THE STATUS OF CRAWFISH FROGS (*LITHOBATES AREOLATUS*)

IN INDIANA, AND A TOOL TO ASSESS POPULATIONS

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ABSTRACT

The conservation status of Crawfish Frogs (*Lithobates areolatus*) in Indiana has changed over the past several decades. Once described as being locally plentiful, declines led to the listing of Crawfish Frogs as a State Endangered Species in 1988. Several records for this species in Indiana are > 50 yrs old and have gone unconfirmed for several decades. However, recent surveys have confirmed the continued presence of Crawfish Frogs in parts of southern Indiana, redefining the perceived range of this species in the state. In an effort to increase survey efficiency in this species, I used automated recording systems and manual call survey techniques to examine the chorusing phenologies of Crawfish Frogs at two sites along the northern extent of their range. Detection probabilities were determined as they related to season and environmental variables and survey duration. I also examined the effect that distance from wetland and position (ground level vs. approximate human ear level) had on call detection in automated recording systems. Correlations between call rates (calls/min) and numbers of male Crawfish Frogs present were used to calculate population estimates at 10 uncensused sites. Detection probabilities were highest when the frogs were breeding and when air temperatures were ≥ 13° C. Initial detection of Crawfish Frogs most frequently occurred during the first five min of sampling. Calls on automated recording units lost resolution as distance from wetland increased, and calls recorded at all distances at human ear level were measurably louder (in decibels) except at the wetland edge. Population estimates at uncensused sites ranged from a low of four to a high of 48. Using

call rates and numbers of male frogs present in wetlands, I present a “rapid assessment” tool that can be used to quickly calculate on-site estimates of Crawfish Frogs in field studies.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

A REVIEW OF THE STATUS AND DISTRIBUTION OF CRAWFISH FROGS (*LITHOBATES AREOLATUS*) IN INDIANA

INTRODUCTION

Crawfish Frogs (*Lithobates* [*Rana areolata*] *areolatus*) are a cryptic and comparatively understudied species distributed in portions of the Midwest, eastern Great Plains, and south-central United States (Lannoo 2005). Parris and Redmer (2005) described their distribution as "disjunct" with populations being "localized in areas of suitable habitat." Crawfish Frogs have experienced declines in Illinois, Indiana, and Iowa (Christiansen and Bailey 1991, Phillips et al. 1999, Minton 2001). In Indiana, Crawfish Frogs are listed as State Endangered. In Iowa, Crawfish Frogs are also listed as State Endangered, but they have not been documented in the state for several decades and may now be extirpated (Christiansen and Bailey 1991).

In their summary on the distributions of amphibians and reptiles of Illinois and Indiana, Smith and Minton (1957) identified Crawfish Frogs as a "western species," noting that most of their range occurs to the southwest of the two states. In Indiana, the majority of historic Crawfish Frog records are located in the western half of the state, extending from Benton County southward to the Ohio River (Minton 2001). An apparently isolated population occurs at Big Oaks National Wildlife Refuge in southeast Indiana (Haswell 2004).

Crawfish Frogs are known to occur in a variety of habitats including open damp areas, wooded mountain valleys, woodlands, and brushy fields (Bragg 1953, Phillips et al. 1999, Minton 2001, Parris and Redmer 2005). However, the northern subspecies *circulosus*, which occurs in Indiana, appears to favor grassland and has been found almost exclusively in this habitat in Oklahoma and Missouri (Bragg 1953, Johnson 2000). While Crawfish Frogs use grassland habitats in Indiana, much of their range appears to occur in areas that were largely forested during pre-settlement times (Jackson 1997, Minton 2001).

Crawfish Frogs are part of a four-species clade contained within the *Nenirana* group of Hillis and Wilcox (2005) that includes Gopher Frogs (*Lithobates* [*Rana*] *capito*) and Federally Endangered Dusky Gopher Frogs (*Lithobates* [*Rana sevosa*] *sevosus*). Both gopher frog species have a southern distribution along the Coastal Plains except for two isolated *L. capito* populations: one in central Alabama and one in Tennessee (Jensen and Richter 2005, Richter and Jensen 2005). Dusky Gopher Frogs have become extremely rare and are currently known from a single site in Harrison County, Mississippi (Richter and Jensen 2005). Gopher Frogs are a protected species in North Carolina, Florida, and Alabama (Jensen and Richter 2005). All three species are ecologically similar, occupying natural and artificial holes, or burrows made by other species (Richter et al. 2001, Parris and Redmer 2005, Bilhovde 2006).

While listed as Endangered in Indiana, the status of Crawfish Frogs in this state is poorly known. As a component of a larger study to understand the conservation biology of Crawfish Frogs in the northern extreme of their range, I provide an overview of the historic distribution of this species in Indiana, building upon the summary of Minton (2001) by incorporating more recent survey data.

HISTORIC OVERVIEW

Early reports of Crawfish Frogs in Indiana date to the latter half of the nineteenth century. Crawfish Frogs were first reported in Indiana in 1878 by F. L. Rice and N. S. Davis from Benton County (Rice and Davis 1878). This specimen was collected by E. F. Shipman and is deposited in the Chicago Academy of Sciences collection (CA 160; Rice and Davis 1878; Table 1). Willis Blatchley reported two additional specimens collected by C. Stewart and H. McIlroy from Vigo County in 1893 and 1894, respectively (Blatchley 1900).

Others contributing early records of this species in Indiana include R. Mumford, A. P. Blair, H. P. Wright and G. S. Myers of Indiana University (Wright and Myers 1927), and David and Paul Swanson, foresters for the Emergency Conservation Works and the Resettlement Administration (Swanson 1939; Table 1). Sherman Minton secured a number of specimens from 1949 to 1954, documenting the presence of Crawfish Frogs in at least seven additional counties. David Rubin reported Crawfish Frogs from a site now known as "Dave's Pond" in northern Vigo County (Rubin 1965). The majority of specimens collected from this site are deposited in the Indiana State University Vertebrate Collection.

Minton (1972, 2001) has provided the most thorough descriptions of the biology of Crawfish Frogs in Indiana. According to Minton, Crawfish Frogs were considered "locally plentiful" in western Indiana until about 1970 when populations began to experience unexplained declines. He noted the disappearance of this species at many localities in Indiana, including sites appearing to have experienced little change in habitat. Evansville resident M. J. Lodato witnessed the extirpation of Crawfish Frogs from three sites near Evansville, Vanderburgh County, all of which were likely extirpated by 1990 (Lodato, *personal communication*). One of these sites, located at Angel Mounds State Historic Site, apparently supported a population of >

100 breeding adults before its numbers diminished during the mid 1980s. Due to their increased rarity in Indiana, Crawfish Frogs were designated a Species of Special Concern in 1984 and elevated to State Endangered status in 1988 (S. Klueh, Indiana Department of Natural Resources, *personal communication*).

In March 2003, Daryl Karns, Joseph Robb, Erin Haswell, and others confirmed the presence of a large population of Crawfish Frogs located within Big Oaks National Wildlife Refuge (Haswell 2004). This discovery added Jefferson, Jennings, and Ripley counties to the Indiana distribution and extended the known range of Crawfish Frogs approximately 90 km eastward. The source of this apparently isolated population is not known and its status as a natural or introduced population has not yet been determined. Despite intensive surveys, no populations have been located outside the refuge.

MATERIALS AND METHODS

To assess the historic status of Crawfish Frogs in Indiana, I compiled a complete list of all known Crawfish Frog records in the state. The historic records of Crawfish Frogs in Indiana are based on locality data from museum and university specimens, literature accounts, Indiana Department of Natural Resources (IDNR) Division of Fish and Wildlife records, IDNR Division of Nature Preserves Heritage Database Center, and other reliable reports. I confirmed these records where possible by examining all known post-metamorphic museum specimens (Table 1). Many of the recent records contained in this report come from an Indiana Department of Natural Resources (IDNR) survey for Crawfish Frogs performed from 2004–2008 (Z. Walker, *personal communication*).

I contacted the following colleges and universities to inquire about possible specimens being stored in their collections: Indiana University-Bloomington, University of Notre Dame, Purdue University, Indiana University-Purdue University Fort Wayne, University of Indianapolis, University of Evansville, Ball State University, University of Southern Indiana, Oakland City University, Hanover College, St. Joseph's College, and Indiana State University.

Crawfish Frog records were obtained from the following sources: Wildlife Diversity Section, Division of Fish and Wildlife, Indiana Department of Natural Resources, (IDNR WDS), Indiana Natural Heritage Data Center, Division of Nature Preserves, Indiana Department of Natural Resources, (INHDC), Indiana State University Vertebrate Collection (ISUVC), Purdue Vertebrate Teaching Collection (PU), University of Michigan Museum of Zoology (UMMZ), Field Museum of Natural History (FMNH), Chicago Academy of Sciences (CA), Harvard University Museum of Comparative Zoology (HUMCZ), Carnegie Museum of Natural History (CM), Texas Cooperative Wildlife Collection (TCWC), California Academy of Sciences (CAS-SU), Hanover College Herpetology Collection (DRK), and the Indiana State Museum (INSM). Data were obtained from records held in the following institutions and accessed through HerpNET data portal (http://www.herpnet.org): TCWC, 16 September 2009; CAS-SU, 16 September 2009; and CM, 20 August 2009.

RESULTS

Minton (2001) included 23 counties in the range of Crawfish Frogs in Indiana. Sixteen of these counties are represented by point localities signifying reliable records and include Benton, Fountain, Vermillion, Vigo, Clay, Owen, Morgan, Sullivan, Greene, Monroe, Daviess, Martin, Pike, Dubois, Vanderburgh, and Warrick. Seven counties not represented by point localities include Warren, Parke, Putnam, Knox, Gibson, Posey, and Spencer. While not necessarily exhaustive (multiple records in a given county may be represented by a single point), Minton's account provides the most thorough compilation of Indiana distributional records for this species in the literature.

Indiana Department of Natural Resources surveys performed from 2004–2008 took place over a large portion of western and south-central Indiana, reaching 17 counties (in part or in whole) including Greene, Owen, Clay, Vigo, Sullivan, Knox, Daviess, Martin, Vermillion, Parke, Fountain, Orange, Lawrence, Pike, Dubois, Morgan, and Monroe (Z. Walker, *personal communication*). Crawfish Frogs were identified in seven of these counties including Vigo, Clay, Owen, Daviess, Sullivan, Parke, and Greene (IDNR, *unpublished data*). The IDNR surveys, however, did not detect Crawfish Frogs in several previously documented counties including Morgan, Monroe, Fountain, Vermillion, Pike, Martin, and Dubois.

The following is a historic (pre-2009) overview of Crawfish Frog records in Indiana based on museum specimens, literature accounts, and several other sources (see Materials and Methods). Accounts are arranged by county and are followed by a discussion and summary.

Benton County

Crawfish Frogs were first reported in Indiana from Benton County in 1878 (CA 160; Rice and Davis 1878, Minton 1998, Minton 2001). This record represents the type locality for the subspecies "*circulosus*" and the northernmost locality for the species in the state. The specific location of this site is unknown and the species has not been recorded in Benton County since (Minton 2001). Minton (2001) saw no reason to doubt the authenticity of this record and stated that he had taken Crawfish Frogs “within 30 miles” of the Benton County border. The specimen is currently deposited in the holdings of the Museum of the Chicago Academy of Sciences.

Clay County

Minton (2001) included two point localities for Crawfish Frogs in Clay County, and noted hearing a “spectacular chorus” on 2 April 1950. A Clay County specimen collected on 2 April 1950 (UMMZ 101623) by Minton likely corresponds to the location of the large chorus heard that night. Russell Mumford collected a Crawfish Frog from northern Clay County on 7 April 1958 (UMMZ 118078). David Rubin and P. Allen collected a specimen near Bowling Green on 18 April 1966 (ISUVC 1492). Indiana Department of Natural Resources personnel reported hearing Crawfish Frogs near Brazil on 26 March 2007.

Daviess County

Paul Swanson provided the earliest report of Crawfish Frogs from Daviess County. He reported frequently hearing Crawfish Frogs from “within the city limits of … Odon” (Swanson 1939). Minton and W. M. Overlease collected a Daviess County specimen on 21 March 1953 (UMMZ 108125). This record likely corresponds to the single point locality Minton (2001) shows on his distribution map and is the only voucher specimen known from the county. Indiana Department of Natural Resources personnel reported a cluster of four call points northeast of Odon between 2004 and 2008 which appear to be distinct from the sites reported by Swanson (1939) and Minton and Overlease. These surveys also revealed a cluster of breeding sites in south-central Daviess County.

Dubois County

Swanson (1939) identified Crawfish Frogs from Dubois County and characterized them as “quite plentiful.” This observation likely corresponds to a point locality given by Minton (2001). Surveys performed by IDNR personnel from 2004–2008 failed to find this species, and therefore the status of Crawfish Frogs in Dubois County is unknown.

Fountain County

Fountain County is represented by a single voucher specimen (FMNH 64663) collected near Kingman by Minton on 18 April 1951. This animal was reported from a shallow pond in a cultivated field (Alan Resetar, Field Museum of Natural History, *unpublished data*). Surveys performed by IDNR between 2004 and 2008 were unable to confirm the presence of Crawfish Frogs at this site and therefore the status of Crawfish Frogs in Fountain County is unknown.

Greene County

At least 17 Crawfish Frog reports come from Greene County, with most arising from the western portion. A number of these records are based on recent IDNR surveys. Minton deposited an animal collected on 25 March 1949 in the University of Michigan Museum of Zoology (UMMZ 100304). This Crawfish Frog represents the only voucher specimen known from Greene County. Minton (2001) includes two points in his distribution map, one of which appears to correspond to the UMMZ specimen.

Crawfish Frogs were reported from the Goose Pond basin, south of Linton in 2002 by Matt Blake and Vicky Meretsky (INHDC). Indiana Department of Natural Resources survey work in the Goose Pond basin from 2004–2008 identified Crawfish Frogs in six areas, including a confirmation of the Blake and Meretsky record. These surveys also identified localities on reclaimed coal mine land in northwestern Greene County.

Martin County

Swanson (1939) included Martin County in a list of counties where Crawfish Frogs were "quite plentiful," and reported frequently hearing them from within the city limits of Loogootee. No voucher specimens are known. The point locality given by Minton (2001) likely corresponds to Swanson's Loogootee observation.

Monroe County

Wright and Myers (1927) reported finding a population "two miles west of Bloomington" on 21 March 1926. This record is supported by specimens deposited in the California Academy of Sciences (CAS–SU 2174-80, 13343–64). Mittleman (1947) reported the collection of one juvenile and an unknown number of tadpoles by H. T. Gier from a small pond "four miles north of Bloomington" on 12 April 1940. These specimens were deposited in the Ohio University collection (OUZ A1126), but appear to have been relocated and may now be lost (S. Moody, Ohio University, *personal communication*). A series of transforming tadpoles (UMMZ 95312) dated 19 July 1940 with the locality description of "Bloomington" were deposited in the University of Michigan Museum of Zoology by A. P. Blair. These specimens may be associated with those collected by Gier that same year (Mittleman 1947). Minton (2001) apparently considered the localities reported by Wright and Myers (1927) and Mittleman (1947) to be the same "colony", even though the collection notes give distinctly different locality descriptions ("two miles west of Bloomington" for Wright and Myers, and "four miles north of Bloomington" for Mittleman). In his 1972 monograph, Minton describes the location as occurring in the "grassy valley of Beanblossom Creek" (Minton 1972). Both populations may be extirpated, as no recent records exist for either of these locations in Monroe County.

A more recent locality for Monroe County was given by Al Parker, who reported sighting two individuals at a wetland near Bloomington along the Beanblossom Creek bottoms on 23 March 1991 (INHDC, Parker, *personal communication*). Indiana Department of Natural Resources personnel were unable to confirm the presence of Crawfish Frogs at this site from 2004–2008, despite numerous visits. Crawfish Frogs are presumed to be extirpated from this location.

The most recent record for Monroe County comes from Brodman (2003), who reported a call record at an unnamed locality. Little is known about this observation, and the status of this population is unknown.

