

Submission ID #: 70856

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Title: A Simple and Precise Feed-Based Approach to Oral Drug Delivery in Fish

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 12

Number of Shots: 34 (+ 2 added shots)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

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. **Justin Kenney:** This study describes a method for precise, non-invasive oral drug delivery to adult zebrafish.

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. **Neha Rajput:** Unlike stressful, invasive or imprecise traditional methods, this protocol enables non-invasive and precise drug delivery to adult zebrafish.

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. **Neha Rajput:** This protocol enables precise drug dosing and allows measurement of behavioral, pharmacological, and physiological responses in adult zebrafish.

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. **Jacob Hudock:** The key challenges are feed homogenization, setting, and confirmed consumption by each fish.

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. **Justin Kenney:** Future studies can extend this method to other compounds, repeated dosing paradigms, diverse behavioral assays, and additional aquatic model organisms.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. Neha Rajput, Postdoctoral Fellow, Wayne State University and Jacob Hudock, PhD student, Wayne State University: (authors will present their testimonial statements live)

What value do you expect this video article to have for other researchers working in your field?

- 1.7. Justin Kenney, Associate Professor, Wayne State University: (authors will present their testimonial statements live)

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Wayne State University

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Fish Weighing and Feed Volume Calculation

Demonstrator: Neha Rajput

- 2.1. To begin, fill a hundred milliliter glass beaker with fifty milliliters of system water [1]. Place it on a digital scale [2] and tare the scale to subtract the beaker weight and reset the scale to zero [3].
 - 2.1.1. WIDE: Talent filling a 100 ml beaker with 50 ml water
 - 2.1.2. Talent placing the beaker with water on the balance
 - 2.1.3. Talent pressing the “Tare” button on the balance **Author’s Note: Shots 2.1.2 and 2.1.3 were combined.**
- 2.2. Use a fine-mesh net to gently remove a single fish from their home tank [1]. Remove excess water by briefly touching the net to a dry paper towel [2] and place the fish carefully into the beaker without spilling water onto the scale [3].
 - 2.2.1. Talent removing a fish using fine-mesh net.
 - 2.2.2. Talent touching the net to paper towel to remove excess water
 - 2.2.3. Talent transferring the fish into the beaker. **Author’s Note: 2.2.1, 2.2.2 and 2.2.3 were combined into one shot.**
- 2.3. Record the weight [1] and calculate the required feed volume as one-percent volume per body weight for each individual fish [2].
 - 2.3.1. Talent recording the weight of the fish.
 - 2.3.2. Talent calculating the required amount of feed based on fish’s body weight.

3. Preparation of Brine Shrimp Extract and Gelatin-based Feed:

Demonstrator: Jacob Hudock

- 3.1. Suspend brine shrimp powder in distilled water to a final stock concentration of two hundred and fifty milligrams per milliliter and a final volume of fifty milliliters [1]. Stir for one hour [2], centrifuge the suspension at 12,500 g for 10 minutes at room temperature and collect the supernatant [3].
 - 3.1.1. Talent adding the pre-weighed brine shrimp powder to water.
 - 3.1.2. Talent placing the brine shrimp suspension on a stirrer. **Author’s Note: Shots 3.1.1 and 3.1.2 were combined.**
 - 3.1.3. Talent centrifuging the stirred suspension.

