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Wetland Ecology Group
Division of Surface Water

FIELD MANUAL FOR THE AMPHIBIAN INDEX OF BIOTIC INTEGRITY FOR WETLANDS

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Field Manual for the Amphibian Index of Biotic Integrity (AmphIBI) for Wetlands.

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ABSTRACT

A field manual has been developed documenting application, sampling, laboratory, and data analysis procedures necessary to calculate the Amphibian Index of Biotic Integrity for Wetlands. It is intended to be used to standardize monitoring techniques for the use of wetland biological assessments using amphibians as an indicator taxa. The methods outlined here can also be used in other situations including monitoring mitigation wetlands or for more general amphibian community characterization. This manual documents methods used in the Ohio Environmental Protection Agency's wetland program. In the years 1996-2010 Ohio EPA has sampled the amphibian communities of over 200 wetlands including targeted reference wetlands, wetlands randomly selected for watershed and land use assessment studies, mitigation bank wetlands, and individual mitigation wetlands. The most typical application of the method employs 10 traps placed evenly around the perimeter of a wetland for a 24 hour period, three times during the amphibian breeding season. For very large wetlands a variation using 10 traps placed equal distances along a transect line is described. Finally, steps for collection and analyzing the data collected are outlined.

INTRODUCTION

This field manual documents the protocols for sampling, laboratory, and data analysis necessary to calculate the Amphibian Index of Biotic Integrity (AmphIBI) for wetlands (Micacchion 2004). It is intended to be used to standardize and document amphibian sampling techniques for the development and use of wetland biological assessments using pond-breeding amphibians as an indicator taxa group. The methods outlined here can also be used in other situations including monitoring of wetlands constructed for compensatory mitigation or for more general amphibian community characterizations.

Ohio EPA began sampling the amphibian communities of Ohio's wetlands in 1996. Natural wetlands that spanned the range of human disturbance levels, from least impacted to severely degraded were selected to provide data from across the entire gradient. Major concerns in selecting a sampling method were ease of use, cost, and reproducibility of results. From the beginning we had been told by several Ohio amphibian experts that using activity traps, specifically screen mesh funnel traps, could provide a good sample of wetland amphibian communities. While we did experiment with other methods it did not take long to determine that using funnel traps was the best way to document the amphibian communities using wetland pools for breeding. Over time we found that the funnel traps were also the best method for sampling the wetland macroinvertebrate community and we were able to monitor the two taxa groups using the same samples.

We have been able to monitor a large number of sites, (20 to 33), distributed throughout an entire ecoregion within a single breeding season. We have found monitoring the amphibian communities of wetlands to be an effective way to determine their ecological condition. The AmphIBI is a reliable wetland assessment tool that is relatively easy to use and provides valuable information often not detected by other assessment methods.

APPROPRIATE TYPES OF WETLANDS FOR AMPHIBI MONITORING

The Amphibian Index of Biotic Integrity (AmphIBI) is designed to be used on a subset of Ohio's wetlands. It should be used to monitor those wetlands that have the features to provide appropriate habitat for pond-breeding amphibian species. Pond-breeding amphibian species have some specific habitat needs that target the types of wetlands they will utilize for breeding. These organisms are adapted to landscapes that are primarily forested and since Ohio was, at the time of settlement, 95 percent forested, this is the natural setting for most of these wetlands. Additionally, pond-breeding amphibians are found in wetland pools that most often have ephemeral hydrology, are free of predatory fish, exist in close proximity to appropriate upland habitats and are near to other pools used by amphibians for breeding (Semlitsch 2000). The most common types of wetlands meeting these criteria are forested and shrub depressions or what are often known as vernal pools. While this type of wetland is by far the most common utilized, many other wetland types are also appropriate breeding sites for these amphibians. In general the characteristics of the type of wetland acceptable for pond-breeding amphibians include:

Seasonal Hydrology

Pools need to maintain inundation, at a minimum, from mid March to late May or early June. Ephemeral wetlands show a great diversity in the average amount of time they remain inundated. It can be as little as two weeks to greater than nine months. Additionally, periods of inundation are influenced by the amount and timing of rain events and air and soil temperatures and can vary greatly by year. Even some permanently inundated wetlands can provide excellent habitat for amphibians if they are, relatively shallow, free of predatory fish and do dry down completely in some years. The shared attribute of these wetlands is that at some point they will not hold water for at least a period of time.

Pond-breeding amphibian species also vary significantly in the amount of time they require to develop from egg to young adults that can leave the pools. Some Anuran species can complete this cycle in less than two months. On the other extreme are species like the Eastern Tiger Salamander, *Ambystoma tigrinum* that require at least four months and prefer to develop over a longer period, four to six months, if their breeding pools will remain inundated throughout that time frame.

Precipitation or Ground Water Hydrology

The pools that pond-breeding amphibians utilize the majority of the time are those that are principally driven by rain water or ground water or a combination of the two. Precipitation driven pools do not require a large watershed to fill up and maintain water for the duration necessary for successful amphibian breeding. Often times the watershed is only slightly larger than the pool itself. When there are local ground water resources near the soil surface ground water can also be involved in a pool's hydroperiod, adding to the inundation when ground water levels are high and allowing percolation and recharge when the water table is low. Pools that are primarily ground water driven can require longer periods for metamorphosis due to the slower maturation rates associated with the cold water temperatures. These two hydrology sources provide pools that are generally seasonal and therefore provide the other habitat features and house other pool inhabitants for which pond-breeding amphibians are adapted to interact.

