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A Comparison of the Efficacy of Survey Methods for Amphibians Breeding in Small Forest Ponds

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Although researchers have studied amphibians for many years, status assessments have been hampered by a lack of standards and protocols for inventory and monitoring. Heyer et al. (1994) and Olson et al. (1997) provide a foundation in their reviews of methods used for measuring and monitoring amphibian biodiversity. It is clear from these reviews that no single method effectively assesses all species and that more information is needed on the efficacy of approaches for surveying and monitoring amphibians in differing geographic regions and habitat types.

Forests and associated wetlands are dominant components of glaciated landscapes of the Upper Great Lakes Region, including the states of Michigan, Minnesota, and Wisconsin, USA (Dahl and Zoltai 1997; Frayer 1997). Because timber harvest rates have increased in recent decades (Vasievich et al. 1997), disturbance from land management activities also has increased. These disturbances affect both wetlands directly and characteristics of the surrounding forest on which wetland communities depend (deMaynadier and Hunter 1995; Pauley et al. 2000; Semlitsch 2000). Wetlands are embedded in, and strongly linked ecologically to the surrounding forest (Palik et al. 2001). This is also true of amphibians in such habitats. They breed in small seasonal forest wetlands and live in the adjacent forest uplands during the remainder of the year (deMaynadier and Hunter 1999; Gibbs 1993; Hecnar and M'Closkey 1996, 1997; Wilbur 1980). In small seasonal forest ponds, water levels can fluctuate widely and the amphibian breeding season is short. There is a need to examine the efficacy of survey methods for assessing amphibians in such habitats.

In this paper, we assess amphibian survey methods in small seasonal forest ponds. Our objectives were to: 1) determine the efficacy of three amphibian survey methods (breeding call, dip net, and funnel trap surveys) for detecting four species (*Rana sylvatica*, *Pseudacris crucifer*, *P. triseriata*, and *Ambystoma laterale*); 2) compare the efficacy of these methods used singly and in combination for documenting species occurrence; and 3) determine the efficacy of two additional methods (egg and metamorph surveys) for *R. sylvatica*.

Materials and Methods.—We conducted the study from 1998 to 1999 at 76 seasonal forest ponds in north central Minnesota in the Sucker Lakes watershed, Cass County, and in the Rice River watershed, Itasca County. In these forested watersheds, ponds are abundant and their morphology, hydroperiods, and animal and plant communities are highly variable. Candidate ponds were identified on 1:15,840 scale color infrared photographs (Brooks et al. 1998). To maximize the likelihood of identifying ponds with seasonal hydroperiods (i.e., ponds in which water is seasonally

present), wetlands that also occurred on the National Wetlands Inventory (Wilén 1990) were excluded from consideration. From the remainder, we randomly choose up to five ponds in each of 13 ecological Landtype Association - Landtype (Albert 1995) combinations found on the watersheds, mostly within undisturbed, mature forest stands. The 76 selected ponds ranged in size from 0.04 to 0.20 ha, 74 had seasonal hydroperiods in 1998, and they occurred within a variety of forest types.

We examined the efficacy of survey methods for four species of amphibians: wood frogs (*Rana sylvatica*); spring peepers (*Pseudacris crucifer*); western chorus frogs (*P. triseriata*); and blue-spotted salamanders (*Ambystoma laterale*). We used three primary methods to survey these species during 1998 and 1999: breeding call (C), dip net (D), and funnel trap (T) surveys.

A modified Wisconsin protocol (Kline 1998; Mossman and Hines 1985; Mossman et al. 1998) was used for the C survey, and was conducted once during each of two sampling periods in both years: 12–15 April to assess wood frogs and western chorus frogs; and 17–19 May to assess other calling amphibians. Each pond was visited between 1915 and 0135 h, and after about a minute of acclimation, one person recorded the presence of all species of amphibians calling during a 3-min period.

The D survey was conducted once each year from 24–28 May and 3 June in ponds containing water to sample amphibian larvae. Larvae were readily identifiable by this point in the season. Ponds were swept with aquatic D-shaped dip nets (30 cm width, 20 cm long canvas bag, 1 mm mesh bottom, with 10 cm long canvas skirt) for a total of 15 person-min, regardless of pond size, between 0800 and 1640 h. An effort was made to first sample all habitat elements in a pond, then habitat elements most likely to produce captures. Captured amphibians were held in buckets until the sampling period ended, then were identified, counted, and released. Although we captured both larvae and occasional adults, only larvae were included in the analysis.

