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Comprehensive *Hydra* Care Protocol

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We use this protocol and it's working

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Abstract

The phylum Cnidaria is a sister group to Bilateria, the clade that includes all bilaterally symmetrical animals, and thus is composed of organisms which offer valuable insight into the evolutionary origins of many fundamental developmental traits. This includes the genus, *Hydra*, which for 300 years has been used to study complex topics such as regeneration, senescence, stem cells, and symbiosis. Given *Hydra*'s enduring prevalence in the field, there is an immense amount of literature for new scientists to dig through in order to decipher the mysteries of establishing a colony for research. While there are many resources, current methods and protocols for *Hydra* care often remain vague or lacking in thorough details.

In the following protocol, I eliminate this barrier to entry for new researchers. With detailed step-by-step descriptions, figures, and video accompaniment, this protocol focuses on expanding pedagogy surrounding Hydra by serving as an educational tool for newcomers to the field.

Guidelines

This protocol was designed using the species Hydra vulgaris "AEP," and while it may act as a foundational care guide it is not guaranteed to be the most effective way to care for other species.

I. Hydra Environment

A. Population Container

When determining how to best keep a *Hydra* population, the first thing to consider is the size of the colony. Starting populations will likely not be too heavily populated, so maintaining large tanks would be wasteful. However, well fed *Hydra* populations reproduce every few days through asexual budding and a population that started with five polyps can rapidly outgrow a dish too small.

Below are options for maintaining two sizes of *Hydra* population:

■ Small Populations (≤ 200 Polyps) (Fig.1)

125 × 125 × 20mm Polystyrene Petri Dishes.

Fill to 1/2 height of dish with Hydra Medium.

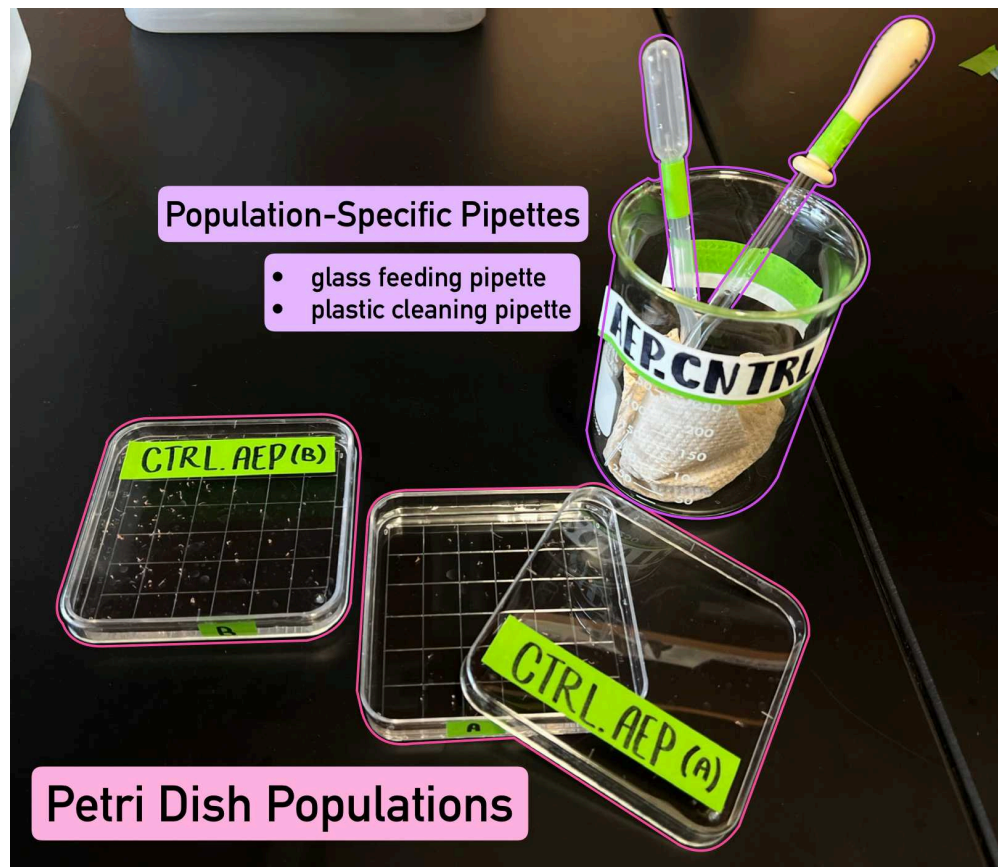


Figure 1 - Two Petri Dish populations and their corresponding population-specific pipettes. Dish, lid, and tools are clearly labelled. Photo depicts CTRL.AEP (A) & CTRL.AEP (B), tools and labelling is clear to communicate populations are not a cross contamination risk so sharing tools is not a hazard.

▪ **Large Populations (≤ 2000 Polyyps)** (Fig.2)

1.48L (6-Cup) Polycarbonate Glass Rectangular Pyrex Dish.

Fill with 300-500mL Hydra Medium.

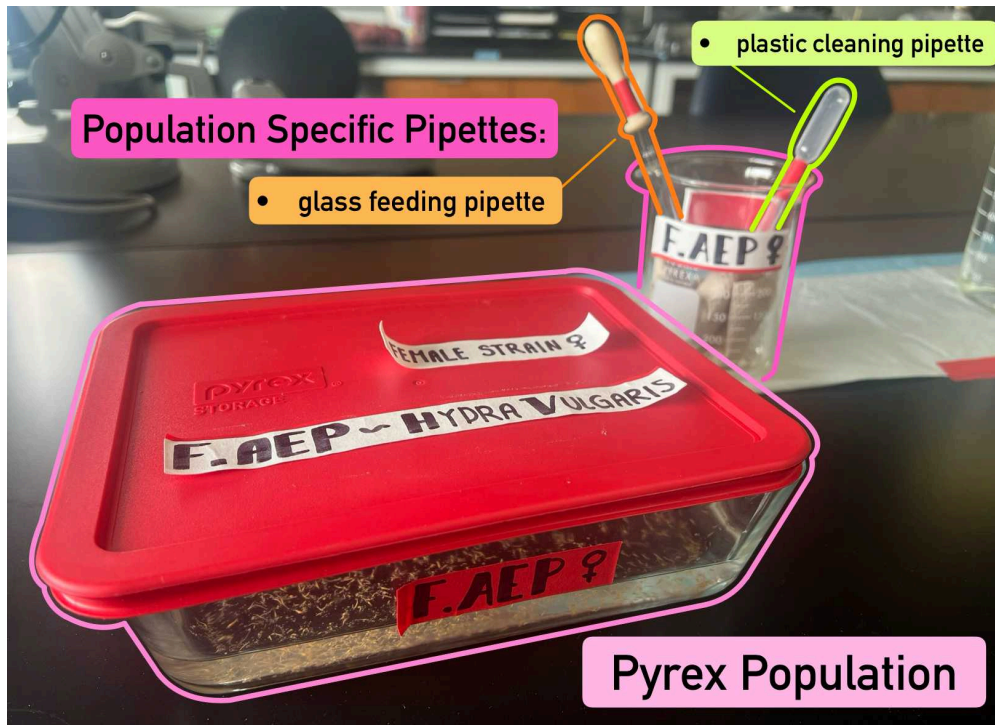


Figure 1 - A Pyrex populations and their corresponding population-specific pipettes. Dish, lid, and tools are clearly labelled. Population F.AEP is sensitive to cross contamination and is thus isolated by distance.