Absence of Predatory Fish Species

Pools should be absent of the fish species that are predacious on adult amphibians and their larvae (Kats et al. 1988). The fish found in wetlands that are most limiting to amphibians are in the families Centrarchidae; most notably Green Sunfish, *Lepomis cyanellus*, Bluegill Sunfish, *Lepomis macrochirus*, several other sunfish species and their hybrids and Largemouth Bass, *Micropterus salmoides*, and Esocidae; most often Grass Pickerel, *Esox americanus* but also occasionally

Chain Pickerel, *Esox niger* and Northern Pike, *Esox lucius*. For wetlands to provide habitat for predaceous fish species they must be associated with a stream. A wetland shares stream hydrology either because the stream feeds or drains the wetland or the wetland is located in the flood plain where it receives periodic overflows from a stream. Some wetlands, especially flood plain depressions, can have predatory fish and still be good pond-breeding amphibian sites. These would be the type of pools that might fill with stream flood waters initially and then revert back to a largely precipitation driven hydrology. In these instances, the numbers of predatory fish in the pools are relatively low, and although they undoubtedly prey on some amphibian larvae, they do not have a significant effect on the number of amphibians able to complete metamorphosis. Some fish species can even be present in large numbers in vernal pool habitats and be of no consequence to the amphibians present. These fish species include Central Mudminnow, *Umbra limi* and Brook Stickleback, *Culaea inconstans*. Stream hydrology sources and extended periods of inundation can also introduce a group of invertebrate predators that can be limiting on pond-breeding amphibians.

Close Proximity to Appropriate Forested Upland Habitats

Amphibians, as their name suggests, have a dual life form. Most Ohio pond-breeding adult amphibians are adapted to,and dependent upon, having a significant percentage of the habitat surrounding their breeding pools dominated by forest plant communities. Pond-breeding amphibian species will not travel far from their preferred upland habitat to breeding pools (Semlitsch 1998). For many species the suitable upland forested habitat needs to be within a 200 meter radius or closer to a breeding pool. Without the correct surrounding habitat a wetland pool is severely limited in its ability to support diverse communities of pond-breeding amphibians.

Close Proximity to Other Breeding Pools

Pond-breeding amphibian species are also adapted to a landscape comprised of numerous wetland breeding pools (Semlitsch 2000). The presence of many pools connected by forested terrestrial habitat promotes amphibian metapopulation dynamics. This results in increased breeding success by avoiding the proverbial "all the eggs in one basket" scenario. Further, it allows for exchange of genetic material as some percent of adults will migrate to adjacent pools and interbreed with those populations. Multiple pools of varying hydroperiods increase the likelihood that at least some of the pools will stay inundated to the end of the amphibian breeding cycle even in dry years and can guard against the complete lost of a breeding effort.

In conclusion, the wetlands that are most appropriate for monitoring using the AmphIBI, and where correlations between wetland condition and AmphIBI scores can be expected, are those pools that are in forested habitats and are forested and shrub depressions, are driven by precipitation and/or groundwater sources and therefore have an ephemeral hydroperiod, and lack predacious fish and other predators associated with semi-permanent to permanent periods of inundation (Porej et al. 2004). While adjacency of forested habitat and other nearby breeding pools is a characteristic of high quality amphibian breeding sites they are not necessary components of those wetlands for which the AmphIBI can be an effective monitoring tool to determine condition.

There are some wetlands that may not fully conform to the criteria described above yet still provide viable habitat for pond-breeding amphibians. Some wetlands that might fall into this group are flood plain depressions, generally those located on the higher elevations or upland edges, depressions in rock outcrops, and headwater stream depressions, to name a few. Knowledge of the habitat needs and tolerances of pond-breeding amphibians must be given full consideration when determining if the AmphIBI is an appropriate monitoring tool for any given wetland.

MONITORING PROTOCOLS

Trap Construction

For the purposes of AmphIBI monitoring, funnel traps should be constructed of aluminum window screen cylinders with fiberglass window screen funnels as each end. These funnel traps are similar in design to commercially available minnow traps. However, the smaller mesh provided by using window screen makes the traps better able to collect and hold a wide range of sizes of larval and adult amphibians as well as a diversity of invertebrates when these taxa are also of interest in evaluation. Aluminum is used for the cylinders to provide maximum structure and fiberglass screening is used for the funnel ends to provide flexibility and ease of funnel inversion and eversion.

The aluminum screen cylinders should be 45 cm (18") long and 20.3cm (8") in diameter. To make the cylinders a piece of aluminum screen 45 cm (18") by 71.1 cm (28") is rolled, and then the two ends are pinched together to form cylinder with a 2.5 cm (1") lip. The lip is then folded over once resulting an 1.25 cm (0.5") fold and generously secured along its length with wire office staples, resulting in a cylinder shape. Figure 1 is a representation of a funnel trap with measurements and construction instructions. Figure 2 is a graphic of a funnel end with measurements. PDFs of the funnel trap construction instructions and a template for the funnel ends (which is to scale, if printed on 11" by 17" paper) can be found Ohio EPA's Wetland Ecology Group Reports webpage: http://www.epa.ohio.gov/dsw/wetlands/WetlandEcologySection_reports.aspx

The template should be used to provide the outline for the funnel ends. Once cut out of the fiberglass window screen both pieces should be rolled into a funnel shape with the ends overlapping by about 1.25cm (0.5"). The area of overlap should be stapled along its length yielding two self sustaining funnels. The bases of the two fiberglass screen funnel ends will be

20.3cm (8”) in diameter and are attached, with wire staples, along the entire perimeters, to both ends of the aluminum screen cylinder such that the funnels direct inward. Each funnel end, if made using the template, will have a circular opening in the middle that is 4.5 cm (1.75”) in diameter that serves as the means of entry into the trap.

The lip on the top of the cylinder of the trap should have a small hole established near each end. A 92 to 100 cm (36 to 39.4”) piece of #36 tarred nylon seine twine should be measured and cut. The ends of the twine should be passed through the holes in the lip and tied in knots. The twine will then provide a workable handle that will tolerate water and weather well. Since ten traps are generally used per site the twine handles of ten traps can be held together with a carabiner to provide a handy means for storage and for carrying the traps into and out of the field.

Trap Placement

In order to capture the diversity and relative abundances of amphibians present in wetlands monitoring should occur three times during the amphibian breeding season. Each pass should be separated from each other by about a six week period. Adult salamanders enter wetlands to breed following the first few warm, rainy nights of late winter to early spring. The actual timing of their arrival is highly weather dependent and varies greatly by year. The occurrence of amphibian breeding runs can also vary greatly from south to north within the state, with southern populations breeding up to several weeks before northern populations. Ideally, weather patterns should be closely followed with monitoring beginning when temperatures and precipitation appear optimal. It is preferable to get out too early and set traps with no captures than to miss the breeding run altogether. If necessary, set traps on each day that seems appropriate until captures occur and use this data for the first pass.

