UNIVERSITI PUTRA MALAYSIA

DEVELOPING A MODEL FOR STUDYING COMPLEX BRAIN FUNCTION USING ZEBRAFISH (DANIO RERIO) AND CAVEFISH (ASTYANAX MEXICANUS).

NABILA IRQIN BINTI AHMAD ZAKI

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

NABILA IRQIN BINTI AHMAD ZAKI

A project submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan

MARCH, 2015

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.

DR. HAFANDI AHMAD

BSc. (UPM), MSc (UPM), PhD (LA TROBE)

Senior Lecturer,

Faculty of Veterinary Medicine,

Universiti Putra Malaysia

(Supervisor)

ASSOC. PROF. DR. HASSAN HJ. MOHD DAUD

DVM (UPM), MSc (STIRLING), PhD (KINGSTON)

Faculty of Veterinary Medicine,

Universiti Putra Malaysia

(Co-supervisor)

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

My supervisor

DR. HAFANDI AHMAD

My co-supervisor

ASSOC. PROF. DR. HASSAN HJ. MOHD DAUD

ACKNOWLEDGEMENTS

Alhamdulillah.

I would never have been able to finish my dissertation without the guidance of my Final Year Project committee members, helps from friends and support from my family.

I would like to express my deepest gratitude to my supervisor, Dr. Hafandi Ahmad for his continuous support and willingness to give me the opportunity to work on this amazing project. Besides that, to my co-supervisor, Assoc. Prof. Dr. Hassan Hj. Mohd Daud for the advice and feedback he gave to me this entire project. My thanks also go to all the Physiology Laboratory staffs for their helps and guidance.

I would like to give my sincere credits to my beloved parents and family members, who have been motivating me spiritually throughout my life.

In addition, I would like to thank my FYP mate, Nur Afiqah Yazid for lending a hand for me during the semester. A special thank you to my lovely housemates, the Rimba 71110 residents, Afiqah, Izzaty, Syahirah, Radiatun Nadwah, Raihan, Diana and Nadia for the love and supports throughout the semesters.

Last but not least, thanks to Faculty of Veterinary Medicine, UPM's lecturer and classmates of DVM 5 2015 that have help me directly and indirectly in this project.

CONTENTS

LIST OF FIGURE

LIST OF APPENDICES

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

NABILA IRQIN BINTI AHMAD ZAKI

2015

Penyelia: Dr.Hafandi Ahmad, PhD

Penyelia bersama: Prof. Madya. Dr. Hassan Hj. Mohd Daud

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 diluangkan oleh cavefish di dalam lengan Y - maze. Walaubagaimanapun ,tempoh masa yang diluangkan 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: Funsi 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

NABILA IRQIN BINTI AHMAD ZAKI

2015

Supervisor: Dr. Hafandi Ahmad, PhD

Co-supervisor: Assoc. Prof. Dr. Hassan Hj. Mohd Daud

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 diebetic (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 known to lack of visual systems, thus this study is to see how their cognition works in the blind state.

2.0 Literature review

2.1 Cognitive function

Learning and memory are closely related in cognitive function concepts. Learning is the acquisition of skill or knowledge (Shacter *et al.*, 2009, 2011) and memory is the process in which information is encoded, stored, and retrieved (Shacter *et al.*, 2010). In animals, cognition, broadly defined, include all in ways which animal takes in information through the senses, process, retain and decide to act in. This cognitive process such as perception, learning, memories and decision making play an important role in mate choice, foraging and many other behaviors (Shettleworth, 2001). 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).

2.2 Cavefish

The cavefish (*Astyanax mexicanus*) is a freshwater fish of the family Characidae of the order Characiformes (Nelson, 2006). These forms have lost their sight and even their eyes. These fish can still, however, find their way around by means of their lateral lines, which are highly sensitive to fluctuating water pressure (Yoshizawa *et al.*, 2012). This species are mainly found native to the Nearctic ecozone, living in underground caves and caverns where the lack of light and predators has made vision un-necessary. Eyes are not the only feature this fish lacks, this unique fish is also without pigmentation, taking on a pink hue from the blood vessels beneath the skin. Growing to a maximum overall length of 12 cm (4.7 in), the cavefish is of typical characin shape. The females tend to be larger and fuller-bodied than males, especially when full of eggs. The anal fin of the male has a slightly curved edge, while that of the female is straight. Its natural diet consists of crustaceans, insects, and annelids, although in captivity it is omnivorous (Froese and Pauly, 2006).

