Zebrafish Husbandry in Laboratory Static Water System - An Alternative, Adaptable and Cost-Effective Protocol for the Model Organism

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Abstract: ***This study describes a simple, low-cost zebrafish (Danio rerio) husbandry system suitable for small laboratories with limited funding. Conventional zebrafish rearing protocols predominantly rely on flow-through or recirculating water systems, which demand substantial space, infrastructure, and maintenance costs. In contrast, the present work documents, for the first time, a complete zebrafish workflow- including rearing, breeding, spawning, hatching, larval nourishment, and long-term maintenance- implemented in a static water system with gradual exchange of water. Adult fish are housed in glass tanks under controlled water quality parameters and a natural day–night light cycle, with additional environmental conditions tailored to approximate natural habitats. Breeding is performed in customized setups at different seasons within the same tanks, and collected eggs are reared under static conditions. Adult zebrafish are fed commercially available dry diets, whereas larvae receive laboratory-hatched Artemia as a live food source. The results demonstrate that this static water protocol can support robust breeding performance and survival rates above 50% from viably collected eggs to adult during monsoon followed by 35-40% in other seasons. This breeding and rearing process is comparable to more complex systems, thereby providing a practical and cost-effective zebrafish husbandry platform for small-scale and resource-constrained laboratories.***

Keywords: Zebrafish; Danio rerio; Static Water System; Laboratory Husbandry; Fish Breeding; Low-Cost Animal Facility

1. Introduction

Zebrafish (*Danio rerio*) have emerged as an indispensable model organism in modern biological research due to their rapid development, high fecundity, genetic, physiological, behavioural attributes, easier laboratory maintenance and observability [1]-[3]. Notably, they are approximately 70% genetically similar to humans with sequenced genome manipulable by advanced gene editing techniques; and external fertilization, transparent embryos, quick growth, selective regenerative capacities etc are making them suitable candidates for developmental and regenerative studies, toxicological studies, drug discovery and chemical safety evaluation [4]-[8]. As zebrafish model provides an effective and sustainable system bringing cellular and molecular biological approaches possible to the major fields of research, they are becoming increasingly popular nowadays and increasingly demanding for laboratory rearing.

Most of the studies delineated protocols for zebrafish maintenance in laboratory which employ artificial light and circulating water in a mechanized or automated setup which advances to recirculating water system [9], [10]. Though, Bhargav outlined three principal approaches of laboratory zebrafish rearing in static water, flow-through and recirculating water system, but present reporting comprises of the open design recirculating and auto-cleaning systems (open-RAC) due to its perfection and scalability, whereas, shows paucity of published protocols and usages of other systems [11]. Avdesh and others published an initial comprehensive video protocol of laboratory zebrafish husbandry with thirty 1.5-liter tanks with an estimated cost of establishment was 9000 USD apart from operational and maintenance costs year around [12]. In 2025, our enquiry for the open-RAC system with twenty 1 litre and twenty 3 litre tanks from Gendanio company is estimated to be approximately 14000 USD as establishment cost. All these systems are efficient in expense of high cost which require guaranteed funding and infrastructural support throughout. These are the real constraints for small scale research and regional laboratories.

In contrast, the static water system may offer a simpler, more cost-effective alternative with establishment and operational simplicity. Modified static water system, if developed with proper quality control and acceptable yield in comparison with the open-RAC system, may act as an acceptable and affordable alternative for the resource-limited laboratory conditions. We are working on such affordable and cost-effective zebrafish rearing system with standard outcome in studying behavioural, toxicological and stress physiological investigations [13], [14]. Presently, we are elaborating the protocol of zebrafish husbandry in our laboratory to furnish it as an affordable and effective alternative operable under limited funding and infrastructural scenario.

2. Protocol

1) Aquarium setup and disinfection procedure:

To successfully rear and breed fish, different tank setups are needed depending on the fish group size. We use glass aquarium of 12″x10″x10″ (LXWXH) dimensions. Make sure to use the right tank size for each fish group.

