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Zebrafish as an Innovative Experimental Model: A Strong Framework for Medical Research in Preclinical Screening

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ABSTRACT
The zebrafish offer numerous advantages over other vertebrate models used in the study of human diseases, particularly for large-scale genetic mutant and therapeutic compound screenings, as well as a range of biomedical research applications. Their use in disease modelling is significantly advancing our understanding of the molecular mechanisms underlying human genetic disorders. These efforts are crucial for the development of precision medicine, providing innovative avenues for diagnosis and treatment. This review explores the zebrafish as a model organism, covering its life cycle, habitat management, and applications in biomedical research—with particular emphasis on developmental disorders, mental health conditions, and metabolic diseases. The zebrafish is an exceptionally valuable vertebrate model for both biomedical research and drug development. In particular, the integration of CRISPR-based knockout technology with extensive data from next-generation DNA sequencing is greatly enhancing the efficiency and accuracy of functional validation of Genome-Wide Association Study (GWAS) candidates in the zebrafish. This advancement is pivotal in identifying causative genes and understanding the molecular mechanisms that drive human genetic disorders. Such initiatives are laying the groundwork for the future of precision medicine by offering novel molecular targets for both diagnostic and therapeutic strategies, especially for rare diseases.

INTRODUCTION

The Cyprinidae family includes the zebrafish, scientifically known as *Danio rerio*, a small shoaling teleost fish with yellow and dark blue stripes that is gaining popularity among fish owners and researchers (Fig. 1). The adult fish is 4– 5cm long, has two sets of barbels, and an incomplete lateral line **(Dal Bosco *et al.*, 2011)**. Males have larger anal fins and more golden colouring, while females have a tiny genital papilla located directly rostral to the anal fin **(Holtzman *et al.*, 2016)**. Zebrafish are hardy freshwater fish native to tropical environments with annual rainy seasons. They are

commonly found in the sluggish currents of rivers, streams, and marshes in South Asian countries such as India, Nepal, and Bangladesh **(Kimmel *et al.*, 1995)**. These environments are typically characterized by clear, shallow waters with bottoms composed of clay, silt, or stones of various kinds **(Holtzman *et al.*, 2016)**. Predominantly feeding on plankton and insects, there is evidence that these fish inhabit the water column and feed at the surface **(Holtzman *et al.*, 2016)**.

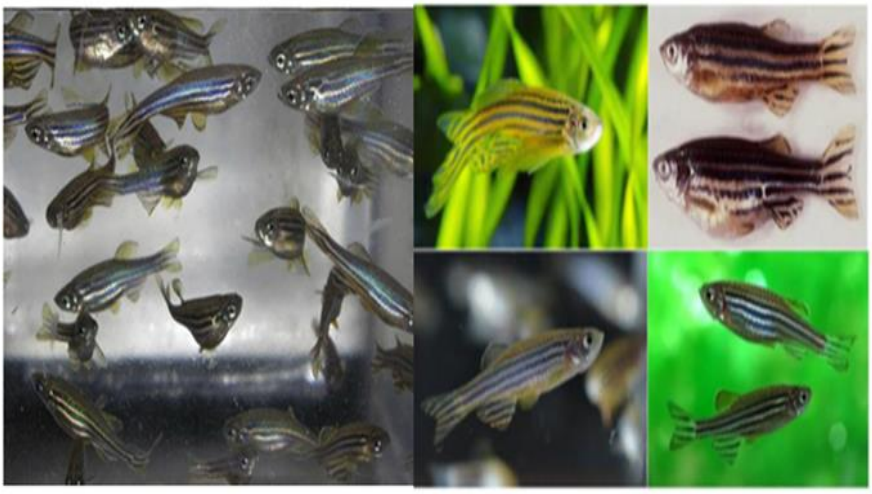

Fig. 1. Male zebrafish have a more stream-lined body with darker blue strips while the females have a white protruding belly

Biology of zebrafish

In the wild, zebrafish typically survive for only one year **(García *et al.*, 2002)**. For most of the year, they inhabit relatively shallow streams. When the monsoon rains begin, zebrafish migrate to rice fields and other densely vegetated, shallow wetlands and floodplains with minimal current and often silty bottoms, where they lay their eggs **(Ahamed *et al.*, 2002)**. The offspring then develop in these temporary waters until they recede at the end of the season. Zebrafish reach sexual maturity as early as two months after fertilisation, demonstrating their rapid developmental timeline **(Howe *et al.*, 2013)**. Although their lifespan in the wild is shorter, zebrafish in captivity commonly live up to 3.5 years, allowing for sustained study and breeding.

In natural habitats, zebrafish spawn in small groups of three to seven individuals. Males display mating behaviors while females deposit eggs along the substrate. As in the wild, zebrafish reared in controlled environments typically spawn at dawn. During courtship, males may swim around females, tap them on the back, or dart back and forth to guide them to a nesting site. Interestingly, zebrafish often prefer to spawn near artificial plants **(Howe *et al.*, 2013)**. Once a male locates a female, he stays close, aligns

his genital pore with hers, and may use rapid tail undulations against her side to synchronize the release of eggs and sperm.

Egg-laying occurs in multiple bouts during interactions that may last up to an hour, with females releasing eggs in clutches of five to twenty. The majority of eggs are laid within the first thirty minutes, with peak production in the first ten **(Kent *et al.*, 2013)**. On average, zebrafish lay between 150 and 400 eggs. These transparent eggs, protected by a chorionic membrane, measure approximately 0.7 mm in diameter. Organ development and initial body movements begin between ten and twenty-four hours after fertilization.

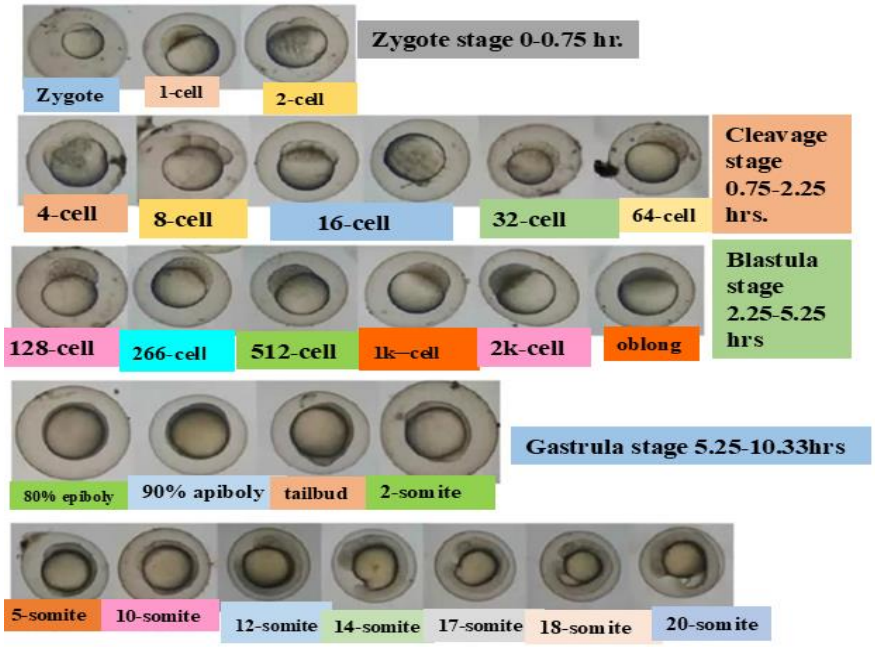

Fig. 2. Segmentation stage (24h)

In the wild, zebrafish typically survive for only about one year. Throughout the year, they spend most of their time in pools and small streams. When the summer rains begin, they migrate to shallow, flooded marshes and floodplains—such as rice fields—that are rich in vegetation, have little to no current, and often feature silty bottoms, where they spawn **(Parichy *et al.*, 2013)**. The young then develop in these seasonal waters until they dry up.

