SideRack: A Cost-Effective Addition to Commercial Zebrafish Housing Systems

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Abstract

Commercially available aquatic housing systems provide excellent and relatively trouble-free hardware for rearing and housing juvenile as well as adult zebrafish. However, the cost of such systems is quite high and potentially prohibitive for smaller educational and research institutions. The need for tank space prompted us to experiment with various additions to our existing Aquaneering system. We also noted that high water exchange rates typical in commercial systems are suboptimal for quick growth of juvenile fish. We devised a housing system we call “SideRack,” which contains 20 large tanks with air supply and slow water circulation. It enables cost-effective expansion of existing fish facility, with a key additional benefit of increased growth and maturation rates of juvenile fish.

Introduction

THE VIRTUES OF THE ZEBRAFISH model system are well documented: relatively simple maintenance and hardiness, large clutch size, optically transparent externally developing embryos combined with vertebrate physiology.^{1–9} These virtues, along with the development of genetic analysis and transgenesis tools, enabled zebrafish to become one of the main animal model systems in developmental genetics. Adaptation of the zebrafish model system at major research centers spurred development of commercial housing systems, which are now available from several suppliers.^{10,11} These commercially available housing systems enable relatively trouble-free and efficient housing for a large number of fish, especially when considering the number of fish per unit (square foot or square meter) of facility floor space. However, convenience and efficiency of the commercial systems come at a significant cost, which often limits the size of new zebrafish facilities being set up as well as expansion possibilities. Furthermore, zebrafish is an excellent model system for undergraduate education. In our experience, undergraduate students can be readily trained and meaningfully contribute to many aspects of research, from facility maintenance to construction of new transgene vectors and establishment of transgenic lines. These experiments require raising a large number of juveniles, preferably in the shortest amount of time possible. In addition to the concerns about the cost effectiveness of commercial systems, we have also noticed that zebrafish larvae grow fastest under low water exchange conditions when food (primarily brine shrimp, but also dry food) remains in the tank for extended time after feeding.

To reduce generation time by raising juveniles more rapidly and to increase the capacity of our zebrafish facility, we built and tested different additions to our commercially supplied Aquaneering system. The SideRack addition described in here enables relatively easy access to tanks for regular observation and maintenance, enables fast growth and maturation of juveniles through low rates of water flow and supplementary aeration, and recycles used water back to the main system. Juveniles raised in SideRack tanks routinely reach sexual maturity at 2½ months of age, speeding up generation and analysis of transgenic lines.

Results and Discussion

Design and construction of the SideRack

Raising multiple clutches of juveniles injected with various constructs for transgenesis, we noticed that larger clutches (>30) of juveniles raised in standard 9.5 L Aquaneering tanks rarely reached sexual maturity in less than 3 months, even when the regular twice daily brine shrimp diet was supplemented with once-a-day dry food. Since our ability to raise and screen fish was limited by the number of tanks rather than floor space of the facility, we started experimenting with different additions to our existing Aquaneering racks. Because we were planning to rear large clutches of juveniles, we chose to use relatively large tanks. We also wanted to have a low rate of water flow so that food (both dry food and live brine shrimp) would stay in the tank and be available for the juveniles to eat for extended amount of time without the need for more than three daily feedings. Low rate

