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Low cost methods for Hydra care V.2

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Hydra is genus of freshwater cnidarian polyp found in lakes, ponds, and streams all over the world. Its remarkable ability to regenerate missing body parts, and even its whole body from fragments, has made Hydra a model system for regeneration and development for over 250 years. Despite their anatomical simplicity, much about Hydra's basic biology and extraordinary regeneration caption remains mysterious.

The simplicity of caring for and doing experiments on Hydra makes it an accessible subject for undergraduate research, high school lab activities, or even a home hobbyist. This collection of protocols describes low-cost methods for maintaining Hydra cultures, including step-by-step instructions for preparing media, growing brine shrimp for feeding to Hydra, and caring for your Hydra colonies. We've focused on equipment that can be found in most high school and college labs, or consumer products that can be purchased online.

Experiments on Hydra can be incredibly simple. Much can be learned from dissecting a Hydra and observing its regeneration. Hydra's response to varying environmental conditions can also be explored in a classroom setting with minimal resources. These simple experiments can be a bridge to cutting-edge molecular biology and stem cell research. Sequencing of the Hydra genome has opened the door for genetic manipulations and large-scale bioinformatics studies. Transgenic lines with fluorescently-labeled cell types and proteins, including neuron and muscle activity reporters, are available.

References:

Howard M. Lenhoff, Ed (1983). Hydra Research Methods (Book).
<https://doi.org/10.1007/978-1-4757-0596-6>

Bossert P, Galliot B (2012). How to use Hydra as a model system to teach biology in the classroom. The International journal of developmental biology.

Contents:

- 1: Storing brine shrimp cysts
- 2: Brine shrimp hatching medium
- 3: Hatch brine shrimp
- 4: Prepare stock solutions
- 5: Prepare Hydra medium
- 6: Harvest brine shrimp
- 7: Feed Hydra
- 8: Routine Hydra tank cleaning
- 9: Full cleaning of Hydra tanks
- 10: Monitor Hydra health
- 11: Emergency antibiotic treatment

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Education, invertebrates, regeneration, aging, animal care, developmental biology, undergraduate research, low cost

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Jan Hamrsky, www.lifeinfreshwater.net

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Tanks for Hydra:

Any glass or plastic container can be used to hold your Hydra. Petri dishes can also be used. For small Hydra colonies, we like "Pruta" food storage containers from Ikea because they have flat, transparent bottoms and are easy to clean. We recommend filling tanks to half their volume with Hydra medium. Set the lid on top of the container loosely to allow gas exchange.

Handling Hydra:

Use glass Pasteur pipettes to move Hydra around. For Hydra stuck to the surface of a container, do not use suction to pull the Hydra off the surface. You might rip it in half! Instead, gently scrape the animal's foot off the substrate with the edge of the pipette. Once it is dislodged you can pick it up with the pipette.

Preventing cross-contamination:

- Some Hydra species and genetic lines cannot be distinguished by eye. For this reason, it is essential to make sure that Hydra do not touch equipment that will be used for more than one species or line.
- Do not touch the edges of beakers, bottles, or other equipment to any container with Hydra. Hydra can stick to the lip of a bottle and be transferred to another colony.
- Do not return animals used for experiments to colonies.
- Follow the cleaning procedure below to scrub and disinfect equipment between uses.
- Some equipment, such as Pasteur pipettes, can be labeled for use with only one line. We label the pipette bulbs with the name of the line and are very careful that the pipette is only used for the intended line. Pasteur pipettes can be used repeatedly if disinfected with ethanol between uses.

Disposing of Hydra:

Avoid putting live Hydra down the drain. Collect all medium containing Hydra in a container and add household bleach at 10% the total volume of the medium. Wait for 10 minutes, then pour down the drain.

Cleaning equipment:

All equipment should be scrubbed with a brush under a stream of tap water, rinsed with DI water, and treated with 70% ethanol (our preference) or 70% isopropyl alcohol to disinfect. You can immerse your equipment in a container of alcohol or use a spray bottle. Set your equipment on a drying rack while the alcohol evaporates. Avoid using soap on equipment that will come in contact with Hydra and Hydra medium.

Types of water:

For preparing media and stock solutions, using Nanopure or MilliQ water if it is available. House DI water from a tap in your lab is probably fine, but monitor your Hydra for health issues that might indicate problems with water quality. DI water from the grocery store or pharmacy can also be used for making solutions. Commercial spring water (e.g. Arrowhead) can be used in place of Hydra medium. Avoid using tap water.