Zebrafish mature quickly, reaching sexual maturity as early as two months after fertilisation. In captivity, zebrafish live for approximately 3.5 years, although they continue to grow and undergo changes for much longer **(Kimmel *et al.*, 1995)**. During the breeding season, they gather in small groups of three to seven. Males seek out females, and eggs are laid along the substrate. Laboratory zebrafish display similar behaviors, often breeding in the early morning. Courtship includes males swimming

around the females, nudging them, or swimming back and forth while guiding them to a nesting site. Interestingly, zebrafish prefer to spawn near artificial plants.

When a male approaches a female, he stays close and extends his fins to align his genital pore with hers. He also rapidly undulates his tail against the female's side to synchronize the release of eggs and sperm **(Pola *et al.*, 2021)**. These interactions can last up to an hour, during which females release eggs in groups of five to twenty. Most eggs are laid within the first 30 minutes, with peak production occurring in the first 10 minutes. Zebrafish can lay between 150 and 400 eggs per clutch. These transparent eggs, protected by a chorionic membrane, are approximately 0.7mm in diameter **(Teame *et al.*, 2019)**. The first body movements and the onset of organ development occur within 10 to 24 hours after fertilisation.

If females are kept away from males for extended periods, they may retain their eggs, which can lead to egg-associated inflammation—a potentially fatal condition.

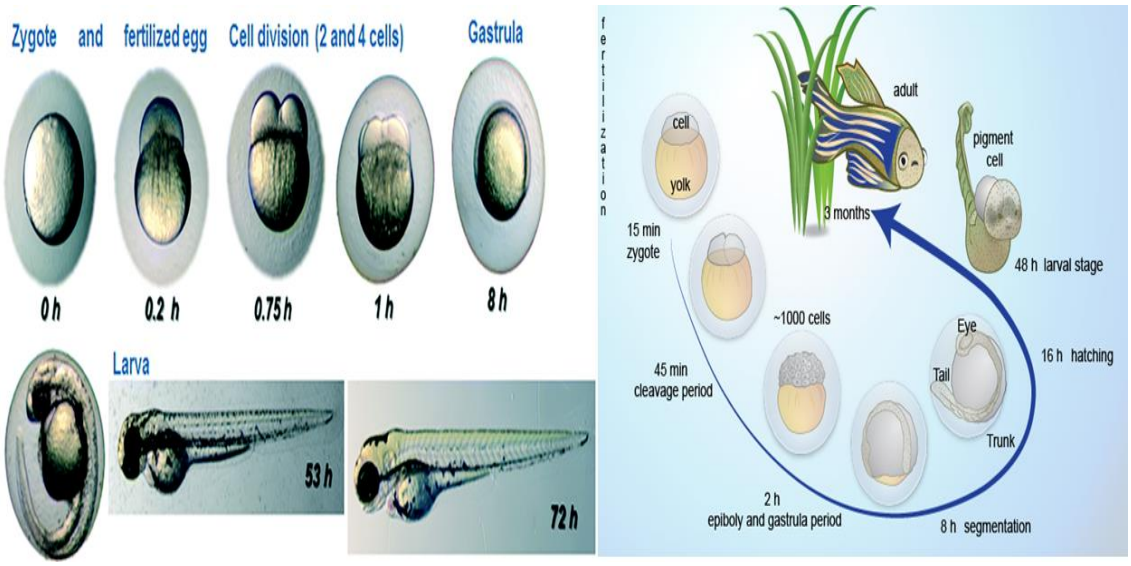

Fig. 3. Stages of embryo

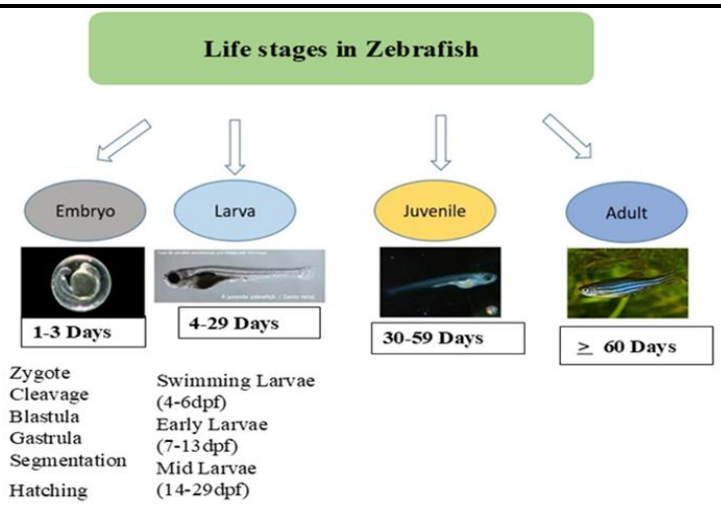

Fig. 4. Stages of zebrafish

Anatomy of zebrafish

Native to Southeast Asia, zebrafish—also known as *Danio rerio* or *Brachydanio rerio*—are tropical freshwater fish now commonly sold in pet shops around the world. They are easy to care for and are known for their striking horizontal blue stripes **(Streisinger *et al.*, 1992)**. Zebrafish are hardy and small, typically measuring 2.5–4 cm in length. They belong to the same family as minnows. Adult male and female zebrafish can be distinguished by several traits: females generally have larger bellies, males have more yellow coloration on the anal fin, and only females have a visibly protruding genital pore **(Mork *et al.*, 2015; Crump *et al.*, 2019)**.

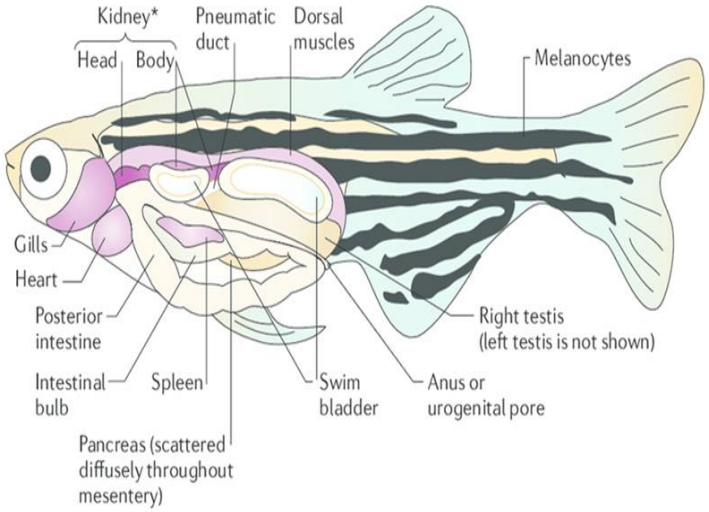

Fig. 5. Zebrafish

Origins of the model

In the 1960s, when biomedical scientists were seeking more complex models than the phage systems previously in use, George Streisinger began employing zebrafish in his research **(Mork *et al.*, 2012; Crump *et al.*, 2015)**. Chuck Kimmel and other researchers at Oregon State University, who were among the first to recognize zebrafish as a valuable model organism for studying neural development, soon acknowledged the broader significance of the zebrafish model **(Haffter *et al.*, 1996)**. Since then, zebrafish research has expanded rapidly, encompassing diverse areas such as toxicity testing, cancer biology, metabolic diseases, and developmental disorders **(Kim *et al.*, 2000)**.

