics and precision breeding, further innovations such as Cas12a, Cas13, base editors, prime editors and CRISPRa/i have been introduced (Komor *et al.,* 2016).

This review provides a thorough overview of the use of CRISPR-Cas for fish genomics, breeding and sustainability. It brings together mechanistic information on a variety of CRISPR systems, along with their applications in important commercial species, regulatory requirements in six jurisdictions, and future outlook for AI-driven and multi-omics strategies, unlike previous literature that was either purely biological or single-species focused. Its comprehensive design connects molecular precision, marketability, regulatory considerations, and sustainability impacts, essential for moving lab innovations into regulations and industry practices.

2. Fundamentals of CRISPR-Cas Systems

2.1 Evolution and Discovery of CRISPR

In 1987, Ishino et al. first identified the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) sequences in Escherichia coli, but it wasn't until almost 20 years later that they were found to have biological significance. Jansen et al. (2002) and Mojica et al. (2005) systemically characterized CRISPR loci in prokaryotic genomes and found that the spacer sequences between repeats are of exogenous phage and plasmid DNA origin indicating an adaptive immune function. Marraffini and Sontheimer (2010) showed that CRISPR systems are DNA targeting and the processing of CRISPR RNA in Streptococcus pyogenes. The breakthrough of the Cas9 nuclease as a programmable endonuclease by and its use for genome editing in human and mouse cells began the revolution in genome editing that now seeps into the field of fish biology.

2.2 Classification of CRISPR-Cas Systems

The CRISPR-Cas universe has been divided into two classes, six types, and more than 30 subtypes, depending on the composition of Cas proteins and their mechanism. Class 1 systems (Types I, III, and IV) are multi-subunit effector complexes, and are currently only used in fish genomics in archaea. Single-effector proteins are almost exclusively used for the eukaryotic genome editing, and are referred to as Class 2 systems (Types II, V, and VI). The most widely used ones in aquaculture research are Type II (Cas9), Type V (Cas12a/Cpf1), and Type VI (Cas13). Although a wealth of engineered variants of SpCas9 exist, such as SpCas9-HF1, eSpCas9 and HypaCas9, which have reduced off-target effects, SpCas9 of S. pyogenes is still the most popular

workhorse. The Cas12a, which has a T rich PAM site and staggered cut, offers complimentary targeting ability especially useful in AT-rich genomic regions that are shared among teleost fish (Makarova *et al.,* 2015; Wang *et al.,* 2025).

2.3 Mechanisms of Genome Editing

The canonical CRISPR-Cas9 editing mechanism involves three major steps: (1) Target recognition by sgRNA, (2) Conformational changes of Cas9 to form an R-loop and sequential nuclease activity by the HNH and RuvC domains yielding a blunt double-strand break (DSB) ~3 bp prior to the PAM motif, and (3) cellular DNA repair, either via error-prone non-homologous end joining (NHEJ) leading to insertion-deletion (indel) mutations for gene disruption, or homology-directed repair (HDR) using the donor template for precise sequence insertion (Jiang & Doudna, 2017; Ran *et al.,* 2013). In the context of editing in post-mitotic and early embryonic cells, where NHEJ is predominant, a fundamental constraint for precision knockin applications is given. Partial improvements have been obtained by active research on HDR enhancement methods such as small molecule inhibition of NHEJ components like SCR7 and M3814, use of asymmetric single-stranded oligodeoxynucleotide (ssODN) donors, and cell-cycle synchronization; however, translation to fish embryo editing remains sub-optimal (Chu *et al.,* 2015)

2.4 Recent Advances: Base Editing, Prime Editing, CRISPRa, and CRISPRi

The precision toolbox of fish genomics has been greatly expanded with evolution beyond classical DSB-mediated editing. Unlike the traditional DNA double-strand breaks (DSBs) associated with homologous recombination, base editors allow single-nucleotide changes. Komor et al. (2016) have developed a method called Cytosine base editors (CBEs) which combine a nickase Cas9 (nCas9) with a cytidine deaminase (APOBEC1) and uracil glycosylase inhibitor in order to achieve precise C.G to T.A conversions. The toolkit for directional base editing has been completed with adenine base editors (ABEs) (Kim *et al.,* 2017) which are based on an evolved TadA deaminase acting on A.T to G.C changes. These were successfully applied in fish, in zebrafish and Atlantic salmon, to correct immune-regulatory mutations and introduce the thermotolerance alleles. Recently developed Prime Editing (PE), which provides even greater versatility. It includes a prime editor (nCas9 with engineered reverse transcriptase) and a pegRNA that guides the editing to the desired site and acts as a template for the edit. PE can cause all 12 types of mutations, without the need for DSBs or donor DNA. Although maximizing efficiency is still required because of competition with endogenous repair pathways, there are success stories in zebrafish and medaka, indicating the potential in commercial species (Anzalone *et al.,* 2019). CRISPRa and CRISPRi use catalytically dead Cas9 (dCas9) that is fused to a transcriptional activator (VP64, VPR, SAM) or repressor (KRAB) as more illustrated in Figure 1 and Table 1. These tools allow for "modulating" the expression of genes without altering them permanently, and without causing mutations. They have been utilized in zebrafish for systematic pathway analysis, identification of growth regulatory networks, and for pre-screening of candidate genes before permanent edits. The precision, safety, and versatility of these advanced CRISPR variants in genome manipulation are greatly improved in aquaculture species.

