

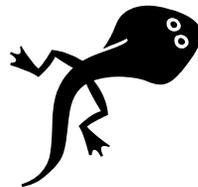


Amphibian Initiative 2007
Survey for Frog Abnormalities on
Tualatin River National Wildlife Refuge

Elizabeth Materna
Oregon Fish and Wildlife Office
Portland, Oregon

Peter Schmidt
Tualatin River National Wildlife Refuge
Sherwood, Oregon

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I. Summary of Survey Efforts

A. Introduction

In response to indications of worldwide declines in amphibian populations, President Clinton and Congress directed Department of the Interior agencies to initiate a national program of amphibian monitoring, research, and conservation. Agencies developed various programs to accomplish this task. The U.S. Geological Survey formed the National Amphibian Research and Monitoring Initiative, while the Fish and Wildlife Service (FWS) has undertaken the National Amphibian Initiative. Other agencies, such as the National Park Service, are also involved. Each agency's efforts vary somewhat in their focus and objectives. The FWS's National Amphibian Initiative surveys for frog abnormalities in metamorphic stages throughout its National Wildlife Refuges (NWRs).

Tualatin River NWR, located in Washington County, Oregon, was selected as one of the refuges for the 2007 National Amphibian Initiative. The Tualatin River NWR was established in 1992 as an urban refuge to provide wetland, riparian, and upland habitats for a variety of migratory birds, threatened and endangered species, fish, other resident wildlife, and for the enjoyment of people. The active acquisition program of the refuge has progressed well, with over one-third of the approved 3,060 acres in ownership by the FWS. When acquisition is complete, this refuge will preserve a floodplain wetland ecosystem, and provide a wildlife center in the shadow of Oregon's largest metropolitan area, Portland. Thus far, more than 625 acres of native floodplain habitats have been restored.

The primary potential sources of contaminants to the refuge are from encroaching urbanization, including nutrient enrichment, pesticides, and other chemicals. We surveyed for Pacific chorus frog (*Pseudacris regilla*), bullfrog (*Rana catesbeiana*), and the northern red-legged frog (*Rana aurora aurora*), a species listed as a "species of concern" by FWS and a "vulnerable" species by Oregon Department of Fish and Wildlife.

B. Summary of Sampling Efforts

1. Conditions

The Tualatin River NWR is situated within the floodplain of the Tualatin River, and comprises less than 1% of the 712 square mile watershed. The refuge supports some of the most abundant and varied wildlife in the watershed and is home to nearly 200 species of birds, over 50 species of mammals, 25 species of reptiles and amphibians, and a wide variety of insects, fish and plants. Refuge habitats are varied and include rivers and streams, seasonal and forested wetlands, riparian areas, grasslands, and forested uplands.

Staff from the Tualatin River NWR and the Oregon Fish and Wildlife Office's Division of Environmental Contaminants surveyed for frogs from mid-May through late August at the refuge.

2. Site Descriptions

Within Tualatin River NWR we collected frogs from three units or sites (Figure 1): the Atfalati Unit (Photo 1), the Riverboat Unit (Photo 2), and the Morand Unit (Photo 3).

Atfalati Unit. The Atfalati Unit is located 1 mile north of Sherwood, Oregon, and is comprised of about 450 acres of managed seasonal emergent wetlands, grassland uplands, and riparian forest. Vegetation in the seasonal wetlands consists primarily of annual wetland plants with some perennial emergent vegetation. Upland grasslands are primarily composed of non-native grasses, scattered shrubs and small ash trees. The riparian forest is mixed coniferous and deciduous trees dominated by grand fir and Oregon ash with an understory of non-native blackberries and native species such as snowberry and hazel.

Approximately 55% of the Atfalait Unit is seasonal emergent wetland managed intensively for migratory and wintering waterfowl, primarily northern pintails and cackling Canada geese. Wetlands are managed by a combination of water management, mowing, discing, and application of FWS-approved herbicide. Dominant plant species include smartweeds, wild millet, and other native and non-native annual plants.

The main water source for the Atfalati Unit is Chicken Creek which is a relatively small watershed that collects water from throughout the Sherwood area and includes the Cedar Creek watershed. Our collection areas were from throughout the Atfalati Unit. This unit was considered our impacted site because of urban influences to the watershed.

Riverboat Unit. The 232-acre Riverboat Unit is about 2 miles from Scholls, Oregon, and includes two wetland areas consisting of one 30-acre managed seasonal emergent wetland and one 11-acre area with no water management capabilities. In addition, about 115 acres are being restored to an oak savanna habitat type, and about 30 acres are fallow agricultural land undergoing pretreatment for restoration activities.

Samples were collected from the smaller wetland which was undergoing restoration to a scrub shrub wetland type. This wetland was heavily disced and sprayed with herbicide during summer 2006, and planted with shrubs and trees during winter 2007. This marsh is filled by rainwater and occasionally from overflow from the Tualatin River during flood events. There is no outlet from this basin once floodwaters recede. The marsh dries up usually during August from evapotranspiration. Although this wetland was sprayed with herbicide the summer prior to our collections, it was considered less impacted than the Atfalati Unit due to the infrequent occurrence of the herbicide spraying and the source of the water.