TABLE 1. LIST OF ITEMS NEEDED TO ASSEMBLE SIDERACK

#	Description	Supplier	Supplier part number	Manufacturer part number	Qty.	Price
1	LASCO 1-1/2-in dia. PVC Sch 40 coupling	Lowes	23901	429015RMC	1	\$0.77
2	LASCO 1-1/2-in dia. × 1-in dia. PVC Sch 40 bushing	Lowes	23917	437211RMC	1	\$1.26
3	1-in × 10-ft 450 PSI Schedule 40 PVC pressure pipe	Lowes	23976	PVC 04010 0600	4	\$13.96
4	Mueller streamline 1-in PVC Sch 40 female in-line ball valve	Lowes	363386	107-235LW	1	\$6.83
5	Watts 1" PVC Spa Flex Hose	Home Depot	222568	RSFN 202257769	3"	\$5.82
6	LASCO 1-in dia. 90° PVC Sch 40 slip elbow	Lowes	23870	406010RMC	6	\$3.96
7	LASCO 1-in dia. 90° PVC Sch 40 Tee	Lowes	23876	401010RMC	5	\$4.30
8	LASCO 1-in dia. PVC Sch 40 Coupling	Lowes	23852	429010RMC	1	\$0.46
9	LASCO 1 × 1/2-in dia. 90° PVC Sch 40 Tee	Lowes	188243	464130RMC	20	\$32.60
10	GF piping systems reducer bushing 1/2" × 1/4" PVC	Grainger	2pmj4	839-072	20	\$111.60
11	Watts 1/4" brass pipe fitting	Lowes	88204	LFA-729	20	\$47.60
12	Tank supply valves 1/4"	Aquaneering	ZMV		20	\$80.00
13	LASCO 1-in dia. PVC Sch 40 adapter	Lowes	23864	435010RMC	2	\$1.52
14	LASCO 1-in dia. PVC Sch 40 plug	Lowes	23525	450010RMC	2	\$3.48
15	LASCO 1-in dia. 45° PVC Sch 40 slip elbow	Lowes	23892	417010RMC	5	\$4.85
	Charlotte pipe 1-1/2-in × 10-ft 330 PSI schedule 40 PVC pressure pipe	Lowes	23830	PVC 07112 0600	1	\$4.97
	Clear Cambro Camwear flat lid for food storage box 12" × 18"	The WEBstaurant Store	2141218CCWCL	1218ccw135	20	\$131.20
	Clear Cambro Camwear polycarbonate food storage box 12" × 18" × 9"	The WEBstaurant Store	21412189CWCL	12189CW135	20	\$345.00
16	Thogus reducer bushing threaded 3/8 × 1/8	Grainger	3GZc6	TRB3818/N-10-G	20	\$40.20
17	The Hillman Group 4-Count 13/32-in × 1-in nylon standard (SAE) flat washer	Lowes	139052	881539	5	\$4.60
18	Watts 1/4-in dia male adapter	Lowes	15118	PL-3004	20	\$63.60
	Tank supply tubing—blue (flexible) 1/4" OD	Aquaneering	PTBLU025		100'	\$60.00
19	Watts 3/4-in × 10-ft PVC clear vinyl tubing	Lowes	20497	SVLK10	1	\$7.98
20	New York wire 36-in × 7-ft charcoal fiberglass screen wire	Lowes	14433	33105	1	\$6.48
21	Watts 1-in × 1-ft reinforced PVC reinforced braided vinyl tubing	Lowes	35766	RBVNL	2'	\$3.44
	Outdoor air pump with 10-outlet adapter, 50 W, 3/8" Barb	Aquatic Ecosystems	9730		1	\$105.04
	Watts 7/16-in × 1-ft PVC clear vinyl tubing	Lowes	22272	RVHF	2"	\$0.72
	Watts 3/8-in Dia Tee CPVC fitting	Lowes	25324	A-291	1	\$2.38
	Thogus hose splicer, 1/8 × 3/8 In, 303 SS	Grainger	4HFK9	TPC7118-5-G	1	\$4.01
	Watts 1/4-in × 20-ft PVC clear vinyl tubing	Lowes	443063	LSVEB20	4	\$11.92
	Lok Tite plastic 5-way air control gang valve	Penn Plax		VN5	4	\$13.96
	Air stones				20	
	Double filter pad tray	Aquaneering	TRAD		1	\$65.00
	Tools and construction supplies					
	Aquarium silicone caulk/sealant					
	Oatey 16 fl oz LO-VOC primer	Lowes	51004	30757L	1	\$9.97
	Oatey 16 fl oz LO-VOC PVC cement	Lowes	23848	30876L	1	\$9.57
	11 inch cable ties					
	Plumber's tape (Teflon Tape)					
	1/4 inch drill bit					
	9/16 inch drill bit					
	PVC cutter					
	Hand saw					
	Drill/driver					
Total						\$1,209.05

Numbers in the first column correspond to numbers in Figures 2 and 3. Also listed are item description (second column), the supplier we purchased from (third column), supplier catalog number (fourth column), manufacturer part number (fifth column), quantity needed (sixth column), and price (seventh column).