Table 1: Comparison of Major CRISPR-Cas Systems and Their Applications in Fish Genomics

CRISPR System	Effector Protein	Editing Mechanism	Advantages	Limitations	Applications in Fish Research
CRISPR-Cas9	SpCas9 / SaCas9	DSB via RuvC + HNH domains; NGG PAM required	High efficiency; well-characterized; broad delivery options	Off-target effects; large size limits AAV delivery	Growth enhancement, disease resistance, knockout studies
CRISPR-Cas12a (Cpf1)	AsCpf1 / LbCpf1	DSB via RuvC domain; T-rich PAM (TTTN)	Staggered cuts; lower off-targets; processes own crRNA	Lower efficiency in some fish species; PAM restriction	Functional genomics in zebrafish; tilapia editing
CRISPR-Cas13	LwaCas13a / CasRx	RNA targeting; no DSB in DNA	RNA knockdown without permanent DNA change	Transient effect; limited fish applications	Gene expression modulation; viral RNA targeting
Base Editing (CBE/ABE)	nCas9-deaminase fusion	C→T or A→G conversion without DSB	Precise single-base changes; no donor template needed	Limited to specific base conversions; bystander edits	Point mutation correction; allele-specific editing in salmon
Prime Editing	PE2/PE3 (nCas9-RT)	pegRNA-guided reverse transcription; versatile edits	All 12 base conversions, small insertions/deletions	Lower efficiency; large construct size	Emerging use in model fish; precise SNP introduction

CRISPR System	Effector Protein	Editing Mechanism	Advantages	Limitations	Applications in Fish Research
CRISPRa/CRISPRi	dCas9-activator / dCas9-repressor	Transcriptional regulation; no DSB	Reversible gene modulation, no permanent edit	Transient; delivery challenges in embryos	Functional genomics; pathway analysis in zebrafish

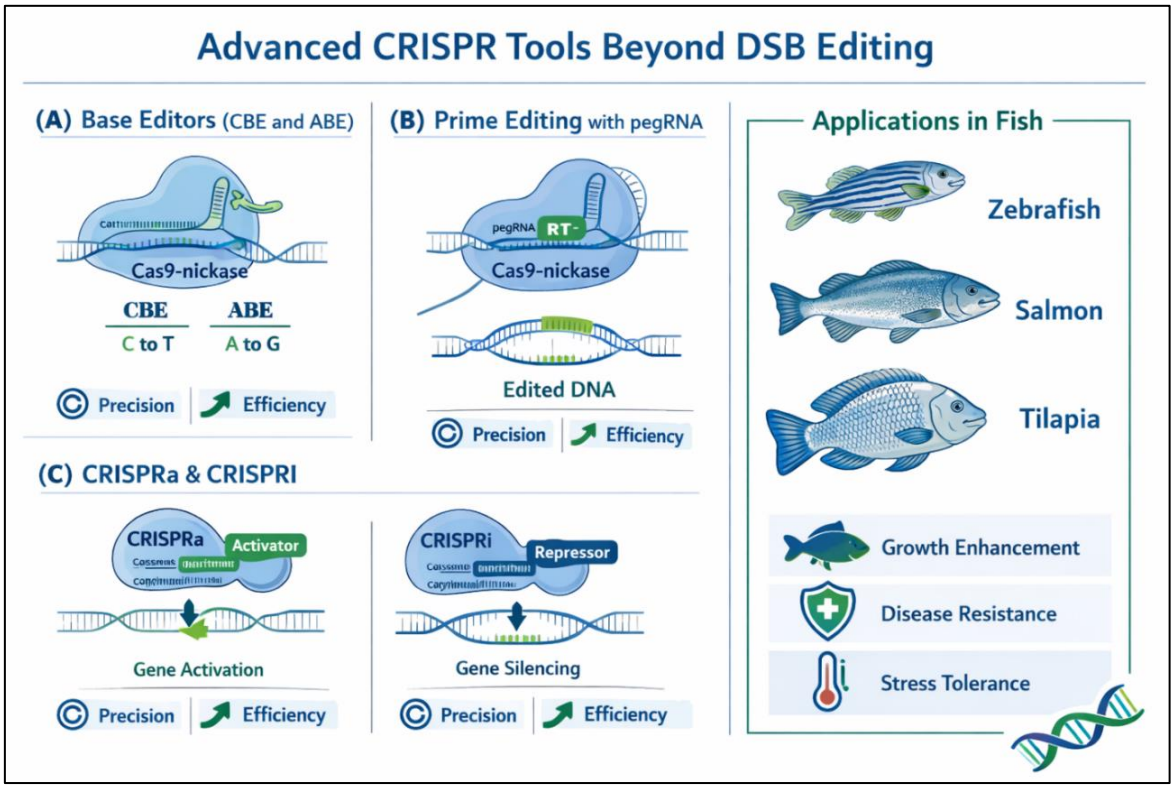


Figure 1: Advanced CRISPR-Cas tools beyond classical DSB editing and their applications in fish genomics

3. Fish Genomics in the CRISPR Era

3.1 Overview of Fish Genomes

Teleost fish are the most species-rich vertebrate group with an estimated 34,000 species, and are characterized by an extraordinary genomic diversity in terms of genome size, ploidy and genome structure. The whole genome duplication event that occurred in teleosts around 350 million years ago generated duplicate copies of genes across the genome that make interpretation of functional genomic data difficult and require multiple approaches to editing to achieve a phenotypic change (Braasch *et al.,* 2008). In fact, salmonids experienced an additional, more recent, whole genome duplication 80 million years ago, creating highly duplicated, partially rediploidized genomes such as the Atlantic salmon genome in which sequence similarity between paralogs is high and poses challenges for designing CRISPR targets (Macqueen *et al.,* 2017). The genome sizes of teleosts vary between about 350 Mb in the pufferfish (Takifugu rubripes) to more than 3 Gb in the Atlantic salmon, with a moderately sized repeat landscape (20-40% of the genome) and a comparable number of protein-coding genes (20,000-30,000) to other vertebrates, with a GC content in the range 40-50% (Lien *et al.,* 2016). All of these parameters can affect the efficiency of CRISPR targeting, off-target landscape, and feasibility of HDR-based knockin approaches.

