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CRISPR-based Genome Editing in Shrimp and Its Potential in Aquaculture

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Abstract In recent years, genome editing technology has developed rapidly and has shown broad prospects in the field of animal and plant breeding. Focusing on the application of CRISPR/Cas system in shrimp genome editing and its potential impact on the aquaculture industry, this study introduces the current development status of the global aquaculture industry and the major challenges faced; outlines the principles and advantages of CRISPR-Cas gene editing technology, and compares it with the early ZFNs and TALENs technologies, pointing out the outstanding advantages of CRISPR in operation simplicity and editing efficiency; expounds the complexity of shrimp genome and the latest sequencing research progress, lists the identification results of major economic trait-related genes and the construction of shrimp transcriptome database. This study focused on reviewing the latest application cases of CRISPR technology in shrimps, including the exploration of using CRISPR/Cas9 to edit the *Vitellogenin* gene of the shrimp to affect ovarian development, and targeting receptor genes susceptible to WSSV to enhance disease resistance. It also explores the prospects of CRISPR in regulating shrimp growth and reproduction, including accelerating growth and gender control, in order to provide a comprehensive reference for in-depth research on shrimp genome editing technology and the application of aquaculture practice.

Keywords Shrimp; Genome editing; Growth and reproduction regulation; Aquaculture; Disease-resistant breeding

1 Introduction

The aquaculture industry is one of the important pillars of the current food supply, but it also faces severe challenges. With the expansion of the scale of breeding and the increase in the degree of intensiveness, various diseases occur frequently, seriously affecting yield and efficiency. Shrimp plays an important role in global aquaculture production. Taking China as an example, the production of farmed shrimps such as white shrimp in South America increased from 1.429 9 million tons in 2013 to 2.098 6 million tons in 2024, an increase of 46.8%, making it the highest-yield farmed crustacean. The high output value of shrimps is accompanied by high risks, and diseases such as leukoplakia syndrome virus (WSSV), early death syndrome (EMS, that is, acute hepatopancreatic necrosis), which reduces the survival rate of shrimp seedlings and huge farming losses (Lee et al., 2022). In addition, the slowdown in growth rates and germplasm degradation caused by genetic degradation also plague the breeding industry. Although traditional breeding methods (such as family breeding, hybridization, etc.) have cultivated multiple shrimp varieties, it is still difficult to make rapid breakthroughs in disease resistance and stress resistance and improvement of growth traits.

The rise of genome editing technology has brought new hope to solve the above problems. Unlike transgenes, gene editing can accurately modify the target gene without the introduction of exogenous genes, so it is theoretically safer and controllable, and has relatively high public acceptance (Strobbe et al., 2023). Among them, CRISPR/Cas9 technology has attracted much attention because of its simplicity and efficiency. Since its introduction in 2012, it has been rapidly applied to the research on gene functions and breeding of various organisms.

In the field of aquaculture, CRISPR technology is gradually expanding from model organisms to economic species, opening up new ways for genetic improvement. For example, researchers have used CRISPR to cultivate new snapper lines with 20% increased muscle yield. For shrimps, the introduction of gene editing is expected to

cultivate varieties that are more resistant to disease and grow faster (Ferdous et al., 2022). In particular, CRISPR can replace traditional eye stalk removal and other measures to promote shrimp maturation and reproduction by directly knocking out or modifying endocrine regulatory genes. This study will systematically discuss the current status and potential of CRISPR gene editing technology in shrimps, and analyze the scientific and social challenges that need to be overcome to achieve this technology.

2 Overview of CRISPR Gene Editing Technology

2.1 Basic principles of CRISPR-Cas system

The CRISPR-Cas system originates from the immune mechanism of prokaryotic organisms. Its basic principle is to use Cas endonuclease and guide RNA (sgRNA) to identify and cause breakage of genome specific sequences, thereby achieving insertion/deletion mutations or other editing at the target site. In application, researchers first designed crRNA that is complementary to the target gene sequence and formed sgRNA with tracrRNA, guiding the Cas9 protein to localize and cleave specific DNA sequences. Following DNA double-strand breaks, cells may undergo non-homologous end joining (NHEJ), resulting in base insertions or deletions that knock out gene function; alternatively, homology-directed repair (HDR) can be utilized to insert exogenous fragments for precise genome editing (Yu et al., 2021). Due to its simplicity of operation and strong targeting, CRISPR-Cas9 is widely used in genetic function research. In model animal zebrafish, CRISPR knockdown of specific genes has become a routine operation, providing an important means for analyzing developmental and disease mechanisms. In contrast, traditional zinc finger endonuclease (ZFN) and TALENs are complex and expensive to build, limiting large-scale applications. It should be noted that there are naturally many types of CRISPR-Cas systems, among which the most widely used is the Cas9 protein derived from Streptococcus, which belongs to the Type II CRISPR system. "Cas9 introduces blunt-ended double-strand breaks, which typically trigger knockout effects mediated by non-homologous end joining (NHEJ). Another commonly used nuclease, Cas12a (also known as Cpf1), generates 5' overhangs upon cleavage, which facilitate the insertion of exogenous sequences through homology-directed repair (HDR).". Cas12a's identification requirements for PAM sequences are different from Cas9, providing complementary options for gene editing (Hillary et al., 2021).

