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Research Article

Modification of Aquarium with Continuous Water Inlet-Outlet System Validated for Longitudinal Stocking of Fish under Laboratory

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Abstract: Unequivocally fish is considered one of the most crucial experimental animal models in basic and applied biomedical research. Fish in the laboratory requires proper housing and handling. The inadequate aquatic facility causes suffering and discomfort to fish. In this article, we discussed the limitations of filtering equipment and their long-term usage associated with hazardous effects on fish health. We attempted to design and construct the aquarium with a simple technique without implementing any filtering equipment. The design resolves to minimize the impact of potential toxins in the aquarium. The design of the proposed aquarium is more appropriate for longitudinal research protocols and housing since it overcomes the constraints of water renewal at the time of experiments without disrupting a test fish's normal behavior. The aquarium provides better functional efficacy for fish welfare. We observed the circadian locomotor activity rhythm of fish and validated it by housing them in test aquarium both singly- and in group-housing conditions under LD 12:12. We concluded that housing in the new test aquarium facilitates better stocking practice when housed for a prolonged duration. The designed aquarium possesses a self-draining facility that helps elude the extent of water deterioration and water turbidity due to suspended wastes, thus enabling a stress-free environment for stocking.

INTRODUCTION

Piscine research is significant and is widespread around the world for centuries. Today, it has become mandatory to provide proper care and comfortable housing to the experimental animals during confinement and experimentation. Many countries have promulgated laws for animal experiments with the prime objective to carry out laboratory tests with appropriate ethical practice. These responsibilities include providing adequate food supply, proper handling, housing, and stress-free stocking of animals while using them in experiments [1, 2]. It is also equally important to outline the related overlooked factors in experimental trials likely to jeopardize fishes under laboratory. The turbidity of water in an aquarium can affect the fish's normal behavior and their capability to interact with other individuals and the environment. When turbidity increases, the fish become less active, and their social interaction alters. Thus, turbidity weakens the fish's ability to use physical cues [3, 4].

The fish movement is also responsible for creating an unclean environment and stress. The fish movement prevents the particles and solid wastes from settling down [5], thereby causing turbidity in the aquarium. The extent of the movement-induced effect is likely to interfere with the outcome of experimental studies. Different fish species require different tank sizes and unusual design. The Prevention of Cruelty to Animals Act, India [6] also emphasizes the animal's housing conditions. It strictly stipulates the following: "Treating animals cruelly - if any person '*keep or confine any animal in any cage or other receptacle that does not measure sufficiently in height, length and breadth to permit the animal a reasonable opportunity for movement*' he shall be punishable." There is a lack of documented research on the need for standardization of tank/aquarium environment and environmental enrichment with the optimum space availability.

The density of fishes is also one of the critical measures for the experimental setting. The reports on fish survivability, growth, and welfare concerning water quality parameters and stocking have revealed an increase in stocking density leads to rapid water quality deterioration and food deprivation, thereby building up stress [7-10]. Improper stocking or crowding can reduce fish welfare and their longevity [11-13]. The accumulation of nitrogenous waste products also increases as an increase in stock [14]. Some adverse effects are also due to the raised amounts of suspended solids. The "Ruptured Intestine Syndrome" (RIS) or "Open Belly Syndrome" has also been experienced by

researchers in fishes. It is caused mainly due to excess feeding or high feed loads by electronic feeders [15].

The water circulation system also influences the quality of water. Because of water resource scarcity and restraint in water supply, recirculation systems were proposed and progressively introduced in fish trials. It is imperative to maintain water quality for longitudinal studies. The available commercial auto-filtering techniques are costly and hazardous too. These filtering techniques rely on water recirculation systems. The latter and oxygenation procedures used in many aquaculture and rearing farms have several adverse effects on fish life [16, 17]. There are many reports on fish diseases due to the faulty water recirculation system. Noble and Summerfelt [17] reported many bacterial, fungal, viral diseases due to the reuse water system. Some significant conditions are: (a) bacterial gill disease, (b) furunculosis, (c) fin rot, (d) proliferative kidney disease, (e) amoebic gill infestation, (f) infectious pancreatic necrosis, (g) viral haemorrhagic septicaemia, and (h) infectious hematopoietic necrosis [18-20]. The recirculation of water is ideal for such diseases due to the suboptimal water condition for microbes and pathogens. The filtering and recirculation system requires a highly specified treatment system, like clarifiers-that remove solid wastes, bio-filters, etc. These treatments also have some limitations, like most conventional clarifiers do not remove colloidal solids (~20 ppm) and support organic solids [21]. The bio-filtration technique consists of microbes that degrade and dissolved wastes metabolically. However, we know that such metabolic activity lowers the oxygen levels and produces carbon dioxide, eventually leading to low water pH [21]. The active carbon dioxide control is essential with a combination of gas transfer techniques and chemical methods [22-24].

