Uttar Pradesh Journal of Zoology

Volume 45, Issue 15, Page 526-540, 2024; Article no.UPJOZ.3765
ISSN: 0256-971X (P)

The Zebrafish (*Danio rerio*): A Dynamic Model Organism for Scientific Research

Rama Krishna Alla [a++*], Anusha Konakanchi [a#], Rajesh Nallakunta [b†], Ramaraju A. V. [c++], Suresh Sajjan M. C. [c++] and Satyanarayana Raju Mantena [c++]

[a] *Department of Dental Materials, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.*
[b] *Department of Oral Medicine and Radiology, Anil Neerukonda Institute of Dental Sciences, Visakhapatnam, Andhra Pradesh, India.*
[c] *Department of Prosthodontics, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i154269

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3765

Review Article

Received: 14/05/2024
Accepted: 16/07/2024
Published: 19/07/2024

ABSTRACT

Zebrafish has become a fundamental model organism in biomedical research due to their genetic similarity to humans, rapid development, and transparent embryos. In developmental biology, zebrafish provides insights into embryogenesis, organogenesis, and genetic manipulability. They

[++] *Professor;*
[#] *Assistant Professor;*
[†] *Associate Professor;*
**Corresponding author: Email: ramakrishna.a@vdc.edu.in, rkdentalbiomaterials@gmail.com;*

Cite as: *Alla, Rama Krishna, Anusha Konakanchi, Rajesh Nallakunta, Ramaraju A. V., Suresh Sajjan M. C., and Satyanarayana Raju Mantena. 2024. "The Zebrafish (Danio rerio): A Dynamic Model Organism for Scientific Research". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (15):526-40. https://doi.org/10.56557/upjoz/2024/v45i154269.*

offer a unique platform for studying vertebrate development and understanding genetic functions through genome sequencing and gene editing techniques. Zebrafish is valuable in disease modelling, particularly in cancer research, neurological disorders, cardiovascular diseases, and infectious diseases. Their regenerative capabilities in tissues like the heart, fin, and spinal cord make them crucial in regenerative medicine studies. Furthermore, zebrafish serves as bioindicators in environmental and ecological studies, assessing the impact of environmental pollutants and behavioural ecology changes. Technological advancements, especially in imaging techniques and genomic resources, have expanded the scope of zebrafish research. Emerging areas like microbiota-host interactions, precision medicine, and artificial intelligence integration hold promise for future discoveries and applications. This review explores the biological characteristics of zebrafish and their significance in various research domains.

Keywords: Zebrafish; biomedical research; developmental biology; disease modelling regenerative medicine; environmental studies.

1. INTRODUCTION

The zebrafish (*Danio rerio*) has emerged as a versatile and powerful model organism in scientific research over the past few decades. Originally native to the freshwaters of South Asia, zebrafish was first introduced to laboratories in the 1970s and has since become indispensable tools in a variety of biological disciplines. Their popularity stems from several advantageous traits, including their genetic similarity to humans, rapid development, and the transparency of their embryos, which facilitates direct observation of developmental processes in vivo [1]. Zebrafish and humans have more than 50% of their genes in common, and many of the genes in humans linked to diseases are also present in the zebrafish genome [2]. These features, coupled with their prolific breeding, ease of genetic manipulation, and relatively low maintenance costs, make zebrafish an attractive alternative to traditional mammalian models such as mice and rats [3]. Furthermore, the external fertilization and development of zebrafish embryos allow for straightforward experimental interventions, making them ideal for high-throughput genetic and chemical screenings [4].

Research using zebrafish has led to significant insights into vertebrate development, organogenesis, and disease mechanisms, providing a deeper understanding of human biology and contributing to the discovery of novel therapeutic targets [5]. For instance, studies on zebrafish have elucidated the molecular pathways involved in heart and fin regeneration, offering potential strategies for regenerative medicine [6]. Additionally, zebrafish is increasingly utilized in toxicology and environmental sciences to assess the impacts of pollutants and chemicals on biological systems [7]. This review aims to explore the multifaceted applications of zebrafish in scientific research, highlighting their contributions to developmental biology, genetics, disease modelling, regenerative medicine, and environmental studies.

2. BIOLOGICAL CHARACTERISTICS OF ZEBRAFISH

2.1 Genetic Features

Zebrafish (Danio rerio) has gained prominence in scientific research largely due to their genetic features, which bear significant similarity to humans. The zebrafish genome, fully sequenced in 2013, reveals that approximately 70% of zebrafish genes have human counterparts, with over 84% of human genes known to be associated with diseases having orthologs in zebrafish [2]. This genetic resemblance allows researchers to model human diseases effectively in zebrafish, facilitating the study of gene function and the genetic basis of disease. Furthermore, Zebrafish possess a high degree of genetic manipulability, enabling precise genetic modifications and studies. Techniques like CRISPR/Cas9 allow targeted gene editing, facilitating the creation of transgenic lines and knockout models to study gene function and disease mechanisms. For example, researchers have used CRISPR/Cas9 to create zebrafish models of human diseases such as muscular dystrophy and cancer, providing insights into disease progression and potential treatments [8-10]. The ease of generating transgenic zebrafish lines, where specific genes can be overexpressed or fluorescently tagged, has significantly advanced research in developmental biology and disease modelling [11]. For instance, the Tg(fli1:EGFP)^{y1} line, which expresses green fluorescent protein in vascular endothelial cells, has been pivotal in studying angiogenesis and vascular development [12]. Further, the Tg(mpx:gfp) line, which labels neutrophils and has contributed to understanding immune responses and inflammation in real-time [13]. These transgenic lines have paved the way for novel insights into disease mechanisms, drug screening, and potential therapeutic targets in medicine.

2.2 Developmental Biology

The developmental biology of zebrafish is remarkable due to their rapid development and the transparency of their embryos, which allows researchers to observe and manipulate embryonic development in real-time [1,14]. This external fertilization and development make it easy to access and study the embryos without invasive procedures. Zebrafish embryogenesis occurs rapidly. Major organs begin to form within 24 hours post-fertilization, and by 5 days post-fertilization, larvae exhibit fully formed structures [1]. The transparency of zebrafish embryos facilitates detailed imaging and tracking of cellular processes during development using advanced microscopy techniques, such as confocal and two-photon microscopy. This transparency allows researchers to observe live, unstained embryos in real-time, providing dynamic insights into cell behaviour, tissue formation, and organ development. For example, the development of the heart can be monitored from the initial formation of cardiac cells to the beating heart, revealing critical processes like cell migration, differentiation, and morphogenesis. Additionally, studies on neural development benefit from this transparency, allowing for the visualization of neural crest cell migration and brain structure formation. These capabilities have led to significant advancements in understanding vertebrate development, organogenesis, and tissue differentiation, providing a robust foundation for investigating developmental disorders and potential therapeutic interventions [14].

