

Next-Gen Aquaculture: Merging CRISPR/CAS9 and eDNA Technologies for Smart Breeding and Environmental Surveillance

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SUMMARY

Aquaculture is revolutionised due to environmental DNA (eDNA) analysis and CRISPR/Cas9 genome editing, which enable precise breeding and advanced environmental monitoring. Compared to the traditional method of breeding, CRISPR/Cas9 comes up with targeted genetic traits, like enhanced growth rate, improved disease resistance, and stress tolerance, in a shorter generation time and a reduction of unwanted mutations, in contrast to traditional breeding. Moreover, eDNA is a highly sensitive method that provides data about changes in the ecosystem and biodiversity, as well as the real-time monitoring of fish species. It facilitates conservation, stock evaluation, and genetic diversity research while reducing the need for physical sampling. These technologies offer a powerful, complementary approach to developing sustainable and productive aquaculture systems.

INTRODUCTION

Aquaculture is rapidly evolving to meet the growing demand for sustainable and effective fish production on a global scale. Conventional breeding and monitoring methods often fail to address problems such as disease outbreaks, genetic limitations, and environmental degradation. Innovative technologies, which include CRISPR/Cas9 genome editing and eDNA surveillance, have taken aquaculture to the next generation. CRISPR/Cas9 enables precise, heritable genomic changes in cultured animals that can enhance growth rates, disease resistance, and stress tolerance.

Potential of CRISPR/Cas9 in the Genetic Improvement of Farmed Fish:

Compared to traditional breeding operations, CRISPR/Cas9 technology can reduce the likelihood of spontaneous mutations by significantly reducing the generation time and offering precise control over genetic alterations. According to Hryhorowicz *et al.* (2023), precision and targeted editing with decreased genetic variety and generation time are important characteristics that lower the likelihood of accidental genetic changes and help reduce natural mutations. Classical breeding introduces a significant amount of genetic variation. For generations, this could lead to unexpected traits and undesirable mutations. In particular, by producing desired genetic alterations in a single generation, CRISPR/Cas9 decreases the likelihood that spontaneous mutations will occur. In addition to all CRISPR/Cas9, it enables the introduction of beneficial mutations and the elimination of harmful mutations. It isn't easy to create a disease-resistant fish population with a high growth rate for potential commercial use at this level of control: A comparison between the new CRISPR/Cas9 genome editing method and traditional selective breeding. A conventional selective breeding, for instance, involves crossing a donor strain with a high growth rate and relatively low disease resistance with a strain with a high growth rate and relatively low disease resistance. The fish population created in this manner will eventually have a high growth rate and be extremely disease-resistant. However, CRISPR/Cas9 creates desired genetic changes in a single generation itself, reducing the number of opportunities for natural mutations to occur (Singer *et al.*, 2021). Backcrossing must be done repeatedly to introduce desirable qualities into the chosen fish. This is followed by the time-consuming and energy-intensive process of screening the following generations for desirable traits. Furthermore, the recipient commercial strain will experience genetic dilution since unwanted genes from the donor strain will be included with the desired gene. CRISPR/Cas9 promotes a fast growth rate and improves disease resistance by deactivating genes and targeting genes that have a quick growth rate. It would take one to two breeding cycles to make a population of homozygous mutant fish. So, CRISPR/Cas9 technology eliminates the genetic dilution that usually happens during normal breeding and speeds up the fish breeding process by editing the genome in a focused and precise way that doesn't take much time.

