Aqualabyrinth – A New Enrichment for Laboratory Zebrafish under Analysis for Future Production

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Abstract: In the field of laboratory animals, there have been considerable improvements in environmental enrichment for mammalian species, but little remains available for aquatic species, in particular fish. Recent publications have shown benefits in providing laboratory zebrafish (*Danio rerio)* certain types of enrichments, such as substrate and plants. This research article describes a novel tank enrichment called the Aqualabyrinth and evaluated the benefit of its use in laboratory zebrafish. The enrichment won 3rd place at the 2020 Janet Wood Innovation Award competition. The Aqualabyrinth was compared to another fish enrichment option. The study was comparing the Aqualabyrinth novel enrichment to the mouse igloo hut by recording the number of interactions by the fish with the enrichments. Results showed a higher rate of interaction with the Aqualabyrinth than the mouse igloo: an average of 10.5 vs 6.25 interactions per 3 fish within a 30-second test interval. In addition, benefits of this novel enrichment include ease of usage, low maintenance, and modular configuration options making it an ideal choice for laboratory fish tanks. With the additional benefits highlighted in this study, the Aqualabyrinth proves to be an efficient and practical environmental enrichment option, pending collaboration with interested manufacturers.

Introduction

Aquatic species, in particular small zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), are being used more and more as animal models in science due to their small size, large transparent embryo, and genetic similarities with human diseases [1]. In addition, their use in neurological science for memory studies is becoming popular [2]. As a result, there have been considerable improvements in environmental enrichment procedures for these aquatic species. Compared to non-aquatic species such as mice, rats, and gerbils, there is still little knowledge on what enrichments those animals need to increase their well-being [3], [4], [5]. It was shown in studies that natural behavior in rodents (nest building, food foraging) was still observed in captivity and the absence of enrichment led to stereotypical behavior [5]. In zebrafish, fearfulness and anxiety have been noticed in the research setting, and fake aquatic background posters on the tanks sides or bottom helped reduce this negative reaction of captivity [5]. Surprisingly plastic plants did not help to reduce aspect of fear and anxiety [5]. Thus, the need of finding new enrichment techniques is still present.

Previous evidence from studies done on singly housed fish showed that social interaction was preferred to inanimate objects, resulting in lowering their anxiety and stress [6]. However, when cohousing is an option, a barren tank does not provide a shelter, nor does it provide other sensory stimuli to the fish. Increasing structural complexity with simulated plants lowered stress as it reduced visibility to the fish, thus preventing dominant ones from easily targeting subordinates [7]. In the case of barren tanks, there is an increasing chance of stress due to dominance among the fish, injuries due to fighting, and possible boredom. Increased cortisol levels can decrease growth rate, fertility, and egg count [8]. In addition, when different types of environmental enrichments were tested to see if there was preference or behavioral changes in laboratory fish, research showed that shaded tanks, barren tanks, PVC material and overcrowding (too many plants) increased stress and aggressivity while plastic material (over PVC), shaded hides inside the tank, and submerged plants reduced aggression or cortisol levels, or increased behavior diversity [8]. A second study testing different types of enrichments concluded that a mirror image of the fish tended to increase aggression after 3 weeks of exposure. Also, the overall conclusion was that the type of enrichment used widely affected the behavior of the zebrafish while growth, fertility, and aggression may or may not be positively affected [9]. Another study evaluated the use of multiple vertical rods into fish tanks to add complexity to the environment of zebrafish in toxicology studies. The researchers reported a decrease in the cortisol level of the fish, and fighting reduced after 2 to 7 days depending on the fish group observed [10]. Taken together, these studies illustrate that enrichment techniques are not equal in their positive effects, and some can be detrimental.

Wild zebrafish are often found in vegetated areas with slow moving water [11]. In the laboratory setting, they serve as a good research model as they are robust and easy to maintain [12]. A new structural enrichment device called the Aqalabyrinth was created to enhance the welfare of Zebrafish and other aquatic fish species, taking into consideration the stress and aggression problems common in many captive aquatic species. The

Aqualabyrinth was fitted to the Aquaneering Inc. tanks (San Diego, CA). The enrichment was made versatile so that it can be easily customized for other types of tanks. The main goal of the Aqualabyrinth product is to help mimic a natural aquatic environment and to provide the fish with a place to explore and hide, as well as a safe environment to be co-housed and to reduce stress and anxiety. The design considered some important specifications that allow for ease of manufacturing and assembly and low cost of the product production. The DFMA technique (Design for Manufacturing and Assembly) was followed in an early design phase of the project to minimize product cost through design and process improvements. The Aqualabyrinth was put under experiment to observe its efficacy and to support manufacturing so that this novel enrichment can be accessible to the many aquatic facilities.

