

Eastern Hellbender Egg Rearing Protocol – Hellbender RCN

Original protocol by J. Greathouse

- Following collection of eggs, the eggs should be transported in a covered container with oxygenated water back to the Hellbender Conservation Center.
- Upon arrival at the Hellbender Conservation Center, each egg should be separated from the clutch by cutting the gelatinous membrane between each egg with scissors while a plastic spoon is under the egg to be separated.
- Create hellbender water/methylene blue mix by adding 0.5 cc of methylene blue to a new gallon of spring water and shake well. (Methylene blue is a color indicator of oxygen level. Water will turn green/yellow if oxygen levels drop.)
- Once the egg has been separated from the clutch, place it into a specimen cup with 60 mL of spring water and methylene blue mix.
- Label the cup with the site name, treatment (shaker, incubator, or room temperature), and its individual ID number.
- While in the cup, inspect the egg for any signs of white cottony fungus and abnormalities in the texture or color of the yolk or yolk sac.
- Using the Harrison chart of larval salamander development, determine the approximate stage of the egg and record this on the appropriate form.
- Place as many cups of eggs that will fit on one half of the shaker platform in the shaker. Set the shaker temperature at 14°C and at 40 revolutions per minute.
- Place the remainder of the cups on the top shelf of the incubator with the temperature also set at 14°C. If space in the shaker and the incubator becomes filled, then specimen cups will be stored in cabinets or in empty aquaria that are covered to prevent light disruption.
- Each day, the egg should be gently removed from the cup with a plastic spoon, and the water and methylene blue should be changed at the same volume as listed above.
- Following water changes, eggs should be staged and the data should be recorded on the appropriate form. Any information such as the presence of fungus or abnormalities in the texture or color of the yolk or yolk sac should be recorded as well.
- Upon hatching of the larvae, the individual should still be removed by spoon until it is too large to handle appropriately with the spoon. At that point, the individual should be placed in the specimen cup lid or a coffee cup lid during water changes.
- Developmental staging of the larvae and inspection for yolk sac abnormalities should continue at this point and should continue to be documented.
- One blackworm per individual will be introduced as prey approximately 3 weeks following hatching, dependent upon yolk sac absorption.