Morand Unit. The Morand Unit is near King City, Oregon, and is about 50 acres consisting of riparian forest, oak/pine savanna, and managed seasonal emergent wetland. This wetland is relatively small at only 6 acres. The unit is primarily managed for neotropical migrant songbirds. The wetland where samples were collected is filled by rainwater and rarely from flooding of the Tualatin River. There are springs and a small abandoned irrigation pond at one end of the wetland, and a deep ditch running the length of the wetland as well. Water typically remains to some extent through summer in the bottom of the ditch. Plant species present on this unit are similar to those found on the Atfalati Unit. This unit was initially considered our

unimpacted site, however, an insufficient number of metamorphosing frogs were found at this site.

3. Amphibians Collected

Visits on May 19 and 23 were conducted to scout areas for presence and developmental stages of larval amphibians. Sampling dates were May 29, June 13, July 2, 11, 18, 24 and 31, August 9 and 22, 2007. Field crews consisted of two to four individuals. Metamorphs were captured with dip nets in areas with submerged and emergent vegetation. At the Atfalati Unit, metamorphs were collected with dip nets and seines. Methods for processing frogs followed the 2007 FWS Standard Operating Procedures for Survey Methods for Frog Abnormalities on Wildlife Refuges (prepared by the FWS's National Conservation Training Center).

Amphibian species found on the refuge units include: rough-skinned newt (*Taricha granulosa*), ensatina (*Ensatina eschscholtzii*), long-toed salamander (*Ambystoma macrodactylum*), Pacific chorus frog (*Psuedacris regilla*), northern red-legged frog (*Rana aurora aurora*), and bullfrog (*Rana catesbeiana*). Because we were unable to get adequate numbers of the same species at the control and treatment sites, two species (*P. regilla*, and *R. catesbeiana*) of amphibians were sampled. In addition, a small number of *R. aurora* were also sampled.

Over 100 individuals were collected from Tualatin River NWR. All data are included in attached tables.

Atfalati Unit

Forty-nine metamorphosing *R. catesbeiana* frogs were collected from the Atfalati Unit from July through August. One malformation was observed (Photo 4) on a tadpole that was not at the metamorphic stage. The left leg was much smaller than the right leg. Subsequent collection efforts failed to recapture this individual. Two potential malformations also were observed at this site. One metamorph was observed with completely black eyes as compared to the golden color of the others. Although we collected this individual, there was no facility or person identified to examine this type of abnormality and it was not submitted for diagnostic work. A tadpole with what appeared to be an infection or injury also was found at this unit and is shown in Photo 5.

Twelve 1-year bullfrog tadpoles were collected from the lateral canal on the Atfalati Unit and sent to Dr. David Green for necropsy at the USGS National Wildlife Health Center in Madison, Wisconsin. In addition, 10 metamorphs and tadpoles were sent to Don Larson for parasitology. We are still awaiting results.

Riverboat Unit

At the Riverboat Unit, *P. regilla* was the most prevalent species and over 50 metamorphs were processed. A much smaller number of *R. aurora* also were processed. Metamorphs were collected at the Riverboat Unit on May 29 and June 16. Results are included in attached original data sheets and table. No abnormalities were observed on frogs collected at the Riverboat Unit.

Morand Unit

R. aurora were collected at this site during two visits (July 2 and 11). Early surveys of this site indicated great promise of collecting adequate numbers of red-legged frog metamorphs. However, fewer and fewer individuals were found during each subsequent visit. Although it is possible that some metamorphosing individuals were missed due to timing, it is also possible that many larvae were preyed upon. During our first site visit, one tadpole collected was visibly injured (a portion of its intestine was outside the body), possibly from attempted predation.

Chytrid Fungus Survey

Tadpoles and metamorphs were sampled for the polymerase chain reaction (PCR) assay for *Batrachochytrium dendrobatidis* (chytrid fungus) during our study. The sampling involved collecting skin swabs and the method is described in attached documentation. Detailed methods of the analysis and sample preparation are also attached. *P. regilla* (n=5) and *R. aurora* (n=3) were swabbed at the Riverboat Unit, *R. aurora* (n=5) was swabbed at the Morand Unit, and *R. catesbeiana* (n=5) were swabbed from the Atfalati Unit. Results were negative for all units.

4. Water Quality

Water quality measurements were not collected during this study effort.

5. Comments and Considerations

Protocols include instruction on what to do in a die-off incident of multiple frogs. However, we encountered instances of a single individual with an unusual condition. In these cases, it would be beneficial to allow submitting a single specimen for diagnostics.

Last year there was a die-off of approximately 80% of the Oregon spotted frog (*R. pretiosa*) population at Conboy Lake NWR. The refuge biologist has good confirmation that the cause was chytrid fungus. Pisces Molecular LLC does the PCR testing at a relatively inexpensive cost and can provide equipment for sampling for an additional nominal expense. Sampling for this fungus is very easy and can be done concurrently with the Amphibian Initiative. Given the threat of this disease to amphibians worldwide, adding this component nationally to the Amphibian Initiative would be highly beneficial and efficient.

