State of Ohio
Environmental Protection Agency

Wetland Ecology Group
Division of Surface Water

Amphibian Index of Biotic Integrity (AmphIBI) for Wetlands

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Testing Biological Metrics and Development of Wetland
Assessment Techniques Using Reference Sites
Volume 3

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1.0 Introduction

A principal goal of the Clean Water Act is to maintain and restore the physical, chemical and *biological integrity* of the waters of the United States. 33 U.S.C. §1251(a). Biological integrity has been defined as "...the capability of supporting and maintaining a balanced integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region (Karr and Dudley 1981). "Integrity" or "Ecological integrity" has been defined as the sum of the earth's *biological diversity* and *biological processes*[1] (Table 1); the converse of ecological integrity is *biotic impoverishment*, which is defined as the systematic reduction in the capacity of the earth to support living systems (Karr 1993). Thus, "A biological system is healthy and has ecological integrity when its inherent potential is realized, its condition is "stable," its capacity for self-repair is maintained, and external support for maintenance is minimal. Integrity implies an unimpaired condition or quality or state of being complete and undivided (Karr, p. 1522, 1993)." The concept of integrity, and its measurement and description by biological surveys, underpins the development of biological criteria.

The factors in natural wetlands which can be degraded by human activity fall into several broad classes: biogeochemistry, habitat, hydrology, and biotic interactions (Table 2). The quantitative measurement (assessment) of the degree of integrity of a particular natural system, and conversely the degree of impairment, degradation or impoverishment, can be attempted in many ways. The State of Ohio has successfully developed a sophisticated system using ambient biological monitoring of fish and macroinvertebrate assemblages to assess the quality of streams in Ohio (the Invertebrate Community Index (macroinvertebrates), the Index of Biological Integrity (IBI)(fish), and the Modified Index of Well Being (fish) (Ohio EPA 1988a, 1988b, 1989a, 1989b; Yoder and Rankin 1995). This type of system has been used and adopted throughout North America and Europe (Karr 1993). *See also* Karr and Kerans (1992); Barbour et al. (1992); Bode and Novak (1995); Hornig et al. (1995); Simon and Emery (1995), Hughes et al. (1998). The statistical properties of Ohio's IBI was investigated and validated by Fore, Karr, and Loveday (1993). They concluded that the IBI could distinguish between five and six nonoverlapping categories of integrity and that the IBI is "...an effective monitoring tool that can be used to communicate qualitative assessments to the public and policy makers or to provide quantitative assessments for a legal or regulatory context based on confidence intervals or hypothesis testing procedures (Fore, Karr, and Loveday, p. 1077, 1993).

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[1] Karr (1993) defines biological diversity as the variety of the earth's naturally occurring *biological elements*, which extend over a broad range of organization scales from genes to populations, species, assemblages, and landscapes; the complement of biological diversity (the elements) are the *biological processes* on which those elements depend.

Table 1. Components of ecological (biological) integrity for wetlands. Adapted from Karr and Kerans (1992), Karr (1993).

Biological diversity	Biological Processes
Elements of biodiversity	Nutrient cycling/biogeochemistry
Genes within populations	Photosynthesis
Populations within species	Water cycling/hydrological regime
Species within communities/ecosystems	Evolution/speciation
Communities/ecosystems within landscapes	Competition/Predation/Mutualisms
Landscapes within biosphere	

Table 2. Factors associated with wetlands that can be negatively impacted by human activities causing wetland degradation. Adapted from lists for flowing waters from Karr and Kerans (1992), Karr et al. (1986), Ohio EPA (1988a).

factor	description	examples of disturbances
biogeochemistry	natural patterns of that type of wetland for nutrient cycling, decomposition, photosynthesis, nutrient sequestration and release, aerobic/anaerobic regimes, etc.	nutrient enrichment, sedimentation, addition of organic or inorganic chemicals, heavy metals, toxic substances, etc.
habitat	natural patterns and structures of that type of wetland for floral and faunal communities.	mowing, grazing, farming, vehicle use, clearcutting, woody debris removal, shrub/sapling removal, herbaceous/aquatic bed removal, sedimentation,, etc.
hydrology	natural hydrologic regime of that type of wetland: frequency, duration, amount of inundation; sources of water, etc.	ditching, tiling, dikes and weirs, additions of storm water, point source discharges, filling and grading, construction of roads and railroad beds, dredging, etc.
biotic interactions	natural patterns of competition, predation, disease, parasitism, etc.	introduction of nuisance or nonnative species (carp, predaceous fish, reed canary grass, purple loosestrife, European buckthorn), etc.

Table 3. Advantages of ambient biological monitoring. Adapted from Karr and Kerans (1992).

#	description
1	Broad based ecologically
2	Provides biologically meaningful evaluation
3	Flexible for special needs
4	Sensitive to a broad range of degradation
5	Integrates *cumulative impacts* from point source, nonpoint source, hydrologic alteration, and other diverse impacts of human society
6	Integrates and evaluates the full range of *classes of impacts* (e.g. hydrologic modifications, habitat alterations, etc.) on biotic systems
7	Direct evaluation of resource condition
8	Easy to relate to general public
9	Overcomes many weaknesses of individual parameter by parameter approaches
10	Can assess incremental degrees and types of degradation, not just above or below some threshold
11	Can be used to assess resource trends in space or time

The State of Ohio's stream indices are codified in Ohio Administrative Code Chapter 3745-1 and constitute numeric "biological criteria" which are a part of the state's water quality standards required under the Clean Water Act. *See* 33 U.S.C. §1313. Biological criteria are numerical values or narrative expressions that describe the reference biological integrity of natural communities (U.S. EPA 1990). It is important to stress that the overall index score resulting from an IBI, as well as each individual metric represent testable hypotheses as to how a natural system responds to human disturbance (Karr 1993). Attributes of natural communities are selected and predictions are made as to how the attribute will respond, e.g. increase or decrease; not change until a particular threshold is reached and then increase quickly; increase linearly, or curvilinearly, etc. Moreover, the existing biological condition of a natural system is the integrated result of the chemical, physical, and biological processes that comprise and maintain the system, and the biological condition of the system can be conceived as the integration or result of these processes over time (Ohio EPA 1988a). The organisms, individually and as communities, are indicators of the actual conditions in that system since they inhabit the system and are subject to the variety of natural and human-caused variation (disturbance) to the system (Ohio EPA 1988a). In this regard, biological monitoring and biocriteria take advantage of this inherent integrative characteristic of the biota of a system, whereas chemical and toxicity monitoring only represents a single point in time unless costly, continuous sampling over time is performed. Table 3 lists some of the advantages inherent in biological monitoring.

"Wetlands" are a type of water of the United States and a water of the State of Ohio under federal and state law. *See e.g.* Ohio Revised Code (ORC) §6111.01(H), OAC Rule 3745-1-02(B)(90), 33 CFR 323.2(c). Until recently, wetlands in Ohio were only generically protected under state's water quality standards. On May 1, 1998, the State of Ohio adopted wetland water quality standards and a wetland antidegradation rule. OAC Rules 3745-1-50 through 3745-1-54. The water quality standards specify narrative criteria for wetlands and created the "wetland designated use." All wetlands are assigned to the "wetland designated use." However, numeric criteria were not proposed since they had not yet been developed

A key feature of Ohio's current regulatory program for wetlands is found in the wetland antidegradation rule. *See* OAC Rule 3745-1-54. The wetland antidegradation rule categorizes wetlands based on their functions, sensitivity to disturbance, rarity and irreplaceability and scales the strictness of avoidance, minimization, and mitigation to a wetland's category. Three categories were established: Category 1 wetlands with minimal wetland function and/or integrity; Category 2 wetlands with moderate wetland function and/or integrity; and Category 3 wetlands with superior wetland function and/or integrity. A wetland is assigned to one of these three categories "...as determined by an appropriate wetland evaluation methodology acceptable to the director." OAC Rule 3745-1-54(C)(1)(a), (C)(2)(a), and (C)(3)(a).

Ohio EPA has developed a rapid qualitative measure of a stream's potential to support levels of aquatic life (Rankin 1989). The Qualitative Habitat Evaluation Index (QHEI) assigns a point total to each of a group of stream attributes, know as metrics, (substrate, in stream cover, channel morphology, riparian zone and bank erosion, and pool/glide and riffle/run quality). The results of the individual scoring of the metrics are summed and result in a composite score for the stream. These QHEI scores correlate strongly to the more intensive measures acquired through biological monitoring of the fish and macroinvertebrate communities. This association allows highly reliable preliminary evaluations of streams to be accomplished using the less resource intensive QHEI.

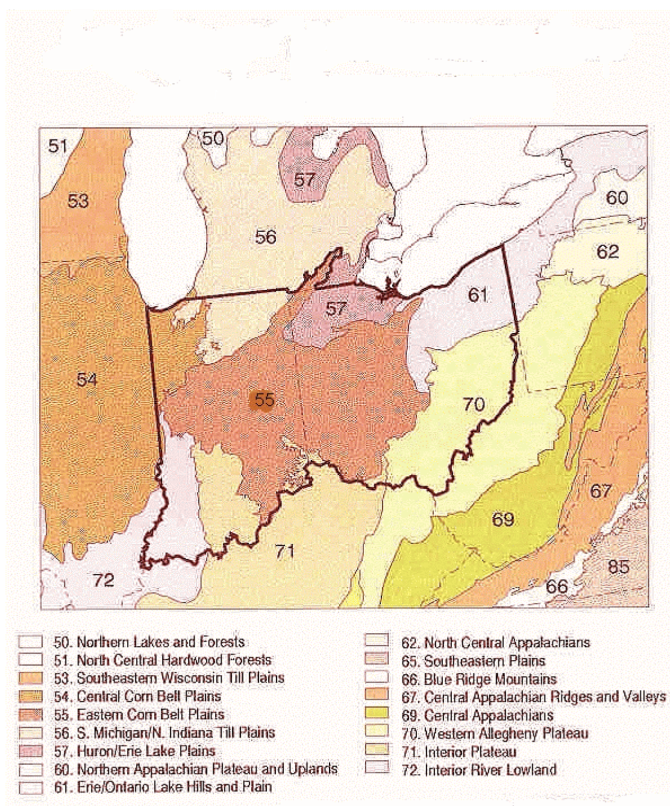

Figure 1. Ecoregions of Ohio, Indiana, and neighboring states. From Woods et al. 1998.

Similarly for wetlands, during the rule

making process, Ohio EPA began developing a rapid wetland evaluation methodology known now as the Ohio Rapid Assessment Method (ORAM) for wetlands. A major rewrite of the ORAM took place with the development of Version 5.0 which became final on February 1, 2001. With the revisions, ORAM Version 5.0 is a rapid wetland ranking tool generally analogous to the QHEI for streams and is used as the human disturbance gradient (x axis) in this study . *See* discussion below and ORAM Manual (Mack 2001a).

The ORAM is designed to categorize a wetland based on whether it is a particular type of wetland (e.g. fen, bog, old growth forest, etc.) or contains threatened or endangered species, or based on its "score." Fennessy et al. (1998a) found significant correlations between a wetland's score on earlier versions of the ORAM and the wetlands biological quality and/or degree of disturbance. The provisional scoring ranges proposed in Fennessy et al. (1998a) were descriptively derived from a sample of wetlands scored using the ORAM and the professional judgment of the Ohio Rapid Assessment Workgroup (Fennessy et al. 1998a). Recalibration of the scoring ranges using actual measures of a wetland's biology and functions has been a continuing need since the adoption of the Wetland Water Quality Standards and Wetland Antidegradation rules and the use of draft versions of the ORAM (versions 3.0, 4.0, and 4.1) in regulatory decision making.