Cleaning and Disinfection Steps:

- **Initial Wash:** Clean all tanks (stocking tanks for new fish, breeding tanks, etc.) thoroughly with regular soap and tap water, finally rinse and wash in running tap water.
- **Tank Disinfection:** Treat the cleaned tanks with a 2 mg/L concentrated potassium permanganate ($KMnO_4$) solution for 24 hours (***Fig 1a***).
- After disinfection, rinse the tanks once with the same water used for fish rearing. Then, dry them completely and store them upside down.

Equipment Disinfection:

Disinfect all equipment like nets, fish measurement tools, baskets, artificial oxygen pump gears, tubes, nozzles and heaters using the exact same procedure described above. Additionally, before using any equipment for fish rearing, always rinse it with fish-rearing water first.

2) Water quality measurements and recycling of water:

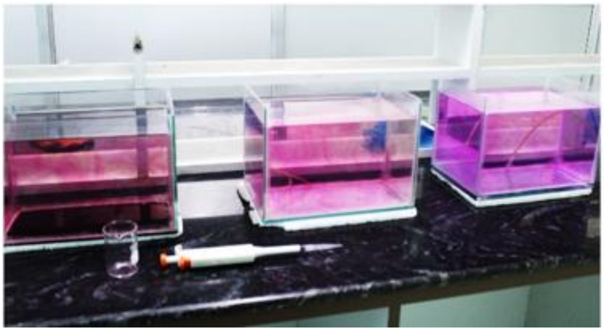
To ensure optimal conditions, water quality is maintained strictly according to OECD guidelines. We utilize treated water supplied by the Kalyani Municipality for all aspects of fish rearing, breeding, and related operations.

The water is pre-aged for two days before being introduced to the fish environment. Water changes are performed regularly. 50% of the tank water is siphoned off and replaced with an equal amount of fresh, two-day-aged water in every 5 days cycle. The entire aquarium system is fully renewed in every 20 days, involving a complete transfer of fish to a fresh, disinfected tank with two-day-aged water (***Fig 1b***).

3) Maintenance of Tank Conditions:

Our protocol ensures the maintenance of a natural day and night cycle. To facilitate this diurnal rhythm, fish tanks are housed in a room equipped with transparent glass windows (***Fig 1c***). The windows are kept open for at least 6 hrs per day during daylight hours to provide natural conditions and air circulation, and closed at night. Our establishment is facilitated by tropical to semi-tropical climate and the fish rearing room positioned adjacent with natural open space.

For crucial seasonal temperature management, in summer, high-speed fans or coolers are employed as per the availability and requirement to reduce heat stress; and in winter, aquarium heaters are utilized to regulate and maintain the water temperature.



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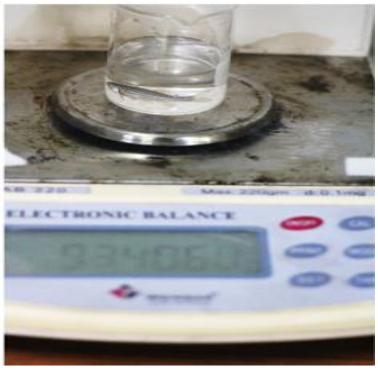

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Figure 1: A– Potassium permanganate treated water filled tank, to disinfect the tanks. B – Water change cycle of tanks, at day 1, fresh water filled tanks are used to rear the fish, after each 7th day 50% tank water was replaced by two-day aged fresh water, while after the 15th day the whole fish group was transferred into a new sterilized tank in two-day aged fresh water. C – Fish tank setup, positioned in a situation where natural air and light is available. D – Measurement of living fish, rectangular glass chamber is prepared to place the fish to measure the length, and the lower glass is graduated in centimetre scale (each with 0.5 cm distance). E – Weighing of fish in living condition (as detailed in text).

4) Fish procurement and handling:

We obtained Wild type Zebrafish from the Centre for Laboratory Animal Research and Training (CLART) at the West Bengal Livestock Development Corporation Limited (WBLDCL).