2.3 Physiological Characteristics

One of the most compelling physiological characters of zebrafish is their remarkable regenerative ability. Unlike mammals, zebrafish can regenerate various tissues and organs, including the heart, fins, spinal cord, and retina [5, 15-20]. This regenerative capacity makes them an invaluable model for studying the underlying mechanisms of tissue regeneration and repair. For example, after cardiac injury, zebrafish can fully regenerate their heart tissue within a few weeks, a process that involves the proliferation of cardiomyocytes and the reactivation of developmental signalling pathways [6]. Similarly, zebrafish can regenerate amputated fins and damaged retinal cells, offering insights into potential therapeutic strategies for human regenerative medicine [5]. In addition to their regenerative abilities, zebrafish exhibit complex sensory systems and behaviours that are comparable to those of higher vertebrates. They possess well-developed visual, auditory, and olfactory systems, which are used in studies of sensory processing and behaviour [21]. Zebrafish display a range of behaviours, including social interactions, learning, and memory, making them suitable for neurological and behavioural research. Studies on zebrafish behaviour have contributed to understanding the genetic and neural basis of social behaviours and neuropsychiatric disorders [22].

3. ZEBRAFISH IN DEVELOPMENTAL BIOLOGY

3.1 Embryogenesis

Zebrafish has become a cornerstone in developmental biology research due to their rapid embryogenesis and transparent embryos, which provide a window into vertebrate development [1,14,23]. Zebrafish go through specific stages during their embryonic development, as shown in Fig. 1. These include the cleavage stage, where the fertilized egg undergoes rapid cell divisions; the blastula stage, where the embryo forms a hollow sphere of cells; the gastrula stage, which involves the development of germ layers; and the segmentation stage, where somites form, contributing to the development of the vertebral column and musculature [1]. By 24 hours post-fertilization, zebrafish embryos exhibit the formation of major organs, and the body plan is established. Within 48 to 72 hours, the larvae begin to exhibit behaviours such as swimming and responding to stimuli, demonstrating functional nervous and muscular systems [1].

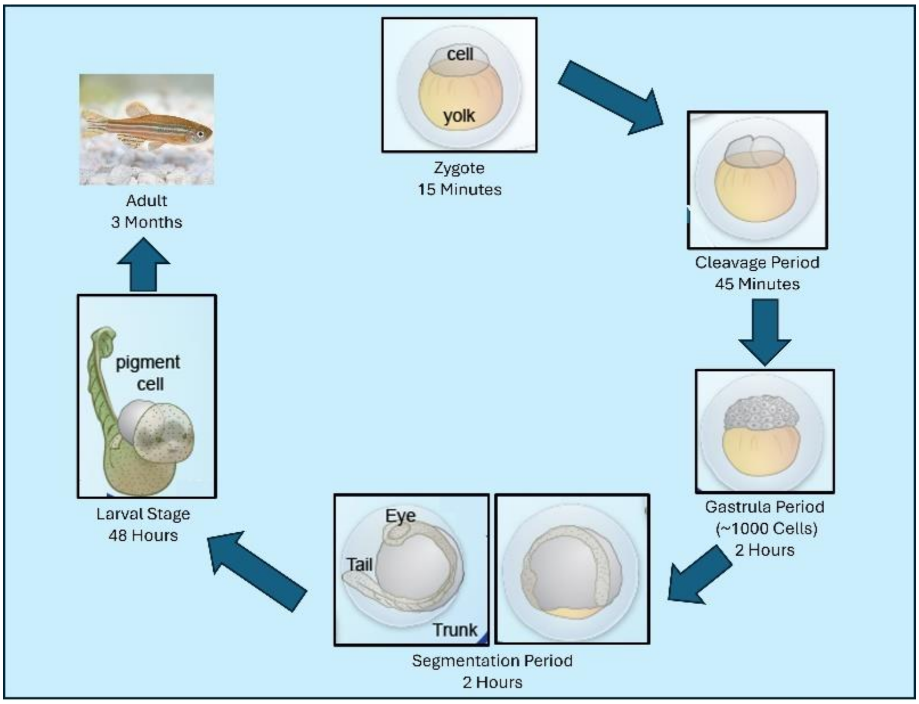

Fig. 1. Embryonic development in Zebra Fish

The transparency of zebrafish embryos allows researchers to employ advanced imaging techniques, such as confocal and live-cell imaging, to observe cellular and molecular processes in real-time. These attributes make zebrafish ideal for studying vertebrate development, as they provide insights into fundamental developmental processes, such as cell differentiation, tissue morphogenesis, and organ formation. A study on zebrafish has elucidated the roles of various signalling pathways, including Hedgehog, Wnt, and Notch, in regulating embryonic development and organogenesis [24]. The ease of genetic manipulation in zebrafish, using tools like CRISPR/Cas9 and morpholinos, has further enhanced our understanding of gene function during development as described in the previous section [8].

3.2 Organogenesis

Zebrafish has significantly contributed to our understanding of organogenesis—the process by which organs form and develop. Several key discoveries in organ development have been made using zebrafish as a model. One notable area of research is heart development [6,20,25]. Zebrafish has a simpler, two-chambered heart, consisting of one atrium and one ventricle. Despite this simplicity, zebrafish heart development closely mirrors that of higher vertebrates in terms of the genetic and molecular pathways involved. Research on zebrafish has identified critical genes and signalling pathways that regulate heart development, such as those controlling the formation and function of cardiac cells, the establishment of heart chambers, and the regulation of heartbeats. This makes zebrafish a valuable model for studying human heart development and disease [25].

In addition to the heart, zebrafish have provided insights into the development and function of other organs, including the pancreas, liver, and nervous system. Studies on pancreatic development in zebrafish have identified key transcription factors, such as Pdx1 (Pancreatic and Duodenal Homeobox 1) and NeuroD (Neurogenic Differentiation 1), that regulate the formation and differentiation of pancreatic islet cells [26]. Pdx1 expression begins in the early stages of pancreas development. It promotes the formation of both the endocrine (islet) and exocrine (acinar) compartments of the pancreas. Knockdown or mutation of pdx1 in zebrafish results in a significant reduction or absence of pancreatic tissue, demonstrating its crucial role in pancreatic development. NeuroD is expressed in the developing pancreatic islet cells and is necessary for the differentiation and maturation of these cells. Studies in zebrafish have shown that the loss of NeuroD function leads to defects in the formation of endocrine cells, including a decrease in the number of insulin-producing β-cells [27,28].