Ohio began working on the development of biological criteria using amphibians in 1996. To date, Ohio has sampled 88 different wetlands mostly located in the Eastern Corn Belt Plains and Erie-Ontario Lake Plains ecoregions (Figure 1) but with some sites in the Huron Erie Lake Plains, the Michigan Indiana Drift Plains, and the Western Allegheny Plateau ecoregions (Table 4). These sites span the range of condition from high degraded by human activity to relatively undisturbed, i.e., the best quality sites

Table 4. Summary of sites sampled by year to develop Wetland IBIs based on amphibians.

year	total	cumulative total	total minus resampled sites	resampled sites
1996	10	10	10	
1997	14	24	11	3
1998	3	27	3	
1999	29	56	29	
2000	19	75	19	
2001	18	93	16	2
totals	93	93	88	5

available or "reference conditions." It should be noted that all sites where amphibian monitoring has occurred have also had their vegetation communities monitored.

However, the amphibian and vegetation surveys have not always occurred in the same year. Vegetation has been surveyed at many sites where amphibian monitoring has not occurred (Mack 2001b). This work has been funded since 1996 by several different U.S. EPA Region 5 Wetland Program Development Grants including CD995927, CD995761, CD985277, CD985276, and CD985875.

The objectives of the wetland biocriteria development project have been to: develop Indices of Biotic Integrity (both interim and final); evaluate ecological integrity of wetlands using vascular plants, macroinvertebrates and amphibians as indicator taxa using an ecoregional approach; and calibrate the ORAM using these IBIs.

Based on preliminary results (Fennessy et al.1998a, 1998b), Ohio EPA concluded that vascular plants, macroinvertebrates, and amphibians could be used as indicator organisms for the development of wetland-specific IBIs. Micacchion et al. (2000) proposed initial amphibian metrics of biotic integrity based on data collected from 1996, 1997, 1997,, and 1999 in the Eastern Corn Belt Plains Ecoregion. This report reevaluates that amphibian data and includes an additional data set of sites from the Erie-Ontario Lake Plains ecoregion sampled in 2000. Additionally, an amphibian index of biological integrity is proposed.

2.0 Methods

Amphibian Community Assessment

Required Supplies
Flagging tape (hot pink)
Triangular ring frame dipnet (#30 mesh size)
Field forceps
White collection and sorting pan
Funnel traps (window screen mesh size)
Sample containers (4oz. wide-mouth glass jars, 1 liter wide-mouth plastic bottles)
Plastic squeeze bottle with 95% ethanol
Preservatives (10% formalin, 95% ethanol)
Salamanders of Ohio (Pfingsten and Downs 1989)
The Frogs and Toads of Ohio (Walker 1946)
A Key to the Anuran Tadpoles of the United States and Canada (Altig et al., in prep.)
Salamanders of the United States and Canada (Petranka 1998)

Quantitative Collection Protocol

We found that funnel traps were effective in sampling both the macroinvertebrate and amphibians found in wetlands. The following methods discussion pertains to the collection of both amphibians and macroinvertebrates since the same sampling protocols are used simultaneously to monitor the two taxa groups. Each time a wetland was sampled we collected a quantitative sample using funnel traps and a qualitative sample collected by using a dip net and by picking natural substrates.

Ohio EPA began evaluating wetland macroinvertebrate and amphibian sampling methods in 1996. A variety of sampling methods including artificial substrate samplers, several types of funnel traps, and qualitative sampling with dip nets were evaluated (see Fennessy 1998a). The use of funnel traps as a method of sampling has been used extensively for amphibians and more recently as a protocol for macroinvertebrate collections in wetlands. A number of different kinds of funnel traps have been described ranging from modified two liter pop bottles to custom-made designs of PVC or clear acrylic plastics to using different types of metal meshes. In addition to the sampling method, the time of year to sample, the intensity, frequency, and duration of sampling were evaluated. At first it was thought that different methods would need to be used for each taxa group. However, the use of window screen mesh funnel traps proved to be affective in collection of both amphibians as well as wetland macroinvertebrates. Since 1997, field collection techniques have become standardized and the same protocols are used at each wetland sampled.

For this project, funnel traps are constructed of aluminum window screen cylinders with fiberglass window screen funnels at each end. The funnel traps are similar in design to commercially available minnow traps. However, the use of window screen, with its smaller mesh, makes the traps better able to collect a wide range of sizes of larval amphibians and macroinvertebrates. Aluminum screening is used for the cylinders to provide maximum structure and fiberglass screening is used for the funnels to allow flexibility to ease funnel inversion and eversion.

The aluminum screen cylinders are 18" long and 8" in diameter and held together with wire staples. The bases of the fiberglass screen funnels are 8" in diameter and attached with wire staples to both ends of the cylinder such that the funnel directs inward. The funnels have a circular opening in the middle that is 1.75" in diameter which serves as the means of entry into the trap. We have also developed a smaller version of the trap that is 5 inches in diameter for use in wetlands with shallower water depths and in other wetlands as they are drying up. When these are used the data is relativized to account for the smaller surface area of the traps' funnel ends and the corresponding expected decrease in trapping productivity.

In the typical application, 10 funnel traps are placed evenly around the perimeter of the wetland. This is done by first pacing around the wetland perimeter to provide a measure of the total wetland perimeter (with practice pacing can be a highly reliable measuring technique). The perimeter total is then divided by 10 and a trap is placed each time that amount is paced off while traversing the perimeter for the second time. Alternatively, for large wetlands or where the placement around the entire perimeter is not feasible, transects along one or several sides of the wetland are used. Also, in some years larger wetlands were monitored with more than 10 traps (12-20). Care is taken to assure that all habitat types within the wetland are represented proportionally within the transect. Each funnel trap location is marked using flagging tape both at the standing water/saturated soil interface and in vegetation above or near the trap. Since flagging is applied before the growing season it is important that an attempt be made to place it where it will not be obscured by new vegetation growth during the later passes. Traps are set at the same locations throughout the sampling season.

Each wetland was sampled three times between March and July. The late winter/early spring sample allows monitoring of adult Ambystomatid salamanders and macroinvertebrates such as fairy shrimp and other early season taxa which are present for a limited time in some wetlands. 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 and location. The timing of amphibian breeding runs can also vary greatly from south to north within the state, with southern populations in some years breeding up to several weeks before northern populations. A middle spring sample (April-May) is conducted in order to collect some adult frog species entering the wetland to breed, to sample early-breeding amphibian larvae and to sample for macroinvertebrates. A late spring/early summer (May-July) sampling is performed to collect relatively well developed amphibian larvae and macroinvertebrates.

The traps are placed on the substrates of the wetland and the trap is almost completely submersed. Traps are placed 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 and oxygen levels in the water decrease. In all cases, the traps are left in the wetland for twenty-four hours in order to ensure unbiased sampling for species with diurnal and nocturnal activity patterns. Limiting trapping time to twenty-four hours also works to minimize the potential for mortality due to individuals being in the traps for extended periods. No bait is used in traps. These are activity traps and designed to collect any amphibians or macroinvertebrates that swim, crawl or float into the funnel openings. Due to the shape of the funnel ends, once an individual organism is inside a trap, it is difficult to impossible for it to find the way out.

Since the traps are modeled after commercially available minnow traps they also are effective in capturing fish. So in addition to amphibians and macroinvertebrates, information on the fish taxa trapped is also recorded. The taxa of fish present are often valuable in explaining trends in the amphibian and macroinvertebrate communities and may themselves be indicators of wetland condition.

Upon retrieval, the traps are emptied by everting the funnel and shaking the contents into a white collection and sorting pan. Organisms that can be readily identified in the field (especially adult amphibians and larger and easily identified fish) are counted and recorded in the field notebook and released. 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 squeeze bottle filled with 95% ethanol. Before leaving the field, generally at the field vehicle, all bottles are supplemented with additional 95% ethanol in proportion to the number of individuals collected. The contents of each trap are kept in separately marked bottles for individual analysis in the laboratory. If large numbers of amphibians and/or fish are kept for identification in the lab, those samples are topped off with 10% formalin in the field to maximize the preservation of identification features.

Laboratory analysis of the funnel trap macroinvertebrate and fish samples follows the standardized Ohio EPA procedures (Ohio EPA 1989). Salamanders and their larvae are identified using keys in (Pfingsten and Downs 1989) and (Petranka 1998). Frogs and tadpoles are identified using keys in (Walker 1946) and (Altig et al., in prep.).

Qualitative Collection Protocol

Qualitative collections of macroinvertebrates and amphibians are made concurrently with funnel trapping at each wetland during the three sampling periods. Qualitative sampling involves the collection of macroinvertebrates and amphibians from all available natural wetland habitat features using triangular ring frame dipnets, collection and sorting trays and also by manual picking of substrates and woody debris with field forceps. Dip net sweeps are made in all habitat types where possible. The collection and sorting tray is often used as a repository for dip net contents to aid in examination and can itself be dipped into the water to yield a sample . Woody debris and other substrate materials are manually collected, searched and picked through with the aid of the forceps. The goal is to compile a comprehensive species/taxa inventory of macroinvertebrates and amphibians at the site. At least one individual of each taxa encountered will be collected or recorded. There is no attempt to make an absolute quantification of organism densities although observed predominant populations will be noted.

Generally, one field crew member will collect the qualitative sample while another crew member deploys or collects the funnel traps (qualitative sampling may occur on either the day of trap deployment or retrieval). A minimum of thirty minutes will be spent collecting the qualitative sample. Sampling will continue until the field crew determines that further sampling effort will not produce new taxa. Samples are deposited in 4 ounce wide-mouth glass bottles marked as qualitative samples and preserved with 95% ethanol. The qualitative field collection and laboratory analysis of these samples for macroinvertebrates and fish will follow the standardized Ohio EPA procedures (Ohio EPA 1989). Salamanders and their larvae will be identified using keys in (Pfingsten and Downs 1989) and (Petranka 1998). Frogs and tadpoles will be identified using keys in (Walker 1946) and (Altig et al., in prep.).

Laboratory Methods

Upon submission to the laboratory, all funnel trap and qualitative samples are assigned a unique lab number for tracking purposes. The contents of each funnel trap are processed individually so that each site has ten quantitative samples to process for each of the three collection dates. Samples preserved in 10% formalin are washed with water under a hood and transferred to 70% ethyl alcohol before the contents are identified.

All organisms within each funnel trap sample are identified and counted. The numbers of each taxa in each trap are entered into our database along with the duration of the trapping effort so that relative abundance, number per hour of trapping, and other metrics can be calculated.

Statistical Analyses
Minitab v. 12.0 was used to perform all statistical tests. Regression analysis, analysis of variance, Tukey's multiple comparison test, correlation coefficients, and t tests were used to explore and evaluate the biological attributes measured for development of an amphibian index of biotic integrity.

3.0 Site Selection

Monitoring of amphibians as indicators of condition in Ohio wetlands has occurred since 1996. During that time Ohio EPA has monitored 88 individual wetlands, including four wetlands that have been monitored in two separate years (County Road 200, Leafy Oak, Rickenbacker and Sawmill), and one wetland (Calamus Swamp) that has been monitored in three separate years. In almost all cases wetlands have been monitored three times during each sampling season. Exceptions would include some wetlands that were monitored up to five times in 1996 when techniques were still being established and some wetlands in other years, principally 1999, that dried up before the final monitoring pass. Also, in 2001, the first sampling run was eliminated to free up staff time to focus on analysis of data collected in previous years.

Of the 88 wetlands monitored 48 have been in the Eastern Corn Belt Plains (ECBP) ecoregion, 35 wetlands in the Erie/Ontario Lake and Drift Plains (EOLP) ecoregion, two wetlands in the Western Allegheny Plateau ecoregion, and two wetlands in the Michigan/Indiana Drift Plains. Additionally, 14 constructed wetlands have been monitored. In all Ohio EPA has monitored the amphibian communities in 102 wetlands since 1996. This document reports on the amphibian communities of 67 natural wetlands monitored between 1996 and 2000 in the ECBP and EOLP ecoregions. Development of an amphibian index of biotic integrity is based on the data from the 40 forested and shrub wetlands in this group.