Lab environment:

Hydra can be kept at room temperature in the lab as long as it stays below 75°F (24°C). Some experiments may require that your colonies be maintained in an incubator at 18°C. For viewing Hydra while feeding and cleaning, an ideal space will be well lit and have dark countertops. If you don't have dark counter tops, you can use a black paper or plastic mat.

These items can be purchased from many vendors, including scientific supply companies. Use the links below as examples. We've made an attempt to find the most affordable examples, but you may be able to find better deals or smaller quantities elsewhere. eBay often has good deals on these items.

Standard lab equipment:

1 L graduated cylinder
lab equipment

Carolina Item # 721606 [↗](#)

Available from many vendors

Plastic funnel
lab equipment

Carolina Item # 972130 [↗](#)

Available from many vendors

Carolina Compact Balance, 220 g
Capacity, 0.1 g Readability
lab equipment

Carolina Item # 702065 [↗](#)

Any electronic lab balance will do

Weigh boats

lab equipment

Eagle Thermoplastic Item # 4YMV5 [↗](#)

Available from many scientific and industrial suppliers.

Dixie cups can be used in place of weigh boats.

Lab scoop

lab equipment

Carolina Item # 702851 [↗](#)

Bottle, High-Density Polyethylene, Narrowmouthed, 1,000 mL

lab equipment

Carolina Item # 716318 [↗](#)

Any glass or plastic bottle for liquid storage will work.

5 gallon HDPE carboy

lab equipment

Tolco Item #: 97028 [↗](#)

Pipette Pump, Plastic, 10ml Size, Green
lab equipment

Grainger **Item # 4YMP8** [↗](#)

Low cost alternative to an automatic pipette pump

Dissecting microscope
lab equipment

AmScope **SE400-Z** [↗](#)

We recommend a binocular stereomicroscope with enough space to view Hydra tanks.

100-1000 µl Micropipette
lab equipment

MiniOne **M2011** [↗](#)

Optional. Only needed for dispensing rifampicin solution.

Reagents:

[↗](#) **Calcium Chloride Dihydrate Fisher**

Scientific Catalog #C79

[↗](#) **Magnesium sulfate anhydrous Fisher**

Scientific Catalog #M65500

[↗](#) **Sodium bicarbonate Sigma**

Aldrich Catalog #S6014

[↗](#) **Potassium chloride Sigma**

Aldrich Catalog #P9333

Optional:

 [Rifampicin, USP Grade Gold](#)

Biotechnology Catalog #R-120

 [Dimethyl sulfoxide \(DMSO\) Bio Basic](#)

Inc. Catalog #D0231.SIZE.500ml

Aquaculture supplies:

San Francisco strain brine shrimp eggs
Biological material

Brine Shrimp Direct SFB8Z 

Imhoff Sedimentation Cone
lab plasticware

BrandTech 388000 

Look for 1 L sedimentation cones.

Imhoff cone stand
Lab equipment

Brine Shrimp Direct RAC 

Flexible air hose
lab equipment

Brine Shrimp Direct AIRHOSE 

Rigid tubing, 0.5 m
lab equipment

Brine Shrimp Direct TUB [↗](#)

Aquarium air pump
lab equipment

Top Fin 5174806 [↗](#)

Brine shrimp seive
lab equipment

Hobby NA [↗](#)

brine shrimp nets and sieves are available from a variety of vendors. Make sure that the one you purchase is for harvesting newly hatched brine shrimp. Mesh size should be <150 microns.

Lab consumables:

50 mL conical screw-cap tube
Plastic consumables

Grenier Bio-One 5622-7261 [↗](#)

Any brand or size of plastic tube with a tight-fitting screw cap will work fine.

Parafilm

lab consumable

Parafilm M **PM996** [↗](#)

Can be purchased from many vendors

Serological pipette, 10mL

10ml serological pipette, sterile, individually wrapped

USA Scientific **1071-0810** [↗](#)



Fisherbrand™ Free-Standing

Microcentrifuge Tubes with Screw Caps
plastic consumable

Fisher scientific **02-682-558** [↗](#)

Any brand of screw cap microcentrifuge tube can be used.

Glass pasteur pipettes, 5 3/4 inch

glass consumables

Fisher Scientific **1367820B** [↗](#)

Any brand of glass Pasteur pipette will do, just make sure you have bulbs that fit. Plastic Pasteur pipettes are not recommended because Hydra tend to stick to plastic.

Rubber bulbs for Pasteur pipette

Lab equipment

Heathrow Scientific HS20622B [↗](#)

Look for 2 mL latex rubber bulbs

Micropipette Tips – 100-1,000 µL

lab consumable

MiniOne M3118 [↗](#)

Optional. Only used for dispensing rifampicin solution.