See **Materials** for Hydra Medium (HM) recipe.

B. Handling Tools

Hydra should not be handled by hand, instead each population is suggested to have the following two "population specific" pipettes:

▪ **One glass "feeding" pipette**

E.g. 5 3/4" Disposable Glass Pasteur Pipette

▪ **One plastic "cleaning" pipette**

E.g. 3mL Disposable Plastic Transfer Pipette

See Fig. 1 & Fig. 2 for visual examples.

Note

Though their names specify a purpose for protocol clarity, in *most* instances tools can be used interchangeably within a population depending on the handler's preference and mobility. The most notable exception being that it is **recommended to *always* use the plastic pipette when handling large volumes of multiple *Hydra* i.e. Post-Feeding Clean (Pyrex)**. This is because of a highly damaging "shredding" effect the glass pipette's fine tip can cause when picking up or delivering large volumes of *Hydra*.

C. Cross Contamination

Identifying species of *Hydra* with the naked eye is often unreliable. This makes establishing precautions to avoid cross contamination between species an essential step when fostering multiple species.

It is due to *Hydra*'s proclivity for attaching to tools (SEE HANDLING HYDRA) that we clarify when using "population-specific" pipettes and recommend avoiding using shared equipment between sensitive populations. It is advised to clearly label all population-specific equipment and in some cases isolating sensitive populations by distance to avoid accidental tool swapping.

See Fig. 1 & Fig. 2 for visual examples.

D. Temperature

Hydra populations raised under this protocol were successfully maintained at room temperature (20-23° C, with slight seasonal fluctuation). Experimental populations were also successfully maintained in Controlled Environment Rooms at 25 and 15° C under described care conditions.

E. Liquid Medium

Hydra are freshwater Cnidarians and can be maintained in anything from pond water, spring water or the *Hydra* Medium (HM) solution described in **Materials**.

■ Cautionary Statement Regarding Salinity

The washes to be described in are essential to maintaining healthy *Hydra* and must be done thoroughly. An inherent incompatibility is between our freshwater specimen and their saltwater food source. ***Hydra* are highly susceptible to illness caused by hypertonic conditions, in some cases saline exposure can even be fatal.** Due to our *Artemia* being fostered in a brine solution, poor or insufficient washes can result in salt being carried over into *Hydra* populations.

It is also important to note, ***Hydra* require living food and will not eat dead *Artemia*.** Washes should be completed relatively quickly, no more than 30 minutes from the time the hatchery bubbler is turned off. This is because over time **as *Artemia* are exposed to cooler, idle, and hypotonic conditions they will begin to die.** The more immediately fatal exposure can occur by leaving *Artemia* in the filtration net without liquid.

II. Handling Hydra

A. Attaching to Surfaces

Hydra attach to surfaces using sticky cells on their basal disk or "foot." This attachment is temporary but very strong and using the suction of a pipette to remove a *Hydra* from a surface can do significant damage to the polyp if it does not detach readily.

To avoid this, use a pipette to gently pulse jets of water toward the *Hydra*'s foot, often this alone is enough to detach the polyp. If not and removal is absolutely necessary, a pipette (glass being most effective) can be used to gently scrape the foot from a surface by wedging between the surface and the foot. Though less damaging, this action does risk damaging the polyp if not done with caution.

B. Attaching to Tools

When transporting *Hydra* in a pipette, polyps can often attach to the inside. This often occurs unpredictably and can be unavoidable.

Many times, one to three rounds of aspirating and dispensing HM through the pipette can be enough to wash off the polyp. However, when a polyp is stubbornly attached we have found that allowing the pipette to rest for a minute without any liquid and *then* repeating the aforementioned aspiration/dispense method is a minimally-stressful method of detaching a polyp.

III. Establishing a Care Routine

Care routines can vary depending on the goal of the population being fostered, experimental design and on the workforce available, however, maintaining health of *Hydra* populations is strongly correlated to maintaining a consistent and effective routine.

A. Feeding Routine

We have found the ideal schedule for basic maintenance or growing *Hydra* populations to be 3-4 feeding days a week. Aiming to reduce the impact of long stretches of starvation by feeding right before weekends and immediately upon return.

Due to the necessity for live food, feeding days must coordinate with timing of brine shrimp hatcheries.

Below, Figures 3 & 4 depict two different weekly care routines our lab populations have been maintained on.

B. Cleaning Routine

Population pipettes should be replaced every 1-2 weeks depending on frequency of use.

Pyrex & Petri dishes should be deep cleaned every 2-3 weeks depending on build up of waste residue. Due to large number of *Hydra*, Pyrex dishes tend to require deep cleaning more frequently.

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Feeding	n/a	Feed	Feed	n/a	Feed	n/a	Feed
Hatchery	n/a	Set up 24hr Hatchery (feeds Tuesday)	Set up 48hr Hatchery (feeds Thursday)	n/a	Set up 48hr Hatchery (feeds Saturday)	n/a	Set up 48hr Hatchery (feeds Monday)

Figure 3 - Weekly schedule depicting 4 Feeding Days per Week, Weekend Availability

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Feeding	n/a	n/a	Feed	Feed	n/a	Feed	n/a
Hatchery	n/a	Set up 24hr Hatchery (feeds Tuesday)	Set up 24hr Hatchery (feeds Wednesday)	Set up 48hr Hatchery (feeds Friday)	n/a	Prepare Brine Solution for Monday Hatchery	n/a

Figure 4 - Weekly schedule depicting 3 Feeding Days per Week, No Weekend Availability

IV. Observing *Hydra*

A. Communicating with Contraction

Since *Hydra* obviously cannot communicate their needs directly, it is essential to familiarize oneself with key polyp behaviors in order to infer the health of a population.

Hydra are very sensitive and reactive to certain stimuli in their environment. Whether it is a change in light, shifting their dish, or touching them with a pipette A *Hydra*'s most common and visible response to stimulus is through **contraction**. (see Fig.5)

All *Hydra* contract, and do so frequently. However, healthy *Hydra* when unstimulated are often seen with both body and tentacles fully elongated. Thus, a clear indicator of stress or poor health is constant contraction. For teaching purposes, I often equate this behavior to clenching your jaw when stressed.

This also means how *Hydra* behave when idle and unstimulated is a helpful way to gauge population health. If an entire population that should be "relaxed" appears to have body and tentacles constantly contracted, this is a sign of stress in their environment.

B. Common Sources of Stress

The most common causes of total population stress are overcrowding, unsanitary conditions, or osmotic stress. Thorough cleaning and increased frequency of fresh HM changes can help alleviate unsanitary or osmotic stress. Note that *Artemia* leftover in a population after cleaning offer significant risk to population health due to bacterial growth, while removing all *Artemia* is in some instances near impossible, avoid leaving dead *Artemia* overnight in dishes as much as possible.