3.2 Genomic Resources and Databases

During the past decade, there has been a revolution in the availability and quality of fish genomic resources. There have been some references genome assemblies that include zebrafish (GRCz11, 1.37 Gb, 2017); zebrafish compared to the reference, Atlantic salmon (ICSASG-v2); zebrafish compared to the reference, rainbow trout (Omyk-1.0); zebrafish compared to the reference, Nile tilapia, channel catfish; common carp; grass carp; medaka, others, and enabled a robust sgRNA design, off-target prediction, and comparative genomic analysis (Houston *et al.,* 2020). Database curation efforts such as Ensembl, NCBI RefSeq, FishDB, and the Functional Annotation of All Salmonid Genomes (FAASG) project have generated standardized annotations for genes, ortholog identification and regulatory element annotations, all of which are essential for CRISPR target identification (Macqueen *et al.,* 2017). Single-nucleotide polymorphism (SNP) panels and high-density genotyping arrays are available for Atlantic salmon (e.g. >900K SNPs), rainbow trout and tilapia and this allows

conducting genome-wide association studies (GWAS) to detect quantitative trait loci (QTL) for further validation by CRISPR (Barson *et al.,* 2015). The contiguity of genome assembly has been significantly enhanced by long-read sequencing technologies (Pacific Biosciences HiFi, Oxford Nanopore Technology), which enables de novo genome assembly of non-model aquaculture species that have a complex repeat landscape that previously could not be assembled at these quality levels.

3.3 Functional Genomics in Fish

Transcriptomic, proteomic and metabolomic platforms are important in identifying candidate genes for CRISPR intervention, as they can reveal patterns of gene expression, protein interaction networks, and responses in metabolic flux to growth stimuli, pathogen challenge or environmental stressors, respectively. RNA sequencing (RNA-seq) has been widely applied to elucidate transcriptional effects of CRISPR-mediated gene knockouts (KO), and to confirm the on-target effects and to detect pathway compensation that can lead to erroneous conclusions about the phenotype. CRISPRi functional screens are mammalian-based and have been adapted to zebrafish, allowing for systematic mapping of the essentiality of genes within the genome relevant to aquaculture trait biology (Iyer *et al.,* 2025).

3.4 Gene Discovery and Trait Mapping

The other economically important traits such as disease resistance to sea lice, pancreas disease, amoebic gill disease, Columnaris disease, and viral hemorrhagic septicaemia have been associated with genomic regions in GWAS and QTL mapping studies, as have growth rate, feed conversion ratio, flesh color, and thermal tolerance. CRISPR validation is increasingly being used to prioritize candidate causal variants identified by GWAS peaks as the variant of interest is either deleted or added in the zebrafish or target species to test if it contributes causally to a difference in a phenotype. This validation paradigm of GWAS discovery and CRISPR validation is a powerful new paradigm in accelerating GWAS discovery into breeding targets (Robledo *et al.,* 2019).

4. Applications of CRISPR-Cas in Fish Improvement

4.1 Growth Enhancement

The most commonly measured economic trait in commercial aquaculture is growth performance, and the mstn gene encodes myostatin, a negative growth factor for skeletal muscle which is the most widely targeted gene in fish species for growth enhancement via CRISPR. The increase in muscle mass in Nile tilapia was up to 30% in F0 and F1 myostatin KO fish, along with a corresponding improvement in feed conversion ratio (FCR). The mstn is the most robust and reproducible growth-enhancing CRISPR target in fish, with similar phenotypes reported in common carp, channel catfish and yellow catfish (*Pelteobagrus fulvidraco*) (Gichana *et al.,* 2026). A simultaneous knockout of two genes, mstn1 and mstn2, was achieved using multiplex Cas9 in rainbow trout, resulting in a clear hyper-muscular effect (hyper-muscular phenotype), but with no reported negative impacts on reproductive viability or survival in tank rearing conditions. In addition to myostatin, the growth hormone receptor and insulin-like growth factor (pathways have been assessed as secondary targets, but the pleiotropic effects of these pathways in reproduction, immune function and bone metabolism need to be carefully considered for commercialisation of this target (Zhu, *et al.,* 2024).

4.2 Feed Efficiency and Nutrient Utilization

Expense of feed accounts for 50-70% of aquaculture production expenses, and reliance on unsustainable fishmeal and fish oil feed creates an economic and ecological burden. CRISPR-based technologies for enhancing the endogenous production of fatty acids are especially promising for decreasing reliance on fish oil in the feed. Atlantic salmon found that gene disruption caused significant inhibition of elongation of EPA and DHA relative to an increase in transcript levels of the compensatory SREBP-1 pathway, thereby laying the groundwork for using CRISPR to manipulate the omega-3 fatty acid profile in salmon. In contrast, optimization approaches using the CRISPRa system have shown to be feasible in tilapia models for increasing the production of omega-3 fatty acids by overexpressing the gene coding for fatty acyl desaturase (fads2) and elongase (elovl5) enzymes (Datsomor *et al.,* 2019).