Consumer products:

Multi-purpose spray bottle

consumer product

CVS Item # 691031 [↗](#)

Available from many pharmacies and home goods stores

70% Ethyl alcohol

consumer product

CVS Health Item # 304659 [↗](#)

Available from many pharmacies and grocery stores.

Household bleach
consumer product

Great value **11463069601** 

Available from any grocery store, pharmacy, or home goods store.

Solar Salt
consumer product

Morton **1691954** 

Sold for water softeners. Can be purchased from many hardware and home goods stores

Baking soda
consumer product

Arm and Hammer **Walmart # 9274239** 

Available from grocery stores and pharmacies.

1 gallon bottle
consumer product

For Your Water **BT110JBADB** 

Use any large plastic bottle or carboy to prepare brine shrimp hatching medium.

Pruta food container
consumer product

Ikea 903.358.43 [↗](#)

Any clear glass or plastic container can be used to house Hydra. We like these food storage containers from Ikea because they are inexpensive and have a smooth inner surface making them easy to clean.

Pyrex 7 Cup Round Storage Dish
consumer product

Pyrex Walmart # 569596797 [↗](#)

You can use any brand of circular glass dish. Pyrex dishes are easy to clean and difficult to break.

Mini glass bowl
consumer product

Duralex Item Number: 2022AF06 [↗](#)

Great for suspending brine shrimp in Hydra medium. Any small glass or plastic bowl will work. Salsa cups are also about the right size.

Glass and Water Bottle Brush
consumer product

Scotch-Brite 449008 [↗](#)

Can be purchased at many grocery stores and home goods stores. Use any brand.

Ethanol and isopropyl alcohol are flammable. Wear gloves and eye protection when handling these chemicals. If you are using a spray bottle, make sure to point it away from your face. Do not allow ethanol or isopropyl alcohol to go down the drain.

Dispose of broken glass, used Pasteur pipettes, and used serological pipettes in a sharps container.

Review the Guidelines section and make sure you have all equipment for keeping Hydra and growing brine shrimp before bringing Hydra into your lab. We recommend writing to a Hydra researcher and requesting the species and genetic lines that you want. Hydra can also be purchased from educational supply companies or collected in the wild.

Brine shrimp cyst storage

1

Store brine shrimp cysts:

Brine shrimp eggs can form thick-shelled cysts that remain dormant for many years until exposed to water. Freshly hatched nauplii (first developmental stage) of the brine shrimp *Artemia franciscana* are a convenient food for Hydra because they are easily stored and grown and the species has no natural defenses against Hydra.

Store unopened containers of brine shrimp cysts in the freezer (-20°C). They will keep for years.



Figure 1. Freshly hatched *Artemia* nauplius.
Image credit: www.oceannutrition.eu

Materials:

- San Francisco Bay brand brine shrimp cysts

- Lab scoop
- Funnel
- 50 mL conical tubes
- Parafilm
- Freezer

Other strains and grades of brine shrimp cysts (such as Great Salt Lake Premium Grade) can be used, but their hatching may be less efficient.

- 1.1 When you are out of cysts and need to open a new container, remove the container from the freezer and allow it to come to **⚡ Room temperature** overnight. This will prevent condensation and absorption of moisture by the cysts.
- 1.2 Open the container and add the brine shrimp cysts to 50 mL conical tubes with a lab scoop and funnel. Cap the tubes tightly, seal with Parafilm, and return to the freezer until ready to use.
- 1.3 When cysts are needed, remove the conical tube from the freezer and allow it to come to **⚡ Room temperature** overnight. Store the cysts at **⚡ Room temperature**, tightly capped when not in use.

Brine shrimp hatching medium 10m

2 Prepare brine shrimp hatching medium:

Brine shrimp cysts are hatched in medium with the same approximate salinity as seawater. Instant Ocean, a standard salt water aquarium mix can be used, but a much less expensive option is "solar salt" that is sold for water softeners. It can be purchased at many home goods and hardware stores. Kitchen baking soda can also be used in place of laboratory-grade NaHCO_3 .

Materials:

- Solar salt
- Sodium bicarbonate (NaHCO_3)
- DI water
- 1 L graduated cylinder
- Electronic balance
- Lab scoops
- Weigh papers or weigh boats
- Glass or plastic bottle, or large carboy

Combine **12.5 g** of solar salt and **1 g** sodium bicarbonate (NaHCO_3) to for every **1 L** DI water and mix until all of the salt is dissolved. NaHCO_3 maintains **pH 8**.

For example, to prepare **3 L** hatching medium, combine:

3 L DI water

3 g NaHCO_3

37.5 g solar salt

Store at **Room temperature**

We prepare 3 L of hatching medium at a time in a plastic jug, cap it and shake to dissolve the salt and NaHCO_3 . Nanopure water, MilliQ water, house DI water from the tap in your lab, or distilled or deionized water purchased from a grocery store will all work for this step. Avoid tap water.