To date, the amphibian study has included wetlands from five hydrogeomorphic (HGM) classes and all three of the major vegetation types: forested; shrub; and emergent. As discussed in the introduction, Ohio EPA has developed a method for rapid assessment of wetland condition. The Ohio Rapid Assessment Method for Wetlands Version 5.0 (ORAM 5.0) has been used to determine the degree of disturbance experienced by wetlands in our data set. This rapid assessment method was developed to differentiate among wetlands based on the level of human disturbance they have experienced. ORAM 5.0 requires the rater to compare the wetland to others of its type. Therefore, in essence, ORAM 5.0 measures the intactness of the wetland assessed and provides information on the relative functional level of that wetland. This method only evaluates the functions that the type of wetland being assessed is capable of performing. Comparisons are to other wetlands of the same type. Therefore, two wetlands can score exactly the same yet perform quite different functions. However, the overall condition (disturbance experienced) of the wetlands will be relatively the same. For this study, ORAM 5.0 scores have been used along the X axis when plotting biological measures of wetland amphibian communities along the Y axis.

Wetlands in this study have been selected to represent the entire gradient of disturbance from those in "reference condition" (least impacted) to those that have been severally degraded. Forested, shrub and emergent vegetation depressional systems have been the focus of study for the monitoring of wetland amphibian assemblages. Depressional systems are the dominant hydrogeomorphic type (Brinson 1993) in Ohio. Of these wetlands, forested systems are the most numerous (Baker and Micacchion 1999). Depressional wetlands also comprise the majority of wetland impacts proposed in Ohio for Section 401 certification and isolated wetland permit reviews. Therefore, by better understanding the dynamics of these systems we have the ability to have an affect on the greatest number of permit decisions. Focusing on depressional systems also limits the number of variables to be considered when comparing the fitness of wetlands to support representative amphibian communities.

The shrub wetlands in this study were located in primarily forested areas. For the wetlands studied for this report the forested and shrub sites are basically ecologically equivalent. Most Ohio wetland tree species will not tolerate growing in standing water for long periods of time. Therefore, trees are generally not found in the parts of wetlands that pond water for several months out of the year. The water tolerant tree species tend to grow on the edges of the areas of extended inundation.

Forested vernal pool wetlands exist where depressions in forested areas are narrow enough that the canopy of the trees that are located on the perimeter of the depression can cover the entire pool. The shrub dominated vernal pools in this study are also situated in forested areas. However, with shrub wetlands the depressions are large enough that the canopy formed by the trees on the edges of the depression cannot fully cover the pool. Underneath these openings shrubs, most characteristically buttonbush *(Cephalanthus occidentalis)* but often a mix of species, become established and provide the canopy. So while, from a vegetation classification stand point, the forested and shrub wetlands are separate, from an amphibian utilization perspective the two resources provide virtually the same within wetland and adjacent uplands habitat values. Therefore, in this study data from these two depressional wetland types have been merged when developing an amphibian index of biotic integrity. Separate analysis of the data from forested and shrub wetlands supports this combining of sites for comparisons.

We have experienced some difficulties in locating forested and shrub wetlands that can be characterized as severally degraded. Although with a thorough search of potential sites we have been able to identify several forested sites that demonstrate a high degree of disturbance. We believe the difficulty in finding highly disturbed forested and shrub sites is due to their responses to most disturbances. When these systems experience most types of severe disturbances the tendency is for them to lose their woody vegetation components and revert to emergent systems. This reversion can be direct as would result from tree and shrub removal or may be indirect in response to other stressors. An example of an indirect response would be a wetland that loses it trees and shrubs when its hydrologic regime is altered. Increases in quantity or duration of water present often results in the elimination of woody vegetation because those species are not adapted to the new hydrologic regime.

Prior to European settlement greater than 95% of Ohio was forested (Lafferty 1979) and therefore forest would have been the primary vegetation class of the vast majority of wetlands and their surrounding uplands. Naturally occurring emergent wetland systems were rare at that time and the largest numbers of these were the Lake Erie coastal marshes although other emergent systems were distributed in small numbers throughout the state. These wetlands included freshwater marshes, peatlands, and wet prairies (Gordon 1966). Therefore, it can be surmised that the much larger percentage of emergent wetlands on Ohio's landscape today is the result of historic disturbances to forested systems. It appears when forested and also shrub wetlands experience severe disturbances it most often results in the dominant vegetation class reverting to an emergent system. This is reflected in the amphibian assemblages in most emergent systems. We found the amphibian residents of Ohio emergent depressional systems to be primarily species that are tolerant of human disturbances.

Only at intact emergent wetland types that occur infrequently in Ohio's landscape have we found species that are intolerant to disturbances. Those wetlands types are the ones that naturally occur on the landscape as emergent systems or with emergent components. These wetlands often are comprised of species that include the common tolerant species found in other emergent systems such as green frogs (*Rana clamitans melanota*), bullfrogs (*Rana catesbeiana*), northern leopard frogs (*Rana pipiens pipiens*), and toads (*Bufo spp.*). In addition these wetlands might be habitat for red-spotted newts (*Notophthalmus viridescens viridescens*), northern cricket frogs (*Acris crepitans blanchardi*) or gray tree frogs (*Hyla versicolor*).

At this point we are not certain how to classify northern cricket frogs. This species was until recently ubiquitious in its distribution in the western two thirds of the state (Walker 1946) and most of the eastern half of the United States (Harding 1997). A major downturn in the numbers of populations and distribution of this species has occurred in the past several years. The reasons behind the drastic reductions in northern cricket frog numbers is not well understood. Today the distribution of the species is extremely spotty, especially in northern Ohio where the species primarily occurs in populations at

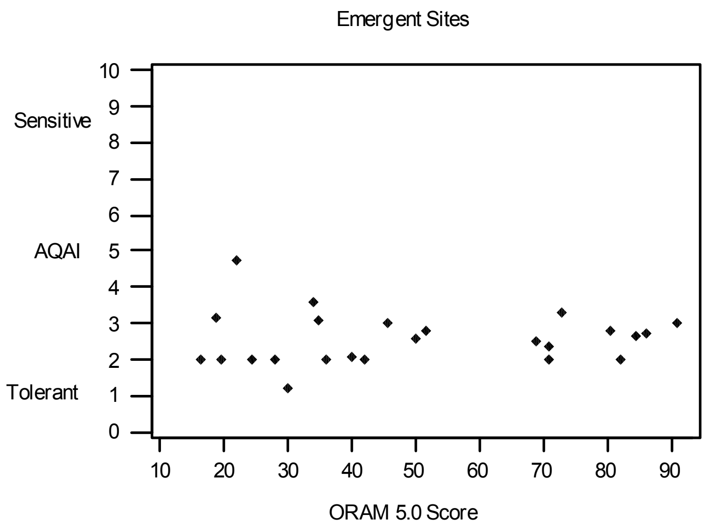

Figure 2. Wetland amphibian community sensitivity to disturbance based on Amphibian Quality Assessment Index (AQAI) scores for emergent depressional wetlands (development of the AQAI is discussed below) plotted against the disturbance gradient (ORAM 5.0 score).

isolated locations (Davis and Menze 2000). For these reasons, and because we have not encountered this species at any of the study sites in this report, even though a large number of our sites have been in its historic range, it is our inclination to consider this a sensitive species.

For some emergent systems we can identify amphibian species whose presence indicate that the wetland has experienced little disturbance. These are wetlands who in their natural state (historic, climax) are comprised of predominantly emergent vegetation.

However, most wetlands that are dominated by emergent vegetation in Ohio are in that condition due to significant levels of human disturbances. For these wetlands we get little resolution on separating the degree of disturbance based on the amphibian community (Figure 2). The amphibian community in most of these emergent systems is totally comprised of disturbance tolerant species as would be expected. Therefore, the metrics of amphibian communities of depressional emergent sites in Ohio are relatively consistent and correspond to the levels of disturbance these systems have incurred. This results in an inability to apply an amphibian index that will work to separate between the conditions (quality) of most emergent depressional sites. For these reasons the development of an amphibian index of biological integrity reported throughout the rest of this report is based on the group of 40 forested and shrub wetlands from the group of 67 natural wetlands monitored in the years 1996, 1997, 1999 and 2000.

4.0 The Amphibian Index of Biotic Integrity (AmphIBI)

A large suite of attributes of the amphibian communities of the study wetlands were examined. Only the quantitative (amphibians trapped) data has been used. An attempt has been made to consider all associations that might be potentially correlated in any way to the disturbance level of the wetlands under study. Data analysis was conducted to determine which attributes showed a change in value along the gradient of human disturbance (Karr and Chu 1999).

Many attributes have been used with other taxa groups as indicators of resource condition. These metrics are used because they correlate well with disturbance levels. However when applied to the amphibian communities of wetlands these attributes did not correlate with disturbance levels. For instance, total amphibian taxa richness and single and multiple taxa dominance attributes displayed no correlation with the level of disturbance being experienced by the study wetlands. Many types of taxa richness have worked out to be key metrics in development of indices of biotic integrity for other taxa groups including fish, macroinvertebrates and plants (Ohio EPA 1988b, Mack 2001, Gernes and Helgen 1999). Many species, genera, family and order associations were analyzed to determine if there were trends that would provide insight into predicting wetland condition. None of these attributes proved to have any strong correlation with wetland disturbance level.

Five attributes of the amphibian community of forest and shrub depressional wetlands (vernal pools) show strong correlation with the human disturbance gradient of the study wetlands. These five metrics then are scored and combined to provide what is being called the Amphibian Index of Biotic Integrity (AmphIBI).

Values for each metric were graphed against ORAM 5.0. The scoring of the two metrics that have absolute values are discussed in their sections. For the other three metrics the 95 percentile of each range of values was determined. Then the remaining range of values was both mathematically and graphically quadrasected (along the y axis). These mathematical and graphical quadrasections were then assessed for ecological relevance. The quadrasections selected were the ones that showed the most ecological significance. Metric scores for individual wetlands were set depending on which quadrasection its metric value occupied. Those metrics were scored as follows: 10 points for first quadrasection; 7 points for second quadrasection; 3 points for third quadrasection; and 0 points for the fourth quadrasection. Discussion on the correlations, development and scoring of the five metric follows.

Amphibian Quality Assessment Index

As reported earlier (Micacchion et al 2000) an amphibian index known as the Amphibian Quality Assessment Index (AQAI) has been developed for wetlands. This is modeled after indices that have been developed utilizing plants to give information on the overall condition of a resource (Andreas and Lichvar 1995, Wilhelm and Ladd 1988, Swink and Wilhelm 1979). In a similar manner we have used the varying sensitivities to disturbance and other habitat requirements to place amphibian species within a range of tolerance coefficients from 1 to 10 (no non-native amphibians have been encountered during our monitoring, therefore no species are assigned a coefficient of conservatism of 0). Lower numbers indicate those species that are adapted to a greater degree of disturbance and a broader range of habitat features. Those species assigned higher numbers are considered to be sensitive to disturbance and have more specific habitat requirements (niche). The tolerance coefficients were assigned after reviewing numerous texts about the autecology of each species and based on the experience of the researchers both through the years of this study and throughout their careers. The species encountered in wetlands and their proposed tolerance coefficients along with some rationale are contained in Table 5.

