**Quarantine and heat treatment for *Hymenochirus boettgeri* infected with Bd**

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**Introduction**

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.

**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 BITESTM Tropical Formula, Mansfield, MA, USA)
* Nitrifying bacterial suspension (ProLine® Aquaculture Bacteria Concentrate, Apopka, FL, USA)
* VirkonTM Aquatic (LANXESS, Pittsburgh, PA, USA)

**Procedure**

1. *Quarantine setup*: 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. Ensure that Bd-positive frogs are completely isolated from Bd-negative frogs to prevent further transmission.
2. *Heat Treatment*: 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. Maintain the water temperature at 30 °C for 10 days. Gradually decrease the water temperature from 30 °C to 25 °C over a period of five days. Monitor and maintain consistent temperature levels throughout the treatment process.
3. *Allocation to permanent aquaria:* Monitor the animals for an additional 10 days after the heat treatment, and then transfer them to permanent aquaria.

**Conclusion**

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