Hatching brine shrimp

2d

3 Set up your brine shrimp hatchery:

Brine shrimp can be hatched in any aerated container. You can buy special brine shrimp hatcheries, but more convenient, lower cost options are available. Imhoff sedimentation cones can hatch a large number of brine shrimp at a time and are easy to clean. An inverted 2 liter soda bottle with a hole cut in the bottom is a low cost alternative.

Brine Shrimp Direct sells stands for Imhoff cones, but anything that will hold an inverted cone will work, including a ring stand or a three liter soda bottle with the top cut off.

The Imhoff cone can be aerated with a standard aquarium pump attached to flexible tubing. You will also need a rigid tube to aerate the bottom on the cone. Brine Shrimp Direct sells long plastic tubes for this purpose, but a 5 mL or 10 mL serological pipette will also work.

Materials:

- Brine shrimp hatching medium (see above)
- Brine shrimp cysts
- Digital balance
- Weigh boat
- Imhoff cone
- Imhoff cone stand
- Aquarium bubbler
- Flexible air line
- Rigid tubing

- Plastic wrap

- 3.1 Assemble your hatchery. Put the rubber plug in the bottom of the Imhoff cone and attach the screw cap. Set the cone in the stand. Attach the air hose to the bubbler and attach the rigid tubing to the air hose. Set the rigid tubing into the Imhoff cone.