FIG. 1. Front and rear views of a 20-tank SideRack unit. (A) Front view of the unit. Red dashed boxes indicate approximate location of close-up views in (B–E). (B) Connection to the main system and routing of air supply, front view. (C) Rear view of the top of the outflow pipe. Yellow arrow indicates the path of $\frac{1}{4}$ " outflow tubing from the tank to the 1" plastic pipe. Note that the $\frac{1}{4}$ " outflow tube does not loop up and down. (D) Rear view of two tanks on a shelf with water inflow (pink arrow), water outflow (yellow arrow), and air supply (red arrow). (E) Rear view of water drainage into the main system (drainage tube indicated by a yellow arrow), onto a double filter pad tray (TRAD, Aquaneering).

of water flow meant that additional aeration would be beneficial. The SideRack addition described here meets these requirements. All the components needed to build it are listed in Table 1. SideRack is housed on a 60" L $\times$ 24" W $\times$ 72" H, four-shelf wire rack (Fig. 1). We have used both Super Erecta

Wire Shelving with MetroSeal3 finish (Metro EZ2460NK3-4) and Trinity TBFC-0904, which provides a more cost-effective alternative (\$199 at Home Depot vs. at least \$369 + shipping). Each shelf fits five 4.75 Gallon Cambro Polycarbonate Food Storage Boxes available through many

FIG. 2. Diagram of inflow (A) and outflow (B) components of SideRack. Numbers next to arrowheads correspond to numbered items in Table 1. Fine arrows indicate holes drilled to insert tank drainage tubes.

online food service suppliers. SideRack water supply is connected to the water supply of the Aquaneering system through 1" ball valve (Fig. 1B, #4 in Fig. 2A and Table 1). Connection of the ball valve to the main system (#1–3 in Table 1 and Fig. 2A) water supply is likely to vary between different facilities and even between racks within a facility. To simplify the design, we decided to utilize two horizontal water supply pipes, with each pipe supplying water to 10 tanks on 2 shelves (Fig. 2A). Thus, water supply plumbing after the ball valve consists of a short fragment of flexible PVC pipe (#5, gray in Fig. 2A), followed by a 90° elbow (#6), a short fragment of flexible pipe again, a 1" T connection (#7), about 1 ft of flexible 1" pipe continuing to standard PVC 1" pipe (#3) through an adapter (#8), and a 90° elbow (#6) again (Fig. 2A). Horizontal water supply lines are made of 58-1/4"-long 1" PVC pipe (#3). To supply water to tanks, 1/2" T connections (#9 in insert of Fig. 2A) were glued to the PVC pipe and Aquaneering ZMV valves (#12) were connected through a pair of adapters (#10, 11).

The outflow system essentially consists of open-ended 1" PVC pipes connected to the vertical downspout through T junctions (#7 in Fig. 2B and Table 1) and 45° elbows (#15 in Fig. 2B and Table 1) attached at an angle (Fig. 1A, E). The water drains into the troth of the main system. Even with relatively low water flow, some debris does get flushed out of the tanks. To provide standard large particle filtration, we placed the same filter assembly as for the water recycled from main system tanks. It consists of Aquaneering Double Filter Pad Tray (Aquaneering TRAD) and a prefilter pad (Aquaneering MAFP622).

To connect tanks to air, water supply, and water outflow, we drill three holes in the back of the tank (Fig. 3A). The large hole about 6.5 cm from the top is drilled with 9/16" drill for waste water outflow. Two smaller holes are drilled about 2.5 cm from the top of the tank with 1/4" drill to supply air and water. The main outflow assembly consists of a 1/4" male adapter, a flat washer, and a reducer bushing (#18, 17, and 16, respectively, in Fig. 3B and Table 1). The three components are assembled into the outflow hole in the tank and sealed with a small amount of aquarium-approved silicone. We tested several different kinds of mesh on outflow mesh assemblies. The version we currently use is diagrammed in Figure 3C. It consists of a diagonally cut 3/4" plastic tubing, an ~10×10 cm square of fiberglass mesh, and a 1–2 cm long fragment of 1" plastic tubing (#19, 20, and 21, respectively, in Fig. 3C and Table 1). The assembly fits tightly onto the reducer bushing on the inside of the tank (Fig. 3D). A standard 1/4" OD plastic tube is connected to the 1/4" male adapter, and the other end of the tube is threaded into a 1/4" hole in the outflow PVC pipe (yellow arrows in Fig. 1C, D, fine arrows in Fig. 2B).

For aeration, we use an air pump (Aquatic Eco-Systems #9730), since our building does not supply compressed air. The output of this pump is sufficient to supply air to four standard size brine shrimp hatchers and at least four SideRacks with 80 tanks total. The air is routed to SideRack on top of system water supply pipes (Fig. 1B, red arrow) in 5/16" ID tubing. This tube is connected to the standard 1/4" OD plastic pipe using a 1" long piece of 3/16" tubing as a reducer. Alternatively, a reducer such as Thogus #TPC7118-5-G can be used. On each shelf, we placed a plastic five-way air control valve (Penn Plax Cat #VN5), to which individual tank air lines are connected (Fig. 1D, red arrow). A standard

FIG. 3. Tank assembly. (A) Tank with holes drilled for air inflow, water outflow, and water inflow. (B) Outflow assembly. Numbers next to arrows pointing to specific items correspond to item numbers in Table 1. (C) Assembly of outflow net. (D) Top view of an inside of a tank with an outflow net.

