

## Protocol

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**Date of Visit:** June 16, 2014–August 22, 2014

**Title:** Promoting interbreeding between surface and cave morphs of *Astyanax mexicanus* to generate surface-cave hybrids

**Rational:** In order to perform genetic analyses to resolve the relationship between genetic factor and behavioral phenotypes, generating F<sub>2</sub> hybrids is essential. Because *Astyanax mexicanus* is a new model system, detail adjustment to optimize water-condition factors is still needed to optimize spawning between two distinct conspecific morphs.

### Protocol

A large flow through system will work best to promote spawning because hormones released by spawning females should encourage further spawning in others.

A rough bottom surface may also encourage spawning behavior. A “breeding net” which is composed of a coarse net (nylon, 5000 micron mesh size, Amazon, Seattle, WA) on top of a frame (made out of Plastic Corrugated Twinwall Sheets, Homedepot) to prevent adult fish to the embryos, and a finer net (nylon 475 micron mesh size, Amazon) underneath the frame to retain the embryos and be flushed out later.

Spawning week (every other week):

- I. Place 2 surface fish males with 1 cavefish female in a 10 g tank. The 2:1 ratio increases likelihood of spawning.
- II. Place breeding net or a separator on the surface to prevent adults from consuming embryos.
- III.

	Temperature (Normal: 72°F)
Day 1	Increase to 74°F
Day 2	Increase to 76°F
Day 3	Increase to 78°F
Day 4	Reduce to 75°F
Day 5	Reduce to 72°F

\*\*Every morning from Day 2, check for embryos. If there is no embryo by noon, remove the breeding nets and feed live black worms then reset net in the afternoon before next temperature increase.

Optimal Conditions:

- I. Adults should be fed the mix of live black worms and other live food that is high in nutrients for 2 weeks prior to planned spawning.
- II. Water change before Day 1 of temperature increase ought to be made
  - a. Ideal conductivity: 700  $\mu$ S
  - b. pH: 7.0
  - c. Pure, filtered water

- III. If not spawning, Ovaprim hormone (Syndel Laboratories Ltd, Qualicum Beach, Canada) may be injected into female in 0.2 mL dosage to the side of the dorsal muscle under the scales.