2.2 Comparison with other gene editing technologies (such as ZFNs, TALENs)

Before the advent of CRISPR, site-directed genome modification mainly relied on artificial nuclease technologies such as ZFN and TALENs. ZFN combines zinc finger protein with FokI endonuclease to form a double-strand cleavage tool that recognizes specific DNA sequences. The disadvantage is that each new target is designed, the corresponding zinc finger protein needs to be screened, which has a large workload and limited target sequence. TALENs use the transcriptional activator (TALE) repeat module of plant pathogens to identify DNA sequences, and also play a role in combination with FokI enzymes. TALENs are more specific and more flexible in design than ZFN, but it is also more cumbersome to build TALE vectors containing a large number of repeat sequences (Yu et al., 2021). In contrast, CRISPR-Cas9 only needs to change the guide RNA sequence to target new targets, greatly simplifying the design process. Literature statistics show that the mutation construction cycle of CRISPR is usually less than half of the traditional methods and the cost is lower. In addition, in terms of editing multiple sites simultaneously (multiple gene knockout), CRISPR can only be achieved by introducing multiple sgRNAs at the same time, while ZFN/TALEN is almost unable to achieve effective multi-site editing. This makes CRISPR an irreplaceable advantage in functional genomics research.

Of course, CRISPR is not without limitations. The first is the off-target effect problem, that is, Cas9 may cause unexpected mutations in other sites in the genome that are similar to the target sequence. Secondly, the transmission and expression efficiency of CRISPR differs in different species. For example, in mammalian cells, Cas9/sgRNA can be efficiently sent to mammalian cells through viral vectors, but in some aquatic invertebrates, the success rate of microinjection is relatively low (Fu et al., 2024).

2.3 Evolution and improvement of CRISPR system (such as Cas9, Cas12, genome precision editing)

Since the advent of the classic Cas9, CRISPR technology has evolved new tools to expand its functions and

applications. One of the important directions is the development of CRISPR systems for different enzymes, such as Cas12a, Cas13, etc. Cas12a not only provides different PAM selection and viscous ends in DNA editing, but also its unique non-specific single-strand DNA degradation activity after cleavage is used in diagnostic fields such as rapid nucleic acid detection (such as novel coronavirus detection) in vitro (Agha et al., 2025). Cas13 acts on RNA and can be used to target degrade specific transcripts and achieve reversible gene silencing. It is known as the "molecular scalpel". There are also Cas protein-based transposase systems (such as Castro) that are exploring for insertion of larger fragments of DNA.

Base editing is another revolutionary improvement in the field of CRISPR. The researchers fuse inactivated Cas9 (dCas9) with cytosine deaminase to construct CBE (cytosine base editor), which can convert specific bases C→T without cutting off DNA. Then there is ABE fusion with adenine deaminase to achieve A→G mutation. The latest original editing is even more powerful. Based on dCas9 fusion reverse transcriptase, it carries the editing template at the same time through a guide sequence to achieve multiple types of point mutation, insertion or deletion editing, which theoretically covers most mutation types. These precision editing tools are of great significance in the medical field and have the potential to improve complex traits of multigene control for aquaculture because they can accurately rewrite functional sites without introducing additional changes.

3 Progress in Genomic Characteristics and Research of Shrimp

3.1 Complexity of shrimp genome and its current sequencing status

The genome of crustaceans is generally large and complex, and shrimp are no exception. Taking the currently dominant breeding of vannamei prawns (*Litopenaeus vannamei*), the diploid genome size is about 1.66 Gb and the encoding genes is about 25,596. The shrimp genome contains a large number of repeat sequences and microsatellites (Simple Sequence Repeats (SSRs), and it is reported that the proportion of repeat sequences exceeds 23%. High proportions of repetition and AT-rich complex regions increase the difficulty of genome assembly, often resulting in unsubsidized or omitted sequence fragments. It was not until the development of high-throughput sequencing and assembly algorithms in recent years that the whole genome sequencing of shrimps has made breakthrough progress. In 2019, Zhang Xiujuan and other research teams used three-generation sequencing (PacBio long reading and length technology) to construct the high-quality sketch genome of South American white shrimp for the first time, with N50 length reaching 0.605 Mb, which was a high level at that time. Subsequently, by introducing Hi-C assisted construction of chromosome-level assembly, tens of thousands of fragments were further anchored to 44 chromosomes, increasing Contig N50 to 42.89 Mb and genome coverage reached ~84.6% (Liao et al., 2025).

