ZEBRAFISH
Volume 12, Number 4, 2015
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DOI: 10.1089/zeb.2015.1087

TechnoFish–Methods

Automated Scalable Heat Shock Modification for Standard Aquatic Housing Systems

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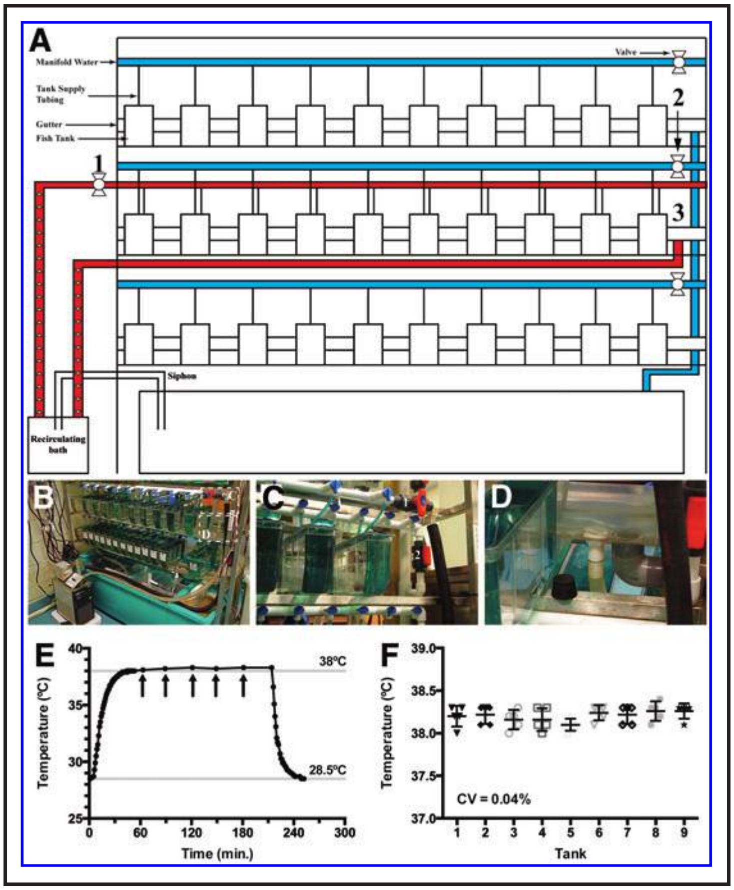

FIG. 1. Modification of the zebrafish housing rack. Schematics of the heat shock modification of a zebrafish housing rack **(A)**. The recirculating bath is shown next to the housing rack and the extra manifold and drain (that circulate heated water to and from the tanks) are shown in *red*. The manifolds and drain of the regular system are shown in *blue*. Pictures of the modified housing rack **(B)**, the extra manifold **(C)**, and the extra drain **(D)**. To expose the fish to the heated water, the recirculating water bath was turned on. Then, the main valve of the regular fish water (valve number 2) was closed and the main valve of the heated water was opened (valve number 1). The main system drain of the gutter of the heat shock tanks' shelf was closed to allow the heated water to return to the water bath through the extra drain (valve number 3 in **A, D**), keeping the rest of the system at normal temperature (28.5°C). To finish the heat shock, the heated water valve was closed (valve number 1) and the recirculating water bath was turned off. Then, the main system drain of the gutter and the regular fish water valve (valve number 2) were opened to gradually mix heated and regular water and return to 28.5°C. Temperature of a fish tank during heat shock **(E)**. Temperature of the nine tanks installed in the modified rack **(F)**, *each dot* represents the temperature of the tank measured at the points marked with *arrows* in **(E)**. Color images available online at www.liebertpub.com/zeb