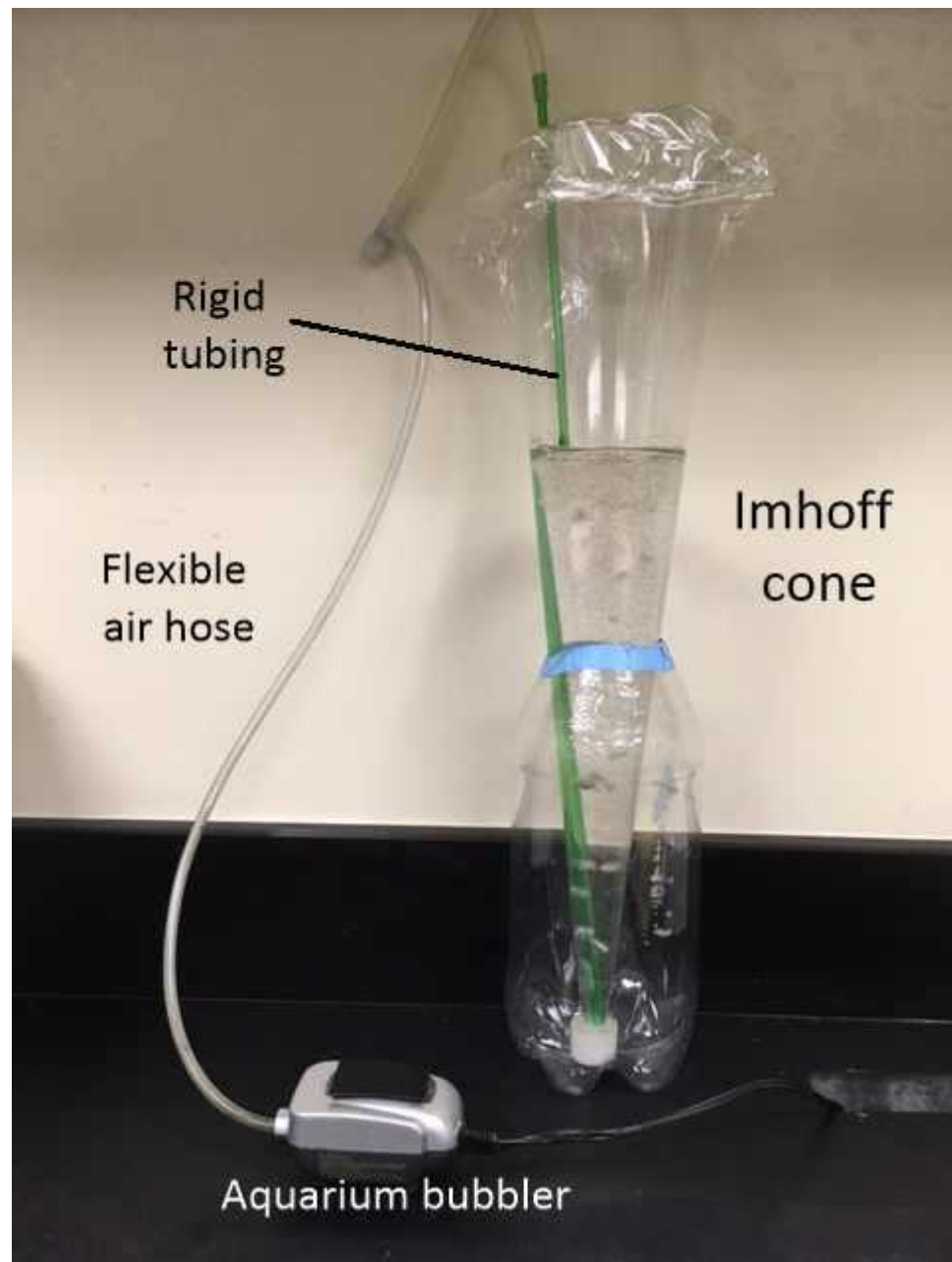


Figure 2. Assembled brine shrimp hatchery.

- 3.2 Add cysts and hatching medium to the Imhoff cone. The quantity of cysts and hatching medium can be adjusted according to your Hydra-feeding needs. Imhoff cones can accommodate up to **1 L** hatching medium and **1 g** of

cysts. **500 mL** hatching medium with **0.5 g** cysts is enough for a few tanks of Hydra.

Plug in the bubbler and cover the top of the cone with plastic wrap. You can also use an appropriately sized food storage container lid or petri dish cover with a hole cut in it.

Increase the rate of aeration if you find that the brine shrimp cysts are settling to the bottom of the cone or sticking to the sides of the cone.

Hydra medium + stocks

30m

4 Prepare concentrated stock solutions:

Hydra medium is a dilute salt solution similar in composition to freshwater ponds and streams. The solution below has a similar composition to pond water and contains essential ions - calcium, magnesium, sodium, potassium, and chloride. The sodium bicarbonate keeps it the pH between **pH 7.5** and **pH 8**.

Materials:

- Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)
- Magnesium sulfate anhydrous (MgSO_4)
- Sodium bicarbonate (NaHCO_3)
- Potassium chloride (KCl)
- DI water
- 1 L graduated cylinder
- Electronic balance
- Lab scoop
- Weigh papers or weigh boats
- 4 x 1 L glass or plastic bottles
- 10 mL serological pipettes
- Automatic or thumb roll pipette pump
- Large carboy

A	B	C	D
Component	Final conc. in Hydra medium	Conc. in 1000X stock	quantity for 1 L 1000X stock
Calcium chloride dihydrate	1.0 mM	1 M	111.56 g
Magnesium sulfate, anhydrous	0.33 mM	330 mM	39.72 g
Sodium bicarbonate	0.5 mM	500 mM	42.01 g
Potassium chloride	0.03 mM	30 mM	2.24 g

Table 1. Chemical composition of Hydra medium

Prepare stock solutions and Hydra medium in MilliQ water or Nanopure water if at all possible. House DI water from the tap in your lab should be used with caution. Distilled or deionized water from the grocery store or pharmacy can be used for making solutions if no other option is available.

Arrowhead Spring Water, which can also be purchased at grocery stores and pharmacies, can be used in place of Hydra medium if necessary.

4.1 Prepare 1000X calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) stock:

Dissolve **111.56 g** $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

in **800 mL** Nanopure water

Bring up to **1 L** with Nanopure water

Store in a screw-cap bottle at **Room temperature**

4.2 Prepare 1000X magnesium sulfate (MgSO_4) stock:

Dissolve **39.72 g** MgSO_4

in **800 mL** Nanopure water

Bring up to **1 L** with Nanopure water

Store in a screw-cap bottle at **Room temperature**

4.3 Prepare 1000X sodium bicarbonate (NaHCO_3) stock:

Dissolve **42.01 g** NaHCO_3

in **800 mL** Nanopure water

Bring up to **1 L** with Nanopure water
Store in a screw-cap bottle at **Room temperature**

4.4 Prepare 1000X potassium chloride (KCl) stock:

Dissolve **2.24 g** KCl
in **800 mL** Nanopure water
Bring up to **1 L** with Nanopure water
Store in a screw-cap bottle at **Room temperature**

5 Prepare Hydra medium:

Hydra medium can be prepared in any large carboy. These can be purchased from scientific supply companies or from a grocery or home goods store. We use a food grade 3 gallon carboy.

Use a graduated cylinder to add **1 L** at a time to the carboy until you have reached it's maximum capacity. Note the number of liters and draw a line on the side of the carboy indicating where to fill to for that volume.

5.1 Fill the carboy to the fill line with Nanopure water. For every **1 L** added, remove **4 mL**.

For example, if your carboy contains **8 L**, remove **36 mL** with either a serological pipette or by draining into a graduated cylinder.