- 3.2. Centrifuge the liquid again to remove any remnant suspended debris and collect the clear supernatant [1]. Store at minus twenty degree Celsius up to six months [2].
- 3.2.1. Talent centrifuging the supernatant again.
- 3.2.2. Talent placing the centrifuged solution in a -20 °C refrigerator.
- 3.3. For preparation of five hundred microliters of gelatin-based feed, thaw the brine shrimp extract [1] and calculate the approximate volume required [2]. Measure and add the appropriate volumes of brine shrimp extract, drug compound, and water to the spirulina-containing tube, recording actual mass to ensure it meets or exceeds the target for desired final volume [3]. Calculate the required weights of components [4] into one-point five milliliters microcentrifuge tubes [5]. **Note: The sentence numbers are adjusted to accommodate the added shot. The VO is replaced from 3.4 to 3.3 for moved shot.**
- 3.3.1. Talent thawing the brine shrimp extract at room temperature
- Added Shot: Talent calculating approximate volumes.**
- 3.4.1. Talent adding brine shrimp extract, drug compound and water to the spirulina tube. **Note: This shot was placed by here by the author**
- 3.3.2. Talent assembling and weighing the drug compound, gelatin and spirulina into separate 1.5 mL tubes.
- 3.3.3. TEXT ON A PLAIN BACKGROUND
Target Final Concentrations of Components
Gelatin (porcine-derived; Bloom $\approx$ 300 g) = 12% (w/v)
Spirulina = 4% (w/v)
Brine shrimp extract (250 mg/ mL Stock) = 80 mg/mL
Drug (10 mg/ mL Stock) = 0.4 mg/ μ L (4 mg/kg at 1% BW)
Water = To adjust to desired final volume
- ~~3.4. Calculate and add the appropriate volumes of brine shrimp extract, drug compound, and water to the spirulina-containing tube [1] Vortex thoroughly to homogenize [1]. Transfer the required volume of the mixture to the gelatin-containing tube and ensure the precise final concentrations of all components [2].~~
- ~~3.4.1. Talent adding brine shrimp extract, drug compound and water to the spirulina tube. **Note: 3.4.1 was moved before 3.3.2.**~~
- 3.4.2. Talent vortexing the mixture to homogenize.
- 3.4.3. Talent transferring the spirulina mixture to the gelatin tube. **Author's Note: 3.4.2 and 3.4.3 were combined.**
- 3.5. Place the tube on a pre-warmed forty-five-degree Celsius heating block for five minutes [1]. Vortex intermittently until the gelatin is completely dissolved, while homogenizing with sterile wood or metal stick if incomplete dissolution is observed [2]. Aliquot hundred microliters of the homogenized mixture into labeled one-point

five milliliter microcentrifuge tubes [3] and solidify at minus twenty degree Celsius overnight [4].

3.5.1. Talent placing the tube on heating block set at 45 °C.

3.5.2. Talent vortexing the mixture intermittently.

3.5.3. Talent transferring 100 µL mixture into labelled 1.5 mL tubes.

3.5.4. Talent placing the tubes in –20 °C refrigerator.

4. Formation of Individual Feed Morsels and Gelatin Feed Administration

Demonstrator: Neha Rajput

4.1. Align a flat sheet of paraffin film in a Petri dish [1]. Reheat and vortex the prepared gelatin feed [2] and pipette the volume corresponding to one percent of the fish's body mass onto the paraffin film to form individual morsels [3].

4.1.1. Talent placing a sheet of paraffin film in a Petri dish.

4.1.2. Talent reheating and vortexing the gelatin feed containing tube. **Author's note:** 4.1.1 and 4.1.2 were combined.

4.1.3. Talent pipetting out the liquified feed on to the paraffin film to make morsels.

4.2. Solidify the morsels by placing the petri dish at minus twenty degree Celsius for at least twenty minutes [1] and keep on ice until feeding [2].

4.2.1. Talent placing the petri dish with morsels at –20 °C refrigerator.

4.2.2. Talent placing the solidified morsels on ice until feeding.

4.3. Transfer fish from the housing rack to the behavior room to acclimatize for one hour [1]. Use transparent barriers to temporarily isolate each fish in the tank for two to five minutes prior to feeding [2]. Use forceps to pick up the feed from parafilm [3] and place an individual gelatin morsel onto the water surface for each fish [4]. **Note:** The sentence numbers are adjusted to accommodate the added shot.

4.3.1. Talent transferring the fish to behavior room for acclimatization.

4.3.2. Talent placing transparent barriers to separate the fishes in the tank.

Added Shot: Talent picking up feed from parafilm with forceps.

4.3.3. Talent placing the feed morsels into the tank for each fish using forceps.

4.4. Observe each fish for five minutes to confirm consumption of the entire morsel [1]. Remove the barriers after feed consumption [2] and return the fish to home tanks or for experimental procedures [3].

