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Title: A Custom-Built Recirculating Zebrafish Housing System

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **04/26/26 or later**

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Current Protocol Length

Number of Steps: 25

Number of Shots: 55

Introduction

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Kaitlyn Poppo:** We designed and tested an affordable recirculating zebrafish housing system for budget conscious research labs and education programs.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research gap or limitation in existing methods and how this protocol addresses them.**~~

- 1.2. **Mikaela Fairbanks:** Many existing zebrafish housing systems are cost prohibitive for smaller research labs, so we designed an inexpensive housing system.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

1.3., Mikaela Fairbanks: This protocol allows researchers to build a zebrafish housing system that they then can use for both research or educational purposes.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the most important consideration or challenge when performing this protocol.**~~

- 1.4. **Kaitlyn Poppo:** Proper tubing connections, slope of return lines, and secure fittings are essential to maintain consistent water circulation and prevent leaks.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Kaitlyn Poppo**: Future studies can modify this system for larger zebrafish colonies and automated water quality monitoring.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committees of Northern Michigan University and Shenandoah University

Protocol

2. Assembling the Vertical Return and Sump Basin

Demonstrators: Mikaela Fairbanks and Kaitlyn Poppo

- 2.1. To begin, assemble the housing rack by fixing the support brackets at the required heights [1] and then install a vertical return pipe [2].
 - 2.1.1. WIDE: Talent showing the assembled support brackets.
 - 2.1.2. Talent installing vertical support with drilled holes.
- 2.2. Using a saw, cut a 3.81-centimeter-diameter polyvinyl chloride pipe into three pieces, each measuring roughly 121.92 centimeters in length [1].
 - 2.2.1. Talent cutting the 3.81 centimeter polyvinyl chloride pipe into three equal 121.92 centimeter sections on a work surface.
- 2.3. Then, make two lengthwise cuts along each of the 121.92-centimeter pipe sections to create a U-shaped trough [1].
 - 2.3.1. Talent cutting lengthwise along a pipe section to form a U-shaped trough.
- 2.4. Place each U-shaped trough onto a shelf with one end inserted into the T joint of the main vertical return [1]. Using the previously inserted string or wire, secure the opposite end of each trough to create a gentle downward slope toward the T joint of the vertical return [2]. Adjust the trough to achieve a 1.5-to-2-degree slope toward the main vertical return [3].
 - 2.4.1. Talent positioning a U-shaped trough on a shelf and inserting one end into the T joint of the vertical return.
 - 2.4.2. Talent tying the opposite end of the trough to the support bracket using the pre-threaded string or wire.
 - 2.4.3. Talent adjusting the trough angle to create a slight downward slope toward the vertical return.
- 2.5. Position the sump basin beneath the assembled rack [1]. Ensure a clearance of approximately 14.29 centimeters between the top of the basin and the underside of

the first shelf [2]. Adjust the basin so that it protrudes slightly from the shelf and aligns with the overhanging vertical return pipe to direct the water back into the basin [3].

2.5.1. Talent sliding the sump basin into position beneath the assembled rack.

2.5.2. Talent measuring and confirming the 14.29 centimeter clearance between the basin and the first shelf.

2.5.3. Talent adjusting the basin position so the vertical return pipe overhangs it.

2.6. After setting up the sump filtration and temperature control systems, position the water pump inside the sump basin aligned with the support bracket of the tank return system [1].

2.6.1. Talent placing the water pump into the sump basin and aligning it with the support bracket.

3. Assembling the Main Water Supply Line

3.1. Cut a 1.905-centimeter vinyl tubing to a length of 69.85 centimeters [1] and connect the tubing to the pump [2].

3.1.1. Talent cutting the 1.905 centimeter vinyl tubing to 69.85 centimeters in length.

3.1.2. Talent attaching the cut tubing securely to the pump outlet.

3.2. Connect a T coupler to the tubing [1-TXT]. Position this T coupler slightly below the second shelf and orient it toward the far side of the sump basin [2].

3.2.1. Talent inserting the tubing into a second T coupler. **TXT: T coupler: 1.905 × 1.905 × 1.27 cm**

3.2.2. Talent adjusting and positioning the T coupler slightly below the second shelf and facing the far side of the sump basin.

3.3. Using a box cutter, cut a 1.905-centimeter vinyl tubing to a length of 36.195 centimeters [1] and fasten the tubing directly to the opposite end of the T coupler from the previous step [2].

3.3.1. Talent cutting the 1.905 centimeter vinyl tubing to 36.195 centimeters in length.

3.3.2. Talent securing the tubing to the open end of the T coupler.

- 3.4. Sequentially connect 36.195 and 36.83-centimeters vinyl tubing to T couplers, adding a new T coupler after each segment and positioning it slightly below the second shelf, facing the far side of the sump basin. [1].
 - 3.4.1. Shot of the assembly after connecting 2 more vinyl tubing and T couplers and talent pointing to the connected segments. **Authors: The repetitive steps have been summarized into 1 line as per our journal guidelines**
- 3.5. Then, connect a 1.905 to 1.27 centimeter reducing elbow with a 1.27 centimeter male adaptor to the tubing [1]. Seat the reducing elbow and male adaptor on the top surface of the fourth shelf [2]. Orient the elbow and adaptor to connect to the vinyl pressure-release line running back down to the sump [3].
 - 3.5.1. Talent attaching the reducing elbow and male adaptor to the tubing. **NOTE: The shots of this step were filmed together**
 - 3.5.2. Talent placing the reducing elbow and male adaptor on the top surface of the fourth shelf.
 - 3.5.3. Talent orienting the elbow and adaptor toward the direction of the vinyl pressure release line leading back to the sump.

