



***DEVELOPING A MODEL FOR STUDYING COMPLEX BRAIN FUNCTION
USING ZEBRAFISH (*Danio rerio*) AND CAVEFISH (*Astyanax mexicanus*)***

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**DEVELOPING A MODEL FOR STUDYING COMPLEX BRAIN FUNCTION
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It is hereby certified that we have read this project paper entitled “Developing a model for studying complex brain function using Zebrafish (*Danio rerio*) and Cavefish (*Astyanax mexicanus*), by Nabila Irqin Binti Ahmad Zaki and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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DEDICATION

I dedicate this thesis to:

My dearest family:

Nik Musalina Nik Mustapha

Ahmad Zaki Alias

Tessa Najiha

Ahmad Nifail

Ahmad Nidzwan

Nayli Idzwati

Jannatul Firdausi

Nur Shahila

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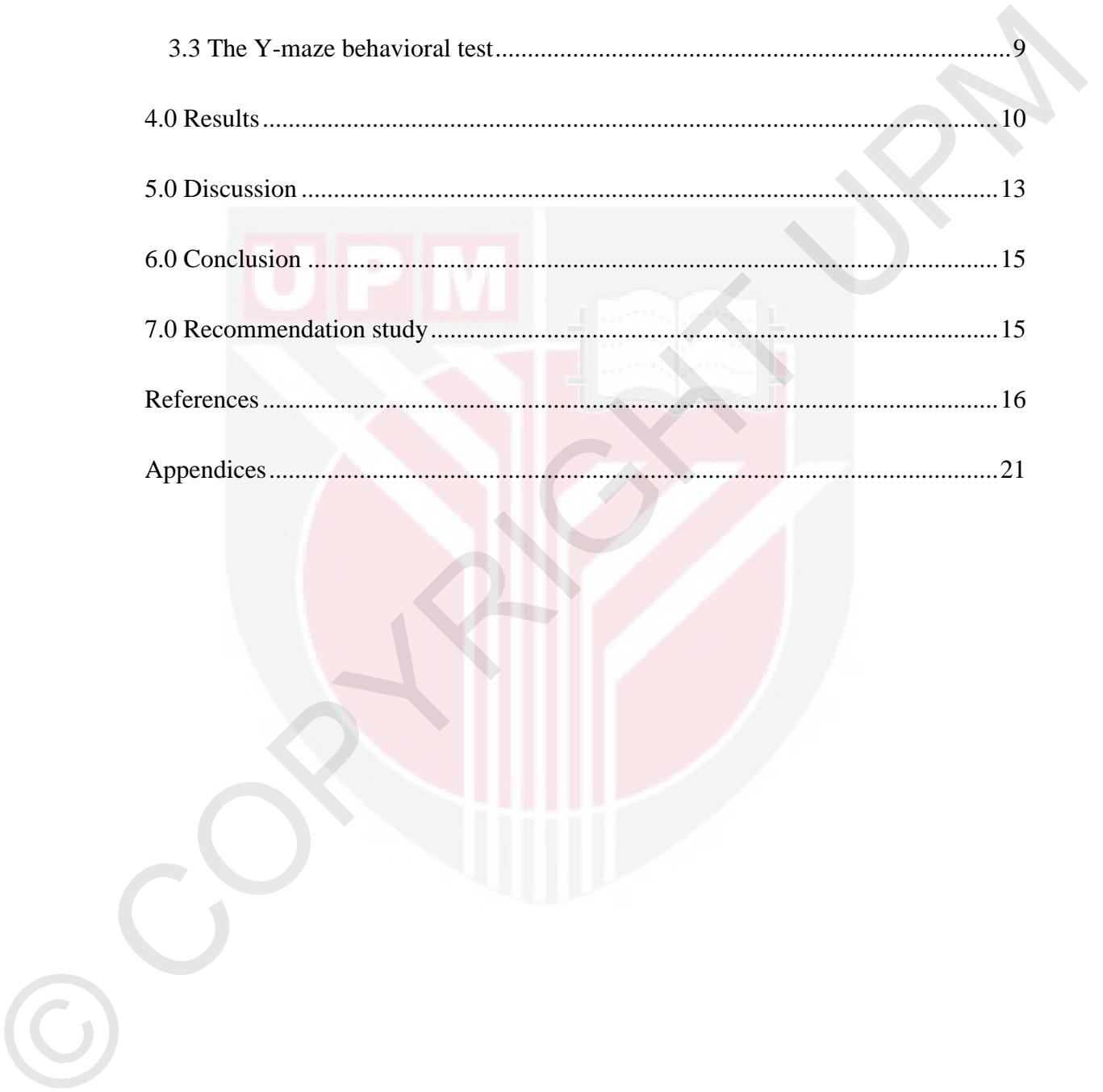
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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kursus VPD 4999 – Projek.