See **V. Disposing *Hydra* & HM Waste** for overcrowding.

V. Disposing *Hydra* & HM Waste

A. Overcrowding

Hydra are excellent at building a colony when fed regularly, however, maintaining a population at a plateau is a less successfully executed feat. Consequently this requires **population culling** to be an essential part of animal care in order to avoid overcrowding stress.

This process includes taking a pipette and removing *Hydra* at random from a population and then processing the removed individuals and HM as waste. Alternatively, populations can be culled during cleaning by washing *Hydra* until population size is preferential, and all individuals remaining can be processed with regular HM waste.

B. Chemically Treating Waste

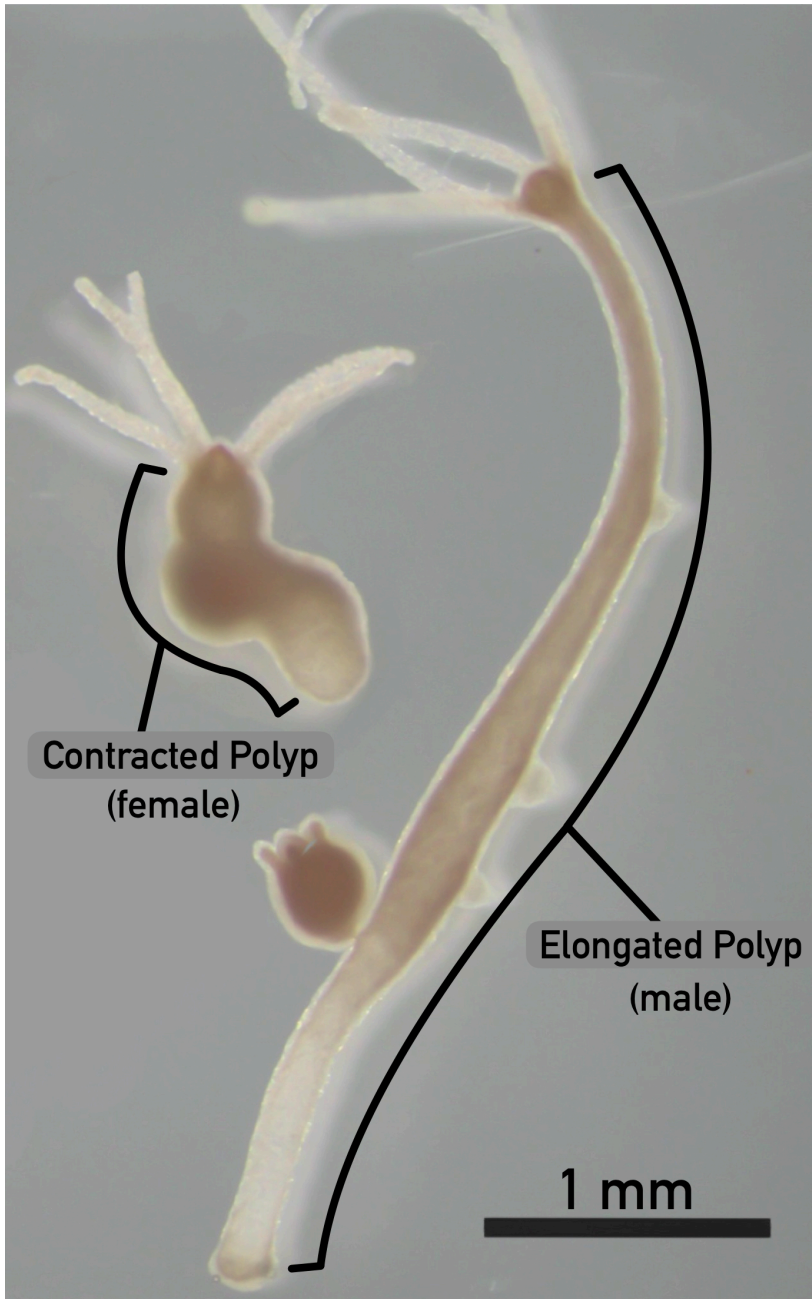


Figure 5 - Depicts a polyp contracting in response to stimulus next to a relaxed elongated *Hydra* polyp

reviewed and you are prepared for the task, your research journey begins by acquiring your specimen. If seeking a particular species of *Hydra*, a viable option is identifying a laboratory actively researching it and requesting to be sent a few individuals. If shipping restraints allow and the lab has organisms to spare, this is a common solution. Alternatively, *Hydra* can also be purchased online from multiple biological supply companies. However, as *Hydra* are common freshwater specimen, they can also be collected in the wild.

Due to *Hydra*'s ability to regenerate at a cellular level and in an effort to avoid introducing lab strain *Hydra* into public water ways, **all HM, and dishware used to contain *Hydra* waste should be chemically treated using Bleach.**

! Firstly, review Warnings for SDS pertaining to handling Bleach and check with your institution's waste management regulations to determine if waste can safely be dumped down drain or should be processed as chemical waste.

Working in a fume hood and wearing all appropriate PPE (gloves, splash goggles, and a lab coat)

1. Add Bleach to liquid HM waste to created a 10% solution by volume e.g. For 900mL of HM Waste, add 100 mL of Bleach.
2. Allow solution to rest in fume hood for at least 10 minutes before dumping or processing.
3. All dishware that has been in contact with bleach should be washed thoroughly with warm soapy water.

VI. Acquiring *Hydra*

Once entire protocol has been

Accompanying video of full protocol can be found here: <https://youtu.be/k7jdAW3nN8c>



Materials

Tools (Reusable)

- 2L Brine Shrimp Hatchery (model used in protocol made by [Brine Shrimp Direct](#))
- 10L Aquarium Bubbler ([example](#))
- 6-Cup Pyrex ([example](#))
- Heat Lamp Bulb ([example](#))
- Heat Lamp Clamp & Aluminum Reflector ([example](#))
- Beakers (400ml x5, 600ml, 1000ml, 2000ml)
- Brine Shrimp Net ([example](#))

(See below for diagram of modified filtration net)

- Zip ties
- Blade

Tools (Consumables)

- 3mL Disposable Plastic Transfer Pipettes
- 5 3/4" Disposable Glass Pasteur Pipettes
- 2mL Rubber bulbs (for Glass Pipettes)
- 125 × 125 × 20mm Polystyrene Petri Dishes
- Brine Shrimp Cysts (*Artemia franciscana* used in protocol purchased from [Brine Shrimp Direct](#))
- Parafilm

Chemicals

- CaCl_2
- Milli-Q H_2O . (Or alternative high purity water.)
- MgSO_4
- NaHCO_3
- K_2CO_3
- NaCl (Non-iodized table salt)
- Germicidal Clorox Bleach
- 70% Ethanol

Premade Solution Recipes:

- **Hydra Medium**

The described recipe was first published by W.F. Loomis in 1953.

1. Prepare the following stock solutions:

- **Stock 1 (1L)**

In a 1L Beaker, add:

 42.18 g CaCl_2

Fill beaker to 1L total volume with Milli-Q H_2O .

Stir until fully dissolved.