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Abstract

Heat shock is a common technique for inducible gene expression system in a variety of organisms. Heat shock treatment of adult zebrafish is more involved and generally consists of manually transferring fish between housing rack tanks and preheated water tanks or the use of timed heaters in stand-alone aquaria. To avoid excessive fish handling and to take advantage of the continuous flow of a standard housing rack, proposed modifications consisted of installing an aquarium heater inside each tank, manually setting the heater to reach heat shocking temperatures (>37°C) and, after that, testing that every tank responded equally. To address the limitations in the existing systems, we developed a novel modification of standard zebrafish housing racks to perform heat shock treatment in conditions of continuous water flow. By adding an extra manifold to the housing rack and connecting it to a recirculating bath to create a parallel water flow system, we can increase the temperature from standard conditions (28.5°C) to heat shock conditions with high precision (38.0–38.3°C, mean ± SD = 38.1°C ± 0.14°C) and minimal variation among experimental tanks (coefficient of variation [CV] = 0.04%). This means that there is virtually no need for laborious pretreatment calibrations or continuous adjustments to minimize intertank variation. To test the effectiveness of our design, we utilized this system to induce enhanced green fluorescent protein (EGFP) expression in *hsp70-EGFP* fish and performed a fin regeneration experiment with *hsp70l:dnfgfr1-EGFP* fish to confirm that heat-induced gene expression reached physiological levels. In summary, our newly described aquatic heat shock system minimizes effort during heat shock experiments, while ensuring the best water quality and fish welfare and facilitating large heat shock settings or the use of multiple transgenic lines for both research and teaching experiments.

Heat shock is a common technique for inducible gene expression system in a variety of organisms, including zebrafish, with over 900 transgenic fish lines carrying a construct under the control of the heat shock promoter 70 (*hsp70*; Zfin transgenic database; http://zfin.org/). Heat shock treatment of zebrafish embryos can be successfully performed with very simple setups because they are fairly stationary and do not require water circulation.[1,2] Heat shock treatment of adult zebrafish is more involved and generally consists of manually transferring fish between housing rack tanks and preheated water tanks[3] or the use of timed heaters in stand-alone aquariums.[4] To avoid excessive fish handling and to take advantage of the continuous flow of standard housing rack, proposed modifications have consisted of installing an aquarium heater inside each tank, manually setting the heater to reach heat shocking temperatures (>37°C) and, after that, testing that every tank responded equally.[5]

To address the limitations in the existing systems, we developed a novel modification of standard zebrafish housing racks to perform heat shock treatment in conditions of continuous water flow. By adding an extra manifold to the housing rack, and connecting it to a recirculating bath (Fig. 1A–D), we can increase the temperature from standard conditions (28.5°C) to heat shock conditions with extreme precision (38.0–38.3°C, mean ± SD = 38.1°C ± 0.14°C) and minimal variation among experimental tanks (coefficient of variation [CV] = 0.04%) (Fig. 1E, F). This means that there is virtually no need for laborious pretreatment calibrations or continuous adjustments to minimize intertank variation. It is, therefore, a clear improvement over the system previously described where heaters and water flow in every tank needed to be manually adjusted and calibrated.[5] Appropriate treatment temperature can be maintained for the desired period of time with no effect on water quality (check the Supplementary Data for further details; Supplementary Data are available online at www.liebertpub.com/zeb).

To test the effectiveness of our design, we utilized this system to induce EGFP expression in *hsp70-EGFP* fish[6] (data not shown) and performed a fin regeneration experiment with *hsp70l:dnfgfr1-EGFP* fish[4] (data not shown) to confirm that heat-induced gene expression reached physiological levels. Importantly, this system is both automated and scalable, and can utilize the existing aquatic rack infrastructure.

In summary, our newly described aquatic heat shock system minimizes the effort during heat shock experiments, while ensuring the best water quality and fish welfare facilitating large heat shock settings or the use of multiple transgenic lines for both research and teaching experiments. If needed, it can be easily automatized installing solenoid valves (like Plast-O-Matic 39P449 or Spartan Scientific 20HL94) and it can be customized to meet the needs of different laboratories without equipment from a specific supplier or brand. In fact, the same modification can be installed in housing systems for other aquatic species, including other fish, xenopus, even sea urchins, jellyfish, or snails.

Acknowledgments

We wish to thank S. Szalay for his excellent technical assistance in building the heat shock system and members of the Kahana laboratory for helpful discussions. This work was supported, in part, by R01 EY022633 from the National Institutes of Health and utilized the Vision Research Core (P30 EY007003) at the University of Michigan.

Disclosure Statement

No competing financial interests exist.

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