Morgan County

Robert Luker collected two individuals from Monrovia in early April 1978 (INSM 71.7.170–171). This record likely corresponds to a point locality given by Minton (2001) and appears to represent the easternmost voucher record in this species' contiguous range in Indiana. Crawfish Frogs may have occurred at more than one site prior to 1980 (IDNR Amphibian and Reptile Technical Advisory Committee 1987), and an INHDC record indicates that the species persisted at Monrovia until at least 1987. Indiana Department of Natural Resources surveys from 2004–2008 failed to detect Crawfish Frogs near Luker's Monrovia site, and populations there may be extirpated.

Owen County

Minton collected Crawfish Frogs from Owen County on 25 March 1954 (UMMZ 110638). Minton (2001) included two records for the southern half of Owen County, one of which is likely the UMMZ specimen. Indiana Department of Natural Resources personnel detected a single population of Crawfish Frogs near the Owen-Clay County line in March 2007. This locality, a cluster of small wetlands on reclaimed coal mine property, represents the only known extant population in Owen County.

Parke County

Indiana Department of Natural Resources surveys identified a single population of Crawfish Frogs in Parke County on 26 March 2007. The locality description associated with this record is somewhat obscure and the exact location of the site is unknown. The status of Crawfish frogs at this site and throughout the county is undetermined.

Pike County

Swanson and Swanson (Swanson 1939) collected a series of Crawfish Frogs from Winslow that are now deposited in the Carnegie Museum of Natural History (CM 13371–13375). John Tritt collected a single Crawfish Frog “near Spurgeon” on 25 June 1963 (ISUVC 2473). Surveys conducted by IDNR from 2004–2008 did not detect Crawfish Frogs in Pike County, and the status of the species there is unknown.

Spencer County

Crawfish Frogs were discovered near Newtonville in Spencer County in 1998 by D. S. Dougas (M. J. Lodato, *personal communication*). Frogs at this site appear to be using a series of breeding ponds situated over several acres on reclaimed mine land (Lodato, *personal communication*). No voucher specimens have been collected. A second locality, located ca 6.5 km from the original site, was discovered in 2008 by Lodato, who identified and photographed a single adult male crossing a highway during a heavy rainstorm (Lodato, *unpublished data*). The breeding wetland has not been identified. Because of the distance between these localities, they appear to represent separate populations. Brodman (2003) reported detecting Crawfish Frogs from an unnamed locality in Spencer County.

Sullivan County

Sullivan County contains at least 26 Crawfish Frog records, with most occurring in the east-central region. Vouchered records include a specimen collected by Minton on 21 March 1952 near Shelburn (UMMZ 105544) and a single adult collected by John Whitaker, Jr. near Sullivan during the first week of June 1969 (ISUVC 2255). Timm (2001) identified 14 Crawfish Frog localities in her report on anuran use of reclaimed and unreclaimed mine areas. She reported Crawfish Frogs from a variety of habitats including a ditch, slough, beaver impoundment, and larger “final cut” strip pits. Voucher specimens are not known from this study and the current status of Crawfish Frogs at these sites is unknown. Brodman (2003) reported Crawfish Frogs from an unnamed locality in Sullivan County.

Surveys performed by IDNR from 2004–2008 reported nine Crawfish Frog localities from Sullivan County including sites near Cass, Hymera, and Dugger. Most of these sites represent call points located along roadways.

Indiana Department of Natural Resources property manager Ron Ronk reported hearing Crawfish Frogs calling from a private wetland complex north of Dugger every year from 2004–2008 (Ronk, *personal communication*). Stuart Smith reported finding a Crawfish Frog after a hard rain near Lake Sullivan on 20 May 2002 (INHDC). Voucher specimens are not available for these records.

Vanderburgh County

A specimen collected by P. L. Swanson and D. C. Swanson on Route 41 in Vanderburgh County on 28 March 1936 is deposited in the Carnegie Museum of Natural History (Swanson 1939; CM 13378). Other sites known to have supported Crawfish Frogs include Angel Mounds State Historic Site near Newburgh. This site, which held a robust population containing an estimated 100 adults in 1980, had shrunk to fewer than 10 breeding individuals in 1987 (IDNR Amphibian and Reptile Technical Advisory Committee 1987). The population was apparently extirpated by 1990 (M. J. Lodato, *personal communication*). Two nearby sites located in Evansville were destroyed by suburban development shortly after the demise of the Angel Mounds site (Lodato, *personal communication*). Extant populations of Crawfish Frogs are not known from any sites in Vanderburgh County.

Vermillion County

Minton collected a specimen on 18 April 1951 from a "shallow pond" near Perrysville in northern Vermillion County (UMMZ 103361); this represents the only known site for Vermillion County. The Vermillion and Benton County records appear to be the two northernmost records in Indiana, and the only populations known to occur west of the Wabash River. The current status of the Vermillion County population is unknown.

Vigo County

Crawfish Frogs were first reported in Vigo County from two sites by Blatchley, who received two specimens collected by C. Stewart at "the south part of the city of Terre Haute" on 8 and 9 October 1893 and a third specimen collected by H. McIlroy "three miles west from where the others were secured" on 9 May 1894 (Blatchley 1900). Locality data for these sites are vague, but a single point locality given by Minton (2001) may represent these two sites. Two Vigo County specimens collected by Blatchley deposited in the Harvard University Comparative Museum of Zoology (HUMCZ A-7043, A-7044) have a collecting date of 09 October 1903. Though specific locality data are not known, these specimens appear to be distinct from the ones previously reported by Blatchley (1900).

An additional locality was identified by Rubin (1965) in northeast Vigo County on 24 March 1964. This area (Dave's Pond) contains at least three distinct wetlands and has been visited numerous times over the past several decades by researchers from Indiana State University. A number of voucher specimens have been collected from this site (ISUVC 395–97, 399–400, 401–403 [eggs only], 937, 2738, 2793, 2822, 3177 [eggs only], 3204–07; PU 8482–83). Crawfish Frogs were present at this site in 2008 (M. J. Lannoo, *unpublished data*). A specimen collected by E. G. Zimmerman on 6 April 1964 (TCWC 66467) contains the locality description "5 mi NE Terre Haute" and may correspond to the Dave's Pond complex. John Whitaker and Rubin collected a specimen about three miles ENE of Dave's Pond near Fontanet on 30 March 1967 (ISUVC 1820). An additional frog was observed in the base of a broken metal pole about three miles west of Dave's pond around the late 1960's (J. O. Whitaker, *personal communication*).

Indiana Department of Natural Resources personnel identified a site near the Parke County line in 2007. This location is situated in a low, flat basin near Raccoon Creek. A specific breeding site has not been identified but a series of small wetlands is present and may be used.

Warrick County

Swanson (1939) includes Warrick County in a list of counties in which Crawfish Frogs are described as being "quite plentiful." However, Minton et al. (1982) note that "some colonies in Vanderburgh and Warrick counties have been destroyed by surface mining, drainage, and urban expansion." Lodato reported Crawfish Frogs from three sites near Elberfeld, Millersburg, and Paradise that were apparently destroyed by mining operations and subsequent housing developments (Lodato, *personal communication*). To my knowledge, no Warrick County specimens have been vouchered, and no extant populations are known from the county.

Jefferson, Jennings, and Ripley Counties

Records for Jefferson, Jennings, and Ripley counties are all located within Big Oaks National Wildlife Refuge, and thus have been placed together here. The suspected presence of Crawfish Frogs at Big Oaks in the spring of 1999 was confirmed in March 2003 (Hauersperger

2005). Three specimens collected by Daryl Karns, Joseph Robb, Erin Haswell, and Diana Schuler on 18 March 2003 have been deposited in the Field Museum of Natural History and Hanover College Herpetology Collection (Jefferson Co: FMNH 262589; Ripley Co: FMNH 262588, DRK 381). Haswell (2004) identified 23 sites at Big Oaks: 21 breeding call locations, two sight records. At least one of these sites is located in Jennings County, which encompasses the northwestern portion of the refuge. Crawfish frogs have been detected in all three counties within Big Oaks every year since their initial discovery at the refuge (J. Robb, *personal communication*). Breeding choruses at Big Oaks tend to be widely scattered and relatively small (typically < 10 individuals, often < 5).

DISCUSSION

With the exception of a few outlying records, Crawfish Frogs historically were known from the southwest quarter of the state, west of the unglaciated region of south-central Indiana. Their recent discovery at Big Oaks National Wildlife Refuge in southeastern Indiana suggests either that the species has a broader, long-undetected, range in the state, or that animals were introduced into the former Jefferson Proving Grounds (Haswell 2004).

Post-glacially, Crawfish Frogs may have become established in the scattered prairies that arose in the region that would become Vigo, Sullivan, Clay, Greene, Knox, and Daviess counties (Betz 1976). While this does not provide an explanation for the existence of populations in the southern two tiers of counties where prairie was apparently not as common (Betz 1976), it is possible that grassy river valleys and unforested flood plains may have supported the species there. Populations occurring in unglaciated, forested areas near Bloomington, Indiana may have existed under similar conditions (Minton 1972). Natural disturbances such as wildfires and bison activity may have also contributed to the eastward expansion of this species into predominantly forested areas of Indiana.

Smith and Minton (1957) suggested that Crawfish Frogs are part of a group of prairie dwelling species that were already declining in numbers prior to Euro-American settlement due to natural changes in the environment. They surmise that relic populations of several western species occurring in Illinois and Indiana "provide almost irrefutable evidence of a retreating grassland fauna." If true, it could explain the occurrence of Crawfish Frog populations in non-grassland habitats in southern Indiana.

In addition to grasslands and seasonal or semipermanent wetlands, Crawfish Frogs also seem to be at least somewhat dependent on the presence of burrowing crayfish. The answer to why Crawfish Frogs were not found in the historic prairie peninsula of northwest Indiana may be that the sandy soils of the "Kankakee Sands Section" (Homoya et al. 1985) do not support burrowing crayfish (Thoma and Armitage 2008). Another possible explanation may be related to the climatic conditions that occur in northwest Indiana. Colder winters in this part of the species' range could be a limiting factor in restricting the northward extension of Crawfish Frogs into other parts of the Prairie Peninsula. The latitude of Indiana's northernmost record in Benton County is similar to that of the northernmost distributional records of Crawfish Frogs in Iowa (Christiansen and Bailey 1991, Parris and Redmer 2005).

Several records occurring along the northern and eastern edge of this species' contiguous range are at least 50 years old and have not been reconfirmed since at least 1955. These records include sites in Benton, Vermillion, Fountain, Martin, and Dubois counties. Though apparently confirmed at a relatively recent date (1987), populations in Morgan County now appear to be extirpated (INHDC, IDNR Amphibian and Reptile Technical Advisory Committee 1987).

Along the southern edge of their Indiana range, Spencer County alone is known to currently support Crawfish Frogs. All other counties in the lower two tiers of southwest Indiana either lack records, have suffered extirpations, or have not had older records (≥ 45 yrs) reconfirmed. The presence of Crawfish Frogs in six Indiana counties (Benton, Fountain, Vermillion, Martin, Dubois, and Pike) has not been verified for at least 45 years (Fig. 1).

Crawfish Frogs appear to be doing well in two areas where, paradoxically, ecosystems were severely degraded in the recent past. Several records have been identified in the large reclaimed coal mine region in western Greene and eastern Sullivan Counties, many of which fall within 11 km of the Greene-Sullivan County line. A recent record for Spencer County (M. J. Lodato, *personal communication*) also occurs on what appears to be reclaimed mine land. Big Oaks National Wildlife Refuge is located at the former Jefferson Proving Grounds. Approximately twenty five million rounds of artillery were discharged there from 1941–1994, and recovery impact fields were subject to herbicide applications, soil sterilents, and disking (K. Knouf, *personal communication*) Despite this history, Haswell (2004) identified 23 Crawfish Frog locality records from Big Oaks. At least one Greene County reclaimed coal mine site and parts of Big Oaks National Wildlife Refuge are currently being maintained as grasslands. Habitat restoration in the form of managed grasslands appears to have favored Crawfish Frogs at these sites. It is evident that once Crawfish Frogs reach these vast grassland sites (whether naturally or anthropogenically), they have the capacity to do well.

In summary, our understanding of the status and distribution of Crawfish Frogs in Indiana has changed over the past several decades. Formerly described as being “locally plentiful” (Minton 2001), declines in this species led to its inclusion on the State Endangered Species List. A lack of recent records in several counties along the northern and eastern periphery of the species' contiguous range and the destruction of several breeding sites further south suggests that Crawfish Frogs may no longer exist in many of the areas they were previously reported to occur.

Habitat destruction resulting from human activities such as mining, suburban development, and farming have likely played a role in the extirpation of localized Crawfish Frog populations. However, the cause of the post-1970 declines noted by Minton (2001) remains unknown. Despite their Endangered status in Indiana, Crawfish Frogs continue to persist in scattered, sometimes clustered, populations in southwestern Indiana. Their discovery at Big Oaks NWR in southeast Indiana extends their range approximately 90 km east of where they were previously known to occur. The presence of this species at sites that have been restored from intense ecological destruction highlights the ability of Crawfish Frogs to colonize/recolonize areas where suitable habitat is present. This stresses the importance of protecting existing populations which can potentially serve as source populations for new colonies, and gives hope for the prospects of successful Crawfish Frog restoration in the future.

CHAPTER 2

A NOVEL USE OF CALLING SURVEYS TO DETERMINE DETECTABILITY AND STATUS OF NORTHERN POPULATIONS OF CRAWFISH FROGS, A SPECIES OF CONSERVATION CONCERN

INTRODUCTION

Over the past two decades, concern over worldwide amphibian declines has led to a considerable global conservation effort (Wake 1991, Wake and Morowitz 1991, Houlahan 2000, Alford et al. 2001, Stuart et al. 2004, Lannoo 2005). Despite these endeavors, the life history and natural history information necessary to conserve amphibian species remains unevenly known. In the New World, for example, North American species are often better known and understood than their Central and South American counterparts (Haddad 2008, Lannoo et al. in press), although this is not true for every species or species group.

Among United States species, Crawfish Frogs and their closest relatives, Gopher Frogs and Dusky Gopher Frogs, are secretive animals, with adults of all three species occupying burrows, including those made by other animals (Richter et al. 2001, Parris and Redmer 2005, Blihovde 2006, Hoffman et al. 2010). This is particularly true of Crawfish Frogs, which occupy crayfish burrows and will quickly retreat into them in response to disturbance (Thompson 1915, Hoffman et al. 2010). Hobart Smith (1950) once noted, "… no other species of *Rana* in this country possess such secretive habits." Because of these cryptic behaviors, Crawfish Frogs are among the least well-known species of North American, indeed New World, ranids.

In an effort to enact management programs to conserve Crawfish Frogs, we need techniques to effectively determine the location and status of their populations. As Smith (1950) implied, Crawfish Frogs are so secretive that surveys of upland adults are impractical. Surveys of breeding adults, on the other hand, offer promise. Gerhardt (1975) found that, when recording at 1 m, Crawfish Frogs produced calls that were more powerful (mean 107.5 decibels [dB]) than any of the twenty other species of North American frogs examined. Indeed, male Crawfish Frogs produce breeding calls that can carry a kilometer (Swanson 1939), and the roar of a large Crawfish Frog chorus has been likened to a distant motor speedway (Minton 2001). Perhaps no other Midwestern amphibian shows such a deeply binary pattern of detectability—Crawfish Frogs are virtually undetectable for most of the year, but heard from long distances when calling during the breeding season (Swanson 1939, Minton 2001).

Call surveys have become widely used for monitoring and assessing frog and toad populations (Zimmerman 1994, Scott and Woodward 1994, Mossman et al. 1998, Weir and Mossman 2005, Steelman and Dorcas 2010). Standardized monitoring programs such as the North American Amphibian Monitoring Program (NAAMP; http://www.pwrc.usgs.gov/naamp/) and Frog Watch (http://www.naturewatch.ca/english/select_province.html) have increased in popularity and have been implemented in several states and provinces in the United States and Canada (Weir and Mossman 2005).

Building on the techniques of manual call surveys, automated recording systems (ARS) such as "frog-loggers" (Peterson and Dorcas 1994, Saenz et al. 2006) and Song Meters® (Wildlife Acoustics Inc., Concord Massachusetts, USA; Waddle et al. 2009) are increasingly being used as a means of surveying anuran species (Bridges and Dorcas 2000, Mohr and Dorcas 1999, Oseen and Wassersug 2002). Automated recording systems allow researchers to compile extensive datasets without being physically present at survey sites. The deployment of numerous ARS allows separate, even distant, sites to be sampled simultaneously, in a uniform way (i.e., without observer bias) and allows researchers to essentially “capture time,” enabling them to evaluate recordings as many times as needed. Automated recording systems may be the most effective way of surveying for species that cease calling in response to disturbance, or with irregular or short breeding seasons (Dorcas et al. 2010).