4. Assembling the Pressure Release and Tank Water Supply Lines

- 4.1. Cut a 1.58-centimeter vinyl tubing to a length of 7.62 centimeters [1]. Connect the tubing to the 1.27-centimeter male adaptor at the top of the shelf [2] and then attach a ball valve to the free end of the tubing [3].
 - 4.1.1. Talent cutting the 1.5875 centimeter vinyl tubing to 7.62 centimeters in length.
 - 4.1.2. Talent attaching the tubing to the 1.27 centimeter male adaptor at the top of the shelf.
 - 4.1.3. Talent securing a ball valve onto the end of the tubing.
- 4.2. Next, using a box cutter, cut a 1.58-centimeter vinyl tubing to a length of 213.36 centimeters [1]. Connect the tubing to the free fitting of the ball valve [2].
 - 4.2.1. Talent cutting the 1.5875 centimeter vinyl tubing to 213.36 centimeters in length.
 - 4.2.2. Talent attaching the tubing to the outlet of the ball valve.

- 4.3. Route the tubing across the top of the rack [1] and then direct it down along the leg of the housing rack into the sump basin [2]. Using fastening straps, secure the tubing along the leg of the housing rack [3].
 - 4.3.1. Talent laying the tubing across the top of the rack.
 - 4.3.2. Talent guiding the tubing down along the leg of the housing rack into the sump basin.
 - 4.3.3. Talent fastening the tubing to the rack leg with straps.
- 4.4. Then, using a box cutter, cut a 1.575-centimeter vinyl tubing into three pieces, each measuring 6.35 centimeters in length [1]. Connect each piece to one of the three free T couplers on the main water supply line [2] and connect a ball joint to the free end of each tubing section [3].
 - 4.4.1. Talent cutting three short sections of 1.575 centimeter vinyl tubing.
 - 4.4.2. Talent attaching each tubing section to a free T coupler on the main supply line.
 - 4.4.3. Talent attaching a ball joint to each of the three tubing ends.
- 4.5. Next, attach three vinyl tubings with 6.35-centimeter length to the free end of a ball joint [1] and connect a 1.58-centimeter to 0.95-centimeter reducer to each 1.58-centimeter tubing section [2].
 - 4.5.1. Talent connecting each tubing section to a ball joint outlet.
 - 4.5.2. Talent attaching a reducer to each tubing section.
- 4.6. Cut a 0.95-centimeter tubing into three pieces, each measuring 5.715-centimeters in length [1]. Connect each piece to a reducer [2] and attach a 0.95-centimeter nylon T coupler to each tubing section [3].
 - 4.6.1. Talent cutting three short sections of 0.9525 centimeter tubing.
 - 4.6.2. Talent attaching each tubing section to a reducer outlet.
 - 4.6.3. Talent attaching a nylon T coupler to each tube.
- 4.7. Obtain six pieces of 0.9525 centimeter vinyl tubing each measuring 5.08 centimeters in length [1] and connect each piece to the nylon T couplers [2].
 - 4.7.1. Talent cutting six short sections of 0.9525 centimeter vinyl tubing.

- 4.7.2. Talent attaching each tubing piece to the outlets of the nylon T couplers.
- 4.8. Attach a hose clamp to each of the six tubing pieces [1]. Slip a steel aquarium valve onto each available tubing end and tighten the hose clamps to secure the valves [2].
 - 4.8.1. Talent placing a hose clamp onto each short tubing section.
 - 4.8.2. Talent fitting a steel aquarium valve onto each tubing end and tightening the hose clamps.
- 4.9. Using fastening straps, secure each steel aquarium valve to the shelf above [1]. Finally, using a box cutter, cut 0.43-centimeter vinyl tubing to the appropriate length required to reach each tank [2].
 - 4.9.1. Talent fastening each steel aquarium valve to the underside of the shelf with straps.
 - 4.9.2. Talent measuring and cutting 0.4318 centimeter vinyl tubing to lengths sufficient to reach individual tanks.

Title Card

Prepare and test the aquatic system by establishing the water circulation, filtration, sterilization, and electrical safety measures before housing the zebrafish.

Results

5. Results

Authors: The results section is slightly re-worded for better clarity

- 5.1. A three-level recirculating zebrafish housing system was created following the protocol [1]. The sump conductivity remained relatively stable throughout the year based on weekly measurements [2].

5.1.1. LAB MEDIA: Figure 1A.

5.1.2. LAB MEDIA: Figure 3A.

- 5.2. Over the 21-day assessment period, the conductivity fluctuated, but remained within 600 to 1000 microsiemens per centimeter, which is safe for the zebrafish [1].