Store in sealed container at room temperature.

- **Stock 2 (1L)**



In a 1L Beaker add:

🧪 8.116 g MgSO_4

🧪 4.238 g NaHCO_3

🧪 1.098 g K_2CO_3

Fill beaker to 1L total volume with Milli-Q H_2O .

Stir until fully dissolved.

Store in sealed container at room temperature.

2. In a 1L Beaker add:

🧪 20 mL Stock 1

🧪 200 mL Stock 2

Fill beaker to 1L total volume with Milli-Q H_2O .

2.1. Let stir for 🕒 00:45:00

3. In a clean, dry 20L Nalgene Carboy, carefully pour the 1L Beaker containing the two stocks.

5. Fill Carboy with Milli-Q H_2O until total volume reaches 20L.

Clean 20L Carboy thoroughly with warm soapy water, sanitize with Ethanol, and allow to dry fully between batches of HM to minimize risk of bacterial or fungal contamination in idle water.

Citation

W. F. Loomis (1953). The Cultivation of Hydra Under Controlled Conditions. Science.

<https://doi.org/10.1126/science.117.3047.565>

LINK

■ Brine Solution

In a 1L Beaker add:

🧪 0.33 g NaHCO_3

🧪 25.0 g NaCl

Fill beaker to 1L total volume with Milli-Q H_2O .

Stir until fully dissolved. (Stirring on hotplate can aid in dissolving salts however ensure temperature is not greater than 28°C when solution is added to hatchery to avoid damaging *Artemia* cysts.)



Note

Storage: Brine Solution can be used immediately or stored for future hatcheries. I recommend making only 1L at a time to ensure correct 1g *Artemia* cysts:1L Brine Solution ratio. If BS is not used immediately, ensure to stir thoroughly prior to adding to hatchery to dissolve any built up sediment.

Modified Brine Shrimp Net Filter

Brine Shrimp Nets are often designed to have the seam interrupting the center of the net, we modified our net to hold the seam out of the way and create a shallower surface for washing.

Note

When Purchasing a Net: Unfortunately we have found some Brine Shrimp Nets marketed to be fine enough for Brine Shrimp collection are too porous for the wash steps described in this protocol. Ours was purchased in person from an exotic pet store and may prove the safer option to avoid spending money on multiple nets.

1. First fold the netting into the desired shape for filtration. Using a permanent marker, then mark where to make the incisions for our zip ties (We used 5)

When identifying where to cut it is important to make sure that the net will not sit so tightly that the Artemia will disperse across the flat surface and risk being lost in the folded fabric along the edges. The ideal filter creates a centered shallow well where the Artemia will collect during washes.

2. Using a razor blade make a small cut in the marked locations

Ensure cuts are just large enough for the zip tie, making too large of incisions can be detrimental to the lifespan of the net and risks coming apart after extensive usage and cleaning.

3. Laced zip ties through holes, encircling the edge of the net, and tighten to fasten the folded fabric around the nets metal frame.

Be cautious not to close the zip ties so tightly though that the netting cannot move otherwise the incisions may risk tearing when washing the net.

Cleaning the Net

Folded fabric creates a potential hazard for collecting dead brine shrimp and other waste. To ensure the net remains sanitary, in a fume hood or other well ventilated area, dip netting in a 10% bleach solution and allow to sit for 5-10 minutes. Then wash thoroughly with warm soapy water and scrub fabric. Check that net does not smell of bleach once dry.



Figure 12 - Filtration Net used in Section Two: Wash Your Food - Feeding a Population

Accompanying video of full protocol can be found here: <https://youtu.be/k7jdAW3nN8c>

Safety warnings

Safety Data Sheets

- Ethanol - C_2H_6O - [SDS](#)
- Sodium hypochlorite - $NaClO$ - "Clorox Germicidal Bleach" - [SDS](#)
- Calcium chloride - $CaCl_2$ - [SDS](#)
- Magnesium sulfate - $MgSO_4$ - [SDS](#)
- Sodium bicarbonate - $NaHCO_3$ - "Baking Soda" - [SDS](#)
- Potassium carbonate - K_2CO_3 - [SDS](#)
- Sodium chloride - $NaCl$ - "Non-iodized Salt" - [SDS](#)

Known Hazardous Interactions

Ethanol + Bleach ($NaClO$) = Chloroform gas formation, neurotoxin **[NEVER ADVISED]**

- Ethanol and Bleach are both used for different sanitization steps, & are never suggested to be used together. Use utmost care to avoid mixing the two.

Calcium chloride ($CaCl_2$) + Water = Strong exothermic reaction **[STOCK 1 CREATION]**

- Calcium chloride is an ingredient used to make Hydra Medium and is added to water at such small quantities the exothermic reaction is negligible. Still, use caution when handling.

Bleach ($NaClO$) + Acid (from bicarbonate) = Chlorine gas release **[DECONTAMINATING HM]**

Bleach ($NaClO$) + Potassium carbonate (K_2CO_3) = Risk of chlorine gas at high pH **[DECONTAMINATING HM]**

- During de-contamination steps at end of cleaning Bleach is added to used Hydra Medium to avoid introducing lab strain *Hydra vulgaris* AEP to public waterways. Potassium carbonate and Sodium bicarbonate are both ingredients in Hydra Medium. **Ensure all steps involving bleach are performed under a fume hood** and allowed time for any potential gases to dissipate prior to dumping.

 **Handling living organisms always poses a risk for infection, allergic reactions, and potential for exposure to harmful substances.** Wearing proper Personal Protective Equipment (PPE) like gloves, lab coats, and eye protection is essential to lab safety as well as practicing aseptic techniques to minimize potential health risks.



Ethics statement

Ethical Animal Care

At the time of publication of this procedure, members of the genus *Hydra* are not subject to any regulation or care standards by the Institutional Animal Care and Use Committee. Living organisms in research should never be subjected to malicious intent or be subject to aimless harm.

Environmental Concern

Hydra vulgaris "AEP" is a lab strain not found naturally, care should be implemented to prevent release of polyps into public water ways. Due to the affinity of *Hydra* for regeneration at a cellular level, used Hydra Medium and culled individuals should be euthanized using a 10% Bleach solution by waste volume to ensure cell death. (ie 100mL Bleach + 900mL Organic Waste Material = 10% Bleach Solution)

Before start

Be sure to review entire protocol, guidelines, warnings, and acquire all necessary materials prior to obtaining *Hydra* specimen.

Accompanying video of full protocol can be found here: <https://youtu.be/k7jdAW3nN8c>

The following protocol is a comprehensive guide to daily care for *Hydra vulgaris*, it describes the process of growing food, washing food, feeding and cleaning your populations. Therefore, it begins under the assumption *Hydra* populations are being kept according to standards outlined in **I. *Hydra* Environment** in **Guidelines**.

