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Short Communication

Zebrafish Housing: The Recirculating and Cost-effective Open Design Aquaria System

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ABSTRACT

The zebrafish's ease of care and high reproductive rate have made them a popular animal model. It is routinely kept and maintained in commercial aquariums. However, the expense of a particular system was prohibitive for researchers with limited budgets or who worked in universities without zebrafish-rearing facilities. Thus, a simple custom-made aquaria system was developed. The recirculating open-design aquaria system was cost-effective and may frequently be improved. This study has developed a distinctive aquaria system that can tackle the issues related to expanding research based on zebrafish.

Keywords: Aquaria system, cost-effective, custom-made, open-design, recirculating, zebrafish

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INTRODUCTION

According to Bhargava (2018), the custom-made system has been termed an open design to distinguish it from a commercial system. Open design was different, especially in its design and holding capacity. The available open designs were simple, modular, and cost-effective, providing zebrafish with chemical-free water. However, the varieties of open designs raised several maintenance issues, thus complicating mass reproducibility.

This study demonstrates the technical development of a manageable and cost-efficient recirculating open design system, showcasing its feasibility and practicality. The system (Figure 1) was constructed in a well-ventilated animal retention room and positioned in a closed area next to the tap water resource at the back of the room. The system was maintained (half an hour daily) by an end user under the Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia. It took nearly six months for the system to be developed, starting from July 2019 and ending in January 2020. It has proven effective in safely housing zebrafish during a series of experiments by Raduan et al. (2023).

THE RECIRCULATING OPEN-DESIGN SYSTEM

An effective recirculating water system should incorporate a filter system, chemical monitoring and regulation, ultraviolet (UV) irradiation, and a light and temperature control unit (Aleström et al., 2020; Lawrence & Mason, 2012; Varga, 2016). This study designated the recirculating open-design system with a built-in filtration and sterilisation unit embedded with single commercial light-emitting diodes (LEDs) (18 W) lamp on the top level of the rack

(a) (b)

Figure 1. Recirculating open-design (custom-made) zebrafish system: (a) Front view of the system; and (b) Back view of the system

row to meet the entire rack's light need using a 14/10 light-dark photoperiod timer. The essential water quality parameters were regularly monitored and maintained. Total ammonia (using AMMONIA TEST KIT, API®, USA), temperature (using thermometer Model SKU: LAB010, Brannan, United Kingdom), pH (using a portable pH meter), and salinity (using a refractometer, Cold-Palmer, USA) were checked daily, whereas dissolved oxygen was checked monthly using a dissolved oxygen meter (Model HD 3030, Trans Instrument, Singapore). The desired total ammonia should be maintained at 0 mg/L, the temperature should be kept within the range of 24–30°C, the pH within 6.8–8.5, and the salinity at 0–5 g/L. The dissolved oxygen level should not be less than 4 mg/L, following the recommendations of Harper and Lawrence (2011).

Figure 2 illustrates the schematic diagram of our recirculating open-design zebrafish system. In concept, the return