Upon transport to the laboratory, we carefully moved the fish into a stock tank. The tank's water was prepared by combining two-day-aged Municipality-supplied water (50%) with the water the fish arrived in from the breeder (50%). This gentle introduction helps them adapt.

We offered food right after the transfer to help reduce their stress. Two days after receiving the fish, their length and weight measurements can be taken.

Transfer of fish to any new tank will be done by using the nylon fish net with ensuring that no other previous food particle, faecal matter or any contaminations pass to the fresh tank. This transfer is carried out with 30% of previous tank water to 70% of two-day-aged supplied water unless any contamination found in the previous tank. In that case only healthy fish are transferred to a properly disinfected tank with 100% of same aged municipal water as mentioned earlier, while regular transfer follows the 50% of previous and 50% of new water aged in laboratory condition. The municipality-supplied tap water used in this study is with standard water parameters (***Table 1***) that worked properly for our purpose.

Table 1: Water parameters used for fish rearing in our laboratory tested by Kalyani State Referral Laboratory, Govt. of West Bengal, India.

Parameters	Result	Acceptable range
pH	7.65	6.5-8.5
Turbidity (NTU)	0.44	1-5
TDS (mg/L)	333	500-2000
Chloride (mg/L)	12.81	250-1000
Total hardness (mg/L)	278	200-600
Total Alkalinity (mg/L)	296	200-600
Calcium (mg/L)	82.25	75-200
Ammonia (mg/L)	0.01	0.01 – 0.05
Dissolve Oxygen (mg/L)	3.6	3-7
Magnesium (mg/L)	17.42	30-100
Temperature (°C)	28 ± 3	

5) Measurement of fish:

The measurement of live fish is a critical task. Length measurements are conducted using a purpose-built scaled glass apparatus (***Fig 1d***), where each marked line corresponds to 0.5 cm, facilitating easy and precise readings by placing the fish in the chamber in water.

For weight determination, fish are initially placed in a beaker containing a volume of water, and the total mass is recorded. Following this, the fish are carefully removed, placed in the beaker and total weight of the system is measured (***Fig 1e***). The fish's individual weight is then derived by subtracting the final weight from the previous one.

6) Zebrafish Rearing:

It is done with a density of twenty fish per ten litres of water in each aquarium. We use a cover for the tanks with a fine mesh. This allows natural air and light to enter while keeping unwanted objects out.

7) Feeding of fish:

Adult fish groups receive **"**TetraBits Complete" (Tetra, Germany), a commercially available dry feed. This diet comprises protein, crude oils, fats, fibre, and moisture. Feeding occurs once daily, with an allowance of 10 to 15 mg of food per gram of fish biomass.

Due to their inability to consume dry feed, newly hatched zebrafish are sustained on live or stored *Artemia*. *Artemia* are introduced to the hatchlings exclusively after 96 hours post-fertilization. (The methodology for *Artemia* hatching will be subsequently elaborated).

8) Procedure for breeding:

Successful fish mating and breeding in a laboratory setting hinge on optimal environmental conditions and a well-maintained water system. In our protocol, fish tanks are positioned by windows to provide a natural day-night cycle (***Fig 2***).

For breeding, we use a clean, disinfected fresh tank filled with 7 to 8 Liters of water, ensuring the water level remains well below the tank's upper edge. A basket is then placed inside the tank, partially submerged, with pores small enough to prevent fish from escaping.

To initiate mating, a group of male and female fish (in a 1:2 proportion) (***Fig 2***) is introduced into the basket area. This transfer occurs during the daytime, after feeding, to allow the fish ample time to acclimate to each other and their new environment. We select female fish with swollen bellies to maximize egg production. The following morning, the fish group is returned to their native tank.

Hatchlings typically become visible after 48 hours and begin moving after 96 hours post-hatching. From the fifth day after hatching, hatchlings are fed living or stored *Artemia*. After 10 days, the new-borns are sieved and transferred to a fresh tank where they continue to grow with regular feeding.

