

DRAFT FOR DISCUSSION

**FINAL REPORT: AMPHIBIAN SURVEY AT
OAK GLEN NATURE PRESERVE 2015**

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1.0 INTRODUCTION

1.1 Purpose

An oil pipeline rupture was discovered at Oak Glen Nature Preserve (OGNP) on March 18, 2014. Approximately 0.5 linear mile of a first order, ephemeral stream and the wetland into which it drains was impacted by up to 30,000 gallons of crude oil. An amphibian survey was conducted by the author and his assistants, beginning May 22, 2014 to establish a baseline of species present at OGNP. Locality and species richness data were collected through November 6, 2014 and summarized in a report to Great Parks of Hamilton County on February 26, 2015 (Davis, 2015). Because the 2014 survey was not initiated until May 22, 2014 the author proposed a 2015 survey to document any early season breeding amphibians or species that were not confirmed during the first year. The 2015 survey was initiated on March 13, 2015 and continued until June 3, 2015. The survey was extended beyond the May 21, 2015 project final date to accommodate for an extended period of below normal temperatures during critical weeks for the breeding period of Fowler's Toad (*Anaxyrus fowleri*), a species suspected to be present at the site and not documented during the 2014 survey.

1.2 Objectives

The main objectives of the survey were to document species overlooked during the 2015 amphibian survey at OGNP, survey portions of the Preserve that were not visited during the 2015 survey, and to determine if two small wetlands at the site were used by ambystomatid salamanders as breeding sites. Special attention was paid to hillsides overlook East Miami River Road which had been suggested as potential mitigation properties for damages incurred as a result of the oil spill.

1.3 Site Description

Oak Glen Nature Preserve consists of two tracts of heavily forested land in Colerain Township, Hamilton County, Ohio (Figure 1). It is owned and managed by the county's park district, Great Parks of Hamilton County. The Preserve is located from the first terrace above the Great Miami River to the hills and plateaus overlooking it east of East Miami River Road and north of Thompson Road. The center of the northern tract of OGNP is at approximately 39.25643° N, 84.68607° W, and the approximate center of the southern tract of land is at 39.25097° N, 84.68962° W. The two tracts are separated by a narrow parcel of privately owned land. The highest and lowest elevations of the northern tract are approximately 875 and 498 feet above sea level respectively. The southern tract's highest point is adjacent to Thompson Road at 845 feet above sea level and the lowest is the floor of the oiled wetland (when it is dry) at 499 feet above sea level.

Amphibian habitats at OGNP are closely linked to topography and geography. Most of the Preserve consists of mature upland deciduous forests on steep slopes and plateaus. The slopes are dissected by deep, often steep-walled ravines, at the bottom of which are ephemeral streams strewn with limestone rocks and slabs. The northwest-facing terraced hillside overlooking East Miami River Road and the Great Miami River valley lacks ravines. The upland forests are dominated by sugar maples (*Acer saccharum*) on the slopes, and oaks (*Quercus* sp.) on the ridges. The plateau of the north tract is dry and dominated by shagbark hickory (*Carya ovata*). The understory throughout consists of paw paw (*Asimina triloba*) and spicebush (*Lindera benzoin*). The invasive Amur honeysuckle (*Lonicera mackii*) is not abundant in the forests except on the privately owned parcel between OGNP's north and south tracts where logging has occurred.

Two larger wetlands are located on the first terrace above the Great Miami River (Figure 2). The wetland located to the south was impacted by the oil spill in March 2014 and is surrounded by early successional bottomland forest dominated by box elders (*Acer negundo*) and silver maples (*Acer saccharinum*). It is located at 39.25549° N, 84.69410° W and is shallow enough to dry completely during dry years, including 2015, when it was completely dry on June 8. The second wetland (referred to as the gravel quarry in this report for the sake of brevity), located to the north, has no canopy on its northwest side but is forested on all others. Most of it is deep and maintains water all year. There is a shallow backwater bay on its southwest corner and another at its western end, both of which provided frog breeding habitat. The gravel quarry It is located at 39.25995° N, 84.68887° W. Two smaller wetlands, each of which dries completely during dry years, including 2015, are situated approximately 7 to 8 meters below the grade of the first terrace. They are located at 39.25841° N, 84.69168° W and 39.25881° N, 84.69140° W (Figure 2). Both are used by spring breeding frogs, toads, and ambystomatid salamanders. Much of the first terrace is in early stages of old field succession and dominated by goldenrods (*Solidago* sp.). There are several depressions and ditches with compacted soils on the first terrace that hold water after rainfall. These probably were created by heavy machinery during the gravel mining period prior to Great Parks' taking ownership, and more recently by heavy equipment used to clean the March 2014 oil spill that impacted one of the ephemeral streams (Stream 3) on the south tract of OGNP and the privately owned parcel of land to its north, and the more southern of the two aforementioned wetlands. Great Parks constructed a small wetland in March, 2015 to provide a site that would act as a sink for breeding amphibians should the oiled wetland not support them. The constructed wetland is located at 39.25020° N, 84.69306° W (Figure 2). Seeps and springs are largely absent at OGNP but one seep, whose head is located at 39.25318° N, 84.68969° W, maintained flow until mid-July, 2014 and was still flowing on June 3, 2015 at the conclusion of the second year of the survey despite the fact that the wetland, excluding the constructed wetland, had dried.

In this report three ephemeral streams are referred to frequently. They are specifically labeled as Streams 2, 3, and 5 (Figure 3). One of them, Stream 5, had minimal flow on June 3 at the conclusion of the survey. Stream 3 had water in it at higher elevations (near the pipeline break) until mid-May but it became increasingly dry by the end of the month unless there had been rain. Even on the terrace it was nearly dry by June. Stream 2 was ephemeral and had water only after substantial rainfall.

1.4 Historical Amphibian Records in Colerain Township and OGNP

Because of its rich geological history, Hamilton County has among the most diverse amphibian faunas in Ohio. Ten anuran species in three families and seventeen salamander species in five families have been recorded in the county. Interestingly, several species of salamanders that occur in Hamilton County only do so in extremely specialized habitats or areas north, east, or west of the deep stage Ohio River valley (outside the area between the Mill Creek and Great Miami River). OGNP is situated immediately south of the deep stage Ohio River valley which is occupied by the present day Great Miami River. Consequently, at least five salamander species that have been documented in Hamilton County (Spotted Salamanders, *Ambystoma maculatum*; Marbled Salamanders, *A. opacum*; Northern Dusky Salamanders, *Desmognathus fuscus*; Kentucky Spring Salamanders, *Gyrinophilus porphyriticus duryi*; Slimy Salamanders *Plethodon glutinosus*) are not present at OGNP. Two species, the Eastern Hellbender (*Cryptobranchus a. alleganienensis*) and the Common Mudpuppy (*Necturus m. maculosus*) are aquatic species that inhabit larger streams and rivers, neither of which is available at OGNP. Red-spotted Newts (*Notophthalmus viridescens*) are only known from a few localities in eastern Hamilton County. Some of these species, Spotted and Marbled Salamanders for example, occur just 2 or 3 kilometers to the north of OGNP on Wisconsinian soils. The much older Illinoian soils at OGNP

are deeply eroded and provide suitable habitat for a limited number of Hamilton County's salamanders. Anurans, because they are so mobile, can disperse much more easily than salamanders. Therefore, while few of its salamanders are known from Colerain Township, most of Hamilton County's frogs and toads can be found in it. Consequently only 52% of Hamilton County's amphibian species have been documented in the Township. Six of these species are salamanders and the remaining eight are anurans (see Table 1).

1.5 Amphibian Habitats at Oak Glen Nature Preserve

Amphibians require habitats that can provide them with moisture. Species that have limited tolerance to desiccation, American Bullfrogs (*Lithobates catesbeianus*) for example, require permanent water sources such as streams or ponds. Others, like American Toads (*Anaxyrus americanus*) and Eastern Red-backed Salamanders (*Plethodon cinereus*) can inhabit terrestrial environments far from a source of permanent surface water. Rocks or logs under which the soil is at least damp will provide the water they need. Cope's Gray Treefrogs (*Hyla chrysoscelis*) are arboreal. They spend much of their time in tree boles where high humidity inside keeps their skin moist and allows them to maintain osmotic balance. Amphibians are typically absent from habitats lacking sources of water or high humidity. Their habitats at OGNP were characterized into six categories based on dominant vegetation, geological features (eroded ravines to expose rocks and outcrops), elevation, and terrestrial vs. wetland (stream vs. pond). Habitat types are summarized and each amphibian species found in association with them is summarized in Table 2.

1.5.1 Upland Deciduous Forests — The mature upland forests at OGNP (Figure 4) are dominated by a variety of hardwood tree species. East of the oil pipeline corridor in the northern tract, where the highest elevation at OGNP (875 feet) occurs, the forests are dominated by oaks and shagbark hickories. In the southern tract of OGNP where elevations are highest (844 feet) near Thompson Road, the forests are dominated by Oaks. In both tracts sugar maples dominate the slopes where soils are somewhat moister. Ashes were once among the dominant hardwoods but many are now dead but still standing. The understory in the southern tract is dominated by paw paws and spice bush. Bush honeysuckle grew in high densities where logging had occurred historically, particularly on the tract of privately owned land between the north and south tracts of OGNP. It was not common in the upland forests of either tract. Because of the steep relief, the soils in these higher elevation forests were dry and the habitat is largely unsuitable for amphibians except where they are dissected by steep ravines.

1.5.2 Wooded Ravines and Ephemeral Streams — The most recent glacier to move across Ohio, the Wisconsin, retreated some 14,000 years ago but it did not reach the area that is now OGNP. The soils at OGNP are much older and are of Illinoian or pre-Illinoian origin. The steep-walled ravines and deep valleys that cut through the deep soils have resulted from at least 125,000 years of erosion. The ephemeral streams at the base of the ravines are littered with Ordovician limestone rocks and slabs (Figure 5). In the upper reaches of these streams there are strata of oolitic limestone sandwiched between fossiliferous limestones. While both provide great refugia for salamanders, oolitic limestone appears to maintain moisture levels more effectively than fossiliferous limestone, a characteristic that is important during extended dry periods. Salamanders, especially Cave Salamanders, were found during all months between May and September. They usually are not found at the surface during July and August. Gaps between rock strata and solution channels on the lower surface of limestone strata provide subterranean habitats for Cave Salamanders. Access to and from the subterranean habitats appear as outcrops on the ravine walls and stream margins at the bottoms of ravines, and places where tree roots may pry at the hidden rock strata to create openings and gaps at the surface. These access points are an essential feature in Cave Salamander habitat.

1.5.3 Bottomland Forests — Most of the bottomland forest habitat at OGNP (Figure 6) is on private property situated between the northern and southern tracts. These forests, around the southern wetlands, are dominated by silver maples and box elders. In the floodplain where the OGNP streams converge, Amur honeysuckle is abundant although some paw paw remains among them, the box elders, and silver maples that are reforesting the area where some logging occurred. Stream 3 in this area, along with Stream 5, is the most important breeding site for Streamside Salamanders at OGNP. The bottomland forests are also important for unisexual *Ambystoma* salamanders, and a host of anuran species.

1.5.4 Wetlands — The northern wetland at OGNP holds water permanently and provides important habitat for American Bullfrogs and Green Frogs (*Lithobates clamitans*). Because fish inhabit the wetland, it is not used for reproduction by ambystomatid salamanders or most of the anuran species at OGNP. The southern wetland does not hold water permanently as evidenced by an aerial photo posted in August 2012 on Google Earth. During May and June, 2014, Cope's Gray Treefrogs chorused from the trees around it and ambystomatid salamanders moving toward it were intercepted by drift fences. The two smaller wetlands (Figure 7) located just south of the East Miami River Road gate also dry during prolonged periods of rainless weather. They were used extensively by anurans, such as Cope's Gray Treefrog, Spring Peepers (*Pseudacris crucifer*), American Toads, and Blanchard's Cricket Frogs (*Acris blanchardi*), all of which are species that breed in ephemeral ponds. Cardno-JF New did not set drift fences around them and the present survey started well after the ambystomatid breeding season was over. It is unknown at this time whether or not either of the smaller wetlands are used as breeding sites by salamanders.

1.5.5 Flooded Depressions and Ditches — At several locations on the terrace near the staging area adjacent to the southern wetland, depressions were filled by rain and held water the entire season. These were used by Green Frogs, juvenile American Bullfrogs, Cope's Gray Treefrogs, and Blanchard's Cricket Frogs. The latter two species bred in the depressions and metamorphs emerged from them. Early in the season, before the present survey started, it is likely that American Toads found these flooded depressions and used them as breeding sites. Metamorphs were found in the southern tract of OGNP and the only other suitable breeding site would be the southern wetland. It was still blocked by drift fences during the species' breeding season. Another pair of flooded depressions formed in the gravel road adjacent to the northern wetland (Figure 8). They were used by Cope's Gray Treefrogs and Blanchard's Cricket Frogs for oviposition but the sandy substrate did not allow the water to stand long enough for the eggs to hatch or tadpoles to develop. Traffic also passed through them on a daily basis.