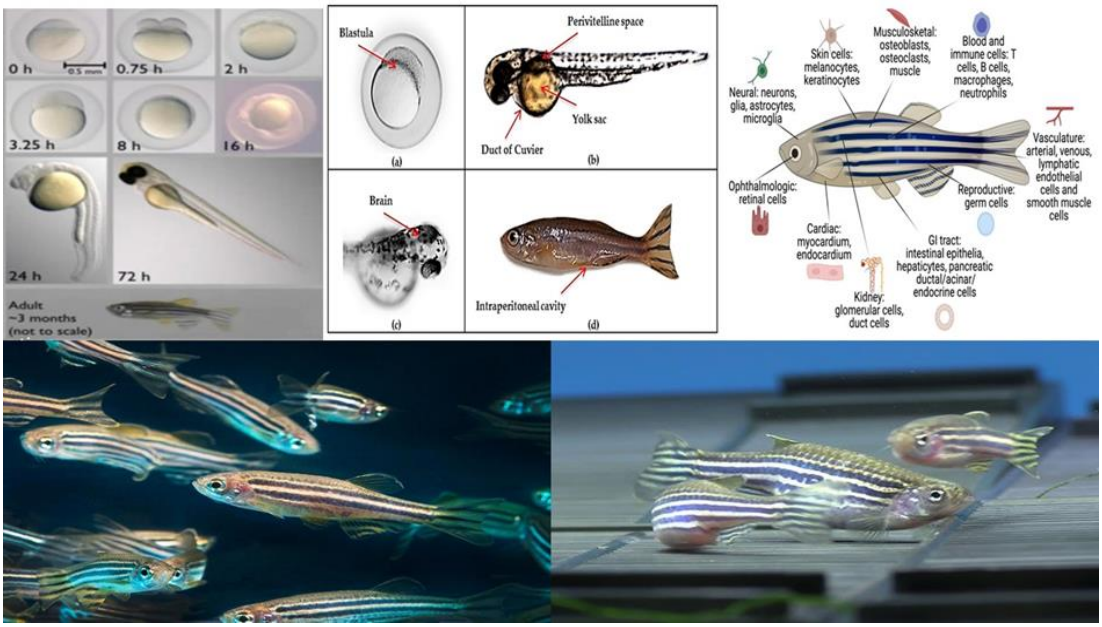

Fig. 6. Origins of zebrafish

Housing and management

Zebrafish are typically maintained for breeding purposes in stationary tanks during the spawning process. A standard spawning cage includes a plastic plant and a tank equipped with a transparent-bottom insert. The insert's grooved base facilitates the easy collection of eggs and is often angled within the tank to create a shallow area that encourages spawning (Fig. 4) **(Doyon *et al.*, 2008)**. After fertilisation, embryos are cultured in petri dishes for at least three to four days at a temperature of approximately 28.5°C **(Meng *et al.*, 2008)**. Following this stage, the larvae are transferred to a tank with minimal or no water flow and are fed rotifers, *Paramecium*, powdered food, or a combination of these.

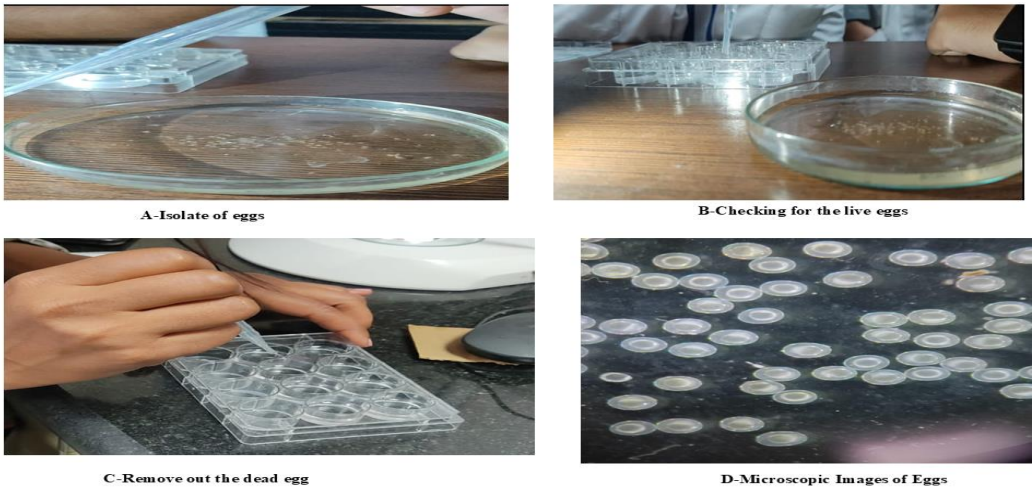

Fig. 7. Ease of egg collection

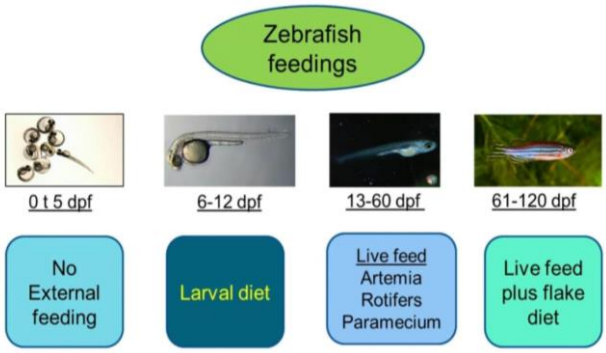

Fig. 8. Feeding

Unfortunately, not much is currently known about the specific dietary requirements of zebrafish, apart from the established need for essential fatty acids. In research settings, zebrafish are typically fed commercial diets, various types of crustaceans—such as rotifers and bloodworms (*Chironomid* larvae)—or a combination of these **(Jarryd *et al.*, 2013)**.

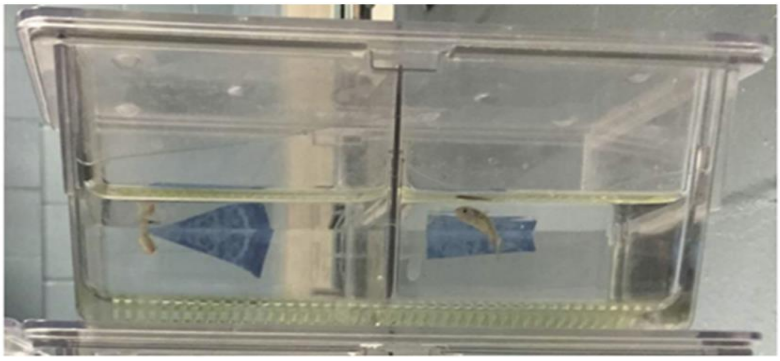

Fig. 9. A zebrafish spawning apparatus is illustrated here. To stop the adult fish from eating the eggs, the mechanism is made to let them fall to the bottom of the tank through a slotted insert

.