T surveys also were conducted to sample amphibian larvae. We used unbaited metal traps of two different mesh sizes in 1998: four (6 x 6 mm) openings per 2.5 cm and eight (3 x 3 mm) openings per 2.5 cm [Cuba Specialty Manufacturing Co., Fillmore, New York; G-40 and G-48M]. Traps were spaced evenly around each pond in shallow water perpendicular to shore. They were placed in water deep enough to submerge the trap entrance, yet provide air for amphibians to breathe. We set two to six traps (one to three of each mesh size) around each pond, alternating mesh sizes from a random start. The number of traps was chosen to approximate an equivalent effort per unit of pond surface area, which averaged about 210 m²/trap. T surveys were conducted once in each pond, for three consecutive days, from 3–25 June in 1998 between 0800 and 1600 h. Captured amphibians were identified to species, counted, and released. Although we captured both larvae and occasional adults in traps, only larvae were included in the analysis. In 1998, we determined that mesh size of traps was of no consequence to wood frog larvae capture rates, but the smaller 3 x 3 mm mesh size produced higher capture rates for the smaller-sized larvae of spring peepers and blue-spotted salamanders (Buech and Egeland 2002). Thus, in 1999, we again trapped each pond for three days between 8 June and 9 July using only 3 x 3 mm mesh traps. Summer precipitation was exceptionally low in 1998 and exceptionally high in 1999; consequently, we were able to trap in

more ponds in 1999. Additionally, because ponds contained more water in 1999 and we wanted to maintain a relatively constant effort per unit of surface area, we used more traps in 40% of ponds in 1999.

Wood frog egg masses are highly visible and often clumped in one location, which suggested that egg mass surveys might be effective for determining occupancy (Crouch and Paton 2000). Surveys for wood frog egg masses (E) (1998 and 1999) and wood frog metamorphs (1999) were conducted to determine if single-visit surveys for these life stages provide useful information. Each pond was visited within a week after the April breeding call survey to look for egg masses. We searched ponds thoroughly between 0800 and 1600 h without time limit, until we either found and recorded the number of egg masses, or were fairly confident that eggs were not present. Similarly, we visited ponds in mid-summer (12–23 July) to conduct a survey for recently metamorphosed wood frogs, and recorded the number observed during a 10 person-min search around the perimeter of each pond.

We summarized data by reducing it to presence-absence information and counted how many ponds a particular method or combination of methods recorded each species as being present for 1998 and 1999. The measure of efficacy was taken as the pond count for a method or combination of methods, expressed as a proportion of the number of ponds in which a species was recorded by all methods combined. For example, if we surveyed 50 ponds and heard wood frogs calling in 30, and we learned by looking at all survey methods that wood frogs were actually present in 40 of the 50 ponds, the efficacy of the calling survey for wood frogs in that year would be stated as 0.75. We combined years by summing the pond counts over 1998 and 1999. In addition to the four amphibian species already mentioned, one other taxon (gray treefrog, *Hyla versicolor*) was included in analyses of species richness. Log-linear and contingency table analyses were used in SYSTAT 8.0 (SPSS, Inc. 1998) to compare the efficacy of methods and where appropriate, Pearson's Chi-square statistic (χ^2) is reported.

Results.—We compared the efficacy of C, D, and T surveys for assessing reproductive activity of the three anurans most frequently found at our ponds (Fig. 1). For wood frogs, these methods yielded similar results in 1998 (Fig. 1a), but efficacies were not homogeneous in 1999 ($\chi^2 = 37.15$, $df = 2$, $P < 0.001$); C surveys recorded wood frogs in fewer ponds than recorded by either D or T surveys. For wood frogs, the combined-year efficacy of D and T surveys was better than for C surveys ($\chi^2 = 10.33$, $df = 1$, $P < 0.001$ and $\chi^2 = 9.39$, $df = 1$, $P < 0.002$, respectively).