Table 5. Wetland Amphibian Tolerance Coefficients and Rationale

species	coefficient	rationale
Ambystoma jeffersonianum complex (includes *A. platineum* and *A. tremblayi*)	5	Jefferson salamanders and associated hybrids require relatively intact wooded habitat adjacent to breeding pools with low to moderate levels of disturbance
Ambystoma opacum	9	Marbled salamanders require intact mature woods and vernal pools that fill in the late fall/early winter
Ambystoma maculatum	8	Spotted salamanders have only been collected in least disturbed wetlands or moderately disturbed wetlands where the disturbance has been recent
Ambystoma texanum	4	Smallmouth salamanders are the most ubiquitous of the ambystomid salamanders and will tolerate wetlands with relatively short hydro-periods
Ambystoma tigrinum	6	Tiger salamanders have been found in a range of wetlands with pools that have deep, long lasting hydrology and nearby uplands that are reasonably intact
Ambystoma laterale	10	Blue spotted salamanders are listed as state “endangered” due to their extremely limited range and can only be found in a few counties in extreme NW Ohio
Hyla versicolor and *Hyla chrysoscelis*	5	Tree frogs require some shrubs or trees adjacent to breeding pools and are less tolerant of other disturbances than most anurans

Table 5. Wetland Amphibian Tolerance Coefficients and Rationale

Bufo spp. (*Bufo americanus* and *Bufo woodhousei fowerli* tadpoles are indistinguishable)	1	American and Fowler's toads require little except enough water to allow for their short reproductive cycle and will tolerate disturbances other amphibians cannot
Hemidactylium scutatum	10	Four-toed salamanders are listed as state "special interest" and have a high fidelity to undisturbed forested sites with vernal pools
Notophthalmus viridescens	9	Red spotted newts are extremely intolerant of disturbance and are found only in well buffered intact wetlands
Rana catesbeiana	2	Bullfrogs which are widely spread, are most common in marshes, but can be found in forested and shrub sites and are tolerant of most disturbances
Rana clamitans melanota	3	Green frogs are found in a wide range of wetlands and are tolerant of most disturbances
Rana pipiens pipiens	2	Leopard frogs breed in a range of sites, the main requirement is enough water for their breeding cycle and some suitable adjacent habitat
Rana sylvatica	7	Wood frogs are dependent on forested wetlands and adjacent areas and require pools within a landscape of minimal disturbance
Pseudacris crucifer	2	Spring peepers breed in a range of sites, main requirement is enough water for breeding cycle and some suitable adjacent habitat
Pseudacris triseriata	3	Western chorus frogs are slightly less tolerant of disturbance than the closely related *P. crucifer*

As calculated for this study, the AQAI is a weighted index that not only takes into account the sensitivity to disturbance of the individual species at a wetland but also includes the number of individuals of each species collected. In doing so the AQAI results in a score that provides information on the overall condition of the amphibian community present and allows for comparisons among wetlands. The index is developed by first summing the number of individuals from all species trapped at a wetland to develop a total. Next the numbers of individuals of each species is multiplied by its corresponding tolerance coefficient to yield a subtotal for each species. The subtotals for each species are then added together to yield a second total. The second total is then divided by the first total to derive the AQAI for that wetland. This index represents the average tolerance coefficient of individual amphibians trapped at that wetland throughout the sampling season (information from all three passes is totaled). Calculation of the AQAI for a hypothetical forested vernal pool wetland is shown in the example below. Information on study wetlands and their AQAI and ORAM 5.0 and other metric scores is presented in Appendix I.

Table 6. Calculation of AQAI for a hypothetical forested vernal pool

Species	Number of Individuals	Tolerance Coefficient	Subtotals
Ambystoma maculatum	50	8	400
Ambystoma jeffersonianum	30	5	150
Ambystoma texanum	20	4	80
Notophthalmus viridescens	25	9	225
Pseudacris crucifer	30	2	60
Hyla versicolor	20	5	100
Rana pipiens pipiens	30	2	60
Rana clamitans melanota	2	3	6
Totals	**187**	**–**	**1081**

AQAI = **5.79** (1081/187)

AQAI scores have been calculated for each of the three runs for each individual wetland for the years 1996, 1997, 1999 and 2000.

The AQAI can be used alone as a measure of the quality (condition) of a wetland community and can be used to compare the relative amphibian community quality of any wetland. In this study the AQAI is also used as one metric in a multi-metric index of biotic integrity. Figure 3 shows the AQAI plotted against ORAM scores and a regression plot. The positive relationship between the AQAI and ORAM 5.0 score is significant (df = 39, F = 31.99, $p<0.001$, R-sq = 45.7%). A comparison of the mean AQAI scores by wetland category has been conducted using a one-way analysis of variance (Figure 4). The means are significantly different based on Tukey's Honest Significant Difference test ($p<0.05$). These results point out the ability of the AQAI to be used as a tool to separate wetlands based on their condition.

As well as developing the AQAI other attributes of the amphibian community of our study wetlands were explored. Analysis of population level attributes was conducted to see which might correlate with wetland condition and provide metrics toward the development of an amphibian index of biotic integrity. Site data was sorted and analysis proceeded in this format to determine if information from one or any combination of the sampling runs would suffice for calculation of a meaningful AQAI and other amphibian metrics. In particular, we hypothesized that the data from the third sampling run would be representative of attributes of the amphibian community over the course of the breeding season. However, analysis to date has revealed that by far the strongest and only meaningful correlations are demonstrated when information from all the sampling runs is pooled together for each wetland. This result illustrates the complexity of developing meaningful indices utilizing wetland amphibians. Further, it points out the need for a significant expenditure of time and other resources to collect the data to compile this index. The level of effort required also increases the desire to be able to correlate the data with a less time and labor intensive rapid assessment method.

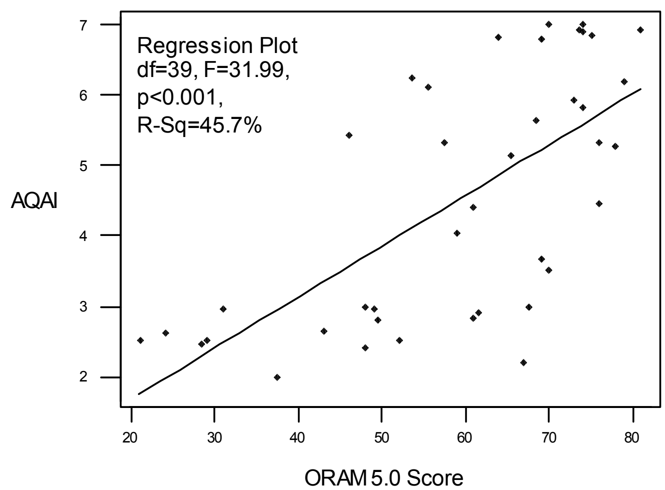

Figure 3. Scatterplot of AQAI scores versus disturbance scale (ORAM 5.0 score) for the forty forested and shrub wetlands.

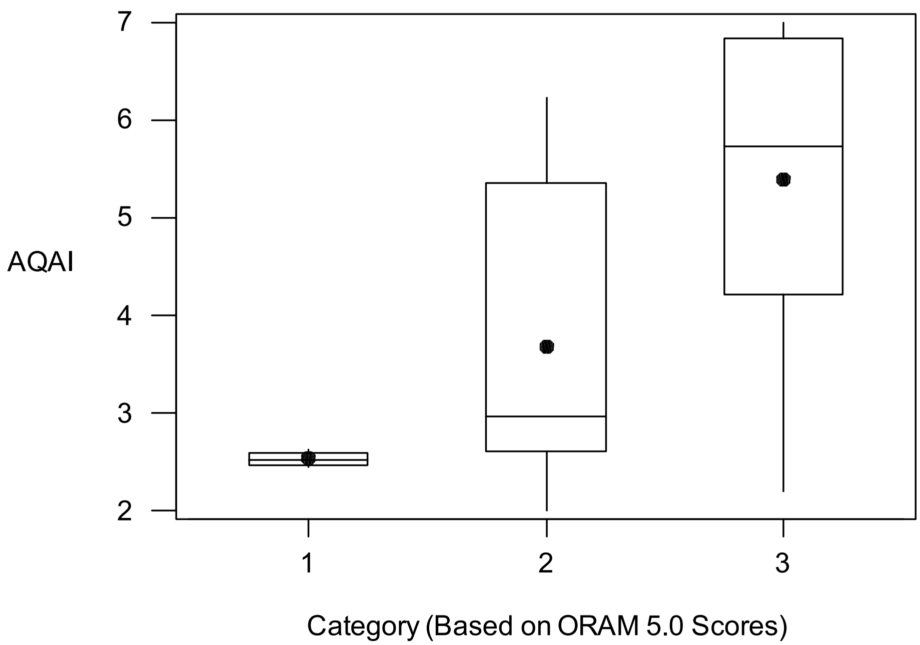

Figure 4. Box and whisker plots of AQAI scores versus wetland category. All means significantly different based on Tukey's Honest Significant Difference test ($p<0.05$). Means are indicated by solid circles. A line is drawn across the box at the median. The bottom of the box is at the first quartile (Q1), and the top is at the third quartile (Q3) value. The whiskers are the lines that extend from the top and bottom of the box to the adjacent values. The adjacent values are the lowest and highest observations inside the region defined by the following limits: Lower Limit = Q1 - 1.5 (Q3-Q1); Upper Limit = Q3 + 1.5 (Q3-Q1).

Number of species of pond-breeding salamanders

Salamander species that utilize primarily ephemeral wetlands for reproduction are referred to as pond-breeding salamander species and are dominated by species in the family Ambystomatidae. This family is also known as “mole salamanders” because all species spend a large percentage of their adult lifes below ground in burrows in the upland areas adjacent to breeding ponds (Petranka 1988). Pond-breeding Ambystomatid species in Ohio are the Jefferson salamander (*Ambystoma jeffersonianum)*, the blue spotted salamander *(A. laterale)*, the spotted salamander *(A. maculatum)*, the marbled salamander *(A. opacum)*, the smallmouth salamander *(A. texanum)* and the tiger salamander *(A. tigrinum)*. There are also at least two Ambystomatid hybrids, the silvery salamander *(A. platineum)* and Tremblay’s salamander *(A. tremblayi)* (Pfingsten and Downs 1989). However, there is difficulty in distinguishing between even adult forms of these salamanders and Jefferson salamanders. To make positive identifications the red blood cells of individuals must be measured to determine ploidy (Pfingsten and Downs 1989) which requires significant expenditures of time, including small scale surgery, and the carrying of special equipment into the field. Therefore, for this study all Ambystomatid hybrids and *A. jeffersonianum* are placed into the *A. jeffersonianum complex* and share the same tolerance coefficient and are considered as a single species for purposes of IBI metric development.

In Ohio, besides Ambystomatid salamanders, we also have two additional species of pond-breeding salamanders. The red spotted newt *(Notophthalmus viridescens)* of the family Salamandridae utilizes wetlands for breeding and a large part of its adult life cycle (Pfingsten and Downs 1989). The four-toed salamander *(Hemidactylium scutatum)*, of the family Plethodontidae, while completely terrestrial in its adult form, lays its eggs in nests located on moss covered logs and woody debris above wetland pools. When larvae emerge from the eggs they then drop into the wetland pools and spend their larval developmental period in the wetlands (Harding 1997).

We have only captured one individual four-toed salamander during the period of this study. That was a larva we collected while conducting the dip-netting qualitative sample at Swamp Cottonwood State Nature Preserve in Medina County on June 27, 2000. Little data is available on the ecology and natural history of four-toed salamander larvae (Petranka 1998). The four-toed salamander is a state listed “special concern” species in Ohio and is rare. However, we would have expected to have encountered them in other wetlands of suitable habitat we have monitored within their range. We believe the larvae may be relatively inactive in the wetlands spending most of their time well concealed in cover. Relatively sessile amphibian larval behavior may be a mechanism to reduce losses through predation (Skelly 1997). This behavior would explain their absence in our funnel traps, even at two sites where there is documentation that a population exists, and at other sites that appeared to be appropriate habitat.