We remain unaware of the drawbacks and hazardous effects of these filtering techniques. Therefore, it is pertinent to look for alternative approaches for maintaining a reasonably good quality of water. To get rid of these shortcomings, we need to design an aquarium integrated with simple techniques of draining wastes that aid in optimizing rapid water deterioration and have higher efficacy for stress-free housing and stocking ailment.

We attempted to design an aquarium that simplifies the filtering process without electronic circuitry, mechanical device, and bio-filters. We planned to make it easy and non-chaotic, taking into consideration space constraints. We picked up the test fish's locomotor activity rhythm as the assay variable to judge the efficacy of the aquarium's proposed new design.

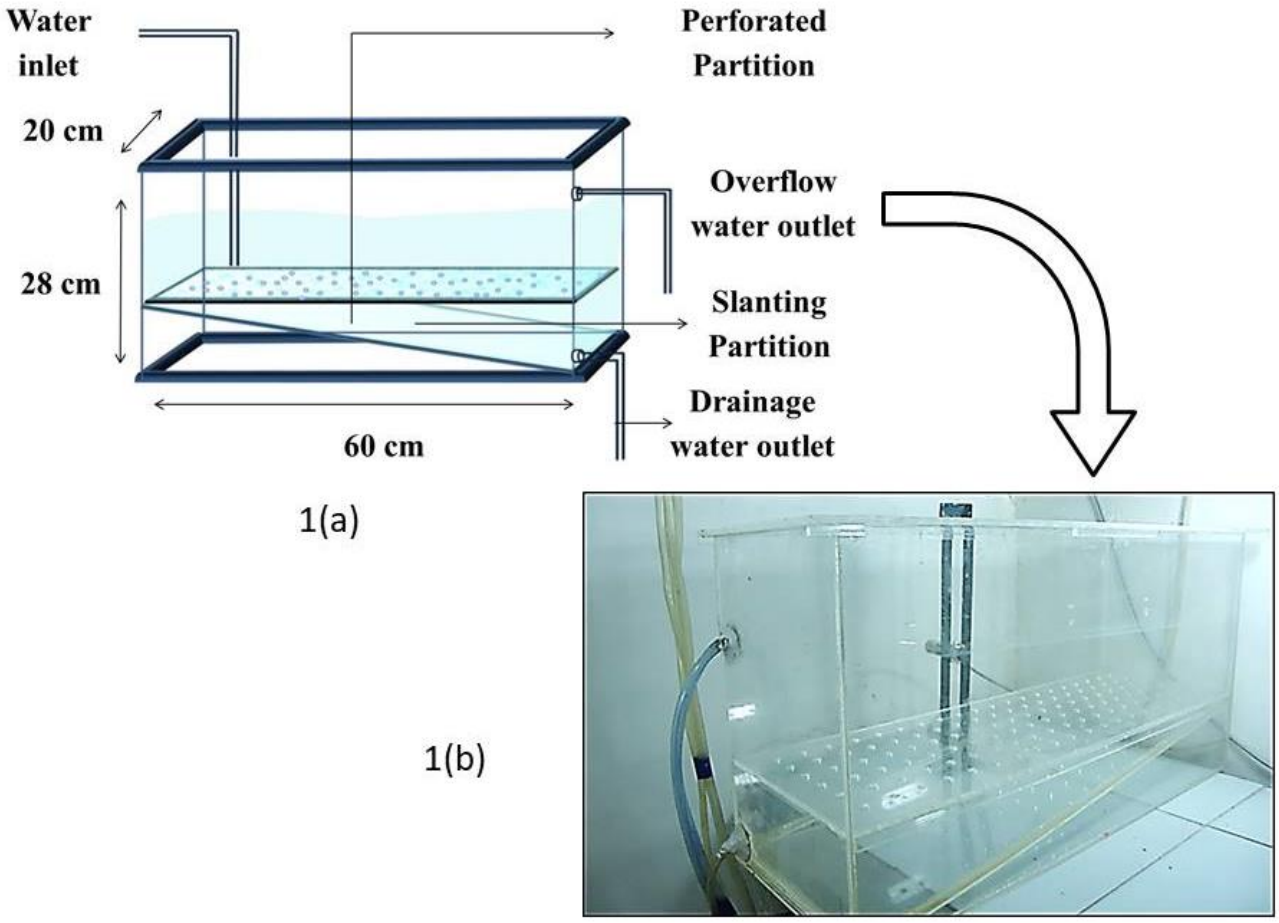

Figure 1. (a) Schematic diagram of the aquarium with perforated partition, single slanting partition, an outlet at the upper side of the edge, and a self-drainage outlet for debris. **(b)** The aquarium's actual image is made of acrylic fibers of 4 mm with perforated partition and slanting partition and has an overflow outlet and self-drainage outlet.