5.2.1. LAB MEDIA: Figure 3B.

- 5.3. Nitrites remained in the low category each day during the 21-day period [1].

5.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the bar for “Nitrites”*

- 5.4. Nitrates stayed in the low category for 18 of the 21 days tested [1] and were in the medium category for 3 days, remaining within the safe range for zebrafish [2].

5.4.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the “Nitrates” bar and focus on the larger dark “low” portion.*

5.4.2. LAB MEDIA: Figure 3C. *Video editor: Then highlight the smaller grey “medium” portion of the same “Nitrates” bar.*

- 5.5. The reproductive success was checked in the system during the same three-week period with a total of 18 mating pairs [1]. Of the 18 total mating pairs, 11 were successful, corresponding to approximately 70 percent mating success [2].

5.5.1. LAB MEDIA: Figure 3D

5.5.2. LAB MEDIA: Figure 3D. *Video editor: Highlight the bar labeled “Mating success”*

5.6. The 24-hour embryo survival rate was nearly 70 percent [1].

5.6.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the bar labeled “24 h survival”*

5.7. The average clutch size for successful matings was approximately 150 embryos [1].

5.7.1. LAB MEDIA: Figure 3E.

1. Polyvinyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyvinyl>
IPA: /ˌpɑːliˈvaɪnəl/
Phonetic Spelling: paa·lee·vai·nuhl
2. Chloride
Pronunciation link: <https://www.merriam-webster.com/dictionary/chloride>
IPA: /ˈklɔːraɪd/
Phonetic Spelling: klor·ide
3. Trough
Pronunciation link: <https://www.merriam-webster.com/dictionary/trough>
IPA: /ˈtrɒf/
Phonetic Spelling: trawf
4. Vertical
Pronunciation link: <https://www.merriam-webster.com/dictionary/vertical>
IPA: /ˈvɜːtɪkəl/
Phonetic Spelling: vur·tuh·kuhl
5. Filtration
Pronunciation link: <https://www.merriam-webster.com/dictionary/filtration>
IPA: /fɪlˈtreɪʃən/
Phonetic Spelling: fil·tray·shuhn
6. Coupler
Pronunciation link: <https://www.merriam-webster.com/dictionary/coupler>
IPA: /ˈkʌplər/
Phonetic Spelling: kuhp·luhr
7. Adaptor
Pronunciation link: <https://www.merriam-webster.com/dictionary/adaptor>
IPA: /əˈdæptər/
Phonetic Spelling: uh·dap·tuhr
8. Reducer
Pronunciation link: <https://www.merriam-webster.com/dictionary/reducer>
IPA: /rɪˈduːsər/
Phonetic Spelling: ruh·doo·suhr

9. Aquarium
Pronunciation link: <https://www.merriam-webster.com/dictionary/aquarium>
IPA: /əˈkwɪəriəm/
Phonetic Spelling: uh·kwair·ee·uhm
10. Conductivity
Pronunciation link: <https://www.merriam-webster.com/dictionary/conductivity>
IPA: /ˌkɑːndʌkˈtɪvəti/
Phonetic Spelling: kon·duhk·tiv·uh·tee
11. Microsiemens
Pronunciation link: <https://www.howtopronounce.com/microsiemens>
IPA: /ˌmaɪkroʊˈsiːmənz/
Phonetic Spelling: mai·kroh·see·muhn
12. Zebrafish
Pronunciation link: <https://www.merriam-webster.com/dictionary/zebrafish>
IPA: /ˈziːbrəˌfɪʃ/
Phonetic Spelling: zee·bruh·fish
13. Nitrites
Pronunciation link: <https://www.merriam-webster.com/dictionary/nitrite>
IPA: /ˈnaɪˌtraɪt/
Phonetic Spelling: nai·trite
14. Nitrates
Pronunciation link: <https://www.merriam-webster.com/dictionary/nitrate>
IPA: /ˈnaɪˌtreɪt/
Phonetic Spelling: nai·trate
15. Embryo
Pronunciation link: <https://www.merriam-webster.com/dictionary/embryo>
IPA: /ˈembriˌoʊ/
Phonetic Spelling: em·bree·oh
16. Reproductive
Pronunciation link: <https://www.merriam-webster.com/dictionary/reproductive>
IPA: /ˌriːprəˈdʌktɪv/
Phonetic Spelling: ree·pruh·duhk·tiv
17. Sterilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilization>
IPA: /ˌsterələˈzeɪʃən/
Phonetic Spelling: steh·ruh·luh·zay·shuhn
18. Circulation
Pronunciation link: <https://www.merriam-webster.com/dictionary/circulation>
IPA: /ˌsɜːkjəˈleɪʃən/
Phonetic Spelling: sur·kyuh·lay·shuhn
19. Electrical
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrical>
IPA: /ɪˈlektrɪkəl/
Phonetic Spelling: ih·lek·truh·kuhl

20. Vinyl

Pronunciation link: <https://www.merriam-webster.com/dictionary/vinyl>

IPA: /'vɪnəl/

Phonetic Spelling: vai·nuhl