Glossary

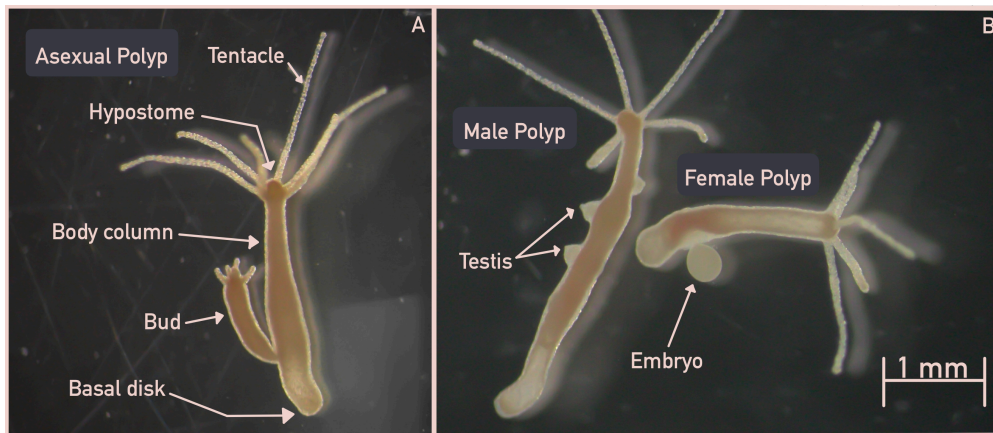


Figure 6 - (A) Shows the anatomy of a *Hydra vulgaris* polyp with attached bud. **(B)** Shows sexually differentiated polyps and their distinct traits.

- **Hydra** - Refers to the genus of carnivorous freshwater hydrozoans within the phylum Cnidaria.
- **Polyp** - Refers to one of two body plans within the Cnidaria phylum, however, is used most frequently in this protocol to refer to an individual of the *Hydra* genus.
- **Hydra vulgaris AEP** - A lab strain of *Hydra vulgaris* developed in the lab of Dr. Hans Bode at UC Irvine. A more detailed explanation of the strain is detailed [here](#). Can be shortened to *H. vulgaris* AEP, or AEP.
- **Lab Strain** - In the context of *Hydra*, a "lab strain" is a lineage of a particular species that does not occur in nature, sometimes strains are notable for transgenic traits or were selectively isolated due to a particular affinity for a naturally occurring behavior (E.g. "AEP" was selected for its ability to produce both male and female polyps.)
- **Asexual** - *Hydra*'s preferred method of reproduction by which offspring is produced by a single parent, in *Hydra* this is done by budding. Also used to describe a polyp that is not actively expressing any sexual traits. (See Fig. 6.A)
- **Budding** - A method of asexual reproduction where a parent *Hydra* produces an outgrowth on the lower region of the body column that develops into a genetically identical clone. Until the offspring detaches it is referred to as a "bud." (See Fig. 6.A)
- **Female/Male** - Used to describe a polyp that is actively expressing sexual traits. Egg field for "Females," and testis for "Males." Due to sex reversal and gametogenesis being a temporary phase, a polyp referred to as



either sex is not guaranteed to always be that sex. (See Fig. 6.B)

- **Population** - In this procedure, a population just refers to a group of *Hydra* polyps in a single Pyrex or Petri dish. (E.g. Population "A" uses population-specific tools to avoid any contamination with Population "B" which are undergoing a different treatment.) (See Fig. 1 & Fig 2)

Food Source - Brine Shrimp Hatchery

2d 0h 10m

- 1 Set up Hatchery area initially by measuring necessary distance between heat lamp bulb and hatchery to maintain incubation temperature of 22°-26°C.

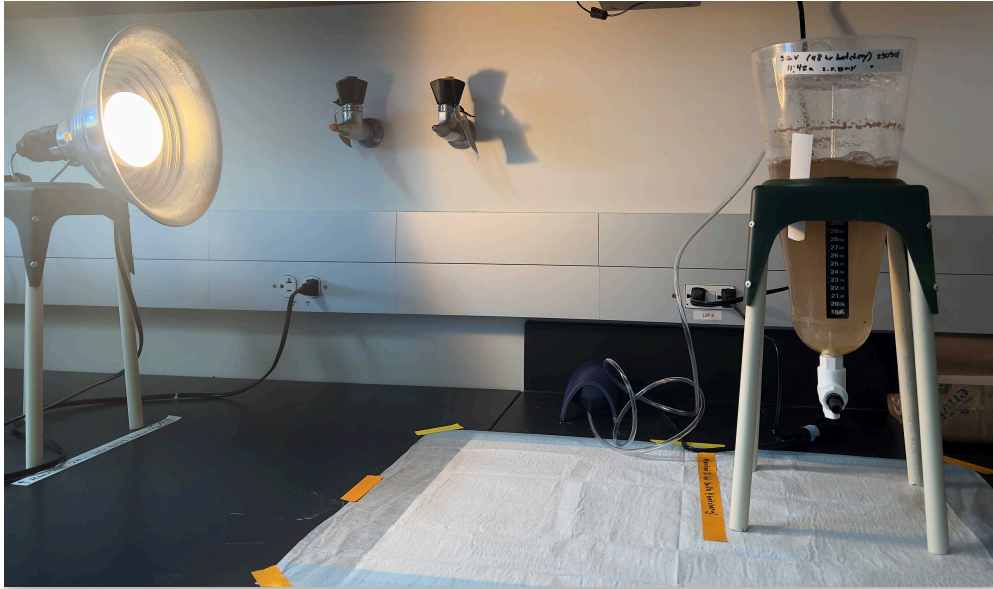


Figure 7 - Depicted is Brine Shrimp Direct hatchery with 10L Bubbler and Heat Lamp. Heat lamp & hatchery distance is pre-measured and marked with tape as to maintain temperature of 22°-26°C.

- 2 Before turning on 10L Bubbler, attach provided rubber tubing to bubbler and opposite end to provided hard plastic straw. Then thread straw through hole in plastic splash lid.
- 3 Fill hatchery cone with 1L of premade Brine Solution then add 1g of *Artemia franciscana* cysts. If a greater quantity is needed maintain 1L:1g ratio.
- 4 Cover hatchery with splash lid and ensure tubing-attached straw is securely inserted into the spigot cavity at the base of the hatchery before turning on bubbler.

10m

Note

Failure to align the straw with the hole will cause directional water flow and unhatched cyst build up within the cavity resulting in inhibited incubation and reduced hatchery output.

- 5 Allow 24-48 hours for cysts to hatch.



Expected result

Artemia are sensitive to light, temperature, water flow, salinity and pH so total hatchery output can be variable if these environmental conditions are inconsistent.

In general, a 24 hour 1L:1g hatchery will produce enough food to feed 2,000-4,000 Hydra (or 1-2 Pyrex populations), 48 hours will produce enough to feed 6,000-8,000 Hydra (3-4 Pyrex populations).

Beyond 48 hours *Artemia* begin to die off and hatchery conditions become unhealthy for feeding.

Harvesting Hatchery

10m

- 6 When ready to feed turn off both heat lamp and bubbler, then remove tubing, and straw.

Complete the following steps in immediate sequence without significant pausing.

- 7 Using a dissection lamp, orient light near the base of the hatchery. *Artemia* are drawn to light, the goal is to direct them to swim toward the spigot. Allow approximately 5 minutes for *Artemia* to congregate toward the light.
- 8 With a 400mL beaker ready, open hatchery spigot and pour out approximately 300ml of brine solution and *Artemia*.