Similarly, zebrafish models have been used to investigate liver development and regeneration, revealing the roles of hepatocyte growth factor (HGF) and Wnt signalling in these processes [29]. Wnt signals help specify liver progenitor cells from the endoderm, the innermost layer of the embryo, during embryogenesis. In zebrafish, Wnt signalling is required for the expression of liver-specific genes. When Wnt signalling is inhibited, the liver fails to form properly, indicating its essential role in liver specification [29,30]. The development of the nervous system in zebrafish, particularly the formation and patterning of the neural tube, has also been extensively studied. Zebrafish models have shed light on the roles of Sonic Hedgehog (Shh) and retinoic acid in neural patterning and axon guidance [31].

Studies on organ function and disease using zebrafish models have led to significant breakthroughs in understanding human diseases. For instance, zebrafish models of muscular dystrophy have helped identify the role of dystrophin and its interaction with the cytoskeleton in maintaining muscle integrity [32]. Zebrafish have also been used to model neurodegenerative diseases, such as Parkinson’s and Alzheimer’s, by expressing human disease-related genes in zebrafish, providing insights into disease mechanisms and potential treatments [33].

4. ZEBRAFISH IN GENETICS AND GENOMICS

4.1 Gene Editing Techniques

The advent of gene editing techniques has revolutionized zebrafish research, with CRISPR/Cas9 emerging as a particularly powerful tool. CRISPR/Cas9 allows for precise and efficient modification of the zebrafish genome, facilitating the creation of targeted gene knockouts and knock-ins. The system operates by introducing a guide RNA (gRNA) that directs the Cas9 nuclease to a specific DNA sequence, where it creates double-strand breaks. These breaks are then repaired by the cell's natural repair mechanisms, often resulting in mutations that can disrupt gene function [8]. This technique has been widely adopted in zebrafish research due to its simplicity, high efficiency, and the ability to target multiple genes simultaneously, which is beneficial for studying complex genetic interactions [34].

In addition to gene knockouts, CRISPR/Cas9 is used to generate transgenic zebrafish lines. These transgenic lines are created by inserting specific DNA sequences into the zebrafish genome, allowing researchers to express genes of interest, including fluorescent reporter genes that label specific tissues or cell types. This capability is instrumental in studying gene function, cellular processes, and tissue dynamics in vivo [11]. The generation of these lines typically involves injecting CRISPR/Cas9 components along with donor DNA into zebrafish embryos, which integrate the donor DNA into their genome during repair processes [35]. The resulting transgenic zebrafish can be used to visualize and track cellular and developmental events in real time, providing valuable insights into biological mechanisms. Transgenic zebrafish, created through techniques like CRISPR/Cas9, express specific genes tagged with markers such as fluorescent proteins, enabling real-time visualization and tracking of cellular and developmental events. These include reporter lines for tissue-specific fluorescence, Cre-Lox lines for controlled gene manipulation, and inducible expression lines for temporal control. Optogenetic lines with light-sensitive proteins allow precise modulation of cellular activities. Mutant lines, with gene mutations induced by CRISPR/Cas9, help dissect gene functions in development and disease [36].

4.2 Genomic Resources

The zebrafish genome has been fully sequenced, offering an extensive resource for genetic and genomic research. The zebrafish reference genome, published in 2013, has enabled researchers to identify orthologs of human genes and study their functions in a vertebrate model [2]. The availability of this genome sequence has facilitated comparative genomic studies, gene mapping, and the identification of genetic variants associated with specific phenotypes.

Numerous genomic databases and resources have been developed to support zebrafish research. The Zebrafish Information Network (ZFIN) is a comprehensive database that provides detailed information on zebrafish genetics, including gene annotations, mutant phenotypes, and expression patterns. ZFIN serves as a central repository for genetic and genomic data, supporting researchers in designing experiments and interpreting results [37].

Mutant libraries represent another critical resource for zebrafish researchers. These libraries contain collections of zebrafish with specific gene mutations, generated using techniques, such as ENU (N-ethyl-N-nitrosourea) mutagenesis, TILLING (Targeting Induced Local Lesions IN Genomes), and more recently, CRISPR/Cas9 mutagenesis. These mutant libraries enable high-throughput screening and functional analysis of genes, allowing researchers to systematically study gene function and identify genetic pathways involved in development, disease, and other biological processes [38]. For instance, the Zebrafish Mutation Project (ZMP) has created a large collection of characterized mutations, providing a valuable resource for functional genomics studies [39].

Mutations in tbx5, leading to heart and limb abnormalities resembling Holt-Oram syndrome [40]; mutations in p53, increasing susceptibility to cancers [41]; and disruptions in the shh pathway affecting neural tube and brain patterning [42]. The ability to manipulate the zebrafish genome with precision, combined with the comprehensive genetic and genomic data available, continues to drive innovations in biomedical research and our understanding of complex biological systems.

5. ZEBRAFISH IN DISEASE MODELLING

5.1 Human Disease Models

Zebrafish has emerged as a powerful model for studying human diseases due to their genetic similarity to humans and their versatile use in various research fields. The transparency of zebrafish embryos and larvae, along with their rapid development and the availability of genetic tools, makes them an ideal model for *in vivo* studies of disease mechanisms and therapeutic interventions.

5.1.1 Cancer research using zebrafish

Zebrafish has significantly contributed to cancer research, providing insights into tumour biology, metastasis, and drug responses. Transgenic zebrafish models expressing oncogenes or harbouring tumour suppressor gene mutations have been developed to study various cancers, including melanoma, leukaemia, and pancreatic cancer [43]. For instance, the overexpression of the human BRAF$^{\wedge V600E}$ mutation in zebrafish led to the development of melanoma, which closely recapitulates the human disease, allowing researchers to study the molecular pathways involved in melanoma genesis and to test potential therapeutic agents [44]. Similarly, zebrafish models of leukaemia have provided valuable insights into the role of specific genetic mutations in leukemogenesis and the effects of targeted therapies [45].

5.1.2 Neurological and cardiovascular diseases

Zebrafish is also used to model neurological and cardiovascular diseases. The transparent embryos and the conserved structure of the nervous system enable detailed studies of neurodevelopmental and neurodegenerative diseases. For example, zebrafish models of Parkinson's disease, generated by expressing human alpha-synuclein, exhibit dopaminergic neuron loss and motor deficits, mimicking the human condition and facilitating the study of disease mechanisms and potential treatments [33]. Additionally, zebrafish models have been used to investigate the genetic basis of epilepsy, autism, and Alzheimer's disease, providing valuable insights into the underlying pathophysiology and potential therapeutic targets [46].