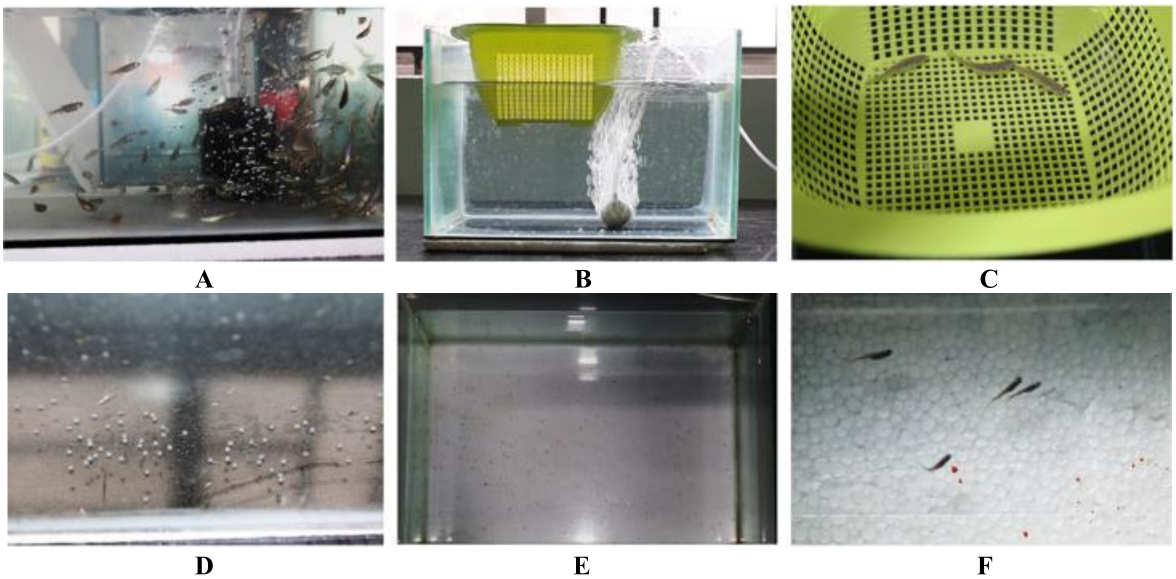

Figure 2: The photographic representation of different stages of adult fish rearing, breeding, nursery and hatchling maintaining and new fry rearing. A- Adult Zebrafish in Stock tank. B- Side view of breeding tank, a 3 mm mesh sized sterilized plastic basket compatible to breeding tank opening is used to conduct mating. After external fertilization eggs are settled down at the bottom of the tank, and adult fish are removed and placed to their original tank. C- Mating behaviour shown by the adult fish in breeding tank. D- Visible eggs at the bottom of the tank. E- 2 days old hatchlings started moving in the tank. F- New adult (1.5 months old) moving freely in fresh water.

9) Zebrafish hatchling maintenance:
After the successful fertilization, it takes 48 hrs to visualize the hatchlings in the tank. In that stage, they look like a small (0.3-0.5 cm) black line, sticks on the glass wall. It is the non-feeding stage of their life. At 5 days post-fertilization (dpf) the hatchlings start to move (***Fig 3***). At the 6 dpf the hatchlings start to feed, and they are fed with live artemia or freeze artemia up to 30 dpf. After 30 days, they can be fed on food pallets. To transfer the hatchlings to a fresh tank, the soft nylon/silk nets of sieve size 120 µm or artemia nets are used. They are transfer very carefully to the new tank upon nets drowned in a beaker full of previous tank water and then released in the new tank water.

10) Artemia production, sieving and storing:
Artemia salina, or brine shrimp, naturally thrive in salinities around 70,000 ppm. Therefore, laboratory hatching requires a similarly high-saline aquatic environment, which we achieve by dissolving NaCl in tank water.