The perpetually dark, nutrient-poor environments inside caves are both novel and stressful, driving the convergent evolution of 'cave-specific' characters in diverse lineages. Cave dwelling species have long been studied by evolutionary biologists, not because of their adaptive characters, but rather for regressive characteristics, which include the loss of pigmentation and eyes. Even in the Origin of Species, Darwin pondered why evolution would favor regressive characters in these lineages, reasoning that eyes should at least be of little harm to cave organisms (Darwin, 1859). Cavefish are a favorite model for studying evolution in caves as this species includes 29 separate populations in Northern Mexico that display convergent (but non-identical) cave phenotypes (Jeffery, 2009).

In addition to their loss of eyes and pigmentation, cavefish display adaptive sensory characters that may promote their survival, such as an increased number of taste buds, larger olfactory bulbs and hypothalamus and larger numbers of neuromasts, cells located in the skin that act as a kind of long distance touch (Wilkens, 2010). The molecular basis of eye loss in cavefish has already been the topic of intensive research, which has shown that overexpression of sonic hedgehog (*shh*) along the embryonic midline leads to apoptosis and resorption of the developing eyes. Interestingly, *shh* overexpression simultaneously causes an increase in the number of taste buds and expansion of the forebrain and hypothalamus, which may lead to improved prey capture ability in the dark cave environment. Taken together, this suggests that natural selection favors sensory expansion, with the secondary loss of eyes occurring as a result of pleiotropic processes (Yamamoto *et al.*, 2004).

This led by Yoshizawa *et al.* (2012) suggested whether an alternative adaptive trait may be linked to eye loss in cavefish. Vibration attractive behavior (VAB), defined as the swimming of a cavefish towards oscillating objects in water, is a potentially adaptive trait that has evolved repeatedly in cave populations. Surface fish rarely display this behavior as it attracts the attention of predators; however, in the context of a lightless cave environment, where starvation is a far bigger threat, this behavior is likely to be adaptive as it aids in catching insects that fall on the surface of the water (Yoshizawa *et al.*, 2010).

The cavefish has also been reported as an excellent model for studying orientation. Its lack of visual sense is an advantage as it prevents recourse to global cues so that the fish is forced to attend only to those cues within the test arena (Burt de Perera, 2004). A number of studies have also shown that cavefish respond to novel arm by increasing their swimming speed (Teyke 1985; Burt de Perera, 2004). As the cavefish glides, it produces a flow-field around itself, which is modified by nearby objects and measured by the lateral line organ. It can increase the stimulus to this organ by swimming faster (Teyke 1988).

2.3 Zebrafish

Zebrafish is a tropical freshwater fish species of *Danio rerio* that belong to the Cyprinidae family of the order Cypriniformes. It is native to the Himalayan region and commonly inhabits streams, canals, water bodies and including rice fields. This is a micro predator fish where it feeds on aquatic crustaceans and other invertebrates such as mosquito larvae. It got its name from the it looks where it has five uniform, pigmented, horizontal, blue stripes on the side of the body extended to the end of the caudal fin that are similar to the zebra's stripes. It has a fusiform and laterally compressed shape, with its mouth directed upwards. The male has torpedo-shaped body, with gold stripes between the blue stripes. While the female has a larger, whitish belly and silver stripes instead. Adult females exhibit a small genital papilla in front of the anal fin origin. The zebrafish can grow to 6.4 cm (2.5 in) in length, although it seldom grows larger than 4 cm (1.6 in) in captivity. Its lifespan in captivity is around two to three years, although in ideal conditions, this may be extended to five years (Spence *et al.*, 2007). As a model biological system, the zebrafish possesses numerous advantages for scientists. Its genome has been fully sequenced, and it has well-understood, easily observable and testable developmental behaviors. Its embryonic development is very rapid, and its embryos are relatively large, robust, and transparent, and able to develop outside their mother (Dahm, 2006).