In cardiovascular research, zebrafish has been instrumental in studying heart development, function, and regeneration. Zebrafish hearts share many anatomical and physiological similarities with human hearts, making them a valuable model for studying congenital heart defects, cardiomyopathies, and heart regeneration. For instance, zebrafish models of congenital heart disease have helped identify critical genes and pathways involved in cardiac development, while studies on heart regeneration have elucidated the molecular mechanisms underlying cardiomyocyte proliferation and tissue repair [6].

5.1.3 Metabolic and infectious diseases

Zebrafish has also been utilized to model metabolic diseases such as obesity, diabetes, and lipid disorders. The conservation of metabolic pathways between zebrafish and humans allows researchers to study the genetic and environmental factors contributing to these conditions. For example, zebrafish models of obesity and diabetes have been developed by manipulating genes involved in lipid metabolism and glucose homeostasis, providing insights into disease mechanisms and potential therapeutic interventions [47].

In the field of infectious diseases, zebrafish models have proven valuable for studying host-pathogen interactions and immune responses. Zebrafish models of bacterial, viral, and parasitic infections have been established, allowing researchers to investigate the molecular and cellular mechanisms of infection and to screen for antimicrobial compounds. The optical transparency of zebrafish larvae facilitates real-time imaging of infection processes, providing a dynamic view of pathogen behaviour and host responses [48,49].

5.2 Drug Discovery and Toxicology

5.2.1 High-throughput screening capabilities

One of the significant advantages of using zebrafish in drug discovery and toxicology studies is their suitability for high-throughput screening (HTS). The small size of zebrafish embryos and larvae, along with their rapid development and ease of maintenance, makes them ideal for large-scale screening of chemical libraries [50-52]. HTS in zebrafish involves exposing embryos or larvae to various compounds and assessing phenotypic changes, such as morphological defects, behavioural alterations, or survival rates. This approach allows for the rapid identification of potential drug candidates and toxicants [53].

5.2.2 Advantages in pharmacological studies

Zebrafish offers several advantages in pharmacological studies, including their genetic and physiological similarities to humans, the ability to perform *in vivo* analyses, and the capacity for real-time imaging of drug effects. The use of zebrafish in pharmacology has facilitated the discovery of novel drugs, Artemisinin, Tacrolimus (FK506), Doxorubicin, etc., and the evaluation of drug efficacy and safety. For instance, zebrafish have been used to identify compounds that modulate specific signalling pathways involved in disease processes, such as the Hedgehog and Wnt pathways, which are implicated in cancer and other diseases [54].

Additionally, zebrafish models have been employed to study drug metabolism and pharmacokinetics, providing insights into drug absorption, distribution, metabolism, and excretion (ADME) processes. The ability to perform genetic manipulations in zebrafish allows researchers to investigate the roles of specific genes in drug metabolism and to assess the impact of genetic variations on drug responses [48].

6. ZEBRAFISH IN REGENERATIVE MEDICINE

6.1 Tissue Regeneration Studies

Zebrafish’s impressive capacity to regenerate different tissues and organs [15-20] provides valuable insights into the mechanisms underlying tissue regeneration, which have significant implications for human health and regenerative therapies.

6.1.1 Mechanisms of fin, heart, and spinal cord regeneration

One of the most well-studied aspects of zebrafish regeneration is fin regeneration. When a zebrafish fin is amputated, the remaining stump rapidly forms a blastema, a mass of proliferating undifferentiated cells. These cells eventually differentiate into various cell types necessary for fin regeneration, following precise spatial and temporal patterns of gene expression and signalling pathways such as Wnt, FGF, and BMP [55]. This process highlights the coordinated cellular and molecular responses essential for regenerating complex structures.

Zebrafish heart regeneration is another area of intense research. Unlike mammals, zebrafish can regenerate significant portions of their heart tissue after injury, such as ventricular resection or cryoinjury. The regenerative process involves the proliferation of existing cardiomyocytes rather than the formation of scar tissue, as observed in mammals. Key signaling pathways, including those mediated by Notch, Nrg1/ErbB2, and Hippo/YAP, play crucial roles in cardiomyocyte proliferation and heart tissue restoration [56]. Understanding these mechanisms offers potential strategies for promoting heart regeneration in humans following myocardial infarction.

Spinal cord regeneration in zebrafish involves the formation of a regenerative bridge at the injury site, composed of glial and neural progenitor cells. These progenitors differentiate into new neurons and glia, restoring the structure and function of the spinal cord. Key factors in this process include the activation of the Sonic hedgehog (Shh) pathway and the involvement of inflammation and immune responses that promote a regenerative environment [57]. Zebrafish spinal cord regeneration provides a model for understanding and potentially enhancing spinal cord repair in mammals.

6.1.2 Comparison with mammalian regeneration

The regenerative capabilities of zebrafish contrast sharply with the limited regenerative responses observed in mammals [58,59]. For instance, while zebrafish can regenerate extensive heart tissue, mammalian hearts primarily form scar tissue after injury, resulting in compromised cardiac function. Similarly, mammalian limbs and spinal cords exhibit minimal regenerative capacity compared to the extensive regrowth seen in zebrafish [58-60]. By comparing the regenerative processes of zebrafish and mammals, researchers aim to identify the genetic and molecular barriers that limit mammalian regeneration and develop strategies to overcome these obstacles.

6.2 Stem Cell Research

6.2.1 Role of zebrafish in understanding stem cell biology

Zebrafish has become a valuable model for studying stem cell biology due to their regenerative capabilities and the accessibility of their embryonic and adult stem cells [61-63]. The transparency of zebrafish embryos and larvae allows real-time visualization of stem cell behaviours, while genetic tools facilitate the manipulation and tracking of stem cells *In vivo.*

Research using zebrafish has provided insights into the maintenance, proliferation, and differentiation of stem cells in various tissues. For example, studies on hematopoietic stem cells (HSCs) in zebrafish have identified key signalling pathways and transcription factors involved in HSC development and self-renewal, such as Notch, Runx1, and Scl/Tal1 [63]. These findings have direct implications for improving bone marrow transplants and developing therapies for blood disorders [63,64].

Neural stem cells in zebrafish have also been extensively studied, particularly in the context of brain and spinal cord regeneration. Zebrafish models have elucidated the role of factors like Sox2 and Ascl1a in neural stem cell activation and neurogenesis following injury [65]. Understanding these processes provides a foundation for developing regenerative therapies for neurodegenerative diseases and central nervous system injuries.

6.2.2 Implications for human regenerative medicine

The insights gained from zebrafish research have significant implications for human regenerative medicine. By uncovering the molecular and cellular mechanisms underlying tissue regeneration in zebrafish, researchers can identify potential targets for promoting regeneration in humans. For instance, the identification of cardiomyocyte proliferation pathways in zebrafish has led to efforts to stimulate similar pathways in human hearts to enhance repair after myocardial infarction.