4.3 Disease Resistance

It is also a necessity to lower the use of antibiotics in fish health management, so that the engineering of resistance to disease has become a key research focus based on CRISPR. Multiplex Cas9 was used to edit channel catfish at two immune-regulatory genes, tlr22 and cxcr3, resulting in genes that were significantly less vital when challenged with the Columnaris disease-causing organism, Flavobacterium columnare, and causing devastating losses in US catfish aquaculture (Elaswad *et al.,* 2018). Interestingly, disruption of the interferon regulatory factor 7 (IRF7) gene by CRISPR/Cas9 in grass carp paradoxically increased GCRV resistance through derepression of constitutive interferon signaling. Tilapia edited at the mucin gene locus exhibited significant alterations in the phenotypes of pathogen adhesion, and zebrafish models with CRISPR-disrupted components of the TLR pathway have helped to clarify conserved mechanisms of innate immune modulation that can be applied to commercial species (Wijerathna *et al.,* 2025). Polygenic and context-dependent nature of host immunity is a major problem in disease resistance breeding: single-gene knock-outs can lead to resistance against one pathogen, and vulnerability to another, thus requiring multiplex and systems-biology approaches, which target multiple nodes of immune regulation.

4.4 Reproductive Performance

One of the most important operational parameters in a breeding program is reproductive management, with unique potential for both sterility and sex control using CRISPR. The CRISPR disruption of dead end in Atlantic salmon results in sterile fish that cannot reproduce in the wild, an approach of great importance for open-cage marine aquaculture operations. Additionally, sterile salmon do not need to use energy diverting towards gonadogenesis as seen in mature fish, which could result in enhanced growth during production cycles (Chu *et al.,* 2023). In salmonids, the prospect of targeting sdY (sex determining region on the Y-chromosome) and amhr2 (amylase, alpha 2 gene) in tilapia would provide the opportunity for the production of monosex populations, growth uniformity and market benefits (Zhu *et al.,* 2024).

4.5 Environmental Stress Tolerance

Engineering thermal and osmotic tolerance via CRISPR has become an important area of research in the wake of climate change influencing the water temperature, dissolved oxygen, and salinity in aquaculture production areas. In zebrafish, targeted editing of the regulatory regions of heat shock factor 1 (hsf1) to boost HSF1 expression has been shown to improve survival during acute heat stress, and these genes are located in the region of the genome that is targeted in this study. For salmonids, the natural population genomics study has identified candidate single nucleotide polymorphisms (SNPs) in genes such as vgll3, six6 and akap11 related to thermal adaptation that have been confirmed as potential targets for CRISPR knockin to introduce thermo-adaptive alleles into production lines (Xiao *et al.,* 2025). Another area of active research is osmotic regulation, with CRISPR disruption of Na^+/K^+ ATPase subunit isoforms in zebrafish models showing evidence of a gene specific role in ion homeostasis for salinity transitions in salmon smoltification and tilapia transferring between freshwater and saline culture systems.

4.6 Flesh Quality and Nutritional Traits

In commercial aquaculture, consumer preference and nutritional value are becoming important selection criteria and applications of CRISPR are rapidly emerging for flesh quality. Mechanistic understanding of flesh structure regulation has been gained from the editing of genes that control muscle fibre composition, such as myosin heavy chain isoforms and titin, in zebrafish models, which can be transferred to production species. The slc45a2 gene has been used as a fluorescent or visual marker for efficiency in confirming the success of the editing in the Atlantic salmon founders prior to trait verification by conventional breeding (Hoshijima *et al.,* 2016). CRISPR approaches that target the expression of fatty acid desaturase and elongase genes for the enrichment of omega-3 polyunsaturated fatty acids (n-3 PUFAs) in fish muscle have direct nutritional value and are closer to the enhancement of lipid quality on plant-based diets, which are important issues in feed sustainability as shown in Table 2 and Figure 2.

Table 2: Applications of CRISPR-Cas in Commercial and Model Fish Species

Fish Species	Target Gene	Trait Improved	Editing Strategy	Outcomes	Reference
Nile tilapia (*Oreochromis niloticus*)	mstn (myostatin)	Growth enhancement	Cas9-mediated knockout	Up to 30% increase in muscle mass; improved feed conversion ratio	Ponzoni et al, 2011
Atlantic salmon (*Salmo salar*)	slc45a2	Pigmentation / Marker	Cas9 knockout	Albino phenotype confirmed; validated editing efficiency >85%	Edvardsen *et al.,* 2014
Rainbow trout (*Oncorhynchus mykiss*)	mstn1/mstn2	Muscle growth	Multiplex Cas9 KO	Significant hyper-muscular phenotype; heavier body weight at harvest	Bayır *et al.,* 2026
Common carp (*Cyprinus carpio*)	sp7 / eda	Scale reduction / body morphology	Cas9 NHEJ	Scaleless phenotype; improved processing efficiency	Wang *et al.,* 2023
Grass carp (*Ctenopharyngodon idella*)	IRF7	Disease resistance	Cas9 KO	Increased resistance to grass carp reovirus (GCRV) infection	Cai *et al.,* 2018
Channel catfish (*Ictalurus punctatus*)	tlr22 / CXCR3	Disease resistance (Columnaris)	Cas9 multiplex	Reduced mortality rates; enhanced innate immunity against F. columnare	Wang *et al.,* 2025
Zebrafish (*Danio rerio*)	tph1a / tph2	Serotonin pathway / model	Cas9 KO + CRISPR screen	Established disease model; serotonin biosynthesis mapped	Li *et al.,* 2016
Medaka (*Oryzias latipes*)	fgfrl1b	Skeletal development	Cas9 KO	Craniofacial defects modeled; ortholog validated for QTL mapping	Kirchmaier *et al.,* 2015