Given the ability of breeding call surveys to detect Crawfish Frogs, the advantages of using ARS to give a complete and accurate record of chorusing, and the tenuous conservation status of this species (Parris and Redmer 2005), I had three goals: 1) To determine the optimal daily and environmental factors for monitoring calling males at two breeding sites where the number of males was known; 2) To determine the effect of distance on Crawfish Frog call detectability; and 3) Using data from the first goal in a novel way, to estimate sizes of populations (using calling males as a proxy) along the current northern extreme of their range. This approach provides a survey and monitoring tool for Crawfish Frogs that provides rapid assessments of population sizes using ARS data.

MATERIALS AND METHODS

Determining Call Rates Based on Seasonal, Daily, and Environmental Factors

Data were collected from two wetlands located in the western section of Hillenbrand Fish and Wildlife Area (HFWA) in Greene County, Indiana. The portion of HFWA utilized by

Crawfish Frogs is situated on a reclaimed surface coal mine, and is managed as prairie by the Indiana Department of Natural Resources (Lannoo et al. 2009). The first site, Nate's Pond, is a shallow (< 0.5 m) seasonal/semi-permanent wetland approximately 0.14 ha in surface area that dries completely during late summer and early fall. Emergent vegetation includes rushes (*Scirpus* sp.), cattails (*Typha* sp.) and small willows (*Salix* sp.). The second site, Cattail Pond, is a relatively shallow (< 1.5 m), semi-permanent wetland approximately 0.33 ha in surface area. Cattails predominate except in the deepest portion, which is open, and in a small, disturbed area along the north edge of the wetland. The two wetlands are approximately 0.9 km apart. These wetlands were chosen because they are known Crawfish Frog breeding sites, and drift fences had been constructed around their perimeters, allowing a census of Crawfish Frog populations (Kinney 2009, Kinney and Lannoo 2009).

Crawfish Frog calling activity was recorded using Song Meter® recording units (models SM1 and SM2, Wildlife Acoustics Inc., Concord Massachusetts, USA). Chorusing data were collected for two months in 2010, from 1 March to 30 April, a time period that encompasses the known breeding season for this species in Indiana (Minton 2001). One recording unit was placed adjacent (< 5 m) to each wetland shoreline on the ground hidden by vegetation, near areas where Crawfish Frog choruses were heard in 2009 (V. C. Kinney, *unpublished data*). Song Meter® units were programmed to record continuously for 8 h segments beginning at 1900 EST and ending at 0300 the following morning. This time frame corresponded to the daily calling period (up to several hours after sunset) noted by Busby and Brecheisen (1997) and Minton (2001).

Environmental variables were recorded, as follows. Air temperature, relative humidity, rainfall amount, and wind speed were logged at 10-min intervals using a HOBO® Micro Station (Onset Computer Corporation, Pocasset, MA) weather station located at a secure site approximately 3.5 km from Nate's Pond and 3.2 km from Cattail Pond. Water temperatures of each wetland were recorded at 30-min intervals using submerged HOBO® Pendent Data Loggers. Data from each of these environmental measurements (with the exception of rainfall) were linearly interpolated to 1-min intervals in order to match the resolution of the Crawfish Frog calling data. Rainfall amounts were averaged to 1-min resolution.

From the drift fence data, counts of breeding Crawfish Frogs present in each wetland were made throughout the study (frogs inside the fence were assumed to be in the wetland, and males were assumed to be contributing to the chorus). In order to process frogs as they moved into and out of breeding ponds, and to prevent injury to the frogs (in an effort to work their way through drift fences Crawfish Frogs often abrade their snouts; Heemeyer et al. 2010), researchers were sometimes present at the wetlands, when frogs were expected to be migrating in and out of breeding wetlands (i.e., during warm rainy nights). Calling data recorded during these times, including a 5-min time lag after researchers had left the area, were censored before datasets were analyzed.

Determining the Effect of Distance on Detectability

I used Song Meter® recording units and a human listener (the author) to examine the effect of distance on ARS results and manual call surveys. Three times during the 2010 breeding season (24 March, 31 March, and 10 April), recording units were placed at four listening stations located 0 m, 50 m, 100 m, and 200 m from a third known Crawfish Frog breeding site (Big Pond) at HFWA. This wetland was used to avoid disturbing frogs and affecting recordings at Nate's Pond and Cattail Pond. Song Meters® were placed at ground level (throughout this study recording units were concealed in vegetation at ground level to prevent detection, and the arrangement of this array mimicked these conditions) and programmed to record simultaneously. A fifth unit was attached to a wooden tripod ~2 m above the ground near human ear level and moved with the listener from station to station (see below). This unit permitted comparisons with ground-level recordings and human perception. All surveys were completed within the daily time frame required by NAAMP protocol (30 min after sunset to 0100; Weir and Mossman 2005).

In addition, 30-min manual call surveys were performed at each ARS station (200 m, 100 m, 50 m, and 0 m, in that order). Each survey was divided into six consecutive 5-min sampling periods (0:00–5:00, 5:00–10:00, 10:00–15:00 … 25:00–30:00). To determine a human perception of calling, Crawfish Frog chorusing was quantified using the NAAMP three-level calling index (Weir and Mossman 2005), with call levels defined as follows:

1. Individuals can be counted, space between calls;
2. Calls of individuals can be distinguished, some overlapping of calls;
3. Full chorus, calls are constant, continuous, and overlapping.

Environmental variables were recorded on site at the beginning and end of each 30-min survey with the aid of a Kestrel® 4000 Pocket Weather Tracker (Nielsen-Kellerman, Co., Chester, Pennsylvania, USA) and included wind speed, sky conditions, air temperature, relative humidity, and moon visibility.

The effect of distance on recording units was quantified by measuring average sound pressure level readings (in dB) from each station. Ground level recordings were analyzed using Song Scope® call recognition software (Song Scope®, Wildlife Acoustics Inc., Concord Massachusetts, USA), and decibel levels of 25 randomly selected calls occurring between approximately 2015 and 2300 hrs EST (times varied between rounds) were recorded for each listening station. This analysis was performed for all three rounds of sampling, and averaged values for each listening station were combined between rounds so that a single value represented each listening station.

This same analysis was performed on the tripod recording units. Because the tripod was moved to different listening stations, randomly selected calls were taken from 30-min recording sessions at each listening station for each round (as opposed to the approximately 2:45 h recordings used on ground level units). Five tripod recordings were compromised by the presence of static-like clicks (at the 0 m listening station during Round 1, and all stations during Round 2). Two tripod recordings during Round 3 had only five and eight measureable calls (at the 100 m and 200 m listening stations, respectively). Thus, the above recordings were not included in the final analysis.

Statewide Surveys of Known Historic and Current Sites

Using Song Meter® recording units, I surveyed for the presence of Crawfish Frogs at localities in western Indiana where Crawfish Frogs had historically occurred, but where recent records were lacking. Surveys took place from 25 March–11 April, 2010, where the most recent known records ranged from 1949 to 1991 (Engbrecht and Lannoo 2010). These sites were Daviess County East, Fountain County, Greene County North, Monroe County, Morgan County, Owen County, Sullivan County North, Vermillion County, and Vigo County East (Appendix A). In cases where the precise locality was unknown (older museum records and literature accounts often contain vague locality descriptions), wetland habitat in the vicinity of the original locality description was selected as the sampling site. Surveys were also performed at nine additional sites where the presence of Crawfish Frogs was confirmed in 2009 (i.e., current sites; N. J. Engbrecht, *unpublished data*): Daviess County North, Daviess County South, Daviess County Southwest, Daviess County Southeast, Greene County Northwest, Green County East, Green County West, Sullivan County East, and Vigo County West. Surveys were performed at one additional site where their presence was suspected (Daviess County Northeast).

Song Meter® units were programmed to record from 1900 to 0300 EST, placed at wetlands (which I term uncensused wetlands because population sizes had not been determined), and checked on a daily basis. Digital recordings were analyzed on site using a laptop computer and Song Scope® Software. Following the technique of MacKenzie and Royle (2005), recording units were removed after Crawfish Frogs were detected. If Crawfish Frogs were not heard after three nights the recording unit was removed.

Other recent records of Crawfish Frogs occur in extreme southern Indiana (two localities in Spencer County) and in southeast Indiana (Big Oaks National Wildlife Refuge; Engbrecht and Lannoo 2010). The population at Big Oaks National Wildlife Refuge is currently being studied by Dr. Joseph Robb, Perry Williams, and Dr. Daryl Karns. Performing surveys at these sites was not logistically feasible for this study; therefore they were excluded from sampling.

Analysis of Recordings

All recordings at Nate's and Cattail Pond were analyzed manually, by visually and audibly inspecting sonograms of the recordings produced by Song Scope® software. Statewide historic and current sites containing Crawfish Frog calls were also analyzed using this method. Because the typical breeding call of Crawfish Frogs consists of a single distinct snore, individual calls could usually be identified (exceptions occurred during especially dense chorusing or when there was interference from outside sources such as road traffic or thunderstorms).

Call counts for each recording bout were compiled by the author or by two trained technicians. The unit of measure was calls/min (Duellman and Trueb 1986, Nelson and Graves 2004), and only the typical, distinct breeding call (as opposed to the elongated aggressive call produced during male to male encounters; Elliot et al. 2009) was included in call counts. This manual approach, while time consuming, gave much more accurate counts than could be obtained through automated call recognition programs (Song Scope® and Raven® [Raven®, www.birds.cornell.edu/raven]; N. J. Engbrecht, *unpublished data*; see Waddle et al. 2009 for a discussion of some of the difficulties associated with using recognition software for anuran call analysis).

Distant chorusing and road traffic noise at four sites (Daviess County North, Owen County, Sullivan County East, and Vigo County West) prevented a comprehensive examination of entire recordings. As a result, when estimating population sizes, call counts were made from periods on the recordings containing the most intense chorusing.

Data Analysis

Detection estimates were established by randomly selecting 100 30-min samples from the calling data recorded at each pond. These analyses were performed using random numbers generated using Program R® (Program R® 2.10.1, The R Foundation for Statistical Computing, Vienna, Austria). Detection estimates were obtained from each pond by examining chorusing rates within four different scenarios, encompassing situations where surveyors would consider searching: 1) March and April (the timeframe for breeding in Indiana Crawfish Frog populations;

Minton 2001); 2) The breeding season (i.e., between the time when chorusing began and ended at each pond: 11 March–14 April at Nate's Pond; 16 March–4 April at Cattail Pond); 3) When temperatures at the beginning of each sampling period were ≥ 13° C during the breeding season (chorusing in Crawfish Frogs has been associated with air temperatures ≥ 13° C [Busby and Brecheisen 1997, Minton 2001]); and 4) When it was raining at the beginning of each sampling period during the breeding season (Smith et al. 1948, Smith 1950). Only 89 samples were available at Cattail Pond when it was raining during the breeding season, and all were used in this analysis. The 30-min samples were sub-divided into six consecutive 5-min periods, allowing me to examine the effect of survey duration on detection probability (Pierce and Gutzwiller 2004).

Population Estimates of Historic and Current Sites

Two approaches were used to calculate population estimates at statewide sites investigated in this study. The first approach was specific to my data set and used the simple ratio:

$$a/b = y/x$$

Where:

a = number of males present at the censused study site;

b = maximum calls/min at the censused study site;

y = number of males present at the uncensused site;

x = maximum number of calls/min at the uncensused site.

To solve for y, I multiplied both sides of the equation by x to get:

$$y = (a/b) \cdot x$$

When calculating these estimates, a and b were date-matched to y. This approach controlled for weather variables, which were assumed to be equal at all sites (the greatest distance between any of these sites was ~ 63 km). The effectiveness of this method was tested using data from Nate's Pond to predict the number of males present in Cattail Pond, and vice versa. In all cases, the results of this test either closely or exactly matched the actual number of males present in each wetland (Table 2).

The second approach used to estimate the number of males present is more generalizable, and was based on the linear regression:

$$y = 0.12\,(x) - 0.38$$

Where:

y = number of calling males in uncensused population;

x = maximum calls/min in uncensused population (Fig. 2).

The constants 0.12 and -0.38 were calculated from data on maximum calls/min and the respective number of males present at Nate's Pond and Cattail Pond during the breeding season. This represented the maximum calling potential for the given number of males present. In one case, when four males were present (which occurred for < 3 h on a single night), the maximum call rate equaled zero. These data were not included in the regression analysis. For this regression, probability that the slope $\neq 0$ was 0.0001, and $r^2 = 0.83$. At statewide sites, to calculate number of males from call data, maximum call rates (x) from uncensused populations were entered and the equation was solved for number of calling males (y) at each site. Statistical analyses were performed using STATISTICA® (STATISTICA® 8.0, StatSoft, Inc., Tulsa, OK).

The regression equation was used in a second way. It was applied to call counts used to establish NAAMP levels, and from these estimates, numbers of calling males based on NAAMP levels could be established.

Two additional steps were required to obtain overall population estimates from numbers of calling males. To estimate the total number of males in the population, I multiplied the number of calling males (y in both equations) by two, representing the average ratio (1:2.5) of calling males to total males during peak calling on each night of the breeding season at Nate's and Cattail ponds (all adults breed; J. L. Heemeyer, *unpublished data*). To obtain total population estimates, I doubled the calculated number of males (because sex ratios approximate 1:1; V. C. Kinney, *unpublished data*) to give an overall estimate of breeding adults in the population.

RESULTS

Determining Call Rates Based on Seasonal, Daily, and Environmental Factors

Using data compiled from Song Meter® recording units, chorusing dates during the 2010 breeding season ranged from 11 March–14 April at Nate's Pond and from 16 March–4 April at Cattail Pond (Fig. 3). Chorusing levels varied within the breeding season at both sites, with peak chorusing (calls/min) occurring in both wetlands during the same 4-d period between 30 March and 2 April (Fig. 3). The beginning of the breeding season at each site was marked by a gradual increase in chorusing levels interrupted by nights of little to no calling. After peak breeding, calling dropped off sharply at the end of the season at both wetlands (Fig. 3).

Within nights, mean calling intensity at both sites increased during the first hour of sampling (0700–0800), and after a period of peak chorusing lasting about 1–2 hrs, grew weaker as the night progressed (Fig. 4). On average throughout the breeding season, calling at Nate's Pond abruptly rose from scattered calling (< 5 calls/min) around 0700, to peak calling (> 25 calls/min) around 0745–0845, then gradually tapered off to < 10 calls/min at 0300 (Fig. 4). Average calling at Cattail Pond steadily rose during the first 30 min of sampling and peaked (> 10 calls/min) for about 2 hrs. Low level calling (< 5 calls/min) continued after 2200.

Crawfish Frogs reduce calling rates, and often stop calling, when disturbed (Wright and Myers 1927, Swanson 1939, Minton 2001; M. Redmer, *personal communication*). To illustrate, I present a plot of a strong chorus recorded on a Song Meter® unit at Nate's Pond on 24 March interrupted repeatedly (3x) by researchers checking drift fences for entering Crawfish Frogs (Fig. 5). A comparison of call levels during and in the absence of disturbance (when chorusing levels were high, between 1930 and 0200) from the data presented in Figure 5 demonstrate that Crawfish Frogs call significantly less during human-induced disturbances (undisturbed = 42.7 calls/min; disturbed = 15.0 calls/min; $p < 0.001$; Independent t-test). For this analysis, I discarded data for five min after each disturbance (because maximum call rates may not resume immediately), and I assumed the number of males present during disturbed and undisturbed periods was equal.

The probability of detecting Crawfish Frogs (DP) varied with season and environmental factors (Table 3; Fig. 6). The environmental factors chosen here were air temperature and rainfall, which are thought to trigger calling or breeding activity in Crawfish Frogs (Smith et al. 1948, Smith 1950, Busby and Brechesien 1997, Minton 2001). Analyses of subsamples taken from Nate's Pond indicated that humidity was not significantly correlated with calling intensity (p = 0.8), nor was wind speed (p = 0.5). Water temperatures were correlated with air temperatures (p < .00001), but because I was interested in the application of easily determined envrionmental variables to call surveys, I focused on air temperatures, which can be generally measured.