The feed must be appropriately sized to match the gape size of zebrafish larvae, which is approximately 100µm. Between 8 and 15 days after fertilisation, both the water flow and feed particle size are increased as the diet shifts to *Artemia* (brine shrimp) or a commercial feed with larger particles **(Patrick *et al.*, 2014)**. By around 29 days post-fertilisation, zebrafish enter the juvenile stage. At this point, housing conditions become more similar to those used for adults, with slower water flow and more frequent feedings to accommodate their smaller size and continued growth **(Jinek *et al.*, 2012)**.

By two months of age, zebrafish are typically sexually mature. Adult zebrafish can be housed either in traditional glass aquaria or in advanced automated systems that monitor and regulate water quality parameters such as salinity, pH, temperature (usually maintained at 28.5°C), nitrogenous waste levels, dissolved oxygen, and water hardness **(Mali *et al.*, 2013)**. Whether managed manually or automatically, maintaining these parameters within optimal ranges is essential for ensuring fish health. Poor water quality can lead to disease and other health complications **(Garcia *et al.*, 2016)**.

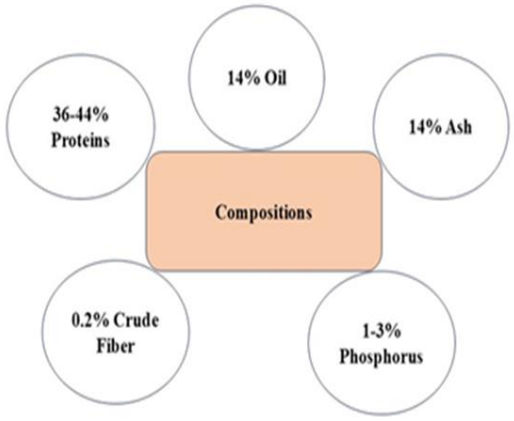

Fig. 10. Composition of minerals

Table 1. Physical parameter and normal values for laboratory

Sr. No.	Parameter	Normal Values
1.	Room Temperature(^{0}C)	28-29
2.	Relative humidity (%)	70
3.	Light Intensity (Lux)	300
4.	Photoperiod (Light: Dark)	14:10
5.	Noise/ Vibration	Free (If possible)

Table 2. Water quality parameters, normal values, testing frequency and management for zebrafish (Sources in biomedical Research; Biology, Husbandry, Disease and Research application, Merian college of Laboratory Animal medicine series, 2020)

Sr. No.	Parameter	Normal ranges	Testing frequency	Management
1.	Water temperature (0c)	26-29 optimal 28.5	Daily	↑ - Start water cooling ↓ -Start water warming
2.	P^H	7-8	Daily	↕ -Slow addition of HCL
3.	Conductivity (Us)/salinity (ppt)	100-200	Weekly	↑ -Slow addition of RO/DI ↓ Slow addition of salt
4.	Hardness (ppm	100-200	Weekly	↑ -Slow addition of RO/DI -Slow addition of NaHCO3
5.	Alkalinity (ppm)	50-75	Weekly	↑ -Slow addition of RO/DI ↓ -Slow addition of NaHCO3
6.	Dissolved oxygen (ppm)	6-8(Near saturation)	Weekly	↑ -Increase water aeration ↓ -Reduce water aeration
7.	Chlorine (ppm)	Zero	Regular	↓ -Vigorous aeration ↑ Sodium thiosulphate, Chemical filteration

Fig. 11. Feeding of zebrafish

Fish tank environment

Transparent plastic zebrafish husbandry tanks are designed for easy handling and can be removed from the system or placed anywhere on a shelf rack. ZEBCARE offers polycarbonate tanks in various sizes, typically ranging from three to ten litres. Additionally, one-litre tanks made of polyethylene are available. Each tank comes with a clear lid that includes openings for feeding and water intake. Upon request, ZEBCARE can also provide tanks made from alternative materials other than polycarbonate **(Sung *et al.*, 2014)**.

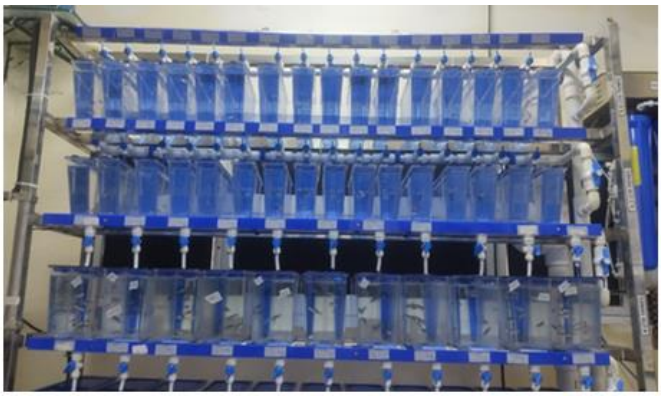

Fig. 12. Fish tank

Table 3. Environment tank capicity

Type	Volume (L)	Effective volume (L)	Length (mm)	Width (mm)	Height (mm)
Single box	1		200	100	85
MC1	3	2.3	240	135	130
MC2A	5		267	207	140
MC2B	5	3	332	150	130
MC3	10		335	235	190
MC3B	8	6.5	345	180	140
MC4A	10		427	267	150
MC4B	13		427	267	180
MC5	27		500	380	208
MC6	39		610	435	215

With the exception of the 1-litre Single Boxes, all clear plastic ZEBCARE zebrafish husbandry tanks are designed to accommodate the attachment of ZEBCARE outflow devices. The wide variety of ZEBCARE outflow devices—available in different colours and mesh sizes—allows animal caretakers to quickly identify the size of the fish in each tank **(Postlethwait *et al.*, 1998)**. Clearly labeling tanks according to life stage further streamlines feeding procedures and significantly reduces feeding time. ZEBCARE outflow sieves are also compatible with other zebrafish tank systems and can be easily removed for cleaning or replacement **(Howe *et al.*, 2013)**.

Zebra fish's importance as an animal model

Contribute to the ongoing advancement of the zebrafish era in biomedical research by utilizing zebrafish as a model organism in biomedical studies **(Angom *et al.*, 2024)**.