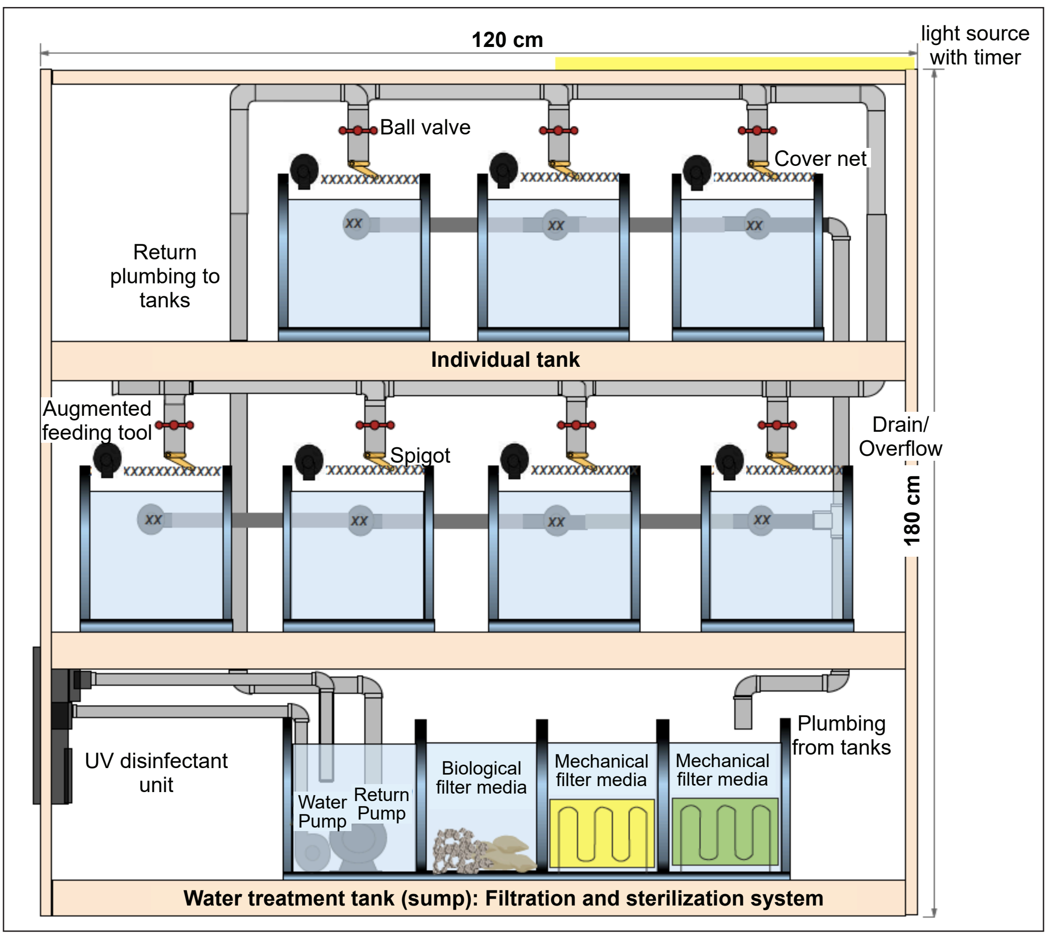


Figure 2. Schematic diagram of recirculating open-design (custom-made) zebrafish system. Before recirculating the clean water (blue arrow) back to the individual tanks, the wastewater (red arrow) that enters the sump must undergo filtration and ultraviolet irradiation

pump pumps water into the various tanks through the regulated water outflow of the manifold, which runs the length of the shelf. Following the principle of an overflow system, water exits the tank through an overflow outlet and drains water (wastewater) to undergo filtration systems before being recirculated. The water treatment tank's water level is checked regularly, and fresh dechlorinated (tap) water is refilled as needed. The water level in the system will typically decrease when residual solid waste from the back of each tank and collected solid waste from the water treatment tank are manually discarded regularly. In addition, natural evaporation occurs and contributes to the drop in water levels in the system. Regular exchanges of fresh (dechlorinated) water are also required to maintain water quality and reduce chemical accumulation (especially nitrogen). Compared to flow-through systems, recirculating systems use much less water overall. Thus, a significant advantage of the recirculating system is that it maintains water quality while minimising water loss (Bhargava, 2018).

CONSIDERATIONS OF COMPONENTS FOR THE RECIRCULATING OPEN-DESIGN SYSTEM

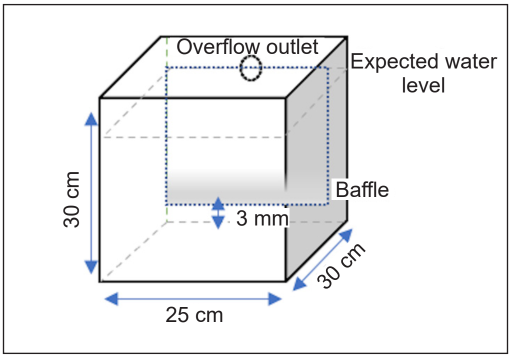
Individual Active Overflow Tank

The tanks are the principal housing enclosures of an aquaculture operation. The design, dimensions, and materials used to construct the tanks will vary based on the scope and scale of the projects. In addition, the approaches to water exchange and specimen confinement will vary (Cockington, 2020). Lawrence and Mason (2012) observed that all these aspects influence the fish's well-being, the system's functionality, and the pace and capability of research. Consequently, selecting a particular system should involve carefully assessing its associated tank types and their contribution to achieving research and husbandry objectives.