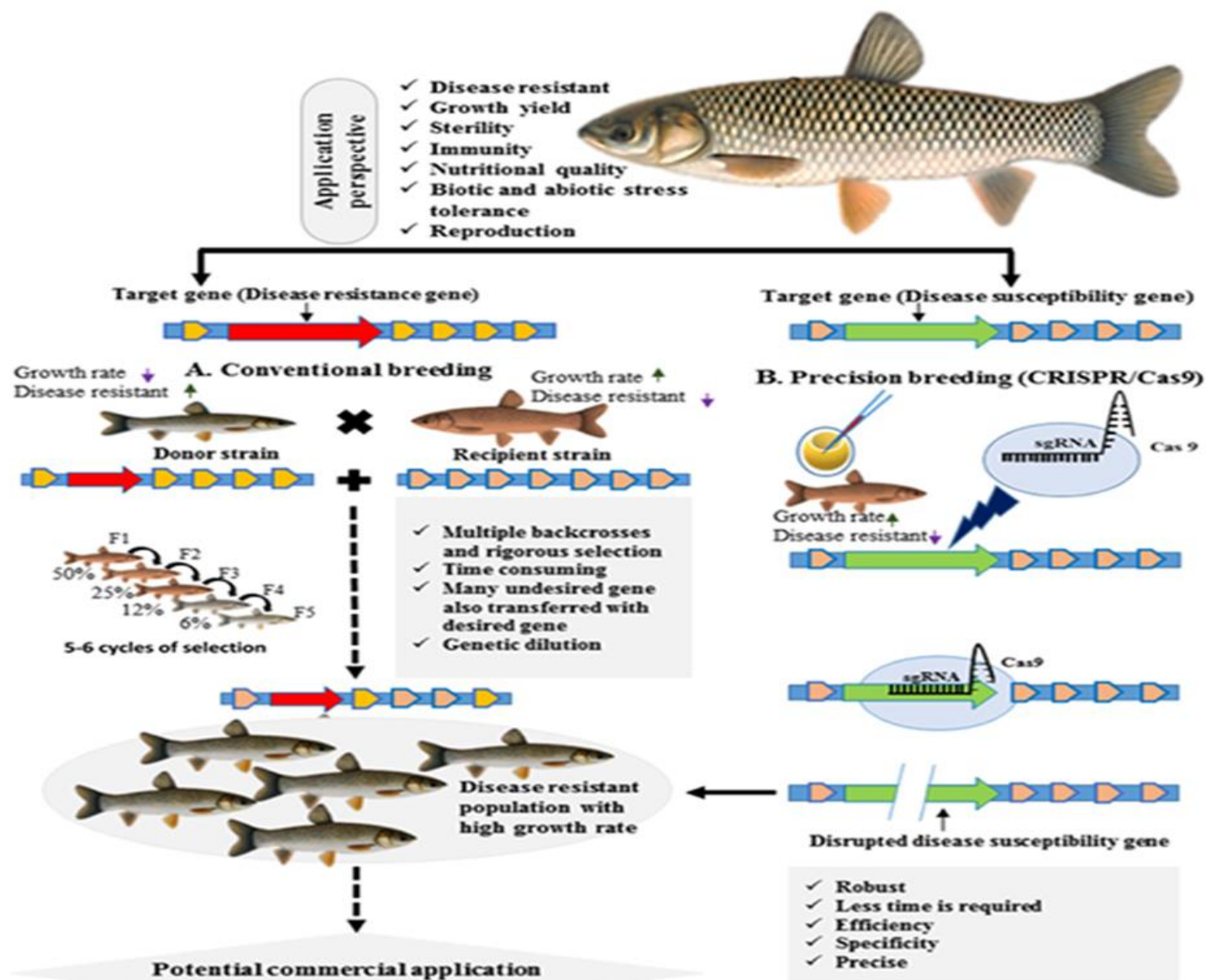


Fig. 1 CRISPR vs. Conventional Breeding (Puthumana *et al.*, 2024).