MATERIALS AND METHODS

Preparation and designing of the aquarium for laboratory purposes

We designed a cost-effective aquarium that helps in getting rid of debris through a self-drainage facility. We used the acrylic fiber sheet (4 mm thick) to construct the aquarium with dimensions: 60 x 20 x 28 cm. It consists of two horizontal bases (60 x 20 cm each) placed parallel to each other with a vertical distance of 8 cm between them. The bottommost floor is a plain sheet of fiber, whereas the layer above it is a perforated sheet. We placed an inclined partition so that the left end of the sheet hangs from the aquarium's left side at the height of 8 cm and touches the bottom platform at the base level. We fitted the aquarium with one inlet for fresh water and two outlets; one located at the bottom for self-drainage and another outlet at the height of [22 cm] for the overflow of excess water (Fig. 1).

Experimental model

Heteropneustes fossilis (Siluriformes, Heteropneustidae) is an indigenous species of Asian sub-continent widely distributed in India, Pakistan, Sri Lanka, Nepal, Bangladesh, Indonesia, parts of Iran, Myanmar, and the Andaman Islands. It is known as Asian stinging catfish or fossil cat and is famous locally as India's *singhi* fish. It is present in swamps, ditches, and ponds. The *H.fossilis* is a night-active species, slightly aggressive and territorial. The fish is quite gregarious with small groups, and it also forms a social hierarchy in the group. It is known to be a bottom feeder and produces lots of waste when kept in tanks; therefore, requires high tank maintenance [25]. It is an aquaculturally and commercially important species in Asia due to its medicinal value and breeds in confined water.

Procurement of fish and acclimatization

The catfish species are famous for experimental trials in the laboratory. We obtained the live specimens of catfish, *Heteropneustes fossilis* from the Directorate of Fisheries, Government of Chhattisgarh, Raipur, India. They were acclimatized to laboratory conditions in stock aquaria for about 15 days. During this period, the fish were exposed to light and dark schedule 12:12 and ambient temperature. *Lighting condition:* An automatic timer (Analog Time Switch FM/1 Quartz) was used to regulate the experimental cubicle's photoperiod. Each aquarium was illuminated using a CFL tube of 20-watt light units. The intensity of light at the surface level of water in each aquarium was ≈250 lux during the photophase. *Water quality*: The quality of water that includes pH, nitrate, nitrite, and ammonia was examined with aquarium water quality kits (API Fresh Water Master Test Kit, India) and maintained within safe limits. *Temperature:* The experimental cubicle's air temperature was regulated as far as possible at a constant level using an air-conditioner with a set value fixed at 25°C. *Food provision:* Fish were provided with commercial fish food. This commercial food is composed of fish meal, shrimps, corn protein, soybean protein, and fish oil with the nutritional status of a minimum of 28% protein. The food was adequately provided at a random time of the day during the photophase of the LD schedule.

Experimental setup

The 20 adult female catfish *H. fossilis* of standard body length (12-17 cm) and body weight (15-20 g) were randomly selected from the stock aquaria and transferred to the experimental room. The fish were housed either in solitude (n = 1) or a group (n = 4) in the specially designed aquaria. The fish under each housing condition were housed in replicates of four (Fig. 2). The locomotor activity rhythm of fish in single- and group-housed has been investigated as a behavioral indicator. The fish were kept under the LD cycle (LD_1 12:12) with the light on time at 05:00 and light off at 17:00. The locomotor activity was recorded continuously for 25 days (day 1st to the day 25th). After that, the fish in single- and group housing were exposed to constant darkness (DD) for 20 days (day 26th to the day 45th) to validate any masking effect due to the water flow system and then were re-entrained to photoperiod for another 25 days (day 46th to the day 70th) in LD cycle (LD_2 12:12) sequentially in a similar specimen.