Zebrafish is used as a model system in many different scientific fields due to its rapid development in combination with a relatively short generation time and ease of genetic manipulation (Dooley and Zon, 2000). However, the most prominent application of zebrafish has probably been within developmental biology. This is due to the ease with which the embryos are obtained, in addition to the transparency of the developing zebrafish embryo, which greatly aids observation of developmental processes. In fact several common and important developmental mechanisms have been identified in zebrafish which are similar in mammals (Jörgens *et al.*, 2012).

Recently, zebrafish have been replacing rodent as vertebrate model in studying neuroscience. The large offspring and easy maintenance in captivity are clear advantages of this animal model compared to rodent (Nasevicius and Ekker, 2001). This make zebrafish reduced the cost of experimentation and significantly increasing research throughput—potentially, more experiments can be run in less time to answer any number of questions (Kandel, 2004).

The clear chorion of the zebrafish allows continuous visualization of neuroanatomy; their rapid development and accessibility to genetic analysis make the zebrafish an excellent model system for molecular and mechanistic studies of neurodevelopment (Guo, 2001). There have been many studies of early regionalization and patterning at the midbrain/hindbrain boundary using zebrafish but surprisingly few on the cerebellar structures that develops from this region; Hibi and Shimizu (2012) show how the developing and mature zebrafish cerebellum has excellent potential for the study of cerebellar circuitry and function.

Zebrafish have excellent vision and the visual system is well studied. Studies by Gestri *et al.* (2012) reported that a broad spectrum of research on the zebrafish nervous system from early development to function and use for modeling human ocular diseases. This makes the emerging field of optogenetics transformed to the way that investigators can monitor and control neuronal activity in vivo.

In the last decade, zebrafish have been gaining popularity in behavioral brain research (Sison and Gerlai, 2010). For instance, zebrafish performed well in several conditioning memory tasks, such as olfactory (Braubach*et al.*, 2009), shuttle box active appetitive (Pather and Gerlai, 2009) and appetitive choice discrimination (Bilotta *et al.*, 2005). Moreover, this small vertebrate showed acquisition in one trial avoidance task (Blank *et al.*, 2009) and achieved good performance in alternation memory tasks and plus maze non-spatial and spatial associative learning tasks (Al-Imari and Gerlai, 2008; Sison and Gerlai, 2010 & 2011). All this data clearly demonstrates the cognitive and mnemonic capabilities of zebrafish.

3.0 Materials and methods

3.1 Experimental design

A five weeks project was conducted to study the comparison of learning and memory function between zebrafish and cavefish. The fish were divided into two groups according to their species; zebrafish (Group 1, n=6) and cavefish (Group 2, n=6). Each group consists of six fish. The fish were tested for learning and memory function by using Y-maze test.

3.2 Experimental location and animal housing

A total of 12 fish (zebrafish and cavefish) were kept in Neurobehavioral Room, Physiology Lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Fish were divided into two groups of fish each and housed in plastic aquarium with dechlorinated tap water and regulated air pump. The water temperature was regulated at an ambient temperature of 25 – 27 °C. Room lighting that consisted of

12 hours of light and ark each were provided. This project was approved by the Institutional Animal Care and Use Committees (UPM/IACUC/FYP.2014/FPV.026).

3.3 The Y-maze behavioral test

As to test and measure the cognitive function affected in this study, Y-maze test was chosen as it induces less stress to the animals (Wang *et al.*, 2009). Y-maze is a model shaped in Y-shape which consist of three equally spaced-arm which are the start arm, familial arm and novel arm. This working memory is based on spontaneous exploration and alternation between arms.

The Y-maze test was conducted in a dim lit room and each fish was placed at the start arm of the maze. During the first trial (training, 5 minutes), fish were allowed to explore only two arms (start and familial arm), with the third arm (novel arm) closed. For the second trial (after 1, 3 and 6 hours), the fish were placed back in the Y-maze with free access to all three arms for 5 minutes. Fish were placed in different arms as starting points in every experiment in order to randomize the maze ques. The time spent in each arm and number of entry of each arm was recorded. The data was tabulated and analysed.

4.0 Results

The data obtained were analysed and shown in the graphs below. In Figure 1, the mean number of entry in each arm by cavefish was higher in the start arm (mean = 9.0) comparing to the novel (mean = 8.85) and familiar arm (mean = 8.95).