Membina model untuk mengkaji fungsi kompleks otak menggunakan Zebrafish

(Danio rerio) dan Cavefish (Astyanax mexicanus).

Oleh

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Zebrafish (*Danio rerio*) telah digunakan selama beberapa dekad sebagai organisma model untuk kajian dalam biologi pertumbuhan.. Malah, beberapa persamaan dan kepentingan mekanisme gen pertumbuhan telah dikenal pasti dalam zebrafish yang sama seperti mamalia. Penyelidikan dalam fungsi kognitif dan memori semakin meningkat dalam haiwan makmal sepanjang dekad lepas, walaubagaimanapun kepentingan dalam ciri-ciri tingkah laku zebrafish adalah masih terhad. Berbeza dengan zebrafish, fungsi pembelajaran dan ingatan untuk cavefish (*Astyanax mexicanus*) adalah berdasarkan kepada sistem mekanosensori dan sisi tepi badan yang sangat sensitif kepada turun naik pergerakan air dan daya tekanan. Kajian evolusi yang telah dilakukan ke atas ikan ini melaporkan bahawa mereka mempunyai deria rasa yang lebih baik dengan mempunyai sensori di seluruh kepalanya yang membantu mereka mencari makanan dengan lebih cepat dalam

kegelapan. Di samping itu, sistem sisi tepi badan membantu mereka untuk melihat persekitaran mereka. Walaupun tikus secara tradisinya telah digunakan untuk mengkaji fungsi kognitif dan memori, zebrafish semakin popular sebagai model yang sangat baik untuk melengkapkan penyelidikan neurosains. Oleh itu, tujuan kajian ini adalah untuk membandingkan fungsi kognitif dan memori kedua-dua watak ikan, zebrafish dan cavefish menggunakan ujian Y-maze. Hasil kajian menunjukkan bahawa tidak terdapat perbezaan yang signifikan di dalam bilangan kemasukan dan tempoh masa yang diluahkan oleh cavefish di dalam lengan Y - maze. Walaubagaimanapun, tempoh masa yang diluahkan dan bilangan kali masuk dalam lengan novel adalah lebih tinggi daripada zebrafish. Oleh itu, fungsi kognitif cavefish adalah berdasarkan ciri-ciri mereka dan zebrafish itu berdasarkan bentuk visual Y- maze.

Kata kunci: Fungsi kognitif, Zebrafish, Cavefish, Y-maze

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Project **Developing a model for studying complex brain function using Zebrafish (*Danio rerio*) and Cavefish (*Astyanax mexicanus*).**

By

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The zebrafish (*Danio rerio*) has been used as a model organism for studies in developmental biology. In fact, several common and important developmental genes mechanisms have been identified in zebrafish, which are similar in mammals. There is also growing research in the cognitive and memory functions in the laboratory animals over last decade, however the interests in the behavioral features of zebrafish are limited. In contrast to zebrafish, the cognitive function for cavefish (*Astyanax mexicanus*) is based on mechanosensory systems and their lateral line, which is highly sensitive to fluctuating water movement and pressure. Many evolution researches have been done to these cavefish reported that they have better olfactory sense by having taste buds all over its head which help them find food more quickly in complete darkness. In addition, the mechanosensory lateral line system helps them to perceive their environment. While rodents have traditionally been used to study cognitive and memory functions, the zebrafish are gaining

popularity as an excellent vertebrate model to complement current translational neuroscience research. Thus, the study purpose in this application is to compare the learning and memory functions of both character of fish, zebrafish and cavefish using the Y-maze task. Results showed that there is no significant differences on cavefish enter and spent time in the Y-maze arms. However, time spent and numbers of entries in the novel arm were significantly higher in the zebrafish. Thus, the cognitive function of cavefish is based on their characteristics and the zebrafish is based on the visual queue of the Y-maze task.

Keywords: Cognitive function, Zebrafish, Cavefish, Y-maze

1.0 Introduction

In animal behavior, cognitive function may apply to assess the ability of the animal to be aware and responsive towards its environment which can happen through thought and experience. Learning is remembering associations. While memory is the capacity to recall previously experienced sensations, information, data and ideas. This is essential for the process of learning by animals (Blood *et al.*, 2007).

Fish has been famous recently as a model for cognitive science studies. A study states that fish are more intelligent than they appear. In many areas, such as memory, their cognitive powers match or exceed those of 'higher' vertebrates including non-human primates (Brown, 2004). Several fish species are capable of learning complex spatial relationships and forming mental maps (Odling-Smee and Braithwaite, 2003) and integrate experiences which enable the fish to generate appropriate avoidance responses (Portavella *et al.*, 2004). Fish behaviour in mazes reveals that they possess spatial memory and visual discrimination (Chung, 2008).