1.5.6 Seeps — Two seeps found at OGNP are associated with Stream 5 (Figure 9). They had constant, but slow flow until mid-July in 2014 and both had flow through June 3, 2015. Southern Two-lined Salamanders (*Eurycea cirrigera*) were found in them and a Cave Salamander was found in one.

1.5.7 Sandy/Gravelly Terrace — The terrace above the Great Miami River is flat and underlain by sand and gravel. The oiled wetland was once a gravel quarry. The sandy, gravelly soil on the terrace are exposed near the northern gravel quarry (Figure 10). It supports sparse vegetation and drains quickly after rain events. Soils between the two smaller wetlands near the East Miami River Road gate and the oiled wetland support a more diverse, meadow-like flora with a few intersperse low, wet areas that have sedges and other hydrophilic plants (Figure 11). The southern end of the terrace, with its meadow-like conditions is inhabited by Northern Leopard Frogs. The entire terrace provides habitat for Fowler's Toads. Neither of these species uses other upland habitats at OGNP.

2.0 METHODS

2.1 Visual Searches/Pedestrian Surveys

Amphibians are secretive animals. Salamanders spend much of their time hiding beneath rotting logs, rocks, and other cover objects in shady areas. Some species are inhabitants of wooded hillsides and others live among the rocks along the margins of streams. During dry conditions many species take refuge in abandoned small mammal burrows. Others are small enough to seek shelter and moisture in earthworm burrows. Where access to underground refuges near rock outcrops is available some species disappear into cracks and fissures between rock strata and move far enough from the surface to avoid dry surface conditions. OGNP has an abundance of the latter and consequently, the Cave Salamander (*Eurycea lucifuga*) is abundant there. Most frogs are usually found in association with water, toads are terrestrial inhabitants in woods and along edges of fields, and treefrogs (Cope's Gray Treefrog, *Hyla chrysoscelis*; at OGNP) live in trees, especially where boles provide them access to water filled cavities. Where visual searches were feasible, they were used to find frogs and toads. Cover objects were lifted, examined for salamanders and returned to their original positions. Salamander larvae in streams that had them could be observed foraging or found hiding beneath submerged rocks. Early in the season, visual searches were used to look for Streamside Salamander (*Ambystoma barbouri*) and Southern Two-lined Salamander eggs on the undersurface of rocks in shallow streams.

2.2 Funnel Traps

During late winter and early spring after the ice has thawed from the surface of vernal pools, ambystomatid salamanders and Spring Peepers migrate from the woods to the pools to reproduce. Migration and reproductive activity occurs at night and it can be difficult to detect migrating adults. Funnel traps were placed in the two small wetlands southeast of the gate on East Miami River Road, in the flooded depression near the staging area parking lot and water retention basins near the staging area parking lot (Figure 12). Traps were set on the bottom with a space at the top out of the water so captured animals could surface for air (Figure 13). Traps were checked daily, frogs and salamanders in them were removed, identified and photographed, then released (Figure 14).

2.3 Mitochondrial DNA Identification

Approximately 3 mm of the tip of the tail of trapped ambystomatid salamanders was clipped and stored in 75% ethanol for positive identification using mitochondrial DNA. Analysis was completed by Dr. Herman Mays in his lab at Marshall University in Huntington, West Virginia. The results are discussed in Section 3.2.2.

2.4 Auditory Surveys

Frogs and toads generally chorus at night, especially later in each species' respective breeding season. Nighttime visits to OGNP were made on nights after or when rain was falling to listen for chorusing frogs. Spring Peepers begin chorusing shortly after pond surfaces thaw and daytime temperatures warm to 55-60°F. Nighttime visits continues through the end of the survey on June 3rd when late spring and summer breeding species (Bullfrogs, *Lithobates catesbeiana*; Green Frogs, *L. clamitans*; Cope's Gray Treefrogs; and Blanchard's Cricket Frogs were chorusing.

2.5 Cave Salamander Monitoring

A monitoring project in Stream 3 was mandated by the Ohio Environmental Protection Agency. Although this project is funded separately, the data are included in this report to provide the most updated distribution for Cave Salamanders at OGNP. The dates for collection of these species may not coordinate with dates when site visits were made exclusively for the amphibian survey.

2.6 Locality Marking

The collection locality of each amphibian that was encountered during the 2015 survey was marked using GPS technology and the localities, dates, and other pertinent ecological information were recorded in an Excel file. In some instances, such as mass collections of breeding ambystomatid salamanders and chorusing frogs, the site was marked and approximate numbers of observed animals was recorded. The coordinates were used to generate species distribution maps.

2.7 Cardno-JF New Data

Cardno-JF New data are used to augment those from the current survey. Cardno-JF New personnel were responsible for monitoring the oiled wetland and the lower stretches of Stream 3. The use of Cardno data allowed for detailed discussions for some species' distributions.

2.8 Scientific Names

Scientific and common names used in this report are after Crother (2012)

3.0 RESULTS AND DISCUSSION

Each species is discussed below. Comments are included where indicated. Distribution maps are provided for each species in Addendum I.

3.1 Frogs and Toads

Seven frog and toad species were documented during the 2014 survey. Fowler's Toad was added to the list as a result of the 2015 survey. All species documented during the 2014 survey were documented again in 2015.

3.1.1 American Toad (Figure 15)

American Toads were found during pedestrian surveys and auditory surveys. They used the constructed wetland extensively for reproduction and eggs, tadpoles, and metamorphs were observed in and around it (Table 3). Adults were observed to be widely distributed throughout OGNP and reproduction occurred in every available body of water (Figure 16). The species is somewhat of a generalist, inhabiting most habitats at OGNP with the exception of dry fields.

3.1.2 Fowler's Toad (Figure 17)

Fowler's Toads were not found during the 2014 survey. However, a single male was heard calling from

the backwaters of the northern gravel quarry and an adult was found moving about during a nighttime pedestrian survey (Figure 18). This is a floodplain and river bottom species that spends the day burrowed into the sand deposited by rivers during high water events. It breeds later than American Toads, although there can be some overlap during May when a few American Toads may still be chorusing when Fowler's Toads are beginning to breed. Although one male was heard calling, no evidence of successful reproduction (i.e. eggs, tadpoles, or metamorphs) was found.

3.1.3 Blanchard's Cricket Frog (Figure 19)

This species was found during visual searches and auditory surveys. It was among the species that used the constructed wetland for reproduction (Table 3). Cricket Frogs are typically associated with floodplain depressions and the margins of streams and rivers. It was found throughout the Preserve on the terrace above the Great Miami River floodplain (Figure 20). Choruses were heard in the small wetlands near the East Miami River Road gate and in flooded depressions in the road leading past the northern gravel quarry. Individuals also bred in the retention ponds near the staging area parking lot and in the oiled wetland. Blanchard's Cricket Frog is listed as a Species of Concern by the Ohio Division of Wildlife (ODW, 2015). An individual was photographed in the northernmost of the two small wetlands near the East Miami River Road gate that was solid green in color, a morph that has only been reported at two other Ohio localities (Figure 11; see Folt and Davis, 2013)

3.1.4 Cope's Gray Treefrog (Figure 21)

This secretive species disappears during daylight hours but becomes extremely vocal during the breeding season. It was heard at numerous localities throughout OGNP, calling from trees after rain events early in the field season. Because the frogs were often some distance from the point where they were heard, specific localities were not recorded. However, by mid-May, on warm humid nights this species was heard in abundance around the oiled wetland, the two smaller wetlands near the East Miami River Road gate, the retention ponds near the staging area parking lot, and in depressions that held water after rain events (Figure 22). Cope's Gray Treefrogs used the constructed pond in numbers. Tadpoles were seined from it on June 3, 2015. This may be among the most abundant amphibians at the Preserve.

3.1.5 Spring Peeper (Figure 23)

This tiny treefrog does not climb into trees but instead inhabits brushy edges along forests. It breeds in fishless bodies of water and at OGNP it was observed or heard at every standing body of water with the exception of the northern gravel quarry. It was seldom seen but usually heard, especially during nighttime auditory surveys. It used the constructed wetland and tadpoles were observed in it several times during May. Individuals and amplexing pairs were captured in the two small wetlands near the East Miami River Road gate, the flooded depression, and water retention ponds near the staging area parking lot (Figure 24). It was the first species to chorus and a few individuals continued to call into mid-May.

3.1.6 American Bullfrog (Figure 25)

This species, the largest of Ohio's frogs, requires permanent water for its tadpoles to develop. Adults observed in small bodies of water are usually transients that took refuge during a dry period. Juveniles were observed in the constructed wetland and a couple of individuals were trapped in the water retention ponds near the staging area parking lot. Males were heard chorusing from the oiled pond but

successful reproduction would not have occurred during 2015 because the wetland was dry by June. Successful reproduction probably occurs in the backwaters of the northern gravel quarry where numerous males were heard chorusing during nighttime visits in late May and into June. The limited permanent water at OGNP probably makes this a relatively uncommon species at the Preserve (Figure 26).

3.1.7 Green Frog (Figure 27)

Most often, Green Frogs require two years for their tadpoles to complete development through metamorphosis. Consequently, they need permanent water. During 2015 the only water bodies to maintain water through the whole season were the northern gravel quarry, the constructed wetland, and the impoundment in Stream 3. Green Frogs chorused from all three in addition to the water retention pond near the staging area parking lot and the constructed wetland (Table 3). Individuals were captured in funnel traps in the latter as well as the two smaller wetlands near the East Miami River Road gate. Transient juveniles dispersing from their natal ponds are often observed in streams during summer. This is a common species everywhere in Ohio and OGNP is no exception. The distribution of the Green Frog at the Preserve is illustrated in Figure 28.

3.1.8 Northern Leopard Frog (Figure 29)

The Northern Leopard Frog (*Lithobates pipiens*) has made a comeback in recent years after disappearing from Hamilton County in the 1980s and 1990s (Boone, 2013). They first re-appeared about 15 years ago in the Great Miami River valley near the Shawnee Lookout boat ramp. They also appeared in the uplands east of Shawnee Lookout. Leopard Frogs were found some 10 years ago at Winton Woods in a wetland near the dam that received sediment dredged from the lake bottom and more recently they have been found around Parky's Farm. Around 2010 they started appearing at the Fernald Preserve, some 3.5 km north of OGNP. They may have followed the Great Miami River and Mill Creek as migration corridors, eventually making it back to the northern part of the county or they may have moved in from the north. The species did not disappear in Butler County as it did in Hamilton County. Two adults were observed on the terrace at OGNP in 2014 and two more were observed in 2015 (Figure 30). One adult was heard chorusing in the constructed wetland during auditory surveys (Table 3). However, no egg masses or tadpoles were observed afterward. Because most of OGNP is densely forested there is not a great deal of Leopard Frog habitat but the species' presence at the preserve is good evidence that it has made a comeback and restoration to the terrace may allow this species' numbers to increase.

3.2 Salamanders

Four salamander species and unisexual *Ambystoma* were documented during the 2014 survey. The unisexual *Ambystoma* are not considered a species because these female biotypes are apparently incapable of reproducing parthenogenetically and require sperm from other *Ambystoma* species, which provide no DNA to the offspring. They were intercepted, during the 2014 survey, by drift fences and pit fall traps as they made their way to the oiled wetland by Cardno-JF New personnel. Each was toe-clipped and subsequently identified using mitochondrial DNA. In 2015, traps were set in the two small wetlands near the East Miami River Road gate and a large sample of breeding adult salamanders was captured, some of which were males. Approximately 3 mm of the tail was clipped from a sample of the salamanders collected in the small wetlands and identified using mitochondrial DNA. Some were confirmed as unisexual *Ambystoma* and others were identified as Jefferson Salamanders (*Ambystoma*

jeffersonianum), a species previously unidentified at OGNP.

3.2.1 Streamside Salamander (Figure 31)

Streamside Salamanders were found to be widespread at OGNP (Figure 32). During 2014, numerous dead egg masses were found in Stream 3 (oiled stream). Live larvae were observed in Stream 5 and Stream 2 just upstream of its confluence with Stream 3. In 2015, egg masses and larvae were observed in Streams 3 and 5, and Stream 2 immediately upstream of its confluence with Stream 3 (see Figure 3). Larvae were observed in Stream 3 to just below the point at which the oil pipeline ruptured in March, 2014. Cardno-JF New personnel captured adults in pit fall traps along segments of Stream 3 on the terrace above the Great Miami River and around the oiled pond. Trapping efforts in the two small wetlands near the East Miami River Road gate resulted in Streamside Salamander captures as well (Figure 2).