3.2 Identification of major economic traits related genes in shrimp

The acquisition of genomic information makes it possible to locate genes that affect the importance of shrimp from a global perspective. In the shrimp farming industry, growth rate, disease resistance, environmental tolerance and reproductive ability are key traits that directly determine yield and profit. In recent years, through genome-wide association analysis (GWAS), quantitative trait loci (QTL) analysis and candidate gene methods, researchers have identified a batch of gene markers related to these traits. In terms of growth traits, Yu et al. conducted a genome scan of vannabinoid shrimp and found that several genomic regions were significantly related to growth traits. One of the prominent candidate genes is MMD2 (a protein related to fungal macrophage migration inhibitor), and subsequent functional studies have shown that the variation of this gene is closely related to shrimp growth differences (Bu et al., 2025). In terms of disease resistance, studies have found that certain microsatellite lengths in the vannabinoid shrimp genome are related to antiviral manifestations: individuals carrying shorter repeats show higher resistance to leukoplakia and iridescent viruses (Yin et al., 2023). This suggests that there are natural disease-resistant alleles in the shrimp population, which can be assisted in breeding through molecular markers. In terms of environmental tolerance, genes have also been reported. The study found that the SNP polymorphism of the *Catalase* gene of vannerbine shrimp is related to hypoxia tolerance. Some heat shock proteins and osmotic regulation-related genes have been shown to be upregulated in high-salt stress responses, suggesting that they may be involved in salt-tolerant traits (Luo et al., 2022). In terms of reproduction

and traits, endocrine hormone genes that control gender differentiation and maturation of shrimps, such as gonad inhibitor (*GIH*), androgen effector gene (*IAG*), have been sequenced and identified in different shrimp species (Ferdous et al., 2012).

3.3 Transcriptomics and the construction of genomic databases

In addition to genome sequencing, transcriptomic research has played a crucial role in elucidating gene functions in shrimp. Transcriptome sequencing (RNA-Seq) can reveal gene expression profiles in specific tissues, developmental stages, or under stress conditions, thereby aiding in the identification of trait-associated genes and regulatory networks. For example, a transcriptomic analysis of Chinese shrimp (*Fenneropenaeus chinensis*) under heat stress identified a series of heat shock proteins and antioxidant enzyme genes, deepening our understanding of the mechanisms underlying high-temperature stress responses (Tang et al., 2022). Active research is also being conducted on reproductive development. One study comparing the ovarian transcriptomes of wild-caught and farmed tiger prawns (*Penaeus monodon*) found that genes related to vitellogenin synthesis were significantly upregulated in wild individuals. Additionally, a transcriptomic analysis of male and female gonads in banana shrimp (*Fenneropenaeus merguiensis*) identified numerous sex-differentially expressed genes, including key enzymes involved in oogenesis and factors regulating male reproduction (Saetan et al., 2016). These data provide valuable insights into sex determination and reproductive regulation in shrimp.

To better manage and utilize the vast amount of omics data, researchers are also building genomic and transcriptomic databases for shrimp. Several international public databases (such as NCBI's Genome and SRA repositories) have included genome sequences and transcriptomic datasets of various shrimp species, enabling researchers to perform comparative analyses and identify candidate genes. In China, some institutions have also developed integrated databases for aquatic animals, combining key gene sequences, QTL information, and expression profiles into unified platforms. Through these databases, breeding experts can query sequence variations and expression patterns of target trait-related genes, thus supporting informed breeding decisions.

4 Current Status of CRISPR Technology in Shrimp

4.1 Research cases of successful application of CRISPR in shrimp

4.1.1 CRISPR/Cas9 edits the *Vitellogenin* gene of *Penaeus monodon* to regulate ovarian development

For a long time, in breeding practice, the eye stalk is often cut off to remove gonad inhibitors, thereby promoting the maturation of the shrimp gonad. However, this method is traumatic and cumbersome to individual shrimps. Using CRISPR gene editing to directly modify genes that control reproduction is expected to become a more refined and effective alternative. One of the key factors in ovarian development of *Penaeus monodon* is Vitellogenin (Vg) and its regulatory pathway. Vg is synthesized in the hepatopancreas of female shrimp and delivered to the ovaries, providing nutrients to oocytes. Generally, gonadal inhibitor (GIH) secreted in the stalk inhibits Vg synthesis, thereby delaying ovarian development (Ferdous et al., 2022). Based on this mechanism, the researchers envisioned that the *Vg* gene itself or the upstream *GIH* receptor gene was knocked out by CRISPR to directly affect the ovarian maturation process.

At present, preliminary studies have tried to apply CRISPR/Cas9 to target reproductive-related genes in pimples. In an editing study on the *P. monodon* yolk proteogen gene, researchers designed multiple exon regions of the sgRNA targeting *Vg* gene, and introduced Cas9 mRNA and sgRNA into the larvae through microinjection, trying to induce the function loss of *Vg* gene. It was found that some CRISPR-treated female shrimp individuals had reduced Vg plasma content and delayed ovarian development, which was consistent with the expected functional deletion effect. There are also studies that select key genes targeting GIH signaling pathways. For example, Thai scholars used RNA interference to knock down the *GIH* gene of *P. monodon* and observed that the development of ovarians of female shrimps was accelerated. Once the functions of these key genes are confirmed by CRISPR, we are expected to cultivate "no eye-cutting" breeding shrimp species to improve reproductive efficiency and reduce damage to individuals.