Figure 2. The experimental set up shows eight aquaria placed on two selves. The fishes were housed either in solitary or in a group. Each aquarium is fitted with a thermometer, water replenishment system, and a pair of IR sensors (Tx & Rx).

Experimental trial by housing fish on test aquarium

We recorded locomotor activity in catfish, *H. fossilis* using infrared electronic switching sensors (Tx and Rx) connected to a specialized data-logger, Stanford Chronobiology Kit (Stanford Software Systems, Santa Cruz, California, USA). The locomotor activity data help us to understand the biological phenomena in the form of continuous periodic events. The rhythmic event was characterized by (a) mean level of activity, (b) the amplitude of the rhythms, and (c) the timing of the peak as an elapsed time from the local mid-night. The direct observation for any stereotypical behavior or any other changes, such as any scars and wounds on the body, and body-color changes were observed manually.

Ethical approval

The Institution Research Committee approved the experimental design, animal model, and study protocol of the Pandit Ravishankar Shukla University, Raipur, India (Notification No. 10891/Acad/Ph.D./2013; dated 17/06/2013) duly on the recommendations of the Departmental Research Committee. Our research methods complied with the mandates and principles outlined by the Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research of the National Research Council (1996).

Statistical analyses

The recorded locomotor activity counts were retrieved using the Chronobiology Kit software and transferred to the Microsoft Excel sheet. The activity counts were log-transformed before computation and parametric statistical analysis. The rhythm parameters, such as (1) circadian mesor (M), (2) amplitude (A), and (3) acrophase (Ø, time of the peak activity) of locomotor activity were computed using time series data analysis software, namely Cosinor rhythmometry [26-28] at the fixed window with a period, τ = 24 h. We hypothesized that the housing in the specially designed aquarium is suitable for singly- and group-housed fish. There are no significant differences in the mesor, amplitude, and acrophase when singly and group-housed fish were compared. The fish were housed in single or group conditions for the total experimental duration of about 70 days in the test aquarium by exposing them to LD_1 followed by DD and again to LD_2. 'LD_1' was the first photoperiod duration that started from day 1st till day 25th. After that day 26th till day 45th was the exposure period to constant condition (DD); then again to second photoperiod duration 'LD_2' that started from day 46th till day 70th. The two-way ANOVA was employed to examine the effects of two factors, namely the photoperiod, i.e., "LD_1 vs. LD_2" and "group condition: "singly vs. group." In this study, we did not include DD data for comparison as our main focus was to validate the design of an aquarium for monitoring of locomotor activity of fish on a longitudinal time scale. We also hypothesized that the housing for a longer duration in the test aquarium does not modulate the locomotor activity behavior of fish in both conditions (singly or group). The paired sample *t*-test was used to compare the difference in mesor and amplitude of locomotor activity between the initial week (LD_1) and the last week (LD_2) of housing. The conventional statistical analyses were also employed with the help of SPSS ver. 20.0 for Windows (SPSS).

RESULTS

Locomotor activity pattern in singly- and group-housed fish

All the replicates of singly- (S1 to S4) and group-housed fish (G1 to G4) displayed significant locomotor activity rhythm in both LD_1 (Table 1a; $p<0.001$) and LD_2 (Table 1b; $p<0.001$) conditions. In both groups, a nocturnal pattern in locomotor activity was evident. Individuals in the former displayed the peaks ranging between 21.96 and 22.66h in LD_1 (Table 1a) and between 20.81 and 22.43h in LD_2 (Table 1b). In group-housed fish, the peak spread ranged from 20.95 to 22.36h in LD_1 (Table 1a) and 22.34 to 22.90h in LD_2 (Table 1b).

Table 1a. Circadian variation in locomotor activity (counts) in fish housed either in solitude (S1 to S4) or a group of four (G1 to G4) under **LD_1 12:12**. The data were sampled at 10-min epoch over 25 consecutive days. The data were log-transformed before subjecting them to Cosinor rhythmometry at the fixed time window with τ = 24 h. There are 3600 data points (144 data points day^{-1}) in each time series