However, this approach is not practicable when monitoring a large number of sites. In those instances average dates of the beginning of amphibian breeding, for that region, should be used and serve as the target for scheduling the start of that year’s monitoring efforts with adjustments made in accordance with the weather and amphibian breeding behaviors that year. The first sampling pass should coincide with the breeding runs of ambystomatid salamanders, red-spotted newts and early breeding frog species, generally in March to early April, depending on which part to the state is being monitored. In some years, when periods of warm weather occur earlier, these breeding runs can begin in February or rarely even earlier.

Data indicate the key to ambystomatid breeding runs is the difference in soil temperatures at the surface and at 30 cm (12”). Ambystomatid salamander breeding runs correspond to when temperatures at the soil surface are warmer than those at 30 cm (12”) below the soil surface and almost always coincide with rain events (Sexton et al 1990). Trapping at these times can yield large numbers of adult salamanders and frogs, therefore traps should not be left for any longer than 24 hours to guard against mortality and to have as little affect on breeding as possible. These adults should be identified in the field and released. Measurements and sex determinations of adults can be made at this time if the survey is part of a larger study but this is not necessary for calculation of an AmphIBI score.

A middle sampling run, five to six weeks after the first run, and generally in late April to early May, is conducted in order to collect some adult frog species breeding within the wetlands at that time and to sample the larvae of early-breeding amphibians. A late spring/early summer sampling, generally in early June to early July, is performed to collect relatively well developed amphibian larvae prior to or corresponding with metamorphosis. It is critical in the timing of the last run to again monitor the weather closely. In a dry spring it may be important to deploy traps for the final pass earlier than would be indicated by using the five to six week window between trapping efforts. Pools can refill after initial dry down so it is important to assure this is not the case with a pool being monitored in the third pass. Not monitoring a pool prior to dry down can result in loss of important data and will skew the final AmphIBI score.

In the typical application, ten funnel traps are placed evenly around the perimeter of the wetland. This is achieved by first pacing around the wetland perimeter to provide a measure of the total wetland perimeter distance (with practice pacing can be a highly reliable measuring technique). The perimeter total is then divided by ten and a trap is placed each time that distance is paced off while traversing the wetland perimeter for the second time. Alternatively, for large wetlands or where placement around the entire perimeter is not feasible (slopes too steep, water too deep, etc.), transects along one or several sides of the wetland can be used. Care should be taken to assure that all habitat types within the wetland are represented proportionally within the transect or transects. The total length of the transect, or transects, can then be divided by ten with a trap placed an equal distance along them.

Each funnel trap location should be marked with flagging tape both at a spot on or near the perimeter and in vegetation directly above or near to the trap location. Additional, I long piece of flagging should be tied to each the twine handle of each trap. Once traps are set this flagging on the handle should be laid out in such a way that it does not block the funnel ends and provides a ready way of spotting the trap location during retrieval the next day. Since the vegetation flagging is applied prior to the growing season it is important that an attempt be made to place it in locations where it will not be obscured by new vegetation growth when the site is visited during the second and third monitoring events. Flagging can often be tied to tree trunks and branches on the perimeter and on shrubs or emergent plants at the trap site. It may be necessary to use artificial structures such as, PVC pipe or wooden stakes, as flagging locations when there are no natural occurring locations that will provide adequate flag visibility. The flagging, at both the wetland perimeter and above the trap, is marked sequentially using a permanent market (i.e. Sharpie) and traps are set at the same locations throughout the sampling season. Putting numbers on the flagging serves as an aid to navigation, both during deployment and retrieval, especially in heavily vegetated sites such as mixed shrub swamps, buttonbush swamps and any other wetlands with areas of dense, tall plant growth. Checking the numbers also serves as further confirmation that all traps are accounted for and placed in the same location each sampling pass. Retrieving all traps is important because, as well as eliminating essential data, traps left in the field can result in high levels of mortality to wetland organisms.

The traps are placed on the substrates of the wetland and the trap is almost completely submersed in the pool water. However, it is important to allow some exposure of air into the upper part of the cylinder. This protocol works to reduce trap mortality by allowing, those organisms that need it, access to fresh air. Placement to allow organisms access to atmospheric oxygen becomes more important as the season progresses, water temperatures rise and oxygen levels in the water decrease.

Once the appropriate depth for trap placement has been reached by walking from the perimeter flagging directly into the pool traps can be placed qualitatively. Trap placement should be chosen to maximize the probability of capturing amphibians given the habitat features present at the location. The habitat structure should be observed and the trap set with the funnel ends aligned to encounter the most likely amphibian travel patterns. In no circumstances should a trap be placed in such a manner that one or both funnel end openings are blocked by being in close proximity to large solid features such as hummocks, woody debris, including downed trees and branches, leaf packs or any other obstructions to amphibian movement through the water column.

In some circumstances, especially when winds are high, or are anticipated to be high during the upcoming 24 hour period, it may be necessary to secure the traps to the substrates or adjacent vegetation as they are light and can at times be moved by wind and the resulting water currents. Traps can be secured to the substrates by utilizing available fallen tree limbs on site or by using stakes made from wood or metal. A limb or stake can be pushed into the substrates, upwind of the trap, until firm. The trap twine handle can then be placed over the stake to hold the trap in place. This method can also be used to keep traps from rolling down slope into deeper water and becoming fully submersed. Displacement down slope can occur when large numbers of adult amphibians are in the traps and moving about. Securing traps during the first pass when large amphibian breeding runs are expected can be a good practice. Also, if large amounts of rain are expected over the period the traps will be deployed, their depth of placement in the water should be adjusted accordingly.

