# Zebrafish Research For Human Diseases

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***Abstract***

*The Zebrafish has been widely used for experimental purposes both in animal and human health research. Various kinds of diseases of human beings are studied in rodents and in Drosophila. Recently Zebrafish has emerged as the best model for studying human diseases. Zebrafish is a tropical freshwater fish, inhabitant of rivers (Ganges mainly) of the Himalayan region of South Asia. It is a very attractive fish with strips and can be kept in aquarium. It is a bony fish (teleost) that belongs to the family Cyprinidae and class Actinopterygii (ray-finned fishes). Compared to other vertebrates, Zebra fish shows biological advantages such as high fecundity, external fertilization, optical transparency, rapid development, and a highly developed immune system similar to that of humans. There are physiological and genetic similarities between Zebra fish with humans, including the brain, digestive tract, muscles, vasculature, and innate immune system. Zebrafish exhibits the power of regeneration even in adulthood. Zebrafish can be used as a model to study developmental biology, cancer, molecular genetics, toxicology, drug development, toxicogenomics and human biomedical applications. Research in vaccine development has been under trial in Zebrafish. There is enormous potential in Zebra fish that has to be explored as more research is needed in this field. In this paper, the potential of Zebrafish as an experimental model for human diseases has been reviewed.*

***Keywords***

*Zebrafish, Health Research, Molecular Genetics, Toxicogenomics, Biomedical & Regeneration*

## Introduction

Animals like mice and drosophila are used as a model organisms to study many diseases. Zebrafish (Danio rerio) have emerged as an ideal model for the study of various kinds of diseases in humans.

The use of the Zebra fish model (Danio rerio) has exploded in recent decades, despite rodents being the most widely used research model. Compared to other vertebrates, Zebra fish have additional biological advantages such as high fecundity, external fertilization, a short generation time of about 3 months, breeding prodigiously (Hundreds of offspring per female per week) and rapid development. Other qualities such as optical transparency, embryos develop externally and can be readily manipulated genetically and highly developed immune system similar to that of humans. Zebrafish (Danio rerio) is one of the best models for studying developmental biology, cancer, molecular genetics, toxicology, drug development, toxicogenomics and human biomedical applications (Lele, Z& Krone, P.H.,1996). Zebrafish as a biological model was first used in the 1970s because it was simpler than the mouse and genetically easy to manipulate. Zebrafish share many physiological and genetic similarities with humans like the digestive tract, muscles, vasculature, brain and innate immune system. Zebrafish exhibits the power of regeneration even in adulthood. The tail fin of Zebra fish shows regeneration. Early development of the cardiovascular system can be observed in transparent embryos and the process of circulation can be monitored. Research in Vaccine development has been under trial in Zebrafish. In this paper the Zebrafish as an experimental model for human diseases has been documented.

## Aim

- To study the characteristics and behavior of Zebrafish
- To find out the potential of Zebrafish as a successful model
- To explore the possibilities of curing diseases while experimenting with Zebrafish

## Methodology

In this systematic literature review, several published articles by eminent scholars/ authors using databases such as Science Direct, Research Gate, Academia, Google Scholar and Pubmed were searched out and collected. Keywords like Zebrafish research, the potential of Zebrafish, Zebrafish as a model, Human diseases, Genomics, Cardiovascular disease etc. were used carefully. Year by year number of publications related to Zebrafish research has been found to increase. Research articles up to 2017 were analyzed thoroughly.

## Discussion

Compared to other vertebrates, Zebra fish have additional biological advantages such as high fecundity, external fertilization, short generation time, breeding prodigiously (Hundreds of offspring per female per week) and rapid development. Other qualities such

as optical transparency, embryos develop externally and can be readily manipulated genetically making them suitable for experiments. Zebrafish have highly developed immune systems similar to that of humans.

**Characteristics**

- Zebrafish (Daniorerio) is a tropical freshwater fish, inhabitant of rivers (Ganges mainly) of the Himalayan region of South Asia. It can be found in India, Nepal, Bhutan, Pakistan, Bangladesh, and Myanmar.
- Danio rerio, in the wild, includes rivers, small streams, channels, and paddy fields from Myanmar, Pakistan, India, Bangladesh, and Nepal.
- It is a bony fish (teleost) that belongs to the family Cyprinidae and class Actinopterygii (ray-finned fishes).
- Zebrafish are small fish of about 6 cm in length on average. On average Zebrafish have a lifespan of 3 to 5 years.
- Zebrafish are generally silvery white in color with blue horizontal lines on either side of their body extending to the caudal fin. The Upper (dorsal) part and belly part may appear pale yellow in color with a golden appearance (Fig:1).