Zebrafish has been a common and useful model organism for studies of vertebrate development and gene function. The zebrafish has been used for decades as a model organism for studies in developmental biology such as in neuroscience (Jörgens *et al.*, 2012) and diabetic (Intine *et al.*, 2013). Comparing to other commonly used laboratory animals (mice and rat), zebrafish have the same metabolic function with less cost of maintenance, easier to breed and faster result obtained. This makes zebrafish a perfect model for this comparison cognitive study.

The cavefish is a unique fish because it does not have visual and even eyes. This fish moves and find their way around by means of their lateral lines, which are

highly sensitive to fluctuating water pressure (Yoshizawa *et al.*, 2012). This cavefish have been a powerful subject for scientists studying evolution. Studies state that the positive genetic benefits of losing their eyes are by not developing eyes they have more energy for growth and reproduction, there remains less chance of accidental damage and infection, since the previously useless and exposed organ is sealed with a flap of protective skin and the lack of eyes disables the body clock, which is controlled by periods of light and dark, conserving energy (Retaux and Casane, 2013). The swimming behaviour of the cavefish has also been studied to see how the spatial parameters encoded in the spatial map of the cavefish. Thus, this could indicate that cavefish have their own cognition in their blind condition making it a perfect model for comparison of the cognitive function with the zebrafish.

1.1 Objective

To compare learning and memory function between Zebrafish and Cavefish using Y-maze test.

1.2 Hypothesis

There are differences in the learning and memory function between the Zebrafish and the Cavefish.

1.3 Justification

In the meantime, zebrafish has long been a premier model organism to study vertebrate development. However, there are little scientific reports regarding cognitive function in the zebrafish (Cognato *et al.*, 2012). The Cavefish is well

References

- Al-Imari, L., Gerlai, R. (2008). Conspecifics as reward in associative learning tasks for zebrafish (*Danio rerio*). *Behavioural Brain Research*, 189, 216–219.
- Bilotta J., Risner M.L., Davis E.C., Haggbloom S.J. (2005) Assessing appetitive choice discrimination learning in zebrafish. *Zebrafish*. 2:259-268.
- Blank, M., Guerim, L. D., Cordeiro, R. F., Vianna, M. R. (2009). A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent longterm memory. *Neurobiology of Learning and Memory*. 92:529–534.
- Braubach, O. R., Wood, H. D., Gadbois, S., Fine, A., Croll, R. P. (2009). Olfactory conditioning in the zebrafish (*Danio rerio*). *Behavioural Brain Research*. 198:190–198.
- Brown C. (2004) Animal minds: Not just a pretty face. *New scientist*. 2451: 42-43.
- Burt de Perera T. (2004) Spatial parameters encoded in the spatial map of the blind Mexican cave fish, *Astyanax fasciatus*. *Animal Behaviour*. 68:291-295.
- Chung S. (2008) Appropriate maze methodology to study learning in fish. *Journal of Undergraduate life sciences*. 2(1):116-133.
- Cognato G.P., Bortolotto J.W., Blazina A.R. and Christoff R.R. (2012), Y-Maze memory task in zebrafish (*Danio Rerio*): The role of glutamergic and cholinergic systems on the acquisition and consolidation periods. *Neurobiology of Learning and Memory*. 98:321-328.
- Dahm R. (2006) learning from small fry: the zebrafish as a genetic model organism for aquaculture fish species. *Marine biotechnology (New York)*. 8(4):329-45.

Darwin C (1859) *On the Origins of Species by Means of Natural Selection*. London, Murray.

Dooley K, Zon L.I., (2000) Zebrafish: a model system for the study of human disease. *Current Opinion in Genetics and Development*. 10:252-6.

Gestri G., Link B.A., Neuhauss S.C (2012) The visual system zebrafish and its use to model human ocular diseases. *Developmental Neurobiology*. 72(3):302-27.

Guo S. (2001) Linking genes to brain, behaviour and neurological disease: What we can learn from zebrafish. *Genes, Brain and Behaviour*. 3:63-74.

Hafandi A., Begg D.P., Premaratna S.D., Sinclair A.J., Jois M., Weisinger R. (2014) Dietary Repletion with ω 3 Fatty Acid or with COX Inhibition Reverses Cognitive Effects in F3 ω 3 Fatty-Acid-Deficient Mice. *Comparative Medicine*. 64(2):106-109.

Helfman G., Collette B., Facey D. (2009) *The Diversity of Fishes: Biology, Evolution and Ecology*, Blackwell Publishing.

Hibi M, Shimizu T. (2012) Development of the cerebellum and cerebellar neural circuits. *Developmental Neurobiology* 72:282–301.