When needed, because the overall pool depth is greatly reduced, due to a dry spring season, traps can be placed in shallower water as long as the entirety of the funnel openings remain submersed during the 24 hour sampling period. In all cases, the traps are left in the wetland for 24 hours in order to ensure unbiased sampling for species with diurnal and nocturnal activity patterns. Limiting trapping time to 24 hours also works to minimize the potential for mortality due to individuals being in the traps for extended periods.

No bait is used in the traps. They are activity traps and designed to collect any organisms that swim, crawl or float into the funnel openings. Due to the shape of the inverted funnel ends, once an individual organism is inside a trap, it is difficult to impossible for it to make its way back out. Since the traps are very similar in design to minnow traps they also are effective in capturing fish. So in addition to amphibians, information on the fish taxa trapped is also recorded. The taxa of fish present are often valuable in explaining trends in the amphibian communities and may themselves be indicators of wetland condition or type.

Trap Retrieval

Traps should remain in the water until they can be processed immediately. Where needed, numbers on the flagging should be checked to assure all traps are removed and in sequence. After retrieving the traps from the water, and while transporting them to the processing area, they should be held level without dipping the ends so specimens remain on the bottom of the trap and are not lost through the funnel end openings. The traps are emptied by everting one of the funnel ends and lightly shaking the contents into a white plastic collection and sorting tray. We have found that the trays that come with many coolers work well as sorting trays. Some amphibians, especially small larvae may be stuck to the trap or funnel walls or in the crease between the two. Close observations should be made to assure all trap organisms are removed before advancing to the next trap. Individual organisms that will not shake out readily may need to be manually removed using fingers and/or forceps. When all remaining organisms are to be preserved the trap and funnel walls can be sprayed with ethanol from a squeeze bottle to wash the “hangers on” out of the trap. A squeeze bottle filled with water can be used to dislodge organisms that will be released. Sometimes a combination of spraying and shaking may be needed to free some specimens from the traps.

Specimen Handling and Identification

Organisms that can be readily identified in the field (especially adult amphibians and larger and easily identified fish) are counted and recorded in a field notebook or on a field form and released in the area where they were captured. The remaining organisms are transferred to wide mouth one liter plastic bottles by washing them out of the collection and sorting tray into the bottles using a plastic squeeze bottle filled with 95% ethanol. The content of each trap is kept separately in a marked wide mouth plastic bottle for individual analysis in the laboratory. The collection tray is then thoroughly rinsed with water from the wetland to remove any trace of alcohol that might adversely affect amphibians to be released into it from the next trap collection.

Before leaving the field, if needed, generally at the field vehicle, the specimen bottles are supplemented with additional 95% ethanol in proportion to the number of individuals collected. Enough ethanol should be added to keep all specimens fully submersed with a little extra added to insure specimens will remain below the liquid surface. Caution should be taken to make sure all lids are secured tightly to eliminate the potential for evaporation of the ethanol. From that point forward the bottles must remain upright to keep all specimens immersed in the ethanol. Amphibians preserved in alcohol retain their identification features for many years after capture. Unlike formalin, preserving specimens in ethanol will not greatly deteriorate the animals' DNA and will allow the opportunity for their later use in genetic studies.

Salamanders and their larvae are identified using keys in Pfingsten and Downs 1989 and Petranka 1998. Frogs, toads and tadpoles are identified using keys in Walker 1946. Any fish samples should be identified using the keys in Trautman 1981.

EQUIPMENT AND SUPPLIES

In order to monitor a wetland using the methods outlined in this manual, the following equipment will be needed:

Waterproof notebook
Waterproof pen
Compass
GPS Unit
10X hand lens
Flagging Tape (flourescent pink recommended)
Funnel Traps (10 per site)
White collection and sorting pan
Squeeze bottles
One liter wide mouth plastic bottles – one per trap
Four oz. wide mouth glass jars – one per trap for long term storage in lab
Field forceps
Heavy duty plastic bags to carry plastic bottles – 10 bottles/bag/by site
2" masking tape for labeling bottles
Fine point permanent marker (Sharpie)
One gallon cubitainer filled with 95% ethanol as a preservative in the field
Duffle bag to carry equipment and bottles in the field
Amphibian keys
Water bottles
Emergency medical kit
Camera with macro capabilities
Chest waders

CALCULATION OF AMPHIBIAN INDEX OF BIOTIC INTEGRITY SCORES

AmphIBI scores are comprised of the total of five individual metrics added together. Those metrics are the Amphibian Quality Assessment Index, the relative abundance of sensitive species, the relative abundance of tolerant species, the number of pond-breeding salamander species, and the presence of Spotted Salamanders and/or Wood Frogs (Micacchion 2004).

A main component of the composition of three of five metrics is the use of amphibian coefficients of conservatism. The coefficients of conservatism are assigned based on a species' sensitivity or tolerance to environmental stressors and whether it has broad or narrow habitat needs. Coefficient of conservatism scores for amphibians range from one to ten. Lower scores are assigned to those species that can tolerate high levels of environmental stress and/or have acceptance of a broad range of habitats. Higher scores are assigned to those species that will not tolerate much stress and/or have specific habitat needs.

A group of amphibian experts has assigned coefficients of conservatism to all Ohio amphibian species. The group was comprised of Robert D. Davic, Jeffrey G. Davis, Ralph A. Pfingsten, Gregory J. Lipps and I. Each Ohio amphibian species was considered and through the consensus of the group a coefficient of conservatism was assigned. There are few differences in the coefficients of conservatism for pond-breeding amphibians from those in Micacchion 2002 and Micacchion 2004. This new group of coefficients of conservatism should be applied for calculating AmphIBI scores. Use of these coefficients of conservatism provides equally strong correlations with human disturbance levels. The list of amphibian species, their common and scientific names, their assigned coefficient of conservatism score, as well as some notes about their ecology, appears in Table 3.

Amphibian Quality Assessment Index

The Amphibian Quality Assessment Index (AQAI) is basically an average coefficient of conservatism score for all individuals collected. The number of individuals of each species is multiplied by that species C of C score. Those results are then summed and divided by the total number of individuals of all species collected. The resulting number is the AQAI. Table 1 provides information on how to assign the 0, 3, 7 or 10 metric score for the AQAI.