Table 4. Different applications of zebrafish

Sr. No.	Types of Fish	Model	Mechanism	Reference
1.	Zebra Fish (Danio rerio)	Neurodegenerative disease For Parkinson's disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an ingredient.	Neuroprotection-Degradation of α-syncline and an increase in genes related to antioxidants (sod1, gss, gpx4a, gclm, and cat) are the two items mentioned. Anti-PD activity was shown to be mediated by the antioxidation pathway.	**(Currie *et al.*, 2007)**
2	Zebrafish (Danio rerio)	Neurodegenerative disease (astaxanthin in zebrafish with AD related with CVD)	Neuroprotection Decreases in MMP-13 activity, acetyl cholinesterase activity, and amyloid beta-peptide aggregation.	(**Kwan *et al.*, 2007)**
3	Zebrafish (Danio rerio)	Neurodegenerative illness (3-HD in adult zebrafish induced by NP)	Reduced reactive astrocytosis, NMDA Antagonist Enhanced expression of the BDNF/tropomyosin-related kinase-B receptor and enhanced vascular density.	**(Halpern *et al.*, 2008)**
4	Zebrafish (Danio rerio)	Metabolic disorder (fructose-mediated glycation with low-density lipoprotein (LDL))	Controlled metabolites connected to the pathways for lipid metabolism, amino acid metabolism, and glycolysis. transcription of a few genes involved in fat and glycolysis metabolism.	**(Wei *et al.*, 2022)**

5	Zebrafish (Danio rerio)	Metabolic disorder (inflammation through lipopolysaccharide (LPS) injection.)	Interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β) are pro-inflammatory cytokines that show a reduction in production in zebrafish induced with lipopolysaccharide (LPS). Prophylactic administration of PSCP to inflamed zebrafish decreased skin haemorrhage, normalised respiration, and prevented caudal fin loss.	**(Montesano *et al.*, 2019)**
6	Zebrafish (Danio rerio)	Endocrine system	The blood levels of glucose, AST, ALT, and ALP were lowered by metformin and silymarin. The fish body needs to raise the absorption level by increasing the amount of acidic goblet cells, which acidifies the environment in the stomach tracts, because a diabetic weakly absorbs nutrients.	**Dysin *et al.*, 2022)**

7	Zebrafish (Danio rerio)	Cancer (xenotransplantation of MCF-7 breast cancer cells and human JF 305 pancreatic cancer cells into zebrafish)	Anti-cancer role Through apoptosis, DNA strand breaks, anti-angiogenesis, and the induction of ROS generation. It has been shown that increased ROS production damages major biological molecules, such as DNA, resulting in apoptosis and DNA strand breaks.Caspases 3/7 interacts with caspase 8 and 9 and is in charge of the proteolytic cleavage of several proteins during apoptosis. The cancer cell Xeno transplanted zebrafish treated with furanodiene showed a significant rise in caspases 8, 9, and 3/7, indicating that furanodiene-induced zebrafish apoptosis is dependent on both caspase 8 and caspase 9, which results in cancer cell death.	**Aharon *et al*., 2021)**
8.	Zebrafish (Danio rerio)	Hepatoprotective (Acetaminophen PAP-induced liver injury in zebrafish)	Hepatoprotective Effect • By controlling targets such as phosphatidylinositol 3-kinase (PI3K), matrix metallopeptidase 9 (MMP9), matrix metallopeptidase 2 (MMP2), and tumour necrosis factor (TNF). The apoptotic signalling	**(Huang *et al.*, 2016*)***

Metabolic disorders

Zebrafish are frequently used as an animal model in metabolic research. Risk factors for metabolic disorders—such as low HDL, high triglycerides, elevated blood glucose, high blood pressure, and abdominal obesity—are especially common among individuals with a family history of such conditions, particularly those who consume

excess calories and lead sedentary lifestyles **(Matthew *et al.*, 2012)**. These risk factors can contribute to the development of metabolic diseases including fatty liver disease, diabetes, and stroke. An imbalance between energy intake and expenditure is also considered a key contributor to these conditions **(Valenti *et al.*, 2020)**.

While zebrafish share general metabolic features with humans, they also possess distinct metabolic traits. During the first five days of life, zebrafish embryos rely on yolk consumption to support growth and prevent starvation. The transition from feeding to fasting, which typically occurs between 5 and 7 days post-fertilisation, has provided valuable mechanistic insights into metabolic homeostasis under conditions of caloric restriction **(Chakrabarti *et al.*, 1983)**.

A unique aspect of zebrafish physiology is the development of adipose tissue. Unlike mammals, zebrafish are poikilothermic and do not appear to require brown adipose tissue. The first adipocyte is typically observed around eight days post-fertilisation, indicating that adipose development occurs relatively late. This period of late adipogenesis provides an opportunity to study the role of adipose tissue in the onset of metabolic diseases. Zebrafish larvae can also be used to develop metabolic models that mimic human disorders, while adult zebrafish allow for phenotypic studies once all major metabolic organs are fully formed **(Howe *et al.*, 2013)**.

Zebrafish models for Parkinson's disease

Parkinson's disease (PD) is one of the most extensively studied neurodegenerative disorders in zebrafish. Recent studies have highlighted the utility of the zebrafish as a model organism in the discovery of new PD therapies **(Xuezhen *et al.*, 2022)**. Numerous genetic, transgenic, and chemically induced zebrafish models have been developed to study PD, largely due to the high conservation of PD-associated genes and the fish's responsiveness to pharmacological agents related to the disease.

Although the zebrafish midbrain lacks dopaminergic neurons, the diencephalic dopaminergic cluster in the posterior tuberculum serves a function similar to the substantia nigra pars compacta (SNpc) in humans. Additionally, the zebrafish share significant similarities with humans in their histaminergic and serotonergic systems **(Nallupillai *et al.*, 2023)**.

While zebrafish do not perfectly replicate the human condition, they serve as a valuable model for studying hypokinetic disorders related to PD. For instance, dopaminergic cell defects in the zebrafish can result in motor impairments resembling bradykinesia—a key symptom of Parkinson's disease in humans.

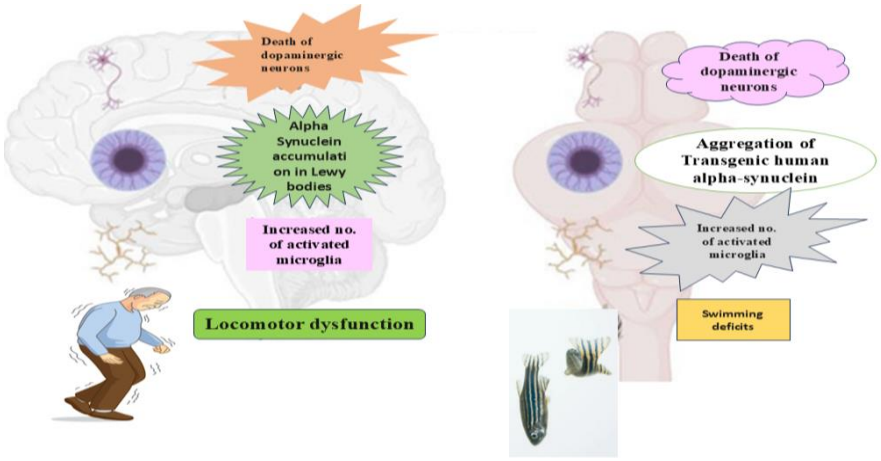

Fig. 13. Mechanism of PD in human and zebrafish

Table 5. Genetic zebrafish models of PD

Methods	**DA neuron loss**	**Other pathologies**	**Motor deficits**	**Other phenotypes**
PINK1 MO knockdown	Yes	the buildup of reactive oxygen species	Positively Affected TEER	Disfigurements based on anatomy. Enhanced death rate
Parkin MO knockdown	No	Enhanced vulnerability Due to stress on proteosomes	Not reported	-
LRRK2 MO knockdown	Yes	Synuclein aggregation	Not reported	Disfigurements based on anatomy.
PINK1 MO knockdown, Micro array analysis	Not reported	Major change to 177 genes. High amounts of reactive oxygen species	Not reported	Reduced heart rate. Enhanced red blood cell production.
Parkin Mok knockdown	Yes	Reduced mitochondrial Activity.	No	-
FBXO7 MO knockdown	Yes	-	Yes—Declined swimming speed	Disfigurements based on anatomy. Enhanced death rate