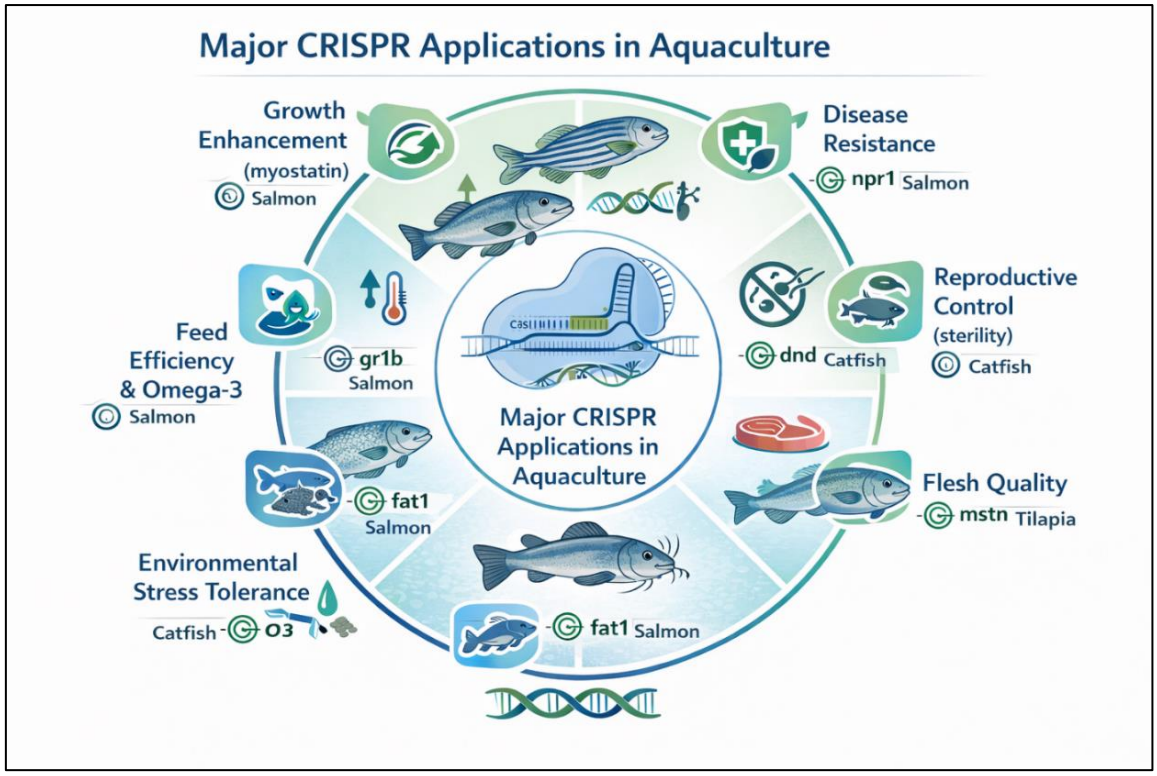


Figure 2: Major CRISPR-Cas applications in commercial aquaculture species

5. CRISPR-Assisted Selective Breeding

5.1 Marker-Assisted Selection versus Genome Editing

The selection process itself has been enhanced with marker-assisted selection (MAS) and genomic selection (GS), which have contributed immensely in improving the efficiency of aquaculture breeding. MAS was first developed in the 1990s and 2010s with the help of genetic markers (microsatellites and later SNPs) associated with quantitative trait loci (QTL), to accelerate selection, with moderate gains, particularly for disease resistance with larger effect QTL. Utilizing genome-wide SNP panels, genomic selection estimates breeding values for all markers and is able to capture a much larger percentage of genetic variance for more complex and polygenic traits. In the more advanced Atlantic salmon programmes, genetic gains of 12-20% per year have been accomplished for growth and disease resistance. But both MAS and GS have disadvantages as they require several generations of recombination to achieve accumulation of desirable alleles and they are unable to add new desirable variants that are not present in the breed. CRISPR-assisted breeding is a method that surmounts these limitations by allowing the incorporation, elimination or alteration of causative variants in a single generation, irrespective of their population occurrence. Combining the power of genomic selection, which is highly effective at exploiting polygenetic variation, with the precision of CRISPR's targeting of specific causal mutations, results in a powerful hybrid paradigm for next-generation precision breeding in aquaculture (Houston *et al.,* 2020; Yáñez *et al.,* 2015).

5.2 Precision Breeding Strategies

The introduction of CRISPR into structured breeding programs can be done in multiple strategic modalities. In a knockin precision breeding approach, the gene of interest is transferred from the locally adapted wild population or related species to the elite breeding line using CRISPR, bypassing the multi-generational introgression process of conventional backcross breeding. This is especially useful for adding disease resistance alleles found by GWAS in a resistant natural population into an elite domesticated population. The growth suppressors and reproductive regulators knockout strategies can be quickly implemented in elite family structures to develop high-value commercial products with a retained genetic merit of the base population. One of the most important points to take into account in the precision breeding design is whether the sequence to be introduced is from another species (transgenic) or whether it is from the same species. Most technologies developed for knockout and knockin in other species, such as species-authentic sequences, are considered as the latter class and are being regarded with increasing positivity from regulatory and public acceptance standpoints, and are relevant for the commercial precision breeding applications (Gratacap *et al.,* 2019).