The number of pond breeding salamanders present in a wetland correlates strongly with the quality of that wetland. In wetlands, we have monitored over the course of this study, the number of species of pond breeding salamanders encountered has ranged from 0 to 5. Figure 5 shows the numbers of species present by the category of wetland in which they occur. As can be seen more pond breeding salamander species are generally present in wetlands that have experienced less disturbance. This would be expected as the number, quality and diversity of habitat features that support salamander populations is greater in least impacted wetlands. Most pond breeding salamanders live in the surrounding terrestrial habitat for the biggest part of the year (Semlitsch 1998). Wetlands in higher categories have generally experienced less disturbance in these surrounding uplands. This intactness provides the diversity of habitats adapted to by the range of pond-breeding salamanders. Therefore, these better habitats would be expected to support higher numbers of species.

Numbers of pond breeding salamanders species was compared by wetland condition (reference versus non-reference) in Figure 5. The difference between the mean numbers of these two conditions was marginally significant ($df = 35$, $t = -1.93$, $p = 0.062$). Only three sites that scored below 57.5 on the disturbance gradient had more than 2 species of pond breeding salamanders. Those sites were Flowing Well, Hempleman and McKinley.

The number of pond breeding salamanders monitored at a site is a metric in the development of the AmphIBI. For this metric points are assigned as follows: 0 pts - no species encountered; 3 pts - one or two species present; 7 pts - three species present; and 10 pts - four or more species present.

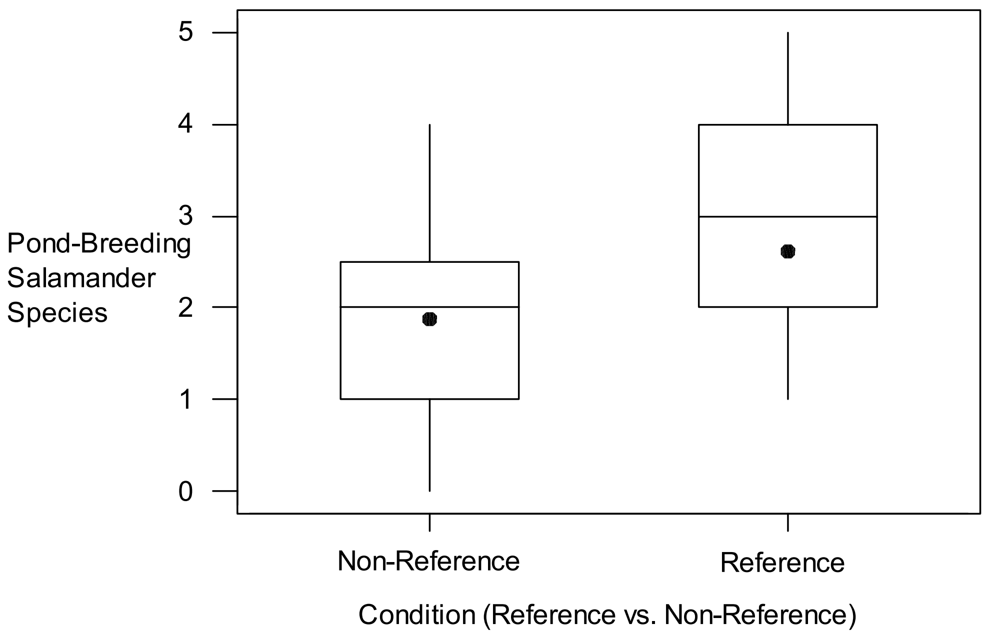

Figure 5. Box and whisker plots showing numbers of pond-breeding salamanders species by wetland condition (reference versus non-reference). Means are indicated by solid circles. A line is drawn across the box at the median. The bottom of the box is at the first quartile (Q1), and the top is at the third quartile (Q3) value. The whiskers are the lines that extend from the top and bottom of the box to the adjacent values. The adjacent values are the lowest and highest observations inside the region defined by the following limits: Lower Limit = Q1 - 1.5 (Q3-Q1); Upper Limit = Q3 + 1.5 (Q3-Q1).

Relative Abundance of Sensitive Taxa

Within the section on development of the AQAI and in (Micacchion et al. 2000) was discussion of the assignment of varying tolerance coefficients to the wetland amphibian taxa. Once species assignments are made the population can be assessed to determine the relative abundance of taxa that fall into ranges of tolerances to disturbances. It would be expected that a larger proportion of taxa with higher tolerance coefficients (sensitive species) would be found in higher category, less disturbed, wetlands. Conversely, in highly disturbed systems, lower category wetlands, you would expect the population to be dominated by individuals with low tolerance coefficients (tolerant species).

Relative abundance measures can be an important indicator of the dynamics of a population. When the relative abundances of the amphibian species that comprise the communities in the wetlands sampled are analyzed strong correlations come to the surface. When looking at all individuals trapped a strong correlation exists between the relative abundance of sensitive species (tolerance coefficients 6-9) and the ORAM 5.0 scores for the wetlands ($p < 0.001$). This is a positive correlation with wetlands having higher relative abundances of sensitive species also having higher ORAM 5.0 scores.

There was a significant correlation between relative abundance of sensitive species and the disturbance gradient (df = 39, F = 19.84, $p < 0.001$, R-sq = 34.3%) (Figure 6). A comparison of the mean relative abundance of sensitive species by wetland category is presented in Figure 7. Mean relative abundances by wetland category were significantly different from one another (df = 37, F = 7.15, $p<0.05$). Some wetlands, Gahanna Woods 4th Pool, Blackjack Road (back), Drew Woods and the Rookery had a far smaller relative abundance of their populations comprised of sensitive species than would have been anticipated based on ORAM scores. Gahanna Woods 4th Pool is a site I have monitored extensively for several years. Based on that information I know it has a much higher quality amphibian community than what was reflected in the monitoring data we collected for the study in 1996. The reasons our 1996 monitoring did not properly characterize the community is uncertain. Blackjack Road (back) is a buttonbush pool located within a forested area that was clear cut 15 to 20 years ago. The forest is on the way to recovery and therefore the wetland scores high on most ORAM 5.0 metrics. However, it appears the impacts to the amphibian community have been longer lasting. Drew Woods is a buttonbush pool located just within the border of a forty acre old growth forest. While the wetland scores highly on ORAM 5.0 it appears the proximity of the wetland to agricultural row cropping is having a limiting effect on the amphibian community. The Rookery is a relatively intact wetland in a forest patch surrounded by a recently constructed golf course community. Before construction the

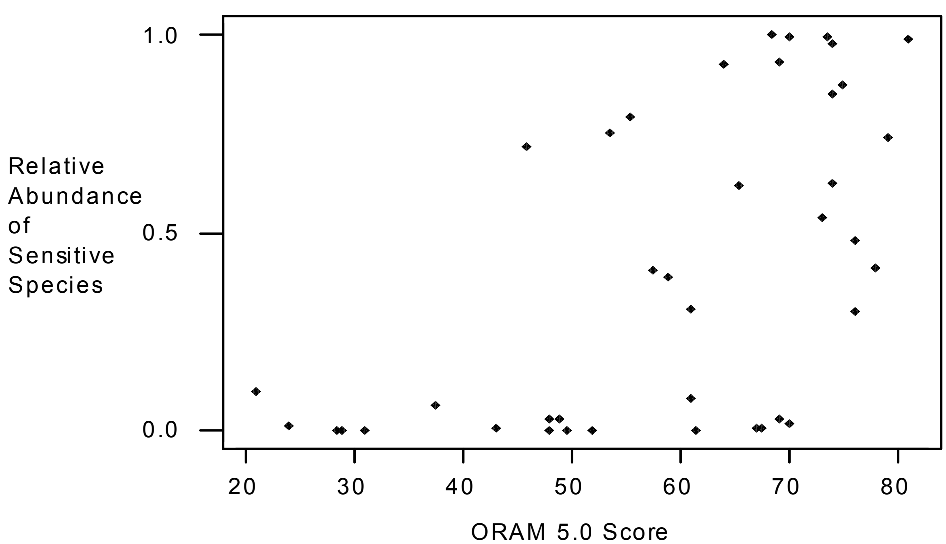

Figure 6. Relative abundance of sensitive species plotted against ORAM 5.0 scores.

surrounding land uses were significantly less intensive and the wetland was part of a much larger forest. Apparently, the changes in surrounding land uses including the reduction in forest size is having a severe negative impact on amphibians.

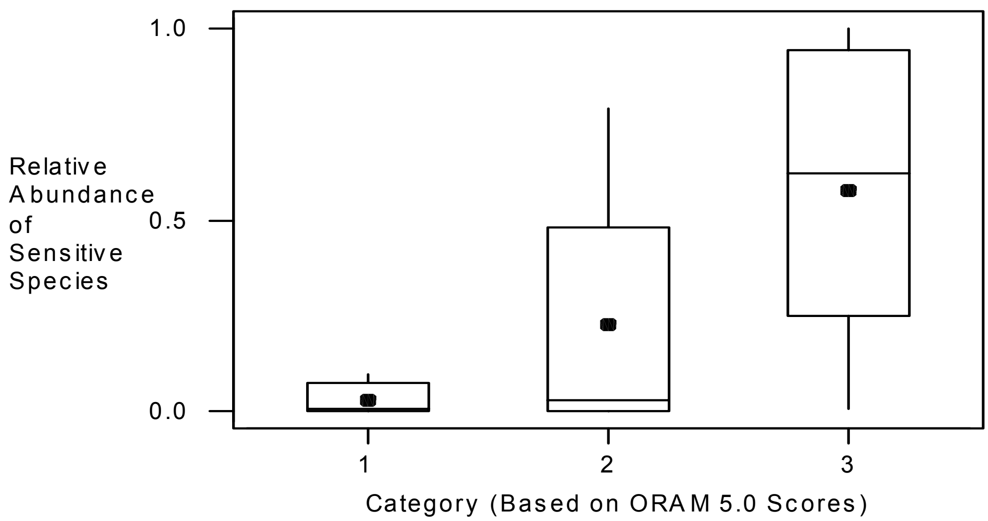

Figure 7. Box and whiser plots of relative abundance of the total population comprised of sensitive species by Category. Means are indicated by solid circles. All means were significantly different from one another (df=37, F=7.15, p<0.05). A line is drawn across the box at the median. The bottom of the box is at the first quartile (Q1), and the top of the box is at the third quartile (Q3) value. The whiskers are the lines that extend from the top and bottom of the box to the adjacent values. The adjacent values are the lowest and highest observationons that are still inside the region defined by the following limits: Lower limit = Q1 - 1.5 (Q3-Q1); Upper limit = Q3 + 1.5 (Q3-Q1).

Relative Abundance of Tolerant Taxa

There is a strong negative correlation between the relative abundance of tolerant species (tolerance coefficients 1-3) and ORAM 5.0 scores (df = 39, F = 20.38, p<0.001, R-sq = 34.9%) (Figure 8). As can be seen the relative abundance of tolerant species increases as ORAM 5.0 scores decrease. This is not surprising since sensitive species would be expected to drop out as wetlands experience increased levels of disturbance. Only those species which are extremely tolerant would be expected to be present in the most impacted wetlands.

It should be noted that almost all the emergent wetlands we monitored had populations with 100% relative abundances of tolerant amphibian species (Figure 1). Even though these wetlands span the range of disturbance, based on ORAM 5.0 scoring and other assessments, they all have relatively similar, highly tolerant amphibian communities.

Again this highlights the difficulties in separating out the condition of emergent wetlands using amphibians as indicators.

In Figure 9 the study wetlands have been divided into three categories based on ORAM scores and the relative abundance of tolerant species in each category is represented in boxplot format.

The means of the relative abundance of each category are significantly different from one another (df = 37, F = 6.65, $p < 0.05$). As can be seen in Figure 9 there is a fair amount of variation in the relative abundance of tolerant species comprising amphibian communities in Category 2 wetlands. However, Category 1 and Category 3 wetlands more clearly separate.