At Nate's Pond, randomly chosen 5-min samples (NAAMP protocol) from March and April, irrespective of knowing whether Crawfish Frog breeding had begun or ended, yielded a 0.30 DP. That is, any 5-min sampling period from 1900–0300 in March or April 2010 at Nate's Pond would have offered a 30% chance of hearing calling Crawfish Frogs. Restricting survey days to when Crawfish Frogs were known to be breeding at Nate's Pond (11 March–14 April), DP rose to 0.75. Further restricting sampling to the time when Crawfish Frogs were known to be breeding and when air temperatures were ≥ 13° C (~55° F, thought to be the temperature minimum for optimally detecting Crawfish Frogs; Busby and Brecheisen 1997, Minton 2001) DP again rose, but only marginally, to 0.76. In contrast, restricting sampling to when Crawfish Frogs were known to be breeding and it was raining dropped DP to 0.41.

At Cattail Pond, DP for March and April was 0.20, and for breeding season (16 March–4 April) was 0.49 (Table 3; Fig. 6). Detection probabilities for samples within the breeding season and when temperatures were ≥ 13° C increased dramatically to 0.91. In contrast, DP for samples selected within the breeding season during rains dropped to zero.

Detection probabilities improved, but only slightly, with duration of sampling period (Table 3; Fig. 6). A second way to state this is that first-time detection of Crawfish Frogs typically occurred during the initial five min of sampling (Table 3). The greatest increases in detectability occurred at Cattail Pond during the breeding season (an increase of 0.06 when extending surveys by five min; an increase of 0.14 when surveys were extended from five min to

30 min). The smallest increase in DP when extending surveys from five min to 30 min occurred at Nate's Pond when raining during the breeding season and at Cattail Pond during March and April. In both cases DPs increased by 0.01. In 11 cases, extending survey duration by five min did not increase DP at all. Regardless of site or sampling parameter, extending survey duration by 5-min increments increased DP values only slightly after the initial five min of sampling.

The Effect of Distance on Crawfish Frog Call Detectability

Crawfish Frogs calls were detected by each of the ground level Song Meter® units (0 m, 50 m, 100 m, and 200 m away from the edge of Big Pond) during all three rounds of sampling (24 March, 31 March, and 10 April). As expected, the resolution of calls on sonograms decreased as distance from wetland increased (Fig. 7). Additionally, calls originating from at least one other site (Nate's Pond) were detected by the recording units. Calls from these two wetlands became increasingly difficult to distinguish with distance away from Big Pond and towards Nate's Pond until at the 200 m station when they became indistinguishable. The 200 m station was approximately 550 m from Nate's Pond, but situated uphill, which likely enhanced the detection of calls from that site.

Recording units placed on the tripod near human ear level (~2 m) averaged higher decibel readings that those placed on ground level at three of four listening stations (Fig. 8). Sound pressure levels of calls measured from tripod recordings were 2.5 dB greater than ground level recordings at the 50 m listening station, 3.9 dB greater at 100 m listening station, and 1.8 dB greater at 200 m listening station. Interestingly, calls from the tripod recording unit at the edge of the wetland averaged 17.7 dB *lower* than the ground (wetland) level unit.

Crawfish Frogs were detected from all four distances from Big Pond (0 m, 50 m, 100 m, and 200 m) by the human listener, though call levels could not be assessed at the 200 m listening station on 24 March and 31 March because chorusing from two other sites blended with Big Pond choruses. Call level indexes show that chorusing was at its highest on 24 March (mean call index of 2.8 compared to 1.6 on 31 March and 1.0 on 10 April). Analysis of ground level audio recordings at the wetland edge allowed for a comparison between calls/min and NAAMP chorusing rank: Level 1 calling ranged from 0–16 calls/min; level 2 from 26–72 calls/min; and level 3 from 54–92 calls/min (Table 4).

Occupancy Surveys of Historic and Current Sites

Crawfish Frogs were detected at one of nine historic sites (Owen County; Fig. 9). At this site, sonograms indicated calling from a distant area, beyond the immediate proximity of the recording unit. A subsequent visit by the author confirmed the presence of Crawfish Frogs in an area south of the original sampling site. Aerial images (viewed using Google Earth®) show a small wetland located 1 km south of the sampling location that may be the source of chorusing detected by the recording unit.

Loud chorusing was heard from eight current sites (Daviess County North, Daviess County South, Daviess County Southwest, Daviess County Southeast, Greene County Northwest, Green County East, Green County West, and Vigo County West) and distant calls were detected from the ninth site (Sullivan County East; Fig. 9). Calls were not heard from an additional site (Daviess County Northeast) suspected to contain Crawfish Frogs.

DISCUSSION

Using ARS techniques at wetlands where the number of males present was known, I was able to: 1) Describe Crawfish Frog call characteristics as they occurred through the duration of the breeding season and during the portion of the night when the majority of calling occurred (Figs. 3, 4); 2) Establish detection probabilities as they related to time, weather variables, and survey duration (Fig. 6); 3) Determine the effect of distance on call detection (Figs. 9, 10); and 4) Develop a model based on maximum call rate that can be used to estimate population at uncensused sites. After first discussing the results of call characteristics and detection probabilities, I will use chorusing data to estimate population sizes at historic and current sites.

Optimizing Detection Probabilities: When Should Crawfish Frog Call Surveys be Conducted?

The data collected here suggest that to optimize detectability, Crawfish Frog surveys should be conducted: 1) After reports of calling have been received (Fig. 6); 2) About an hour after sunset (Fig. 4); 3) When temperatures are ≥ 13° C (~55° F; Fig. 6, Table 3); 4) When it is not raining (Fig. 6); and 5) Under conditions where frogs are not disturbed (Fig. 5).

At Nate’s Pond, DP during 5-min samples increased from 0.30 during March and April to 0.75 during breeding season, and marginally increased to 0.76 when temperatures were ≥ 13° C (Table 3; Fig. 6). At Cattail Pond, detection increased from 0.20 during March and April to 0.49 during the breeding season, and further increased to 0.91 when temperatures were ≥ 13° C (Table 3; Fig. 6).

Rainfall decreased detection probabilities (Table 3, Fig. 6). Five-minute samples from Nate’s Pond when rain was falling during the breeding season yielded a DP of only 0.41, and decreased DP at Cattail Pond to zero. These numbers are lower than what would be expected given the information provided in the literature regarding rainfall and breeding in this species (Smith 1950, Barbour 1971, Minton 2001). Careful interpretation of these results should be considered, however, as low detectability during rainfall in this study may be due to at least two factors. First, researchers studying Crawfish Frogs at the two study sites were often present on rainy nights. Because these pond disturbance times were censored during data analyses, removing these times resulted in a portion of data associated with rainfall being excluded from the analyses, creating a partial bias against rainfall. Secondly, only 89 data points (minutes) when rainfall occurred during the breeding season at Cattail Pond were available as a pool from which random samples were to be selected; all of these points were drawn from 21 and 22 March, nights without chorusing at Cattail Pond.

Rainfall may play a more significant role in initiating the Crawfish Frog breeding season itself by triggering migration and filling up breeding wetlands (Barbour 1971, Busby and Brecheisen 1997). Smith et al. (1948) noted “warm weather (exceeding 10° C) in spring is insufficient alone to stimulate breeding activity; a certain amount of rainfall is required in addition.”

Optimizing Detection Probabilities: How Long Should Crawfish Frog Surveys Last?

Detection probabilities improved only slightly when survey length was extended (Table 3; Fig. 6). Initial detection of Crawfish Frogs most frequently occurred during the first five min of sampling, and in the majority of samples, occurred during the first min.

Standardized surveys have used 3-min (Shirose et al. 1997), 5-min (NAAMP; Weir and Mossman 2005), and 10-min (Hemesath 1998) listening periods. My data show that, when detected, Crawfish Frogs were usually heard during the first five min of sampling, and increasing sampling effort (by 5-min increments) provided comparatively little gain in terms of detection (Fig. 6). This was true across all survey conditions. Shirose et al. (1997) found that 3-min surveys appeared adequate to sample for the presence/absence and calling intensity of species in their study. They also found that in the majority of instances, all species identified at a given site were heard within the first min of surveying. A similar trend in early detection was observed in this study.

Determining the Effect of Distance on Detectability

Crawfish Frog calls recorded at short distances can be easily recognized using sonograms produced by Song Scope® software. As expected, calls viewable on sonograms generally decrease in quality as the distance from the sampling wetland increases. Distant calls originating from other wetlands can also appear on sonograms, and using ARS to distinguish between the sources of different calls is difficult. Calls originating from Big Pond are easily recognized at the 0 m and 50 m listening stations (Fig. 7). At the 100 m station, these calls become more difficult to distinguish from calls originating from other wetlands. At 200 m, they are indistinguishable from these calls.

The inability to detect Crawfish Frog calls by ear from the 200 m listening station in this study highlights an interesting aspect of call detection, and is likely due to a combination of at least two factors. While the overall terrain at the listening stations is relatively level, the 200 m listening station is located down a gently sloping decline, with a short portion of land occurring between the listening station and Big Pond. Conversely, Nate's Pond is located further down the incline, about 550 m in the opposite direction of Big Pond. Despite being over two times greater than the distance of Big Pond, the landscape between the 200 m listening station and Nate's

Pond provides for an amphitheater-like arrangement, with the listening station being raised above the pond. As a result, calls from Big Pond were likely weakened by land and vegetation interference, and calls from Nate's Pond carried more freely to the listening station, drowning out calls from Big Pond. During Round 3, when overall chorusing was less intense, calls coming from Big Pond could be detected at the 200 m listening station by the human listener.

One limitation to using ARS recording units is their inability to discern the directional origin of sound. As noted above, calls lose visual and audible resolution as distance from the call source increases. When analyzing sonograms in this study, researchers most always (except under certain circumstances) counted strong calls present on sonograms, excluding distant sounding calls that were understood to be coming from another wetland. This allowed for an estimate of calls occurring in the immediate vicinity (wetland of interest). This approach would likely become less practical at larger wetlands, where frogs could potentially call from distant parts of the wetland. In a few cases during this study, calls at Big Pond detected by the human listener were not detected (counted) during analyses of recordings. This resulted in underdetection using call counts from ARS recordings. Conversely, analyses of call counts using ARS recordings identified calls that were not detected during on-site manual call surveys. In these cases, the manual call survey approach underdetected Crawfish Frog chorusing, and the ARS unit aided in more accurate detection.

The effectiveness of using ARS techniques for anuran sampling depends largely on the objectives of the research. Studies focusing on calling metrics at specific wetlands may need to consider the effects of external calls originating from other sites. In such cases, recording units should be placed close to the wetland of interest to eliminate the possibility of data contamination (via external calling), with consideration of the size of the wetland as noted above.

This is less of an issue for studies that are not “pond-specific,” but that focus on detecting any and all species within “earshot” of the recording unit.

The results of this study indicate that Song Meter® recording units have the ability to detect Crawfish Frogs from over 500 m distance. Detection of distant calls recorded on a Song Meter® was also observed at the Owen County study site. The author visited this site on 9 April and heard Crawfish Frog calls coming from an area ≥ 600 m from the original recording site. The specific location was not identified, but the chorus appeared to have possibly been coming from a small wetland 1 km from the original recording site. This audible distance is consistent with values reported by Swanson (1939) and Minton (2001) for detection with the human ear.

Conclusions About Occupancy Based on Detection

Crawfish frogs were heard at only one of nine historic sites surveyed. The detection of Crawfish Frogs at the Owen County site reconfirms a 46 year-old record (Engbrecht and Lannoo 2010), and represents one of only two known extant populations in the county. The non-detection of Crawfish Frogs at the eight other historic sites may reflect Minton’s (2001) observations of the disappearance of Crawfish Frogs from several sites in Indiana; six of my sampled historic sites were based on location data accompanying Minton’s museum specimens (Engbrecht and Lannoo 2010).

Imprecise locality descriptions did not improve chances of detectability. However, the carrying power of Crawfish Frog breeding calls and the recording units’ ability to pick up distant chorusing would have improved opportunities for detection.

Crawfish Frogs were heard calling at all areas where they were found in 2009. This result documents the ongoing presence of this species in a region where declines are known to have occurred (Minton 2001). It also validates the effectiveness of ARS techniques in detecting Crawfish Frogs at sites where calling occurs at low levels, representing potentially smaller populations (i.e., Daviess County North, Daviess County Southeast, and Greene County Northwest; Fig. 9). These are populations that might have been missed using standardized manual call survey techniques.

Population Estimates

Two methods of different utility and precision were used to determine population estimates in this study, and a third method is examined for its potential use in estimating populations. The first method was specific to this study and consisted of a simple, ratio-based approach using detailed population data from an on-site study. Using this technique, I was able to estimate the number of calling males at uncensused statewide historic and current sites by taking call rate and number of males present from Nate's and Cattail ponds and date-matching them with call data from uncensused sites. Date-matching allowed me to control for weather variables occurring that night. This value was doubled, once to account for the ratio of males present to males in the entire population (based on approximate ratios at Nate's and Cattail ponds; V. C. Kinney, *unpublished data*), then doubled again to account for females (based on an estimated 1:1 sex ratio; V. C. Kinney, *unpublished data*). A test of this approach using data from Nate's Pond to predict number of males present at Cattail Pond, and data from Cattail Pond to predict number of males present at Nate's Pond yielded accurate population assessments (Table 2).

Using this approach I obtain estimates for uncensused statewide historic and current sites (Table 5; Fig. 9). From these data, it appears that Greene County Northwest, Daviess County

Southeast, Daviess County North are most in peril (estimates between four and eight breeding adults), while Sullivan County East, Greene County West, Owen County, and Vigo County West are more robust (estimates between 16 and 48 breeding adults). All of these populations are considerably smaller than those reported from southern Illinois (Cagle 1942), and these population sizes warrant concern.

The second approach used in this study is more generalizeable, and involves the simple regression equation: $y = 0.12(x) – 0.38$, where y = population at the uncensused site, and x = maximum call rate at the uncensused site. By reducing this equation researchers can take the number of calls per six seconds, and subtract 0.4 to get an estimate of calling males—multiply by four to get an estimate of population size. If this rapid assessment approach is used, I suggest repeating the survey several times and using the maximum call rate observed.

Because this formula is based on maximum call rates, and because Crawfish Frog chorusing is variable even with the same number of males present, this population estimate provides the researcher with a minimum estimated number of frogs. Frogs can always call less than their maximum potential; they can never call more. Therefore, estimating populations based on call counts that are below maximum provide researchers with conservative population estimates.

Using this regression approach, I obtained population estimates that were often less than those obtained using the ratio approach (Table 5). This could be, in part, because the regression approach does not control for seasonal or weather variables. Differences in population estimates between the ratio and regression methods should be considered by researchers and weighed against the benefit of quick data collection. The ratio approach requires a reference site at which the number of males present and call rate is known, and obtaining these numbers (particularly population estimates) can be time consuming. Additionally, due to regional differences in season and weather, this method is most appropriately used to sample populations occurring in the same region as the reference site. The advantage of this method is that it may produce more precise population estimates because it helps control for weather variables. The design of the regression approach does not require data from a reference site (the data is built in from this study), and requires only the call rate from the uncensused site. Because of its design, this approach has the capacity to be used across a broader geographic range; though because it does not control for weather variables, it may result in less precise population estimates.

Another approach examined in this study is also generalizable, but less precise. It relies on NAAMP survey calling indices. In this approach, NAAMP calling indices were matched with maximum call rates (calls/min) obtained from ARS units placed at the wetland edge during manual call surveys. This allowed for a comparison of how call rate (calls/min) corresponded with each of the NAAMP calling index levels (Table 4). The imprecision of this technique lies partially in the qualitative nature of using call index values as opposed to counting numbers of calls. Additionally, considerable overlap occurs between call rates for Level 2 and Level 3 (Table 4). This approach, however, could be used by NAAMP coordinators with data that has already been collected by survey volunteers, and would require relatively little effort to apply. Additional research on the relationship between call rate and call index value should further understanding as to how population estimates can be calculated using NAAMP survey protocols.

