EXPLORE THE ISSUE BEING INVESTIGATED

Amphibian Eggs Hatching in Shallow Ponds Thirst for Oxygen

Most amphibians lay their eggs in water, eggs which hatch into aquatic larvae. A very real problem with this sort of aquatic reproduction is predation. Predatory fish can rapidly clear an area of amphibian eggs and larvae, which offer a rich, easily-harvested food supply. For this reason, successful amphibian reproduction is usually restricted to fish-free breeding sites, typically temporary shallow ponds. These ponds often support rich algal growth that can increase oxygen availability through photosynthesis. However, when algae die they are decomposed by bacteria, a process that can deplete dissolved oxygen to levels that are dangerous to amphibians--there is just not enough dissolved oxygen in these waters to support the metabolism of amphibian larvae.

The survival of amphibians in oxygen-poor habitats has not been studied intensively. Most of the studies examining the amphibian response to hypoxia involve the overwintering behavior of amphibians. In ice-covered ponds, oxygen levels drop dramatically, forcing amphibians to endure prolonged periods of severe hypoxia. Adult amphibians tolerate prolonged hypoxic conditions by reducing their metabolic activity.

However, while decreasing metabolic activity to low levels can work in adults to offset hypoxic conditions, this approach would be fatal in developing embryos. While it is known that acute oxygen starvation can cause death, little or nothing is known about the effects of chronic hypoxia on the embryo itself. Researchers have shown that chronic exposure to low oxygen levels can slow embryonic growth and delays hatching -- but is the hypoxic embryo smaller at hatching? Or has amphibian reproduction adapted to hypoxia, slowing development to accommodate lower oxygen availability, but not harming the fitness of the larvae? It is this question that M. Christopher Barnhart of Southwest Missouri State University and then graduate student Nathan Mills set out to address.

The researchers selected four different amphibian species to examine, two frog species (*Rana sphenocephala*, commonly known as the southern leopard frog and *R. palustris*, commonly known as the pickerel frog) and two salamander species (*Ambystoma maculatum*, commonly known as the spotted salamander and *A. annulatum*, commonly known as the ringed salamander). All four species breed and lay their eggs in shallow ponds or temporary pools. Barnhart and Mills collected the four kinds of amphibian eggs and placed them in oxygen-controlled environments where the oxygen levels could be adjusted from low to high levels of oxygen. The developing embryos were monitored for their rates of development and date of hatching under different levels of hypoxia.

Egg mass of *Ambystoma maculatum* The eggs of *Ambystoma maculatum* develop within a massive jelly matrix which helps protect the eggs but may also interfere with oxygen availability (courtesy of M. Christopher Barnhart).

In order to accurately compare the rates of development between experimental treatments, the researchers used standardized developmental staging systems. The jelly matrix surrounding the eggs is transparent, which allowed the researchers to visually categorize each embryo's stage of development. Development occurs along a continuous scale of morphological change. A developmental staging system establishes steps in development based on the physical changes in an embryo.

By comparing the rate of development (the time it takes an embryo to reach a certain stage of development) and the time of embryo hatching in the different oxygen conditions, the researchers were able to directly access the effects of hypoxia on amphibian embryo development.