3.2.2 Unisexual *Ambystoma* and Jefferson Salamander (Figure 33)

Historically, salamanders found in Colerain Township identified as Jefferson Salamanders could have been this species or an all-female, triploid hybrid of the Jefferson Salamander. The female hybrids reputedly have longer, flatter toes than Jefferson Salamanders. In recent years it has been determined that the hybrids are “biotypes” possessing the mitochondrial DNA of two or more closely related species. The female biotypes require sperm from Jefferson, Small-mouthed (*Ambystoma texanum*), Tiger (*A. tigrinum*), Blue-spotted (*A. laterale*) or Streamside Salamander males (Owen and Juterbock, 2013). The males do not contribute DNA, but their sperm cells stimulate cell division, development, and growth of the female biotypes’ eggs. Now referred to as unisexual *Ambystoma* salamanders, the all-female biotypes cannot be assigned a species name because they may possess mitochondrial DNA from as many as five species of *ambystomatid* salamanders. It has been suggested that this reproductive strategy, which is limited to these salamanders, affords them an advantage as they populate new areas because a single female can move into a breeding population of one of the other species and quickly build her biotype’s population. Results from the mitochondrial DNA analysis of the tissue samples taken from salamanders at OGNP in 2014 by Cardno-JF New personnel concluded that they are unisexual *Ambystoma* salamanders. Random tissue samples were selected from toe clips collected by Ohio EPA personnel and nitrogen base pairs ranging in length from 400–500 nucleotides of the 16-S gene were compared to known sequences for unisexual *Ambystoma* salamanders. The OGNP specimens’ DNA were a 99–100% match to known samples.

In 2015 ambystomatid salamanders were trapped from the two small wetlands near the East Miami River Road gate (Figure 34), 3 mm tail clips were taken and their DNA was analyzed by Dr. Herman Mays in his lab at Marshall University in Huntington, West Virginia. He analyzed them using the 16S gene in sequences of nearly 600 nucleotide base pairs. The results concluded that both unisexual *Ambystoma* and Jefferson Salamanders were present in the ponds. Jefferson Salamanders were not identified in 2014 and represent a new species at OGNP.

3.2.3 Southern Two-lined Salamander (Figure 35)

The Southern Two-lined Salamander is a common species in southwest Ohio that can be found hiding beneath rocks and logs at the margin of streams and seeps. Larval development occurs over two seasons; hence the streams in which it lives and breeds must have perineal flow. Cardno-JF New personnel caught this species in their pit fall traps around the oiled pond and in the lower stretches of Stream 3 in 2015. Efforts to look for this species during my 2015 amphibian survey in Stream 3 yielded

no eggs, larvae, or adults. The portion of Stream 3 on the terrace downstream of its confluence with Stream 2, along with short segments of Stream 5 and two seeps associated with Stream 5 were the only places at OGNP preserve that maintain water long enough to support the species' 2-year larval development period. During 2014 and the spring of 2015, only one small area was found where Two-lined Salamanders were found with any regularity (see Figure 9). The intermittent nature of the streams at OGNP do not provide high quality breeding habitat for this species. Those individuals of this species caught in pit fall traps around the oiled wetland are inexplicably out of their normal habitat and the standing water in the wetland does not provide breeding habitat for the species. These adults may have been flushed downstream during high water and perhaps they moved along the drift fences after being displaced. The distribution for Two-lined Salamanders at OGNP is illustrated in Figure 36.

Cardno-JF New personnel captured only two or three adult Southern Two-lined Salamanders from the lower stretches of Stream 3 through June 3, 2015. Two possible explanations for the apparent rarity of this species are 1) Stream 3 is ephemeral and does not hold water long enough to support a large population of this species. The same certainly appears to be the case in most of Stream 5 and all of Stream 2, neither of which were contaminated by the oil spill. 2) Perhaps historically there was a more significant population in Stream 3 as it flows over the terrace but the impact to Southern Two-lined Salamanders by the oil spill might have been sufficiently significant that the population was severely impacted. There would have been no reproduction in Stream 3 for this species in 2014, which would explain the lack of larvae in 2015. While Streamside Salamanders bred in Stream 3 and their larvae were abundant in 2015, the adults do not live in close association with water and indeed most had probably moved back into surrounding woods before the oil spill occurred in 2014. Conversely, Two-lined Salamanders do not move away from the water but live along the margins. Unlike Streamside Salamanders, they would not have been able to escape the oiled water and their population would have declined as a result.

3.2.4 Cave Salamander (Figure 37)

The Cave Salamander is listed as "Endangered" by the Ohio Division of Wildlife. This species was widespread in the appropriate habitats at OGNP. It is important to recognize that this is not a stream dwelling species. It spends up to 90% of its life in subterranean habitats and only occasionally does it make its way into the limestone strewn, intermittent streams that occupy the bottoms of limestone ravines in southwest Ohio (Davis, 2013). It also does not inhabit hillside or ridge top forests unless there are rock outcrops on them. Reproduction and larval development occurs underground. Larvae are rarely found in streams and even then they are typically adjacent to a seep or spring. Because OGNP has not been glaciated since the Illinoian glacier moved through the area perhaps 250,000 years ago, the rivulets and streams in the preserve have cut deep into the hillsides creating outcrops that provide access into underground recesses that provide optimal habitat for Cave Salamanders in Ohio. Consequently, the species is very common at OGNP. During the 2015 amphibian survey efforts were made to get into streams and rivulets that were not examined in 2014 or needed to be re-examined to document presence. Nearly every stream that was visited in 2015 had Cave Salamanders (Figure 38). At most Cave Salamander sites in Hamilton and Butler counties, encountering two or perhaps three individuals in one day can be considered successful. On two occasions at OGNP, eight Cave Salamanders were found in one day suggesting that it provides the best habitat for the species in Ohio excepting habitats augmented by humans such as springhouses and cisterns. Adults, subadults, and juveniles were found, the latter being found less often, which is not uncommon in any Cave Salamander population. As part of the protocol for monitoring Cave Salamanders in Stream 3, 12 inch² ceramic floor tiles were positioned near outcrops in Streams 2, 3, and 5. These have provided important data regarding prey species availability to Cave Salamanders and a preliminary list of species found under

cover tiles at OGNP is provided in Table 4. Additional preliminary data from the 2015 Cave Salamander monitoring project compare the number of Cave Salamanders found in the oiled stream (Stream 3) to the number in the two adjacent streams; Streams 2 and 5 (Table 5).

3.2.5 Eastern Red-backed Salamander (Figure 39)

Because the 2014 survey did not begin until May 22, most sightings of this species occurred in September. The 2015 spring amphibian survey confirmed that this species is common as it was found frequently. The mature forests at OGNP provide a great deal of habitat for Red-backed Salamanders. Because of the steep relief, the ridge tops drain quickly and are too dry for this species. The distribution of the Red-backed Salamander at OGNP is primarily associated with the ravines and shaded hillsides (Figure 40). Another note of interest is that in southwest Ohio there are two color morphs of the Eastern Red-backed Salamander. One, the red-backed phase has a deep reddish-brown dorsal stripe on a gray background. The second, called the lead-backed phase has no dorsal stripe and the same gray ground color. One hundred percent of the Red-backed Salamanders found at OGNP in both 2014 and 2015 are of the red-backed phase.

4.0 DISCUSSION

Among the goals of this survey was to attempt to find species that might occur at OGNP but were not identified during the 2014 survey. Two species were added to the species list. Fowler's Toad and Jefferson Salamander were documented during the 2015 survey. Only one species that could inhabit OGNP that has not been confirmed and that is the Northern Ravine Salamander (*Plethodon electromorphus*), a close relative of the Red-backed Salamander. Ravine Salamanders more typically inhabit drier, south facing hillsides closer to ridgetops than Red-backed Salamanders. They have not been documented in Colerain Township but have been found in Crosby Township approximately 4.7 km north of OGNP. In the coming years, should the author continue to monitor Cave Salamanders at OGNP, efforts will be made to find Northern Ravine Salamanders.

Discussed multiple times in this report is the abundance of Cave Salamanders at OGNP. Of 30 Cave Salamanders captured at OGNP during April, May, and June 2015 only three were captured in Stream 3 between the oil line easement and the terrace upon which the oiled wetland is located. These data are only preliminary and monitoring will continue in Streams 2, 3, and 5 until October, 2015.

Streamside Salamanders reproduced in Stream 3, eggs hatched, and larva metamorphosed and moved from the stream to terrestrial habitats, a positive sign toward the stream's recovery. However, this is a resilient species that is often found living in suburban neighborhoods, parks, and even in agricultural areas. No evidence for Two-lined Salamander reproduction was detected in Stream 3 between its confluence with Stream 2 and the oiled wetland.

OGNP has an impressive diversity of amphibian species inhabiting it. The lowland wetlands are important for frogs and toads and as ambystomatid salamander breeding sites. The uplands, especially the deep, moist, rock strewn ravines are important to plethodontid salamanders. No efforts have been made to target reptile species at the preserve but Eastern Gartersnakes (*Thamnophis s. sirtalis*), Northern Ring-necked Snakes (*Diadophis punctatus edwardsii*), Midland Painted Turtles (*Chrysemys picta marginata*), Red-eared Sliders (*Trachemys s. scripta*), Eastern Box Turtles (*Terrapene c. carolina*), and Snapping Turtles (*Chelydra s. serpentina*) have been identified in the Preserve. Concentrated efforts targeting various snakes and lizards would probably add to the reptile species list and knowledge of their distribution at OGNP.

5.0 RECOMMENDATIONS

Clearly, Cave Salamander monitoring in Stream 3 needs to continue. Likewise, based on the use of atypical habitats by Southern Two-lined Salamanders and the lack of evidence of reproduction in oiled areas for the species, monitoring should focus on them.

The portion of the preserve on the terrace is underlain by gravelly and sandy soil through which water percolates easily. In some of the wetlands, for example the two smaller ones near the East Miami River Road gate, the oiled wetland, and the retention pools near the staging area parking lot, water pools and remains for extended periods. However, even in some of these, water may drain out before the metamorphosis is complete for some of the amphibians that breed in them. At the north end of the preserve, American Toads and Cricket Frogs bred in both 2014 and 2015 in depressions in the sandy, gravelly road adjacent to the gravel quarry and in the graveled area just inside the East Miami River Road gate. During nighttime auditory and pedestrian visits, these pools were being used extensively by toads and Cricket Frogs. However in neither 2014 nor 2015 did metamorphs emerge from them. While they are attractive breeding sites, they will always result in an annual loss of recruitment because they do not hold water (Figure 41). The gravel quarry, which holds water permanently, has fish and is therefore not suitable for either species. The two smaller wetlands near the East Miami River Road gate failed to hold water long enough for the development of the amphibians that bred in them (see Table 3). In short, the north end of the preserve has limited reproductive sites that insure a reasonable chance for metamorphosis and emergence. I would recommend the construction of a wetland or two at the north end of the preserve, similar to the one constructed in March, 2014 near the oiled wetland (Figure 42). It would likely be used by Cricket and Green Frogs, Cope's Gray Treefrogs, American and Fowler's Toads, Spring Peepers, and perhaps by ambystomatid salamanders.

Because preliminary data have already been collected for reptiles at OGNP, a complete survey for that taxon would be advisable to document the entire herpetofauna at the Preserve. My field experiences in southwest Ohio are extensive, and species such as Ring-necked Snakes, which are infrequently seen in Hamilton County, appear to be abundant at OGNP, while other species which are often seen have not been documented at the Preserve.

6.0 ACKNOWLEDGMENTS

I appreciate the opportunity from Great Parks to conduct this study. Danielle Thompson (Cardno-JF New) provided and confirmed data regarding amphibians captured in the pit fall traps she and her colleagues check after rain events. Niki Gustufson assisted with early season funnel trapping efforts and Herman Mays analyzed mDNA from tissue samples collected at OGNP. Eric Chapman's help as a field technician was invaluable.

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Addendum I

Figures

Figure 1. Oak Glen Nature Preserve consists of two tracts. The center of the northern tract is at approximately 39.25643° N, 84.68607° W, and the approximate center of the southern tract is at 39.25097°N, 84.68962° W. The Preserve is located immediately south of the Great Miami River and the Deep Stage Ohio River valley, and the Wisconsin glacial boundary. Inset shows location of Hamilton County.

