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Information Sheet #4

THE TREMATODE *RIBEIROIA ONDATRAE* IN SOUTHEASTERN AMPHIBIANS

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Amphibian Die-offs:

In the last 20 years, scientists have documented an increasing trend of amphibian population declines and extinctions (1). Potential causes for these declines include: climate change, habitat loss, diseases, and introduced species (2). In 1995, public perception and concern of amphibian declines increased greatly when a group of middle school students in Minnesota found a pond where approximately half of metamorphic leopard frogs (*Rana pipiens*) had malformations (3). Since this initial discovery, over 60 species of frogs, toads and salamanders have been reported with malformations in 46 states. Extra, missing or misshaped hind limbs are the most common malformations observed. Most of the reported malformations have been from the Western, Midwestern, and Northeastern United States (3). Populations in the Northeast and Midwest were assumed to be affected by water contamination from pesticides as Canadian ponds in agriculture areas displayed the highest rates of malformations (4). However, other hypotheses were suggested including increased UV-B radiation from ozone depletion, retinoids in waste water, and parasite infection.

In 1986, Pacific treefrogs (*Hyla regilla*) and long-toed salamanders (*Ambystoma macrodactylum*) were reported with high prevalence of limb abnormalities, especially extra limbs (5). The malformations were thought to be caused by trematodes, which are parasitic worms. Trematodes burrow into tadpoles around developing limb buds and form cysts (metacercariae), which can mechanically interfere with development (5). Almost 10 years later, Pieter Johnson and colleagues (6) examined Pacific treefrogs in 13 ponds and found severe malformations in 4 ponds. Water analysis revealed no indication of PCBs, pesticides, or heavy metals. However, all 4 ponds with malformed treefrogs had one characteristic that was missing from ponds where malformations were not observed – high abundance of a certain species of aquatic snail (*Planorbella tenus*, 6). As it turns out, this snail is the first host of the parasitic trematode *Ribeiroia ondatrae*. In the laboratory, Johnson exposed Pacific treefrog tadpoles to *Ribeiroia ondatrae* and 85% of metamorphic frogs developed malformations. This initial research demonstrated that *R. ondatrae* caused malformations (6), which may impact amphibian populations by reducing the chance of juvenile frogs reaching adulthood.

Malformations caused by *R. ondatrae* infection have been documented in the western toad (*Bufo boreas*), rough-skinned newt (*Tarcha granulose*), California newt (*Taricha torosa*), northern red-legged frog (*Rana aurora*), cascade frog (*Rana cascade*), American bullfrog (*Rana catesbeiana*), Columbia spotted frog (*Rana luteiventris*), Great Basin spadefoot toad (*Scaphiopus intermontanus*), and the Oregon spotted frog (*Rana pretiosa*; 7,8). The prevalence of amphibian malformations due to *R. ondatrae* appears to be increasing, but may be an artifact of heightened awareness and increased monitoring of wetlands for malformed amphibians. The first report of malformed frogs in North American was in 1899 and subsequent reports were published through the 1960s. In a pond in western Montana, extra-legged treefrogs composed 20-25% of metamorphosing individuals from 1958-1961 (4). Johnson and colleagues re-surveyed the same pond in the 1990s and found 50% of the treefrogs had malformations with high rates of *R. ondatrae* infection (4). Further, two of four species that had bred at this pond in the 1960s were no longer present (4). Another study in Minnesota ponds reported that the types of malformations in resident amphibians increased from 2 to 12 malformation types between the 1960s and 1990s (9). Based on these studies, it appears that malformation rates are increasing as well as the types of malformations observed in amphibian populations. Interestingly, a search of the literature revealed no reported cases of malformations due to *R. ondatrae* in the southeastern United States; however, extensive surveys have not been performed.

Pathogen Characteristics:

Ribeiroia ondatrae has a complex life cycle that includes three hosts: primary host (birds or mammals), first intermediate host (snails), and second intermediate host (amphibians or fish). Adult *R. ondatrae* worms live in the gastrointestinal track of birds and mammals, and reproduce sexually. Fertilized eggs are released into the environment when the primary host defecates. If the feces is deposited in the water, the eggs will hatch into miracidium that burrow into planorbid snails and reproduce asexually. Thereafter, free-swimming cercariae are released from the snail in large numbers. The cercariae burrow into tadpoles usually around the limb buds or vent and encyst in the host as metacercariae (4). The metacercarial cysts can mechanically or chemically disrupt limb development. If the infected amphibian is consumed by a bird or mammal, the metacercariae develop into adult worms and the life cycle is completed. It is believed that malformations from *Ribeiroia* infection may benefit the trematode by making capture of the amphibian easier by the primary host (4,10). Known primary hosts of *Ribeiroia* include great blue herons (*Ardea Herodias*), osprey (*Pandion haliaetus*), muskrats (*Ondatra zibethicus*), red fox (*Vulpes vulpes*), mink (*Mustela vison*), raccoons (*Procyon lotor*), and stripped skunks (*Mephitis mephitis*, 9).