5.3 Integration with Genomic Selection

The strongest near-term paradigm for implementation of CRISPR in aquaculture is its incorporation into current genomic selection systems. Houston et al. (2020) proposed a breeding strategy that involves targeted introgression of high-frequency

favorable alleles discovered by GWAS into the germplasm using CRISPR editing and a selection of the appropriate polygenetic background via genomic selection, which was dubbed 'hybrid genomic-CRISPR breeding'. This approach is theoretically expected to yield genetic gains significantly higher than those for each of these technologies, especially for complex traits, such as disease resistance, that rely on multiple, large-effect genes and the polygenic background as illustrated in Figure 3. Target prioritization is further improved by integration of multi-omics; Integrating the results of the three different omics layers gives a multi-layered evidence base from which candidate targets can be prioritized for CRISPR applications, with the transcriptomic data used to identify immune genes that are differentially expressed in fish challenged with the pathogen, the proteomic data to identify rate limiting biosynthetic enzymes in muscle tissue during growth and the metabolomic data to identify individuals that exhibit superior feed efficiency.

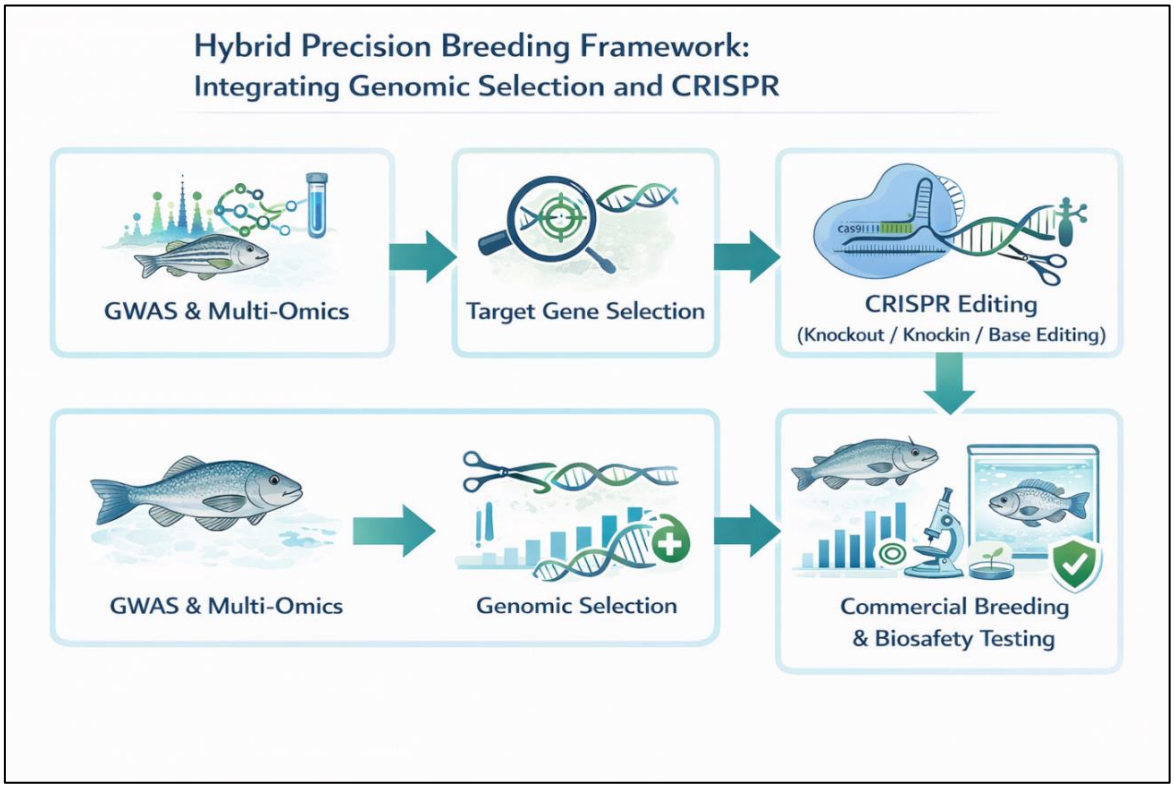


Figure 3: Hybrid precision breeding framework integrating genomic selection, multi-omics, and CRISPR editing

5.4 Case Studies in Commercial Species

Several research programmes have been undertaken in Atlantic salmon focusing on germ cell ablation (dnd knockout) for sterile production, fatty acid biosynthesis (elovl2 knockout) for lipid quality, pigmentation markers (slc45a2) for editing verification and immune regulatory genes for disease resistance. The research pipeline from mstn knockout to generation of functional F1/F2 population is one of the most advanced for any non-model food fish, and tilapia edited populations have been successfully demonstrated under laboratory conditions with consistent phenotypic enhancements in controlled environments. A convincing case exists for commercial disease resistance breeding for channel catfish, and multiplex CRISPR targeting of immune loci has a clear path to a reduction in mortality in the US catfish industry as discussed in Table 3 (Wargelius *et al.,* 2016; Jin *et al.,* 2020).

Table 3: Comparison of Conventional Breeding and CRISPR-Assisted Breeding Approaches

Parameter	Conventional Selection	Marker-Assisted Selection (MAS)	Genomic Selection (GS)	CRISPR-Assisted Breeding
Precision	Low–Moderate	Moderate	High	Very High (single-gene level)
Cost	Low	Moderate	High (genotyping arrays)	High (initial R&D); decreasing rapidly
Time Requirement	Multiple generations (5-10 yrs)	2-4 generations	1-2 generations	1-2 generations; immediate trait fixation
Genetic Gain	Slow; limited by recombination	Moderate	High; captures genome-wide effects	Highest; direct allele modification

Parameter	Conventional Selection	Marker-Assisted Selection (MAS)	Genomic Selection (GS)	CRISPR-Assisted Breeding
Trait Specificity	Low; polygenic traits only	Moderate; depends on marker density	High for complex traits	Maximum; targets causal mutations
Regulatory Complexity	Minimal	Minimal	Minimal	High; country-specific GMO/GEO regulation
Commercial Adoption	Widespread	Widespread	Growing (salmon, tilapia)	Limited; regulatory bottleneck