5.2 For every liter, add **1 mL** of each 1000X stock solution.

For example, if your carboy contains **8 L**, add:

8 mL 1000X $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ stock

8 mL 1000X MgSO_4 stock

8 mL 1000X NaHCO_3 stock

8 mL 1000X KCl stock

Shake the carboy vigorously to mix.

Store at **Room temperature**

6 Harvest brine shrimp for Hydra feeding:

After 48 hours growth, your brine shrimp nauplii should be ready to feed to Hydra! Follow the steps below to harvest live nauplii from your hatchery.

Materials:

- Hydra medium
- 70% ethanol
- DI water
- Brine shrimp hatchery (see above)
- 10 mL serological pipette
- Automatic or thumb roll pipette pump
- Brine shrimp net
- Small dish or beaker

Avoid feeding brine shrimp less than 48 hours old to Hydra. Brine shrimp less than 48 hours old contain a chemical that may make Hydra sick.

- 6.1 Turn off the bubbler and remove the tube from the Imhoff cone. Wait for 5-10 minutes for the nauplii to settle to the bottom of the cone. Unhatched cysts will sink to the bottom, shells from hatched cysts will float to the top and live shrimp will settle above the unhatched cysts.

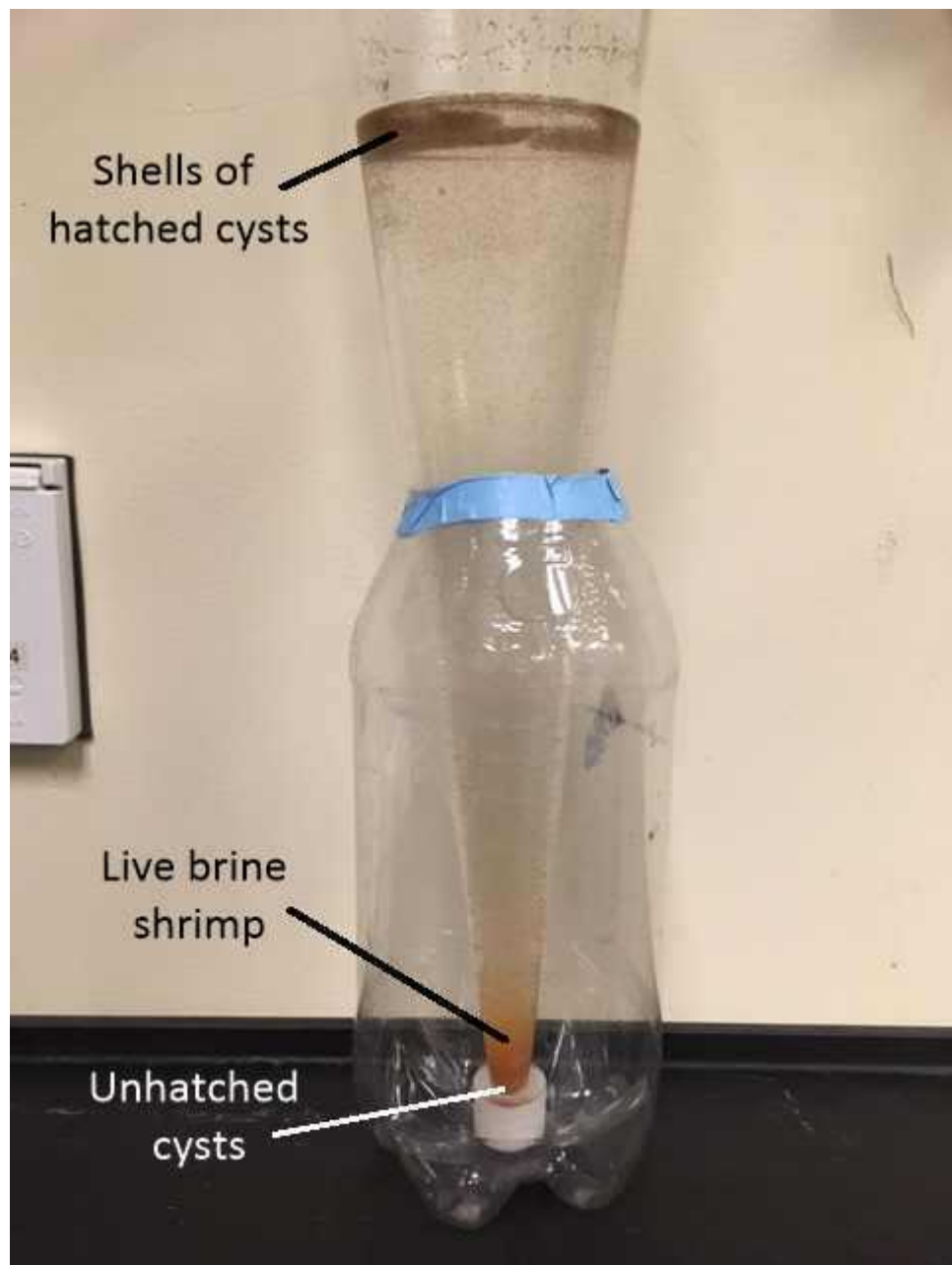


Figure 3. Brine shrimp hatchery after settling.