To grow *Artemia*, we use transparent, clean glass jars filled with saline water at a concentration of 50,000 to 70,000 ppm (achieved with NaCl). Jars are supplied with artificial oxygen and illuminated by a high-power bulb positioned above. This bulb provides both the necessary light and water warmth for *Artemia* cyst for hatching. We add 10 mg of cysts per litre of water, and activate the system (***Fig 4***) for 30 to 40 hours.

After hatching, live, reddish *Artemia* will be visible in the jar. We sieve the *Artemia* using an arrangement of a pair of the mentioned nets. The sieved *Artemia* can then be fed directly to new-born zebrafish or stored at 4°C for 5 to 10 days.

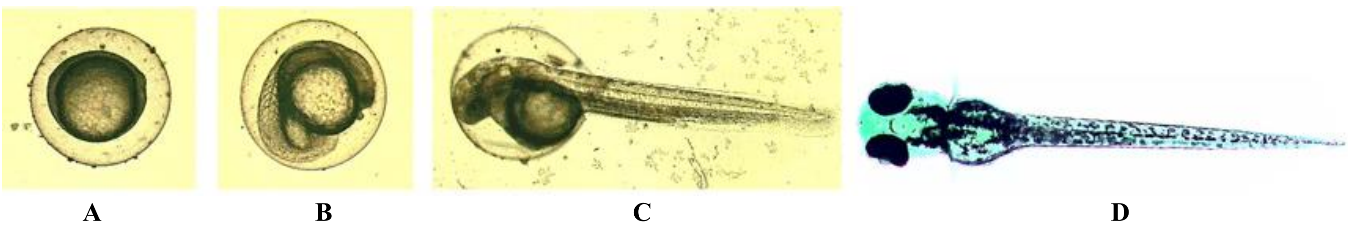

Figure 3: Different life stages of Zebrafish under microscope. A- Zebrafish egg immediate after the lay. B- Fertilized egg, C - One day old hatchling, D – Five days old hatchling with visible eye, pigmented cells, heart pumping, operculum etc.

A B

Figure 4: Artemia grown in the laboratory condition with maximum yield. A- the set up for hatching of artemia from commercially available cyst. B- Artemia after the hatching, sieved by using the nylon artemia nets. Two different types of mesh used. At the top, egg shells accumulated and at lower net living artemia settled.

Cost estimation of static water zebrafish husbandry setup: An approximate cost for setting up a laboratory Zebrafish housing system (consist of 11-12 numbers of aquarium) has been estimated here. The following set up is suitable for stocking 200 fish at a time, while in running experimental condition 200 to 250 fish can be easily maintained in this set up.

Required items	Description	Quantity	Uses	Price (INR)
Wooden Table	Wooden table with 12ftx2ftx3ft (LxWxH) size	1	To set the aquarium in a proper way	8000
Stock Tanks	Glass tank with the dimension (LxWxH) 30″x12″x18″	1	Use for stocking the fish after procured from the certified seller	1000
Small tanks	Glass tank with the dimension (LxWxH) 12″x10″x10″	10	Use for experiments, breeding and as nursery	5000
Glass container	Glass container having capacity of 4 L	2	Use for growing Artemia	200
Net	Measure as the upper opening size of glass tanks		To cover the tanks, to prevent to enter unwanted things in the water	500
Oxygen pump	Multichannel	1	Generating oxygen to supply into the fish tanks	2000
Pipe	Nylon transparent pipe		To supply the aeration to all the tanks	200
Nozzle	Both side or triple side opening plastic nozzle	30	To connect the pipes for aeration to the tanks	300
Mesh	5 ″/5 ″ nylon mesh	1	To capture the fish from tanks	100
Plastic Basket, mug, bucket and others	Varies	1 each	Multipurpose	300
Siphon	Commercially available multipurpose Siphon	1	To exclude the water from the tanks	100
Thermostats	75 W glass water heater	12	To maintain the temperature of water in winter	2400
	Total			20100

This costing is approximately INR 20100, which is estimated only around USD 230 as the establishment cost of the setup. There is a minimal operational and recurring cost and needs involvement of one non-technical human resource who shall be trained for a couple of days to handle the fish and the setup.