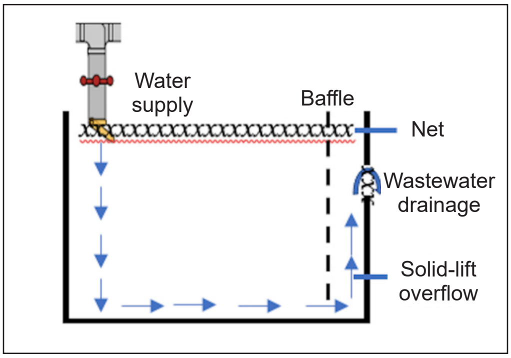
Standard zebrafish tanks are often made of polycarbonate, polysulfone, high-quality glass, or acrylic (Matthews et al., 2002). One of the downsides of polycarbonate and polysulfone is their susceptibility to release bisphenol-A (BPA), an acknowledged oestrogen mimic (Howdeshell et al., 2003). That flaw is particularly characteristic in standard tanks. Additionally, the glass is relatively affordable, does not scratch easily, does not produce biofilms or other types of tank fouling, and is chemically inert, so there is no chance of chemicals leaking into the inhabitants (Cockington, 2020). As a result, this study's aquaria system is outfitted with premium transparent glass tanks (using standard glass grade [5 mm thickness]) sealed with SN-503 RTV neutral silicone sealant by Soon Lee Frame & Glass Works, Terengganu) that are net-covered. Each tank is designed to be flexible and can be easily reassembled from the water pipelines. Since most pet stores carry inexpensive rectangular (box) glass aquariums, glass tanks are the most popular type of housing for small-scale operations (Cockington, 2020).

Researchers who wish to conduct small-scale experiments but do not have access to a large-scale production facility still rely heavily on the traditional box tank despite its decreased prevalence in modern times. These tanks range in size from 20 to 50 L and offer a comprehensive approach to fish care. The considerable water volume provides sufficient capacity to buffer against variations in water quality and permits fish to display their natural schooling behaviour (Cockington, 2020). The schematic diagram of the rectangular individual tank is shown in Figure 3. With a total capacity of 17 L and dimensions of 30 × 25 × 30 cm^3 (height × width × depth), each tank can hold around 85 adult zebrafish. The standard recommendation for animal density was five fish per litre (Liu & Zhong, 2017; Vargesson, 2007). Following the desired fish capacity and research requirement, the tank may be set with varying sizes and shapes. As Lawrence and Mason (2012) mentioned, there are no established specifications for the size and form of zebrafish aquariums. Depending on requirements, the fitted glass tanks can be altered, disconnected, and reconnected to the main water supply.

The passive overflow within the individual tanks hinders the capacity to maintain tank hygiene, needing periodic cleaning interventions to keep the tanks functioning as intended (Cockington, 2020). In assisting the removal of waste or solids, tanks should be equipped with specific rear baffles or siphons (Hammer, 2020). A tank's "self-cleaning" capability to remove waste solids is essential when selecting a tank (Timmons & Ebeling, 2013). Typically, the commercial tank manufacturer has a particular design that employs the solids-lift (active) overflow principle while simultaneously preventing fish from fleeing the specific tank (Hammer, 2020). In this study, the individual tank was custom-designed with a partitioning glass baffle (Figure 3). The 5 mm baffle thickness (25 × 25 cm^2; width × length) was positioned so that there was a 3 mm gap at the tank's base. It aims to change the tank's



(a)



(b)

Figure 3. The primary hydrodynamic design of an active (solids-lift) tank. A spigot on the front of the tank pumps water into the tank. (a) The ingenious design of the separating baffle at the rear of the tank creates a pressure differential between the holding space and the overflow; and (b) Particulate waste is drawn to the back of the tank by a strong undertow current (blue arrows) and lifted to the tank overflow

hydrodynamics so that an undertow current can develop, producing an active draw across the tank base (Cockington, 2020). In the meantime, with continuous water circulation in the system, it was determined that the overflow outlet of the tank would be closed with a net (200 µm mesh size) to prevent small zebrafish (2-3 mm) from escaping. The net segregates fish to prevent mixing with other treatment or study groups in a specific experimental design.