Replicate	*p*-value*	M ± SE	A (95% CL)	Ø in h (95% CL)
S1	<0.001	0.53 ± 0.006	0.52 (0.50, 0.55)	22.29 (22.13, 22.46)
S2	<0.001	0.23 ± 0.005	0.25 (0.23, 0.27)	22.37 (22.08, 22.65)
S3	<0.001	0.14 ± 0.004	0.10 (0.09, 0.12)	21.96 (21.39, 22.52)
S4	<0.001	0.44 ± 0.006	0.45 (0.43, 0.47)	22.66 (22.46, 22.86)
G1	<0.001	0.34 ± 0.007	0.31 (0.29, 0.34)	21.83 (21.52, 22.15)
G2	<0.001	0.55 ± 0.007	0.47 (0.44, 0.50)	22.36 (22.13, 22.58)
G3	<0.001	0.81 ± 0.01	0.42 (0.38, 0.46)	21.92 (21.55, 22.29)
G4	<0.001	0.67 ± 0.009	0.28 (0.24, 0.31)	20.95 (20.51, 21.39)

*p from an F-test of null amplitude rejection hypothesis; [M]rhythm-adjusted average of the best-fitted Cosine function ± 1 standard error; [A]half of the difference between the maximum and the minimum of the Cosine function (95% confidence interval); [Ø]time in hour of the maximum in the Cosine function (95% confidence interval) referenced to local midnight.

Table 1b. Circadian variation in locomotor activity (counts) in fish housed either in solitude (S1 to S4) or group of four (G1 to G4) under LD2 12:12. The data were sampled at 10-min epoch over 25 consecutive days. The data were log-transformed before subjecting them to Cosinor rhythmometry at the fixed time window with τ = 24 h. There are 3600 data points (144 data points day-1) in each time series.

Replicate	*p*-value*	M ± SE	A (95% CL)	Ø in h (95% CL)
S1	<0.001	0.39 ± 0.006	0.23 (0.20, 0.25)	21.83 (21.44, 22.22)
S2	<0.001	0.38 ± 0.007	0.38 (0.36, 0.41)	20.81 (20.55, 21.06)
S3	<0.001	0.47 ± 0.007	0.41 (0.38, 0.43)	22.43 (22.19, 22.68)
S4	<0.001	0.47 ± 0.007	0.47 (0.44, 0.50)	22.14 (21.92, 22.36)
G1	<0.001	0.63 ± 0.009	0.45 (0.41, 0.48)	22.34 (22.07, 22.61)
G2	<0.001	0.97 ± 0.01	0.49 (0.45, 0.53)	22.90 (22.62, 23.17)
G3	<0.001	1.06 ± 0.01	0.40 (0.36, 0.44)	22.67 (22.28, 23.05)
G4	<0.001	0.95 ± 0.01	0.33 (0.29, 0.37)	22.66 (22.21, 23.11)

*p from an F-test of null amplitude rejection hypothesis; [M]rhythm-adjusted average of the best-fitted Cosine function ± 1 standard error; [A]half of the difference between the maximum and the minimum of the Cosine function (95% confidence interval); [Ø]time in hour of the maximum in the Cosine function (95% confidence interval) referenced to local midnight

Effects of the factors' photoperiod (LD_1 vs. LD_2)' and 'group condition (singly vs. group)' on the locomotor activity

Both the factors' photoperiod' and 'group condition' produced statistically significant effects on the circadian mesor only (p<0.05 and p=0.001, respectively Table 2). However, they did not provide a statistically significant individual impact on the amplitude and the acrophase of the rhythm in locomotor activity. Interestingly a significant interaction effect (p=0.016) was witnessed on the acrophase of locomotor activity rhythm (Table 2).

Effect of prolonged housing in test aquarium on locomotor activity rhythm

The prolonged housing in the test aquarium for about 70 days might influence locomotor activity's mesor and amplitude. Table 3 depicts the summary of circadian mesor and amplitude of locomotor activity for the first week (days 1-7) of LD_1 and the last week (days 64-70) of LD_2 for the singly-housed (coded S1FW to S4LW) and the group-housed fish (coded G1FW to G4LW). Comparisons of circadian mesor and amplitude were made between the first week (FW) and the last week (LW) for both the groups separately. Results revealed a statistically significant difference between the circadian mesors of FW and LW in the group-housed fish only (0.56 ± 0.19 and 0.86 ± 0.22; t_3 = 13.910; p = 0.001). Further, statistically significant difference between the FW and the LW could not be validated for the mesor (0.28 ± 0.17 and 0.54 ± 0.10; t_3 = 1.863; p = 0.15,) or amplitude (0.32 ± 0.22 and 0.46 ± 0.15, t_3 = 0.827; p = 0.46) of the singly-housed fish; and the amplitude of group-housed fish (0.34 ± 0.09 and 0.33 ± 0.03, t_3 = 0.117; p = 0.91).