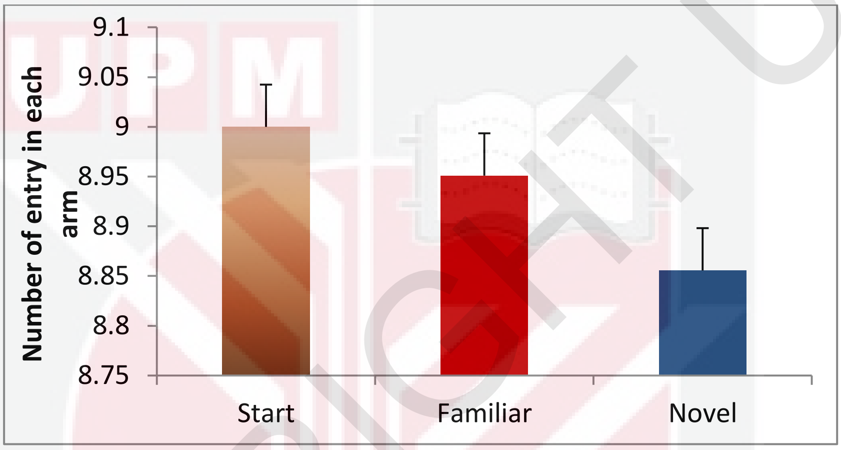


Figure 1: Mean number of entry in each arm by cavefish

Figure 2 showed that the mean time spent in each arm by cavefish was also higher in the starting arm (mean = 1.40 mins) compared to the familiar (mean = 1.33 mins) and novel arm (mean = 1.21 mins).

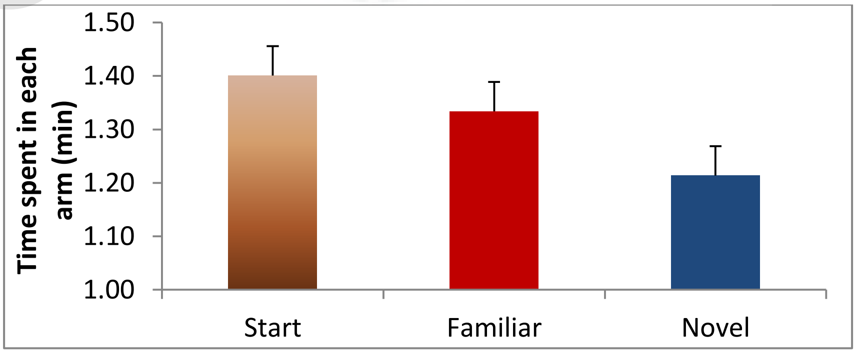


Figure 2: Time spent in each arm by cavefish.

On the zebrafish, Figure 3 showed the mean number of entry in each arm was higher in the novel arm (mean = 13.5) compared to the start mean = 8.4) and familiar arm (mean = 10.1).

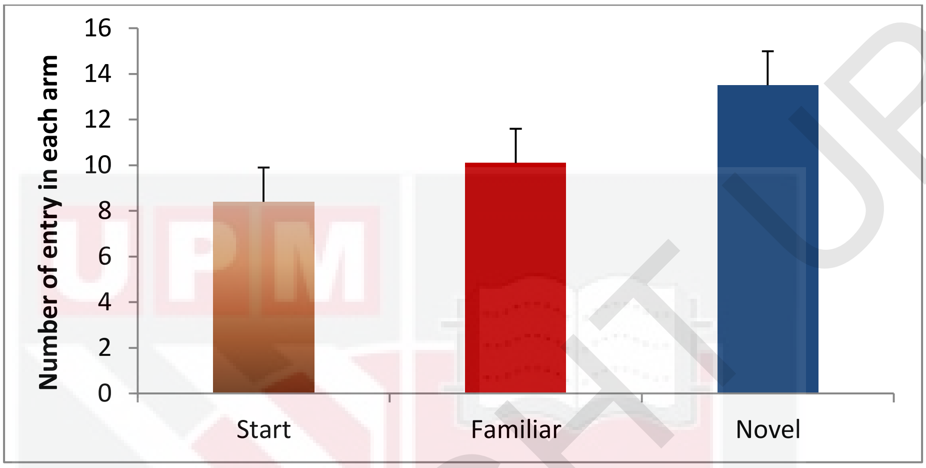


Figure 3: Number of entry in each arm by zebrafish

The time spent in each arm by zebrafsh is shown in Figure 4. The results showed that the zebrafish spent longer time in the novel arm (mean = 2.98 mins) comparing to the start (mean = 1.86 mins) and familiar arm (mean = 2.27 mins).