**Fig: 1 Zebrafish**

**Sexual Dimorphism**

- This fish shows sexual dimorphism, the female Zebrafish tend to be larger in size with a rounded belly whitish in color (Fig:2a).
- Females have lighter body coloring than males.
- Females (adults) have a small genital papilla located in front of the anal fin.
- Male Zebrafish are more slender with a golden appearance on their belly, pelvic and ventral fin. They have darker body coloring than females(Fig: 2b).
- Males have dark yellow tints to the underside of their fins(Chilling, T.F., 2002).

**Fig: 2a: Female zebra fish**

**Fig: 2b: Male zebrafish**

Source : Kimberly Halverson

## Lifecycle

The main stages of the lifecycle of a Zebrafish include:

- Unfertilized egg
- Embryo
- Larval stage
- Juvenile
- Adult A female Zebrafish can produce as many as several hundreds of eggs per spawning (Fig: 3).

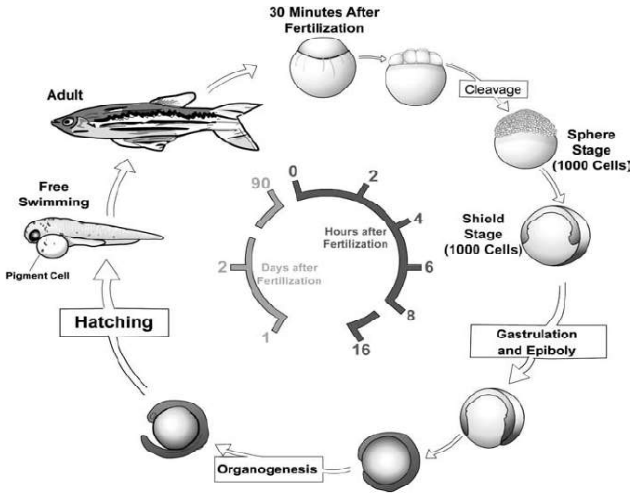



**Fig: 3: Lifecycle**

## Zebrafish as Ideal Model

Main characteristics that make Zebrafish as ideal for research studies in include:

- Enormous egg production the Zebrafish can lay about 200 eggs per week (Brand, M et al.,2002).

- External fertilization
- Embryos can be manipulated from this early stage.
- They can be easily bred and maintained in the laboratory (Fig: 4).

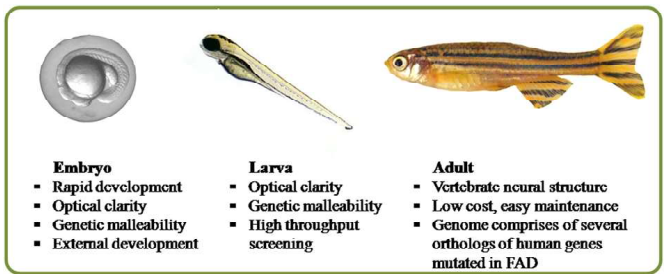



**Fig: 4 Embryo, larva, adult**

- Zebrafish prefers to be housed together in groups and thus can be placed in small space or aquarium.
- The transparent larvae of Zebrafish allow for easier imaging of internal structure and function (Fig: 5)).

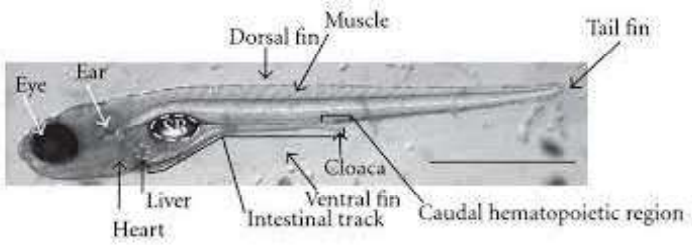



**Fig: 5 : Transparent Larva**

- Their embryos are transparent, gene expression can be monitored in various tissues and organs in vivo without harming the embryos.
- Their genome sizes are approximately 20 to 40 % of the mammalian genome, making them the only vertebrates available for large-scale mutagenesis.
- Their maturation time takes only 2 to 3 months, which is relatively less laborious and time-saving for generating transgenic lines.