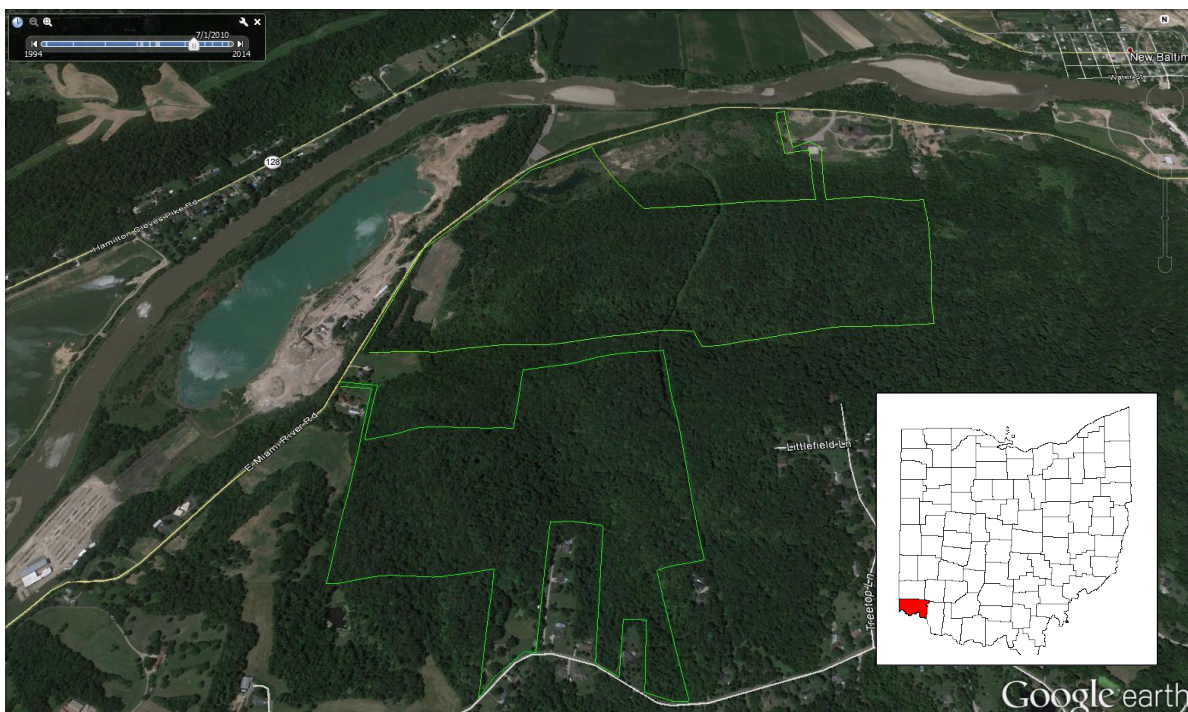


Figure 2. Two wetlands are located on the terrace above the Great Miami River. The southern wetland dries completely during some years, including 2015. Oil from a pipeline break in March 2014 drained into and contaminated it. There are two smaller wetlands between the larger north and southern wetlands that are near the East Miami River Road gate. The white dot represents the approximate location of a wetland that was constructed by Great Parks in March, 2015.

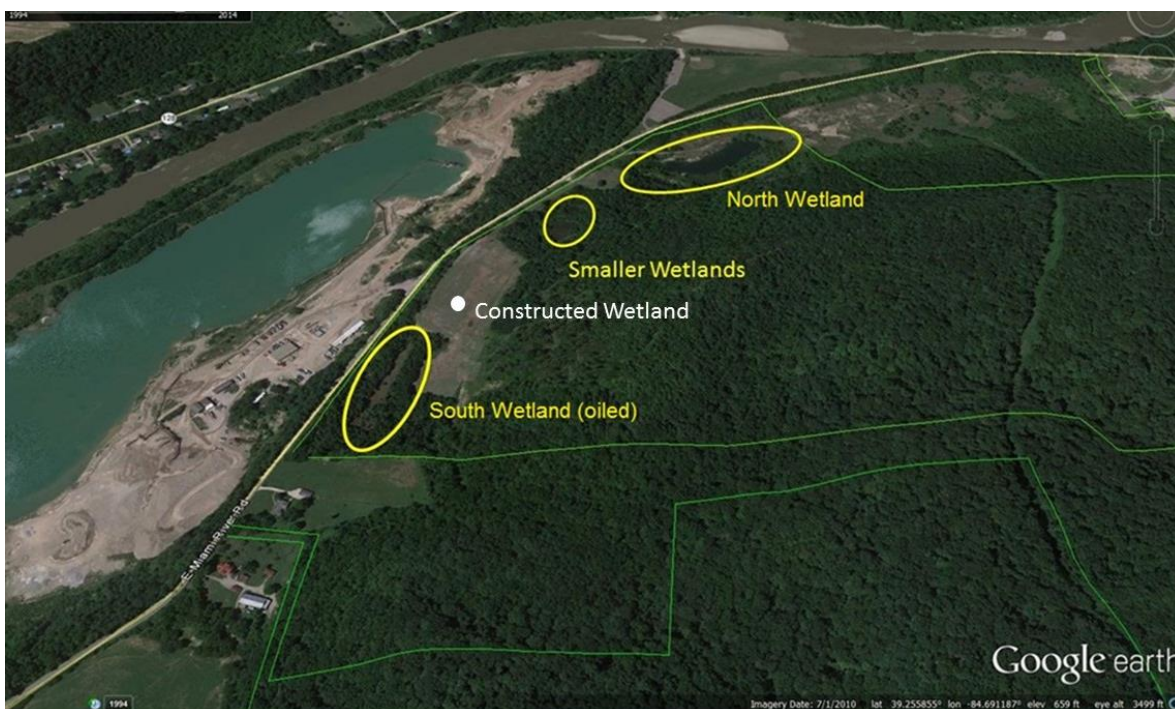


Figure 3. There are six ephemeral streams at Oak Glen Nature Preserve. Five are marked here and the sixth is located at the southwest corner of the south tract (red line; referred to as “Stream 6” in the report). Stream 3 was contaminated by March, 2014 leak in an oil pipeline.

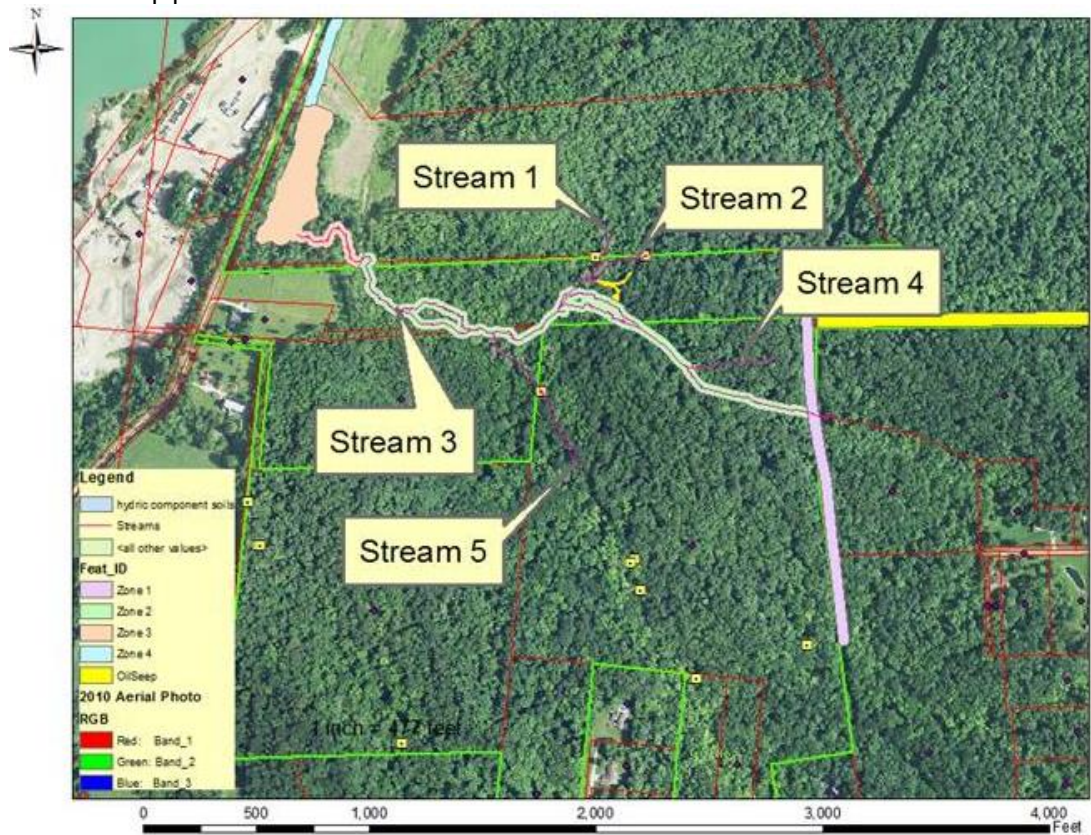


Figure 4. Upland deciduous forest at OGNP between Streams 3 and 5.



Figure 5. Wooded ravine with ephemeral stream (Stream 5) at OGNP.



Figure 6. Bottomland Forest at OGNP near the oiled wetland.



Figure 7. Wetland at OGNP. This wetland is one of the two small wetlands near the East Miami River Road gate (see Figure 2). This photograph was taken on March 15, 2015. By June 3, 2015 it was dry.



Figure 8. Depressions in a road at OGNP fill with water after rain events. These are used by American Toads, Blanchard's Cricket Frog, and Cope's Gray Treefrogs as breeding sites but they dry, stranding and killing the tadpoles before they can complete metamorphosis.



Figure 9. This seep is located in Stream 5, just west of the oil pipeline easement.



Figure 10. Sparse vegetation grows on the sandy, gravelly soil on the terrace near the gravel quarry.



Figure 11. The soil on the terrace near the oiled wetland supports forbs and grasses, and sedges and other hydrophilic plants grow in depressions where the soil is saturated. The edge of the constructed wetland is at the right.



Figure 12. Funnel traps were set in four wetlands to target the capture of ambystomatid salamanders. Blue and red dots are the sites of the small wetlands near the East Miami River Road gate. The yellow dot is in a flooded depression near the staging area parking lot and the white dot represents the approximate location of two water retention pond. Streamside and Jefferson Salamanders, as well as unisexual *Ambystoma* were trapped from the ponds marked by the red and blue dots.



Figure 13. Funnel traps were set to target the capture of adult ambystomatid salamanders immigrating to several wetlands to breed (see Figure 13).



Figure 14. Funnel traps were pulled from the water daily and trapped amphibians were removed, identified, and released into the pond from which they were captured. Pictured is a mix of 52 ambystomatid salamanders including Streamside and Jefferson Salamanders as well as unisexual *Ambystoma* taken from one trap. Other species captured in funnel traps included Spring Peepers, Green Frogs and Bullfrogs.



Figure 15. American Toads were widespread at OGNP. Tadpole, metamorphs, and adults were observed.



Figure 16. Distribution of the American Toad at OGNP. Squares represent records from 2015 and circles from 2014.

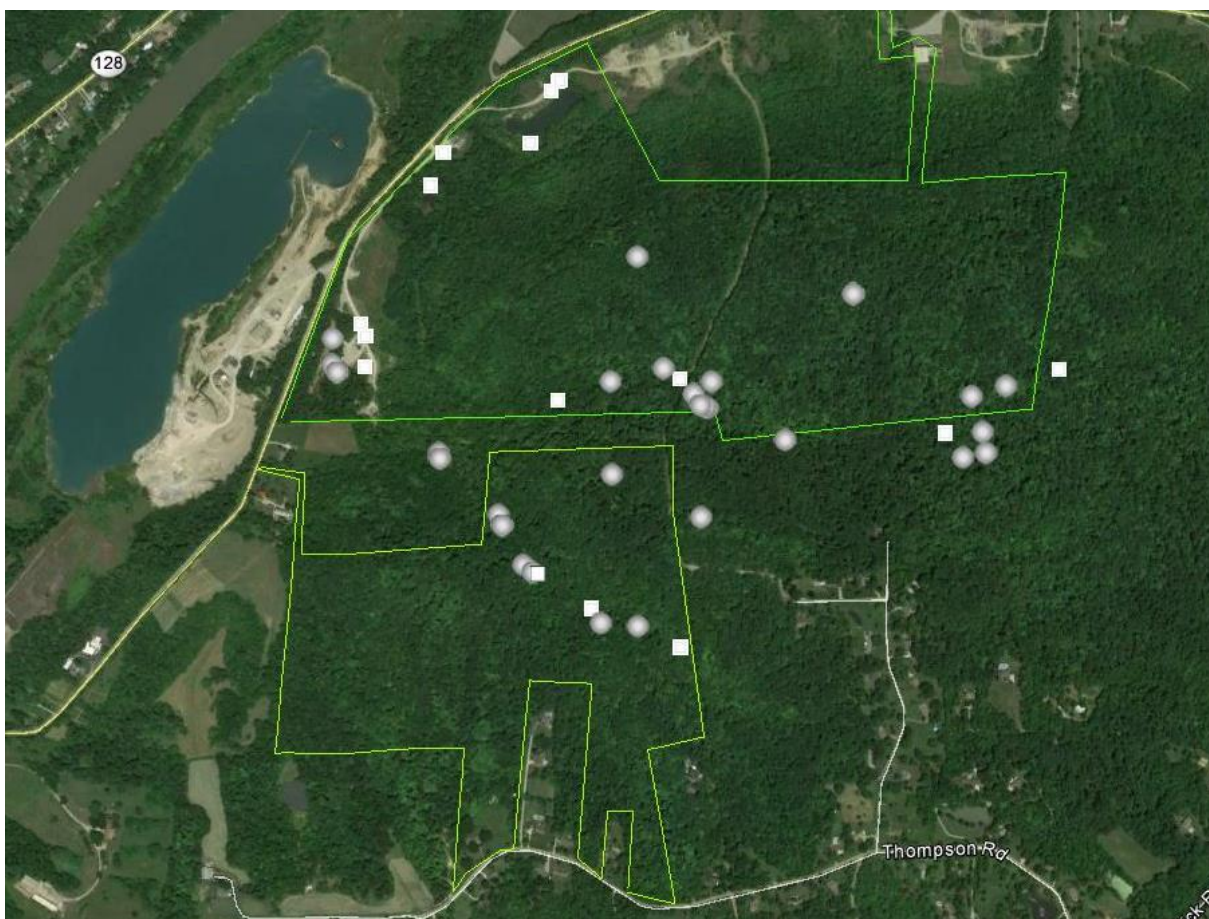


Figure 17. Fowler's Toads were documented only twice during the 2015 survey, once during an auditory survey and a second time during a pedestrian survey. Fowler's Toads are distinguished from American Toads by smaller dorsal warts that usually number three or more per dark, dorsal spot (left) and a single dark spot on the center of their white chest (right). American Toads have only one or two, larger warts per dorsal dark spot and mottled, cream-colored chest.