Relative Abundance of Sensitive Species

Sensitive species are those that have been assigned coefficient of conservatism scores of 6 to 10. This metric is calculated by dividing the number of individuals with coefficient of conservatism scores of 6 to 10 by the total number of individuals collected and yields a percentage measurement. Table 1 provides information on matching relative abundance percentages for sensitive species with the appropriate metric score.

Relative Abundance of Tolerant Species

Tolerant species are those that have been assigned coefficient of conservatism scores of 1 to 3. This metric is calculated by dividing the number of individuals with coefficient of conservatism scores of 1 to 3 by the total number of individuals collected and yields a percentage measurement. Table 1 provides information on matching relative abundance percentages for tolerant species with the appropriate metric score.

Number of Pond-Breeding Amphibian Species

The number of species of pond-breeding salamanders collected at each site provides the number to use to determine the score for this metric. Those species which are considered pond-breeding salamanders for this metric are Red-spotted Newt, *Notophthalmus viridescens viridescens*, Four-toed Salamander, *Hemidactylium scutatum*, Streamside salamander, *Ambystoma barbouri*, Jefferson Salamander, *Ambystoma jeffersonianum*, Blue-spotted Salamander, *Ambystoma laterale*, Spotted Salamander, *Ambystoma maculatum*, Marbled Salamander, *Ambystoma opacum*, Small-mouthed Salamander, *Ambystoma texanum*, Eastern Tiger Salamander, *Ambystoma tigrinum* and Unisexual Hybrids, *Ambystoma sp.* Table 1 shows how the number of salamander species relates to the scoring for this metric

Presence of Spotted Salamander or Wood Frog

When either one of these species, Spotted Salamander or Wood Frog, *Lithobates sylvaticus* is present ten points are added to the AmphIBI score for the wetland. No additional points, beyond the first ten, are added if both species are present.

AmphIBI Score

AmphIBI scores can be calculated as described above and in Micacchion 2004. Table 1 below provides the breakpoints for scoring the five AmphIBI metrics. Table 2 provides information on how AmphIBI scores relate to wetland categories and proposed aquatic life uses.

Automated Spreadsheet for Calculation of Metrics and AmphIBI Scores

To make the process easier an Excel spreadsheet has been developed that allows for calculation of AmphIBI scores from the raw amphibian data collected and aggregated from the three sampling passes during the breeding season. This spreadsheet uses the new set of coefficients of conservatism. If the data for each species is input into the appropriate cell in the spreadsheet (highlighted), metric scores and an AmphIBI score will be automatically calculated for each site. Copying the worksheet and pasting it to additional worksheets will allow for generation of AmphIBI scores for multiple sites. The template for this spreadsheet can be accessed on the Ohio Environmental Protection Agency, Division of Surface Water, Wetland Ecology Group Reports webpage: http://www.epa.ohio.gov/dsw/wetlands/WetlandEcologySection_reports.aspx

USING THE AMPHIBIAN INDEX OF BIOTIC INTEGRITY AS A PERFORMANCE STANDARD FOR DETERMING WETLAND CONDITION

The AmphIBI can be used as performance goal for wetland restoration (re-establishment) or enhancement (rehabilitation). When wetlands are constructed or modified, and they are the types of wetlands that meet the monitoring criteria for use of the AmphIBI, this index can do an excellent job of measuring the success of the restoration or enhancement efforts.

For most restorations a goal is set that reflects that, at least, good ecologic condition based on the AmphIBI score that has been met. This goal can be assured by using an AmphIBI score of 20 as the performance standard. Table 2 shows that an AmphIBI score of 20 is the minimum necessary to place a wetland within the good range of ecological condition and also the minimum score required to meet the proposed Wetland Habitat aquatic life use. From a regulatory standpoint this places the wetland in the middle of category 2 and reflects that the project has been successful.

For enhancement, we would require a baseline AmphIBI assessment occur prior to the enhancement activities being implemented. We would then set the performance standard for the enhancement project as an AmphIBI score of 20, or an AmphIBI score that is ten points higher than the baseline AmphIBI score, whichever is the larger number. The minimum score assures the wetland is of, at least, good ecological condition and the maximum score assures that enough ecological lift has occurred to consider the project successful.

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Table 1. Scoring breakpoints for assigning metric scores for AmphIBI.

Metric	Score O	Score 3	Score 7	Score 10
Amphibian Quality Assessment Index	<3.00	3.00 - 4.49	4.50 - 5.49	≥ 5.5
Relative Abundance of Sensitive Species	0%	0.01-9.99%	10-49.99%	≥50%
Relative Abundance of Tolerant Species	≥80%	50.01-79.99%	25.01-50.00%	<25%
Number of Pond-Breeding Salamander Species	0	1-2	3	>3
Spotted Salamanders and/or Wood Frogs	Absent			Present

Table 2. Aquatic Life Uses, corresponding categories and AmphIBI scores.

Aquatic Life Use	**Category**	**AmphIBI Score**
Limited Wetland Habitat (LWLH)	1	<10
Restorable Wetland Habitat (RWLH)	Modified 2	10-19
Wetland Habitat (WLH)	2	20-29
Superior Wetland Habitat (SWLH)	3	30-50

Table 3. Ohio amphibian species, C of C scores and ecology notes.