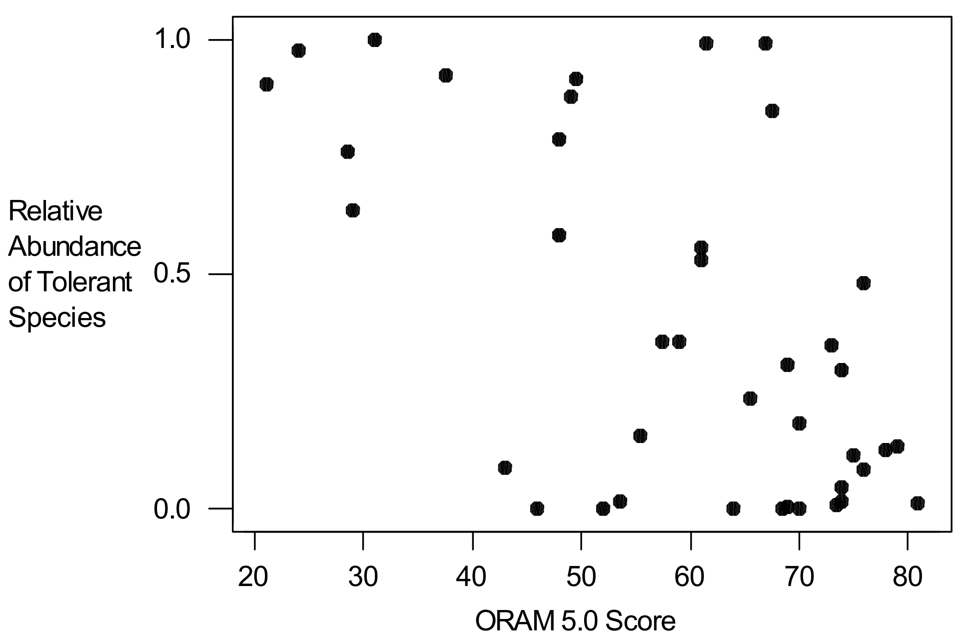

Figure 8. Relative abundance of tolerant species graphed against ORAM 5.0 scores.

Three wetlands that have relatively high ORAM 5.0 scores (> 60), yet still have a large proportion of the population comprised of tolerant species, are Gahanna Woods 4th Pool, Blackjack Road (back) and Area K. The reasons Gahanna Woods 4th Pool and Blackjack Road (back) are outliers here are likely the same as the factors that appear in the discussion of the relative abundance of sensitive species above. Area K is a relatively large buttonbush pool located within an area of some forest and agriculture within a state wildlife area.. There has been significant beaver activity in and around the wetland. We trapped one species of fish, black bullhead (*Ameiurus melas*) at Area K. Black bullheads are opportunistic feeders and will utilize whatever food is available and it has been documented that larger individuals will prey on frogs (Becker 1983). It appears at Area K that black bullheads may have been predatory on amphibians and had a limiting effect on their populations.

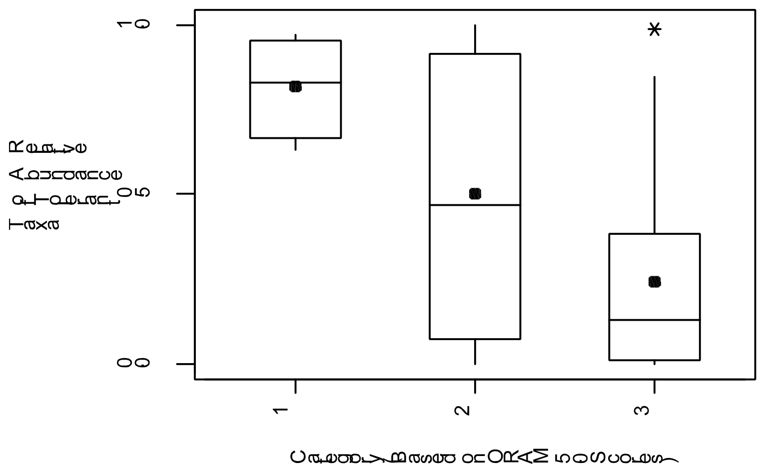

Figure 9. Box and whisker plots of relative abundance of the total population comprised of tolerant species by Category. Means are indicated by solid circles. All means were significantly different than each other (df=37, F=6.65, p<0.05). A line is drawn across the box at the median. The bottom of the box is at the first quartile (Q1),and the bottom of the box is at the third quartile (Q3) value. The whiskers are the lines that extend from the top and bottom of the box to the adfacent values. The adjacent values are the lowest and highest observations that are still inside the region defined by the following limits: Lower limit = Q1 - 1.5 (Q3-Q1); Upper limit = Q3 + 1.5(Q3 - Q1). Outliers are points outside of the lower and upper limits and are plotted with asterisks(*).

Flowing Well, Killdeer Plains, Lawrence Low #2 and Sawmill are wetlands that, based on ORAM 5.0 scores (43-53.5), would be predicted to have larger relative abundances of tolerant species comprising their amphibian communities. Flowing Well, Killdeer Plains and Lawrence Lo #2 are located within large forest patches. So while the wetlands themselves are demonstrating some levels of disturbance, their landscape position and habitat features allow them to perform better than would otherwise be expected. Sawmill is a mature forested wetland that has been completely encircled by urban development. Only narrow buffers (< 25 meters) remain around the perimeter of the wetland. The approximately five acre wetland still has good habitat features. The only amphibians trapped, when this wetland was monitored in 1997, were individuals of a remnant population of smallmouth salamanders (*Ambystoma texanum*), which have been assigned a tolerance coefficient of 4.

Spotted Salamanders or Wood Frogs

Two species of amphibians, in particular, appear to be especially good indicators of relatively undisturbed wetland condition. The spotted salamander *(Ambystoma maculatum)* and the wood frog *(Rana sylvatica)* were almost exclusively found in those wetlands that had experienced relatively low levels of disturbance. When graphing their presence against our disturbance gradient these species almost without exception do not show up except in wetlands that score well into the upper areas of the range are monitored (see Figures 10 and 11). The literature is clear about the need for undisturbed forested uplands adjacent to suitable breeding pools for the occurrence of these two species (Pfingsten and Downs 1989, Petranka 1998, Harding 1997, Wright and Wright 1995, Walker 1946).

Data from the wetlands where spotted salamanders were present and their corresponding forested buffer widths are summarized in Table 7. Using a binary logistic regression the percentage of wetlands that are likely to have the presence of spotted salamanders is calculated. As can be seen as forested buffer widths of the wetlands increases there are much higher odds of the wetland providing habitat for spotted salamanders. The odds of finding a spotted salamander population in a wetland with an average buffer width of over 50 meters are eight times the probability of finding a population in a wetland with average buffer widths of 25 to 50 meters. The odds of finding a spotted salamander population in a wetland with an average buffer width of over 50 meters is 70 times the odds of finding a population in a wetland with average buffer widths of 10 to 25 meters.

Table 7. Presence of Spotted Salamanders and Buffer Widths

	< 10 meters	10 - 25 meters	25 - 50 meters	> 50 meters
Presence of A. maculatum (%)	0	6	37	83

Spotted salamanders were present at 14 sites and wood frogs were presence at 16 sites. At only five sites were both spotted salamanders and wood frogs found together. Those five wetlands: Fowlers Woods; Grand River Terraces; Pallister; Slate Run; and Swamp Cottonwood were all least impacted sites that are examples of the best levels of integrity that can be expected from wetland amphibian communities in Ohio today.

It appears that wood frogs were once present across all of Ohio (Walker 1946). However, since the time of European settlement there has been significant disturbance to the original condition of the Eastern Corn Belt Plains and Huron/Erie Lake Plain ecoregions of Ohio. Historic land uses have been so intensive and the affects have been so long lasting that the wood frog has been eliminated from much of its range there. Today the wood frog is absent from even areas in these ecoregions that have had many years to recover and appear to have large tracts of suitable habitat. Spotted salamanders have a range that extends across a large portion of the state. Although this species has not been documented in each of Ohio's 88 counties it probably occurs in all of them (Pfingsten and Downs 1989).

This metric scores 10 additional points for those wetlands were either spotted salamanders or wood frogs are collected. No additional points are assigned to those wetlands which have both species present. Sites with both of these species absent receive a score of zero.

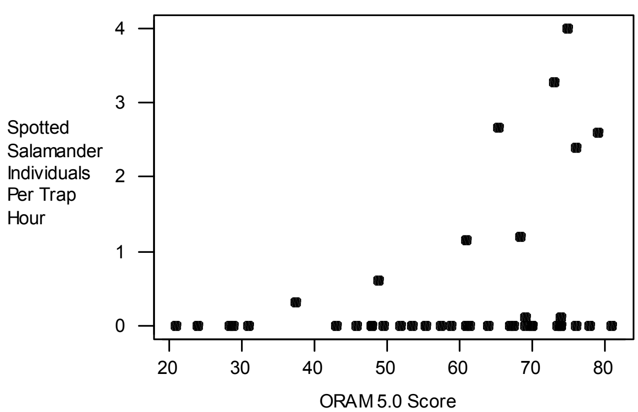

Figure 10: Spotted Salamanders per trap hour versus ORAM 5.0 Scores.

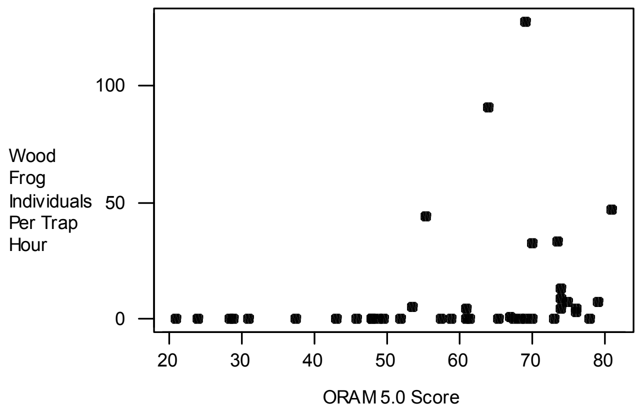

Figure 11: Wood frog individuals per trap hour versus ORAM 5.0 Scores.

Total AmphIBI Score

In order to compile an AmphIBI score for the wetlands the total of the five metrics: AQAI; number of pond-breeding salamanders species; relative abundance of sensitive species; relative abundance of tolerant species; and presence of spotted salamanders or wood frogs is summed. This results in a composite score for each site than can range from zero to fifty. Scoring of the metrics has proceeded such that higher AmphIBI scores correspond to a higher level of biological integrity.

The AmphIBI is graphed against ORAM 5.0 scores in Figure 12. There is a significant positive relationship between AmphIBI and ORAM 5.0 scores (df = 39, F = 48.01, $p < 0.001$, R-sq = 55.8%).

The AmphIBI scores for individual wetlands are graphed against the corresponding wetland categories based on ORAM 5.0 scores in Figure 13. A one way analysis of variance was performed comparing the means of the three groups. For the forested and shrub wetlands in this study the mean AmphIBI scores of the three categories were significantly different from each other after using Tukey's Honest Significant Difference test(df = 37, F = 16.92, $p < 0.05$). The overall behavior of the AmphIBI is satisfactory. Significant differences between the mean AmphIBI scores by category indicates the appropriateness of its use as a tool for determining biological condition that corresponds to wetland categories.

In Figure 14 the AmphIBI scores for individual wetlands are graphed against wetland condition (reference versus non-reference). Reference wetlands are those which have no blatantly obvious human disturbances. For study consistency purposes the determinations that were made for reference versus non-reference in the vegetation analysis (Mack 2001b) were also used for the amphibian analysis. A two sample T test was performed comparing the means of AmphIBI scores for the two groups. The means were found to be significantly different (df = 34, t = -4.86, p < 0.0001). This result again points out that the AmphIBI can be used as a tool to differentiate between wetlands based on overall wetland condition.