Figure 18. Distribution of Fowler's Toad at OGNP. The northern observation was an advertisement call and the southern record was a visual encounter during a nocturnal pedestrian survey.

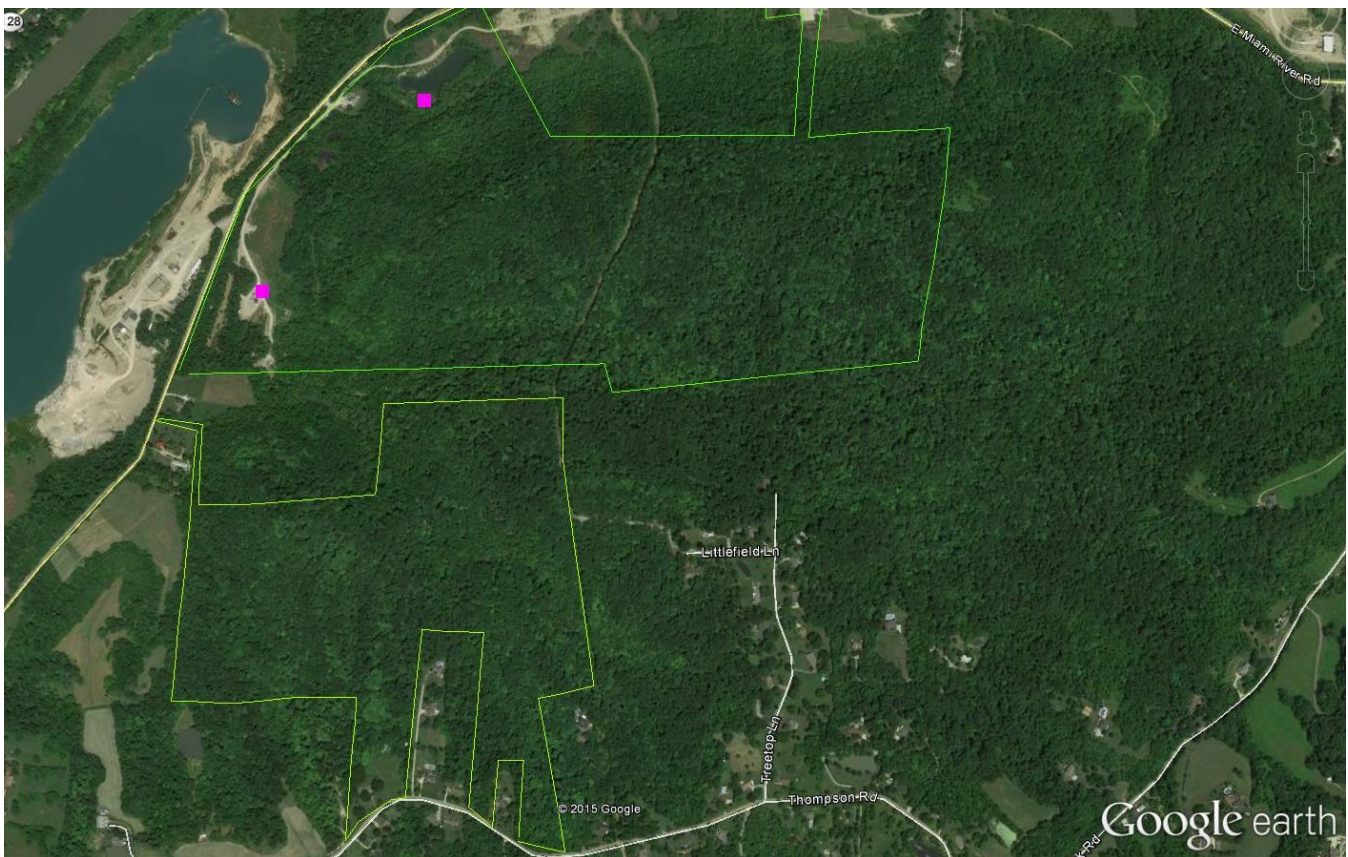


Figure 19. Blanchard's Cricket Frog in the constructed wetland (left) and the rare green color morph of the species in one of the small wetlands near the East Miami River Road gate (right).



Figure 20. Distribution of Blanchard's Cricket Frog at OGNP. Squares represent records from 2015 and circles from 2014.

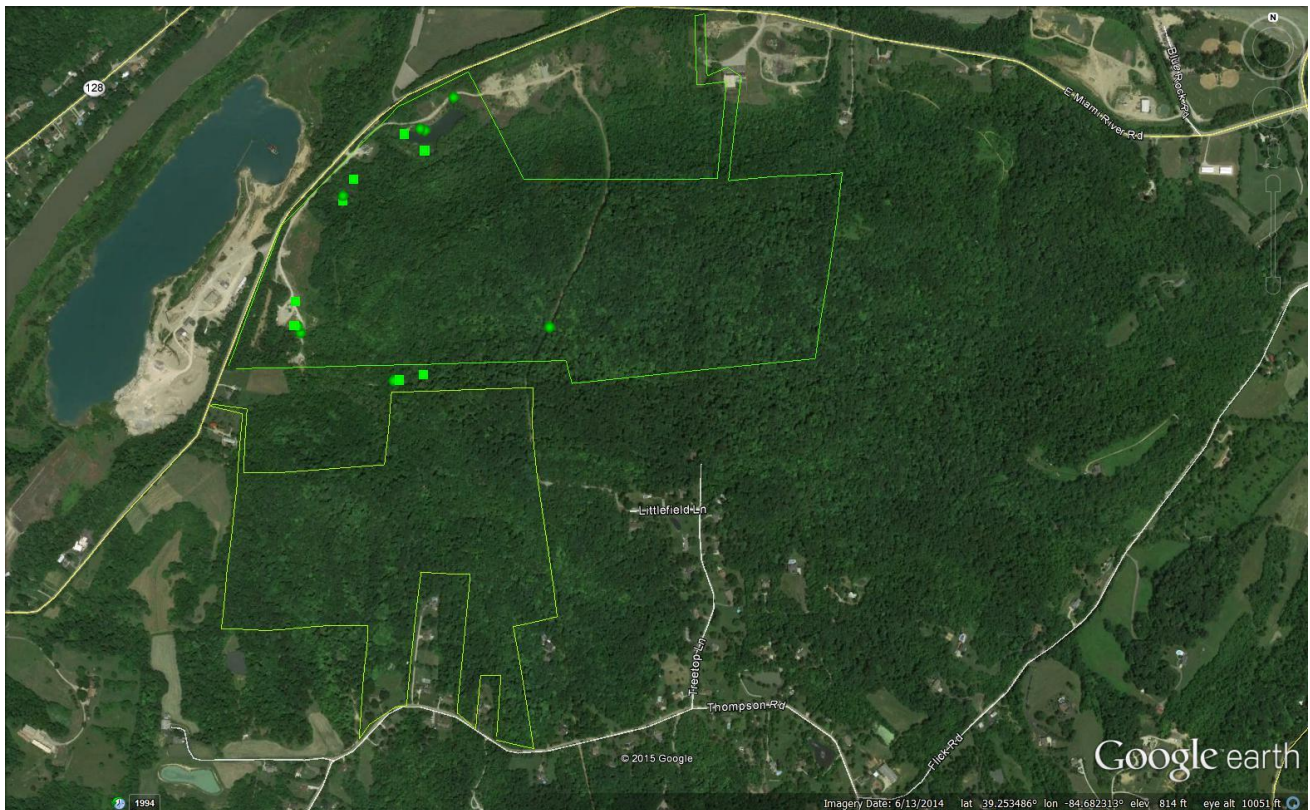


Figure 21. Cope's Gray Treefrog is a common, although rarely seen inhabitant of OGNP. This adult female was photographed in the constructed wetland in 2015 (left). Metamorph photographed in September, 2014 (right).



Figure 22. Distribution of Cope's Gray Treefrog at OGNP. Squares represent records from 2015 and circles from 2014. They were heard calling periodically from trees on the forested hillsides west of breeding sites after rainfalls before the breeding season. This common species is rarely seen due to its cryptic coloration and arboreal habits. They are more often heard than seen. The majority of the records represented on this figure are at breeding sites.

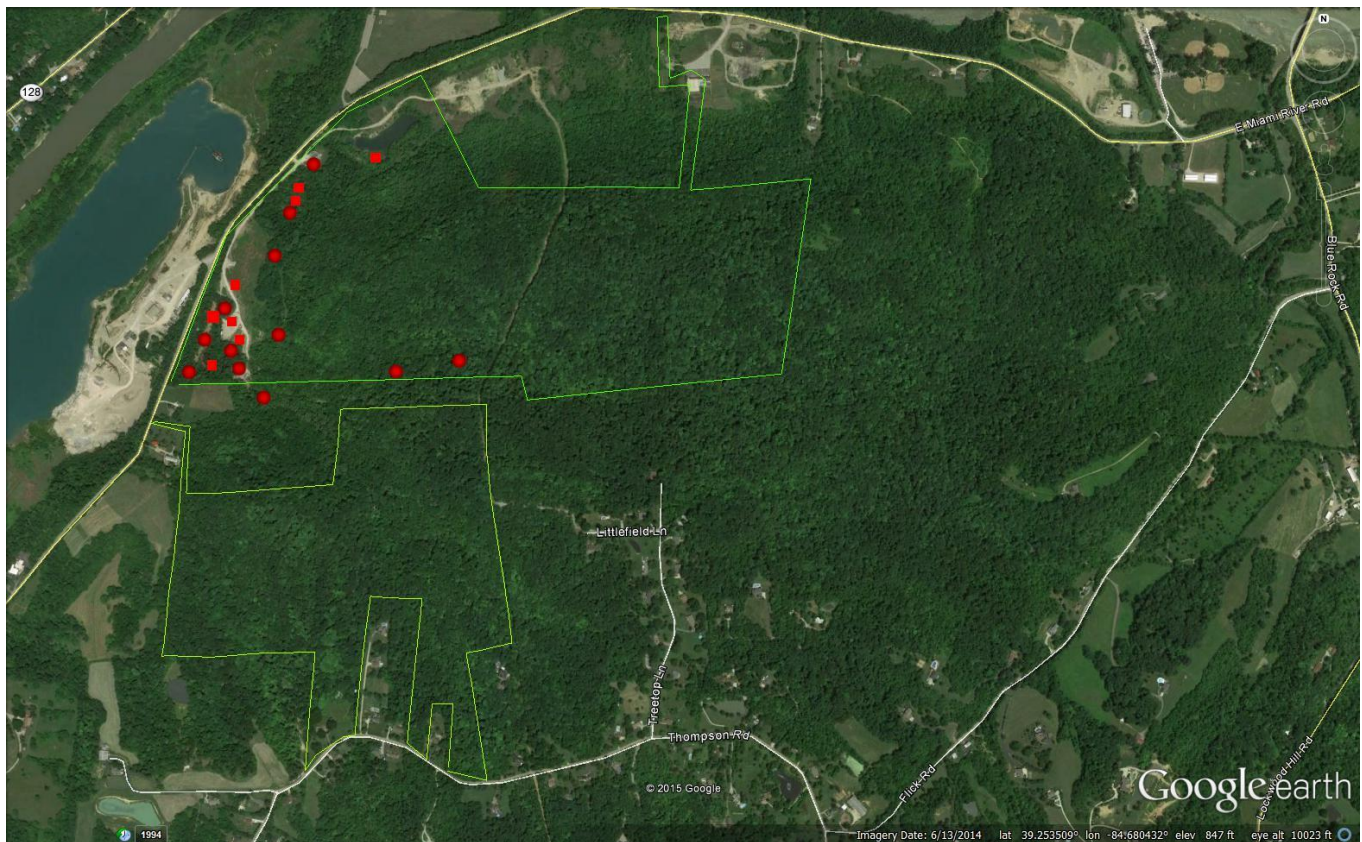


Figure 23. The abundance of Spring Peepers at OGNP was most evident in the spring when they were chorusing. Individual frogs and amplexant pairs were frequently captured in funnel traps (right).