4.1.2 CRISPR/Cas9 targets the white spot virus (WSSV) receptor gene to improve disease resistance

In addition to breeding, disease-resistant breeding is another most attractive application direction for gene editing. In the shrimp farming industry, white spot disease caused by the white spot syndrome virus (WSSV) is one of the most harmful diseases. Regarding WSSV, studies have pointed out that the Rab7 protein on the host cell membrane binds to the viral encapsulation protein VP28, which is an important way for WSSV to enter cells (Sritunyalucksana et al., 2006). In addition, some transcription factors (such as YinYang1, YY1) in shrimp are suspected to be involved in the viral replication cycle. Based on these understandings, multiple scientific research teams have tried to use CRISPR technology to knock out related genes to verify the anti-disease effect. In his master's study, Bermúdez et al. reported the attempt to target mutations of WSSV receptor genes in vannabinoid shrimp sperm cells using CRISPR/Cas9, hoping to cultivate anti-WSSV offspring by obtaining mutation-bearing sperm. Although this idea faces technical challenges (such as how to effectively edit germ cells), preliminary results show that the binding ability of CRISPR-treated semen cells to leukoplakia virus particles is reduced, suggesting that receptor gene function has been affected.

4.2 Selection and design strategies of target genes

Successful gene editing experiments are inseparable from reasonable target gene selection and careful experimental design. In the application of shrimp CRISPR, researchers generally select genes closely related to the target trait and have clear functions as editing objects based on the aforementioned genomic and transcriptome studies. For example, in experiments that set to improve disease resistance, host genes that are proven to be necessary for pathogen invasion or replication will be preferred. For example, in terms of trait improvement, endocrine hormone genes and growth inhibitor genes have become the first target of choice because they have a significant impact on growth and reproduction (Miao et al., 2023). The ideal target gene should be non-essential and trait-specific so that its knockout is not fatal but can lead to expected phenotypic changes. In terms of design, 2 to 3 sgRNAs are usually designed for the functional key regions of the target gene (such as catalytic domain or ligand binding sites) to increase the probability of mutation hitting the critical position. sgRNA design requires the help of shrimp genome sequences to avoid complete matches of more than 3 to 4 bases with other sequences in the genome, thereby reducing the risk of off-targeting. Some specialized software and tools can help predict potential off-target sites on the shrimp genome, and even if shrimp are not a model species, basic evaluation can be performed as long as the genome sequence is provided.

In addition to sequence design, the delivery strategy of editing tools is also an important part of the design process. In shrimp, the most commonly used method is to mix the mRNA or protein of Cas9 with sgRNA synthesized in vitro and inject it into the fertilized egg or single-cell embryo through microinjection. Microinjection requires an appropriate period of time: fertilized eggs of penaeid shrimp possess a chorion and initiate cleavage shortly after fertilization, so it is generally chosen to complete the injection before the first cleavage. There are also some explorations that try to inject CRISPR reagent through the semen to edit germ cells or pass them into shrimp oocytes through gene guns, electroporation, etc., but the efficiency still needs to be improved.

4.3 Editing efficiency and off-target effect issues

The application of CRISPR technology in shrimps is still an emerging field, and a prominent challenge is the low editing efficiency. Compared with the high-efficiency mutation acquisition rate of more than 50% of mammalian and insect-mode species, shrimp gene editing often faces problems such as low injection survival rate and high chimera ratio. In the early study of *Chi4* knockout in the spinal tail white shrimp, although hundreds of fertilized eggs were injected, only a handful of larvae that successfully grew into and had mutations. Crustaceans such as vannabinoid shrimp develop rapidly and are sensitive to operation and are prone to injury or infection during microinjection (Xu et al., 2019), thereby reducing survival. Even if the larvae hatch, the mutations introduced by CRISPR are often in a chimeric state (different cells carry different genotypes), resulting in a non-obvious phenotype and cannot be directly and stably passed on to offspring. All of these make screening and breeding of gene-edited shrimp more difficult.

Off-target effect is another highly concerned issue. Because the shrimp genome is huge and the information is not as comprehensive as that of model organisms, in existing studies, sequencing is mostly used to verify whether the expected sites are mutations to confirm the effect of CRISPR, but rarely comprehensively detect possible off-target sites. This brings hidden dangers of safety: Unexpected mutations caused by off-targeting may affect other gene functions and even lead to recessive negative traits. To address this problem, the conventional approach is to avoid highly homologous sequences in the genome when designing sgRNA and adopt the lowest effective concentration of Cas9/sgRNA combination. High-fidelity Cas9 variants (such as eSpCas9, SpCas9-HF1, etc.) developed in recent years have been shown to significantly reduce off-target frequency in some model animals (Matsumoto et al., 2020). In the future, these high-fidelity enzymes are also expected to be applied to shrimp gene editing experiments to improve specificity.