Scientific Name	Common Name	C of C	Ecology Notes
Scaphiopus hobrookii	EASTERN SPADEFOOT	10	The Eastern Spadefoot is the only state endangered Anuran species and very few populations are known. It is dependent on sandy floodplains of large rivers where it is nocturnal and spends the bulk of its life underground. This species relies on soft, sandy soils to excavate its burrows. Heavy spring or summer rains trigger breeding that occurs in the resulting ephemeral pools in the floodplains. Metamorphosis is extremely rapid and allows utilization of pools that are, at times, only inundated for a few weeks.
Anaxyrus americanus americanus	EASTERN AMERICAN TOAD	2	The Eastern American Toad is a generalist that occupies a wide range of habitats across the entire state. For breeding it prefers sites with open canopies and warm, sun-drenched vegetated pools. This species is tolerant of polluted waters and most disturbances and can be found in highly urbanized areas.
Anaxyrus fowleri	FOWLER'S TOAD	4	Fowler's Toad is most often found in areas of loose and sandy soils, especially river bottoms, or areas where the land use is predominantly agricultural. It is less tolerant of human disturbances than the closely related Eastern American Toad.
Acris crepitans	NORTHERN CRICKET FROG	7	The Northern Cricket Frog prefers areas of open canopy along the edges of streams, ponds and wetlands where emergent vegetation and algae are abundant. Once common in the northern and central parts of Ohio, few of those populations remain and it is currently only common in southwestern Ohio.
Hyla chrysoscelis	COPE'S GRAY TREEFROG	4	Cope's Gray Treefrog and the identical in appearance, Gray Treefrog, require the presence of trees and shrubs in their habitats, although only a limited number of woody plants are needed. Compared to other pond-breeding frogs it is a late breeder and requires pools that stay inundated into early summer.
Hyla versicolor	GRAY TREEFROG	4	A species that is intermediate in its habitat requirements, the Gray Treefrog requires at least some trees or shrubs to provide suitable habitat. Like Cope's Gray Treefrog it breeds later than most other pond breeders and needs pools with periods of inundation from mid-spring to early summer.

Scientific Name	Common Name	C of C	Ecology Notes
Pseudacris brachyphona	MOUNTAIN CHORUS FROG	5	The Mountain Chorus Frog only occurs in southeastern Ohio and is not found in areas of deforestation and strip mining. It is often in the same breeding pools as the closely related Western Chorus Frog where it can be identified by its raspier song.
Pseudacris crucifer crucifer	SPRING PEEPER	3	The Spring Peeper utilizes a range of habitats for its breeding and non-breeding. The main requirements are pools that remain inundated long enough to allow metamorphosis close to some suitable field or forest cover.
Pseudacris triseriata	WESTERN CHORUS FROG	4	Somewhat selective in habitat use, the Western Chorus Frog is still found in a wide array of environments. Most breeding pools are shallow with dense cover around the borders.
Lithobates catesbeianus	AMERICAN BULLFROG	2	The American Bullfrog is relatively indiscriminate in its habitat selection other than only inhabiting permanent water bodies. This species is found in both ponds and streams in or near deep water areas having ample vegetation or other cover.
Lithobates clamitans melanota	NORTHERN GREEN FROG	1	The Northern Green Frog is likely the most commonly encountered anuran species in the state. It can tolerate high levels of disturbance and other pollution and therefore is found in habitats where other species cannot exist.
Lithobates palustris	PICKEREL FROG	7	Groundwater hydrology is a key element of the habitats where the Pickerel Frog is found as it prefers clear, cold waters. Additionally, this species requires its habitats to be fairly undisturbed.
Lithobates pipiens	NORTHERN LEOPARD FROG	4	While somewhat of a generalist and pioneer, the Northern Leopard Frog's range appears to be shrinking, especially in southeast Ohio where recent sightings are a rarity. This species is an early breeder and will utilize ephemeral pools as well as more permanent water bodies.
Lithobates sphenocephala utricularius	SOUTHERN LEOPARD FROG	8	The Southern Leopard Frog, the southern equivalent of the Northern Leopard Frog, is only known from Scioto County. No breeding populations have been documented in the state.
Lithobates sylvaticus	WOOD FROG	7	The Wood Frog, as it name implies, is a woodland species requiring upwards to a square kilometer of landscape adjoining fish-free, ephemeral pools be predominately forested. Breeding at the pools lasts only a few days in late winter to early spring and it spends the rest of its life in moist adjacent woodlands.

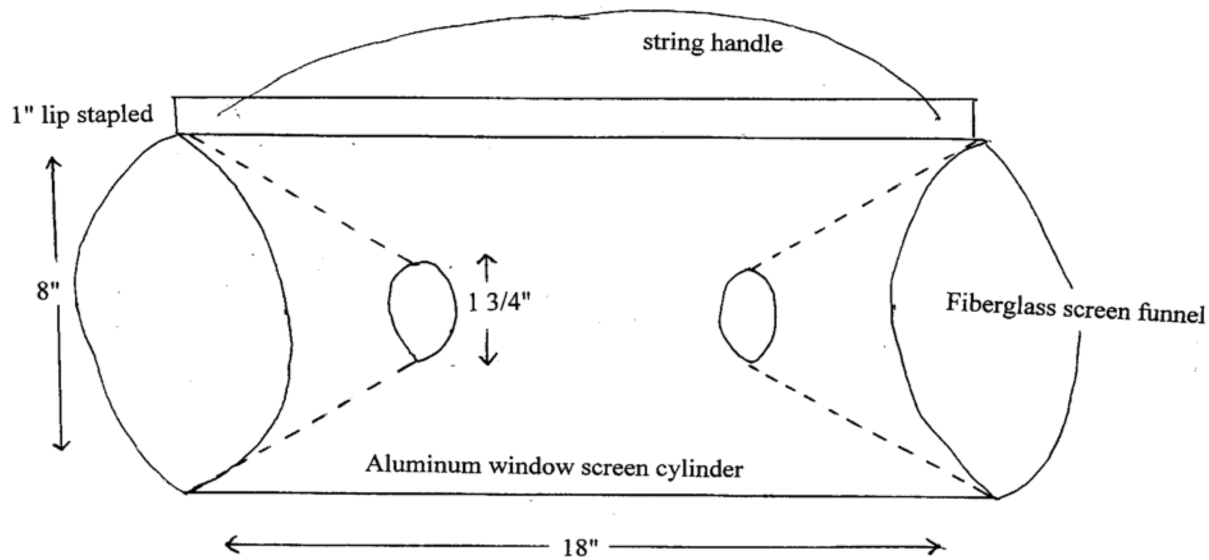
Scientific Name	Common Name	C of C	Ecology Notes
Cryptobranchus alleganiensis alleganiensis	EASTERN HELLBENDER	10	The Eastern Hellbender, North America's largest salamander, has very specific habitat needs. This state endangered species requires streams of clear, swift running, relatively shallow, highly oxygenated water with riffle areas having large rock slabs, logs and other similar cover for feeding, breeding and brooding behaviors.
Necturus maculosus maculosus	COMMON MUDPUPPY	7	The Common Mudpuppy is a large salamander that inhabits permanent water bodies including streams and lakes. It needs large rocks, logs, or other cover for den and nest sites and will not tolerate high levels of pollution.
Notophthalmus viridescens viridescens	RED-SPOTTED NEWT	6	The Red-spotted Newt, Ohio's only newt species, is dependent on highly forested landscapes with scattered pools. Adults and larvae are aquatic, the juvenile stage, Red Eft, is terrestrial. This species is rare in most parts of the state but is common in the Western Allegheny Plateau ecoregion of eastern and southeastern Ohio.
Ambystoma barbouri	STREAMSIDE SALAMANDER	5	The Streamside Salamander is generally limited to breeding in stream habitats free of predacious fish species. This species, until recently, thought to be stream breeding populations of the Small-mouthed Salamander, only occurs in southwest Ohio in headwater stream systems.
Ambystoma jeffersonianum	JEFFERSON SALMANDER	6	The Jefferson Salamander requires relatively intact wooded habitat adjacent to breeding pools with low to moderate levels of disturbance in the surrounding landscapes.
Ambystoma laterale	BLUE-SPOTTED SALAMANDER	10	The Blue-Spotted Salamander is listed as state endangered due to its extremely limited range and can only be found in a few counties in extreme northwest Ohio. This species is much more common in rest of its range which is north of Ohio and prefers habitats with soils having a high percentage of sand.
Ambystoma maculatum	SPOTTED SALAMANDER	8	The vernal pools where the Spotted Salamander breeds need to be in or in close proximity to areas with a high percentage of forested habitats. This is a sensitive species and only occurs where few disturbances to the pools and surrounding landscapes have occurred.