This will be the **Working Food Volume** for the rest of feeding, Hatchery cone is no longer needed.

- 9 Using a dissection lamp, orient light near the base of the beaker. The aim now is to localize all of the living *Artemia* into one area to maximize the amount of brine shrimp collected via pipette in **Step 10**.

5m

Allow approximately 5 minutes for *Artemia* to congregate toward the light.

Note

In this smaller volume, the degree of light diffusion significantly effects the way *Artemia* localize. Minimize light diffusion by changing light direction downward.

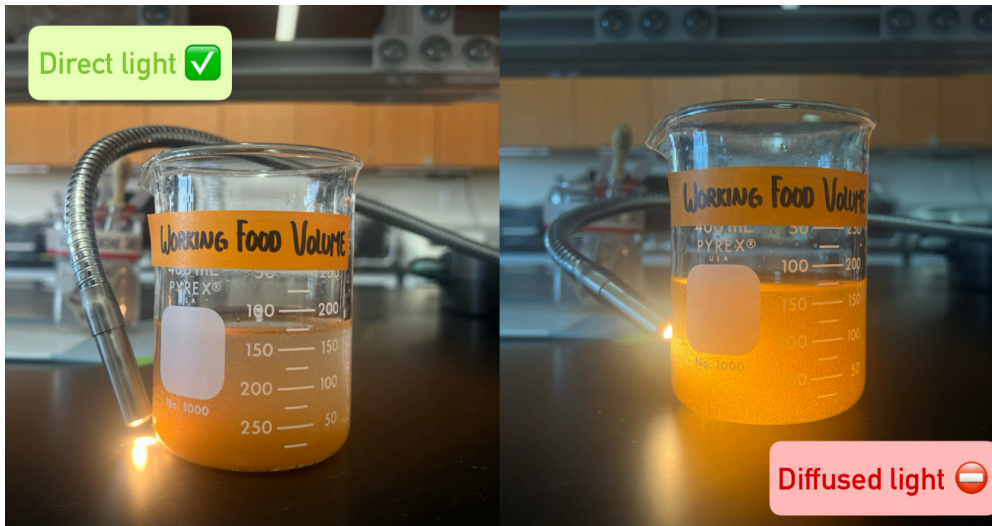


Figure 8 - Direct light draws the *Artemia* to a more specific location. When the light diffuses through the liquid the *Artemia* will disperse more sporadically.

- 9.1 While *Artemia* are swimming towards light prepare set up for next steps.
- Clearly label a satellite beaker and disposable plastic pipette for warm tap water washes.
 - Clearly label a satellite beaker and disposable plastic pipette for Hydra Medium washes.
 - Clearly label a disposable plastic pipette for collecting Brine Shrimp. (Referred to as the "Saline Pipette," note that no other tools should contact Brine Solution or Saline Conditions)
 - Place an additional 400mL beaker for waste produced from washes under brine shrimp net filter.
 - Prepare glass feeding pipettes for populations about to be fed.

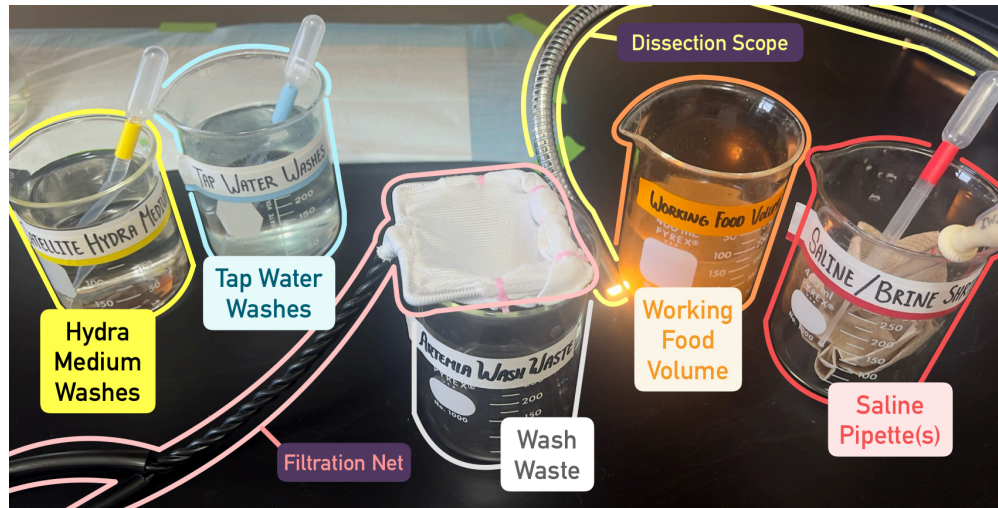


Figure 9 - Population-specific glass feeding pipettes not depicted, see Figures 1 & 2 in **Guidelines**

Wash Your Food - Feeding a Population

2h 10m

- 10 Ensure familiarity with **Guidelines** > **I. Hydra Environment** > **E. Liquid Medium** > **Cautionary Statement Regarding Salinity** before proceeding.

2m



The following section is recommended to be completed per single *Hydra* population.

Ensure following steps are completed immediately in sequence and without significant pausing. Leaving *Artemia* in net for too long can result in suffocation, reducing the volume of live food available for populations.

Using the Saline Pipette pick up *Artemia* from the **Working Food Volume** that have congregated near the light & deliver to filtration net.

Repeat until net holds enough *Artemia* to feed desired population.

- If feeding a Pyrex Dish population ($\leq 2,000$ Polyps) - Repeat 3-4 times
- If feeding a Petri Dish population (≤ 200 Polyps) - Repeat 1-2 times

! *These quantities can vary considerably depending on success and age of hatchery. Listed volumes are averages based on consistent high hatchery output, increase or decrease as suits your populations.*

**Note**

The **Working Food Volume** of *Artemia* will always have some amount of hatched cysts at the bottom of the beaker. These provide no nutrition to the Hydra. To avoid picking up cysts aspirate slowly and avoid pressing the pipette too close to the floor of the beaker.

Put away Saline Pipette, do *not* use for wash steps.

- 11 Using appropriate Tap Water pipette, rinse brine solution off of *Artemia* using warm tap water wash. Repeat 5-10 times.

2m



⚠️ Avoid touching filtration net with Tap Water pipette.

- 12 Using appropriate Hydra Medium pipette, rinse tap water off of *Artemia* using Hydra Medium wash. Repeat 5-10 times.

2m



⚠️ Avoid touching filtration net with Hydra Medium pipette.

- 13 Using a population-specific glass feeding pipette in dominant hand and the Hydra Medium plastic pipette in off-hand. Simultaneously deliver Hydra Medium into brine shrimp filtration net while aspirating *Artemia* from net with glass feeding pipette.

1m

**Note**

Adding HM to filtration net acts as a liquid “vehicle” to pick up the brine shrimp with the glass pipette, pipetting must be simultaneous to avoid loss of liquid through the mesh.

- 14 With washed *Artemia* collected in population-specific glass feeding pipette, deliver to corresponding. population. Gently release droplets of *Artemia* uniformly around dish.