**Human Diseases**

- A number of human diseases have been studied in Zebrafish, for example, Duchenne muscular dystrophy, Human melanoma, and Human neurodegenerative diseases.
- Most of the tissues and organs of Zebrafish are similar to humans except for lungs, prostrate and mammary glands(Fig:6). There are physiological and genetic

similarities between Zebra fish with humans, including the brain, digestive tract, muscles, vasculature, and innate immune system(Gore, et al .,2012; Zhao, et al ., 2015).

- The haploid Zebrafish genome has 25 chromosomes, whereas the Human genome has 23 pairs of chromosomes(Postlethwait, et al., 2000) (Fig: 7). These chromosomes contain about 1.7 X $10^9$ base pairs of DNA, about half the mammalian genome size. (Fig: 8).

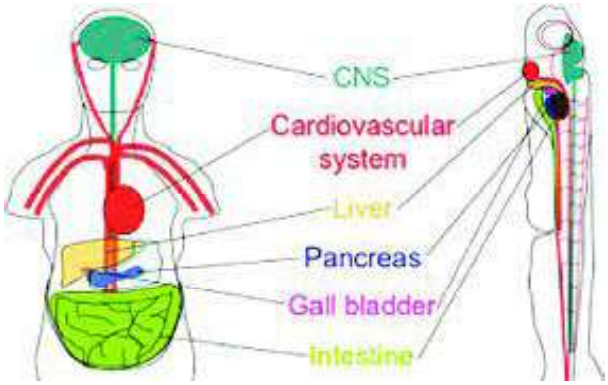
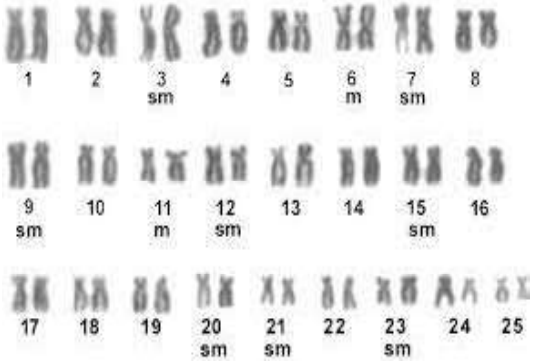



**Fig: 6: Organs of Humans&Zebrafish**



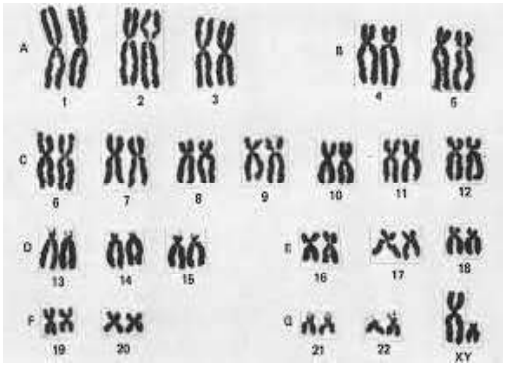



**Fig:7:Genome of Zebrafish** **Fig: 8: Genome of Human**

The Zebrafish genome contains 154 psuedogenes (non functional)only as compared to 13,000 psuedogenes in humans. Almost 70 percent of protein-coding human genes are related to genes found in the Zebrafish (Daniorerio) (Santoriello et al., 2012). About 84 percent of genes known to be associated with human disease have a counterpart in Zebrafish(Fig:9).

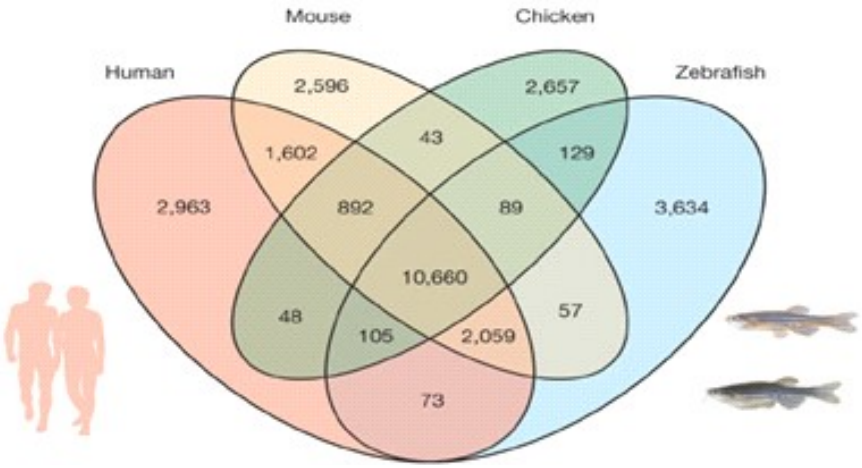



**Fig:9 (Orthologue genes shared between the zebrafish, human, mouse and chicken genome) (Kerstin Howe, et al.,2013).**

About 82% of human genes concerned with genetic-related diseases have their orthologs in the Zebrafish model (Howe et al., 2013). Scientists have successfully developed nearly 15,000 mutations in Zebrafish models. By understanding both the similarities and the differences between the human genome and the Zebrafish genome we can understand the behavior of mutations in the genome that results in disease which will be helpful in the exact treatment of the disease.