TABLE 2. REGULAR MAINTENANCE OF SIDERACK TANKS

<i>Maintenance item</i>	<i>Frequency</i>
Check/adjust water inflow	Every week
Check/adjust air supply	Every week
Clean/replace particle filter	Every week/as needed
Siphon off excess food	Every other week/as needed
Check/wash outflow net assembly	Every other week/as needed
Clean/replace outflow tubing	Every other month/as needed

small air stone is placed in each tank. Central air supply is a much safer alternative to drawing electrical wiring to power air pumps in the vicinity of tanks.

Regular maintenance of SideRack tanks

We typically transfer juveniles into SideRack tanks at about 1 month of age and follow maintenance procedures outlined in Table 2. The single most important difference from commercial systems is that SideRack tanks are not self-cleaning. Due to low flow rate, excess food debris tends to build up on the bottoms of tanks, primarily during the first 2 weeks on SideRack when juveniles are still small. We siphon off excess food and debris using ¼" plastic tubing attached to a 1-mL plastic pipette. If appropriate amount of food is fed, this needs to be done 2–3 times (every other week) before the fish grow up and should not take more than 15 min for a 20 tank rack.

We also observe a significant buildup of algae over time, particularly when fish are kept in SideRack tanks for more than the 1½ months required to reach sexual maturity. It can be, in part, attributed to the fact that Cambro lids are clear, while most commercial systems use darker blue or green lids. To reduce this problem, we started applying a static cling window tint (GILA CS78) to the lids and sides of Cambro tanks.

Potential problems with SideRack tanks

We find SideRack system to be trouble free if the maintenance schedule outlined in Table 2 is followed. The most likely problem to occur is water overflow. The following conditions may lead to water overflow:

- Excess food and debris is not siphoned off and clogs up the tank outflow filter.
- Outflow water line is far too long and circles up and down. Surface tension produced by air bubble/water interface in a very long water line may overcome water pressure and block flow.
- Outflow line is tightly pressed against the inner wall of the outflow PVC pipe, blocking flow. Solution: cut the end of outflow tubing at an angle.
- Outflow tubing is blocked by algal growth. In our facility, this only occurs when tubing is not replaced for several months.

Overall, SideRack tanks provide an excellent alternative for rearing large batches of juvenile fish for line propagation in transgenesis experiments. The cost is much lower as compared with commercially available systems. Just as importantly, the amount of time that needs to be spent feeding and maintaining SideRack tanks to achieve maximum larval growth rates is not significantly higher compared with commercial systems. Since the rate of water flow is relatively low, SideRacks can be added to most existing multitrack facilities without upgrading the water pumps or filtration to account for increased water flow. Our facility has eight double-sided, six shelf Aquaneering racks (ZAD660) and we have added four SideRack systems (80 tanks) without any negative impact on the rate of water flow on the main system. The total cost of adding 80 4.75 gallon (18-L) tanks was ~\$6,000, or about half the cost of purchasing one additional double-sided six-shelf rack with about 70 9.5-L tanks. We do not recommend

adding it to a single standalone rack though, because water pressure generated by standalone rack water pumps may not be sufficient to supply 20 additional tanks even at a low flow rate. To cost-effectively expand facilities without multitrack systems, a recently described standalone module would be more appropriate.¹² Notably, the SideRack system can be easily modified (more shelves, smaller tanks) to meet the needs of a specific laboratory. Furthermore, it may be possible to install a SideRack in a temperature-controlled room adjacent to the main fish facility, either to expand the facility or to have fish on an alternative light–dark cycle.

The utility of a fish housing system is best measured by user preference. Over the 2 years since we started installing SideRack tanks, facility users interested in fastest possible rearing of juveniles have been choosing them over the standard 9.5 L tanks on the main system. The small amount of additional effort in maintaining the tanks is offset by lower daily feeding requirements and shorter maturation time, which in turn saves time and effort. If we were to order a new fish housing system today, we would specify a system consisting mostly of smaller (up to 2.8 L) tanks and add SideRacks in place of most 9.5 L tanks.

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Disclosure Statement

The authors declare no conflict of interests.

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