Scientific Name	Common Name	C of C	Ecology Notes
Ambystoma opacum	MARBLED SALAMANDER	8	The Marbled Salamander requires large tracts of mature woods and breeding pools that fill in the late fall to early winter. Because it breeds in the fall and eggs begin to develop in late fall or early winter the larvae become the top predators in their ephemeral breeding pools. This species' range is comprised of isolated populations in all but the southeastern portion of Ohio.
Ambystoma sp.	UNISEXUAL HYBRIDS	5	These all female populations of ambystomatids, known as Unisexual Hybrids, can exist in landscapes that have experienced a moderate amount of human disturbance and contain low amounts of forest cover. These hybrids are dependent on pure breed males of the genus being present at breeding pools to contribute sperm and initiate reproduction.
Ambystoma texanum	SMALL-MOUTHED SALAMANDER	4	The Small-mouthed Salamander is the most ubiquitous of the ambystomatids. This species has a moderate tolerance to many landscape disturbances and the ability to breed successfully in wetlands with shallower depths and shorter hydroperiods than those used by other species in its family.
Ambystoma tigrinum	EASTERN TIGER SALAMANDER	9	The Eastern Tiger Salamander, Ohio's largest land-dwelling salamander, requires seasonal to semi-permanent pools that are free of predacious fish. The extended hydroperiods associated with the deeper breeding pools selected by this species are needed to accommodate the relatively long time larvae need to metamorphose. This species' range is restricted to north central and western Ohio.
Aneides aeneus	GREEN SALAMANDER	10	The Green Salamander is dependent on moist crevices in rock outcrops that are shaded by mature forest and can also be found in trees. It only occurs in extreme south central Ohio at the northern extent of its range and is a state endangered species.
Desmognathus fuscus	NORTHERN DUSKY SALAMANDER	4	The Northern Dusky Salamander is a common stream side salamander dependent on the moist environment and cover provided by headwater streams. This species can tolerate moderate levels of surrounding landscape disturbance.
Desmognathus ochrophaeus	ALLEGHENY MOUNTAIN DUSKY SALAMANDER	3	The Allegheny Mountain Dusky Salamander, a close relative of the Northern Dusky Salamander, is more terrestrial and is found along streams but also more upland habitats. This species is limited to eight counties in extreme northeastern Ohio.

Scientific Name	Common Name	C of C	Ecology Notes
Eurycea bislineata	NORTHERN TWO-LINED SALAMANDER	5	The Northern Two-lined Salamander and the closely related Southern Two-lined Salamander are the most common stream salamanders in Ohio. While both species are found under rocks, logs and debris at the edge of brooks and streams or in cold springs and seeps, this species is limited to northeast Ohio.
Eurycea cirrigera	SOUTHERN TWO-LINED SALAMANDER	5	The Southern Two-lined Salamander and the closely related Northern Two-lined Salamander are the most common stream salamanders in Ohio. While both species are found under rocks, logs and debris at the edge of brooks and streams or in cold springs and seeps, this species, as its name suggests, is limited to the southern half of Ohio.
Eurycea longicauda longicauda	LONGTAIL SALAMANDER	6	The Longtail Salamander utilizes a range of habitats both aquatic and terrestrial. It can often be found in woodland seeps and springs with rock outcroppings and breeds and lays eggs in springs.
Eurycea lucifuga	CAVE SALAMANDER	10	This Cave Salamander is state endangered, has a narrow niche and generally is found in the twilight zone of caves. However, it can also be found in crevices in limestone rocks around springs and under logs, wood slabs, stones and debris in moist environments near caves. This species' range is limited to extreme southern and southwest Ohio.
Gyrinophilus porphyriticus porphyriticus	NORTHERN SPRING SALAMANDER	9	The Northern Spring Salamander lives in or around clear, cold, highly oxygenated waters, such as headwater streams without fish or springs in wooded areas, or in caves. This species occurs in east and southeastern Ohio with isolated populations in Hamilton County.
Gyrinophilus porphyriticus duryi	KENTUCKY SPRING SALAMANDER	9	The Kentucky Spring Salamander lives in or around clear, cold, highly oxygenated waters such as headwater streams with no fish or springs in wooded areas, or in caves. The Northern Spring Salamander is more northern in distribution while this species only occurs in the extreme south central part of the state.
Hemidactylium scutatum	FOUR-TOED SALAMANDER	10	The Four-toed Salamander is an inhabitant of mature forested woodlands that have ephemeral pools with ample moss, often sphagnum, covered hummocks, tussocks and woody debris. Nesting occurs on structures above the water, the females brood the eggs and the aquatic larvae drop into the water as they emerge from the eggs.