Intine R.V, Olsen A.S., Sarras M.P. (2013) Azebrafish model of diabetes mellitus and metabolic memory. *Journal of visualized experiments*. (72):e50232.

Ingham P.W. (2009) The power of the zebrafish for disease analysis. *Human Molecular Genetics*. 18:R107-12.

Jeffery W.R. (2009) Regressive evolution in *Astyanax* cavefish. *Annual Review of Genetics*. 43:25-47

Jörgens K, Hillebrands JL, Hammes HP, Kroll J. (2012), *ExpClinEndocrinol*

Diabetes. 120(4): 186-7

Kandel E.R (2004) The molecular biology memory storage: A dialog between genes and synapses. *Bioscience Report*. 24:475-522.

Keller E.T., Murtha J.M. (2004) The use of mature Zebrafish (*Danio rerio*) as a model for human aging and disease. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*. 138:335-41.

Nasevicius, A., Ekker, S. C. (2001). The zebrafish as a novel system for functional genomics and therapeutic development applications. *Current Opinion in Molecular Therapeutics*, 3, 224–228.

Nelson J.S. (2006) *Fishes of the world*, Fourth Edition. John Wiley and Sons Inc.

Odling-Smee L., Braithwaite V. A. (2003). The role of learning in fish orientation. *Fish and Fisheries*. 4: 235–246.

Onaolapo OJ, Onaolapo AY, Mosaku TJ, Akanji OO, Abiodun OR. 2012. Elevated plus maze and y-maze behavioral effects of subchronic, oral low dose monosodium glutamate in Swiss albino mice. *J Pharmacy BiolSc* 3:21–27.

Pather, S., Gerlai, R. (2009). Shuttle box learning in zebrafish (*Danio rerio*). *Behavioural Brain Research*, 196, 323–327.

Protas M., Tabansky I., Conrad M., Gross J.B., Vidal O., Tabin C.J., Borowsky R. (2008) Multi-trait evolution in a cavefish, *Astyanax mexicanus*. *Evolution Development*. 10:196-209.

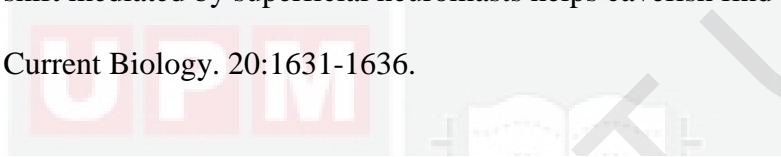
Portavella M., Torres B., Salas C. (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *The Journal of Neuroscience*. 24: 2342–2335.

- Retaux S., Casane D. (2013) Evolution of eye development in the darkness of caves: adaptation, drift or both? *Evolutionary developmental biology*. 4:26.
- Schacter D.L., Addis D.R. (2009) Remembering the past to imagine the future: A cognitive neuroscience perspective. *Military Psychology*. 21:S108-S112.
- Shettleworth S.J. (2001) Animal cognition and animal behaviour. *Animal Behaviour*. 61:277-286.
- Sison, M., & Gerlai, R. (2011). Associative learning performance is impaired in zebrafish (*Danio rerio*) by the NMDA-R antagonist MK-801. *Neurobiology of Learning and Memory*, 96, 230–237.
- Spence R., Gerlach G., Lawrence C., Smith C. (2007) The behaviour and ecology of the zebrafish, *Danio rerio*. *Biological reviews of the Cambridge Philosophical Society*. 83(1): 13-34.
- Teyke, T. 1985. Collision with and avoidance of obstacles by blind cave fish *Anoptichthys jordani* (Characidae). *Journal of Comparative Physiology*, 157, 837e843.
- Teyke, T. 1988. Flow-field, swimming velocity and boundary layer: parameters which affect the stimulus for the lateral line organ in fish. *Journal of Comparative Physiology A*, 163, 53e61.
- Teyke, T. 1989. Learning and remembering the environment in blind cave fish *Anoptichthys jordani*. *Journal of Comparative Physiology A*, 164, 655e662.
- Wang Y., Li S., Hui-ping D., Shen L., Yi-yuan T. (2009) Differential impairment of spatial and non-spatial cognition in a mouse model of brain aging. *Life Sciences*. 85:127-135.

Wilkens H. (2010) Genes, modules and the evolution of cavefish. *Heredity*. 105:413-422.

Yamamoto Y., Stock D.W., Jeffery W.R. (2004) Hedgehog signalling controls eye degeneration in blind cavefish. *Nature*. 431:844-847

Yoshizawa M., Goricki S., Soares D., Jeffery W.R. (2010) evolution of behavioural shift mediated by superficial neuromasts helps cavefish find food in darkness. *Current Biology*. 20:1631-1636.



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