Combination of Water Treatment Tank with Filtration and Sterilization System

The water treatment tank (sump), or the reservoir, is positioned at the bottom of a self-contained recirculating system. It provides a place to store water circulated throughout the system. The sump was configured to a total capacity of 70 L and dimensions of $38 \times 38 \times 76.5$ cm^3 for our recirculating system (width, height, and length) (Figure 4). The sump's material composition is comparable to the individual tank's grade. According to Bhargava (2018), the sump may have a single large chamber or numerous small chambers to hold the pump, aeration assembly, charcoal bag, heating and cooling coils, and other components. As for this study, the sump was split into four interconnected compartments by baffles, as shown in Figure 4.

Technically, when the water level in the sump decreased (below the ideal capacity) due to natural evaporation, the fresh dechlorinated (tap) water was manually refilled (using a syphon) into the sump. The tap water directly from the public water supply system was initially kept (without filtration) in the 5 L polypropylene (PP) bucket for two days to allow the chlorine to evaporate naturally before being into the system. The bucket was placed near the tap water source. Any rust-prone or leaching

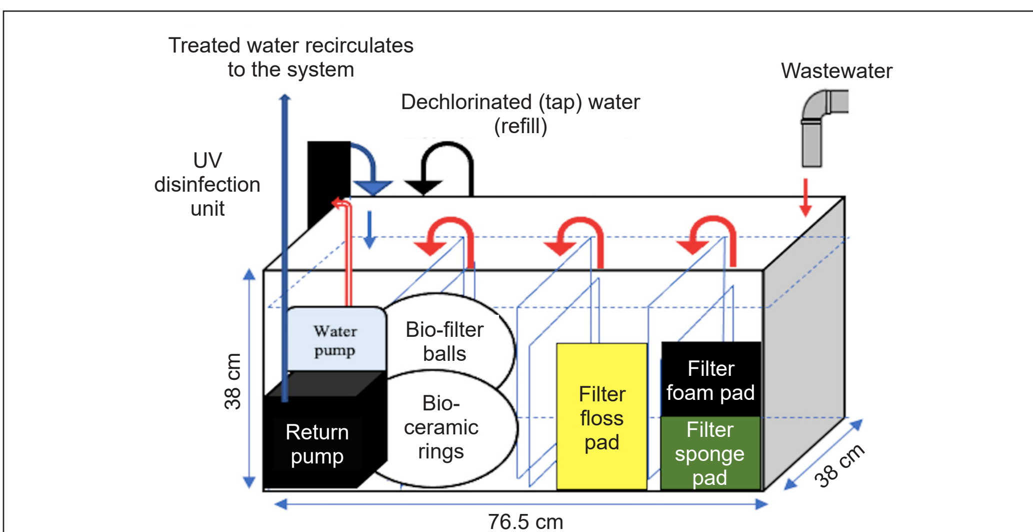


Figure 4. Wastewater (red arrow) undergoes filtration and UV treatment in the sump before recirculating as clean water (blue arrow) to individual tanks. The water pump transfers water to the sterilisation unit, while the return pump brings clean water back to the system.

material, i.e., iron hooks, should not come into direct contact with the sump water, as even trace levels of copper and zinc are toxic (Hernandez et al., 2011; Ribeyre et al., 1995). The sump should be frequently cleaned. A separate syphon eliminates debris from filtration media, uneaten food, and fish waste from the sump as needed. Excessive cloudiness indicates an accumulation of waste.

Mechanical filtration is often the initial step in the filtration assembly of a recirculating system, and its function is to separate and remove suspended solids from the effluent water. Solid waste, such as uneaten food and faeces pellets, must be removed from the circulating water as quickly as possible. It will then disintegrate, providing food for heterotrophic microorganisms. Subsequently, dissolved oxygen concentrations may decline drastically, followed by increased ammonia concentrations. Particle filters for mechanical filtration may be depth or surface filters that collect solids as water passes (Cockington, 2020). As referred to in Figure 4, the coarse and fine mechanical filters were used to fill the sump's first two compartments, consisting of filter foam, sponge, and floss pad (commercial grade supplied by Perniagaan Moon Lai, Pahang). According to Bhargava (2018), scientists rely on foam, sponge or polyester fibres (beginning at 10 microns) for mechanical filtering.