5 The Application Potential of CRISPR in Shrimp Disease-Resistant Breeding

5.1 Main diseases of shrimp and their genetic background

There are many types of diseases in shrimp farming, including viral, bacterial and parasitic diseases, among which viral diseases are the most harmful. In addition to the widely prevalent white spot syndrome virus (WSSV), common viral diseases include taola syndrome virus (TSV), infectious subcutaneous and hematopoietic tissue necrosis virus (IHHNV), yellow head virus (YHV), etc. Among bacterial diseases, acute hepatopancreatic necrosis (AHPND, also known as EMS) is caused by Vibrio parahemolyticus carrying virulence plasmids, which has caused serious losses in Asia since its emergence around 2010 (Seibert and Pinto, 2012). The death-stealing disease (EHP) caused by microsporidium has also attracted much attention in recent years. The frequent occurrence of these diseases is often related to factors such as breeding density and environmental stress, but from the perspective of the breeding varieties themselves, there are significant differences in the susceptibility of different strains to the disease, which shows that disease resistance has a certain genetic basis.

With the development of molecular biology, the immune response mechanism of shrimp to major pathogens has been gradually revealed. In the breeding program in Thailand, a line of platypal shrimp with high resistance to WSSV was selected, which is presumably possible to carry certain favorable alleles. From the perspective of immune genes, the innate immune system of shrimps includes multiple links such as pattern recognition receptors (such as β-1,3-glucan binding proteins, TLR-like receptors), signal transducers (such as MyD88, NF-κB), and effector molecules (such as antimicrobial peptides, phenol oxidases) (Sanguanrut et al., 2018). Functional mutations in any link may affect the overall anti-infection ability (Figure 1) (Lee et al., 2022).

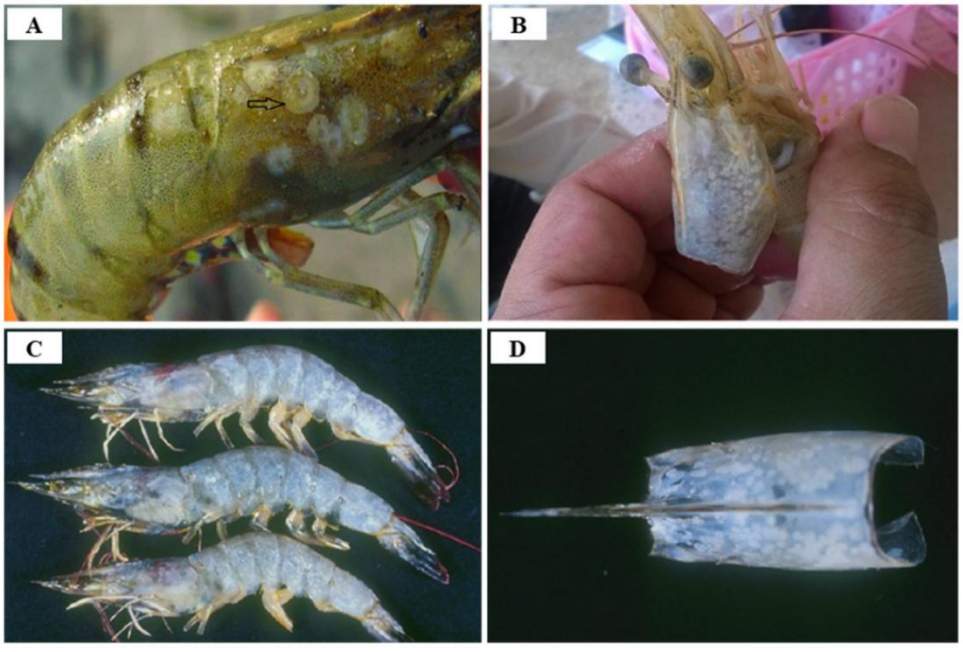

Figure 1 External white spot symptoms indicating white spot syndrome virus (WSSV) infection (Adopted from Lee et al., 2022)

5.2 Research and editing of the functions of disease-resistant genes

After digging out disease-resistant candidate genes, they need to verify their role through functional research. CRISPR is the weapon in this link. In the past, RNAi was used to silence genes in shrimps for short-term shrimps, but the efficiency and specificity of RNAi in shrimps often affect the interpretation of the results. In contrast, CRISPR knockout can produce stable gene deletion effects, which more effectively demonstrates the causal relationship between gene and phenotype. In recent years, CRISPR research on shrimp disease-resistant genes has gradually begun.

The C-type Lectin family of shrimps, which encode pattern recognition receptors, are involved in pathogen recognition and phagocytosis. There are reports that knocking down a C-type lectin will increase shrimp's resistance to leucorrhea virus, suggesting that its normal function may be used by the virus to help invade (Lai et al., 2013). If the corresponding *Lectin* gene is knocked out with CRISPR, shrimp may become resistant to the virus. Some experiments are currently underway. For example, antioxidant enzyme genes play a role in protecting cells in oxidative stress caused by pathogen infection. Research has used CRISPR to knock out the stress response factor gene in vannabine shrimp to activate the Nrf2 pathway, thereby improving the antioxidant ability of cells. The results showed that the mortality rate of gene-edited cells was reduced under Vibrio infection (Gui et al., 2016). These attempts show that the idea of enhancing shrimp's autoimmune defense through gene editing is feasible.