6. Implications for Fisheries Sustainability

6.1 Sustainable Aquaculture Production

The sustainability argument for CRISPR in aquaculture is that the technology can be used to do so. A disease resistant fish does not need to be subjected to so many antibiotics and chemotherapeutic treatments that would lead to contamination of the water column and sedimentation of pharmaceutical substances near the production sites. In an industry responsible for around 0.6% of global GHG emissions and a major proportion of coastal eutrophication, the shorter production cycles that enable faster growth of the edited fish species offer a critical efficiency improvement, by reducing cumulative feed inputs, waste outputs and energy consumption per unit of fish protein produced. Another major ecological concern for open-net salmon farming is the risk of escape into and hybridisation with wildstocks; whereas, dnd knockout salmon eliminate it (Houston *et al.,* 2020; Qasim *et al.,* 2026).

6.2 Reduction of Antibiotic Dependence

Antimicrobial resistance (AMR) has become one of the greatest health challenges of the 21st century and aquaculture systems are one of the important drivers of AMR in coastal and freshwater ecosystems. Disease resistant fish strains created through CRISPR could be a biological solution to infection control, rather than a pharmaceutical solution, and could reduce the need for regular prophylactic antibiotics use in intensive fish farming in Southeast Asia and South America. Norwegian salmon farming has successfully shown that antibiotic use can be reduced 99% from 1990 levels, by selectively breeding and vaccinating against disease resistance; thus, genome-edited disease resistance will be able to achieve similar, or potentially better, reductions in systems where vaccines are not available (Cabello *et al.,* 2016; Watts *et al.,* 2017; Chaudhary *et al.,* 2025).

6.3 Climate Change Adaptation

Elevated water temperatures, changes in precipitation, and increases in disease pressures are projected to impact the viability of current aquaculture production systems in major production areas. CRISPR-mediated engineering of thermal tolerance alleles, confirmed by natural population genomics of fish from warm-water refuges, is a scientifically defensible, targeted strategy for future-proofing aquaculture genetics to climate-driven changes in environment. This is in line with the broader IPCC guidance to address food system adaptation through genetic resource management and ecosystem-based approaches (Barange *et al.,* 2018). Importantly, the improvement of the thermotolerance trait by the introduction of validated natural alleles in a knockin type of CRISPR is conceptually similar to fast-track adaptation but without the ecological hazards of introducing completely new genetic constructs.

6.4 Conservation of Fish Genetic Resources

The paradoxical application of the CRISPR technologies is in Conservation genetics which is concerned with the preservation and restoration of wild fish populations, threatened by genetic erosion, hybridization and habitat loss. In contrast, propagation of rare species using a more abundant surrogate host (using the germ cell transplantation of endangered donor species into a sterile host fish produced by CRISPR technology), has been successfully demonstrated in salmonid and medaka model species. The ecological risks of "self-propagating" drive systems require extraordinary regulatory caution and international governance frameworks, as the population reduction of invasive species and the restoration of locally adaptive alleles to threatened wild populations are possible uses of gene drive systems that could be developed in the future (Piaggio *et al.,* 2017; Allendorf *et al.,* 2010).

6.5 Ecosystem-Level Impacts

Environmental risks to the ecosystem of the release of genome edited fish, either through intentional release for conservation or accidental (aquaculture escape) need a robust environmental risk assessment conducted at the ecosystem level. If the editing is coupled with poor physical or biological containment, then, in principle, edited traits providing competitive advantages (such as improved growth, disease resistance) may change competitive relationships and exclude wild conspecifics. Sterility based containment is a continuing research area in biosafety, but no single containment method is proven to be absolutely safe at commercial population scale. The full environmental impacts from the aquaculture product to the plate should be quantified through LCA studies of CRISPR-edited products to determine the net benefits of CRISPR technologies and inform policy frameworks.

7. Challenges, Risks, and Regulatory Frameworks

7.1 Off-Target Effects

An issue with the precision of CRISPR editing is that the Cas nucleases can cut genomic sequences that are somewhat similar to the sgRNA, resulting in off-target mutations. The rate of off-target cleavage is found to be highly variable across sgRNAs, Cas protein variant,

chromatin context of the target sequence, and Cas protein concentration, as demonstrated by high throughput methods such as GUIDE-seq, CIRCLE-seq, DISCOVER-seq, and CAST-seq. This lack of detailed off-target profiling in fish, compared to mammalian systems, is an empirical knowledge gap. High fidelity variants of Cas9 (SpCas9-HF1, eSpCas9, HypaCas9) have been developed to significantly decrease off-target effects, but may have decreased activity in chromatin-rich genomic regions (Fu *et al.,* 2013; Hsu *et al.,* 2013). For regulatory approval and commercialization of edited fish lines, rigorous whole genome sequencing is necessary for multiple generations, however, this is not readily available for many aquaculture research programs due to the high cost.

7.2 Ethical and Welfare Considerations

The use of genome editing for food animals involves a unique set of ethical questions related to animal welfare, intrinsic value and the place of humans in the natural biological process. Although the commercially desirable super-muscled phenotypes generated by mstn knockout are beneficial, such fish would be considered welfare issues should they have locomotor impairments, compromised immune systems or reduced reproductive fitness in their natural environment. Ethical principles for considering genome edited animals in aquaculture should take into consideration the difference between eliminating constraints on natural growth potential, such as reducing susceptibility to disease, and setting artificial performance demands that reduce wellbeing, such as overcrowding, genetic uniformity or overproduction (Zhang *et al.,* 2020; Wang *et al.,* 2025). Many bioethicists and environmental groups have called for cautious evaluation and incremental introduction of products, accompanied by thorough long-term monitoring of multiple generations prior to commercialization.