The individual tanks and biological filters of recirculating systems are home to various microorganisms (Rurangwa & Verdegem, 2015). All recirculating systems contain and sustain populations of various microorganisms, i.e., bacteria and viruses. Many species are safe or valuable for fish (e.g., nitrifying bacteria), but some can be harmful, especially in huge numbers. Recirculating systems are designed to preserve water by exchanging a negligible portion of the overall system volume to enable the removal of nitrates. This conservation strategy will accumulate many of these co-occurring organisms in a system over time. In order to maintain low nitrate levels in recirculating systems, it is recommended to utilise a biological filter (commercial grade biofilter balls and bio-ceramic rings/granules supplied by Perniagaan Moon Lai, Pahang) with regular fresh (dechlorinated) water exchange (5-10%/day) (Lawrence & Mason, 2012). Without this filtering, any recirculating zebrafish system would be incomplete (Bhargava, 2018). This process uses fluidised bed biofilters to reduce the total nitrogen content in the system water by bacterial action (Leveque et al., 2016). It is essential because zebrafish are exceedingly harmful to high nitrogen loading, particularly ammonia and nitrite (Harper & Lawrence, 2011; Kroupová et al., 2018). Biological filtration is a process that harnesses the capabilities of chemolithotrophic bacteria, including *Nitrosomonas* and *Nitrobacter*, to cleanse nitrogenous waste by oxidising ammonia to nitrite and nitrate. An effective open-design system should have recirculating water with ammonia levels of 0 mg/L, nitrite levels of 10 mg/L, and nitrate levels of 75 mg/L (McNabb et al., 2012).

The aquaria system employs a disinfection procedure in which water passes through a disinfection unit after solids removal (mechanical filtration), biological filtration, and chemical filtration (optional) to control the populations of the particular organisms (Lawrence & Mason, 2012). UV disinfection unit, also known as an ultraviolet steriliser, use light to produce ultraviolet C (UV-C) ultraviolet radiation with a short wavelength (250-280 nm) that has disinfectant qualities, destroying organisms' DNA and killing or rendering them inactive. There is no established standard for the level of irradiation required for disinfection (Lawrence & Mason, 2012). Following Emperor Aquatics Inc.'s operating and service instructions, an 18-W UV lamp can kill algae, bacteria, and protozoa in an aquarium with 200–250 L of water (Bhargava, 2018). Our system employed 36 W of UV-C power and a maximum flow rate of 4,500 L/hr (model CUV-136, Sunsun, China) to cover a 189 L water storage capacity. Installing the UV assembly in the water inlet line of recirculating systems is typically recommended rather than in the line collecting the waste (Harper & Lawrence, 2011). Figure 4 demonstrates that the disinfection step was performed immediately after mechanical and biological filtration, ensuring that the disinfection unit was supplied with nearly clean water before recirculating throughout the system. Since a UV lamp's lifespan is limited, regularly inspecting its output is recommended (Avdesh et al., 2012).

Pumps and Circulation

All recirculating systems require a route to transport water to higher elevations to serve individual tanks, enhance system pressure, and move water for filtering, disinfection, or other treatment (Timmons & Ebeling, 2013). Water pumps are essential to provide fish with oxygenated, clean water. It aids in the elimination of solid and nitrogenous wastes from individual tanks. The oxygenated water and nitrogenous wastes are transferred to aerobic nitrifying bacteria in the biological filter (Hammer, 2020).