3m



- If feeding a Pyrex Dish population ($\leq 2,000$ Polyps) - 1-3 pipettes full per dish
- If feeding a Petri Dish population (≤ 200 Polyps) - 2-4 drops per dish

⚠️ *These quantities can vary considerably depending on ratio of Artemia to Brine Shrimp collected in **Step 13**. Listed volumes are averages based on personal experience, increase or decrease as suits your populations.*

**Note**

Brine shrimp tend to sink to the bottom of the dish, using the same glass pipette, and being careful not to pick up any Hydra, gently pulse liquid within the dish to disperse the brine shrimp and ensure all polyps catch food.

- 14.1 After 10 minutes, check-in with population to determine if extra food is needed. Using glass feeding pipette either spot feed individual polyps that have not caught food or gently pulse liquid to further disperse not-captured brine shrimp.



- 15 **Repeat Steps 10-14** until all populations have been fed.

- 16 Allow *Hydra* at least 2 hours to feed before cleaning. Then, depending on population containment, Proceed to either Post-Feeding Clean (Pyrex) or Post-Feeding Clean (Petri Dish)

2h

**Note**

If cleaning happens too early, even minimal suction from pipettes can result in food being unintentionally pulled out of the *Hydra* body cavity before they are able to digest.

Post-Feeding Clean (Pyrex)

58m

- 17 Acquire necessary materials prior to cleaning.

Note

The following list of materials is per Pyrex.

If populations are contamination risks to each other, **non-consumables should be fully cleaned and sanitized between populations**. If cross contamination is not a concern, tools can be reused without cleaning.

- At least 2L of Clean Hydra Medium (HM)
- Clean and clearly labelled satellite beaker for Hydra Medium & designated pipette.
- Population-specific pipettes

5 clean, dry Beakers (suggested volumes are recommended but not necessary)

- 2000mL - For Waste Collection
- 1000mL - For Working Volume
- 600mL for Wash 1
- 400mL for Wash 2
- 400mL for Wash 3 (Optional)

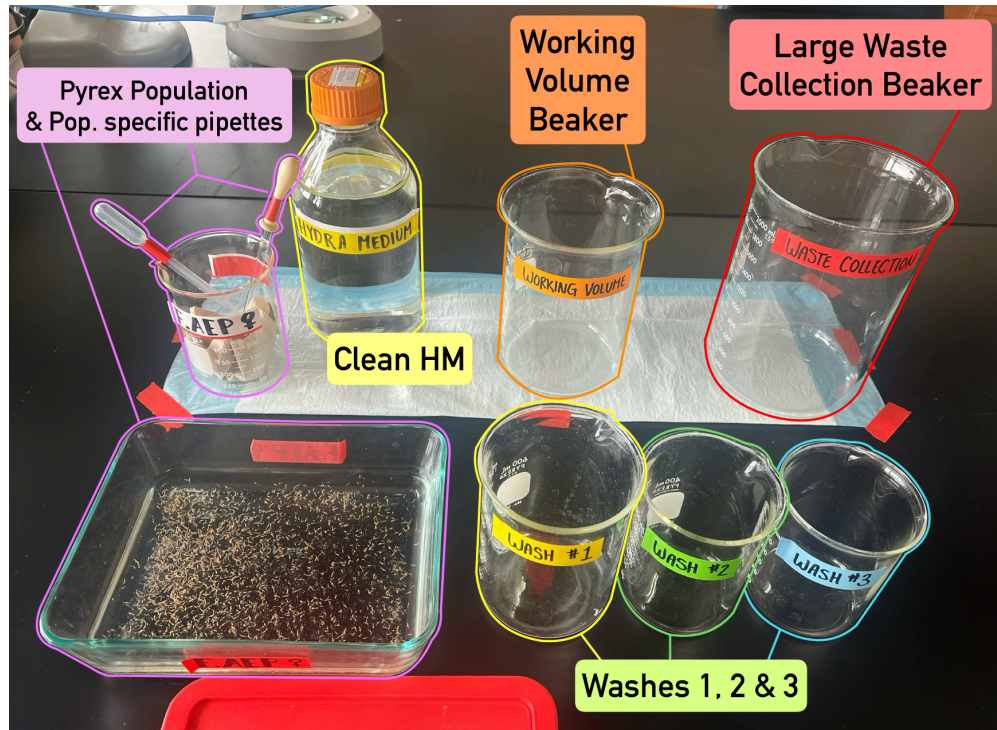


Figure 10 - Materials required for cleaning Pyrex population.

- 18 Pour contents of Pyrex carefully into 1000mL beaker, this is our **Working Volume**.
- 19 Gently rotate the beaker to create a vortex, then allow the beaker to sit for 1-2 minutes while the centrifugal force on the spinning liquid pulls the *Hydra* together in the middle of the beaker. 2m
- 20 While the **Working Volume** is settling, hold the Pyrex at a 45 degree angle and with the HM Pipette, gently rinse the inside of the Pyrex with clean HM. 4m

Aim to knock loose any remaining dead *Artemia*, pellets or film from the bottom surface. *Hydra* that had previously clung to the floor of the dish will likely also come loose.

As liquid accumulates on one end of the Pyrex, pour it off into the 600mL beaker for **Wash 1** & repeat the spinning process described in **Step 19** but now with **Wash 1**.



- 21 With the Pyrex now rinsed of waste, pour a shallow layer of HM approximately 1cm deep.

1m

Then, to ensure no polyps dry out, use the HM pipette and clean HM to gently detach any lingering *Hydra* clinging to the Pyrex walls above the liquid level.

- 22 Add 100mL of HM to both of the 400mL beakers for **Wash 2** and **Wash 3**.

Note

If using alternative volume beakers or other vessel, note the exact volume of HM being added is not important, it is only relative to what we need it for. The intention is to add enough HM to be able to accomplish the spinning effect created in Step 19. Volumes too small will not pull *Hydra* to the center of the beaker, volumes too large risk splashing and losing polyps.

- 23 In **Wash 1** (600mL Beaker)... Use population-specific plastic pipette to slowly and gently pick up *Hydra* accumulated in center of the beaker. Avoid picking up waste.

2m

Then, immediately deliver *Hydra* to **Wash 2** (400mL beaker) and repeat spinning process described in **Step 19** but now with **Wash 2**.

Note

Collecting *Hydra* while **Wash #** is still spinning can help in reducing the amount of waste collected as heavier mass will be brought to the center first.

- 24 In **Wash 2** (400mL beaker)... Use population-specific plastic pipette to slowly and gently pick up *Hydra* accumulated in center of the beaker. Avoid picking up waste.

2m

Then, immediately deliver *Hydra* to **Wash 3** (other 400mL beaker) and repeat spinning process described in **Step 19** but now with **Wash 3**.

Note

If at this step **Wash 3** does not appear adequately clean, replicate wash steps can be added as needed.

Dump the waste from **Wash 2** into **Waste Collection Beaker** and add 100mL of clean HM. Transfer *Hydra* from **Wash 3**, to clean medium in **Wash 2**, and spin.