Scientific Name	Common Name	C of C	Ecology Notes
Plethodon cinereus	EASTERN RED-BACKED SALAMANDER	3	The Eastern Redback Salamander is a common inhabitant of forested landscapes and has an extremely small home range. It will not be found in drier habitats that cannot satisfy its moisture requirements.
Plethodon glutinosus	NORTHERN SLIMY SALAMANDER	4	The Northern Slimy Salamander prefers drier upland forests and is not found in bottomland hardwood forests. In the forest it has a limited range and is generally found under rocks and logs.
Plethodon electromorphus	NORTHERN RAVINE SALAMANDER	4	The Northern Ravine Salamander is dependent on forested areas. As its name implies, it prefers the slopes and ravines of woodland landscapes.
Plethodon wehrlei	WEHRLE'S SALAMANDER	10	Wehrle's Salamander is only known from two locations in eastern Ohio. One of these sites is in Monroe County and the other in Washington County. It is a woodland salamander often found in more rocky habitats than other salamanders. Its presence in Ohio is questionable, both sightings are from prior to 1950 and intensive investigations in subsequent years have yielded no additional individuals.
Pseudotriton montanus diastictus	MIDLAND MUD SALAMANDER	10	The Midland Mud Salamander is an animal of muddy lowland springs, sluggish floodplain brooks and the swampy forested areas along these streams. It has a limited range in south central Ohio and is rare even within its range.
Pseudotriton ruber ruber	NORTHERN RED SALAMANDER	9	The Northern Red Salamander can be found in both upland and lowland springs, generally in constant shade, as well as in clean, clear streams within mature, deciduous forest. It is limited to cold waters and seeps found in the sandstone areas of eastern Ohio.

Funnel Trap Design



Aluminum window screening 28" x 18" is rolled into a cylinder 18" long and stapled through a 1" lip to form a tube 8" in diameter. Fiberglass screening is cut out and stapled to form a funnel with a opening of 9" on the wide end and 1 3/4" on the narrow end. The narrow end of the funnel is placed inside the cylinder as indicated in the figure. The wide end of the funnel is rolled over the outside edge of the cylinder and stapled every 1/2". A string handle is attached to the lip. The trap is emptied by everting the fiberglass funnel and dumping and shaking the contents into a pan.

Figure 1. Funnel trap design with construction notes.

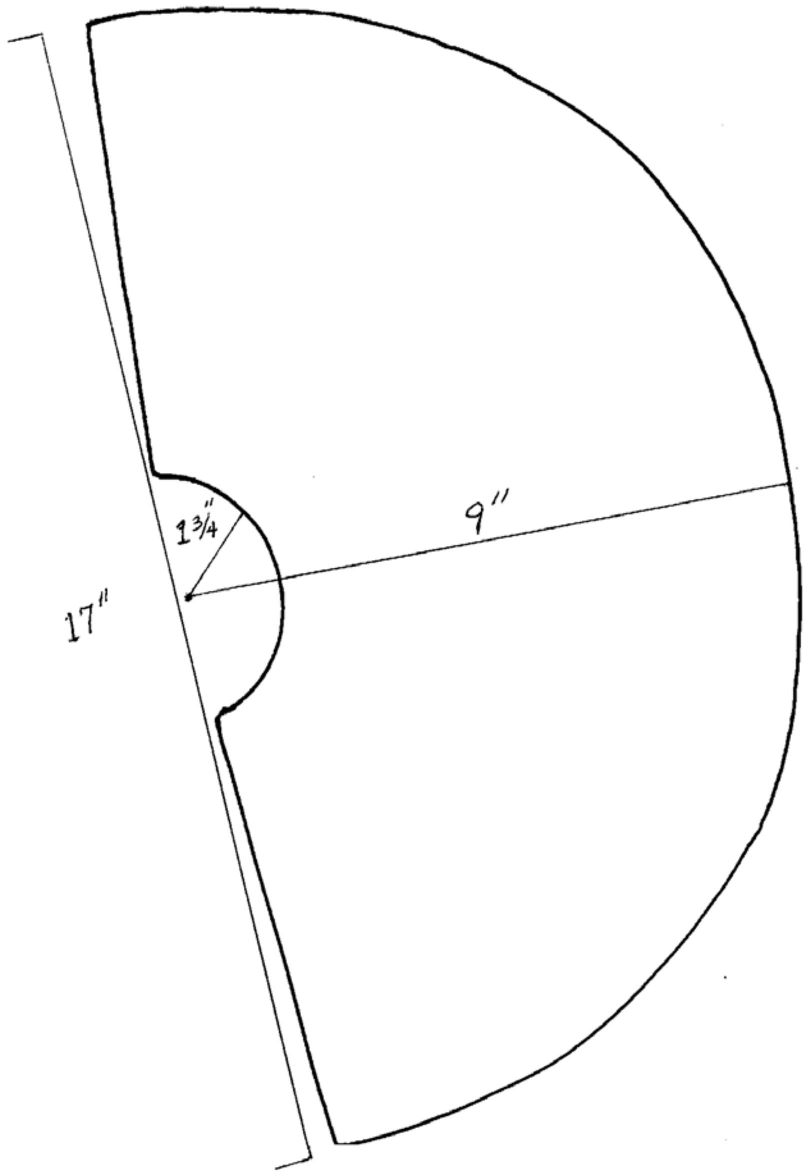


Figure 2. Funnel end shape and measurements (**not to scale –** base=17", long radius=9", short radius=1¾ "). PDF with scaled funnel end template, if printed on 11" by 17" paper, available at: http://www.epa.ohio.gov/dsw/wetlands/WetlandEcologySection_reports.aspx