Table 2. ANOVA summary: Effects of the factors "photoperiod" (LD_1 *vs.* LD_2) and "group condition" (single *vs.* group); and their interaction (GC x LC) on the circadian rhythm characteristics of locomotor activity of *H. fossilis*

Source*	Variable	df	SS	MS	*F*	*P*
Light (LC)	Mesor	1,12	0.162	0.162	5.88	0.032
	Amplitude	1,12	0.008	0.008	0.54	0.47
	Acrophase	1,12	0.130	0.130	0.52	0.48
Group (GC)	Mesor	1,12	0.537	0.537	19.50	0.001
	Amplitude	1,12	0.007	0.007	0.48	0.50
	Acrophase	1,12	0.081	0.081	0.33	0.57
GC x LC	Mesor	1,12	0.047	0.047	1.72	0.21
	Amplitude	1,12	0.00002	0.00002	0.002	0.96
	Acrophase	1,12	1.946	1.946	7.923	0.016

*Source of the variation in the data; [df]degrees of freedom; [SS]sum of squares; [MS]mean squares; [F]*F*-statistic; [P]*P*-value

Table 3. Summary of circadian mesor (M) and amplitude (A) of locomotor activity in fish housed in solitude (S1FW to S4LW) and group-housed (G1FW to G4LW). The data code S1FW to S4FW and G1FW to G4FW was the first week's (LD_1) activity data, and sequentially S1LW to S4LW and G1LW to G4LW were the last week's (LD_2) data of mesor and amplitude of locomotor activity of fish. The data were sampled at 10-min epoch over seven consecutive days. The data were log-transformed before subjecting them to Cosinor rhythmometry at a fixed time window (τ = 24 h). There are 1008 data points (144 data points day^{-1}) in each time series.

Code	*p*-value*	M ± SE	A (95% CL)
S1FW	<0.001	0.53 ± 0.01	0.62 (0.58, 0.66)
S2FW	<0.001	0.18 ± 0.008	0.21 (0.18, 0.24)
S3FW	<0.001	0.16 ± 0.009	0.16 (0.13, 0.19)
S4FW	<0.001	0.30 ± 0.009	0.35 (0.32, 0.39)
S1LW	<0.001	0.44 ± 0.01	0.28 (0.23, 0.33)
S2LW	<0.001	0.59 ± 0.01	0.65 (0.59, 0.70)
S3LW	<0.001	0.66 ± 0.01	0.46 (0.42, 0.51)
S4LW	<0.001	0.47 ± 0.01	0.47 (0.42, 0.51)
G1FW	<0.001	0.29 ± 0.01	0.30 (0.25, 0.34)
G2FW	<0.001	0.56 ± 0.01	0.48 (0.43, 0.53)
G3FW	<0.001	0.71 ± 0.01	0.25 (0.18, 0.31)
G4FW	<0.001	0.68 ± 0.01	0.33 (0.27, 0.39)
G1LW	<0.001	0.55 ± 0.01	0.35 (0.30, 0.40)
G2LW	<0.001	0.87 ± 0.01	0.30 (0.24, 0.37)
G3LW	<0.001	0.99 ± 0.02	0.37 (0.30, 0.45)
G4LW	<0.001	1.04 ± 0.02	0.31 (0.23, 0.39)

*p from an F-test of null amplitude rejection hypothesis; [M]rhythm-adjusted average of the best-fitted cosine function ± 1 standard error; [A]half of the difference between the maximum and the minimum of the cosine function (95% confidence interval).

Assessment of Supplementary variables of the experimental trials

The water quality parameters in the designed aquarium were ascribed qualitatively per week and other stereotypic behaviors were also monitored at random time of the day during photophase schedules. The parameters are illustrated in Table 4.

Table 4. The water quality parameters and ethological variables of fish which were examined are illustrated

Parameters	Analysis	Observations and Inference
pH	Qualitatively (Kit Based)	Within safe limit
Nitrate	Qualitatively (Kit Based)	Within safe limit
Nitrite	Qualitatively (Kit Based)	Within safe limit
Ammonia	Qualitatively (Kit Based)	Within safe limit
Turbidity	Not tested	Constant water renewal
Oxygen Demand	Not tested	Freshwater replenishment
Cortisol level	Not tested	Invasive techniques were eluded throughout the experiments
Sociability	Manual Observation	Group cohesion and neighboring were observed
Air gulping behavior	Manual observation	Stable
Vertical swimming	Manual observation	Not found
Food Anticipatory activity	Standford Chronokit software (Actogram)	Found less frequent
Lesions	Manual observation	Occurred often and self-recovered
Survivability	20 specimens	All survived throughout the experiment of 70 days