Additionally, the study of stem cell biology in zebrafish contributes to the development of stem cell-based therapies for various diseases. The ability to manipulate and expand specific stem cell populations, informed by zebrafish research, holds promise for treating conditions such as spinal cord injuries, neurodegenerative diseases, and hematopoietic disorders.

7. ZEBRAFISH IN ENVIRONMENTAL AND ECOLOGICAL STUDIES

7.1 Ecotoxicology

Zebrafish (Danio rerio) has become a prominent model in ecotoxicology, playing a crucial role in assessing the impact of environmental pollutants and serving as bioindicators of ecological health [66-68]. Their sensitivity to various contaminants, combined with their well-characterized physiology and genetic makeup, makes them an invaluable resource for environmental monitoring and toxicological research [66-69].

7.1.1 Assessing the impact of environmental pollutants

The use of zebrafish in assessing the impact of environmental pollutants is well-established, with numerous studies utilizing their embryos and larvae to evaluate the toxicity of chemicals, heavy metals, pharmaceuticals, and other pollutants [70,71]. Zebrafish offers a rapid and cost-effective method for screening toxic effects, providing data on lethal and sub-lethal endpoints such as mortality, malformations, and behavioural changes.

One significant application of zebrafish in ecotoxicology is the evaluation of endocrine-disrupting chemicals (EDCs). EDCs, such as bisphenol A (BPA) and phthalates, can interfere with hormonal systems and cause adverse developmental and reproductive effects. Studies have shown that exposure to these chemicals in zebrafish leads to alterations in gene expression, reproductive dysfunction, and developmental abnormalities, highlighting their potential risks to aquatic ecosystems and human health [72].

Additionally, zebrafishes have been employed to assess the toxicity of emerging contaminants, such as nanoparticles and microplastics. These studies have demonstrated that exposure to nanoparticles can induce oxidative stress, inflammation, and developmental defects in zebrafish embryos, providing insights into the environmental impact of nanomaterials [73]. Similarly, research on microplastics has revealed their potential to accumulate in aquatic organisms and cause physical and chemical harm, emphasizing the need for further investigation into their ecological effects [74].

7.1.2 Zebrafish as bioindicators

Zebrafishes serve as effective bioindicators due to their sensitivity to environmental changes and their ability to reflect the health of aquatic ecosystems. Bioindicators are species used to monitor the health of an environment or ecosystem, providing early warning signs of pollution or ecological degradation. For example, the presence of zebrafish in a contaminated water body can indicate the presence of pollutants and their potential impact on the aquatic community. Changes in zebrafish populations, such as reduced survival rates, altered reproductive success, or developmental abnormalities, can signal the presence of toxic substances and prompt further investigation [75].

Moreover, zebrafish have been used in biomonitoring programs to assess the effectiveness of pollution control measures and environmental remediation efforts. By monitoring the health and behaviour of zebrafish populations before and after remediation, researchers can evaluate the success of interventions and identify any remaining risks to aquatic life [76].

7.2 Behavioural Ecology

7.2.1 Studies on social behaviour and neural circuits

Zebrafish is increasingly used in studies on behavioural ecology, particularly in understanding social behaviour and the neural circuits underlying these behaviours. Their complex social interactions and well-defined behavioural repertoires make them a suitable model for investigating the genetic and environmental factors influencing behaviour. Research on zebrafish social behaviour has provided insights into the mechanisms of aggression, mating, and group dynamics. For instance, studies have shown that specific genes and neurotransmitters, such as serotonin and oxytocin, play crucial roles in regulating social behaviours in zebrafish [77]. Understanding these mechanisms can shed light on the evolution of social behaviour and the neural circuits involved in these processes.

Additionally, zebrafish is used to study the impact of environmental changes on behaviour. For example, exposure to pollutants or altered environmental conditions, such as changes in water temperature or pH, can affect zebrafish behaviour, including their feeding, mating, and schooling activities. These studies provide valuable information on how environmental stressors influence animal behaviour and the potential ecological consequences [78].

7.2.2 Impact of environmental changes on behaviour

Environmental changes, whether natural or anthropogenic, can have profound effects on the behaviour of aquatic organisms. Zebrafish is particularly useful for studying these impacts due to their sensitivity to environmental variations and their well-characterized behavioural responses. For example, changes in water temperature can affect zebrafish behaviour, influencing their activity levels, feeding patterns, and reproductive success. Studies have shown that elevated temperatures can increase metabolic rates and alter the timing of developmental milestones in zebrafish, highlighting the potential impacts of climate change on aquatic ecosystems [79].

Similarly, exposure to chemical pollutants, such as heavy metals and pesticides, can disrupt normal behavior in zebrafish. Research has demonstrated that sub-lethal concentrations of these contaminants can impair cognitive functions, alter predator-prey interactions, and reduce overall fitness, providing insights into the ecological risks associated with pollution [80].

8. TECHNOLOGICAL ADVANCES AND FUTURE PROSPECTS

8.1 Advancements in Imaging Techniques

The development of advanced imaging techniques has significantly enhanced the utility of zebrafish in biomedical research, allowing for real-time visualization of cellular processes and providing deeper insights into developmental biology, disease mechanisms, and therapeutic responses [81].

8.1.1 Real-time imaging of cellular processes

One of the major advancements in zebrafish research is the ability to perform real-time imaging of cellular and molecular events in live organisms. The transparency of zebrafish embryos and larvae makes them particularly suitable for live imaging studies. Techniques such as time-lapse fluorescence microscopy enable researchers to track the dynamic behaviours of cells and tissues over time, providing crucial information on processes such as cell migration, division, and differentiation. For instance, real-time imaging has been instrumental in studying the mechanisms of angiogenesis (the formation of new blood vessels) in zebrafish. Researchers can visualize the growth and branching of blood vessels in living embryos, shedding light on the cellular and molecular interactions that drive this essential process [82]. This has important implications for understanding vascular diseases and developing new therapeutic strategies.

8.1.2 Advances in microscopy and imaging technologies

Technological advancements in microscopy and imaging technologies have further expanded the capabilities of zebrafish research. High-resolution confocal microscopy and two-photon microscopy allow for detailed imaging of zebrafish tissues at subcellular resolution, enabling the study of intricate cellular structures and interactions. These techniques have been crucial in visualizing the architecture of neural circuits, the organization of cellular organelles, and the dynamics of protein-protein interactions. Moreover, the development of light-sheet fluorescence microscopy (LSFM) has revolutionized zebrafish imaging. LSFM provides rapid, high-resolution, and three-dimensional imaging of entire zebrafish embryos and larvae with minimal phototoxicity, making it ideal for long-term studies of developmental processes [83]. This technique has facilitated large-scale imaging studies, enabling researchers to generate comprehensive atlases of zebrafish development and organogenesis.