II. Budget

Equipment – waders-2 at \$ 49.00 each, totaling \$98.00

Staff – Oregon Fish and Wildlife Office - 2 biologists totaling approximately 250 hours

Tualatin River NWR - 1 biologist at approximately 16 hours

Hourly wage- approximately \$43/hour (cost to government)= \$11, 438

Chytrid testing- sampling supplies \$50

3 pooled tests \$250

Total \$300

Photos



Photo 1. Collection site at the Atfalati Unit of the Tualatin River NWR (TLT03-NE-07312007).



Photo 2. Collection site at the Riverboat Unit of the Tualatin River NWR (TLT01-NE-06132007).



Photo 3. Collection site at the Morand Unit of the Tualatin River NWR (TLT02-W-06132007).



Photo 4. Bullfrog tadpole with a malformation to the left rear leg.



Photo 5. Bullfrog tadpole with abnormal skin, reason unknown.

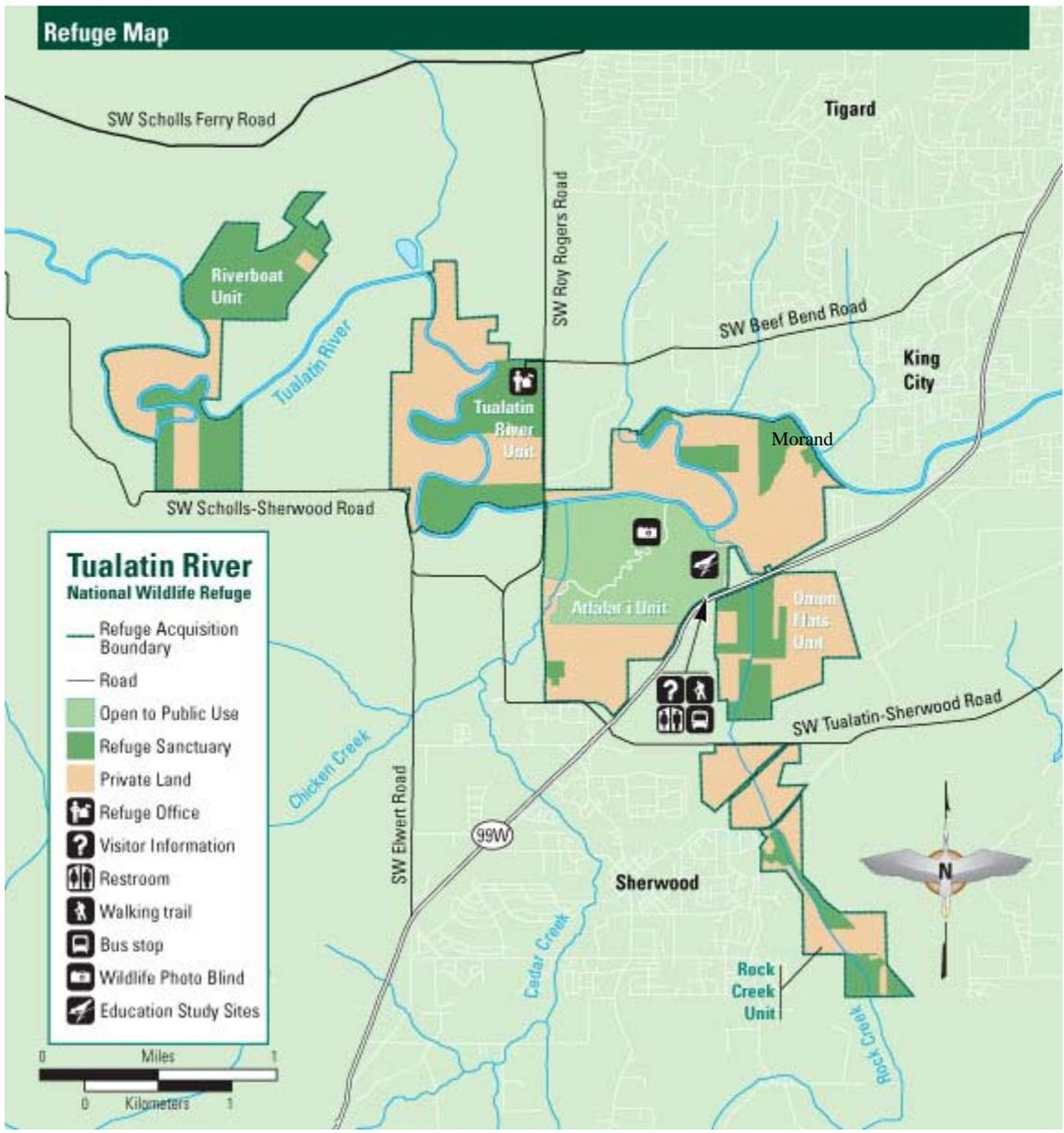


Figure 1. Map of Tualatin River NWR.