Figure 24. Distribution of Spring Peepers at OGNP. Squares represent records from 2015 and circles from 2014. Most of the records were documented during the breeding season when this species was chorusing. It is difficult to find outside the breeding season. The 2014 records (circles) near the center of the figure were not in the vicinity of breeding sites and support that Spring Peepers are widespread throughout OGNP.

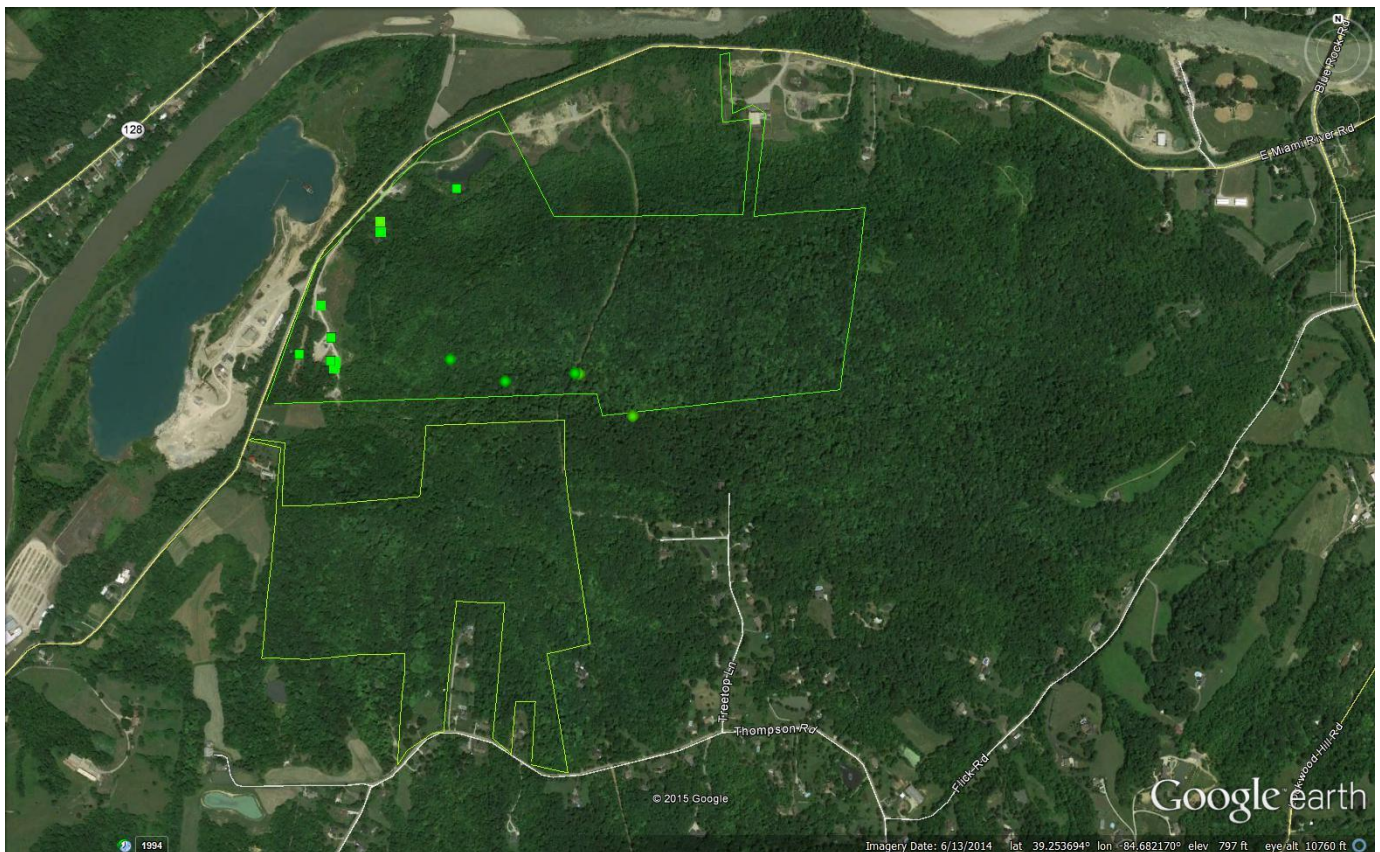


Figure 25. American Bullfrogs are not common at OGNP due to the lack of permanent water. Many of the observations were juvenile that are transient between water bodies during rain events. Adult males were heard chorusing from the gravel quarry and the oiled wetland. The latter was dry by June and any tadpoles in it would have died. Because the quarry holds water permanently, it is probably the only body of water that sustains Bullfrogs at OGNP.



Figure 26. Distribution of American Bullfrogs at OGNP. Bullfrogs require permanent water for successful reproduction. Juveniles are often found in small bodies of water, which they use while dispersing between rain events. The only site at OGNP that holds water permanently is the gravel quarry at the northern end of the preserve. No other water at the preserve has proven to be permanent.



Figure 27. Northern Green Frogs are common at OGNP. They are more transient than Bullfrogs and juveniles will utilize small streams as a water source. Left – A juvenile photographed in Stream 5. Right - These two adults were funnel trapped from the settling pond near the staging area parking lot.



Figure 28. Green Frogs are widely distributed at OGNP. This species is less apt to stay in permanent water. Instead it will migrate along stream corridors and consequently it is found throughout much of OGNP with the exception of well drained hillsides.



Figure 29. Only four Northern Leopard Frogs have been observed at OGNP. Twenty years ago this species appeared to have become extirpated from Hamilton County. The only habitat for this species at the Preserve is on the terrace above the Great Miami River.



Figure 30. Because most of OGNP is densely forested and hilly, it does not provide suitable habitat for the Northern Leopard Frog. The species is more typical of meadows and flood plains. The terrace between East Miami River Road and the hillsides to its east provides the only habitat for it.

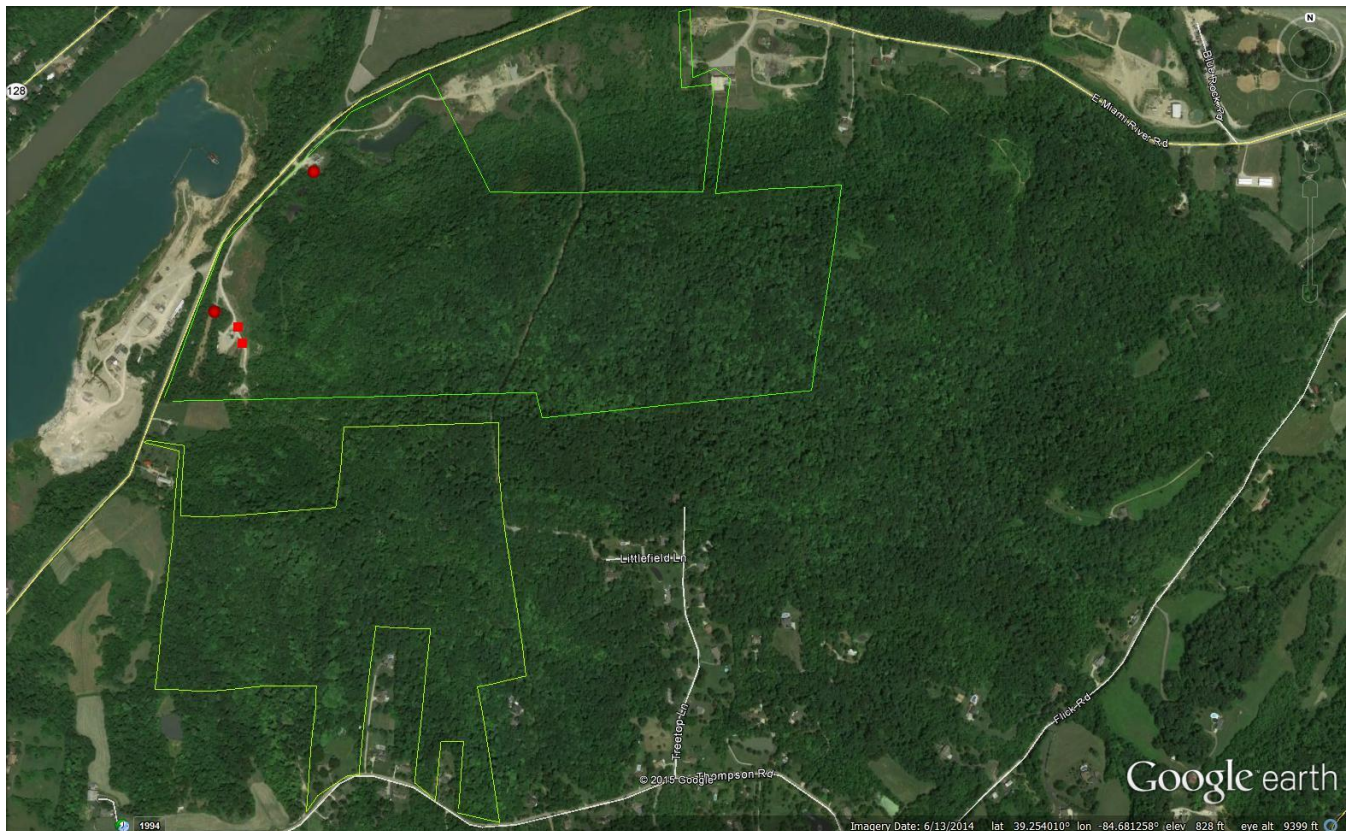


Figure 31. Top Left – Adult Streamside Salamander from Stream 3 on the terrace. Top Right – First year Streamside Salamander from Stream 5. Bottom Left – Streamside Salamander eggs from Stream 5. Bottom Right – Streamside Salamander larva from Stream 2 just upstream from its confluence with Stream 3 on the terrace at OGNP.



Figure 32. Distribution of the Streamside Salamander at OGNP. Squares represent records from 2015 and circles from 2014.

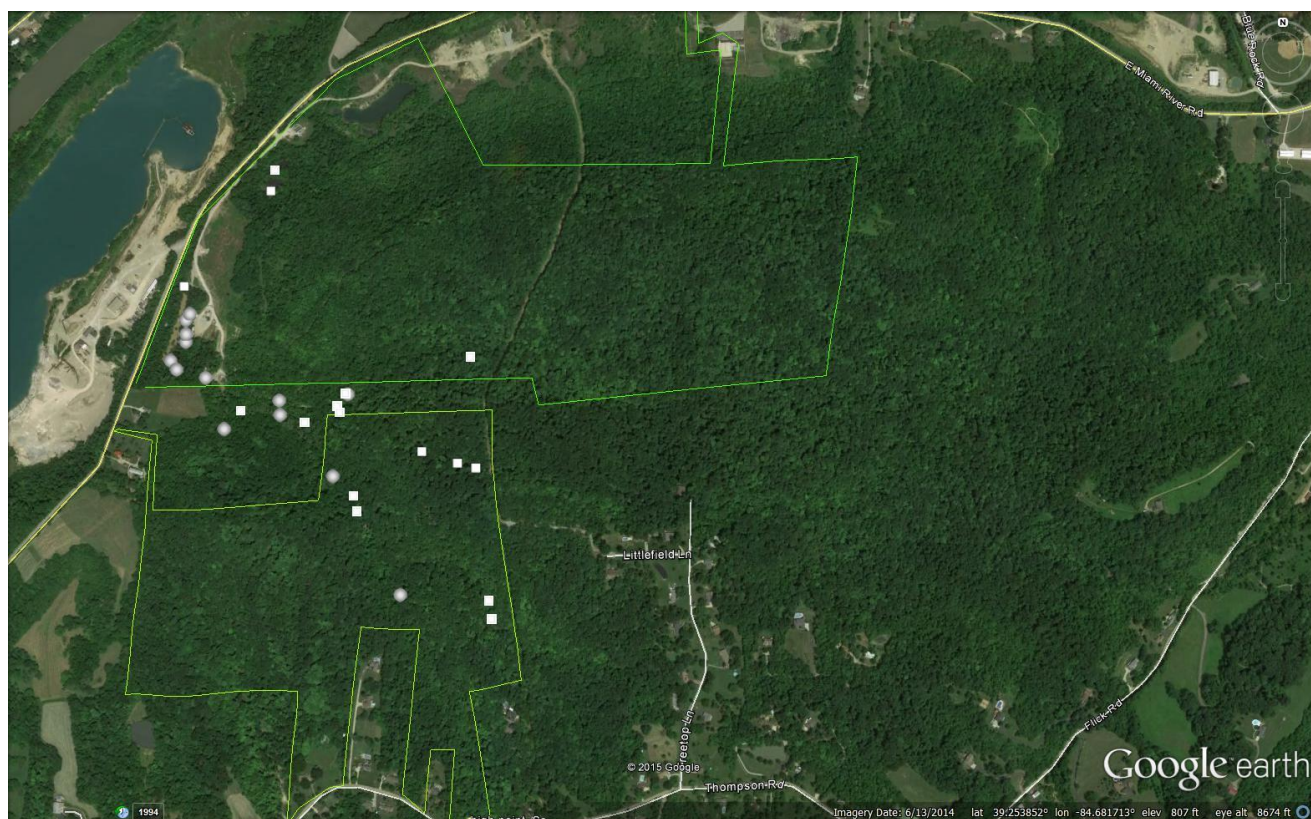


Figure 33. Jefferson Salamanders and unisexual *Ambystoma* are so similar that they can only be accurately identified via DNA analysis. Only unisexual *Ambystoma* were found during 2014. In 2015 both adult unisexuals and Jefferson Salamanders were captured in funnel traps in the small wetlands near the East Miami River Road gate (see also Figure 14). Left – A Jefferson Salamander or unisexual *Ambystoma* between two Streamside Salamanders. Note that Streamside Salamanders have a cylindrical, as opposed to a laterally flattened tail. Streamside Salamanders also have a blunter snout, smaller head, and the costal grooves along the sides are visible right onto the tail. Jefferson Salamanders and unisexual *Ambystoma* have a longer head, a more discernable neck, and their costal grooves do not cut as deep into their sides.