Recent advances in super-resolution microscopy, such as structured illumination microscopy (SIM) and stochastic optical reconstruction microscopy (STORM), have pushed the limits of spatial resolution, allowing researchers to visualize molecular complexes and nanoscale structures within zebrafish cells [84]. These technologies have provided unprecedented insights into the organization and function of cellular components, contributing to a deeper understanding of fundamental biological processes.

8.2 Future Directions

The future of zebrafish research is promising, with emerging research areas and technological innovations poised to drive new discoveries and applications.

8.2.1 Emerging research areas

One emerging area of zebrafish research is the study of microbiota-host interactions. The zebrafish model is increasingly being used to investigate the role of the gut microbiota in health and disease. Researchers are exploring how microbial communities influence host physiology, development, and immune responses, with potential implications for understanding human diseases such as inflammatory bowel disease and metabolic disorders [85].

Another exciting area is the use of zebrafish in precision medicine. The generation of patient-specific zebrafish models through techniques such as CRISPR/Cas9 genome editing, and xenotransplantation allows for the personalized study of disease mechanisms and therapeutic responses. This approach has the potential to accelerate the development of tailored treatments for cancer, genetic disorders, and other diseases [86].

8.2.2 Potential for new discoveries and applications

The continued integration of cutting-edge technologies and multidisciplinary approaches in zebrafish research holds great potential for new discoveries and applications. Advances in single-cell sequencing technologies are enabling researchers to dissect the heterogeneity of cell populations and uncover novel cell types and states in zebrafish tissues. This has important implications for understanding development, tissue homeostasis, and disease progression [87].

Furthermore, the development of high-throughput screening platforms in zebrafish is facilitating the discovery of new drugs and therapeutic targets. Automated imaging and analysis systems allow for the rapid screening of large compound libraries, accelerating the identification of potential therapeutics for a wide range of diseases [45]. This approach is particularly valuable for identifying small molecules that can modulate biological pathways and provide new treatment options.

In addition, the integration of artificial intelligence (AI) and machine learning (ML) with zebrafish research is poised to enhance data analysis and interpretation. AI-driven image analysis tools can automate the quantification of complex phenotypes and identify subtle patterns in large datasets, improving the efficiency and accuracy of zebrafish studies [87].

9. CONCLUSION

Zebrafish (Danio rerio) is a pivotal model organism in biomedical research due to their genetic similarity to humans, rapid development, and transparent embryos. These features facilitate profound insights into developmental biology, genetics, disease mechanisms, and environmental impacts. Advanced imaging techniques allow real-time visualization of cellular processes, enhancing the study of embryogenesis, organ development, and gene function through tools like CRISPR/Cas9. Zebrafish revolutionize disease modelling for cancers, neurological disorders, and cardiovascular conditions, and their high-throughput drug screening accelerates pharmacological research. Their regenerative abilities provide valuable insights into regenerative medicine, while their role as bioindicators aids environmental studies. Technological advancements and emerging research areas, including microbiota-host interactions and precision medicine, promise continued breakthroughs and applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn. 1995;203(3):253-310.
2. Howe K, Clark M, Torroja C. et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013;496:498–503.
3. Lawson ND, Wolfe SA. Forward and reverse genetic approaches for the analysis of vertebrate development in the zebrafish. Dev Cell. 2011 Jul 19;21(1):48-64.
4. Peterson RT, Fishman MC. Designing zebrafish chemical screens. Methods Cell Biol. 2011;105:525-41.
5. Gemberling M, Bailey TJ, Hyde DR, Poss KD. The zebrafish as a model for complex tissue regeneration. Trends Genet. 2013; 29(11):611-20.
6. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. Science. 2002;298(5601):2188-90.
7. Hill AJ, Teraoka H, Heideman W, Peterson RE. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci. 2005;86(1):6-19.
8. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JJ, Joung JK. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature Biotechnology. 2013;31(3):227-9.
9. Hoshijima K, Jurynec MJ, Grunwald DJ. Precise editing of the zebrafish genome made simple and efficient. Developmental Cell. 2016;36(6):654-67.

