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# 🌐 Quarantine and heat treatment for Hymenochirus boettgeri infected with Bd

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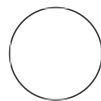
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Chytrid Fungi - Molecular and Genomic Techniques

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## ABSTRACT

This protocol outlines the procedures for conducting heat treatment for clearing Batrachochytrium dendrobatidis (Bd) infection of H. boettgeri. It ensures that individuals are free of Bd for use in subsequent experiments.

## ATTACHMENTS

[n4ub5hd6.docx](#)

## GUIDELINES

### Conclusion:

This protocol outlines the steps for quarantining and conducting heat treatment on H. boettgeri frogs infected with Bd to eliminate the infection.

### DOI:

[dx.doi.org/10.17504/protocols.io.4r3l22b9jl1y/v1](https://dx.doi.org/10.17504/protocols.io.4r3l22b9jl1y/v1)

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

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MATERIALS

- H. boettgeri adult frogs that are Bd-positive
- 38 L glass tanks (25 × 30 × 50 cm) with plastic lids containing holes smaller than 1 cm in diameter for air circulation
- 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)
- Virkon™ Aquatic (LANXESS, Pittsburgh, PA, USA)


Quarantine setup

- 1 Place Bd-positive frogs in 38 L standard system glass tanks, condition the water, feed the animals, and conduct water changes as described in “Housing and Care for Hymenochirus boettgeri” protocol.



Note

Ensure that Bd-positive frogs are completely isolated from Bd-negative frogs to prevent further transmission.

Heat Treatment

- 2 Gradually increase the water temperature in the quarantine standard system tanks from room temperature to  30 °C over a period of 10 days using an aquarium heater.

- 3 Maintain the water temperature at  30 °C for  240:00:00 . 1w 3d

- 4 Gradually decrease the water temperature from  30 °C to  25 °C over a period of five days.
- 5 Monitor and maintain consistent temperature levels throughout the treatment process.

### Allocation to permanent aquaria

- 6 Monitor the animals for an additional 10 days after the heat treatment, and then transfer them to permanent aquaria.