- 6.2 While your cone is settling, rinse your brine shrimp net or sieve with DI water and add Hydra medium (see step 8 above) to a small dish or beaker.
- 6.3 With a 10 mL serological pipette and pipette pump, gently suck up the live shrimp from bottom of the cone. Get as many as you can while avoiding the unhatched cysts.



Figure 4. Use a 10 mL serological pipette to harvest brine shrimp.

Unhatched cysts are not digestible by Hydra and will cause fouling in the Hydra tanks. Try to avoid collecting unhatched cysts as you pipette the live brine shrimp out of the cone.

- 6.4 Dispense the shrimp from your pipette into the net or strainer and allow the medium to filter through. Rinse the shrimp with DI water to remove all of the salt.
- 6.5 If you are using a sieve, just set the sieve with the brine shrimp into the dish of Hydra medium. If you're using a net, turn the net inside out and submerge the brine shrimp in the dish of Hydra medium.

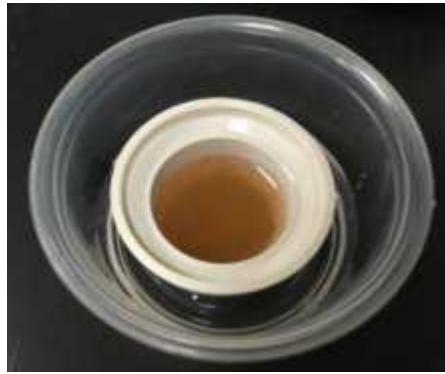


Figure 5. Brine shrimp in a sieve suspended in Hydra medium.

- 6.6 Clean up: Empty the hatchery, scrub it under tap water, rinse with DI water and spray it with ethanol to sterilize. Rinse the net and plastic tube with DI water and sterilize with ethanol. Leave the net, hatchery, and plastic tube on the bench for the ethanol to evaporate.

Hydra feeding

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- 7 With your freshly hatched brine shrimp rinsed and suspended in Hydra medium, it's time to feed your Hydra.

Materials:

- Brine shrimp nauplii in Hydra medium
- Hydra tanks
- Plastic or glass Pasteur pipette

Use a plastic or glass Pasteur pipette to suck up some of the shrimp in the dish of Hydra medium. Add the shrimp drop-wise to each of your Hydra tanks, making sure to disperse them throughout the tank. Add as much as the Hydra can consume with little excess. Excess shrimp can contribute to fouling. Getting the quantity right will take some practice.

After Hydra eat, it will take 6-8 hours for them to regurgitate pellets of undigested material. A good schedule is to feed your Hydra in the morning and clean their tanks in the afternoon before you leave for the day. You will remove both uneaten shrimp and pellets at the same time.

If you have observed poor health of your Hydra or fouling in the tanks, you might want to clean twice on each feeding day - once 30 minutes after feeding to remove uneaten shrimp, and once in the afternoon to remove pellets.

Hydra tanks do not have to be perfectly clean. Remember, Hydra's natural environment is a pond, so a sterile environment may not be ideal.

8 Clean your tanks after every feeding:

Clean Hydra tanks to remove uneaten shrimp and regurgitated pellets on the same day that you feed your Hydra. While cleaning, take note of any changes to the health of your Hydra and the accumulating debris in the tanks that might indicate that it's time for a full cleaning (see below).

Materials:

- Hydra medium
- Large glass bowl
- Pasteur pipettes and bulbs

- 8.1 Gently swirl the medium in the tank to dislodge uneaten shrimp, pellets, and other debris. Pour the medium out of the tank into a large glass bowl.
- 8.2 Add a little more Hydra medium to the tank, swirl again, and pour into the glass bowl. Replace the Hydra medium in the tank with fresh medium.

While dispensing the medium into the tanks, avoid pouring the medium directly on top of the Hydra. Hold the tank at an angle and dispense the medium down the side of the tank.

While pouring the medium out of the tank, some Hydra may stick to the side of the tank above the level of the medium. Use the tip of your Pasteur pipette to push the Hydra back into the medium.

