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Housing and Care for *Hymenochirus boettgeri*

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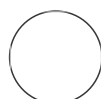
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Chytrid Fungi - Molecular and Genomic Techniques

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ABSTRACT

This protocol provides an overview of housing and care procedures for *H. boettgeri* in a controlled laboratory environment, encompassing feeding at all life stages and the maintenance of water quality in both a closed recirculating system (standard aquarium) and a static closed system (used for individual housing). Housing can also be done in a semi-open recirculation system (ZebTec, Tecniplast) that is compatible with zebrafish.

ATTACHMENTS

[n4t85hd6.docx](#)

GUIDELINES

Conclusion

This protocol offers comprehensive guidance on housing and caring for *H. boettgeri* in a laboratory environment, including instructions on aquatic systems, environmental conditions, feeding, and water quality maintenance. The equipment used in this protocol can be updated as needed, as long as the basic requirements for animal safety are maintained. Adhering to these procedures ensures the well-being and safety of the frogs throughout various experiments and research studies.

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Protocol status: Working
We use this protocol and it's working

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MATERIALS

- *H. boettgeri* specimens
- 38 L glass tanks (25 × 30 × 50 cm) with plastic lids containing holes smaller than 1 cm in diameter for air circulation
- 1 L plastic tanks (16 cm × 6.5 cm × 11 cm) with plastic lids containing holes smaller than 1 cm in diameter for air circulation
- Penn Plax Deluxe Net Breeder (17.15 cm × 12.07 cm × 13.34 cm; Hauppauge, NY, USA)
- Aquarium heater (EHEIM thermocontrol 75 W, Deizisau, Germany)
- Aquarium filter (Tetra, Blacksburg, VA, USA)
- EcoTestr CTS1 meter (Oakton, Vernon Hills, IL, USA)
- Water testing kit (API Liquid Test Kits, API®, Chalfont, PA, USA)
- Gravel substrate (diameter > 1 cm to prevent ingestion by the animals)
- Artificial plants
- PVC pipes
- Reverse osmosis (RO) water
- Synthetic sea salt mixture (Instant Ocean® Sea Salt, Blacksburg, VA, USA)
- Sodium bicarbonate
- Brine shrimp (*Artemia* sp., BIO-MARINE®, Hawthorne, CA, USA)
- Frozen gamma-irradiated bloodworms (*Chironomus* sp., Tropical Marine Center, UK)
- Powdered fry food (Sera® Micron Nature, Heinsberg, Germany)
- Small-granule fish food (BUG BITES™ Tropical Formula, Mansfield, MA, USA)
- Nitrifying bacterial suspension (ProLine® Aquaculture Bacteria Concentrate, Apopka, FL, USA)
- Tricaine methanesulfonate (MS-222; Syndel, Ferndale, WA, USA)
- Virkon™ Aquatic (LANXESS, Pittsburgh, PA, USA)

Housing

20m

- 1 Juvenile or adult frogs
- 1.1 Animals can be kept in groups or individually as needed. House animal groups in 38 L glass tanks (standard system; Figure 1A) with a density of at least 1.5 L of water per individual. House animals individually in 1 L plastic tanks (static system; Figure 1B).



Figure 1. Examples of standard (A) and static (B) *H. boettgeri* housing systems.

- 1.2 Keep the tanks covered to prevent animal escapes.
- 1.3 Maintain a minimum 1 cm space between the lid and the water surface for frogs to access the surface for breathing.
- 1.4 Enrich standard tanks with 1 cm layer of gravel substrate, artificial plants, and PVC pipes. Add at least gravel substrate to static tanks.
- 1.5 Sterilize all enrichment items with 1% Virkon for 00:20:00 and rinse thoroughly with sterile water. 20m



1.6 Ensure all enrichments are free from rough surfaces or sharp edges.

1.7 Maintain a 13:11 light-dark cycle.

2 Eggs and tadpoles

2.1 Keep eggs and tadpoles inside net breeder tanks to prevent them from being preyed by larger animals or getting sucked into the filter.





2.2 Place a Net Breeder tank inside the standard tank. Position the Net Breeder near the surface so that eggs and tadpoles can access the water's surface.

2.3 Maintain up to 80 eggs or 30 tadpoles of similar age in each Net Breeder tank. This number can be updated if larger breeding tanks are used.

2.4 Larger tadpoles may prey on smaller tadpoles or eggs, so relocate tadpoles by size to another tank if necessary.

Water Conditions

3 Fill the tanks with RO water.

- 4  Adjust water conductivity to $900 \pm 100 \mu\text{S}/\text{cm}$ by adding  0.5 undetermined of formulated synthetic sea salt mixture.
- 5 Adjust pH levels to 7 ± 0.4 by adding sodium bicarbonate when necessary.
- 6 Maintain a constant water temperature, ranging from  20°C to  25°C .
- 7 Position the aquarium filter in the middle of the tank for effective water circulation.
- 8 Initiate the biological cycle by adding 1 ml/L of nitrifying bacterial suspension (ProLine® Aquaculture Bacteria Concentrate).

Monitoring

- 9 Monitor pH and conductivity three times per week.
- 10 Check nitrogenous compounds once a week in cycled tanks (i.e., established nitrifying bacteria in sufficient abundance to maintain ammonia and nitrite values near 0 and increase nitrate values).
- 11 Check nitrogenous compounds at least three times a week in tanks with cycling in progress.
- 12 Always check parameters before water changes.

Water Changes

- 13 In cycled tanks, ammonia should be ≤ 0.25 , nitrite should be 0, and nitrates will steadily increase, necessitating water changes.
- 14 Perform a 50% water change in cycled tanks when nitrates exceed 40 ppm.
- 15 In non-cycled tanks, perform a 50% to 70% water change when necessary (i.e., ammonia $\square 1$ ppm, nitrite $\square 1$ ppm, or nitrate $\square 40$ ppm).
- 16 Condition water temperature, pH, and conductivity before water changes. If water changes coincide with feeding days, perform them at least 4 hours after feeding.

Feeding

- 17 Juvenile and adult frogs
 - 17.1 Feed the frogs ad libitum twice daily three times per week.
 - 17.2 In the morning, feed with newly hatched brine shrimp.
 - 17.3 At least 5 hours later, feed with frozen gamma-irradiated bloodworms, or small-granule fish food.

17.4 Remove uneaten food from the tank at the end of the day.

18 Tadpoles and newly metamorphosed frogs

18.1 Begin feeding tadpoles when they start free swimming (typically 1-2 days after hatching).

18.2 Provide ad libitum daily feedings of powdered fry food (Sera® Micron Nature) for one week.

18.3 Transition to a diet of newly hatched brine shrimp ad libitum twice daily, which should continue for at least 4 weeks post-metamorphosis. Afterward, switch to the same diet as juvenile and adult frogs.

18.4 Remove uneaten food from the tank at the end of the day.

Health Monitoring

19 Continuously observe and monitor the health and behavior of *H. boettgeri*.

20 Inspect all animals daily for signs of disease (e.g., lethargy, red spots, bloating, abnormal floating or swimming patterns, and lack of righting response).

- 21 Isolate any sick animals from healthy ones.
- 22 If euthanasia is necessary, immerse the animal in a solution of tricaine methanesulfonate (MS-222; 0.5 g/L; Syndel, Ferndale, WA) for at least 15 minutes. The time required in MS-222 solution may vary per animal.