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Snail husbandry for maintaining the *Schistosoma mansoni* life cycle

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We use this protocol and it's working

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Abstract

Maintenance and general husbandry of schistosome-susceptible snails, including but not limited to *Biomphalaria glabrata* fresh water snails. The purpose of this SOP is to outline the weekly protocols required for general husbandry of a *Schistosoma mansoni* life cycle through the use of *Biomphalaria glabrata* snails.



Materials

Coarse grit poultry oyster shells

Aquariums (maximum density of snails is ~1 adult snail per 1L water volume)

Air compressor or air source

Marina blue airline tubing (pet store or Amazon)

25mm air stone cylinders (pet store or Amazon)

Artificial lotus leaves (pet store or Amazon)

Temperature-regulated room, aquarium heaters, or incubators to house snail tanks (temperature at 25°C)

☒ MilliQ water

☒ Blunt featherweight forceps wide tip **BioQuip Catalog #4750**

☒ Sodium Alginate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W201502**

☒ Calcium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C1016**

☒ MgSO₄ × 7H₂O **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M2773**

☒ Potassium sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0772**

☒ Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5761**

☒ Iron(III) chloride hexahydrate **Catalog #236489**

Snail gel food (see recipes)

1x Aquarium Water (diluted from 10X; see recipes)

10X AQUARIUM WATER

5.56g CaCl₂

12.28g MgSO₄-7H₂O

0.43g K₂SO₄

4.2g NaHCO₃

480μl FeCl₃-6H₂O (0.5g/100ml water)

Fill to 10L and store in 1L bottles

Dilute to 1x: 9L MQ water + 1L 10x

SNAIL GEL FOOD <https://www.afbr-bri.org/schistosomiasis/standard-operating-procedures/gel-snail-food-preparation/>

1L diH₂O

4g sodium alginate

16g barley grass powder (health food store or Amazon)

4g wheat germ (health food store or Amazon)

4g fish flakes/pellet pre-ground mix (pet store or Amazon)

2g powdered milk (health food store or Amazon)



2 liter 2% CaCl_2 solution (40g CaCl_2 + 2 liter H_2O)

1. Add sodium alginate to 1L diH_2O and heat on magnetic stirrer with large stir bar, stirring rapidly (temp should be $\sim 80^\circ\text{C}$)
2. Add barley grass powder and stir until homogeneous
3. Add the pulverized wheat germ/fish flakes/powdered milk mixture
4. Stir for several minutes until thoroughly mixed
5. Divide mixture between 2 trays so that it is about 15mm deep and cool at 4°C 3-4hrs until gel is set
6. Gently flood with 2% CaCl_2
7. Place at 4°C overnight (can combine multiple slabs of gel into one tank to save space)
8. Pour off CaCl_2 and rinse 2x with diH_2O
9. Slice into cubes of approximately 15mm x 15mm and store in re-sealable bag at 4°C . Use within 1 week and do not overfeed.

Troubleshooting

Safety warnings



- ! For all procedures described, wear appropriate PPE:
 - Lab coat
 - Long-cuff gloves
 - Waterproof vinyl apron (when working with pre-patent and patent snails)
 - Face shield (when working with pre-patent and patent snails)

IMPORTANT. ALL pre-patent and patent snails must be treated as if they are infectious (i.e. wear face shield and waterproof vinyl apron). Infected snails will release small amounts of cercariae all the time, even if kept in the dark.

Before start

Thoroughly rinse and autoclave oyster shells (can be re-used indefinitely by soaking in 10% bleach overnight, rinsing thoroughly, and autoclaving)

Preparing a new aquarium tank

- 1 Label clean aquarium with appropriate information in permanent marker (i.e. date, owner, age, numbers of individuals, exposure date, expected patent date, experiment number, etc). Example labels are attached  infected_new tanks.avery
 uninfected_new tanks.avery
- 2 Rinse a generous handful of autoclaved oyster shells with water until it runs clear and add to the base of the tank
- 3 Fill aquarium 1/2-3/4 full with 1x Aquarium Water (see recipe in "MATERIALS" section)
- 3.1 For infected snails, keep water level at or just below 1/2 full to reduce chance of splashing when handling
- 4 Place a clean airline and air stone in aquarium and put in appropriate heated incubator
- 5 Connect airline and assure that the appropriate amount of air is going to the tank and all other tanks connected to the same airline. *Note that changing the number of tanks in a connected airline results in decreased or increased air pressure to other tanks so adjusted accordingly*
- 5.1 Add one 15ml falcon tube styrofoam rack to breeder tanks (snails also love to lay eggs on artificial lotus leaves)
- 6 Add snails to the tank (<100 baby snails/10L water; <10 adult breeder snails/10L; <30 juvenile parasite-exposed snails/10L)

Snail feeding (2x/week)

- 7 Place one cube (15mm x 15mm) of snail gel per 6-8 adult snails or two per 100 baby snails (use size and number of snails to estimate amount of snail gel to give to each tank)
- 8 To prevent overgrowth of bacteria, do not overfeed snails. If any snail gel is leftover >1 day after a feeding, then the snails are being overfed and reduce as necessary



Snail tank housekeeping

- 9 Visually inspect the snail tanks during feedings for heavy bacterial and/or waste contamination (turbid and/or smelly water, large amount of debris collected in oyster shells) and/or heavy rotifer infestations. If any of these is detected, rinse the snails thoroughly with fresh 1x Aquarium Water and prepare a new tank, airline, and bubbler for the snails
- 10 Once a week top up water on tanks to their appropriate levels using MilliQ H₂O. To top up the water use the hose connected to the MilliQ water source
- 11 Replace 10% of the dirtiest tanks each week

Snail egg collection and baby snail maintenance

- 12 Fill a 100ml beaker or small dish with 1x Aquarium Water
- 13 Working with one styrofoam rack at a time, use featherweight forceps to gently remove the egg masses from the rack and place in the beaker (slide forceps under egg mass and lift away gently)
- 14 Repeat for all styrofoam racks from breeder tanks
- 15 Check the egg masses under the microscope for rotifer infestation. Discard individual egg masses with heavy rotifer infestations. Rinse egg masses if rotifers are present in the water until none are seen under microscope
- 16 When finished collecting, empty the beaker into a prepared nursery/baby tank for that particular week (1 new nursery/baby tank per week) rinse container well with 1x Aquarium Water to remove stuck baby snails/egg masses
- 17 Split any tanks that have >100 baby snails to promote growth

Decontaminating infected and uninfected snail tank and tank water

- 18 Remove airline with air stone and place in tank. If applicable, keep styrofoam rack in tank.



- 19 Remove any snails that might be kept – leave dead shells. Add 20ml of 5000ppm Klorsept and leave overnight (minimum 6 hours)
- 20 After this period, dispose of the decontaminated water using the sieve or colander to collect oyster shells and dead snails
- 21 Rinse tank with diH_2O and then spray tank with 70% EtOH. Scrub tank with green scouring pad, rinse thoroughly with diH_2O and allow to air dry
- 22 Styrofoam racks, air stones, and airline should be sprayed with 70% EtOH, rinsed liberally with diH_2O , and air dried
- 23 Put decontaminated and rinsed oyster shells and dead snails in an autoclave tray and autoclave when full



Protocol references

Citation

Barbosa ND, Pimentel-Souza F, Sampaio IB (1987)

. The effect of seasonal temperature and experimental illumination on reproductive rate in the snail *Biomphalaria glabrata*.

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