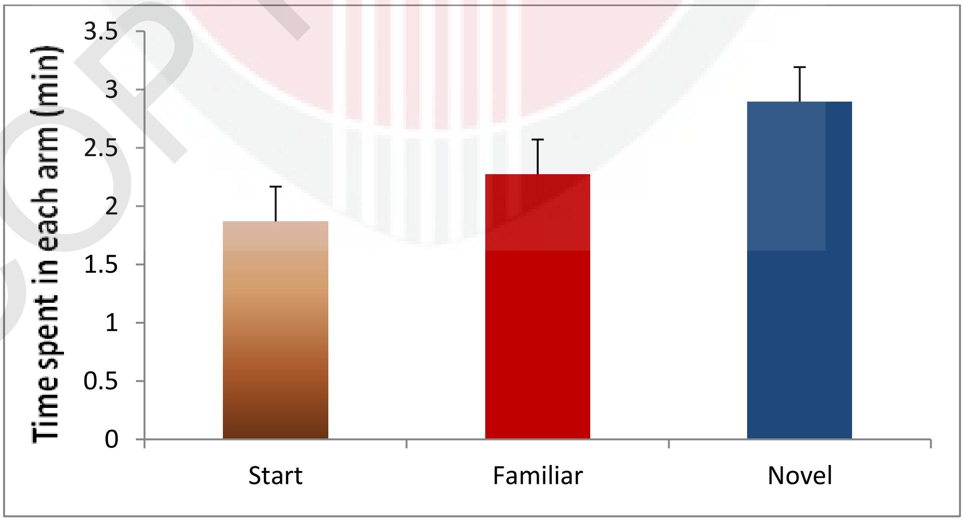


Figure 4: Time spent in each arm by zebrafish

By comparing between the cavefish and zebrafish in Figure 5, we can see that the mean number of entry in novel arm by zebrafish was significantly higher (mean = 13.5) compared to cavefish (mean = 8.85).

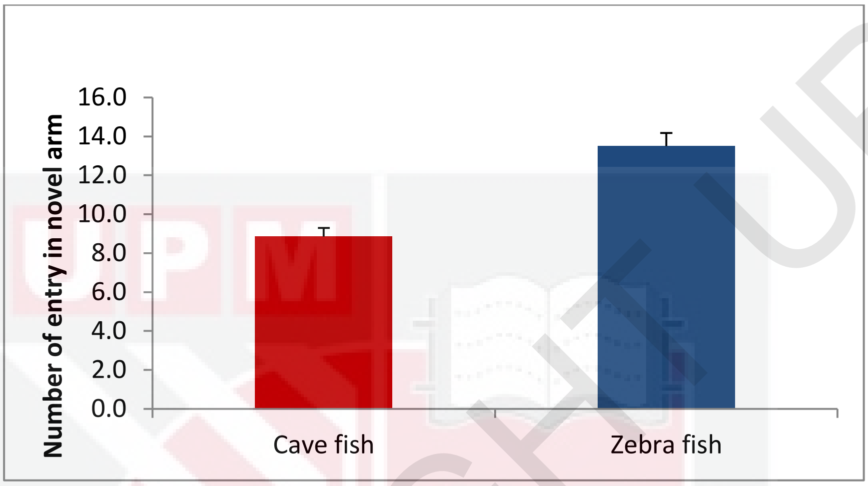


Figure 5: Number of entries in the novel arm by zebrafish and cavefish

Lastly, the comparison of the time spent in the novel arm between cavefish and zebrafish are shown in Figure 6. The results also showed that zebrafish spent more time in the novel arm (mean = 2.89 mins) compared to cavefish (mean = 1.21 mins).