Efficacy patterns of C, D, and T surveys for spring peepers (Fig. 1b) and western chorus frogs (Fig. 1c) were similar in both years. In 1998, 1999, and both years combined, efficacies of C, D, and T surveys were not homogeneous for both spring peepers ($\chi^2 = 70.94$, $df = 2$, $P < 0.0001$; $\chi^2 = 76.84$, $df = 2$, $P < 0.0001$; $\chi^2 = 134.85$, $df = 2$, $P < 0.0001$; respectively) and western chorus frogs ($\chi^2 = 35.40$, $df = 2$, $P < 0.0001$; $\chi^2 = 12.35$, $df = 2$, $P < 0.002$; $\chi^2 = 43.06$, $df = 2$, $P < 0.0001$; respectively). In both years combined, C surveys detected these frogs more frequently than either D (spring peepers $\chi^2 = 134.75$, $df = 1$, $P < 0.001$; western chorus frogs $\chi^2 = 31.16$, $df = 1$, $P < 0.001$) or T surveys (spring peepers $\chi^2 = 44.66$, $df = 1$, $P < 0.001$; western chorus frogs $\chi^2 = 31.16$, $df = 1$, $P < 0.001$). D and T surveys generally yielded similar results except the T survey

recorded spring peepers in many more ponds than did the D survey (combined-years analysis: $\chi^2 = 37.36$, $df = 1$, $P < 0.001$), especially in 1999.

Combinations of survey methods, especially combinations that included a C survey, did a better job of detecting all three species (Fig. 1). Call plus dip net surveys (CD) and call plus funnel trap surveys (CT) yielded similar results. These combinations were better than dip net plus funnel trap (DT) surveys for spring peepers and western chorus frogs (χ^2 range in 4 comparisons: 30.00 to 62.45, $df = 1$, $P < 0.001$). For wood frogs, only CD surveys differed from DT surveys ($\chi^2 = 5.48$, $df = 1$, $P < 0.019$). C surveys detected spring peeper and western chorus frog reproductive activity almost as well as CD and CT surveys, however, C surveys detected spring peepers in fewer ponds than CT surveys ($\chi^2 = 7.27$, $df = 1$, $P < 0.007$). For wood frogs, the addition of either D or T