5.3 Feasibility and challenges in building a disease-resistant strain

Using CRISPR to obtain disease-resistant mutant individuals is only the first step, and cultivating them into a stable genetic disease-resistant strain is the ultimate goal. There are already some successful cases in this regard: researchers have cultivated animal strains such as parasite-resistant cattle, pigs, etc. through gene editing, proving that the route from mutants to strains is feasible (Islam et al., 2020). Similarly, in shrimps, once a founding individual (F0 generation) that resists disease mutations is obtained, the mutations can be fixed to the population in a homozygous state through appropriate hybridization and breeding. Specifically, male shrimp carrying disease-resistant mutations can be mated with wild-type female shrimp to obtain F1 heterozygotes, and then mates are mated or backcrossed to screen out individuals with homozygote mutations. Due to the large amount of egg laying in a single time, this screening is affordable in quantity. In terms of screening methods, individual genotypes can be quickly identified by PCR and sequencing, without the need to take generations of time like traditional breeding. However, the challenge cannot be ignored. Disease resistance is usually a complex trait that often involves the synergy of multiple genes. Mutations in a single gene may not be sufficient to provide comprehensive resistance. Secondly, disease-resistant mutations may pay a certain price to grow or reproduce. According to evolutionary biology theory, increased disease resistance is sometimes accompanied by a decrease in growth rate or a change in energy distribution. For example, a continuously activated immune system consumes additional resources. When building disease-resistant strains, it is necessary to observe whether mutations bring negative traits, and if so, they need to be balanced in breeding. There are also factors from regulation and the public that are part of the challenge. Once the disease-resistant gene-edited shrimp strain is successfully cultivated, its promotion and application needs to be approved by policy and market recognition. Regulators will pay attention to the performance of these shrimps in the ecological environment, and are worried about whether they will have a competitive advantage or genetic infiltration to the wild population if the disease-resistant genetically modified shrimp escapes. Although gene-edited shrimp does not contain exogenous genes, it is still necessary to prove that it will not become a risk of invasive species because disease-resistant mutations may improve survival.

6 Application Prospects of CRISPR in the Regulation of Shrimp Growth and Reproduction

6.1 Editing of genes related to control growth rate

Growth speed directly determines the length of the breeding cycle and the yield, and is one of the important traits of aquatic animal breeding. Studies have shown that crustacean ecstasy inhibitor hormone (MIH) plays a key role in maintaining molting cycle and body length growth. In the MIH knockout experiment of ridge-tailed white shrimp, the frequency of molting of mutant shrimp increased and the development period of larval bodies was

shortened. This suggests that disrupting the MIH pathway through gene editing may shorten the time for farmed shrimp from hatching to product specifications. Gao et al.'s study knocked out the *MIH* gene of the ridge tail white shrimp by co-injecting *EcILP-CRISPR*, and the results were obtained with accelerated larvae (Gao et al., 2022) (Figure 2). If this strategy is applied to breeding varieties such as vannabinoid shrimp, it may achieve faster growth and metabolism rhythm, so that shrimps can reach market specifications earlier. In addition to endocrine regulation, muscle growth inhibitors are also popular targets. The insulin-like growth factor (IGF) pathway is generally involved in the growth regulation of invertebrates. There have been studies that knocking out an insulin-like peptide encoding gene in the spinal tail white shrimp will cause individual growth retardation, indicating that the normal function of this gene is to promote growth. Conversely, if IGF binding proteins or downstream negative feedback factors can be found to inhibit growth, destroy their genes, or accelerate growth.