Estimated yield of fish (monsoon breeding):

Subject	Numbers	Photo
No of fish breed	3 (2 female, 1 male)	

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Egg yield	~ 100	
Fertilized Eggs	~ 70-75	
Hatchling	~ 60	
Adult	~ 55	

Seasonal variation of Breeding success and survival

To understand the suitable breeding season for laboratory rearing fish, they are introduced for mating and laying eggs in summer, monsoon and winter separately. A variation in egg yield and survival of adult individuals in each group are represented in the ***Table 2*** (shows follow-up survival data up to 450 days), where we found best result in monsoon followed by summer and winter. While they are reared in the laboratory condition for more than a period of 2 years, their mortality rate decreased less than 5 % yearly after reaching maturity. Till date, 3 cycles of zebrafish breeding and rearing have been maintained in the laboratory with the yield utilized in different experiments.

Table 2: Fish breeding, hatching success and survival variation in different seasons

	Breeding Day	Day 0	Day 1	Day 30	Day 90	Day 270	Day 450
Season	No of fish bred	Egg yield	Fertilized egg	Hatchling survive	Adult	Adult	Adult
Summer (May)	3 (2 female, 1 male)	~ 90	~ 60-65	~ 45	44	44	42
Monsoon (September)	3 (2 female, 1 male)	~ 100	~ 70-75	~ 60	56	55	53
Winter (January)	3 (2 female, 1 male)	~ 70	~ 45-50	~ 40	37	35	33

Modifications done

Previously documented Zebrafish housing setups are arranged in a closed-door system where natural air circulation is absent, while in the present study it was modified. All the tanks are positioned such a way that it would be supplied with mostly natural conditions, such as air circulations, temperature, bright or dimmed light weather due to sunny or cloudy days and diurnal light and dark cycle, and these were maintained by using no artificial light. Modifications made in the regular maintenance cycle of the tanks, in water exchange volumes and seeding density. The conditions were standardized as described in the protocol. An adaptable procedure of simple, cost effective, yet highly efficient breeding chamber and collecting tank setup had been employed which produced about 50 adults per sets of 1:2 breeding pairing of male to female. Our system thrives on the local municipality supplied running water which maintains the standard parameters (***Table 1***) and a periodic partial to full exchange of tank water as described in the protocol. The whole system needs minimal equipment and operational costs.

3. Conclusion

Minimal previous reports are present where zebrafish rearing in static water system were performed while the protocol for the same is not available as such [15], [16]. Present study showed that the above-mentioned protocol is suitable for the zebrafish rearing in laboratory condition in controlled natural influences. As our setup is established in tropical to semi-tropical climate, and natural environmental surroundings with air circulation and light conditions exists in the room, these may provide a natural support system for the zebrafish. We can connect these conditions with the natural evolutionary history and conditions of zebrafish where this model organism is naturally occurring in the north-eastern region of the Indian subcontinent [17], [18].

This study presents a practical and economical static-water zebrafish husbandry protocol suitable for laboratories with limited infrastructure and financial resources. The system supported routine maintenance, breeding, hatching, and juvenile development using simple equipment and periodic water replacement. The protocol demonstrated satisfactory fish survival and reproductive performance under standardized conditions. Owing to its low establishment and operational costs, this approach may provide a useful alternative for small-scale zebrafish facilities and educational or research laboratories. Further comparative studies with recirculating systems would help validate its broader applicability.

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Ethical considerations:
The experimental analysis with the Animal model is approved by Institutional Animal Ethics Committee of Visva-Bharati University (Regn No. 1819/GO/Re/S/15/CCSEA) in accordance with the guideline of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Govt. of India with approval Ref. No. IAEC/VB/2025-II/02.

Declaration of conflicting interest
The authors declare no competing interests.

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