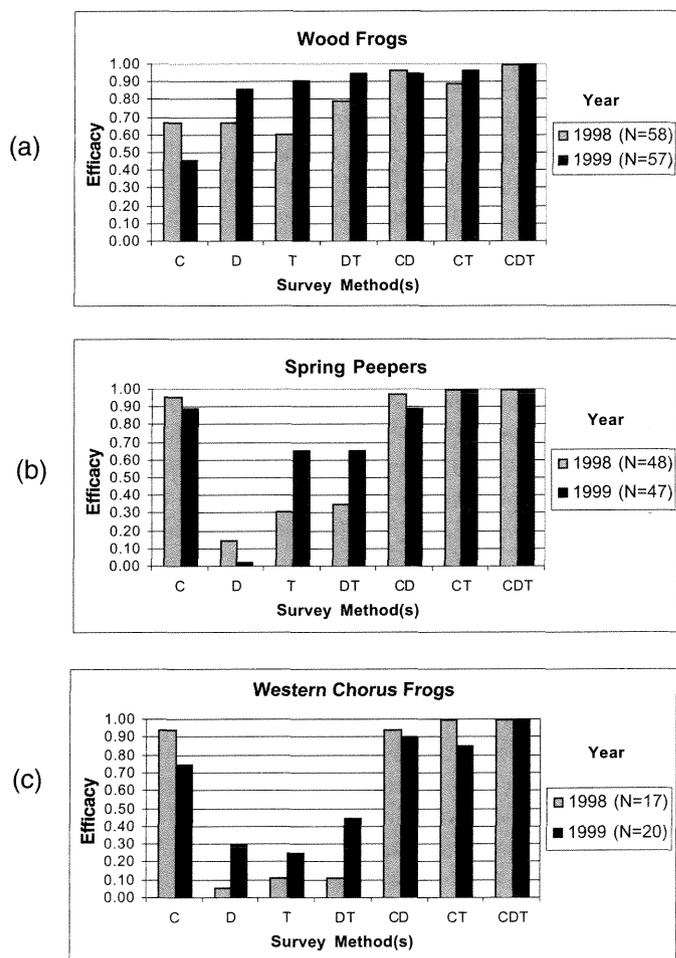


FIG. 1. A comparison of the efficacy of three survey methods for assessing occurrence of reproductive activity of anurans, including (a) wood frogs, (b) spring peepers, and (c) western chorus frogs, in 76 seasonal forest ponds in north central Minnesota in 1998 and 1999. Methods used were breeding call (C), dip net (D), and funnel trap (T) surveys, which were analyzed singly and in combination. The measure of efficacy was taken as the pond count for a method or combination of methods expressed as a proportion of the number of ponds in which a species was recorded by all methods combined. The sample size listed for each year is the total number of ponds in which reproductive activity was recorded for the species by any of the three methods.