Materials and Methods

Housing & Husbandry

The zebrafish involved in the experience were of AB strained originally supplied by a donating laboratory and housed in Aquaneering 2.8 and 6 liters tanks in reverse osmosis water supplied with balanced salt and bicarbonate solutions, heated at 27º Celsius with light:dark cycle 10:14 exposure 10Am to 12AM. Water pH, temperature, and conductivity was monitored daily, while nitrate, nitrites, hardness and alkalinity was monitored weekly, to ensure acceptable water quality. Fish were fed twice daily with Gemma Micro 300 (Skretting, USA). Fish were housed in groups of 3 fish or more. All experiments were performed under Institutional Animal Care and Use Committee-approved protocol at the Research Institute of McGill University Health Centre and followed the CCAC guidelines [13], [14].

Equipment

2 x Vertical Large Panels (A) (Figure 1)
2 x Vertical Small Panels (B) (Figure 1)
2 x Horizontal Small Panels (C) (Figure 1)
2 x Red Igloos (Figure 2)
5 x 2.8 liters Aquaneering Inc. Laboratory Fish Tanks
1 x Metal Tally Counter

Testing Conditions

(1) Vertical Large + Vertical Small Panels in a 2.8 liter tank
(2) Vertical Large + Horizontal Panels in a 2.8 liter tank
(3) Horizontal Panel in a 2.8 liters tank
(4) Igloo in a 2.8 liters tank

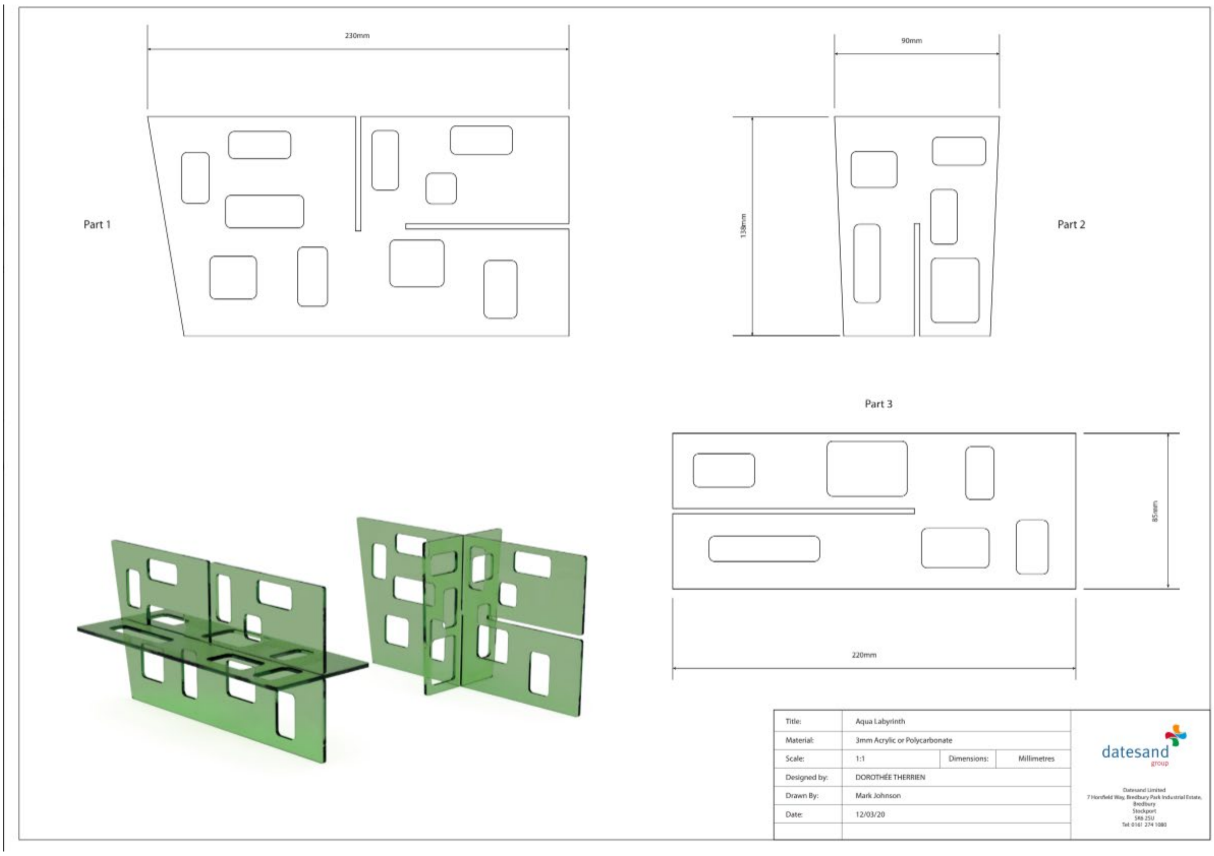


Figure 1. Technical Drawing of the Aqualabyrinth

Figure 2. Mouse Igloo (L) and Aqualabyrinth (R)

