

MINI-REVIEW

Unlocking Potential: Gene Expression and Regulation in Aquaculture

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The authors assert that the manuscript was developed without any commercial or financial associations that could be interpreted as a potential conflict of interest.

Authors Contribution

All listed authors have contributed significantly, directly, and intellectually to the work and have endorsed it for publication.

Abstract

Aquaculture's increasing role in global food security, particularly in addressing the increasing demand for seafood, depends on a deep comprehension of the intricate mechanisms governing gene expression and control. The conversion of genetic code into functional proteins, known as gene expression, is crucial in determining the traits of an organism. Gene regulation, on the other hand, controls the timing and amount of gene expression. In the realm of aquaculture, grasping these genetic mechanisms is essential for identifying physiological traits relevant to production, choosing suitable breeding stocks, and adapting fish populations. Advanced techniques such as gene mapping, microarrays, and real-time PCR have been pivotal in researching genes linked to growth, maturity, and other life-history traits. These molecular techniques play a significant role in aquaculture research, particularly in studying toxicity, early development stages, and the overall health of fish

KEYWORDS

Aquaculture, Fish Genetics, Gene Expression, Fish Genome

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INTRODUCTION

The burgeoning growth of aquaculture, which involves cultivating diverse aquatic organisms like fish, shrimp, and algae, is crucial for global food security by supplying key protein sources. In this sector, genomic studies have focused on exploring evolutionary genomics, behavioral adaptations, and physiological responses, significantly aiding in the conservation and management of fish species. An essential element in this context is regulating gene expression in aquatic life, involving various aspects such as controlling immune-related genes critical for disease resistance and health (Liu *et al.*, 2022). Understanding how environmental factors affect the gene expression patterns of these species is essential for their adaptation, metabolism, and overall physiological health. Gaining insights into these genetic processes is vital for improving aquaculture practices and tailoring gene expression to enhance growth, reproduction, disease resistance, and the sustainable management of aquatic environments (Torres *et al.*, 2020).

GROWTH MEDIATED GENES

The main objective of aquaculture is to achieve rapid growth in less time and cost with more profit. The growth rate, size, and overall development of aquatic organisms are influenced by a variety of genes. Among all those genes, growth hormones similar to insulin and growth Hormone are pivotal in regulating growth by regulating the Hypothalamic-Pituitary-Somatic axis which has been vital throughout vertebrate evolution. Dietary factors with environmental changes affect these growth genes which have been used as a key formula to get maximum yield in aquaculture with healthy and wealthy aquatic animals (Dehkhoda *et al.*, 2018). Single growth hormone gene is an inherent quality of vertebrates with some exceptions like Gilthead seabream, European seabass, and salmonids possessing multiple copies because of the phenomenon of gene duplications (Kamenskaya *et al.*, 2015; Velez *et al.*, 2017). Hypothalamic neurohormones, Other variables influencing the regulation of the HPS axis include Growth Hormonal-Releasing Hormone, Somatostatin, Growth Hormone, and Insulin-like Growth variables-I (Table 1) (Wojcik *et al.*, 2018).

Genes associated with the immune system

Non-specific immunity is characterized by its ability to recognize and respond to foreign entities, identifying pathogen-associated molecular patterns (PAMPs) to combat pathogens through cellular immunity. This forms the cornerstone of the natural immunity system, delivering the initial line of barrier against microbial invaders. Fishes are prone to bacterial, viral, and parasitic infections easily, so developing safe and effective treatments is essential for sustaining health and productivity in aquaculture for maximum profit. Pro-inflammatory cytokines like IL-1 β activation trigger an inflammatory reaction and orchestrate barrier actions against infections. Such type of activation leads to the upregulation of genes related to the acute phase response which results in the synthesis of transferrin and ceruloplasmin-like proteins. These proteins bind iron, creating an environment that hampers bacterial growth. Other gene-derived substances, like lysozyme, protease inhibitors, and complementary proteins, also contribute to preventing the proliferation or harmful effects of pathogens. Pressley *et al.* (2005), demonstrated that in

zebrafish, the inflammatory response, marked by increased levels of IL-1 β , TNF α , and mRNA, intensifies following *Edwardsiella tarda* infection. Teleost fish are particularly valuable for immune studies as they exhibit both innate and adaptive immune systems. In catfish, extensive research has led to the identification of numerous immune-related and antigen genes, including cathepsin, ceruloplasmin, calreticulin, heatadaptation-related 65 kDa protein (wap65), and matrix metalloproteinase-13 (Table 1), as indicated in studies by Sha et al. (2008), Jiang et al. (2010), Liu et al. (2011), and Feng et al. (2012).