Estimating numbers of calling males and overall population sizes using these methods rests on certain assumptions: 1) The relationship between the number of males present and call rate (calls/min) is linear; 2) Half of all males in the population are present; 3) All adults breed; 4)

Sex ratios are 1:1; 5) Disturbances did not occur; and 6) Chorusing is at maximum call rate. These assumptions are discussed below.

1. *Call rate is linearly proportional to number of males present*. Data from Nate's Pond and Cattail Pond showed a linear relationship (Fig. 2). It is not known if this linear trend would continue in large populations (an aggregation estimated to be 500 Crawfish Frogs was reported by Philip Smith in southern Illinois [Cagle 1942]). In a study of Green Frogs (*Lithobates clamitans*), Nelson and Graves (2004) found that the relationship between number of males and calls was roughly linear when up to 10 males were present, but as density increased (> 50 males) calling became asymptotic, with call rates peaking at about 45 calls/min. It may be that at higher densities of male Crawfish Frogs calling in this species becomes asymptotic.

2. *At any given time half of all males in the population are calling.* This may be the weakest of the five assumptions since the number of males present in a wetland is known to change throughout the breeding season. To assume that all males in the population are present would underestimate the total population, but assuming that only a small percentage of males are present (such as 10%) would grossly overestimate the population values. In this study, assuming a 1:2 ratio of males present to total number of males represents a balance, and reflects the overall ratio of 1:2.5 that occurred during peak calling on each night of the breeding season at Nate's and Cattail ponds (V. C. Kinney, *unpublished data*).

3. *All adults breed*. Studies at HFWA indicate that all adult Crawfish Frogs breed each year, and that breeding takes place once per season (J. L. Heemeyer, *unpublished data*). This

may not hold true in southern populations, where warmer climates and tropical storms may permit fall breeding, as is the case with closely related Gopher Frogs (*Lithobates captio*) and Dusky Gopher Frogs (*L. sevosus*; Richter and Seigel 2002, Richter et al. 2003).

4. *Sex ratios are 1:1*. Research at our study site show overall adult sex ratios at Nate's and Cattail ponds approximate 1:1, although numbers varied between wetlands and between years (2009, 2010; V. C. Kinney, *unpublished data*).

5. *Calling is not influenced by disturbance.* Human disturbance lowers calling rates in Crawfish Frogs (Fig. 5). To eliminate the effects of human interference, periods when researchers were present at Nate's and Cattail ponds were censored before analyses. Steps to help eliminate disturbance when performing onsite surveys include avoiding approaching breeding wetlands, turning off vehicle headlights and stereos, and, when possible, giving a time lag of up to five min before conducting call counts.

6. *Chorusing is at maximum call rate*. Population estimates in this study are based on maximum call rates; thus, chorusing levels detected during surveys are assumed to be at peak levels. Because Crawfish Frogs are capable of calling below their maximum potential (i.e. at less than maximum call rates), estimating populations from less than maximum chorusing would result in more conservative (lower) population estimates. The approaches examined in this study assume that calling rates detected during surveys are at maximum.

Implications

This study offers a rapid assessment tool that can be used to monitor frog populations in a practical and novel way. Certain characteristics of Crawfish Frog breeding make this tool particularly useful. The loud, distinct breeding call allows for the calculation of call rates (via manual counts or using ARS) from proximal or remote distances. The relatively short breeding season in which peak chorusing is even further compressed (Fig. 3) demands that researchers make good use their time; this tool allows for short visits to numerous sites. Repeated visits to study sites over the course of the breeding season should help account for changing call intensities, providing researchers with opportunities to hear peak chorusing and thereby improving population estimates.

While this tool was developed to study Crawfish Frogs, it has the potential to be used with other species when certain assumptions are met. The purpose of this tool is to provide (minimum) population estimates, however, and does not provide the absolute counts that methods such as drift fence/pitfall trapping can provide. Is this tool final? The answer of course, is “no”. Research-based tools can most always be refined. This tool was built on extensive data sets from two wetlands (Nate’s Pond and Cattail Pond), and increasing the number of wetlands would certainly improve the model. As in all studies, however, time, resources, and knowledge of the species were limited.

This study represents a new step toward understanding a species and how to save it. It also represents a step toward developing a tool that can be used in studying other anuran species using a rapid assessment approach that saves time and allows researchers to expand their search area by visiting (or revisiting) more sites. In situations where populations (or species) are at risk, time can be the most limiting factor.

Because of their propensity to call, frogs present researchers with an opportunity to locate, study, and—in dire circumstances—save them. Identifying how and when to detect frogs, and identifying the status of populations are important components to anuran conservation. Finding answers to these questions (or finding out how to answer them) is an ongoing process. The application of these answers, however, may reach beyond the confines of a given species, and can potentially benefit anuran conservation as a whole.

REFERENCES

Alford, R. A., P. M. Dixon, and J. H. K. Pechmann. 2001. Ecology: Global amphibian population declines. Nature 412:499–500.

Barbour, R. W. 1971. Amphibians and Reptiles of Kentucky. University Press of Kentucky, Lexington, Kentucky, USA.

Betz, R. F. 1976. The prairies of Indiana. Prairie Conference Proceedings. Pages 25–31 *in* D. C. Glenn-Lewin and R. Q. Landers, Jr., editors. Prairie Conference Proceedings. Iowa State University, Ames, Iowa, USA.

Blatchley, W. S. 1900. Notes on the batrachians and reptiles of Vigo County, Indiana (II). Annual Report to the Indiana Department of Geology and Natural Resources 24:537–552.

Blihovde, W. B. 2006. Terrestrial movements and upland habitat use of Gopher Frogs in Central Florida. Southeastern Naturalist 5:265–276.

Bragg, A. N. 1953. A study of *Rana areolata* in Oklahoma. The Wasmann Journal of Biology 11:273–319.

Bridges, A. S., and. M. E. Dorcas. 2000. Temporal variation in anuran calling behavior: Implications for surveys and monitoring programs. Copeia 587–592.

Brodman, R. 2003. Amphibians and reptiles from twenty-three counties of Indiana. Proceedings of the Indiana Academy of Science 112:43–54.

Busby, W. H., and W. R. Brecheisen. 1997. Chorusing phenology and habitat associations of the Crawfish Frog, *Rana areolata* (Anura: Ranidae), in Kansas. Southwestern Naturalist 42:210–217.

Cagle, F. R. 1942. Herpetological fauna of Jackson and Union counties, Illinois. American Midland Naturalist 28:164–200.

Christiansen, J. L., and R. M. Bailey. 1991. The Salamanders and Frogs of Iowa. Iowa Department of Natural Resources. Nongame Technical Series, Number 3, Des Moines, Iowa, USA.

Dorcas, M. E., S. J. Price, S. C. Walls, and W. J. Barichivich. 2010. Auditory monitoring of anuran populations. Pages 281–298 *in* C. K. Dodd, editor. Amphibian ecology and conservation. Oxford University Press, Oxford, U.K.

Duellman,W. E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill, New York, New York, USA.

Elliot, L., C. Gerhardt, and C. Davidson. 2009. The Frogs and Toads of North America: A Comprehensive Guide to Their Identification, Behavior, and Calls. Houghton Mifflin Harcourt, Boston, Massachusetts, USA.

Engbrecht, N. J., and M. J. Lannoo. 2010. A review of the status and distribution of Crawfish Frogs (*Lithobates areolatus*) in Indiana. Proceedings of the Indiana Academy of Science 119:64–73.

Gerhardt, H. C. 1975. Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. Journal of Comparative Physiology 102:1–12.

Haddad, C. F. B. 2008. Amphibian Declines: The Conservation Status of United States Species. By Michael Lannoo, editor. Copeia 1:245–246.

Haswell, E. 2004. Northern Crawfish Frogs (*Rana areolata circulosa*) at Big Oaks National Wildlife Refuge in Southeastern Indiana. Unpublished Independent Study. Hanover College, Hanover, Indiana.

Hauersperger, B. A. 2005. Breeding Ecology of the Northern Crawfish Frog, *Rana areolata circulosa*. Unpublished Independent Study. Hanover College, Hanover, Indiana.

Heemeyer, J. L., V. C. Kinney, N. J. Engbrecht, and M. J. Lannoo. 2010. The biology of Crawfish Frogs (*Lithobates areolatus*) prevents the full use of telemetry and drift fence techniques. Herpetological Review 41:42–45.

Hemesath, L. M. 1998. Iowa's frog and toad survey, 1991–1994. Pages 206–216 *in* M. J. Lannoo, editor. Status and Conservation of Midwestern Amphibians. University of Iowa Press, Iowa City, Iowa, USA.

Hillis, D. M., and T. P. Wilcox. 2005. Phylogeny of the new world true frogs (*Rana*). Molecular Phylogenetics and Evolution 34:299–314.

Hoffman, A. S., J. L. Heemeyer, P. J. Williams, J. R. Robb, D. R. Karns, V. C. Kinney, N. J. Engbrecht, and M. J. Lannoo. 2010. Strong site fidelity and a variety of imaging techniques reveal around-the-clock and extended activity patterns in Crawfish Frogs (*Lithobates areolatus*). Bioscience 60:829–834.

Homoya, M. A., D. B. Abrell, J. R. Aldrich, and T. W. Post. 1985. The natural regions of Indiana. Proceedings of the Indiana Academy of Science 94:245–268.

Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature 404:753–755.

Indiana Department of Natural Resources. 1987. Amphibian and Reptile Technical Advisory Committee Meeting Notes. Unpublished data. Indianapolis, Indiana.

Indiana Natural Heritage Data Center. 2009. State of Indiana, Department of Natural Resources, Division of Nature Preserves. Indianapolis, Indiana.

Jackson, M. T. (editor) 1997. The Natural Heritage of Indiana. Indiana University Press, Bloomington, Indiana, USA.

Jensen, J. B., and S. C. Richter. 2005. *Rana capito*. Pages 536–538 *in* M. J. Lannoo, editor. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, California, USA.

Johnson, T. R. 2000. The Amphibians and Reptiles of Missouri. Second edition. Missouri Department of Conservation, Jefferson City, Missouri, USA.

Kinney, V. C. 2009. Breeding biology of Crawfish Frogs (*Lithobates areolatus*) in Southwestern Indiana. Indiana Academy of Science, Meeting Abstracts, pp. 81–82. Accessible at http://www.indianaacademyofscience.org/Events-Meetings/Annual-IAS-Meetings-Archive.aspx

Kinney, V. C., and M. J. Lannoo. 2010. *Lithobates areolatus*. Breeding. Herpetological Review 41:197–198.

Lannoo, M. J. (editor) 2005. Amphibian Declines. The Conservation of United States Species. University of California Press, Berkeley, California, USA.

Lannoo, M. J., V. C. Kinney, J. L. Heemeyer, N. J. Engbrecht, A. L. Gallant, and R. W. Klaver. 2010. Mine spoil prairies expand critical habitat for endangered and threatened amphibian and reptile species. Diversity 1:118–132.

Lannoo, M. J., A. L. Gallant, and R. W. Klaver. In press. Conservation Status of North American Amphibians *in* H. Heatwole, editor. Amphibian Biology. Vol. 9. Surrey Beatty & Sons, Baulkhlam Hills, Australia.

Levell, J. P. 1997. A Field Guide to Reptiles and the Law. Revised Second edition. Serpent's Tale, Lanesboro, Minnesota, USA.

MacKenzie, D. I., and J. A. Royle. 2005. Designing occupancy studies: General advice and allocating survey effort. Journal of Applied Ecology 42:1105–1114.

Minton, S. A., Jr. 1972. Amphibians and Reptiles of Indiana. Indiana Academy of Science. Monograph Number 3, Indianapolis. Indiana, USA.

Minton, S. A., Jr. 1998. Observations on Indiana amphibian populations: a forty-five-year overview. Pages 217–220 *in* M. J. Lannoo, editor. Status and Conservation of Midwestern Amphibians. University of Iowa Press, Iowa City, Iowa, USA.

Minton, S. A. Jr. 2001. Amphibians and Reptiles of Indiana. Second Edition. Indiana Academy of Science, Indianapolis, Indiana, USA.

Minton, S. A., Jr., J. C. List, and M. J. Lodato. 1982. Recent records and status of amphibians and reptiles in Indiana. Proceedings of the Indiana Academy of Science 92:489–498.

Mittleman, M. B. 1947. Miscellaneous notes on Indiana amphibians and reptiles. American Midland Naturalist 38:466–484.

Mohr, J. R., and M. E. Dorcas. 1999. A comparison of anuran calling patterns at two Carolina bays in South Carolina. The Journal of the Elisha Mitchell Scientific Society 115:63–70.

Mossman, M. J., L. M. Hartman, R. Hay, J. R. Sauer, and B. J. Dhuey. 1998. Monitoring long-term trends in Wisconsin frog and toad populations. Pages 169–198 *in* M. J. Lannoo, editor. Status and Conservation of Midwest Amphibians. University of Iowa Press, Iowa City, Iowa, USA.

Nelson, G. L., and B. M. Graves. 2004. Anuran population monitoring: Comparison of the North American Amphibian Monitoring Program's calling index with mark-recapture estimates for *Rana clamitans*. Journal of Herpetology 38:355–359.

Oseen, K. L., and R. J. Wassersug. 2002. Environmental factors influencing calling in sympatric anurans. Oecologia 133:616–625.

Parris, M. J., and M. Redmer. 2005. *Rana areolata*, Crawfish Frog. Pages 256–258 *in* M. J. Lannoo, editor. Amphibian Declines: The Conservation of United States Species. University of California Press, Berkeley, California, USA.

Peterson, C. R., and M. E. Dorcas. 1994. Automated data acquisition. Pages 47–57 *in* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster, editors. Measuring and Monitoring Biological Diversity—Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC, USA.

Phillips, C. A., R. A. Brandon, and E. O. Moll. 1999. Field Guide to Amphibians and Reptiles of Illinois. Illinois Natural History Survey. Manual 8, Champaign, Illinois, USA.

Pierce, B. A., and K. J. Gutzwiller. 2004. Auditory sampling of frogs: Detection efficiency in relation to survey duration. Journal of Herpetology 38:495–500.

Rice, F. L., and N. S. Davis. 1878. *Rana circulosa*. Page 355 *in* D. S. Jordan, editor. Manual of the Vertebrates of the Northern United States. Jansen and McClurg, Chicago, Illinois, USA.

Richter, S. C., and J. B. Jensen. 2005. Dusky Gopher Frog. Pages 584–586 *in* M. J. Lannoo, editor. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, California, USA.

Richter, S. C., and R. A. Seigel. 2002. Annual variation in the population ecology of the endangered Gopher Frog, *Rana sevosa*, Goin and Netting. Copeia 2002:962–972.

Richter, S. C., J. E. Young, G. N. Johnson, and R. A. Seigel. 2003. Stochastic variation in reproductive success of a rare frog, *Rana sevosa*: Implications for conservation and for monitoring amphibian populations. Biological Conservation 111:171–177.

Richter, S. C., J. E. Young, R. A. Seigel, and G. N. Johnson. 2001. Postbreeding movements of the Dark Gopher Frog, *Rana sevosa* Goin and Netting: Implications for conservation and management. Journal of Herpetology 35:316–321.

Rubin, D. 1965. Amphibians and Reptiles of Vigo County, Indiana. M. A. Thesis, Indiana State University, Terre Haute.

Saenz, D., L. A. Fitzgerald, K. A. Baum, and R. N. Conner. 2006. Abiotic correlates of anuran calling phenology: The importance of rain, temperature, and season. Herpetological Monographs 20:64–82.

Scott, N. J., and B. D. Woodward. 1994. Surveys at breeding sites. Pages 118–125 *in* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster, editors. Measuring and Monitoring Biological Diversity—Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC, USA.

Shirose, L. J., C. A. Bishop, D. M. Green, C. J. MacDonald, R. J. Brooks, and N. J. Herlferty. 1997. Validation tests of an amphibian call count survey technique in Ontario, Canada. Herpetologica 53:312–320.

Smith, H. M. 1950. Handbook of Amphibians and Reptiles of Kansas. University of Kansas Museum of Natural History, Miscellaneous Publication, Number 2, Lawrence, Kansas, USA.