Field, Gross, and Histological Signs:

When *R. ondatrae* metacercariae encyst in the tadpole around the pelvic griddle, malformations almost always occur. Typical amphibian malformations due to *R. ondatrae* include: polymelia (extra limbs; Fig. 1), polydactyly (extra digits), amelia (missing limb), ectromelia (missing digits), apody (missing foot), polypody (multiple feet), polyphalangy (multiple phalanges; Fig. 1), skin webbing, anophthalmy (missing eye), and mandibular hypoplasia (malformed jaw, 10). Most (95%) malformations occur on the hind limbs and are usually (89%) asymmetrical in frogs. Salamanders experience a more equal distribution of malformations between forelimbs and hind

limbs (4). The U.S. Geological Survey (USGS) published a field guide to amphibian malformations in 2005, which is available at: http://www.nwhc.usgs.gov/publications/fact_sheets/pdfs/frog.pdf. Amphibian malformations can be reported to USGS at: http://www.nbii.gov/portal/server.pt.

Factors Contributing to Emergence:

Prior to the discovery of *R. ondatrae*, pesticides and UV-B radiation were thought to be the primary causes of amphibian malformations. After learning that *R. ondatrae* cause malformations, researchers began investigating what mechanisms might contribute to higher parasite infection rates. Johnson and colleagues led a series of aquatic ecosystem studies that demonstrated that factors that contribute to the proliferation of snails in the family Planorbidae will increase *R. ondatrae* densities and the number of amphibian malformations (11,12). Factors that were identified were those that contribute to euthrophication (i.e., excessive algal production), such as allowing cattle to deposit waste in wetlands or high amounts of fertilizers in runoff. In addition, pesticides, such as Atrazine, Malathion and Esfenvalerate, can affect the immune system and mobility of tadpoles, which can increase the likelihood of infection by *R. ondatrae* cercariae (13). Inasmuch as fish can be infected by *R. ondatrae* also, it is possible this parasite could be introduced into aquatic systems when fish are released from hatcheries. *Ribeiroia ondatrae* was discovered in American bullfrog larvae at a warm-water fish hatchery in the southeastern United States (14).

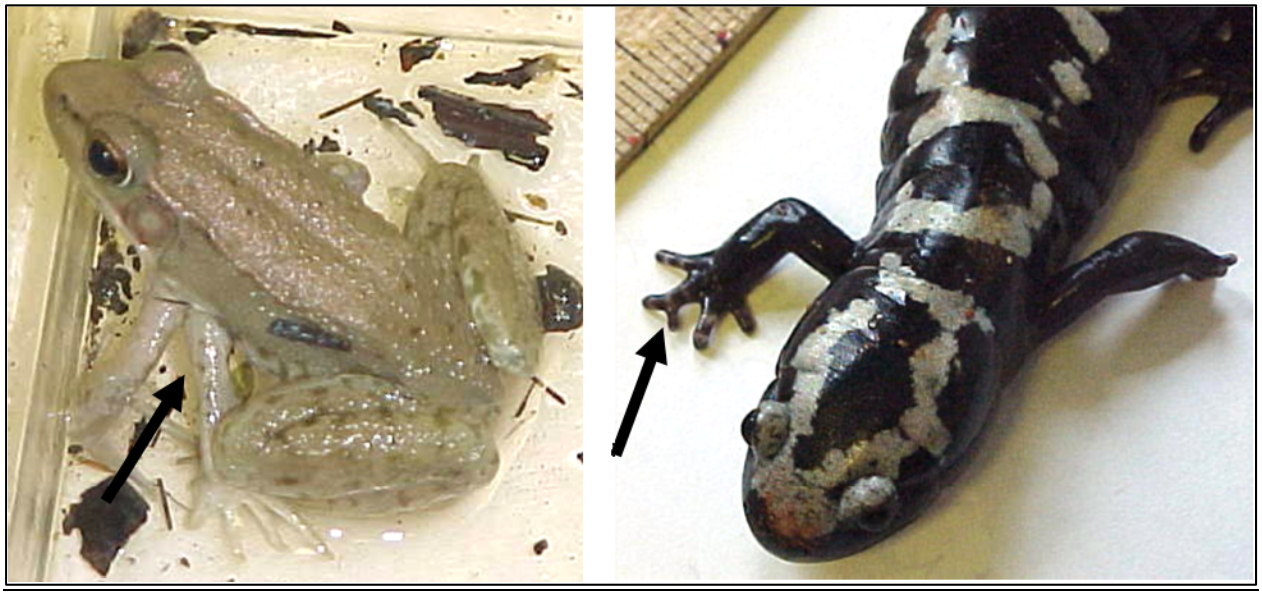

Figure 1. Polymelia in a juvenile green frog (left) and polyphalangy in an adult marbled salamander (right) found near Norris, Tennessee. Photos by Jeff Harshbarger.

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