Table 6. Zebrafish models of Parkinson's disease using chemical approaches

Methods	DA neurone decease	Different diseases	Motor deficits	Different phenotypes
MPTP	Not reported	-	Sure enough, swimming speed and distance were down, crossings were down, and freezing spells were up in both frequency and duration.	-
6-OHDA	Not reported	-	Yes. There was a decrease in swim distance, speed, and maximum acceleration; there was also an increase in absolute turn angle and immobility time.	Reduced head and total length.
Paraquat	Not reported	-	Yes—decreased line crossings, decreased swimming distance and speed, and impaired motor coordination.	-

Dyslipia and atherosclerosis in zebrafish models

Atherosclerosis often develops as a result of dyslipidaemia, which is caused by elevated levels of cholesterol, triglycerides, or HDL cholesterol. Due to an expanding understanding of the zebrafish nutritional requirements, several researchers have developed experimental models by modifying the standard zebrafish diet. For instance, a high-fat diet has been used to induce metabolic stress in zebrafish, leading to obesity, hyperglycaemia, and dyslipidaemia. Zebrafish fed a high-cholesterol diet exhibit histological changes that closely resemble human atherosclerotic pathology.

Developing high-cholesterol dietary protocols is essential for studying dyslipidaemia. **Kumar *et al.* (2022)** used the metabolism of zebrafish embryos to identify stages of lipid and lipoprotein metabolism. Their findings indicated that the ability to form lipoproteins is essential for the circulatory system's capacity to absorb exogenous fatty acids **(Balkrishna *et al.*, 2021)**.

Zebrafish as a model for glucose metabolism and Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is primarily caused by insufficient insulin production due to dysfunction of pancreatic β-cells. Zebrafish share key pancreatic functions and metabolic pathways with humans. When fed a high-fat, high-calorie diet, zebrafish rapidly develop obesity and metabolic abnormalities similar to those in humans.

In zebrafish, the pancreas responds to dietary glucose by producing insulin, which downregulates gluconeogenesis by suppressing associated genes. When blood glucose levels drop, glucagon triggers gluconeogenesis **(Jin *et al.*, 2009)**. Immersion in high-glucose solutions (e.g., 111 mM for 14 days) induces hyperglycaemia in zebrafish, decreases muscle mRNA expression for insulin receptors, and increases levels of glycated proteins like fructosamine in the eyes **(Mohammadi *et al.*, 2020)**.

Researchers created a T2DM model by overfeeding zebrafish with a daily intake of 408 calories per fish. Gene expression analyses in the pancreas and liver revealed metabolic mechanisms closely resembling those found in human T2DM. Age-related studies showed that hyperglycaemia developed more slowly in younger zebrafish (4–11 months old) compared to older ones **(Vrieze *et al.*, 2014)**.

Glucose levels in key homeostatic organs can also be elevated by immersing zebrafish embryos in glucose solutions. Adults exposed to 1% glucose for 24 hours exhibited blood glucose levels of up to 400mg/ dL. Two transgenic insulin resistance models have been developed: one expressing a dominant-negative IGF-I receptor in skeletal muscle and the other using CRISPR/Cas9 to knock down insulin receptor genes in the liver **(Zhu *et al.*, 2019)**.

Additionally, zebrafish larvae administered human recombinant insulin showed signs of hyperinsulinaemia. These larvae often expressed increased levels of tyrosine phosphatase non-receptor type 6, a protein known to act as a negative immune regulator. In another study, mutant zebrafish lacking *insulin receptor a* and *b* genes and fed a high-carbohydrate (41%) diet developed symptoms including hyperglycaemia, reduced growth hormone signalling, increased visceral fat, and fatty liver—traits that strongly resemble human lipodystrophy **(Park *et al.*, 2019)**. Blood glucose in zebrafish can be effectively monitored using two commercially available portable glucose meters designed for diabetic patients.

Zebrafish models of Alzheimer's disease

Alzheimer's disease (AD), a major cause of dementia, is characterized by two pathological hallmarks: intracellular amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) composed of aggregated hyperphosphorylated tau proteins, both derived from cleaved amyloid precursor protein (APP). The disease leads to progressive atrophy of brain regions including the hippocampus and parietal cortex **(Choi *et al.*, 2014)**.

Genome-wide association studies (GWAS) have identified multiple high-risk loci linked to immune regulation, suggesting that microglia may play a role in AD pathogenesis **(Rawls *et al.*, 2009)**. Notably, Aβ plaques may appear in the brain before cognitive symptoms develop, whereas NFTs are more directly associated with neurodegeneration, neuronal loss, and cognitive decline **(Barroso *et al.*, 2013)**.

Recent PET imaging studies and biomarker meta-analyses have shown strong correlations between elevated tau levels in the blood and cerebrospinal fluid and cognitive decline in AD patients **(Gut *et al.*, 2015)**. Zebrafish, with their genetic

tractability and transparent embryonic development, offer a valuable platform for investigating the cellular and molecular mechanisms underlying Alzheimer's disease.

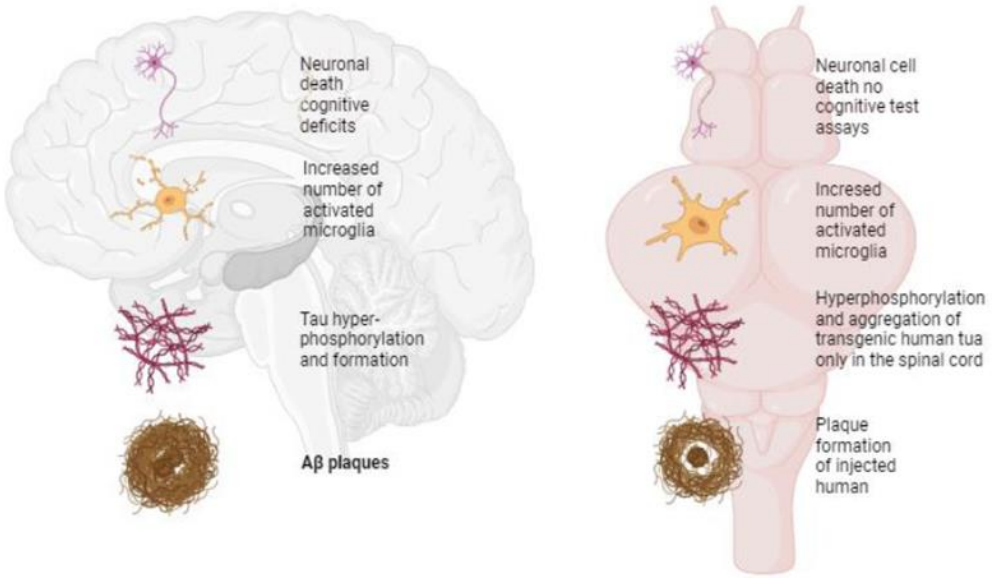

Fig. 14. Mechanism of AD in human and zebrafish

Table 7. Zebrafish models of tauopathy

Method(s)	**Target of tau phosphorylation**	**NFT formation**	**Another phenotype**
MAP-Tau4R mutation	Enolase-2promoter–Neurons	Yes	-
Tau P301L mutation	her4.1promoter–NPCs (with radial glial identity) and neurons	No–Investigated in adult zebrafish	
TauP301Lmutation	PanN: Gal4VP15driver Pan-neuronal	Yes	Increased loss of neural cells. Compromised activity of proteasomes.
TauP301Lmutation	HuC promoter–Neurons	Yes	Increased neuronal cell death.
FTDP-17mutation	GATA-2promoter–Neurons	Yes	Cytoskeletal filament disruption in the cell axon