10. Hu Y, Liu L, Jiang Q, Fang W, Chen Y, Hong Y, Zhai X. CRISPR/Cas9: a powerful tool in colorectal cancer research. J Exp Clin Cancer Res. 2023;42(1):308.
11. Auer TO, Del Bene F. CRISPR/Cas9 and TALEN-mediated knock-in approaches in zebrafish. Methods. 2014;69(2):142-50.
12. Lawson ND, Weinstein BM. *In vivo* imaging of embryonic vascular development using transgenic zebrafish. Developmental biology. 2002;248(2):307-18.
13. Renshaw SA, Loynes CA, Trushell DM, Elworthy S, Ingham PW, Whyte MK. A transgenic zebrafish model of neutrophilic inflammation. Blood. 2006;108 (13):3976-8.
14. D'Costa A, Shepherd IT. Zebrafish development and genetics: introducing undergraduates to developmental biology and genetics in a large introductory laboratory class. Zebrafish. 2009;6(2):169-77.
15. Smith KA, Mommersteeg MTM. Talkin' 'bout regeneration: New advances in cardiac regeneration using the zebrafish. Curr Opin Physiol. 202;14:48–55.
16. Sehring IM, Weidinger G. Recent advancements in understanding fin regeneration in zebrafish. Wiley Interdiscip Rev Dev Biol. 2020;9(1):e367.
17. Thomas ED, Cruz IA, Hailey DW, Raible DW. There and back again: development and regeneration of the zebrafish lateral line system. Wiley Interdiscip Rev Dev Biol. 2015;4(1):1–16.
18. Cigliola V, Becker CJ, Poss KD. Building bridges, not walls: spinal cord regeneration in zebrafish. Dis Model Mech. 2020;13 (5):dmm044131.
19. Marques IJ, Lupi E, Mercader N. Model systems for regeneration: zebrafish. Development. 2019;146(18):dev167692.
20. Beffagna G. Zebrafish as a smart model to understand regeneration after heart injury: how fish could help humans. Frontiers in Cardiovascular Medicine. 2019;6:107.
21. Nicolson T. The genetics of hearing and balance in zebrafish. Annu Rev Genet. 2005;39:9-22.
22. Chia K, Klingseisen A, Sieger D, Priller J. Zebrafish as a model organism for neurodegenerative disease. Frontiers in molecular neuroscience. 2022;15:940484.
23. Veldman MB, Lin S. Zebrafish as a developmental model organism for pediatric research. Pediatric Research. 2008;64(5):470-6.
24. Schier AF, Neuhauss SC, Helde KA, Talbot WS, Driever W. The one-eyed pinhead gene functions in mesoderm and endoderm formation in zebrafish and interacts with no tail. Development. 1997;124(2):327-42.
25. Liu J, Stainier DY. Zebrafish in the study of early cardiac development. Circulation research. 2012;110(6):870-4.
26. Biemar F, Argenton F, Schmidtke R, Epperlein S, Peers B, Driever W. Pancreas development in zebrafish: early dispersed appearance of endocrine hormone expressing cells and their convergence to form the definitive islet. Developmental Biology. 2001;230(2):189-203.
27. Zecchin E, Filippi A, Biemar F, Tiso N, Pauls S, Ellertsdottir E, Gnügge L, Bortolussi M, Driever W, Argenton F. Distinct delta and jagged genes control sequential segregation of pancreatic cell types from precursor pools in zebrafish. Developmental Biology. 2007;301(1):192-204.
28. Dong PD, Munson CA, Norton W, Crosnier C, Pan X, Gong Z, Neumann CJ, Stainier DY. Fgf10 regulates hepatopancreatic ductal system patterning and differentiation. Nature Genetics. 2007 Mar;39(3):397-402.
29. Ober EA, Verkade H, Field HA, Stainier DY. Mesodermal Wnt2b signalling positively regulates liver specification. Nature. 2006;442(7103):688-91.
30. Goessling W, North TE, Lord AM, Ceol C, Lee S, Weidinger G, Bourque C, Strijbosch R, Haramis AP, Puder M, Clevers H. APC mutant zebrafish uncover a changing temporal requirement for wnt signaling in liver development. Developmental Biology. 2008;320(1):161-74.
31. Varga ZM, Wegner J, Westerfield M. Anterior movement of ventral diencephalic precursors separates the primordial eye field in the neural plate and requires cyclops. Development. 1999;126(24):5533-46.
32. Bassett DI, Currie PD. The zebrafish as a model for muscular dystrophy and congenital myopathy. Human Molecular Genetics. 2003;12(suppl_2):R265-70.
33. Xi Y, Yu M, Godoy R, Hatch G, Poitras L, Ekker M. Transgenic zebrafish expressing green fluorescent protein in dopaminergic neurons of the ventral diencephalon. Developmental dynamics. 2011;240(11): 2539-47.

34. Jao LE, Wente SR, Chen W. Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proceedings of the National Academy of Sciences. 2013;110(34): 13904-9.
35. Ranawakage DC, Okada K, Sugio K, Kawaguchi Y, Kuninobu-Bonkohara Y, Takada T, Kamachi Y. Efficient CRISPR-Cas9-mediated knock-in of composite tags in zebrafish using long ssDNA as a donor. Frontiers in Cell and Developmental Biology. 2021;8:598634.
36. Suster ML, Kikuta H, Urasaki A, Asakawa K, Kawakami K. Transgenesis in zebrafish with the tol2 transposon system. Transgenesis Techniques: Principles and Protocols. 2009:41-63.
37. Ruzicka L, Bradford YM, Frazer K, Howe DG, Paddock H, Ramachandran S, et al. The zebrafish model organism database: Updates and new directions. Genesis. 2015;53(8):498-509.
38. Varshney GK, Carrington B, Pei W, Bishop K, Chen Z, Fan C, Xu L, Jones M, LaFave MC, Ledin J, Sood R. A high-throughput functional genomics workflow based on CRISPR/Cas9-mediated targeted mutagenesis in zebrafish. Nature Protocols. 2016;11(12):2357-75.
39. Kettleborough RN, Busch-Nentwich EM, Harvey SA, Dooley CM, De Bruijn E, Van Eeden F, Sealy I, White RJ, Herd C, Nijman IJ, Fényes F. A systematic genome-wide analysis of zebrafish protein-coding gene function. Nature. 2013;496 (7446):494-7.
40. Garrity DM, Childs S, Fishman MC. The heartstrings mutation in zebrafish causes heart/fin Tbx5 deficiency syndrome. Development. 2002;129(19): 4635–4645.
41. Berghmans S, Murphey RD, Wienholds E, Neuberg D, Kutok JL, Fletcher CD, Morris JP, Liu TX, Schulte-Merker S, Kanki JP, Plasterk R. tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors. Proceedings of the National Academy of Sciences. 2005;102(2):407-12.
42. Krauss S, Johansen T, Korzh V, Moens UL, Ericson JU, Fjose A. Zebrafish pax [zf-a]: A paired box-containing gene expressed in the neural tube. The EMBO Journal. 1991;10(12):3609-19.
43. White RM, Sessa A, Burke C, Bowman T, LeBlanc J, Ceol C, Bourque C, Dovey M, Goessling W, Burns CE, Zon LI. Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell stem cell. 2008;2(2):183-9.
44. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, Berghmans S, Mayhall EA, Traver D, Fletcher CD, Aster JC. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Current Biology. 2005;15(3): 249-54.
45. Stosik M, Tokarz-Deptuła B, Deptuła W. Haematopoiesis in zebrafish (*Danio rerio*). Frontiers in Immunology. 2022;13: 902941.
46. Baraban SC. Forebrain electrophysiological recording in larval zebrafish. JoVE-Journal of Visualized Experiments. 2013;24(71):e50104.
47. Zang L, Maddison LA, Chen W. Zebrafish as a model for obesity and diabetes. Frontiers in Cell and Developmental Biology. 2018;6:91.
48. Chakraborty C, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: a complete animal model for in vivo drug discovery and development. Current Drug Metabolism. 2009;10(2):116-24.
49. Tobin DM, May RC, Wheeler RT. Zebrafish: A see-through host and a fluorescent toolbox to probe host–pathogen interaction. PLoS Pathogens. 2012;8(1):e1002349.
50. Cassar S, Adatto I, Freeman JL, Gamse JT, Iturria I, Lawrence C, Muriana A, Peterson RT, Van Cruchten S, Zon LI. Use of zebrafish in drug discovery toxicology. Chemical Research in Toxicology. 2019;33(1):95-118.
51. Hill A. Zebrafish in drug discovery: Safety assessment. Drug discovery and evaluation: Safety and Pharmacokinetic Assays. 2013:615-6.
52. Cassar S. Zebrafish in Drug Discovery: Safety Assessment. In: Hock FJ, Gralinski MR, Pugsley MK. (eds) Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays. Cham: Springer International Publishing. 2023;1-21.
53. Peterson RT, MacRae CA. Systematic approaches to toxicology in the zebrafish. Annual review of pharmacology and toxicology. 2012;52:433-53.
54. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, FitzGerald GA. Prostaglandin E2 regulates vertebrate haematopoietic stem cell