Smith, H. M., C. W. Nixon, and P. E. Smith. 1948. A partial description of the tadpole of *Rana areolata circulosa* and notes on the natural history of the race. American Midland Naturalist 39:608–614.

Smith, P. W., and S. A. Minton Jr. 1957. A distributional summary of the herpetofauna of Illinois and Indiana. American Midland Naturalist 58:341–351.

Steelman, C. K., and M. E. Dorcas. 2010. Anuran calling survey optimization: Developing and testing predictive models of anuran calling activity. Journal of Herpetology 44:61–68.

Stuart, S. N, J. S. Chanson, J. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Swanson, P. L. 1939. Herpetological notes from Indiana. American Midland Naturalist 22:684–695.

Thoma, R. F., and B. J Armitage. 2008. Burrowing Crayfish of Indiana. Final Report to the Indiana Department of Natural Resources.

Thompson, C. 1915. Notes on the habits of *Rana areolata* Baird and Girard. Occasional Papers of the Museum of Zoology, Number 10, University of Michigan, Ann Arbor, Michigan, USA.

Timm, A. 2001. Frog and toad populations in reclaimed and unreclaimed areas of southwestern Indiana, Sullivan and Pike counties. Unpublished Report, Indiana University, Bloomington.

Todd, M. J., R. R. Cocklin, and M. E. Dorcas. 2003. Temporal and spatial variation in anuran calling activity in the western Piedmont of North Carolina. Journal of the North Carolina Academy of Science 119:103–110.

Waddle, J. H., T. F. Thigpen, and B. M. Glorioso. 2009. Efficacy of automatic vocalization recognition software for anuran monitoring. Herpetological Conservation and Biology 4:384–388.

Wake, D. B. 1991. Declining Amphibian Populations. Science 253:860.

Wake, D. B., and H. J. Morowitz. 1991. Declining amphibian populations—A global phenomenon? Findings and recommendations. Report to Board on Biology, National Research Council, Workshop on Declining Amphibian Populations, Irvine, California. Reprinted in Alytes 9:33–42.

Weir, L. A., and M. J. Mossman. 2005. North American Amphibian Monitoring Program. Pages 307–313 *in* M. J. Lannoo, editor. Amphibian Declines, the Conservation of United States Species. University of California Press, Berkeley, California, USA.

Wright, H. P., and G. S. Myers. 1927. *Rana areolata* at Bloomington, Indiana. Copeia 159:173–175.

Zimmerman, B. L. 1994. Audio strip transects. Pages 92–97 *in* W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster, editors. Measuring and Monitoring Biological Diversity—Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC, USA.

Table 1. Museum specimens of *L. areolatus* from Indiana. Specimens CAS-SU 13390–93 were found to be misidentified and are not included. Specimen OUZ A1126 could not be located and may be lost.

County	**Year**	**Collection Number**
Benton	pre-1879	CA 160
Clay	1950	UMMZ 101623
	1958	UMMZ 118078
	1966	ISUVC 1492
Daviess	1953	UMMZ 108125
Fountain	1951	FMNH 64663
Greene	1949	UMMZ 100304
Jefferson	2003	FMNH 262589
Monroe	1926	CAS-SU 2174–80, 13343–64
	1940	OUZ A1126, UMMZ 95312
Owen	1954	UMMZ 110638
Pike	1936	CMNH 13371–75
	1963	ISUVC 2473
Ripley	2003	FMNH 262588, DRK 381
Sullivan	1952	UMMZ 105544
	1969	ISUVC 2255
Vanderburgh	1936	CMNH 13378
Vigo	1903	HUMCZ A-7043, A-7044
	1964	ISUVC 395–97, 399–403, TCWC 66467
	1965	ISUVC 937
	1966	PU 8482–83
	1967	ISUVC 1820
	1969	ISUVC 2822
	1972	ISUVC 2738, 2793, 3204–3207
	1974	ISUVC 3177

Table 2. A test of the ratio method used to estimate number of calling males as a function of maximum call intensity (calls/min). Here I use the formula y = (a/b) • x, where in the Nate's Pond estimate, a = number of males present at Cattail Pond, b = maximum calls/min at Cattail Pond, x = maximum number of calls/min at Nate's Pond, solved for y, which equals the number of males present at Nate's Pond. The number of males predicted at Cattail Pond was calculated the same way from Nate's Pond data. Values representing predicted number of males were rounded to the nearest whole number. In each case, note that predicted number of males closely matched number of males present. Comparative data were collected on the same night to control for season and weather.

Nate's Pond		
Max Call Rate	**# Males Present**	**# Males Predicted by Cattail Pond Data**
60	9	11
64	9	10
54	11	11
71	12	12

Cattail Pond		
Max Call Rate	**# Males Present**	**# Males Predicted by Nate's Pond Data**
45	8	7
50	8	7
45	9	9
54	9	9

Table 3. Detection probabilities at different survey durations using four different sampling parameters. Probabilities were determined using 100 randomly selected 30-min samples for each parameter. The exception was at Cattail Pond when rain was falling during the breeding season, where all available samples (n = 89) were used. Crawfish frogs were not detected in any samples within this parameter.

Site	**Survey parameters**	**5 min**	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
Nate's Pond	March and April	0.30	0.32	0.34	0.37	0.40	0.41
Nate's Pond	Breeding Season	0.75	0.80	0.84	0.85	0.86	0.86
Nate's Pond	Breeding Season + Air Temp ≥ 13° C	0.76	0.81	0.83	0.84	0.85	0.86
Nate's Pond	Breeding Season + Rainfall	0.41	0.42	0.42	0.42	0.42	0.42
Cattail Pond	March and April	0.20	0.20	0.20	0.20	0.20	0.21
Cattail Pond	Breeding Season	0.49	0.55	0.60	0.61	0.62	0.63
Cattail Pond	Breeding Season + Air Temp ≥ 13° C	0.91	0.96	0.99	1.00	1.00	1.00
Cattail Pond	Breeding Season + Rainfall	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Maximum calling rates and corresponding call index values for 30-min (each with six 5-min periods) call surveys at four distances from Big Pond during three rounds of sampling. Means are for each category are presented at the bottom of each column. Calls could not be detected at the 200 m listening station during rounds 1 and 2. Estimated number of males calling was calculated using the regression method.

Call Index	Max Calls/min	Estimated # Males Calling
1	0–16	0–1
2	26–72	2–7
3	54–92 (∞)	6–9 (∞)

Table 5. Population estimates for Crawfish Frogs at 10 sites in southwest Indiana. Estimates were independently based on maximum call rates (calls/min) and number of males at Nate's Pond and Cattail Pond, and by combining data from both sites using regression analysis. The asterisk indicates that < 0.5 individuals were estimated, but because chorusing occurred at the site, it was given a value of 1.

Site	**Date**	**Max Calls/Min**	**Estimated # Males (Nate's Pond Data)**	**Estimated # Males (Cattail Pond Data)**	**Estimated # Males (Regression Analysis)**	**Estimated Population (by Ratio)**	**Estimated Population (by Regression)**
Greene Co. Northwest	26-Mar	6	1	1	1*	4	4
Sullivan Co. East	27-Mar	46	6	4	5	16–24	20
Daviess Co. South	28-Mar	32	6	6	4	24	12
Daviess Co. Southwest	28-Mar	16	3	3	2	12	8
Daviess Co. Southeast	28-Mar	10	2	2	1	8	4
Greene Co. West	29-Mar	39	7	6	4	24–28	16
Greene Co. East	29-Mar	21	4	4	2	16	8
Owen Co.	5-Apr	84	12	N/A	10	48	40
Daviess Co. North	7-Apr	22	2	N/A	2	8	8
Vigo Co. West	9-Apr	57	6	N/A	6	24	24

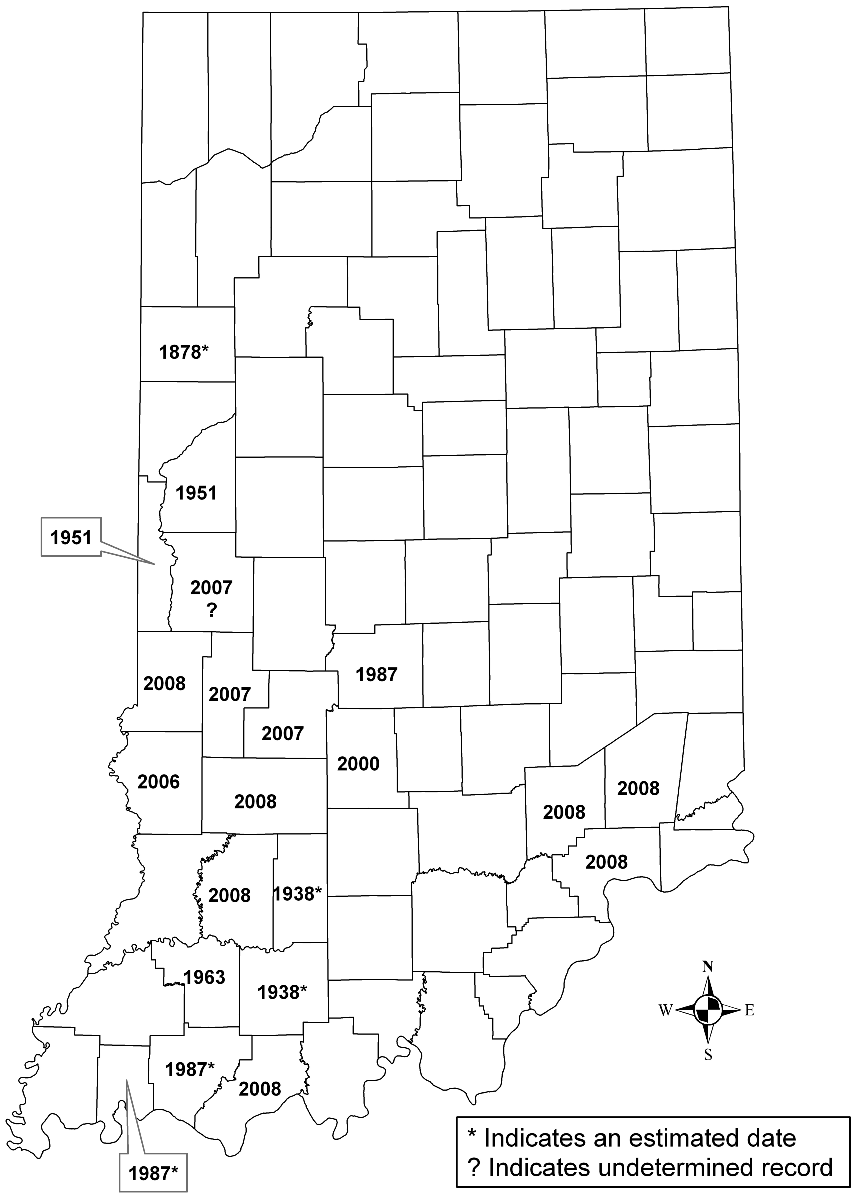


Figure 1. Most recent records (pre-2009) for Crawfish Frogs in Indiana by county.

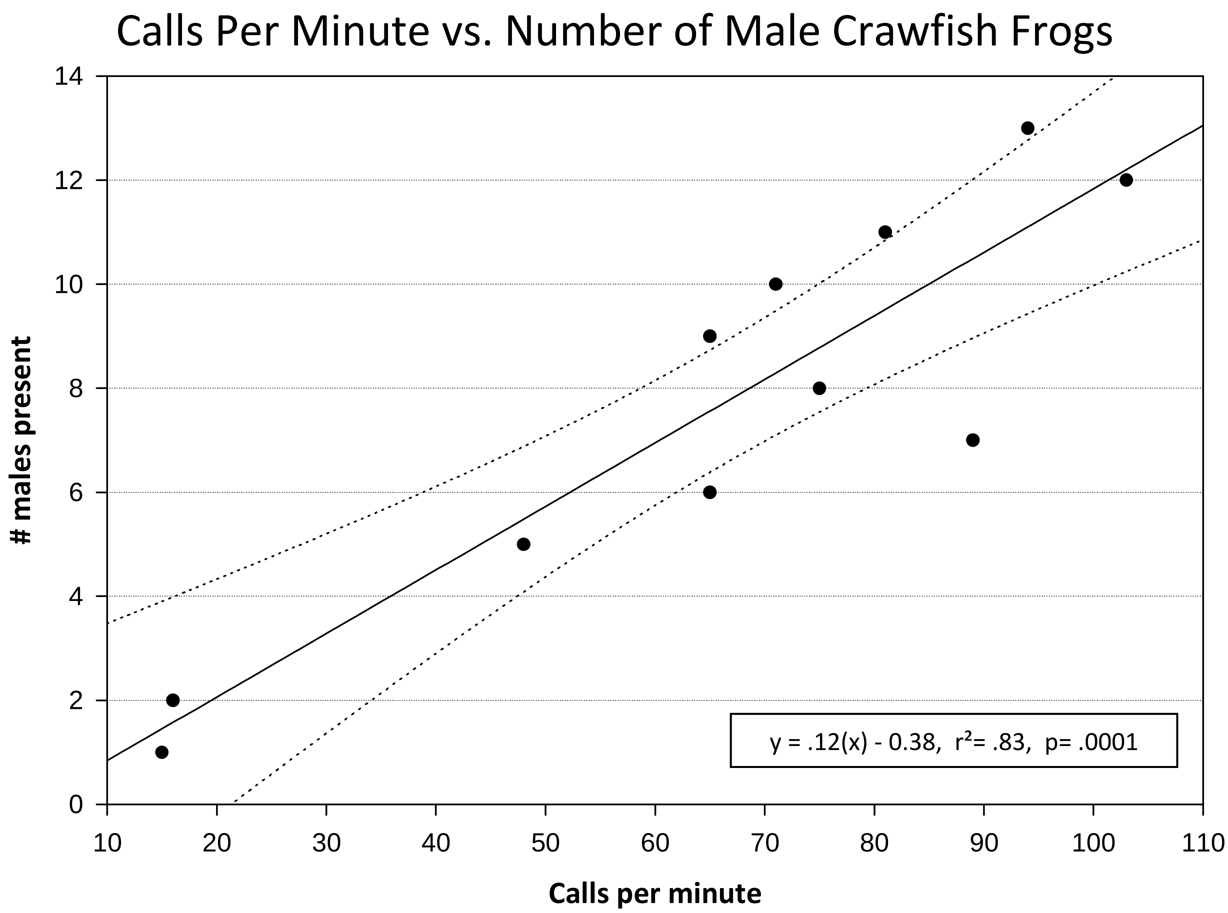
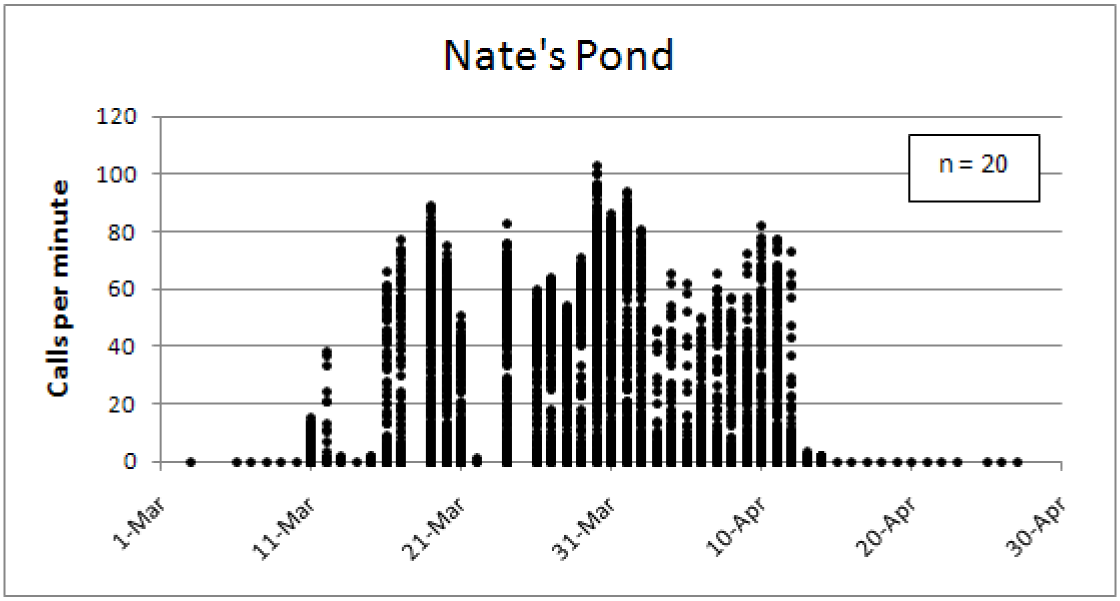


Figure 2. Graph showing the relationship between call rate (calls/min) and number of males present in wetland. Linear regression shows a highly significant correlation between number of males present and maximum call rate ($p = .0001$, $r^2 = 0.83$). Data from Nate's Pond and Cattail Pond were combined for this analysis.