Testing Aquariums & Recording

The components of the Aqualabyrinth were produced by Datesand Group (UK) as part of the Janet Wood Innovation Award. The prototypes were made with acrylic polymer plastic. The different panels consisted of a small vertical panel, a large vertical panel, and a small horizontal panel (Figure 1). As a comparison, we used mouse Igloos (Bioserv, NJ, USA). In each 2.8L tank, 3 adult fish were housed together (males and females mixed together).

The tanks were placed in such a way that they all had neighbor tanks on opposing sides to rule out side preference during the experiment. The fish were provided one type of enrichment and were acclimated to it at least 5 days before the recording of data.

To record and compare the different enrichment interactions, one video of 2.5 minutes for each type of enrichment was captured during a non-feeding time of the day (1 hour after the first feeding of the day was performed) and the camera was set to record without human presence to avoid distracting the fish. For all the enrichments types (1)(2)(3)(4), 1 tank for each was set up to gather data.

Analysis

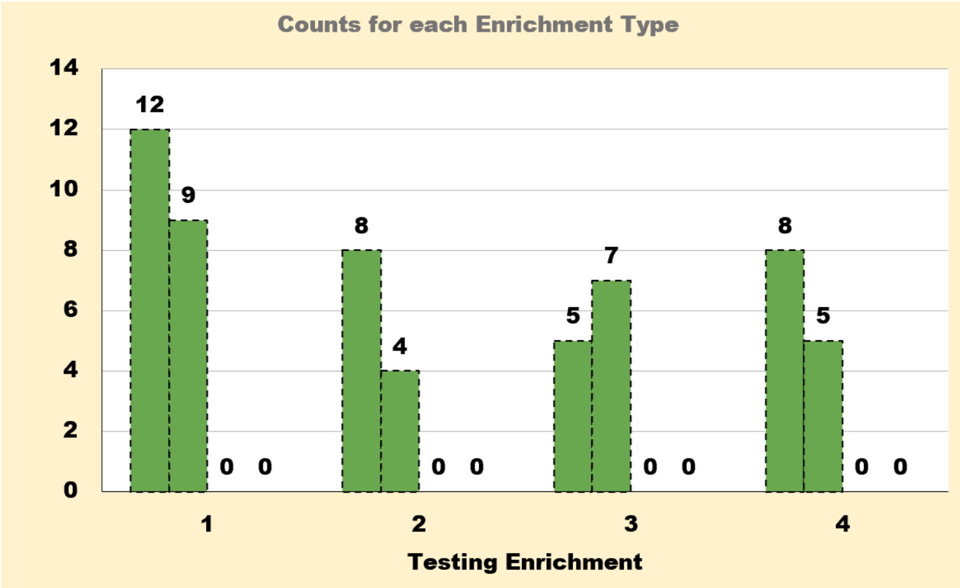
From each 2.5 minute video recording, 30 seconds was viewed at t= 60 seconds and at t=1.5 minutes and the average of interactions was calculated to analyze the behavior. Interaction with the enrichment was recorded. For the mouse igloo, the number of times a fish passed at a minimum distance of 1 cm of the enrichment and above or below was counted and for the Aqualabyrinth configurations, the number of times a fish passed through an opening was counted.

Results

The Aqualabyrinth configuration (Table 1) gave the highest averaged interactions of 10.5. All other configura-tions, including the mouse igloo gave between 6-6.5 interactions for a 30-second interval.

Table 1. Results of the Counts of each 30 Second Window.

Testing Enrichment	Counts during 30 sec	Average Counts
(1) Vertical Large + Vertical Small Panels in a 2.8 liters tank	at t= 60 seconds: 12 at t= 90 seconds: 9	10.5
(2) Vertical Large + Horizontal Panels in a 2.8 liters	at t= 60 seconds: 8 at t= 90 seconds: 4	6
(3) Horizontal Panel in a 2.8 liters tank	at t= 60 seconds: 5 at t= 90 seconds: 7	6
(4) Igloo in a 2.8 liters tank	tank #1 at t= 60 seconds: 8 at t= 90 seconds: 5 tank #2 at t= 60 seconds: 7 at t= 90 seconds: 5	6.25



Graph 1. Counts for each Enrichment Type.