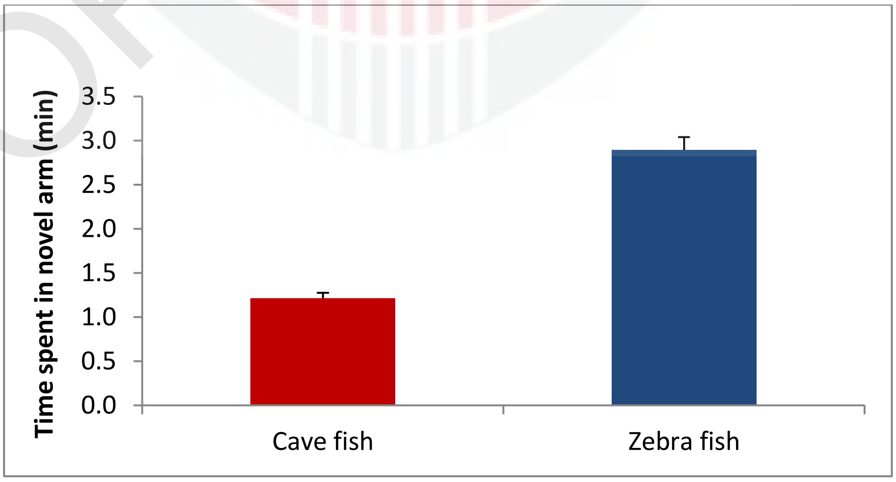


Figure 6: Time spent in novel arm by zebrafish and cavefish

5.0 Discussion

This study is associated with tendency of the fish to explore novel arm or new environment than familial arm and start arm. The Y-maze performance was favourable when the number of entries and time spent in the novel arm were greater than those in the other arms. The total number of arm entries and time spent in the novel arm are indicator of spatial working memory (Onaolapo, 2012). Therefore it is appropriate to use this model to assess the cognitive function of the cavefish and zebrafish.

Cavefish shows high number of entry and time spent equally in all arm of the Y-maze. This could be due to cavefish just explore the arm based on their lateral mechanosensory and olfactory sensory bulb making it not significant between all arms. Their mechanosensory and olfactory bulb are more for the usage of finding food in the complete darkness. In fact, they are very sensitive to fluctuating water movement pressure and they will detect the vibration created by their prey and will move towards the source of direction for feeding (Yoshizawa *et al.*, 2012). Previous studies reported that they have better olfactory sense by having olfactory buds all over its head which help them find food more quickly in complete darkness (Helfman and Facey, 1997). In this study no stimulus (vibration or smell) was done to attract the cavefish to a certain area. Plus, as this fish cannot see and they swim following the wall, we might say that they do not even know the difference of each arm in the Y-maze.

For the zebrafish, higher number of entry and time spent in the novel arm can be seen in this study. It shows that the zebrafish have a high exploratory behaviour indicating that this fish have a good learning function. Then, when preferential exploration of novelty is established, it memorizes the novel arm and keeps on exploring the arm even after several hours (Al-Amari and Gerlai, 2008). The zebrafish can also recognize visual cues and can developed preferential on the visual cues. This was supported by previous study showed that they spent less time in the y-maze arm with cross visual ques, but have equal time spent in square, triangles and circles visual cues (Cognato *et al.*, 2012). Thus, in this study we used square, triangle and circle for the arm cues to avoid visual cues preferential.

Comparing the number of entry into the novel arm between cavefish and zebrafish, we can see that zebrafish have a significantly higher number of entries into the novel arm comparing to the cavefish. This can be due to the blind state of the cavefish, making them unable to differentiate the novel arm from the other two arms and travel to all these three arms equally. Thus, the average number of entries in the novel arm is lower. For the zebrafish, as it can see and differentiate the novel arm, and due to its high exploratory behavior, they travel frequently to the novel arm making them have higher average number of entries into the novel arm.

In the parameter of time spent in the novel arm between the cavefish and zebrafish, we can also see that zebrafish spent more time in the novel arm comparing to the cavefish. As for cavefish a study state that cavefish respond to novel environment by increasing their swimming speed (Teyke 1985; Burt de Perera, 2004). As the cavefish glides, it produces a flow-field around itself, which is modified by nearby objects and measured by the lateral line organ. It can increase the stimulus to this organ by swimming faster (Teyke 1988). Thus this makes the average time spent in the novel arm lower. The high exploratory behavior of the zebrafish makes them spent more time in the novel arm to explore the novel arm. Clearly, these two species of fish have significantly different in their characteristic (blind and eye-sighted) of cognitive function.