- 8.3 It's likely that many Hydra were poured off along with the debris into the glass bowl. If there are only a few, or you have enough remaining in your tanks, you can discard the ones you've poured off.

If you want to recover these Hydra, a convenient method is to gently swirl the medium in the glass dish then set the dish on your bench until it stops swirling. The Hydra will collect in the center of the dish where they can easily be picked up with a pipette and transferred back into their tank.



Figure 6. Hydra collect in the center of the bowl after gentle swirling.

If you have only a few Hydra in your tanks, you simply fill a new tank with Hydra medium and transfer your Hydra one by one with a Pasteur pipette.

- 8.4 Pour discarded Hydra medium into a bottle or beaker and treat with 10% bleach for 20 minutes before pouring down the drain. This is a precaution against releasing Hydra into the environment, which is especially important when you are working with transgenic lines.

Full cleaning of Hydra tanks

30m

- 9 Approximately every month, or whenever you observe scum accumulating on the surface of your Hydra tanks, you will need to do a full cleaning of the tanks.

Materials:

- Hydra medium
- Latex or nitrile rubber gloves
- Large glass bowl
- Pasteur pipettes and bulbs

- 9.1 With a rubber glove on your hand, use your finger to scrape the Hydra off the surface of the tank until they are floating loose in the medium.
- 9.2 Pour the contents of the tank into your glass bowl and swirl to collect the Hydra in the center of the bowl (see Step 8.3 above).
- 9.3 Fill a clean tank halfway with Hydra medium. Use a Pasteur pipette to transfer the Hydra back into the tank, being careful to avoid any debris.

You may need to pipette the Hydra up and down or transfer them to another container with fresh Hydra medium to get all of the debris off them.

- 9.4 Wash the dirty tank by scrubbing with a brush under tap water. Rinse with DI water then spray with ethanol. If you are going to add Hydra directly back into the tank, wipe with a paper towel to remove residual ethanol.

Monitor Hydra health

- 10 As you are feeding and cleaning your Hydra, keep an eye out for any changes in the appearance of the animals that might indicate a decline in health. Make sure to examine your Hydra once per week under a dissecting microscope.

Things to look for:

- Tentacles look short and pointy.
- Hydra are contracted and don't extend when left to rest.
- There is an excessive amount of debris accumulating on the surface of the tank.
- Hydra look like they are wearing fuzzy sweaters. This is a sign of a bacterial infection. Hydra normally have colonies of bacteria that live on their feet but sometimes the bacteria can start colonizing other parts of the animals. Usually this requires antibiotic treatment (see protocol below).

See Figure 2 from Bossert & Galliot (2021) for a detailed description of the stages of Hydra health degeneration.

Bossert P, Galliot B (2012). How to use Hydra as a model system to teach biology in the classroom. The International journal of developmental biology.

Emergency antibiotic treatment

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11 Treat infected Hydra with rifampicin:

These steps are only needed for treating bacterial infections that may occasionally appear in your tanks.

Materials:

- Rifampicin powder
- Dimethyl sulfoxide
- Serological pipettes

- Automatic or thumb roll pipette pump
- Screw cap plastic microtubes
- Refrigerator

11.1 Prepare rifampicin stock:

Prepare **150 mg/mL** rifampicin stock solution in DMSO by adding the DMSO directly to the bottle of rifampicin.

For example, if you have a **1 g** bottle of rifampicin powder, add **20 mL** of DMSO.

Cap the bottle and invert it a few times to make sure all rifampicin in the bottle is dissolved.

11.2 Aliquot your rifampicin stock solution in screw cap plastic tubes and cap tightly. Store in the dark in the refrigerator at **4 °C**. DMSO becomes solid at **4 °C** so it will need to be thawed before use.

12 Prepare Hydra medium with rifampicin for treating infected Hydra.

Materials:

- Rifampicin stock solution (**150 mg/mL** in DMSO)
- Hydra medium
- Adjustable volume micropipette and tips
- Glass or plastic bottle

Remove a tube of rifampicin stock from the refrigerator and allow it to thaw at **Room temperature**.

Dilute the rifampicin stock 1:1000 in Hydra medium for a final concentration of 50 µg/mL.

For example, to make **1 L**, add **1 mL** rifampicin stock to **999 mL** Hydra medium. Store medium with rifampicin in the dark.

13 Treat infected Hydra:

Use Hydra medium with rifampicin in place of regular Hydra medium. Feed and clean your Hydra on your regular schedule, replacing the medium with fresh rifampicin-containing medium. Store Hydra being treated in the dark.

Continue treating with rifampicin for at least two weeks. If the infection is still there after two weeks, you can treat for longer.