- 25 When *Hydra* are rinsed and clean, use population-specific plastic pipette to slowly and gently transfer clean polyps from **Wash 3** beaker to Pyrex.

2m

Then, empty all waste from **Washes 1, 2, & 3** into 2000mL Waste Collection Beaker.

- 26 Add 200mL of clean HM to **Wash 1**.

3m

Then, using population-specific plastic pipette, slowly and gently transfer 1-2 pipette volumes of *Hydra* from **Working Volume** (1000mL Beaker) to clean medium in **Wash 1** and repeat spinning process described in **Step 19**.

- 27 Repeat **Steps 22-26** until all *Hydra* from **Working Volume** are washed and have been added back to their Pyrex.

40m

- 28 Spot clean any notable waste from the Pyrex with a glass pipette.

2m

- 29 Fill Pyrex with 300-500mL Hydra Medium.

*Process all Hydra waste & dishware as described in **V. Disposing Hydra & HM Waste** found in **Guidelines**.*

Deep Cleaning (Pyrex)

10m

- 30 Follow **Steps 17-20** in Post-Feeding Clean (Pyrex) protocol except rinse all *Hydra* from the Pyrex.

- 31 Cover **Working Volume** and **Wash 1** beakers with Parafilm and label with population, date, initials.

- 32 Transfer empty Pyrex to the fume hood, fill Pyrex with 1.4L of 10% Bleach & let sit for 10 minutes.

10m



- 33 Dump bleached waste liquid, remove tape labels on Pyrex, wash both dish and lid thoroughly with sponge and warm soapy water. Allow Pyrex & lid to air dry.





Safety information

Smell Pyrex to ensure no scent of bleach lingers on the dish. If the dish still smells of bleach, re-wash.

Following step introduces Ethanol, Bleach & Ethanol produce Chloroform, a neurotoxin. Ensure no bleach remains before proceeding to next step.

- 34 Sanitize Pyrex using 70% EtOH and a paper towel. Do not fill, just wipe the lid and surfaces & allow to air dry.
- 35 When EtOH has fully evaporated, re-label the dish & continue regular cleaning procedure at **Step 21**.

Post-Feeding Clean (Petri Dish)

- 36 Acquire necessary materials prior to cleaning.
 - Short term waste beaker
 - Waste Collection beaker (If cleaning multiple Petri dishes)
 - Population-specific pipettes
 - A satellite beaker for clean Hydra Medium and designated pipette.

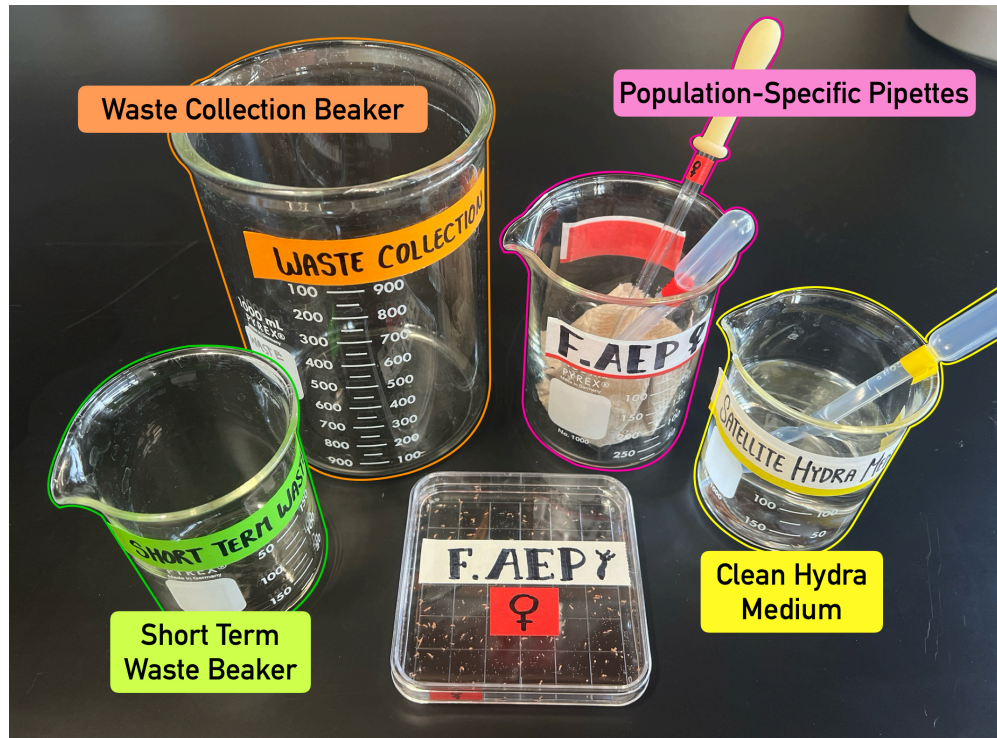


Figure 11 - Materials required for cleaning Petri Dish population.

- 37 Using population-specific pipettes, "vacuum" the dead *Artemia* from around the *Hydra* within the dish. Deliver waste from pipette to the **Short-Term Waste** beaker.
- Alternate between population-specific plastic/glass pipettes and replenish HM with small volumes as needed.
- 38 Be sure to check **Short-Term Waste** beaker for any accidentally removed *Hydra* and add them back to their dish.
- 39 When dish is clear of *Artemia* waste & **Short-Term Waste** beaker is clear of *Hydra*, refill dish to half its height with clean HM and ensure all polyps are below water level.
- 40 *If cleaning one population, skip this step and proceed to Step 41...*
- If cleaning multiple populations, dump **Short-Term Waste** beaker in **Waste Collection** beaker after ensuring no *Hydra* remain attached inside.
- Then, continue cleaning remaining petri dishes repeating **Steps 36-40** until all remaining populations are complete.
- 41 Process all waste as described in **V. Disposing Hydra & HM Waste** found in **Guidelines**.

Deep Cleaning (Petri Dish)

- 42 Follow Steps **36-40** in Post-Feeding Clean (Petri Dish).

Take **Short-Term Waste** beaker, rinse with a small volume of clean HM to remove waste and relabel with population being cleaned, date, initials.

- 43 Transfer *Hydra* from Petri Dish into beaker, then cover with Parafilm.

Note

Hydra attach to Petri Dishes much more strongly than Pyrex glass, this process can take some time and be stressful on the polyps. Use pipettes to gently rinse the *Hydra* from the dish with HM to encourage detaching.

- 44 Dry petri dish with a paper towel, then using 70% Ethanol, add a small volume to the dish and use an additional paper towel to wipe down the surfaces of the dish with the Ethanol. Allow to air dry.

- 45 When dish is fully dry of Ethanol, add a shallow pool of clean HM and using a plastic population specific pipette, add *Hydra* back to the dish from the beaker.

When all *Hydra* have been returned to their dish, fill dish to half its height with clean HM.

Process all waste as described in **V. Disposing *Hydra* & HM Waste** found in **Guidelines**.

Citations

W. F. Loomis. The Cultivation of *Hydra* Under Controlled Conditions

<https://doi.org/10.1126/science.117.3047.565>