Table 8. Zebrafish models of Aβ toxicity

Method(s)	**A collection of beta**	**Normal cell death**	**Expanding NSPCs and fostering new neurones**	**Another phenotype**
Injections of human Aβ42 into the ventricles	Yes	Yes	Yes	Producing Aβ sheets within cells. Impairment in conditioning and reduced taught behaviour
Ventricular injections of human TR-Aβ 42	Yes	Yes	Yes	Higher level of microglia activation. Higher levels of synaptic degeneration.
(Ventricular injections) Aβ 1–42	Not reported	Yes	Not Reported	Elevated phosphorylation of tau. impeded evasion of Unpleasant stimuli.
Human A 42 (The Mitfa promoter controls expression in melanophores.	Not reported	Not reported	Not Reported	Unusual pattern and skin pigmentation loss

Zebrafish models of Huntington's disease

In an effort to determine the physiological function of *HTT* (huntingtin), numerous research teams have examined the effects of *HTT* depletion on early zebrafish development. Although the zebrafish homolog of human *HTT* contains 3,121 amino acids and shares approximately 70% similarity with mammalian *HTT*, it only includes four glutamine residues—compared to up to 35 in humans and seven in mice **(Salmi *et al.*, 2019)**.

Similar to the human brain, the zebrafish brain exhibits high levels of *HTT* expression, which is essential for the development of telencephalic progenitor cells and preplacodal tissues. It has been suggested that the zebrafish telencephalon may serve as an anatomical equivalent to the human striatum. Additionally, the progressive olfactory deficits observed in Huntington's disease (HD) patients closely correspond to the loss of

placode-derived tissues in zebrafish, including lateral line sensory neurons and olfactory neurons **(Razali *et al.*, 2021)**.

Focusing on central nervous system (CNS) regions where *HTT* is expressed, researchers investigated how *HTT* deletion affects different brain structures in zebrafish **(Wullimann *et al.*, 2001)**. Inhibition of genes typically expressed in the anterior neural plate (*six1*, *dlx3b*, and *emx3*) led to decreased expression, suggesting that impaired *HTT* mRNA translation disrupts anterior brain development. The anterior neural plate gives rise to key forebrain structures, including telencephalic precursors and preplacodal cells, which are critical for early neural development **(Angom *et al.*, 2024)**.

Further studies have demonstrated that homotypic connections between neuroepithelial cells require *HTT*, as shown using both *HTT*-depleted zebrafish embryos and *HTT*-null mouse embryonic stem cells **(Kaslin *et al.*, 2001)**. Inhibiting *HTT* translation blocks rosette formation and neurulation—effects that are similar to those seen with *N-cadherin* ablation. Notably, the loss of the apical marker ZO-1, which is essential for proper synthesis and distribution, led to the formation of cellular aggregates in the brain ventricles and mispositioned cells in the diencephalic neural tube just 24 hours post-fertilisation **(Fang *et al.*, 2012)**.

Compared to controls, *HTT* morphants displayed altered ventricular spaces and reduced cephalic regions. Interestingly, while *HTT*-depleted cells remained confined to more basal areas, *N-cadherin* mutants showed structural disorganization across the entire forebrain. Additional phenotypes resulting from *HTT* knockdown included reduced haemoglobin levels, lower iron concentrations in the yolk, and increased expression of erythroid and ubiquitous transferrin receptor transcripts **(Oka *et al.*, 2010)**. These findings suggest that *HTT* plays a role in cytosolic iron release from endocytic compartments, functioning downstream of transferrin receptor-mediated iron endocytosis. This aligns with previous studies demonstrating that HD patients often present with iron deficiencies and disruptions in iron metabolism **(Narasimha *et al.*, 2021)**.

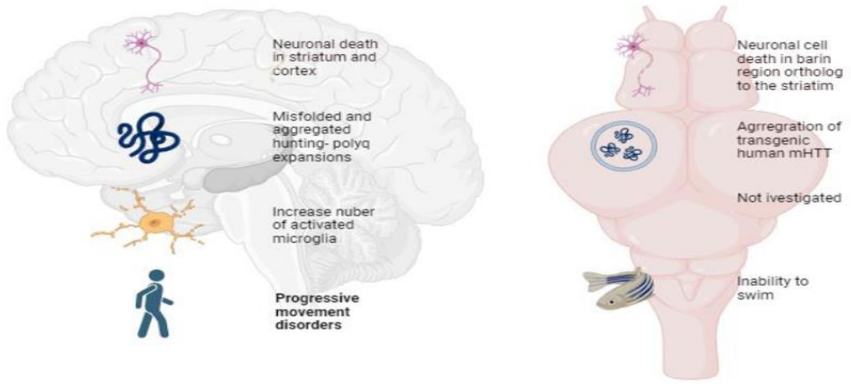

Fig. 15. Mechanism of HTT in human and zebrafish-Huntington disease

Table 9. Zebrafish models of Huntington's pathology

Method	**Neuronal loss**	**Impaired metabolism**	**Motor deficits**	**Another phenotype**
AMO knockdown	Yes	Reduced BDNF levels.	Not reported	Disfigurements based on anatomy. Enhanced death rate
AMO knockdown	Too early	Not reported	Not reported	Problems with brain development. Morphological misshapen bodies.
AMO knockdown	Not reported	IncreasedADAM 10 Activity. Increased Cadherin Cleavage.	Not reported	Impaired brain development.
AMO knockdown	Not reported	Impaired iron metabolism. Reduced haemoglobin production.	Not reported	Developmental retardation and morphological deformities.
4Q,25Q, and102Q POLYQEXPANSION	Yes–Only in 102Q	Not reported	Not reported	Physical abnormalities and an increased risk of death Question number 102.
CRISPR/Cas9deletion	No	No	Not reported	Declining fitness and adult survival

Zebrafish as a model for biomedical research: Brain-to-organ communication

The intricate interactions among various endocrine glands are regulated by the hypothalamic-pituitary-gonadal (HPG) axis, a system that plays a crucial role in initiating and coordinating the physiological changes associated with human puberty. Beyond its role in reproductive function, the HPG axis is vital for proper development and

maturation **(Valera *et al.*, 1994)**. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which travels through the anterior pituitary hypophyseal portal system and binds to receptors on adenohypophysis secretory cells **(Robin *et al.*, 2015)**. In response, these cells release follicle-stimulating hormone (FSH) and luteinizing hormone (LH) into the bloodstream **(Olsen *et al.*, 2012)**. By the time adolescence is reached, these hormonal processes result in the full sexual maturation of the individual **(Giovanni *et al.*, 2010)**.