homeostasis. Nature. 2007;447(7147): 1007-11.
55. Poss KD, Keating MT, Nechiporuk A. Tales of regeneration in zebrafish. Developmental Dynamics: an official publication of the American Association of Anatomists. 2003;226(2):202-10.
56. Gemberling M, Karra R, Dickson AL, Poss KD. Nrg1 is an injury-induced cardiomyocyte mitogen for the endogenous heart regeneration program in zebrafish. Elife. 2015;4:e05871.
57. Becker CG, Becker T. Neuronal regeneration from ependymo-radial glial cells: cook, little pot, cook!. Developmental Cell. 2015;32(4):516-27.
58. Schwarzer S, Rekhade DR, Machate A, Spieß S, Geffarth M, Ezhkova D, Hans S. Reactivation of the neurogenic niche in the adult zebrafish statoacoustic ganglion following a mechanical lesion. Frontiers in Cell and Developmental Biology. 2022;10:850624.
59. Massoz L, Dupont MA, Manfroid I. Zebra-Fishing for Regenerative Awakening in Mammals. Biomedicines. 2021;9(1) :65.
60. Tsata V, Wehner D. Know how to regrow—axon regeneration in the zebrafish spinal cord. Cells. 2021;10(6):1404.
61. Wattrus SJ, Zon LI. Stem cell safe harbor: The hematopoietic stem cell niche in zebrafish. Blood Advances. 2018;2(21): 3063-9.
62. Ceci M, Mariano V, Romano N. Zebrafish as a translational regeneration model to study the activation of neural stem cells and role of their environment. Reviews in the neurosciences. 2018;30(1) :45-66.
63. Chen AT, Zon LI. Zebrafish blood stem cells. Journal of Cellular Biochemistry. 2009;108(1):35-42.
64. Bertrand JY, Kim AD, Violette EP, Stachura DL, Cisson JL, Traver D. Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo. Development. 2007; 134(23):4147–4156.
65. Kroehne V, Freudenreich D, Hans S, Kaslin J, Brand M. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development. 2011;138(22):4831-41.
66. Spence R. Zebrafish Ecology and Behaviour. In: Kalueff, A., Cachat, J. (eds) Zebrafish Models in Neurobehavioral Research. Neuromethods, Vol 52. Humana Press, Totowa, NJ. 2011:1-46.
67. Dai YJ, Jia YF, Chen N, Bian WP, Li QK, Ma YB, Chen YL, Pei DS. Zebrafish as a model system to study toxicology. Environmental Toxicology and Chemistry. 2014;33(1):11-7.
68. Parichy DM, Postlethwait JH. The biotic and abiotic environment of zebrafish. In; Behavioral and neural genetics of zebrafish 2020; Academic Press. 2014;3-16.
69. Chen P, Huang J, Rao L, Zhu W, Yu Y, Xiao F, Yu H, Wu Y, Hu R, Liu X, He Z. Environmental effects of nanoparticles on the ecological succession of gut microbiota across zebrafish development. Science of the Total Environment. 2022;806:150963.
70. Bambino K, Chu J. Zebrafish in Toxicology and Environmental Health. Curr Top Dev Biol. 2017;124:331-367.
71. Lei P, Zhang W, Ma J, Xia Y, Yu H, Du J, Fang Y, Wang L, Zhang K, Jin L, Sun D, Zhong J. Advances in the Utilization of Zebrafish for Assessing and Understanding the Mechanisms of Nano-/Microparticles Toxicity in Water. Toxics. 2023;11(4):380.
72. Gorelick DA, Halpern ME. Visualization of estrogen receptor transcriptional activation in zebrafish. Endocrinology. 2011;152(7):2690-703.
73. Chen TH, Lin CC, Meng PJ. Zinc oxide nanoparticles alter hatching and larval locomotor activity in zebrafish (Danio rerio). Journal of Hazardous Materials. 2014;277:134-40.
74. Pitt JA, Trevisan R, Massarsky A, Kozal JS, Levin ED, Di Giulio RT. Maternal transfer of nanoplastics to offspring in zebrafish (Danio rerio): A case study with nanopolystyrene. Science of the Total Environment. 2018;643:324-34.
75. Jiang YX, Zhang JG, Fang GZ, Huang GY, Shi WJ, Ying GG. Transgenerational effects of androstadienedione and androstenedione at environmentally relevant concentrations in zebrafish (Danio rerio). Journal of Hazardous Materials. 2022;423:127261.
76. Li H, Liu Y, Chen Q, Jin L, Peng R. Research progress of zebrafish model in aquatic ecotoxicology. Water. 2023;15(9): 1735.
77. Löhr H, Hammerschmidt M. Zebrafish in endocrine systems: recent advances and implications for human disease. Annual Review of Physiology. 2011;73:183-211.

78. Kern EM, Robinson D, Gass E, Godwin J, Langerhans RB. Correlated evolution of personality, morphology and performance. Animal Behaviour. 2016; 117:79-86.
79. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic toxicology. 2004;68(4):369-92.
80. Hallare AV, Köhler HR, Triebskorn R. Developmental toxicity and stress protein responses in zebrafish embryos after exposure to diclofenac and its solvent, DMSO. Chemosphere. 2004;56(7):659-66.
81. How Zebrafish Imaging Can Support Precision Medicine. Available:https://idea-bio.com/how-zebrafish-imaging-support-precisionmedicine/#:~:text=zebrafish%20have%20been%20successfully%20used,way%20to%20practice%20personalized%20therapy, Accessed on 20th June 2024.
82. Isogai S, Horiguchi M, Weinstein BM. The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. Developmental Biology. 2001;230(2):278-301.
83. Keller PJ, Schmidt AD, Wittbrodt J, Stelzer EH. Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. science. 2008; 322(5904):1065-9.
84. Schermelleh L, Heintzmann R, Leonhardt H. A guide to super-resolution fluorescence microscopy. Journal of Cell Biology. 2010;190(2):165-75.
85. Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell. 2006;127(2):423-33.
86. Farrell JA, Wang Y, Riesenfeld SJ, Shekhar K, Regev A, Schier AF. Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. Science. 2018;360(6392): eaar3131.
87. Vashishat A, Patel P, Das Gupta G, Das Kurmi B. Alternatives of Animal Models for Biomedical Research: A Comprehensive Review of Modern Approaches. Stem Cell Rev and Rep. 2024;20:881–899.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3765