Environmental DNA (eDNA): A Game Changer in Aquatic Monitoring

Environmental DNA (eDNA) is a game-changing method for studying marine fisheries because it can monitor aquatic biodiversity without harming it and is very sensitive. It makes it easier to identify species, find elusive or rare species, and observe how the composition of communities changes across time and place. Although this application demonstrates variability in dynamic environments such as rivers and oceans, eDNA can also assess biomass or abundance. Elements like species behaviour, DNA transport, and degradation complicate its accuracy. Nevertheless, eDNA holds great promise for biodiversity conservation and fisheries stock management. By preserving genetic diversity and defining management units, eDNA is a more cost-effective option for population genetic analysis than traditional tissue collection. Studies on whale sharks show that high-throughput seawater eDNA sequencing (e.g., mitochondrial markers) can accurately quantify genetic diversity and reflect abundance/biomass trends. However, it is essential to calibrate against traditional methods due to catchability biases. To support wider biodiversity monitoring, eDNA's great sensitivity allows it to discover rare, invasive, or endangered species without causing disruption. However, the inability to determine age or size classes, database flaws, and the unknown eDNA geographic distribution in water make interpretation difficult. For applications in fisheries and conservation, robust sampling design, replication, and customised reference databases are advised to increase dependability. We will soon witness standardized workflows from field sampling to laboratory processing and bioinformatics tailored to particular ecosystems rather than a universal approach, demonstrating the rapid advancement of fish eDNA monitoring toward becoming a standardized, autonomous and globally scalable system; Even in remote waters, real-time collection, filtration and analysis (such as onboard qPCR) will be

possible because to in situ autonomous equipment; Fish eDNA is poised to become a routine tool for biodiversity monitoring, stock assessment and ecosystem-informed policy worldwide, once enough data and experience are gathered. CRISPR-based diagnostics promise quick, highly specific, and portable fish detection in the field; meta-omics techniques will expand the scope beyond single species to capture community-level diversity and ecosystem health; and ecological modelling of eDNA concentration, accounting for shedding, transport, and degradation, is being refined to quantify abundance.