4.4.1. Fish consuming the entire morsel. **Author's Note:** 4.4.1 was combined with 4.3.3, as the fish ate the morsel immediately.

4.4.2. Talent removing the barriers after feed consumption.

4.4.3. Talent returning the fish to their home rack.

Results

5. Results

5.1. When adult AB (A-B) and TL (T-L) zebrafish were given gelatin feed for five mornings instead of their usual meal, all TL zebrafish consumed the morsels within five minutes on the first day [1], whereas AB fish required four days of acclimation to reach a consistent consumption rate for fifteen out of sixteen individuals [2]. TL fish of both sexes consumed the feed rapidly from the first exposure within four to twenty seconds [3], whereas AB fish showed longer initial consumption times ranging between seven to one hundred and forty-four seconds, but improved to under 30 seconds over repeated days [4].

5.1.1. LAB MEDIA: Figure 2A: *Video Editor: Please emphasize the red line.*

5.1.2. LAB MEDIA: Figure 2A: *Video Editor: Please emphasize the gray and brown lines.*

5.1.3. LAB MEDIA: Figure 2B: *Video Editor: Please emphasize the red and pink lines.*

5.1.4. LAB MEDIA: Figure 2B: *Video Editor: Please emphasize the gray and brown lines.*

5.2. Following two days of acclimation to gelatin morsels, male and female adult AB fish were divided into control and treatment groups, then fed with vehicle or MK-801 containing morsels [1]. Behavior was analyzed as distance from the bottom in a novel tank test and both male [2] and female drug-treated fish spent more time near the top of the tank compared to control indicating reduced anxiety-like or predator-avoidance behavior [3].

5.2.1. LAB MEDIA: Figure 3: *Video Editor: Please emphasize the two purple and two green boxes in the figure sequentially.*

5.2.2. LAB MEDIA: Figure 3: *Video Editor: Please emphasize the fourth moss green box in the figure.*

5.2.3. LAB MEDIA: Figure 3: *Video Editor: Please emphasize the second emerald green box in the figure.*

Pronunciation Guide:

1. Centrifuge

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

IPA: /'sentrəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

2. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /su:'pə:nətənt/
Phonetic Spelling: soo·pur·nuh·tuhnt
3. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>
IPA: /sə'spenʃən/
Phonetic Spelling: suh·spen·shuhn
4. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊˌli:tər/
Phonetic Spelling: my·kroh·lee·terz
5. Gelatin
Pronunciation link: <https://www.merriam-webster.com/dictionary/gelatin>
IPA: /'dʒələtɪn/
Phonetic Spelling: jel·uh·tin
6. Spirulina
Pronunciation link: <https://www.merriam-webster.com/dictionary/spirulina>
IPA: /ˌspaɪrəˈlu:nə/
Phonetic Spelling: spy·ruh·loo·nuh
7. Homogenize
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /hoʊ'mɑ:dʒəˌnaɪz/
Phonetic Spelling: hoh·maa·juh·nyze
8. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'æliˌkwɑ:t/
Phonetic Spelling: al·ih·kwaat
9. Paraffin
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraffin>
IPA: /'pærəfɪn/
Phonetic Spelling: pa·ruh·fin
10. Acclimatize
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimatize>
IPA: /əˈklaɪməˌtaɪz/
Phonetic Spelling: uh·kly·muh·tyze
11. Distilled
Pronunciation link: <https://www.merriam-webster.com/dictionary/distilled>

IPA: /dɪ'stɪld/

Phonetic Spelling: dih·stild

12. Concentration

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

IPA: /ˌkɒːnsən'treɪʃən/

Phonetic Spelling: kaan·suhn·tray·shuhn

13. Vortex

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

IPA: /'vɔːrˌtɛks/

Phonetic Spelling: vor·teks

14. Microcentrifuge

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

IPA: /ˌmaɪkroʊ'sentrəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

15. Acclimatization

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

IPA: /əˌklaɪməˌtaɪˈzeɪʃən/

Phonetic Spelling: uh·kly·muh·ty·zay·shuhn