Due to many small-diameter valves and connections that deliver water to several separate tanks in the recirculating system, pumps designed for high water pressures are required. Our system utilised a commercial submersible pond pump (model SOBO WP-12,000 DW, China, with the specification of 175 W and 12,000 L/hr), in line with other commercial systems, as a return pump installed in the sump's last chamber (Figure 4). In addition, a water pump (model Dophin P-2000, China, with specifications of 14.8 W and 700 L/hr) was installed in the last chamber, which connected to the UV sterilisation unit. It helps to move post-filtered water through the UV sterilisation process. The submersible pond pump's motor (model SOBO WP-12,000 DW, China) is a powerful magnetic drive in a waterproof plastic housing that rotates a magnetised impeller blade in the pump head (Malone, 2013). Tiny air leaks on the pump head close to the impeller housing are the most common cause of gas bubble disease (GBD), which leads to a significant mortality rate among zebrafish. GBD can also occur if insufficient water is in the sump near the pump's intake, forcing it to draw in air and water, thus compressing the gases. To prevent GBD, the sump's water level must be regularly maintained. GBD symptoms include tiny bubbles in the water spigots and on the tank walls and fish remaining at the tank's bottom (Hammer, 2020).

The Rack

The rack aims to hold the entire system in place securely (Figures 1 and 2). The zebrafish's housing requirements will define the tank's dimensions, typically housed in groups whose numbers are set by the study. The size and shape of the tank determine the requirements for the rack. Its designation should consider the safety of humans, the benefits of fish housing, and the appropriateness of fish housing. The rack's appropriate design allows for the three-dimensional grid organisation of the tanks and integrates critical aquaria infrastructures. The infrastructure consists of water pipelines for the influent and effluent streams, aeration, lighting, and monitoring capabilities (Lawrence & Mason, 2012).

The single-sided commercial rack was employed in this study. It was measured at $180 \times 120 \times 45$ cm^3: height × width × depth. The rack can be as tiny as a single tabletop unit or as vast as the available space in the room. It is typically designed with four to six regularly spaced shelves to provide easy access to the individual tanks for personnel (Lawrence & Mason, 2012). This rack was configured with three evenly spaced shelves to store seven 17 L tanks (up to a maximum of eight), including the 70 L sump tank.

This rack system adopted a standalone design with a water treatment tank at the bottom, following Lawrence and Mason (2012), who favoured a rack design that utilised space beneath the lowest rack row to house filters, pumps and UV sterilisers. Above each tank, water delivery polyvinyl chloride (PVC) pipes (supplied by ZNE Hardware, Terengganu and sealed using SS100 PVC pipe adhesive) were built into the rack to transport water from a spigot (metal brass was used and supplied by Dynamic Expansion Sdn. Bhd., Pahang) into the tank. Water was collected from each rack, sent to a joint water return pipe, filtered in a separate unit, and recirculated back to the racks (Figures 1 and 2). Aquaneering Incorporated commercial aquatic system (Aquaneering, n.d.) was comparable to our water delivery system. A physically isolated water treatment section considerably reduces noise and vibration in the fish holding or culture area, which is the actual value of this technique (Hammer, 2020).

The water in the zebrafish system contains salts and minerals capable of corroding a range of metals (Lawrence & Mason, 2012). The high salinity of the water (> 300 ppm) could corrode the rack if water spillage or leakage happens during zebrafish handling; consequently, the rack material must be rustproof (Bhargava, 2018). The materials chosen by top commercial providers, such as stainless-steel racks, plastic-coated metal racks, and PVC-coated corrosion-resistant racks, have resolved this issue (Aquaneering, n.d.; Iwaki Aquatic, n.d.; Tecniplast, 2022). In addition, the rack material selected for open-design systems must sustain the whole weight of filled zebrafish tanks and withstand seismic waves (Bhargava, 2018). In the current setup, the rack is made of metal that underwent the most advanced epoxy coating technique during its manufacturing process. The decking material is composed of durable high-density fibreboard that can support up to 200 kg/level (TTF Group, n.d.).

CONCLUSION

Open-design systems are gaining popularity among zebrafish researchers because they are inexpensive, flexible, scalable, and effective. These configurations can also create opportunities for independent work for technical specialists who wish to construct and sell them to regional or national academic institutions or laboratories. In our system, water exchange is currently done manually. Consequently, introducing a programmable and automated water exchange system could be the subject of future research. It would be intriguing to have automation in open-design systems, particularly for cost-effectively measuring oxygen and pH levels and nitrogen content. However, skilled, well-trained, knowledgeable, and experienced human labour remains essential.

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