Discussion

In this study, both the mouse Igloo and the Aqualabyrinth were used as structural enrichment for Zebrafish. Interaction with the enrichments was used here as a simple way to measure its use in fish. In this brief study, mouse Igloo interactions were not as frequent as the ones for the complete Aqualabayrinth. Both types of enrichment allowed similar interactions in similar conditions. However, the fish used the enrichments in different ways. For the mouse igloo, most fish passed close to it, but did not usually pass in it or explore it. This may be due to the shape of the enrichment, which is not an enrichment that was meant to be for aquatic species, but rather for small rodents. For the Aqualabyrinth, the fish often moved through the partitions up, down, right, or left. The interactions were easier to assess and record as being a fish-enrichment interaction.

Both types of enrichment allowed proper monitoring of the health of the fish with their semi-transparent finish, but the cleaning of the igloo was more labor intensive due to the black rubber material and the dome shaped. Indeed, the rubber may increase dirt and algae accumulation and the dome shape can be tricky to clean on the concave side. The Aqualabyrinth design is flat and made only from acrylic which makes it easy to clean and handle.

It is interesting to note the difference in terms of space and shape of the enrichment options. Igloos have limited configurations (either right side up or upside down). Aqualabyrinth (even using one panel) can be very versatile and can fit in different angles and in different sized tanks. As shown in the methodology section, with only 3 different types of panels, laboratory facilities can easily create at least 5 different configurations and with those configurations, the different size of tanks also gives different appearance outcomes and may influence how the fish will behave. In this study, the simpler designs resulted in fewer interactions. Given that novelty has been related to improved animal welfare in many species, Aqualabyrinth could potentially prevent stereotypical behavior as the variety of configurations allows the fish to have a new experience as frequently as needed [4]. More research is needed to confirm the full impact of structural enrichment on laboratory fish.

It is important to note that depending on the fish habituation to the enrichment, the fish may interact differently. One tank was analyzed during the experiment with the horizontal panel in the 2.8L tank and they were not utilizing the enrichment. Rather, they were staying at the top of the enrichment and chasing each other. This tank had male and female fish and was observed during a 2 day-period. A hypothesis could be that some configurations may induce egg laying behavior as it may mimic shallow waters. Also, like for any other animals, fish who grow up in environments without enrichment may have difficulty adapting to different enrichment structures, such as navigating through openings [15]. Indeed, captive animals, unlike wild animals, do not have the same basic hunting instincts [15]. Early exposure to enrichment and complex environments is important so that the animals can develop more nerve connections making them more apt to interact with their environment, develop better coping mechanisms and be less stressed, and avoid stereotypical behaviors [15].

Zebrafish tend to interact with a novel enrichment visually and by touching it with its mouth. This behavior decreases, as it becomes no longer novel [16]. To be effective, an enrichment needs to be used in positive, species-appropriate ways. Namely, overcrowding, single housed fish, and small tank size can negatively affect the fish response to the enrichment. An observer may think that fish do not enjoy or use an enrichment but the cause may be the overall housing condition which is not conducive to enrichment use. Careful selection of the enrichment is important depending on the fish environment. One study found that the flow of water was very important for the fish and the space to swim as well [17]. The North American 3Rs Collaborative recommends having no more than 5-20 fish per liter to avoid stress [18]. In the author's own personal home tank, zebrafish were seen to stop fighting when using the Aqualabyrinth. To further evaluate the potential effect on the fighting, longer, controlled studies should be performed.

Conclusions

The common arguments of lack of environmental enrichment options for aquatic species often revolve around impact of water quality, potential risk of disease spread due to the biofilm accumulation on the structures, and that diverse environmental enrichment may lead to experimental variation [8]. However, careful selection of the material used to put into the water or use of outside backgrounds will not affect water quality [19]. Using proper tank cleaning as well as using good material for enrichment will reduce biofilm accumulation. The benefits of enrichment to fish can be substantial. Research has shown that proper housing and enrichment of the fish increased survival rate [11], [20]. Lastly, experimental variation is possible, and there are ways to make the study consistent by having the same enrichment for all tanks of the experiment. It is important to remember that in the most common laboratory animals, non-aquatic animals such as rats and mice, enrichments are mandatory unless justified. Happy animals make good science and this leads to quality scientific data. Namely, if enrichment does work, preventing stress and fight wounds, then the investment becomes worth it [6]. In addition, enrichment to fish during studies is highly recommended by the AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) [21].

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Research Institute of McGill University Health Center (protocol code 7124 and date of approval of 02/01/2019).

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Conflicts of Interest: The authors declare no conflict of interest. Upon reading this article, the author hopes that the laboratory animal community can see the same potential and decide to invest in the manufacturing the product with her collaboration so that it becomes readily available on the Laboratory Market.

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