Both humans and the Zebrafish are vertebrates that show similarities in many major organs and tissues. There is resemblance between the muscle, blood, kidney and eyes of Zebrafish with human systems. Many other mutants showing phenotypic similarities to human diseases like neurological disorders, hema-tological disorders, muscle disorders, cancer and cardiovascular diseases have been studied and identified (Gama et al., 2012; Berman et al., Liu,2011; 2012; Lin,2012 ).

Many diseases have been studied in this fish. The first human disease identified in Zebrafish was a blood disorder that involved a specific defect in hemoglobin production because of the ALAS2 mutated gene (Chitramuthu, B.P., 2013). Cardiovascular diseases of human beings have been studied in Zebrafish successfully, as the heart of Zebrafishis in primitive form that develops completely within 48 h post-fertilization (HPF). The cardiac development can be easily observed in the transgenic line possessing an FP-tagged heart.The embryos of Zebrafish with a defective cardiovascular system can grow in water by taking dissolved oxygen. While studying the early development of the cardiovascular system of Zebrafish we can observe the flow of blood (Chavez, M.N., et al., 2016). Genes involved

in heart development can be studiedby mutation method (Scheer, N., Campos-Ortega, J.A.,1999). Heart rate in Zebrafish resembles to human heart rate, even ECG patterns of adult Zebrafish show P, QRS and T waves, with a QT duration (Milan, et al., 2006), There is similarity in electrophysiology of the adult Zebrafish heart to that of human (Lin, et al., 2018; Liu et al., 2016; Nemtsas, et al., 2010).

Renal diseases Polycystic kidney disease (PKD), nephronophthisis, acute kidney injury (AKI), and ciliopathies have been studied in Zebrafish (Swanhart, L.M., 2011).In the case of renal cystitis, Zebrafish can be used as potential therapeutic agents (Tobin, J.L., Beales& P.L., 2008) . Regulation of glucose metabolism by insulin in Zebrafish also shows the structure and functional similarity to those in mammals. Zebrafish are exploited for carcinogenic treatment, transplantation of mammalian tumor cells, and transgenic regulations (Mizgirev.I&Revskoy. S.,2010).The Zebrafish can be used to study melanoma development, progression, drug screening, and treatment. Zebrafish research has been useful in detecting the causal gene in human muscular dystrophy and in understanding of skin cancer. We can recognize the key molecules responsible for the development of cutaneous squamous cell carcinoma (cSCC) and head and neck squamous cell carcinoma (HNSCC) (Shin, Y.S., 2016).

Many neurological disorders such as anxiety and post-traumatic stress disorder can be studied in the Zebrafish model. This fish is a suitable organism for studying cognitive deficits of depression (Kalueff, et al., 2014; Stewart, et al ., 2014 ). Depressive disorders and social aspects of depression can experiment on in Zebrafish (Tele, Karnik, I., Gerlai, R.,2012).

The regeneration process can be studied in this model as Zebrafish have been shown to regenerate lost fins as well as organs such as the retina, spinal cord, and the heart. Full regeneration of the caudal fin used to take place within a couple of weeks (Azevedo, A.,2011). Zebrafish have the ability to repair heart muscle, if part of their heart is removed they can grow it in a few weeks. They regenerate tissue and recover and regain normal functions in these organs, which may help to deal with human heart diseases and potentially prolong life.

We can predict and test the toxicity of drugs for mammals in Zebrafish embryos. The Zebrafish is being used as a model to study drug and chemical toxicology (Spitsbergen &Kentm, 2003; Rubinstein,2006). Drugs like gentamicin, cisplatin, vinblastine, quinine, neomycin, doxorubicin, dexamethasone, cyclosporin A, caffeine, camptothecin, MPA, fluorouracil, etc. have been tested. (Zhang, C.,2003; Langheinrich, U., 2002). We get the information about the disease in Zebrafish and test new drugs on the fish that could be applied to human beings. Even the trial for vaccination is on cards for the human immune system.