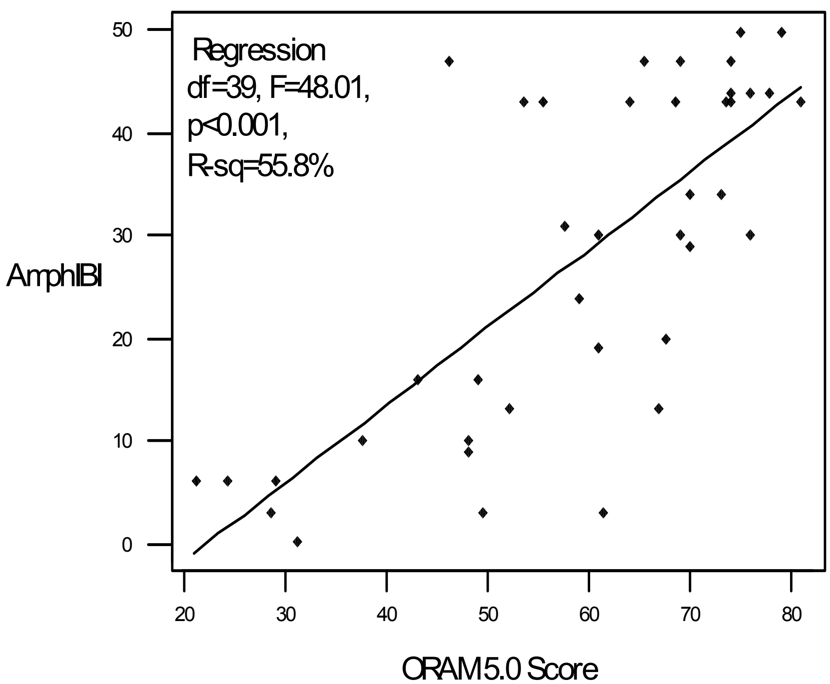

Figure 12. Regression plot of AmphIBI scores versus disturbance scale (ORAM 5.0 score).

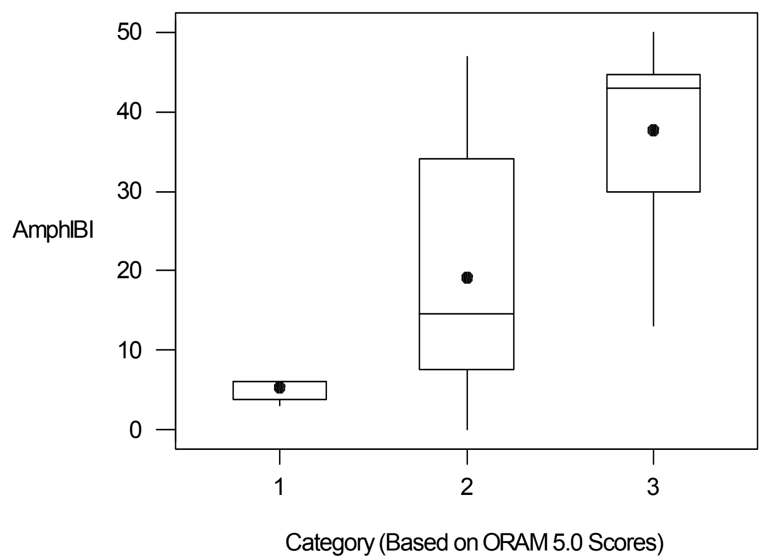

Figure 13. Box and whisker plots of Amphibian IBI scores by category. Means are indicated by solid circles. A line is drawn across the box at the median. All means were significantly different from each other (df=37, F=16.92, p<.005).The bottom of the box is at the first quartile (Q1), and the top is at the third quartile (Q3) value. The whiskers are the lines that extend from the top and bottom of the box to the adjacent values. The adjacent values are the lowest and highest observations inside the region defined by the following limits: Lower Limit = Q1 - 1.5 (Q3-Q1); Upper Limit = Q3 + 1.5 (Q3-Q1).

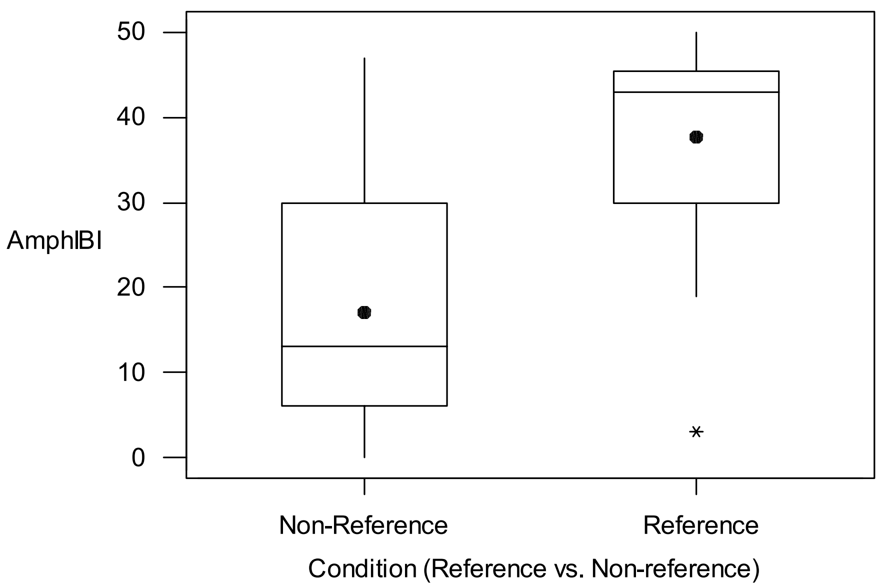

Figure 14. Box and whisker plots of Amphibian IBI scores by wetland condition (reference versus non-reference). Means are indicated by solid circles. A line is drawn across the box at the median. Means are significantly different from each other (df=34, t=-4.86, p<0.0001). The bottom of the box is at the first quartile (Q1), and the top is at the third quartile (Q3) value. The whiskers are the lines that extend from the top and bottom of the box to the adjacent values. The adjacent values are the lowest and highest observations that are still inside the region defined by the following limits: Lower Limit = Q1 - 1.5 (Q3-Q1); Upper Limit = Q3 + 1.5 (Q3-Q1). Outliers are points outside of the lower and upper limits and are plotted with asterisks (*).

The scoring breakpoints for the metrics that make up the AmphIBI are summarized in Table 8. With breakpoints assigned for the various metrics an AmphIBI can be developed for any wetland in question. Investigators would follow the protocols laid out in the Methods section. Trapping would need to occur for three twenty-four periods during the amphibian breeding season. Then all amphibians would need to be identified and counted. Once that is accomplished it is merely a process of matching up the numbers from any site with the corresponding scores for each of the five metrics. Those metric scores are then totaled to provide the AmphIBI score for the site.

Based on how the AmphIBI scores graph compared to ORAM scores breakpoints for category determinations can be proposed. On that basis it is recommended that wetlands with an AmphIBI score less than ten be assigned to Category 1. Wetlands that have an amphibian community resulting in AmphIBI scores between ten and twenty-nine should be assigned to Category 2. Assignment to Category 3 would be recommended for wetlands with AmphIBI scores of 30 and above.

At times, after reviewing all other available information, including other wetland assessment methods, the appropriate antidegradation category for a wetland under review is uncertain. In these situations, for wetlands that match up with the class of wetlands in this study (seasonally inundated shrub and forested depressions), the AmphIBI is a tool that can be used to provide an answer. By monitoring the amphibian community and doing the necessary math an AmphIBI score can be derived. The AmphIBI score will then dictate, based on the breakpoints for categories, what is the appropriate antidegradation category for the wetland in question.

Table 8. Scoring breakpoints for assigning metric scores for AmphIBI.

Metric	Score 0	Score 3	Score 7	Score 10
AQAI	<3.00	3.00 - 4.49	4.50 - 5.49	$\geq$ 5.5
Rel. Abundance Sensitive Spp.	0%	.01 - 9.99%	10 - 49.99%	$\geq$ 50%
Rel. Abundance Tolerant Spp.	>80%	50.01 - 79.99%	25.01 - 50%	$\leq$ 25%
# of Pond-Breeding Salamander Spp.	0 -1	2	3	> 3
Spotted Salamanders or Wood Frogs	absent	-	-	present

5.0 Amphibians, Wetlands and Landscape Factors

Monitoring amphibian communities in wetlands provides an opportunity to collect information on a combination of wetland and upland attributes. While some amphibians spend their entire life in wetlands, most only utilize the wetlands for part of their life cycle. The majority of the amphibians species utilize wetlands for breeding, egg laying and metamorphosis yet spend the bulk of their lives in the adjacent uplands. This is especially true of seasonal wetlands that only remain inundated for several months of the year, generally from late fall or winter through early summer, and then are dry for the rest or year.

Therefore, monitoring amphibians in wetlands provides an indication of the integrity of the wetland but also gives direct measures of the condition of the adjacent uplands. Without appropriate upland areas to support many amphibian species there can be no possibility of their occurrence in wetlands (Semlitsch 1998). However, the amphibians that utilize wetlands for their breeding cycle generally will not travel far from their upland habitats to reach breeding pools. This means that the amphibians monitored in wetlands provide information on the condition of uplands in the immediately vicinity. So, to a larger degree than other taxa groups amphibians provide information on the condition of the wetland/upland system rather than just the condition of what is within the wetland jurisdictional boundary. The amphibians reflect more what is going on from an ecological wetland boundary rather than the more limited “jurisdictional” boundary. Yet, there is no chance of survival of these species without the presence of the jurisdictional wetland. The inundated wetland is essential to their breeding efforts and some amphibian species (i.e. newts) utilize them for much longer periods.

Species that are considered to be sensitive to disturbance require intactness not only in the wetland but also the adjacent uplands. There are several wetland species that require wetlands that are in good condition and show up only in wetlands that have been minimally impacted. These species include spotted salamanders (*Ambystoma maculatum)*, red-spotted newts *(Notophthalmus viridescens viridescens)*, marbled salamander *(Ambystoma opacum)*, four-toed salamanders *(Hemidactylium scutatum)*, and wood frogs *(Rana sylvatica sylvatica)*. When these species occur in a wetland it can also be surmised that there is a good level of intactness to the immediately adjacent uplands and that a large percentage of that upland is forested.

The Ohio Rapid Assessment Method for Wetlands Version 5.0 (ORAM 5.0) was used as the disturbance gradient in this study and requires information about the condition of the uplands that comprise the buffers and surrounding land uses as well as important within wetland attributes. ORAM 5.0 metric 2a evaluates the “Average Buffer Width” with choices of **wide**, >50m (7 pts), **medium**, 25m to <50m (4pts.), **narrow** 10m to <25m (1pt.) and **very narrow**, <10m (0 pts.). Metric 2b assesses the “Intensity of predominant surrounding land uses” with choices of **very low**, second growth or older forest, prairie, savannah, wildlife area, etc. (7 pts.), **low**, shrubland, young second growth forest, etc. (5 pts.), **moderately high**, residential, fenced pasture, park, conservation tillage, new fallow field (3 pts.), and **high,** urban, industrial, open pasture, row cropping, mining, construction, etc. (1 pt.). This means the disturbance gradient accounts for the condition of uplands around wetlands as well as the condition of the wetlands themselves. The fact that amphibians are dependent on areas in close proximity to wetlands then does not affect our ability to make correlations between resource condition and the health of the amphibian community since our disturbance gradient assesses adjacent uplands.

Many sensitive amphibian species require large contiguous areas of forested uplands in order for populations to remain viable. Additionally, less intensive surrounding land uses may allow other wetlands to be in close proximity in order to facilitate metapopulation dynamics (Semlitsch 2000). Without other wetlands nearby the gene pool has the potential to become stagnant and there is no refuge if a wetland encounters a limiting stochastic event (Gibbs 1998). One or several bad years can place the entire population in jeopardy and can lead to local extinctions. Other wetlands ideally should be situated within the same large forest patch. If the landscape is fragmented then intact travel corridors need to be present to allow organisms to travel from wetland to wetland with adequate cover and appropriately moist microclimates. So for amphibians the buffer width and intensity of surrounding land uses are extremely important factors.