A



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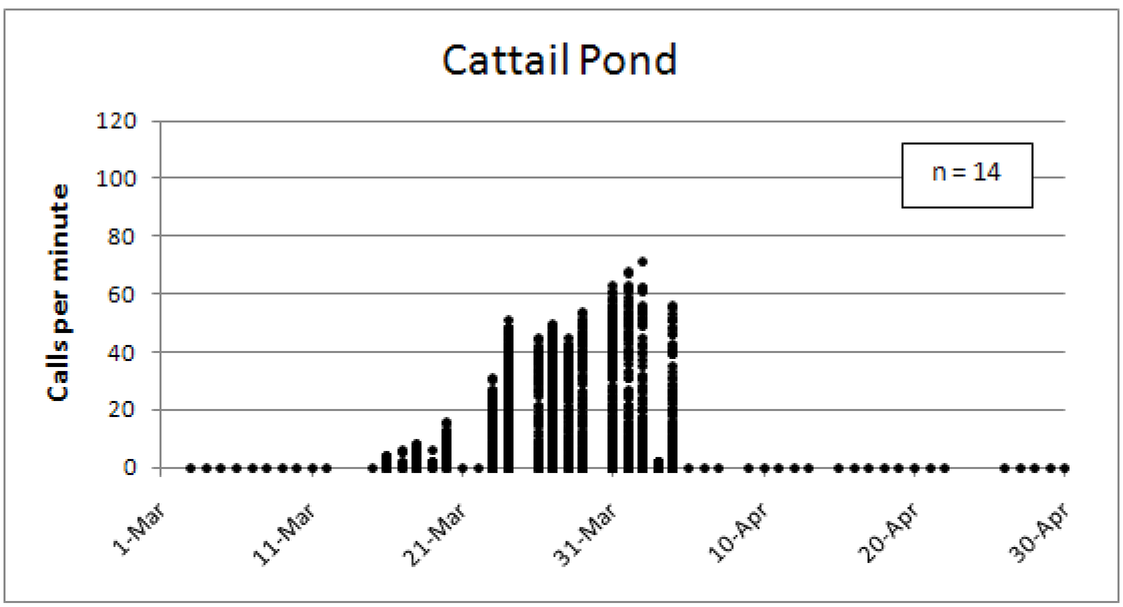
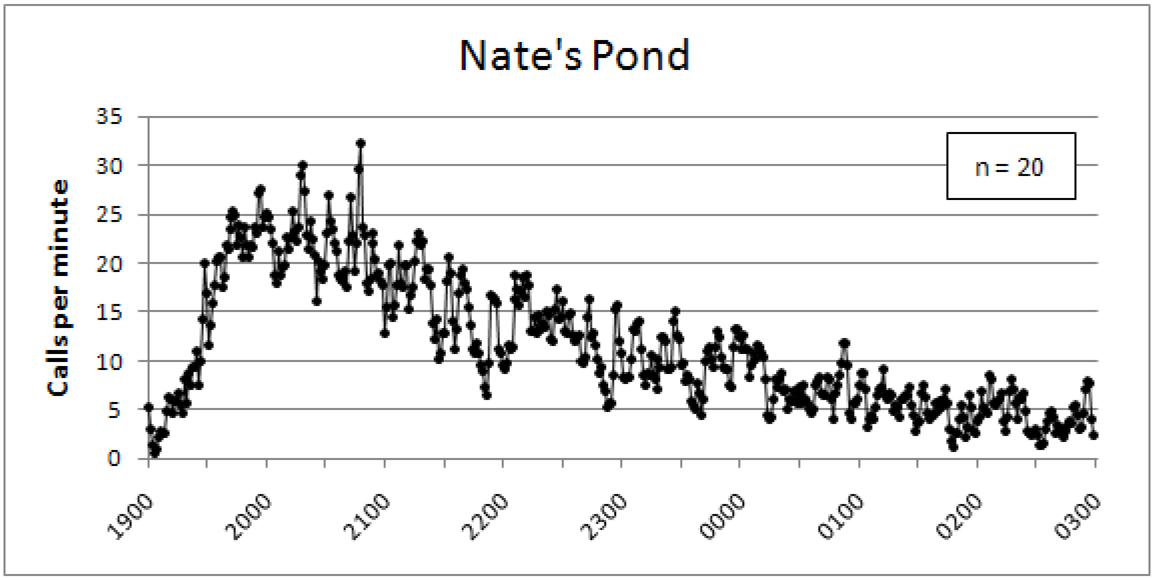


Figure 3. Call rates (calls/min) as a function of date at A) Nate's Pond and B) Cattail Pond during the 2010 breeding season. Gaps in the x-axis are nights when recording units malfunctioned or when times were removed due to human disturbance at the study site.

A



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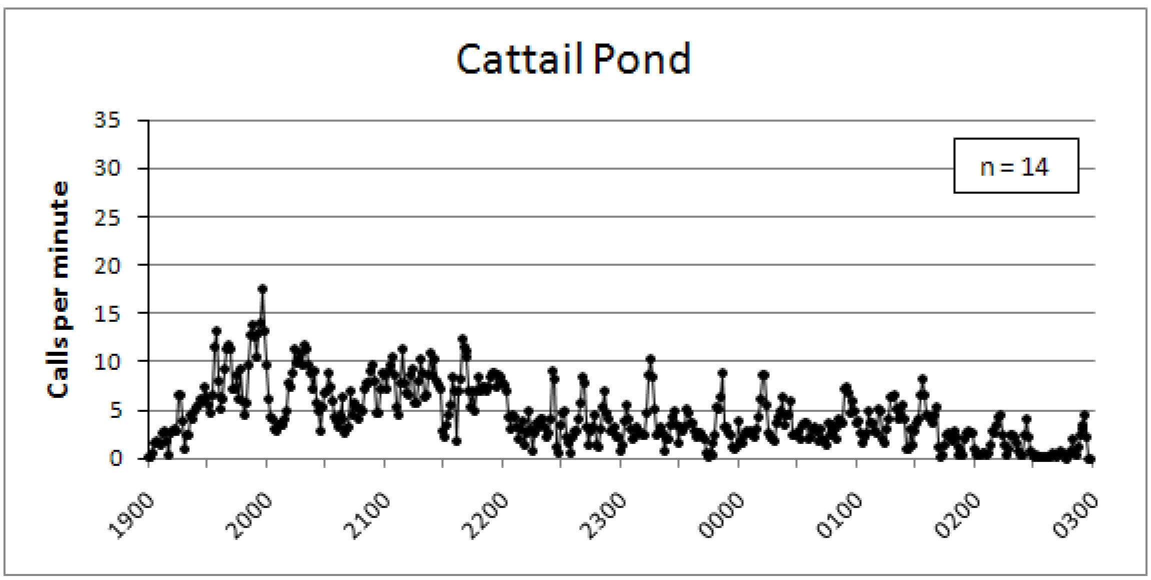

Figure 4. Mean call rates (calls/min) during the daily sampling period examined in this study (1900–0300) at A) Nate's Pond and B) Cattail Pond. Call rates were averaged for each minute across the breeding season at each site (11 March–14 April at Nate's Pond, and 16 March–4 April at Cattail Pond). Note that calling intensity increases during the first hour, and after 1–2 hrs of peak chorusing, decreases gradually through the night.

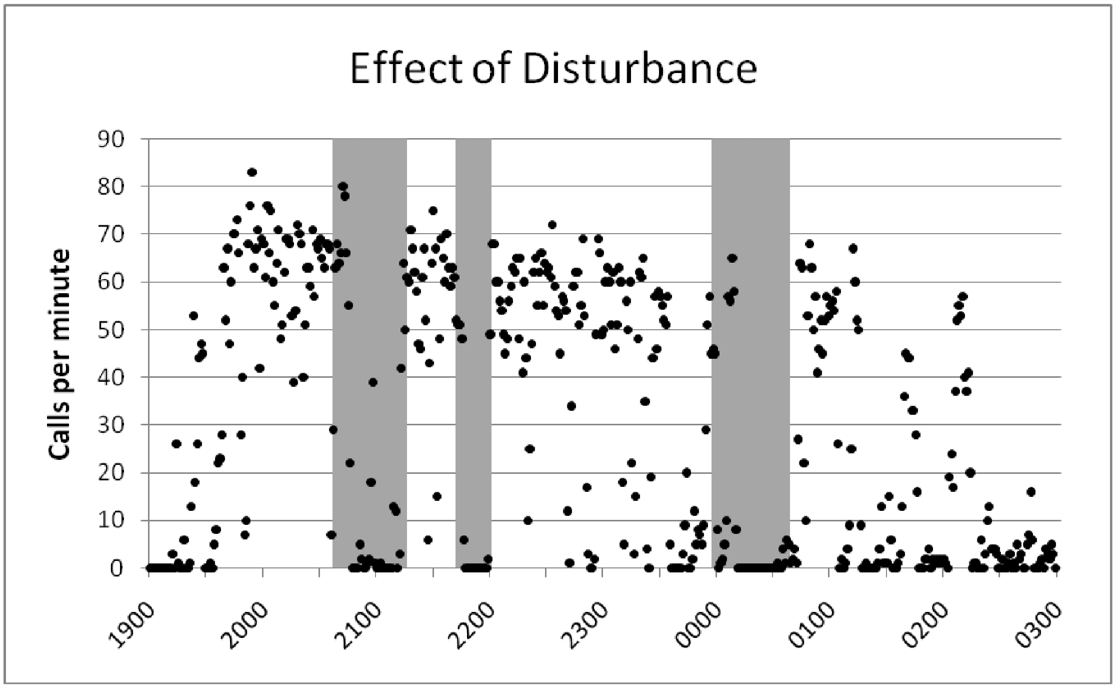

Figure 5. An example of the effect of human disturbance on Crawfish Frog calling intensity (Nate's Pond, 24 March 2010). Human presence is indicated by gray bars.

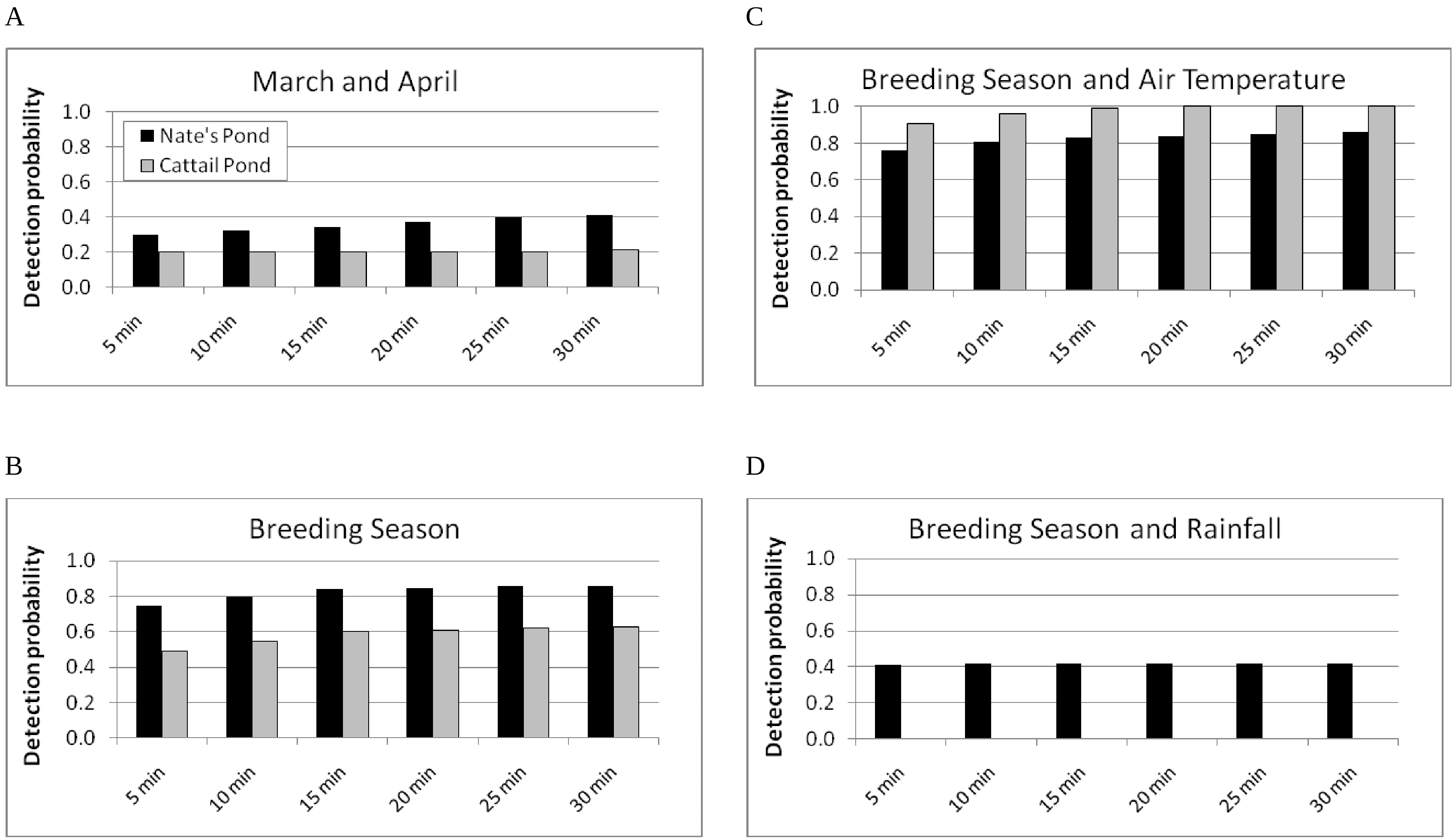

Figure 6. Detection probabilities under different survey conditions and durations at Nate’s Pond and Cattail Pond. Survey conditions include the A) Months of March and April; B) Breeding season (11 March–14 April at Nate’s Pond; 16 March–4 April at Cattail Pond); C) Breeding season when air temperature was ≥ 13° C (~55° F); and D) Breeding season when rainfall occurred.