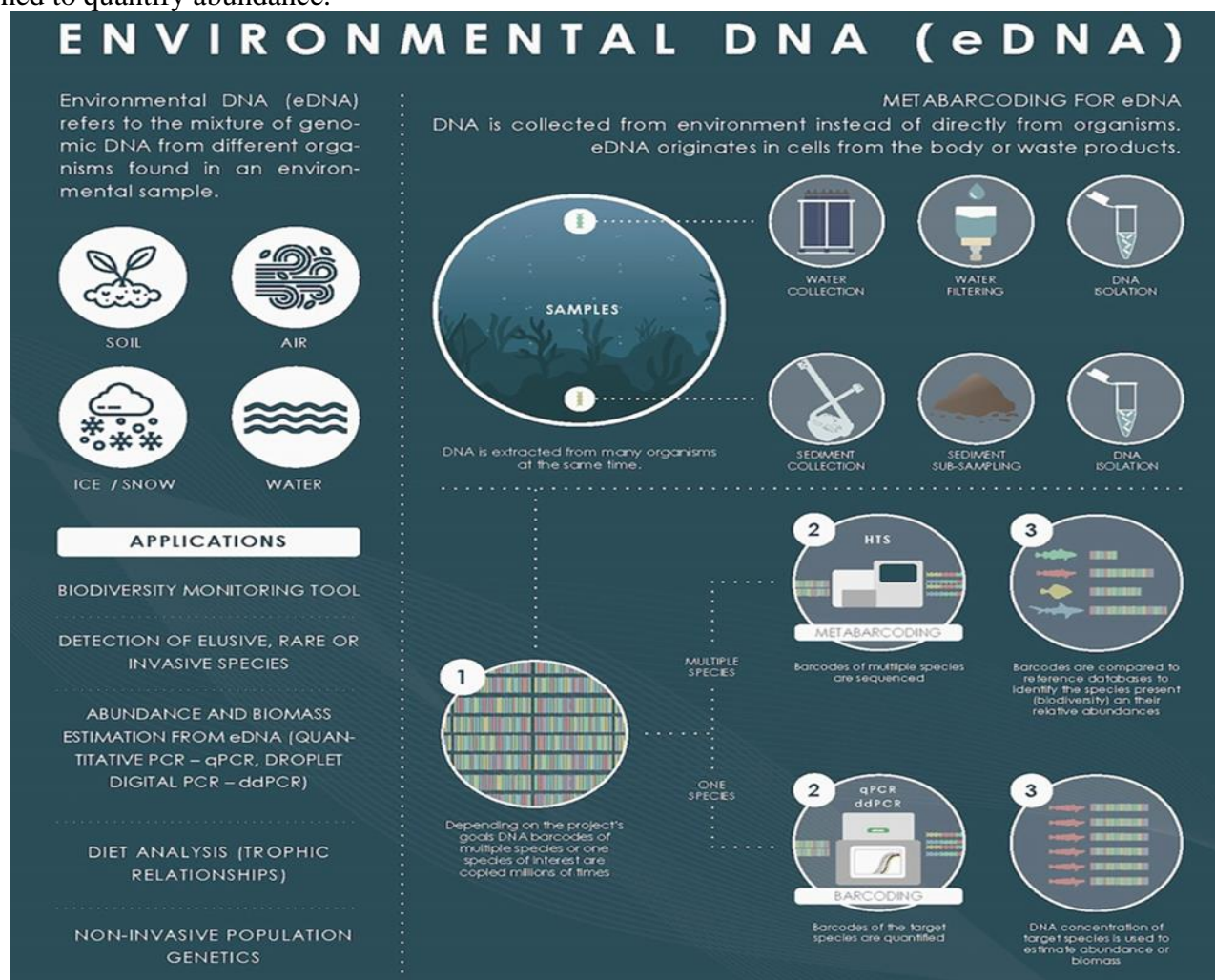


Fig. 2 Collection and application of eDNA (Ramírez Amaro et al, 2022)

Integration of CRISPR and eDNA: Synergistic Potential

Combining CRISPR and eDNA helps in the effective monitoring of biodiversity and the practices of sustainable aquaculture. CRISPR/CAS9 genome editing promotes desired traits like disease resistance, growth rate, and reproductive control. It can improve aquaculture production and effectively address various environmental sustainability issues. Through integrating advanced technologies, pathogens identification and management of the aquaculture system can be improved, and the ecological impacts minimised. However, the challenges, such as limited public acceptance, legal framework, and technical constraints, point out the need for effective research and open communication for implementing innovative technology in fisheries.

Smart Breeding:

CRISPR /CAS9 can be used for sustainable aquaculture practices, which promote the desired traits like improving growth rate and disease resistance by permitting the genetic changes in fish. The responsiveness of eDNA, particularly the use of RPA-CRISPR/CAS12a, is very high in detecting the low-abundance fish species, which further helps in real-time biodiversity monitoring and conservation. SHERLOCK, which uses CRISPR-based techniques, has high species identification accuracy, which helps manage and conserve aquatic ecosystems (2020). These innovations and technologies provide sustainable fisheries practices, which are much needed for fisheries and management.

CONCLUSION:

The synergetic effects of eDNA and CRISPR/Cas9 are a most important step in smart aquaculture. CRISPR/Cas9 techniques improve the productivity and quality of farmed fish, while eDNA enhances biodiversity conservation and environmental monitoring. Together, they stimulate faster breeding cycles, precisely analyze the health condition of fishing, and promote ecologically friendly practices. These techniques to overcome the problems like public perception, technical limitations, and regulatory barriers must be addressed through constant research and open communication. This combination strategy lays the foundation for aquaculture's powerful and sustainable future.

REFERENCES:

- Baerwald, M. R., Goodbla, A. M., Nagarajan, R. P., Gootenberg, J. S., Abudayyeh, O. O., Zhang, F., & Schreier, A. D. (2020). Rapid and accurate species identification for ecological studies and monitoring using CRISPR- based SHERLOCK. *Molecular ecology resources*, 20(4), 961-970.
- Hryhorowicz, M., Lipiński, D., & Zeyland, J. (2023). Evolution of CRISPR/cas systems for precise genome editing. *International Journal of Molecular Sciences*, 24(18), 14233.
- Okoli, A. S., Blix, T., Myhr, A. I., Xu, W., & Xu, X. (2022). Sustainable use of CRISPR/Cas in fish aquaculture: the biosafety perspective. *Transgenic Research*, 31(1), 1-21.
- Singer, S. D., Laurie, J. D., Bilichak, A., Kumar, S., & Singh, J. (2021). Genetic variation and unintended risk in old and new breeding techniques. *Critical Reviews in Plant Sciences*, 40(1), 68-108.