Figure 34. Jefferson Salamanders and unisexual *Ambystoma* were only captured in or near breeding sites. Outside the breeding season they live on the wooded hillsides east of the breeding sites. Toe-clipped individuals captured in 2014 were captures in pit fall traps by Cardno-JF New personnel at the oiled wetland in 2015. They had been released in the woods several hundred yards to the east in April, 2014.

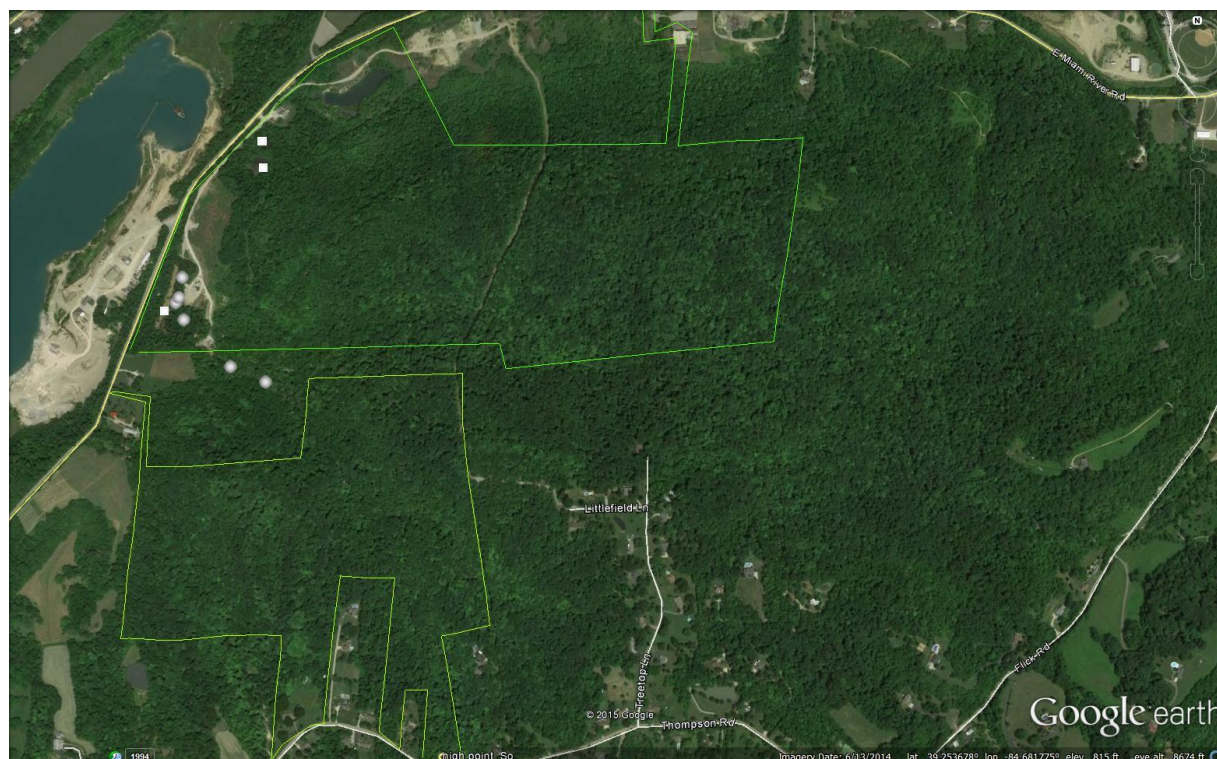


Figure 35. Southern Two-lined Salamanders are common in southwest Ohio but at OGNP they are found at just a couple of sites where permanent water persists long enough for their two-year larval development. The adult (left) and larva (right) were found at the only site where they can be found with certainty at OGNP (see Figure 28).



Figure 36. Distribution of Southern Two-lined Salamanders at OGNP. Squares represent records from 2015 and circles from 2014. Most of the records are from Stream 5. The two circles are records from Cardno-JF New's pit fall traps in the lower reaches of Stream 3. Circles and squares representing those Two-lined Salamanders capture in pit fall traps around the oiled wetland are not included because that habitat is atypical for the species. They likely ended up there when moving along the drift fences.

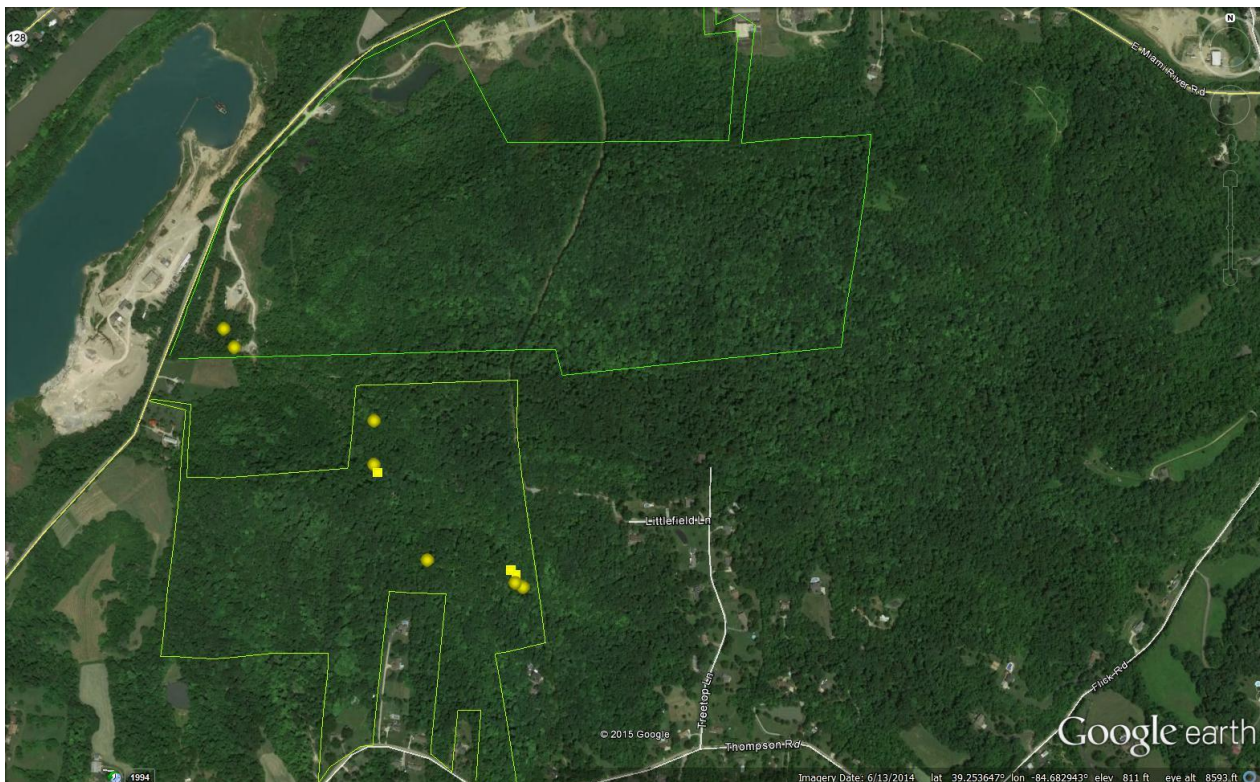


Figure 37. Cave Salamanders are abundant at OGNP, perhaps more so than elsewhere in southwest Ohio. Adults, subadults and one metamorph have been found at the Preserve. Larvae are subterranean and therefore are only seen in caves. These five adults were found under one rock in Stream 1.



Figure 38. Distribution of the Cave Salamander at OGNP. Squares represent records from 2015 and circles from 2014.

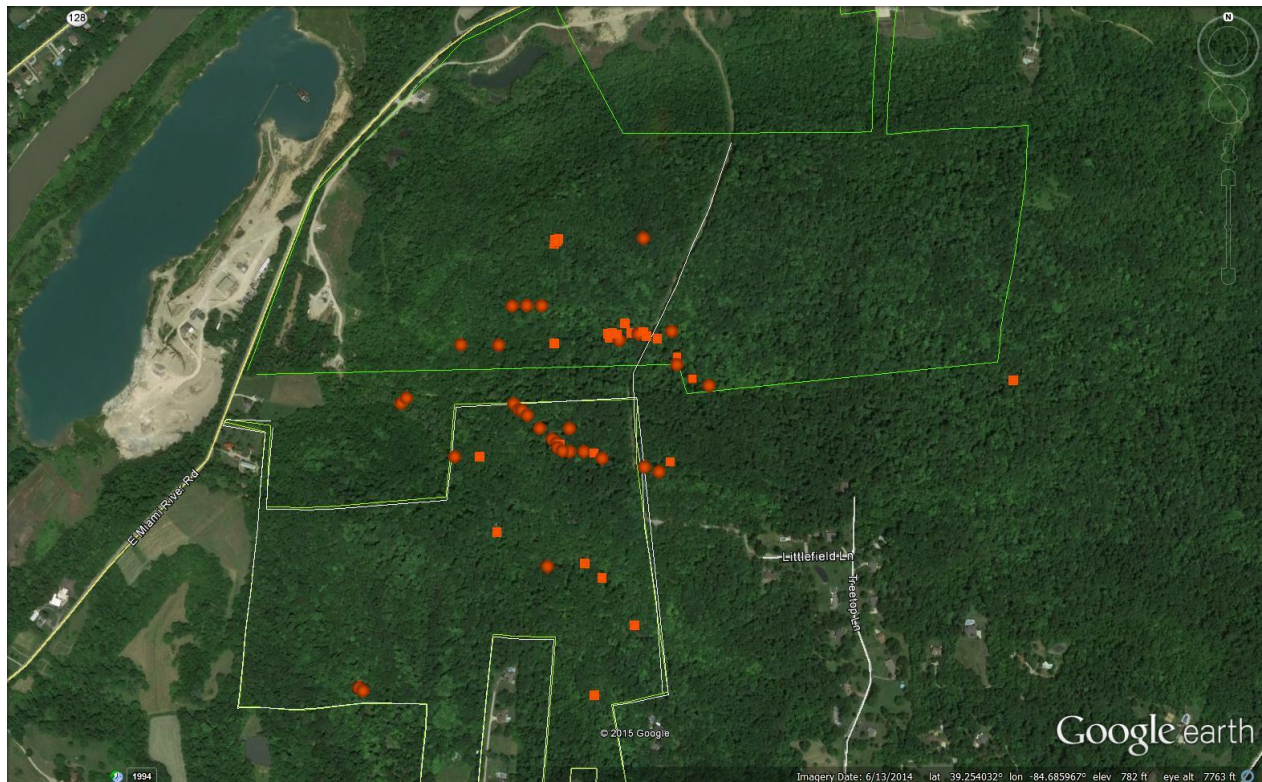


Figure 39. Eastern Red-backed Salamanders are common at OGNP, but like other amphibians there, with the exception of American Toads, the ridgetops are too dry for them. Consequently they are most closely associated with ravines at the Preserve. The Red-backed color morph of this species comprised 100% of the Red-backed Salamanders found at OGNP.



Figure 40. Distribution of the Eastern Red-backed Salamander at OGNP. Squares represent records from 2015 and circles from 2014.