In Kallmann syndrome (KS), a genetic disorder, puberty fails to occur. Research has implicated mutations in the *WDR11* gene in the pathogenesis of KS **(Harold *et al.*, 2013)**. Expression of *WDR11* in the zebrafish brain suggests that interaction between WDR11 and EMX1 proteins may influence this developmental pathway. In the adult zebrafish brain, injury initiates an inflammatory response followed by neuroregeneration. A combination of leukotriene C4 (LTC4) and cysteinyl leukotriene receptor 1 (Cysltr1) is sufficient to enhance both neurogenesis and cell proliferation. The receptor Cysltr1, which binds LTC4, is upregulated on radial glial cells following traumatic brain injury, linking the inflammatory response to central nervous system recovery mechanisms **(McQuade *et al.*, 2019; Miao *et al.*, 2023)**.

The NADPH oxidase (NOX) family of enzymes is responsible for producing reactive oxygen species (ROS) in response to environmental cues. One such enzyme, dual oxidase (DUOX), functions in the thyroid gland and has been associated with congenital hypothyroidism in humans due to *DUOX2* mutations. In zebrafish, *duox*-deficient models exhibit symptoms such as impaired social behavior, anxiety, growth retardation, and thyroid goiters **(Cass *et al.*, 2017)**. These phenotypes indicate that *duox*-knockout zebrafish may be a valuable model for studying thyroid maturation and related neurological disorders, including intellectual disability and autism.

Zebrafish in autisim and gut-brain axis research

Many children diagnosed with autism spectrum disorder (ASD) experience gastrointestinal issues such as abdominal pain, constipation, and diarrhea. Emerging research on the brain-gut axis suggests that host-associated microbial communities play a role in neurological health. Microbial interactions may influence the host directly through microbial metabolites or indirectly through immune, endocrine, or metabolic pathways. Gut-derived chemical signals are believed to affect brain function and have been associated with symptoms of anxiety, depression, cognitive impairment, and ASD **(Dickson D. *et al.*, 2019)**.

Furthermore, environmental signals—alongside microbial stimuli—modulate internal signaling pathways, such as those that stabilize β-catenin. This stabilization stimulates proliferation of intestinal epithelial cells, underscoring the close connection between gut health and brain development **(Molinuevo *et al.*, 2018)**.

Zebrafish cancer models

Numerous zebrafish-based cancer models have been developed to investigate tumor biology. Due to space constraints, only transgenic tumor models are discussed here. Tables (10, 11) summarize the models, with Table (10) organizing them by affected organ and cancer type. Most models employ transgenic strains that express oncogenes under ubiquitous or tissue-specific promoters. Table (8) details genetic mutants—identified through TILLING or forward genetic screens—that function as tumor suppressors and have since been studied for their role in carcinogenesis.

Two alternative approaches in zebrafish cancer research include the xenotransplantation of human cancer cells into zebrafish hosts and the functional analysis of oncogenes and downstream signaling pathways in various cancer types. These approaches are described in depth in several recent reviews **(Hampel *et al.*, 2020)**.

There is a noticeable bias in the selection of tissue-specific promoters in early transgenic zebrafish cancer models, reflecting the focus of the laboratories involved in their creation **(Karlovich *et al.*, 1998)**. The use of human oncogenes, often combined with fluorescent reporters, has allowed real-time *in vivo* imaging of tumor initiation, progression, and metastasis. These studies have demonstrated that human oncogenes can transform zebrafish cells, validating their cross-species oncogenic potential **(Lumsden *et al.*, 2007)**.

Table 10. Zebrafish cancer model

Organ/ System Cancer type	Strategy	Onset (months)	Main advantages
T-ALL	c-Myc conditional	4	Delayed onset allows propagation of line
T-ALL	c-Myc transgenic	2	childhood leukaemia (CD10+B-ALL) Highly penetrate
CA exocrine pancreas	KRASV12 transgenic	6	ptf1 a promoter. Similar to human disease
Testicular cancer	ENU mutagenesis	7	Highly penetrant. Susceptibility gene still unknown
Melanoma	HRASV12 transgenic	6	MITFA promoter – late onset

Table 11. Cancer predisposition mutants

Responsible protein	Type of mutation	Type of cancer
p53	TILLING mutant	Yes, MPNST
p53	ENU mutant	Yes, sarcoma
Ribosomal proteins	Insertional mutagenesis	Yes, MPNST
Genomic stability genes	mutagenesis ENU	Yes, papilloma and others

Uses in research

Due to their small size, ease of maintenance in large populations, frequent spawning, large egg clutches, transparent and non-adherent eggs, rapid development, and well-characterized genome, zebrafish have become an attractive model organism for biomedical research. Remarkably, approximately 70% of zebrafish genes have human orthologs **(Ghosh *et al.*, 2016)**.

Scientific publications describing zebrafish in research date back several decades **(Bhat *et al.*, 2023)**. Fewer than twenty zebrafish-related studies were published annually until the early 1970s, when usage began to increase. By the mid-1970s, annual publications reached around 40. This figure doubled during the 1980s, reached 200 in the early 1990s, and surged to 1,929 publications by 2012 **(Lele *et al.*, 2002)**.

Initially, zebrafish research was concentrated in developmental biology. However, over the past several decades, zebrafish have increasingly been used in genetics, cell biology, neuroscience, biochemistry, and molecular biology.

Disease and health management in zebrafish

Many pathogenic organisms affecting zebrafish are environmental opportunists that remain asymptomatic unless the fish experience stress, often due to poor husbandry conditions **(Dubey *et al.*, 2023)**. To prevent disease, effective health monitoring and quarantine programs are essential, alongside good water quality, proper housing maintenance, and avoidance of overcrowding **(Rajeshwari *et al.*, 2024)**.

To date, no viruses have been identified as naturally occurring disease agents in zebrafish **(Gautam *et al.*, 2024)**. Among bacterial pathogens, infections caused by *Mycobacterium* species are the most frequently reported **(Dubey *et al.*, 2022)**.

CONCLUSION

Zebrafish offer several advantages over other vertebrate models used for studying human diseases, particularly in large-scale screening of therapeutic compounds and genetic mutants. Their utility in biomedical research extends across diverse fields. With the rapid advancement of disease modeling in zebrafish—alongside transformative technologies such as CRISPR and next-generation sequencing—we are accelerating our understanding of the molecular mechanisms behind hereditary human disorders.These innovations are essential to the future of precision medicine, as they introduce novel

approaches to both diagnosis and treatment. This article focuses primarily on the use of zebrafish disease models in biomedical research, especially in relation to developmental disorders, mental health conditions, and metabolic diseases. In both biomedicine and pharmaceutical development, the zebrafish stands out as an invaluable vertebrate model. The ability to functionally validate Genome-Wide Association Study (GWAS) candidates in zebrafish is greatly enhancing our capacity to identify causative genes and elucidate the molecular pathways involved in the pathogenesis of human genetic diseases. These advancements are made possible through CRISPR-based gene knockout techniques and the integration of extensive datasets generated by next-generation DNA sequencing.

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