ORAM 5.0 metric 3e evaluates the “Modifications to the natural hydrologic regime” with choices of **none or none apparent** (12pts.), **recovered** (7 pts.), **recovering** (3 pts.), and **recent or no recovery** (0 pts.). “Habitat alteration” is the focus of ORAM 5.0 metric 4c with choice of **none or none apparent** (9 pts), **recovered** (6 pts.), **recovering** (3 pts.), and **recent or no recovery** (1pt.). It is critical for wetland breeding amphibians to have hydroperiods that match the requirements of their life cycle. Water must not only be present in adequate depths and for appropriate amounts of time it must also be present at the right time of year. Any alteration to the natural hydrologic regime that these amphibians are adapted to can have a severely limiting effect. Also, intact, high quality habitat, also plays a major role in the ability of a wetland to support a healthy amphibian community. The types of vegetation, the interspersion and the microtopography of a wetland all play important roles.

Metrics 2a, 2b, 3e and 4c measure factors that are vital to the ability of a wetland to support amphibian populations. The AmphIBI is graphed against the total of these four metrics for the study wetlands in Figure 15 . The resulting correlation is stronger than the relationship between the AmphIBI and the total ORAM 5.0 score (Figure 12).

The strong correlation of the AmphIBI to the characteristics these four metrics evaluate points out their importance to the amphibian community. Some of the outlying wetlands, when the AmphIBI is compared to the total ORAM 5.0 score, may be due to these wetlands over scoring or under scoring on other metrics. However, overall ORAM 5.0 serves as a good predictive tool of wetland biological condition.

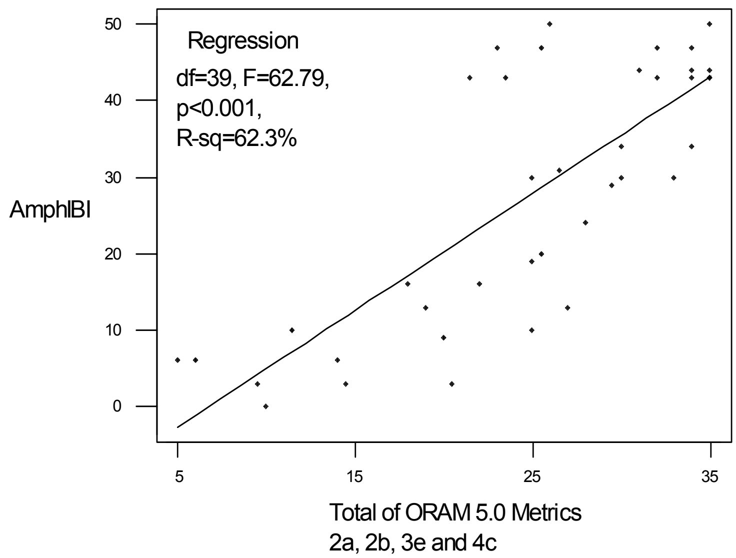

Figure 15. AmphIBI scores plotted against the total points for ORAM 5.0 metrics 2a, average buffer width, 2b, intensity of predominant surroundingland uses, 3e, modifications to the natural hydrologic regime, and 4c, habitat alteration.

6.0 Presence of Fish

Fish have been present in numerous wetlands in this study. Fish were encountered largely in emergent wetlands connected hydrologically to stream systems. Wetlands attached hydrologically to streams harbored populations of predatory and non-predatory fish species. However, some amphibian species are extremely susceptible to predatory fish species. Most Ambystomatid salamanders and some of the frog species find the presence of predatory fish to be a limiting factor and do not occur in habitats where they are present (Hecnar and M'Closkey 1997). At times amphibians may attempt breeding in pools where the recruitment or stocking of predatory fish species has been recent. These breeding attempts generally result in a complete elimination of the larval amphibians of some species and in the long term can decimate populations. This occurs because the those amphibian species have not developed defensive mechanisms to allow them to coexist with predatory fish species (Kats et al., 1988).

Isolated depressional wetlands were almost always devoid of any fish. However, when isolated depressional systems had fish they were generally non-predatory. These non-predatory fish species are often compatible members of high quality amphibian wetlands. For instance, brook sticklebacks *(Culaea inconstans)* were found in one isolated wetland (Fowler Woods) and central mudminnows *(Umbra limi)* in another (Oyer Wood Frog) that had superior amphibian populations. It is somewhat surprising that these two species were found in wetland pools that are seasonal. Apparently, these fish species have the ability to utilize extremely shallow temporary connections between wetlands and other surface waters. It may be that during storm events these species swim up gradient, sometimes in overland flows that are only a few inches deep, and populate the pools.

How those populations deal with the eventual drying up of the pools is uncertain. While standing water is temporary, perhaps enough moisture remains in the highly organic soils to allow the fish to survive until the pools are recharged. This certainly seems to be a strategy of central mudminnows that are able to spend significant periods of time aestivating, burrowed into the substrates during times of drought (Trautman 1981). For the brook sticklebacks, which have a high affinity for groundwater fed habitats, and perhaps the central mudminnows, as well, this may be a pioneering adaption of these species that allows for recruitment into new habitats. Some populations benefit by selection of suitable permanently inundated wetlands or streams while others pay the price for living on the edge. In the end it is a behavior that results in species being able to extend their range and take advantage of otherwise unavailable suitable habitats.

Of the 40 wetlands that comprised the forest and shrub wetland classes from which the AmphIBI is derived, only ten wetlands had fish. Of those only six contained what we considered to be predatory fish species. Of the 24 emergent wetlands that comprised this study, fifteen contained fish. Twelve of those wetlands had species of predatory fish. The negative effects of predatory fish species presence on some amphibian species may be an additional factor why the amphibian communities of emergent sites are depressed when compared to forested and shrub sites.

7.0 Conclusions

In Ohio there are 16 species of wetland dependent amphibians. Because such a large percentage of the state was historically forested most of the diversity of this Class has developed within a forested setting. This leads to difficulties when trying to use the amphibian community to determine wetland condition for wetlands dominated by emergent vegetation. However, for isolated depressional forest and shrub wetlands the Amphibian Index of Biotic Integrity (AmphIBI) is a tool that effectively utilizes their amphibian communities to separate out wetlands based on their overall condition.

The AmphIBI uses five metrics: the Amphibian Quality Assessment Index (AQAI); the number of pond-breeding salamander species; the relative abundance of sensitive species; the relative abundance of tolerant species; and the presence of spotted salamanders or wood frogs. Each metric scores 0, 3, 7, or 10 points and the scores from the five metrics are summed to provide the AmphIBI (see Appendix 1). An AmphIBI score of less than ten indicates low quality (Category 1), a score between 10 and 29 indicates moderate quality (Category 2) and a score 30 or greater indicates high quality (Category 3).

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APPENDIX 1 - AmphIBI wetland study sites, metrics and scores.

Wetland Name	year	CLASS	Oram Score (V 5.0)	Cat	AQAI	AQAI metric	Tol. Rel. Abund.	Tol. Metric	Sens. Rel. Abund.	Sens. Metric	Pond Sal. spp.	Pond Sal. Metric	Spotted Sal.	Wood Frog	AmphIBI
2 Meadows	1999	Shrub	49	2	2.97	0	0.880	0	0.028	3	2	3	10	0	16
Ackerman	1997	Forest	24	1	2.62	0	0.976	0	0.012	3	2	3	0	0	6
Area K Wetland	1999	Shrub	61.5	2	2.90	0	0.992	0	0.000	0	1	0	0	0	3
Big Woods	1999	Forest	68.5	3	5.63	10	0.000	10	1.000	10	2	3	10	0	43
Blackjack Rd (back)	2000	Shrub	67	3	2.19	0	0.993	0	0.005	3	0	0	0	10	13
Blackjack Rd (front)	2000	Forest	55.5	2	6.12	10	0.154	10	0.794	10	1	0	0	10	43
Blanchard Oxbow	1996	Shrub	48	2	3.00	3	0.582	3	0.000	0	2	3	0	0	9
Callahan	1997	Shrub	57.5	2	5.32	7	0.356	7	0.402	7	4	10	0	0	31
Cessna	1996	Shrub	61	3	2.85	0	0.557	3	0.081	3	2	3	10	0	19
Collier Woods	1999	Forest	73.5	3	6.93	10	0.007	10	0.993	10	1	0	0	10	43
Drew Woods	1999	Shrub	70	3	3.52	3	0.182	10	0.017	3	2	3	10	0	29
Eagle Cr Bog	2000	Shrub	81	3	6.93	10	0.013	10	0.988	10	1	0	0	10	43
Eagle Cr Vernal	2000	Forest	64	3	6.83	10	0.002	10	0.923	10	2	3	0	10	43
Flowing Well	1997	Forest	46	2	5.43	7	0.000	10	0.714	10	4	10	10	0	47
Fowler Woods	2000	Forest	79	3	6.20	10	0.131	10	0.738	10	4	10	10	10	50
Frieds Bog	2000	Shrub	76	3	4.46	3	0.481	7	0.481	7	1	0	0	10	30
Gahanna 4th	1999	Forest	67.5	3	2.99	0	0.848	0	0.004	0	4	10	10	0	20
Graham Rd	1999	Forest	28.5	1	2.45	0	0.763	0	0.000	0	2	3	0	0	3
Grand R Terraces	2000	Shrub	74	3	5.83	10	0.297	7	0.624	10	3	7	10	10	44
Hebron	1997	Forest	49.5	2	2.80	0	0.918	0	0.000	0	1	0	0	0	3
Hempelman	1997	Forest	48	2	2.42	0	0.790	0	0.026	3	3	7	0	0	10
Johnson Rd	1999	Forest	21	1	2.52	0	0.904	0	0.095	3	1	0	0	0	6
Keller High	1997	Shrub	65.5	3	5.15	7	0.234	10	0.618	10	5	10	10	0	47
Killdeer Plains	1999	Forest	53.5	2	6.23	10	0.017	10	0.750	10	2	3	0	10	43
Lawrence High	1997	Forest	73	3	5.92	10	0.350	7	0.538	10	3	7	0	0	34
Lawrence Low 2	1999	Forest	43	2	2.64	0	0.089	10	0.007	3	2	3	0	0	16
Leafy Oak	1999	Forest	78	3	5.28	7	0.127	10	0.413	7	4	10	10	0	44
McKinley	1996	Shrub	37.5	2	1.99	0	0.923	0	0.062	3	3	7	0	0	10
Oyer Wood Frog	1999	Shrub	69	3	6.80	10	0.003	10	0.934	10	3	7	0	10	47
Pallister	2000	Forest	74	3	6.99	10	0.044	10	0.853	10	3	7	10	10	47
Pawnee Rd	2000	Forest	70	3	7.00	1	0.000	10	0.997	10	1	0	0	10	34
Route 29	1997	Shrub	59	2	4.05	3	0.356	7	0.390	7	3	7	0	0	24
Sawmill	1997	Forest	52	2	2.52	0	0.000	10	0.000	0	1	0	0	0	13
Slate Run	1999	Shrub	76	3	5.33	7	0.083	10	0.303	7	4	10	10	10	44
Swamp Cottonwood	2000	Shrub	75	3	6.84	10	0.114	10	0.871	10	4	10	10	10	50
The Rookery	1999	Shrub	69	3	3.67	3	0.306	7	0.028	3	3	7	10	0	30
Tipp-Elizabeth Rd	1999	Forest	29	1	2.52	0	0.635	3	0.000	0	2	3	0	0	6
Towners Woods	2000	Shrub	74	3	6.88	10	0.015	10	0.977	10	2	3	0	10	43
Townline Rd	2000	Forest	61	3	4.39	3	0.529	7	0.306	7	2	3	0	10	30
US 42	2000	Forest	31	2	2.97	0	1.000	0	0.000	0	0	0	0	0	0