Table.1. Some important genes from fishes and shellfishes and their function

Genes Involved	Function	References
GH	Growth Related	Tang <i>et al.</i> , 1993; Almuly <i>et al.</i> , 2000 Dehkhoda <i>et al.</i> , 2018; Wojcik <i>et al.</i> , 2018
GHRH		
SST		
IGF-1		
IL-1 β	Immune Related	Pressley <i>et al.</i> , 2005, Feng <i>et al.</i> , 2012, Liu <i>et al.</i> , 2011, Sha <i>et al.</i> , 2008
TNF α		
cathepsin		
ceruloplasmin		
calreticulin		
wap65		
DMY	Sex Determination and Regulation	Matsuda <i>et al.</i> , 2002, Myosho <i>et al.</i> , 2012, Yano <i>et al.</i> , 2012, 2013
DMRT1		
GsdfY		
Gsdf		
AMH		
Amhr2		
Hsd17b1		
hsp90	Environmental Regulated	Quinn <i>et al.</i> , 2011, Geng <i>et al.</i> , 2014, Yuan <i>et al.</i> , 2016 Wang <i>et al.</i> , 2017; Zhong <i>et al.</i> , 2017; Fu <i>et al.</i> , 2017a, 2017b, 2017c Sun <i>et al.</i> , 2015, Li <i>et al.</i> , 2022
HIF		
MAPK		
PI3K/Akt/mTOR		
Ras		
VEGF		
CC chemokines		
Bcl-2		

FIH-1		
STAT3		
Claudin		

Environmental Influences on Gene Expression

Ecological parameters such as surface water heat, quality, oxygen levels, seasonal variations, and diet are the foremost and important factors that play a crucial role in gene modification and its regulations. Liu et al. (2013), have shown that temperature differences can lead to varied expression patterns in thousands of genes. The challenge of heat stress, intensified by global climate change, is particularly threatening for species accustomed to colder waters. The challenge of heat stress, intensified by global climate change, is particularly threatening for species accustomed to colder waters. Research, like that conducted by Quinn et al. (2011), has pinpointed essential genes, such as small heat shock proteins and hsp90, which play a role in enhancing resistance to temperature stress. Selective breeding efforts have been effective in improving temperature tolerance in species like rainbow trout, as evidenced by the work of Chen et al. (2015) and Tan et al. (2016). The identification of QTL related to temperature tolerance in species like turbot, as reported by Ma et al. (2021), provides promising directions for future research. Furthermore, the variation in dissolved oxygen levels in aquatic ecosystems presents a constant challenge, requiring ongoing adaptation from the residing organisms.

Common hypoxia arises in aquaculture because of both natural (weather, temperature, and water flow) and artificial (water pollution, eutrophication, overcrowding, and improper aeration techniques) phenomena. The root cause of this phenomenon has been invented at the molecular level. This research is crucial for decoding the intricate molecular processes that allow these species to survive in less-than-ideal oxygen conditions. Investigations into catfish, for instance, have involved detailed analyses of QTL, identifying genes linked to low oxygen endurance. These studies have included both within and between-species comparisons, enriching our comprehension of the genetic traits that enable catfish to cope with hypoxic environments (Wang et al., 2017; Zhong et al., 2017). Such research has revealed the role of multiple gene pathways, like HIF signalling, MAPK signalling, and others, in the catfish swim bladder's hypoxic response. The discovery of genes shared between hypoxia and disease responses, such as the claudin gene, CC chemokines, and the Bcl-2 gene, highlights the complex interplay of these physiological conditions (Sun et al., 2015; Fu et al., 2017a, b, c; Yuan et al., 2016). HIF-1 and FIH-1 are the responsible factors in case of catfish for their response to low oxygen (Geng et al., 2014). Moreover, Li et al. (2022) illustrated a connection between reduced methylation in the promoter regions of STAT3 and VEGFA genes and their increased gene alteration under a hypoxic environment, suggesting the significance of epigenetic changes in modifiable genetic structure in response to reduced oxygen levels in catfish.