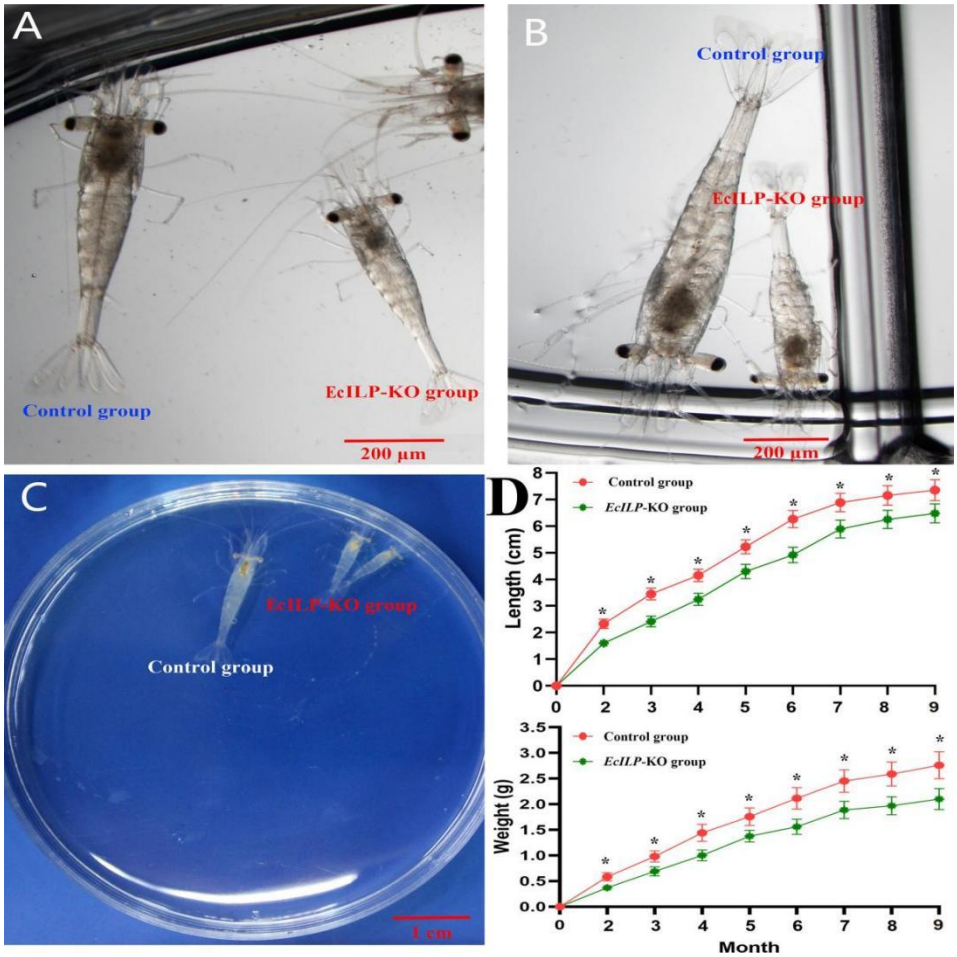

Figure 2 Growth comparison of *EcILP-KO* and control group individuals (Adopted from Gao et al., 2022)

Image caption: (A) 18 days after hatching, (B) 22 days after hatching, (C) One month after hatching, (D) Statistical changes in body length and body weight from 2 to 9 months after hatching. Error bars refer to the standard deviation. *P* values are denoted: **P* < 0.05. (Adopted from Gao et al., 2022)

6.2 Targeted modification of genes for reproductive development regulation

In addition to accelerating growth, regulating the reproduction and development of shrimps is also a way to improve breeding benefits. Many farmed shrimps have problems with reproductive control, such as the difficulty of maturing gonads of the shrimps and the decline in fertility. Gene editing can intervene in the key genes of the shrimp's reproductive axis to achieve the goal of artificially controlling the reproductive cycle and maturity age. Among several shrimp species, the phenomenon of eye stalk removal promotes maturation indicates that the role of inhibitors exists conservatively. Therefore, knocking out the *GIH* gene by CRISPR or weakening its receptor signal may produce shrimp that can mature and lay eggs early without removing the eyes. This will be of great application value for breeding difficulties such as prawns. Currently, some studies have begun to verify the

functional verification of *GIH* genes, and the results of using RNA interference and gene editing methods support their inhibitory effects on yolk protein synthesis (Kluebsoongnoen et al., 2020). If the *GIH* gene can be completely knocked out in vivo and the phenotypes such as early maturity or increased egg laying times of female shrimp will be further proved. Another gene closely related to reproduction is the composition of the vitamin A metabolic pathway. Research has found that retinoic acid signaling plays an important role in the development of crustacean ovary. Some experiments are trying to intervene in retinoic acid synthase or degradation enzyme genes through gene editing to prolong the reproductive activity period of shrimp.

6.3 Research progress on monosexual reproduction and gender control

Many breeding species have significant gender 2 phenomenon, that is, there are obvious differences in growth rate and body size of male and female individuals. Some shrimps grow slowly while females grow faster (or vice versa), so obtaining a single-sex population helps to increase yield and neatness. If a single-sex group escapes to the wild and cannot reproduce, it can reduce the risk of ecological invasion. Traditionally, methods to achieve single-sex groups include manual identification sorting and sexual reversal processing, but these methods are time-consuming and labor-intensive or have technical bottlenecks. CRISPR technology provides new ideas for gender control.

The gender-determining mechanism of crustaceans is similar to that of insects, and generally secretes *IAG* from male-specific androgen glands to induce male traits. Knocking out the *IAG* gene of male shrimp by CRISPR can cause female phenotypes to be produced by genetically XY (or ZZ) and achieve sexual reversal. Gui et al.'s research on the spine-tailed white shrimp has confirmed this: the genetic male shrimp not only females in appearance, but also grows ovaries and lays eggs. This means that through gene editing, we can turn male shrimp into functional female shrimp. If these sexual reversal females are mated with normal males, it is possible to give birth to all male offspring (because the reversal females do not have female chromosomes, only male components are provided). The next generation that reproduces in this way will manifest as normal males if they are still XY and have no *IAG* defects (Gui et al., 2016). Therefore, a whole male shrimp population can be mass-produced for breeding, thereby increasing yield by taking advantage of the gender-type 2 advantages. Similarly, if the target is a total female population, acquired reversal of male shrimp (genetic female to male).