**Conclusion**

Zebrafish show biological advantages such as high fecundity, external fertilization, optical transparency, rapid development, and a highly developed immune system similar to

that of humans. There are physiological and genetic similarities between Zebra fish with humans, including the brain, digestive tract, muscles, vasculature, and innate immune system. Zebrafish exhibits the power of regeneration even in adulthood. Zebrafish can be used as a model to study developmental biology, cancer, molecular genetics, toxicology, drug development, toxicogenomics and human biomedical applications Research in vaccine development has been under trial in Zebrafish. Zebrafish have some disadvantages because they are not mammals and lack mammalian organs (e.g. lungs and mammary glands etc.). Variation can be observed in some of the diseases caused by orthologous genes. The genome of Zebrafish contains many gene duplications resulting in subfunctionalization and neofunctionalization. Even then similarities are more between Zebrafish and humans, that's why scientists are working on the Zebrafish model for studying diseases. There is enormous potential in Zebra fish that has to be explored as more research is needed in this field.

**References**


1. Azevedo, A., Grotek, B., Jacinto, A., Weidinger, G., Saude, L., Karl, M. (2011). "The regenerative capacity of the Zebrafish caudal fin is not affected by repeated amputations". PLoS One.;6:e22820. DOI: 10.1371/journal.pone.0022820.
2. Berman, J., Payne, E., Hall, C. (2012). "The zebrafish as a tool to study hematopoiesis, human blood diseases, and immune function". *Journal of Advances in Hematology. 2.* Article ID 425345. https://doi. org/10.1155/2012/425345.
3. Brand, M., Granato, M., Nusslein- Volhard, C. (2002). "Keeping and raising zebrafish.: A Practical Approach". Oxford University Press: Oxford. **Pg. 7-37.**
4. Chakraborty, C., Hsu, C.H., Wen, Z.H., Lin, C.S., Agoramoorthy, G. (2009). "Zebrafish: A complete animal model for in vivo drug discovery and development". *Journal of Current Drug Metabolism. 10.* **Pg. 116-124.**
5. Chavez, M.N, Aedo G, Fierro F.A., Allende, M.L, Egana, J.T. (2016). "Zebrafish as an emerging model organism to study angiogenesis in development and regeneration". *Journal of Frontiers in Physiology. 7.* **Pg. 56.** DOI: 10.3389/ fphys.2016.00056.
6. Chilling, T.F. (2002). "Zebrafish: A practical approach". Oxford University Press: New York, NY.
7. Chitramuthu, B.P. (2013). "Modeling human disease and development in zebrafish". Journal of Human Genetics Embryology. 3. e108. DOI: 10.4172/2161-0436.1000e108 electrocardiogram platform for cardiovascular drug assay and electrophysiology research in adult Zebrafish. Scientific Reports. 8. 15986.
8. Gama Sosa, M.A., DeGasperi, R., Elder, G.A. (2012)."Modeling human neurodegenerative diseases in transgenic systems". *Journal of Human Genetics. 131.* **Pg. 535-563.**

9. Gore, A.V., Monzo, K., Cha, Y.R., Pan, W., Weinstein, B.M. (2012). "Vascular development in the Zebrafish". *Cold Spring Harbor Perspectives in Medicine. 2(5).* a006684. https://doi.org/10.1038/s41598-018-33577-7.
10. Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphrey, S., McLaren, K., Matthews, L., et al.(2013). "The zebrafish reference genome sequence and its relationship to the human genome". *Journal of Nature 496(7446).* **Pg. 498–503.**
11. Kalueff, A.V., Echevarria, D.J., Stewart, A.M. (2014)." Gaining translational momentum: more Zebrafish models for neuroscience research". *Journal ofProgNeuropsychopharmacol Biology Psychiatry. 55.* **Pg. 1–6.**
12. Karnik, I., Gerlai, R. (2012). "Can Zebrafish learn spatial tasks? An empirical analysis of place and single CS-US associative learning". *Journal of Behaviour Brain Research. 233.* **Pg. 415–21.**
13. Langheinrich, U., Hennen, E., Stott, G., Vacun, G. (2002). "Zebrafish as a model organism for the identification and characterization of drugs and genes affecting p53 signaling". *Journal of Current Biology. 12.* **Pg. 2023-2028.**
14. Lele, Z., Krone, P.H. (1996). "The Zebrafish as a model system in developmental, toxicological and transgenic research". *Journal of Biotechnology Advances.. 14(1).* **Pg. 57-72.**
15. Lin, M.H., Chou, H.C., Chen, Y.F., Liu, W., Lee, C.C., Liu, L.Y., Chuang, Y.J. (2018). "Development of a rapid and economic in vivo electrocardiogram platform for cardiovascular drug assay and electrophysiology research in adult zebrafish". *Journal of Scientific Reports. 8.* 15986. https://doi.org/10.1038/s41598-018-33577-7.
16. Lin, Y.Y. (2012). "Muscle diseases in the zebrafish". *Journal of Neuromuscular Disorders. 22.* **Pg. 673-684.**
17. Liu, S & Leach, S.D. (2011). "Zebrafish models for cancer". *Journal of Annual Review of Pathology. 6.* **Pg. 71-93.**
18. Milan, D.J., Jones, I.L., Ellinor, P.T., MacRae, C.A. (2006). "In vivo recording of adult Zebrafish electrocardiogram and assessment of drug-induced QT prolongation". *American Journal of Physiology. Heart and Circulatory Physiology. 291.* H269–H273.
19. Mizgirev, I., Revskoy, S. (2010). "Generation of clonal Zebrafish lines and transplantable hepatic tumors". *Journal of Nature Protocols. 5(3).* **Pg. 383-394**.
20. Nemtsas, P., Wettwer, E., Christ, T., Weidinger, G., Ravens, U. (2010). "Adult Zebrafish heart as a model for human heart? An electrophysiological study". *Journal of Molecular and Cellular Cardiology. 48.* **Pg. 161–171.**