Genes related to sex determination and regulation:

The determination of sex in fish is a complex and diverse mechanism, significantly different from the mammalian XX and XY chromosomal systems. It is subject to a variety of influences, including genetic and environmental factors. In teleost fish, for example, the sex determination process includes a wide type

of mechanisms where both genetic makeup and environmental conditions (temperature) are crucial. A notable breakthrough in this area was the identification of the DMY gene in *Oryzias latipes*, a variant of the DMRT1 gene located on the Y chromosome (Matsuda et al., 2002), and the discovery of the GsdfY gene as a key determinant of sex in another medaka species, *O. luzonensis* (Myosho et al., 2012). These findings highlight the rapid evolution and diversity of primary sex-determination genes in teleosts. Often, new pivotal genes are identified within the genetic network responsible for sex determination, with genes like Gonadal Soma-Derived Factor, Doublesex and mab-3 related transcription factor 1, Anti-Müllerian hormone, 17 β -Hydroxysteroid dehydrogenase 1, and Anti-Müllerian hormone receptor being significant, although there are unique instances such as the Sdy (sexually dimorphic on the Y-chromosome) gene in Atlantic salmon and rainbow trout (Yano et al., 2012, 2013). In channel catfish, innovative studies have uncovered an epigenetically marked site within the sex-determination region, demonstrating different methylation patterns on X and Y chromosomes that affect the activity of sex-determining genes (Yang et al., 2022). Additionally, the Siamese fighting fish (*Betta splendens*) demonstrates the variety in sex determination systems, utilizing an X/Y mechanism with the Doublesex and mab-3 related transcription factor 1 gene playing a central role, indicating the importance of further research into the evolutionary background and functionality of these intricate processes.

Technological Advances in Studying Gene Expression

Research into fish gene expression and regulation has undergone a significant transformation due to technological advancements, enabling scientists to delve into the complexities of molecular biology with unprecedented precision. Key technical developments in this field include:

Transcriptomics responses using Microarray:

In aquaculture, the intestinal immunity of fish is of paramount importance due to their increased susceptibility to infections at elevated densities, where the gut often serves as a major site for pathogen entry (Salinas and Parra, 2015). The use of commercial pelleted feeds enriched with dietary supplements, medications, and vaccines plays a significant role in enhancing fish health by boosting their immune systems by lowering the risk of diseases (Caipang and Lazado, 2015). The gastrointestinal immune system in teleost fish is also key in establishing beneficial microbial relationships, which can help control pathogen levels and bolster overall health and resilience (Ringø et al., 2014). The adaptability of gut microbiota to dietary variations opens avenues for optimizing fish health (Llewellyn et al., 2014; Merrifield and Rodiles, 2015; Rurangwa et al., 2015). Grasping the complex interactions among diet, gut functions, and immune regulation is crucial in devising strategies for disease prevention and treatment.

Recent advancements in high-throughput RNA analysis technologies have enabled a deeper investigation of the fish intestine at a transcriptomic level, moving beyond the constraints of older methods such as Northern blotting or quantitative PCR (Li and Li, 2014; Qian et al., 2014). The transcriptome, comprising both protein-coding mRNAs and non-coding RNAs, undergoes changes depending on the developmental stage, physiological state, and environmental conditions of the fish (Lindberg and Lundberg, 2010). This complexity, including aspects like alternative splicing and epigenetic regulation, underscores the importance of transcriptomics in elucidating the detailed relationship between genes and

observable traits, influencing a broad spectrum of scientific disciplines (Wang et al., 2009; Mastoridis et al., 2015). Therefore, utilizing transcriptomics approaches is essential for uncovering the intricacies of the fish gut and developing innovative treatments to protect the health of these vital aquatic organisms.