7 Ethical, Safety and Regulatory Considerations of CRISPR Applications

7.1 Biosafety and ecological risk assessment

When applying gene editing technology to farm shrimp, the first thing to consider is biosecurity and ecological impact. Although gene-edited shrimp does not carry exogenous transgenic fragments themselves, their artificially induced genetic variations may give them some significant advantages (such as faster growth or stronger disease resistance), and these characteristics may have an impact on the ecosystem if they spread to nature under uncontrolled conditions. To ensure biosecurity, scientific research and breeding units should establish strict biosecurity systems. During the experimental stage, gene-edited shrimps should be cultivated in a controlled circulating water system, and wastewater can only be discharged after treatment to prevent embryos or individuals from leaking. Transportation and use of gene-edited shrimp seedlings must have a complete traceability and isolation mechanism. In addition to ecology, in terms of food safety, it is necessary to confirm that gene editing will not introduce harmful substances or sensitizing ingredients (Zhao and Wang, 2024). Typical variations produced by CRISPR are base deletions or substitutions, which are essentially no different from natural mutations and generally do not produce completely new proteins. However, it is not ruled out that some edits may alter metabolic pathways, resulting in accumulation of specific substances. Therefore, before the new product is promoted, it should be tested for abnormal components in its muscles, hepatopancreas and other edible tissues according to the food safety assessment process. Bioethical aspects (Parra et al., 2021), involve the public's acceptance of animal genetic modification, such as whether it is considered acceptable to modify organisms for human use. Compared with mammals, the public usually has fewer concerns about modifying invertebrates, but it also needs to introduce the purpose and process of gene editing transparently and openly to avoid causing unnecessary panic.

7.2 Regulatory policies and global comparisons of gene editing products

Regulatory policies for gene-edited organisms in countries around the world are still evolving. Compared with traditional GMOs, gene editing, especially edited organisms without exogenous DNA insertions, have been proposed to implement relatively loose supervision in many countries. The United States considered exempting some gene-edited crops from genetically modified regulations around 2020, but the regulation of animals is still relatively strict and the FDA will manage it according to the review of new animal drugs. In 2022, the US FDA approved a case of gene-edited cattle for food for the first time (Eriksson et al., 2019), and its editing only causes gene deletion in natural mutant forms. This case shows a trend of regulators' recognition of security. However, gene-edited shrimp as aquatic animals may be more cautious in regulation. The EU currently tends to treat gene-edited organisms equally with genetically modified organisms, requiring cumbersome approval procedures. In contrast, Japan, Brazil and other countries have taken a more open attitude towards gene-edited foods that do not contain exogenous fragments: Japan has launched gene-edited puffer fish and tomatoes, and the government regards such products as non-traditional genetically modified. China also pays great attention to gene editing in the agricultural field. In the revised draft of the safety management measures for agricultural genetically modified organisms released in 2022, it was proposed for the first time that gene-edited organisms can be different from genetically modified organisms. This means that if a new gene-edited aquatic product does not contain exogenous genes and its mutations can occur in nature, the approval process is expected to be simplified (Yang et al., 2023). In fact, the Ministry of Agriculture and Rural Affairs of China has accepted several safety evaluation applications for gene-edited aquatic animals and is organizing expert reviews.

The differences in regulation among countries have led to challenges in international trade in gene-edited products. If one country recognizes it but another country does not, market access will arise. Therefore, coordination is also being sought internationally, such as the International Organization for Standardization and Codex Alimentarius Commission, which began to discuss guidelines for gene-edited foods, striving to unify standards based on scientific risk assessments.

7.3 Issues of public awareness and social acceptance

Any promotion of emerging biotechnology cannot be separated from public understanding and support. Gene-edited shrimp, as a new product on the table, is based on how consumers think about gene editing. Research shows that when the public learns about the health and environmental benefits of gene editing, acceptance will be significantly improved. Especially in China, a recent consumer survey showed that more than 80% of respondents expressed willingness to try gene-edited foods and trusted the government and scientific institutions to check such foods. This provides a good social foundation for the promotion of gene-edited aquatic products. However, on the other hand, some consumers still have doubts about "genetic modification" and are concerned about food safety or ethical issues (Plate-Church, 2019). Therefore, it is very necessary to strengthen popular science publicity, and it is necessary to clarify the difference between gene editing and genetic modification. Again, authoritative endorsement is important. Let well-known scientists and nutritionists explain the safety of gene-edited foods to increase public trust. At the same time, when the product is launched, the regulatory authorities should do a good job in labeling and traceability, and the information is open and transparent.

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Conflict of Interest Disclosure

The author confirms that the study was conducted without any commercial or financial relationships and could be interpreted as a potential conflict of interest.

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