21. Postlethwait, J.H., Woods, I.G., Ngo-Hazelett, P., Yan, Y.L., Kelly, P.D., Chu, F. (2000). "Zebrafish comparative genomics and the origins of vertebrate chromosomes". *Journal of Genome Research. 10.* **Pg. 1890–1902.** DOI: 10.1101/gr.164800.
22. Rubinstein, A.L., (2006). "Zebrafish assays for drug toxicity screening". *Expert Opinion on Drug Metabolism & Toxicology. 2(2).* **Pg. 231-240.**
23. Santoriello, C., Zon, L.I. (2012). Hooked! "Modeling human disease in Zebrafish". *Journal of Clinical Investigation. 122(7).* **Pg. 2337-2343.**
24. Scheer, N., Campos-Ortega, J.A. (1999). "Use of the GAL4-UAS technique for targeted gene expression in Zebrafish". *Journal of Mech Dev. 80.* **Pg. 153–8.**
25. Shin, Y.S., Cha, H.Y., Lee, B.S., Kang, S.U., Hwang, H.S., Kwon, H.C. (2016). "Anti-cancer effect of luminacin, a marine microbial extract, in head and neck squamous cell carcinoma progression via autophagic cell death". *Journal of Cancer Research and Treatment. 48*. **Pg. 738-752.**
26. Spitsbergen, J.M., Kent, M.L. (2003). "The state of the art of the zebrafish model for toxicology and toxicologic pathology research, Advantages and current limitations". *Journal of Toxicologic Pathology. 31.* **Pg. 62-87.**
27. Stewart, A.M., Braubach, O., Spitsbergen, J., Gerlai, R., Kalueff, A.V. (2014 ). "Zebrafish models for translational neuroscience research: from tank to bedside". *Journal of trends Neuroscience. 37.* **Pg. 264–78.**
28. Swanhart, L.M.,Cosentino, C.C., Diep, C.Q., Davidson, A.J., Caestecker, M. de., Hukriede, N.A. (2011). "Zebrafish kidney development: basic science to translational research" Birth Defects Res. *C. Embryo Today. Vol. 93(2)*. **Pg. 141-156.**
29. Teles, M.C., Dahlbom, S.J., Winberg, S., Oliveira, R.F. (2013). "Social modulation of brain monoamine levels in Zebrafish". *Journal of Behaviour Brain Research. 253.* **Pg. 17–24.**
30. Tobin, J.L., Beales, P.L. (2008). "Restoration of renal function in Zebrafish models of ciliopathies". *Journal of Pediatrics Nephrology. Vol. 23(11)*. **Pg. 2095-2099**.
31. Zhang, C., Willett, C., Fremgen, T. (2003). "Zebrafish: An animal model for toxicological studies". Journal of Current Protocols in Toxicology. DOI: 10.1002/0471140856.tx0107s17.
32. Zhao, S., Huang, J., Ye, J. (2015). "A fresh look at Zebrafish from the perspective of cancer research". *Journal of Experimental & Clinical Cancer Research. 34.* **Pg. 80.** DOI: 10.1186/s13046-015-0196-8.