Genome Editing:

Within TILLING, the task of isolating specific gene mutants through detailed screening often faces the challenging reality of not always yielding the desired mutants. This situation demands extensive and strenuous effort, thus hindering the efficiency of the process. Nevertheless, the field of reverse genetics has experienced a significant transformation with the advent and progress of genome editing technologies, promising to bring about a radical change in the area. The essence of genome editing lies in its innovative strategy, which involves the strategic placement of endonucleases at targeted gene sequences. This accurate positioning enables the direct cutting of DNA at these specified locations, facilitating the intended genetic alterations (Fig.1).

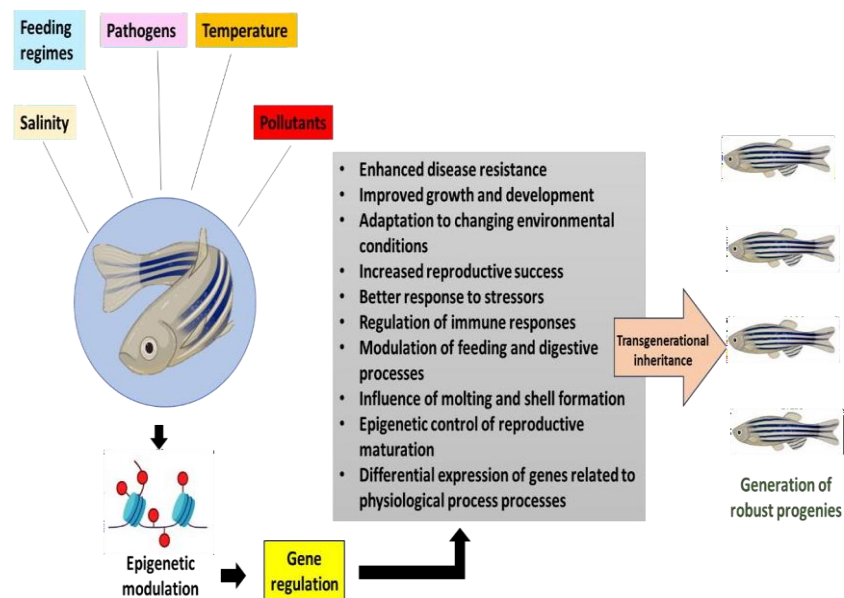


Fig.1. Epigenetic modulation for gene regulation to produce desirable progenies

Currently, three main genome editing techniques have gained prominence: ZFN, TALEN, and CRISPR. The process of DNA cleavage initiates a cellular gene repair response, which includes mechanisms like the error-prone NHEJ and the more accurate HDR. NHEJ, though prone to introducing errors, often results in mutations like insertions or deletions at the repair site, making it a useful method for disrupting gene functions. This aspect of gene repair has been particularly beneficial in inducing mutations in species such as zebrafish, demonstrating its practicality (Raby et al., 2020). The presence of a DNA fragment with a matching sequence during the cleavage event allows for the precise and effective use of HDR to introduce new genes, marking the advent of new possibilities in genetic manipulation. These groundbreaking genome editing techniques show promise in developing animal models that closely

replicate human genetic mutations, thus offering unprecedented insights into genetic disorders and diseases affecting humans.

CONCLUSION

Despite various challenges in aquaculture, such as ethical considerations, environmental impacts, and public perception, the potential of gene expression and regulation in this field is highly promising. It is crucial to thoughtfully navigate these factors to achieve a balance between harnessing genetic technologies for improved production and addressing concerns related to biodiversity and ecosystem health. Advances in molecular biology, especially CRISPR-Cas9 gene editing, have opened new possibilities for precise genetic modifications to enhance traits in aquaculture species. However, the responsible and transparent use of these technologies is essential to mitigate risks and ensure sustainable practices. In summary, delving into gene expression and regulation in aquaculture holds immense promise for meeting the increasing global demand for seafood. By understanding the genetic basis of traits and regulatory mechanisms, researchers and farmers can significantly enhance aquaculture's efficiency, productivity, and sustainability. Moving forward, it's paramount to adopt a holistic approach that considers the environmental, ethical, and societal implications of these advancements, ensuring responsible use of gene expression and regulation tools for a resilient and sustainable future that balances human needs with ecological health.

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