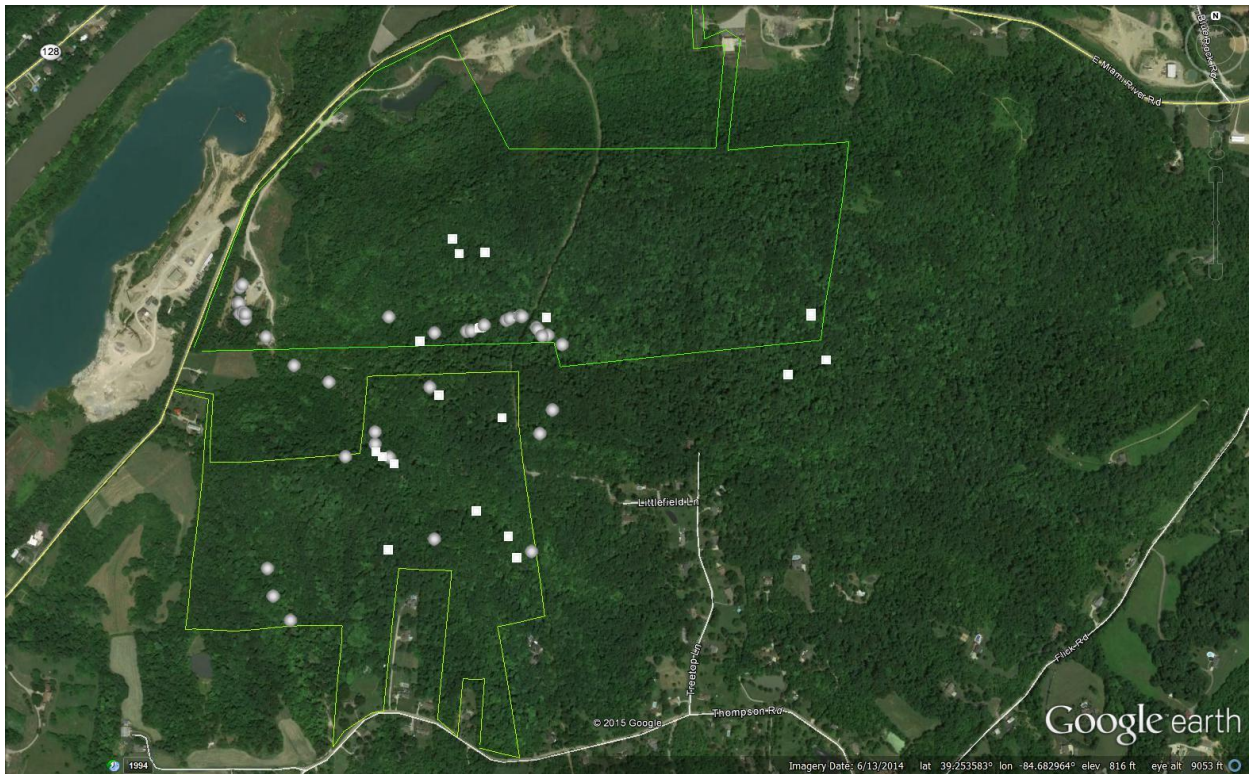
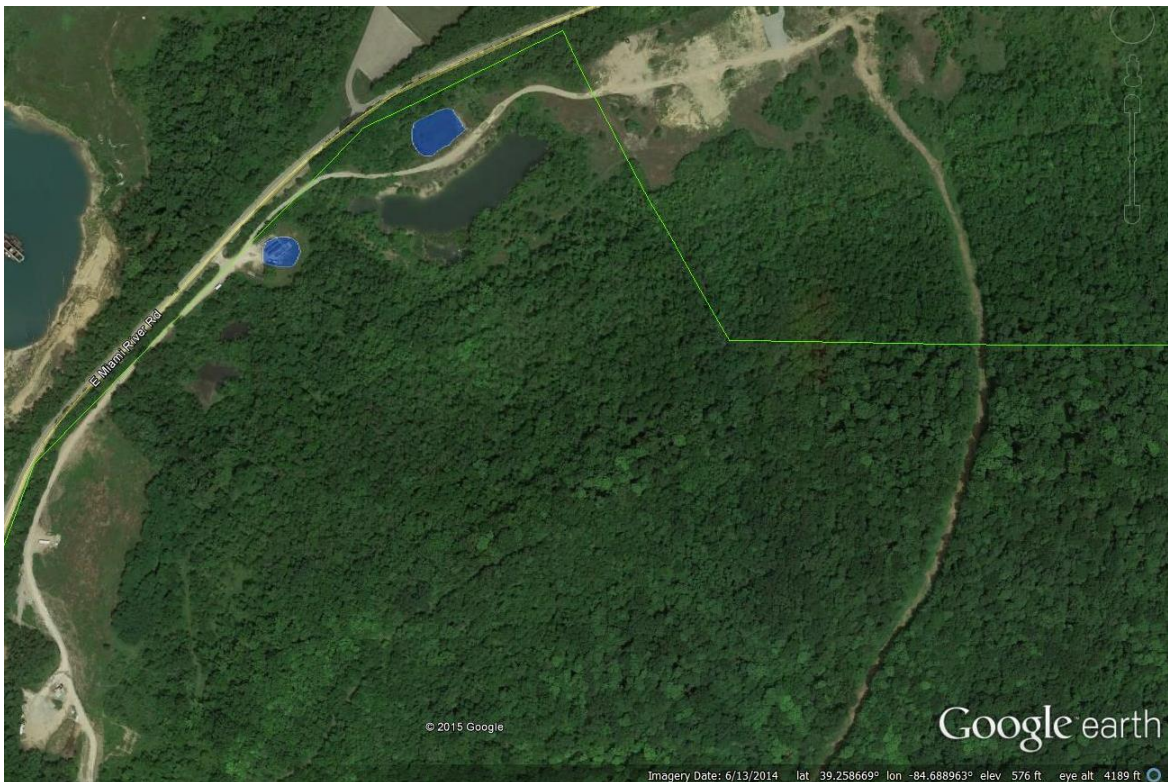


Figure 41. Dried American Toad tadpoles in a dried depression in the road that was filled with water during the breeding season. The sandy, gravelly soils on the terrace at the north end of the preserve offer little opportunity for completion of development among amphibians. This results in an almost predictable loss of annual recruitment for some species. The two piles of dead tadpoles are in the two lowest spots in the puddle. The tadpoles were stranded in them, then became more and more concentrated until the last of the water evaporated or percolated down through the substrate.



Figure 42. Suggested sites for more permanent wetlands at the north end of OGNP to increase the probability of successful recruitment of amphibians into the amphibian populations at that end of the Preserve are marked in blue.



Addendum II

Tables

Table 1. Museum vouchers for six species of salamanders, unisexual *Ambystoma* salamanders, and eight species of frogs and toads collected in Colerain Township (Hamilton Co., Ohio) were found in four museum collections (CMC = Cincinnati Museum Center, KU = University of Kansas, MU= Miami University, OSM = Ohio State University). Records for OGNP were observed during the 2014 and 2015 amphibian surveys. Only one Colerain Township inhabitant, the Long-tailed Salamander was not found at OGNP. It does not occur sympatrically with Cave Salamanders.

Species	Colerain Township	OGNP	Museum
Streamside Salamander <i>Ambystoma barbouri</i>	✓	✓	CMC, KU, MU, OSM
*Jefferson Salamander <i>Ambystoma jeffersonianum</i>	✓	✓	CMC, KU,
*Unisexual <i>Ambystoma</i> salamander <i>Ambystoma biotypes</i>	✓	✓	OSM
Southern Two-lined Salamander <i>Eurycea cirrigera</i>	✓	✓	CMC, KU, MU, OSM
Long-tailed Salamander <i>Eurycea longicauda</i>	✓		KU
Cave Salamander <i>Eurycea lucifuga</i>	✓	✓	CMC, KU,
Eastern Red-backed Salamander <i>Plethodon cinereus</i>	✓	✓	CMC, KU, OSM
American Toad <i>Anaxyrus americanus</i>	✓	✓	CMC, KU
Fowler's Toad <i>Anaxyrus fowleri</i>	✓	✓	CMC, KU
Blanchard's Cricket Frog <i>Acris blanchardi</i>	✓	✓	CMC, KU, OSM
Cope's Gray Treefrog <i>Hyla chrysoscelis</i>	✓	✓	CMC, KU
Spring Peeper <i>Pseudacris crucifer</i>	✓	✓	CMC
American Bullfrog <i>Lithobates catesbeianus</i>	✓	✓	CMC, KU, OSM
Green Frog <i>Lithobates clamitans</i>	✓	✓	CMC, KU
Northern Leopard Frog <i>Lithobates pipiens</i>	✓	✓	CMC, KU

*Confirmed by mitochondrial DNA analysis.

Table 2. Five salamander species, unisexual *Ambystoma* salamanders, and eight frog and toad species were documented from six habitat types at Oak Glen Nature Preserve during the 2015 amphibian survey. Wooded ravines and their associated ephemeral streams were inhabited most frequently. Wetlands were used by American Bullfrogs and Green Frogs all year, but only as breeding sites for other species found in them.

Species	Mature, upland forest	Wooded ravines & ephemeral streams	Bottom-land forest	Wetlands	Flooded depressions & ditches	Seeps	Sandy Terrace
Streamside Salamander <i>Ambystoma barbouri</i>		✓	✓	✓			
Jefferson Salamander <i>Ambystoma jeffersonianum</i>			✓	✓			
Unisexual <i>Ambystoma</i> salamander <i>Ambystoma biotypes</i>			✓	✓			
Southern Two-lined Salamander <i>Eurycea cirrigera</i>		✓				✓	
Cave Salamander <i>Eurycea lucifuga</i>		✓					
Eastern Red-backed Salamander <i>Plethodon cinereus</i>	✓	✓				✓	
American Toad <i>Anaxyrus americanus</i>	✓	✓	✓	✓			✓
Fowler's Toad <i>Anaxyrus fowleri</i>							✓
Blanchard's Cricket Frog <i>Acris blanchardi</i>		✓		✓	✓		
Cope's Gray Treefrog <i>Hyla chrysoscelis</i>	✓	✓	✓	✓	✓		
Spring Peeper <i>Pseudacris crucifer</i>	✓	✓	✓	✓			
American Bullfrog <i>Lithobates catesbeianus</i>				✓	✓		
Green Frog <i>Lithobates clamitans</i>		✓		✓	✓		
Northern Leopard Frog <i>Lithobates pipiens</i>				✓	✓		✓

Table 3. Breeding sites used by amphibian species at Oak Glen Nature Preserve. Check marks indicate that breeding amphibians (including chorusing frogs) were documented at a respective breeding site. Check marks followed by a plus symbol indicate that larvae/tadpoles emerged from or were observed very near completion of metamorphosis at a respective breeding site. The check marks of some species are followed by minus symbol to indicate that reproduction occurred in 2014 and 2015 but the site dried completely stranding eggs and/or tadpoles causing losses of reproductive gain on a regular basis. Some sites might be used but no evidence of reproduction or metamorphosis was observed in 2014 or 2015. The left column does not include species that were not observed breeding. Jefferson Salamanders were not identified using mitochondrial DNA in the oiled wetland in 2014. Reproduction occurred in the oiled wetland in 2015 but tissue samples were not taken from salamanders using that wetland. It remains unknown whether or not that species uses the oiled wetland.

	Stream 3	Stream 5	Oiled Wetland	Gravel Quarry backwater	Small Wetland North	Small Wetland South	Water Retention Pond	Flooded Depression (parking lot)	Constructed Wetland	Flooded depressions in gravel road near Gravel Quarry
Cricket Frog			✓	✓	✓	✓	✓ +	✓	✓	✓ -
Spring Peeper			✓		✓	✓	✓ +	✓	✓	
Cope's Gray Treefrog			✓	✓	✓	✓	✓ +		✓ +	✓ -
American Toad				✓	✓	✓	✓ +	✓	✓ +	✓ -
Fowler's Toad				✓						
Bullfrog			✓	✓						
Green Frog			✓	✓	✓	✓	✓		✓	
No. Leopard Frog									✓	
Streamside Salamander	✓ +	✓ +	✓ +		✓	✓				
Jefferson Salamander			?		✓	✓				
Unisexual <i>Ambystoma</i>			✓ +		✓	✓				
So. Two-lined Salamanders		✓ +								

Table 4. Preliminary list of potential Cave Salamander prey species found under 12 inch² ceramic tiles placed near outcrops in Streams 2, 3, and 5. Taxa marked with an asterisk have been reported in the literature as confirmed Cave Salamander prey species (Davis, 2013).

ARTHROPODA Insecta	*Ants *Beetles Field Crickets *Cave Crickets *Springtails *Thrips Wood Roaches
Diplopoda	*Millipedes
Chilopoda	*Centipedes
Arachnida Opiliones Araneae	*Harvestmen *Spiders
Crustacea	*Isopods
MOLLUSCA Gastropoda	*Snails Slugs
ANNELIDA Oligochaeta	*Earthworms
CHORDATA Amphibia	*Red-backed Salamanders (juveniles)

Table 5. Cave Salamanders were discovered in five streams (and their tributaries between March 15 and June 3, 2015. Preliminary data are presented herein. The “New Stream” is near the southeast corner of the Northern Tract of OGNP (see Figure 38).

Stream	Number/s Observed	Dates Observed
1	8	April 27 May 5
2	14	May 12, 22, 27 June 3
3	3	May 27 June 3
5	5	May 4, 5, 21, 27 June 3
New Stream	1	May 22