DISCUSSION

The attempt was to develop a new perspective of aquarium designing and stress-free housing of fish in the laboratory, keeping in view the shortcomings of filtering equipment and their limitations. Concerning ethical endpoints, the prime concern must be for maintaining better survivability and fish welfare in the laboratory as the unfed food material and excretion in the aquarium make the water quality toxic (high pH, high ammonia, high NO_2, etc.). Secondly, the restricted monitoring and nominal human presence are desirable to avoid any masking effect during the experiment. Thirdly, the practices of handling, collecting, and sorting of fishes have shown a significant impact on the physiology and survivability of fish when such activities exceed [29]. These are confinement-related stresses, which lead to debilitating fish conditions. These stresses can suppress their immune systems that produce effects on the overall health and well-being of fish [29].

The present test aquarium designed for laboratory purposes is mainly based on the technique used in aquaculture and fish farming in a large arena. The aquaculture and rearing farm employ two different water management systems, i.e., (a) open system and (b) closed or semi-closed system. In the open system, the natural water is used for rearing and is implemented directly in water bodies (pond, river, lakes, etc.), providing a natural condition. This system requires the least monitoring. In a closed system, there is no provision for water exchange, and the water recirculation system is used that is extensive and expensive [30]. Comparatively, the open water system provides aeration, which is more efficient than aeration using air-pumps/lifts [31]. The water flows in tanks provide aerobic swimming for fishes, which is considered better than anaerobic swimming activity called burst-type swimming, usually during stagnant water. In aerobic swimming, fish uses oxidative red muscles, whereas, in anaerobic swimming activity, they utilize glycolytic white muscle [29]. Burst swimming causes physical exhaustion, leading to a decline in blood pH, metabolic, endocrine disturbances and respiratory problems [32, 33]. However, the excessive flow rate of water should be avoided as this may fatigue the fish due to exhaustive and intense swimming [34, 29].

Hence, we designed the test aquarium based on a freshwater flow-through open system. It has automated draining, freshwater inlet, and overflow outlet that reduces monitoring and handling frequency. The water present in the aquarium is continuously aerated due to constant fresh water inlet. The waste material (excreta and residual food particles) settles down at the slanting bottom due to the perforated base and drain out via water flowing in the aquarium. The designed aquarium has an outlet both on the lower and the upper part of the aquarium, facilitating both settled and floating particles expulsion through the drainage outlets. The toxic waste remaining does not mix up by the fish movements; hence, water remains free from turbidity. In a conventional or traditional aquarium, the fundamental drawback and chance of deterioration of the water quality could be ascribed to the mixing up of waste matter by the fish's movement itself [5]. The housing in the present test aquarium benefited the fishes by saving energy, aerobic swimming activity, and their gills' ventilation due to reduced oxygen demand on naturally flowing water [29]. However, in the present study the water parameters were examined qualitatively. Using a water analysis kit, we found the water quality remains within the safe limit throughout the experiment. Additionally, the study was proposed with the objective to house the fishes both singly and in group for prolong period in designed aquarium, hence it is not feasible to house the fish (singly/group) in conventional aquarium for longitudinal period of 70-72 days without water renewal therefore, the comparative assessment of water quality and turbidity in conventional aquarium was not conducted due to ethically abide. The present investigation was performed successfully and all the 20 fishes kept either in solitude or in the social group were found to be survived at the end of the experiment trial.

The behavioral indicators of excellent or poor fish welfare (acute to chronic) are swimming activity changes, food-anticipatory behavior, feed intake, and ventilation rate [35, 36]. We determined the locomotor activity as one of the behavioral assays of fish using an automated data logger (Standford Chronobiology Kit). Examining the fish movement or locomotor activity provides important non-invasive identification for stress responses. The fish can perceive a variation in the environment and an initial sign of potential welfare problems [36]. The study of rhythms in locomotor activity has been used to explore fishes' physiological behavior [37-39]. Therefore, we studied the circadian variation in locomotor activity as an indicator since water quality might produce an immediate effect on the rhythm in locomotor activity. The stocking density might also have a confounding impact on the locomotor activity of fish.