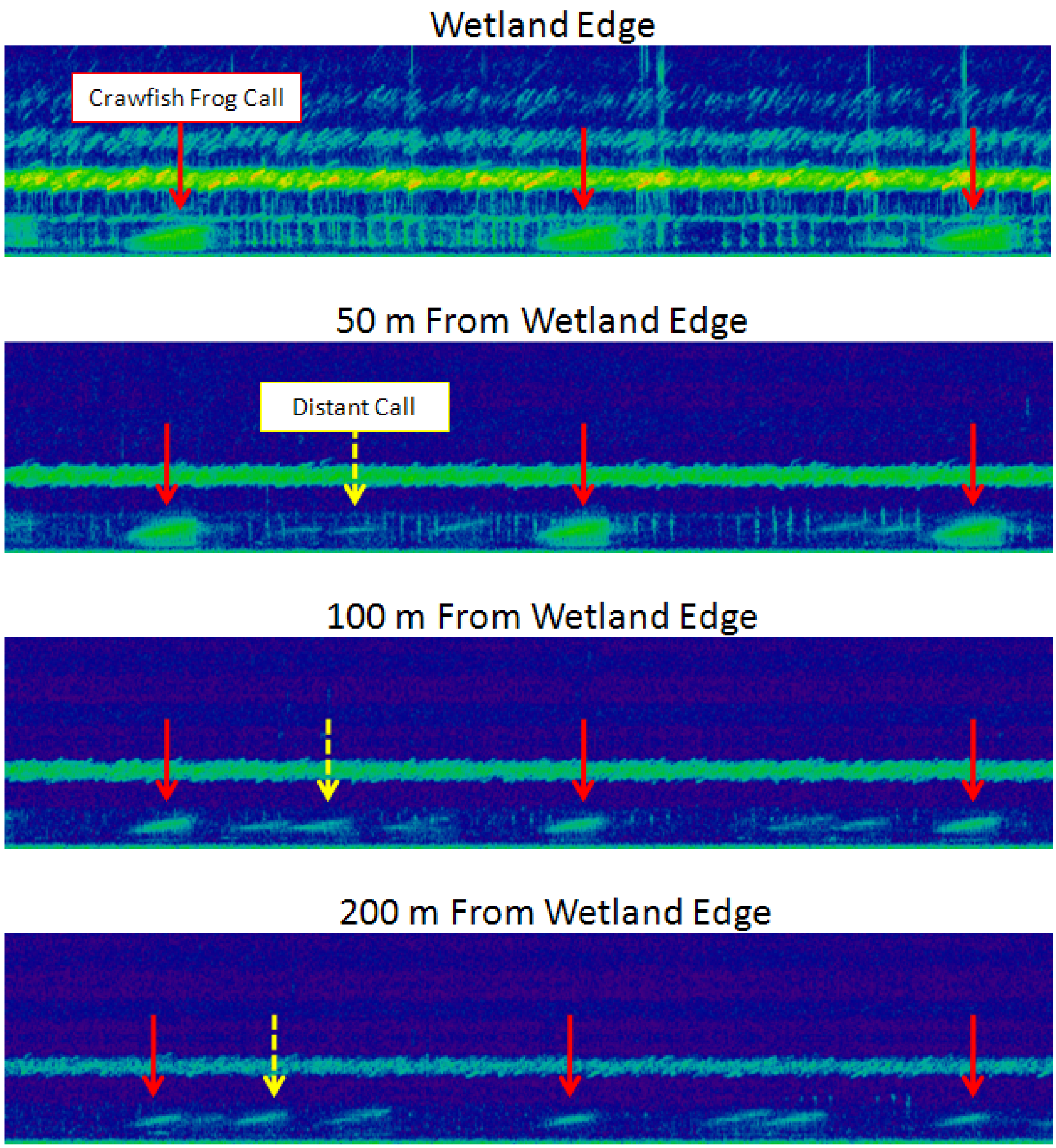

Figure 7. Sonograms showing the effect of distance on Crawfish Frog call detection using Song Scope® software.

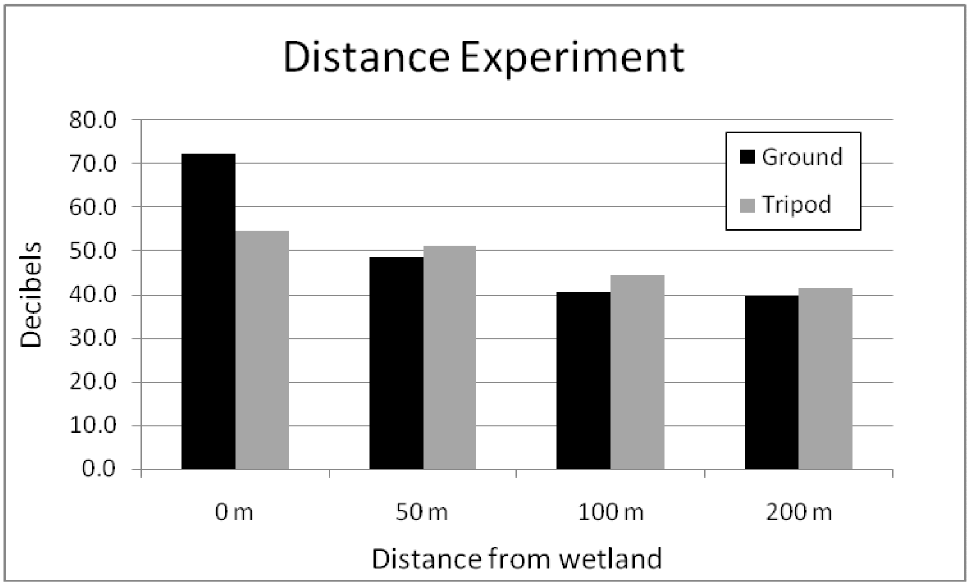

Figure 8. Sound pressure levels (in decibels) from recording units placed at ground level and on a tripod.

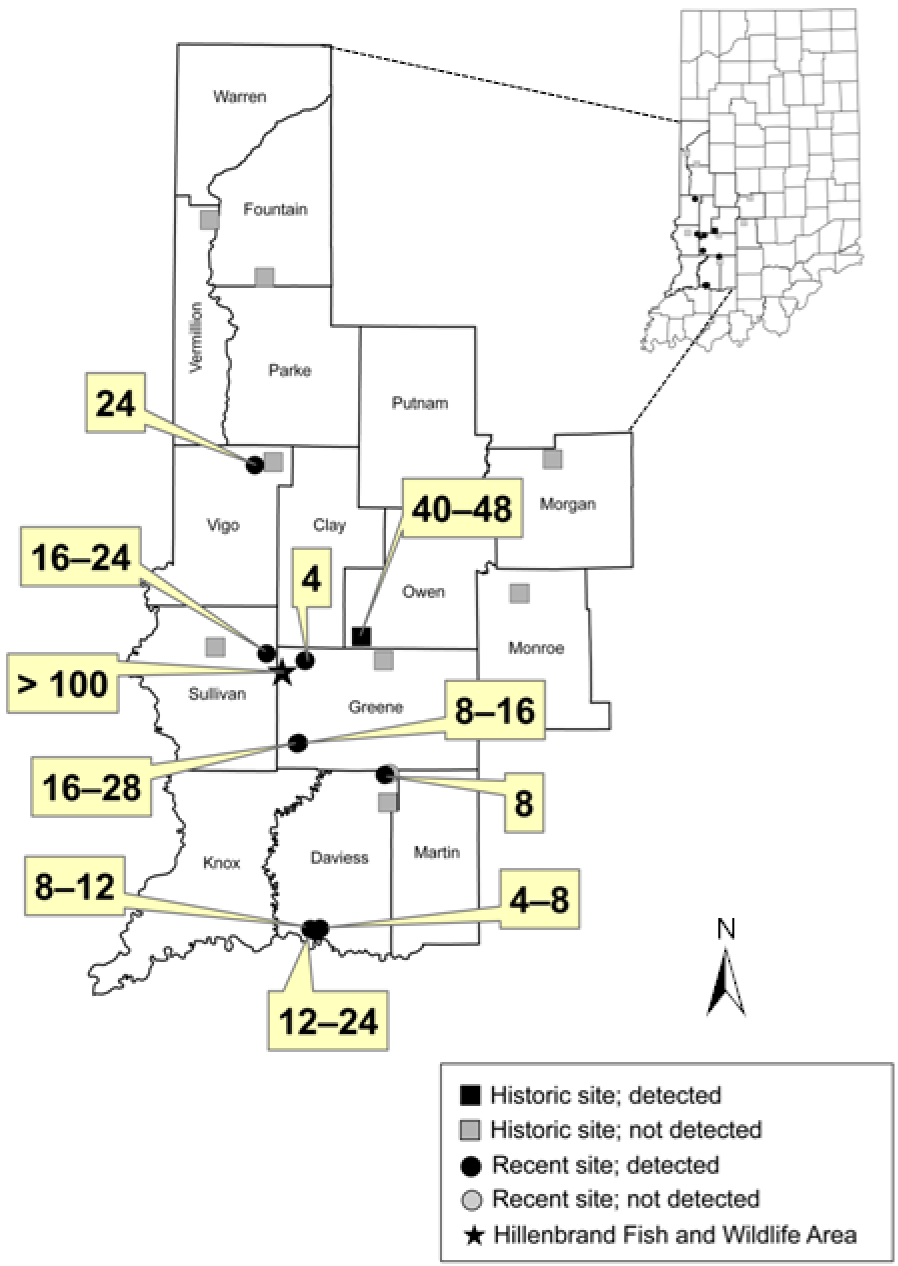

Figure 9. Map showing survey sites visited during this study. Crawfish Frogs were detected at or near all nine recently documented (2009) sites, and at one of nine historic sites. Population estimates are given for all sites where Crawfish Frogs were detected.

APPENDIX A: HABITAT DESCRIPTIONS OF HISTORIC CRAWFISH FROG SITES

Here I provide a description of the habitat at historic sites based on personal observation and remote sensing analyses using aerial photographs as viewed using Google Earth® and Acme Mapper 2.0 (http://mapper.acme.com/). A more general description of these sites can be found in Engbrecht and Lannoo (2010). Notes on the suitability of habitat for Crawfish Frogs at each site are given.

Daviess County East

The collection locality for this site is located in northern Daviess County, east of the town of Odon. The habitat is predominately agriculture with interspersed parcels of grassland and forest. Large portions of rolling forest are located to the east. A recording unit was placed at a small wetland located on private property that appears to have been managed for wildlife. Crawfish Frogs were not documented at this site but habitat (both aquatic and terrestrial) in the immediate vicinity appears adequate. Additional pasture land south of this site could also serve as terrestrial habitat.

Fountain County

The collection locality at this site is located in southern Fountain County, and is described as a “shallow pond in a cultivated field” (Alan Resetar, Field Museum of Natural History, *unpublished data*). The majority of the landscape at this site consists of agriculture, though bottomland forest and a residential neighborhood are located nearby. Small areas of grassy habitat occur in the area. The original collection locality could not be identified. A recording unit was placed at a small pond located in the forested bottoms of a small creek north of Kingman. Because the original collection locality occurred in a cultivated field, the breeding site has probably been destroyed, though open grassy habitat located north of the sampling site could potentially serve as terrestrial habitat. Crawfish Frogs were not detected at this locality and their status at this there remains unknown.

Greene County North

This collection locality is situated in an area of rolling hills near the conjunction of the Eel and West Fork White River. The landscape immediately surrounding this area is dominated by pasture. Floodplain, agricultural fields, and tracts of forest also occur there. A recording unit was placed at a small pond located between a pasture and an agricultural field but Crawfish Frogs were not detected. The abundance of open, grassy habitat (pasture) and the presence of several small ponds may make this area suitable for Crawfish Frogs. Additional survey work in nearby areas may reveal the presence of this species.

Monroe County

This site is located in a large wetland complex in the bottoms of Beanblossom Creek (Al Parker, *personal communication*) in northern Monroe County. The wetland basin consists of both grassland and forest, and is at least partially bordered by cattle pasture. Both terrestrial and aquatic habitats appear adequate, though the presence of fish may be a limiting factor. A recording unit was placed in a relatively open, shrubby area within the wetland complex. Crawfish Frogs were not detected at this site, and their status remains unknown. Indiana Department of Natural Resources personnel failed to detect this species despite visiting the site several times from 2004–2008 (Engbrecht and Lannoo 2010). Because it has not been detected in recent surveys, Crawfish Frogs are thought to possibly be extirpated at this site.

Morgan County

Crawfish Frog records from this region are noted by several sources, and were collected here as recently as 1978 (Engbrecht and Lannoo 2010). This site is situated in northern Monroe County, in an area characterized by heavy agriculture to the northwest and large tracts of forest to the southeast. Suburban developments also occur in the region, though pasture exists adjacent to the sampling site. Because the original collection site is in a developed area, a small wetland approximately 0.4 km east of the collection locality was selected for sampling. This wetland consists of a small, shallow oxbow adjacent to a stream in a narrow woodlot. This area was being used to pasture horses at the time of sampling. Crawfish Frogs were not detected at this site. Livestock pasture located near the original collection site could serve as terrestrial habitat for a remaining population, however, because Crawfish Frogs have not been detected in recent surveys (by the author and by IDNR personnel; Engbrecht and Lannoo 2010), they are suspected to be extirpated at this site.

Owen County

This site is located in southwest Owen County and is characterized by a matrix of forest and agriculture. A recording unit was placed at a farm pond in a grassy pasture ca. 1.4 km SW of the original locality description. Though calls were not heard from this site, distant chorusing was heard on two of the recordings on the nights of 5 April and 6 April, 2010. The author subsequently visited the area on 9 April and heard sporadic calling coming from an area at least

0.6 km south of the sampling site. The chorusing site could not be identified, but the calls appeared to be coming from an area approximately 1 km to the south. A small wetland located within a large pasture may be serving as a breeding site for this species. The grassy pastures around the wetland could serve as terrestrial habitat during the non-breeding season and be a key component to Crawfish Frogs' ongoing existence at this site.

The last known report of Crawfish Frogs from this vicinity was by Sherman Minton on 25 March 1954 (Engbrecht and Lannoo 2010). In March 2007, IDNR personnel located a population of Crawfish Frogs over 4 km from this site near the Clay-Owen County border (Engbrecht and Lannoo 2010). The IDNR site appears to be distinctly different from the early locality description given by Minton, and it is not known if these populations represent the remnants (or the persistence) of a larger metapopulation. Further investigation is needed in order to confirm the relationship of Crawfish Frog populations at these sites.

Sullivan County North

This site is situated in an area heavily dominated by agriculture, with small woodlots and housing establishments located nearby. A recording unit was placed at an artificial pond located within a small woodlot ca. 0.9 km south of the original collection locality. Crawfish frogs were not detected at this site. Though the overall landscape in this area is dominated by agriculture and rural development, grassy fields and a nearby cattle pasture could serve as potential habitat. The status of this population is unknown, but the overall lack of habitat suggests it may be extirpated.

Vigo County East

This site is located in northeast Vigo County near an agricultural field by North Branch Otter Creek. The habitat of this area is characterized by a mosaic of forest, shrub land, agriculture, and rural development. A recording unit was placed adjacent to a forested wetland near the original locality description, but Crawfish Frogs were not detected. A network of grasslands (including a small pond) west of this area could serve as potential habitat, though past mining activity and other habitat disturbances may have rendered the original collection site unsuitable for Crawfish Frogs.

Vermillion County

The original locality description for this site places it in a flat, open area approximately one mile west of the Wabash River in northern Vermillion County. The landscape is predominantly agriculture, with sizeable tracts of forest located to the east along the edge of the Wabash River flood plain. Several ponds are located in the area, but the pond noted in the original locality description (Engbrecht and Lannoo 2010) has not been identified and may no longer exist. A moderately sizeable (~ 9 ha) grassland/savannah-like complex along with a (presumably artificial) pond are located very near the original locality description, and could serve as habitat for a remaining population. A recording unit was placed at a small pond located in the front yard of a farm house approximately 0.75 km north of this area. The pond appeared to be spring-fed, and contained fish. Crawfish Frogs were not detected at this site, though a Cricket Frog (*Acris crepitans*), which is listed as a Species of Special Concern in Indiana, was documented. This site represents one of only two Indiana records for Crawfish Frogs occurring on the west side of the Wabash River, and contains the most descriptive locality data for any of these sites. The current status of Crawfish Frogs at this locality is not known.

APPENDIX B: A PROTOCOL FOR PERFORMING CRAWFISH FROG SURVEYS

The following is a protocol designed to maximize detection of Crawfish Frogs using manual call survey techniques. Call surveys should begin no sooner than 30 min after sunset, and conclude no later than three hours after sunset. Additionally, surveys should be performed when temperatures are ≥ 13°C and it is not raining.

1.) When approaching the site, minimize disturbance by turning off car stereos and, after arriving at the site, immediately turn off vehicle headlights. With windows rolled down, briefly listen for calling from within vehicle.

2.) Exit vehicle and quietly shut door. Find a comfortable place to stand where you can remain for the duration of the survey. Avoid talking, and, when necessary, only in a quiet whisper.

3.) Wait 3–5 min before starting surveys to allow frogs to recover from any disturbance caused by your presence.

4.) Begin survey. Listen for five min. Note the presence of Crawfish Frogs and the intensity of chorusing using the three-level NAAMP calling index given by Weir and Mossman (2005):

1. Individuals can be counted, space between calls;
2. Calls of individuals can be distinguished, some overlapping of calls;
3. Full chorus, calls are constant, continuous, and overlapping.

5.) Take note of which direction and how far the calls appear to be coming from. Describe the various landscape features associated with the area the calls are coming from.

6.) To obtain population estimates (after the 5-min survey has been completed), count the number of calls per six seconds. Repeat this technique 10 times. Using the maximum value obtained in these counts, multiply the number by two. This is the minimum population estimate at the sampling site. To obtain a more robust population estimate, multiply the maximum call count by four.