6.0 Conclusion

From this study we can conclude that, there are differences in zebrafish and cavefish of their cognitive function. We can conclude that zebrafish have a higher exploratory behaviour indicating that this fish have a good learning and memory function. However, cognitive function on the cavefish depends only on its olfactory sensory and mechanosensory lateral line.

7.0 Recommendation study

Future work can be done on giving treatment to the fish to see the effects on cognitive function development in the fish such as treatment with dietary supplement omega-3 fatty acid. In addition, a molecular study of the brain gene expression can be performed to give more insight on the zebrafish and cavefish brain associated with the cognitive function.

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) Assesing 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 (Daniorerio).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) Saptial parameters encoded in the spatial map of the blind Mexican cave fish, Astyanaxfasciatus. Animal Behaviour. 68:291-295.

Chung S. (2008) Appropriate maze methodology to study learning in fish. Journal of Undegraduate 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 (DanioRerio): 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 Nehaviour. 3:63-74.

Hafandi A., Begg D.P., Premaratna S.D., Sinclair A.J., Jois M., Weisinger R. (2014) Dietary Repletion with ω3 Fatty Acid or with COX Inhibition Reverses Cognitive Effects in F3 ω3 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 bilogy memory storage: A dialog betwee genes and synapses. Bioscence Report. 24:475-522.

Keller E.T., Murtha J.M. (2004) The use of mature Zebrafish (Daniorerio) 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 (Daniorerio).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, Astyanaxmexicanus. Evolution Development. 10:196-209.

Portavella M., Torres B., Salas C. (2004). Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephaticpallium. 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 (Daniorerio) by the NMDA-R antagonist MK-801. Neurobiology of Learning and Memory, 96, 230–237.

Spence R., Gerlach G., Lawrence C., Smith C. (2007) Thebehaviour and ecology of the zebrafish, Daniorerio. Biological reviews of the Cambridge Philosophical Society. 83(1): 13-34.

Teyke, T. 1985. Collision with and avoidance of obstacles by blind cave fish Anoptichthysjordani (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 Anoptichthysjordani. 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.

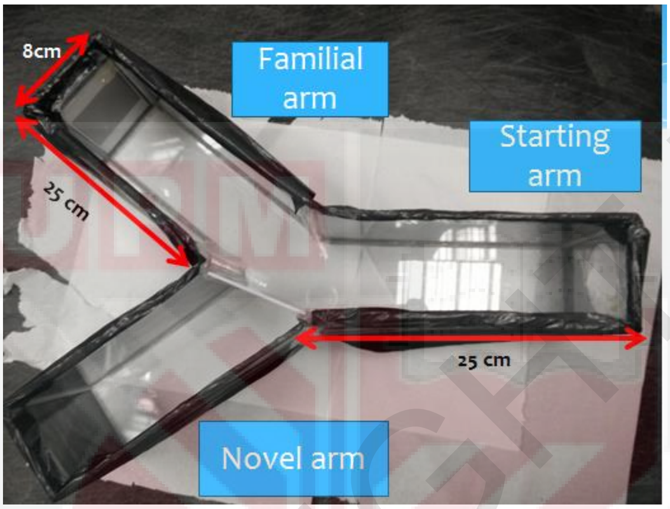
Wilkens H. (2010) Genes, modules and the evolution of cavefish.*Heredity*.105:413-422.

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.

Appendices

Appendix A: The Y-Maze Model



Appendix B: Average number of entry in each arm by Cavefish

Arm	Number of entry
Start	9.00
Familiar	8.95
Novel	8.86

Appendix C: Average time spent in each arm by Cavefish

Arm	Time (min)
Start	1.40
Familiar	1.33
Novel	1.21

Appendix D: Average number of entry in each arm by Zebrafish

Arm	Number of entry
Start	8.4
Familiar	10.1
Novel	13.5

Appendix E: Average time spent in each arm by Zebrafish

Arm	Time (min)
Start	1.868
Familiar	2.273
Novel	2.895

Appendix F: Number of entry in novel arm: Cavefish vs Zebrafish

Fish	Cavefish	Zebrafish
Number of entry	8.86	13.50

Appendix G: Time spent in novel arm: Cavefish vs Zebrafish

Fish	Cave fish	Zebra fish
Time (min)	1.214	2.895