7.3 Biosafety Concerns

In addition to animal welfare, biosafety issues include possible ecological effects arising from the escape of edited organisms, horizontal gene transfer and disruption of the genetic structure of wild organisms. Although gene editing, in contrast to transgenesis, does not integrate foreign DNA in most NHEJ-based knockout applications, the fitness effects of these edited traits in the wild is poorly described in most target organisms (McGinnity *et al.,* 2003). In a situation where edited fish mix with wild counterparts, as happens all too often in commercial aquaculture operations worldwide, the mix of edited and wild fish needs to be empirically assessed using experiments in mesocosms and semi-natural ponds that better replicate the complexity of the environment than laboratory tank experiments.

7.4 Public Acceptance

Consumer and societal acceptance is a key and frequently overlooked hurdle for commercialization of genome edited aquaculture products. The results from surveys conducted across North America, Europe, and Asia-Pacific show a generally more positive attitude towards the technology of CRISPR-edited food compared to traditional GMOs, especially if the editing is within the same species (no interspecies transfer), and is associated with other benefits besides commercial productivity, such as disease prevention or sustainability benefits. But considerable doubt arises, especially in the European markets where the precautionary principle is entrenched in the regulatory culture (Nguyen *et al.,* 2023). The elements of transparent communication strategies, participatory governance mechanisms with consumer involvement and clear labeling policies are considered important enablers for more general societal acceptance.

7.5 Global Regulatory Landscape

The regulatory status and regulatory control of genome-edited fish are highly divergent in different jurisdictions, resulting in a complex regulatory landscape across the globe that hinders international trade and commercialization of genome-edited fish.

In the United States, the US Food and Drug Administration (FDA) regulate genome-edited animals under the New Animal Drug (NAD) authority, using a risk-based approach which is not process dependent but rather based on the nature of the genome-edited animal itself and the specific edit made. In 2022, the Low-Risk Determination pathway was established, which offers a relatively favorable context for the commercialisation of fish products derived from edited fish for the market of food products; small insertions or deletions identical to naturally occurring variants can be subjected to this pathway, but no edited food fish has yet received full market approval by FDA.

European Union: Most genome-edited organisms are currently regulated as GMOs by the European Union under the most stringent pre-market assessment regime in the world, that is, Directive 2001/18/EC. The Court of Justice of the European Union (ECJ) decision in 2018 that organisms created by mutagenesis methods such as CRISPR are considered GMOs, subject to GM regulation, has been bitterly contested by the scientific community as being scientifically unsound. The proposal by the EU Commission for a new regulation on new genomic techniques (NGTs) for the production of plants in 2023 suggests a move towards product-based, process-independent regulation – aquatic animals are not mentioned. In 2023, the EU Commission proposed a new regulation on new genomic techniques (NGTs) for plants, which shows a shift towards product-based and process-independent regulation, but does not explicitly refer to aquatic animals.

China:

China has a dual regulation system for intraspecies gene editing, as sequences that are not derived from other organisms do not require all the conditions associated with GMO registration and several edited crop varieties have been approved. CRISPR-edited catfish, carp, and tilapia are being cultivated in China, and the regulatory pathways for aquatic genome-edited organisms (GEOs) are being developed (Chen *et al.,* 2019).

In 2019, Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) and Consumer Affairs Agency created a notification-based system for genome-edited organisms that does not require pre-market approval for SDN-1 (site-directed nuclease 1, which is equivalent to NHEJ knockout) edits without insertion of exogenous DNA, making this framework one of the most permissive worldwide and potentially favorable for the fast-track commercialisation of CRISPR aquaculture products (Matsuo & Tachikawa (2022).

Australia:

Food Standards Australia New Zealand (FSANZ) considers SDN-1 organisms to be non-GM if no novel traits are introduced that could create a safety concern only organisms with introduced foreign DNA are considered GM (Thygesen (2024).

Canada:

Health Canada uses a trait-based, product-centric regulatory philosophy where they assess the product, not the process. Fish with novel traits, but developed with the CRISPR method, that are not substantially different from non- CRISPR versions might be classified as novel foods or feeds, which would require pre-market notification, rather than as GMOs, which would require the full GMO process (Singer & Michaud (2025).

9. CONCLUSIONS

After years of research into the genetics of fish using the CRISPR-Cas system, this review shows how this technology could be used to change the face of fish selective breeding and sustainable fisheries. The most promising applications of CRISPR-Cas systems in aquaculture have been the knockout of a growth-promoting gene such as myostatin, the knockout of an immune gene for disease resistance and the ablation of germ cells for sterility. The combination of CRISPR with genomic selection and multi-omics platforms is a powerful hybrid paradigm that can contribute to increased genetic gain of both a monogenic trait and a complex polygenetic trait. The benefits of CRISPR-assisted precision breeding align directly with the UN SDGs 2 and 14, in terms of antibiotic usage reduction, environmental footprint decrease and climate resilience. But significant hurdles, such as off-target effects, mosaicism, ecological risks and the lack of a coherent regulatory framework, must be overcome to unlock this potential. The key to future success will be systematic evaluations across generations, design innovation with the help of artificial intelligence, harmonisation of regulatory frameworks and public involvement. In the right hands and with equitable access, CRISPR technologies can contribute to sustainable, resilient aquaculture systems that can help to feed the global protein needs while protecting aquatic ecosystems.

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