The present findings revealed that fish's locomotor activity pattern remains consistent and stable throughout the housing, evidently supporting no masking and disruption due to turbidity of water or unfed food. The Cosinor rhythmometry analysis on locomotor activity pattern of *H. fossilis* also depicted consistency in circadian rhythmicity in respect of mesor, amplitude, and acrophase in all replicates of singly- and group-housed fish under LD photoperiod. However, Chen et al. [40] and Vera et al. [41] reported that circadian self-feeding and locomotor activity in fish differs among individuals and in the group, and there could be diel variability among individuals regarding feeding and locomotor activity pattern. The social grouping could influence the pattern from diurnal to nocturnal or *vice versa* [42, 41]. Vera et al. [43] also reported the crepuscular activity in catfish *Clarias gariepinus* at a different stocking density of 5 and 10 fishes. However, our findings did not show variability in the pattern of locomotor activity. The results demonstrated a significant difference in activity level, i.e., circadian mesor of singly- and group-housed fish. It could be explained as a consequence of group density, but there was no difference in locomotor activity rhythm amplitude. Crawford [44] and Clayton [45] defined that increment in the group's activity level is a kind of socially facilitated behavior. The behavior exhibited in a social situation by the group results from cohesive interaction among the group members. Hence, the increase in group-housed fish activity showed the social facilitated behavior and not because of stress due to stocking.

One of the common problems with teleost or freshwater fishes is that their procurement is very limited, and they rarely survive in laboratory conditions for a more extended period. We housed the freshwater benthic fish *H. fossilis* in laboratory conditions for a prolonged duration of about 70 days without filtering equipment. We observed a statistically significant circadian locomotor activity rhythm

under both LD_1 and LD_2 conditions. It was revealed that photic sensitivity [46], light responsiveness [47], interaction [4], and endogenous properties were not modulated and influenced by water turbidity. However, it has been reported that water quality affects the fish's activity, including interaction behavior with conspecifics and their social dynamics [3, 4]. The circadian mean locomotor activity and amplitude during the initial/first weekdays (1-7) and the last weekdays (65-70) did not reveal any significant differences and variation on singly-housed fish. In contrast, the group-housed fish showed variability in mean activity level only due to prolonged housing, but amplitude remained stable.

In the conventional aquarium, it was revealed that increased stocks of either of the same species or mixed-species may yield spurious data on behavioral output since group size could affect fish's behavior and welfare [48]. It has been noticed that in conventional aquarium, the stocking density also subjects to acute and chronic stress in fish, mainly in response to water deterioration due to increased oxygen consumption and hypoxia-like condition [16, 49]. The water becomes turbid within 7-10 days in conventional aquarium as they do not have facility for the removal of leftover food. In the absence of draining system, the water becomes gradually more turbid and toxic with the progress of time along the longitudinal scale (5). However, in the present study, it is evidently reported that the draining of wastes and cleaning can be possible through outlets in the aquarium. The water flow can be regulated by the valves connected directly to tap (inlet) and to lower (outlet) pipes. Since water is aerated and renewed continuously at the constant and slow flow rate in the designed aquarium, an increase in the stocking density did not produce any adverse changes in behavioral outcomes. It has been examined in traditional aquaria that changing the sudden amount of water 50% or 25% can affect the fish welfare by having a detrimental impact on social signals. Water renewal can dilute the chemical signals of fishes and exaggerate their aggression level [50]. It has recently been reported that changing water in large volumes at a time in the aquarium, a commonly used practice, could deteriorate fish's social communication [50]. Therefore, this new aquarium provides a unique perspective of water renewals and cleaning management in the laboratory. However, considering as future perspectives the proposed designed aquarium with more appropriate modifications can be applied by the users for better stocking and welfare of fish during experiments. The present aquarium offers a stress-free environment with limited space constraints and facilitates aerobic conditions.

CONCLUSION

This aquarium is suitable for collecting observations on behavioral parameters by applying visual-based techniques and methodology. The aquarium is appropriate for longitudinal study as they enable data capture without masking, less interruption on account of cleaning, and disturbing the fish inside the aquarium. The aquarium that we presented here is simple, inexpensive, durable, and easy to handle. The aquarium helps to avoid confinement related stresses. Using these perspectives for aquarium designing, the researchers can develop a low cost and capable fish tank for analyzing a large variety of behavioral parameters in fishes by excluding confounding interferences and masking factors.

EXPERIMENTAL LIMITATIONS

The analysis of stress in fish should have been based on the cortisol measurements. The observations on water parameters should also have been carried out quantitatively. The water flow rate has not been determined that could strengthen the proposed design. The comparative assessment in conventional aquarium with parallel condition could have been a profound approach for validating the current findings.

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CONFLICT OF INTEREST

The author declared no potential conflicts of interest concerning the research, authorship, and publication of this article.

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