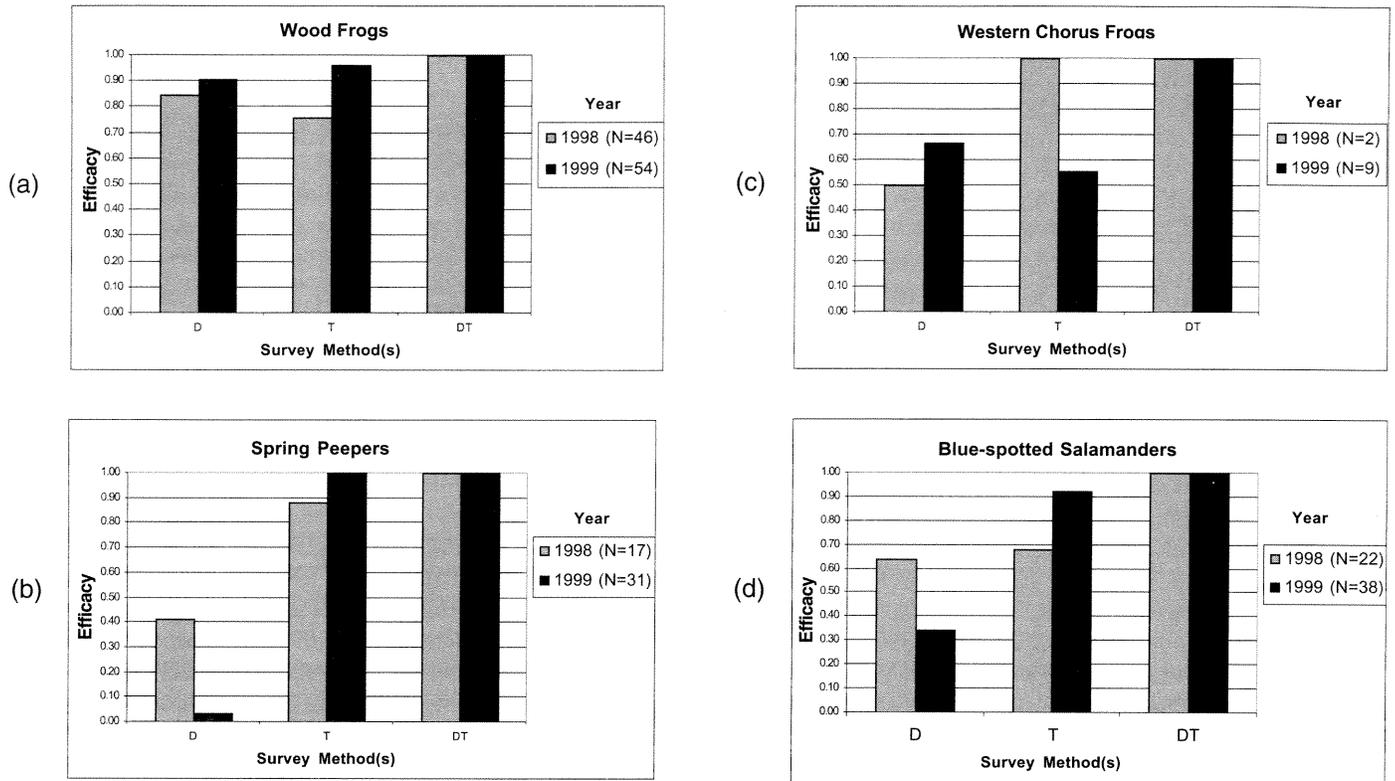


FIG. 2. A comparison of the efficacy of two survey methods for measuring the presence of (a) wood frog, (b) spring peeper, (c) western chorus frog, and (d) blue-spotted salamander larvae in 76 seasonal forest ponds in north central Minnesota in 1998 and 1999. Methods used were dip net (D) and funnel trap (T) surveys, which were analyzed singly and in combination. The measure of efficacy was taken as the pond count for a method or combination of methods expressed as a proportion of the number of ponds in which a species was recorded by all methods combined. The sample size listed for each year is the total number of ponds in which a species larva was recorded by either of the two methods.

surveys to C surveys detected their presence more effectively ($\chi^2 = 48.39$ and 40.67 , $df = 1$, $P < 0.001$).

In a second analysis, we disregarded C surveys and reanalyzed data from D and T surveys for wood frog, spring peeper, western chorus frog, and blue-spotted salamander larvae (Fig. 2). These methods might be chosen to assess non-calling amphibians. T surveys did a better job than D surveys in documenting the occurrence of larvae for two of the four species (Fig. 2b and 2d) ($\chi^2 = 61.12$ and 19.17 , $df = 1$, $P < 0.001$, respectively, for spring peepers and blue-spotted salamanders); however, the western chorus frog (Fig. 2c) sample size was low.

Multiple visits contributed to the efficacy of T surveys (Fig. 3). In both years combined, 94% of 86 ponds known to contain wood frogs were identified after only one day of trapping, and 99% after two days of trapping. However, we also captured a total of 9395 wood frog larvae in these ponds during both years. For less abundant species [blue-spotted salamanders ($N = 48$ ponds; 272 larvae) and spring peepers ($N = 46$ ponds; 377 larvae)], about 70% of ponds known to contain the species were identified after the first day and 90% after the second day. Results were similar for the chorus frog, which was found in only 6 ponds (10 larvae) that we trapped. The known complement of species in ponds also was identified in about 70% of ponds after one day and in 90% of ponds after two days of trapping.

Survey data for wood frogs were reanalyzed to include E surveys to document reproductive activity (C, E, D, and T surveys in

Fig. 4a) and presence of only their eggs or larvae (E, D, and T surveys in Fig. 4b). Efficacy of E surveys tended to be more variable among years compared to other survey methods. Furthermore, with the exception of the 1999 C survey, E surveys documented the presence of wood frogs in fewer ponds than any of the other survey methods. In fact, E surveys identified only 40 and 70% of ponds known to contain either wood frog eggs or larvae, respectively, in 1998 and 1999. As in Fig. 1, the best two-method survey

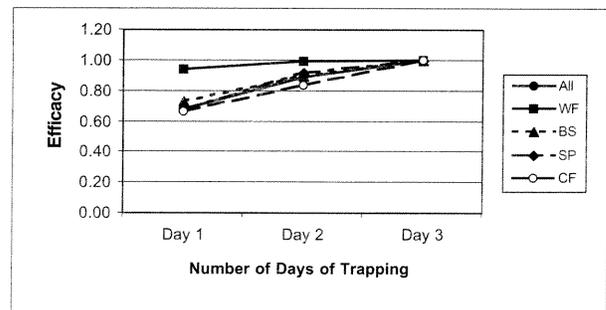


FIG. 3. Efficacy of T surveys for identifying ponds known to contain wood frogs ([WF]; $N = 86$ ponds), blue-spotted salamanders ([BS]; $N = 48$ ponds), spring peepers ([SP]; $N = 46$ ponds), chorus frogs ([CF]; $N = 6$ ponds), and the known-species complement ([All]; $N = 91$ ponds), in relation to the number of days trapped. The measure of efficacy is the cumulative proportion of ponds known to contain the species or the known complement of species after 1, 2, and 3 days of trapping.

sets for documenting reproductive activity of wood frogs were CD or CT surveys (Fig. 4a). Of these, only the CT survey combination was worse than the three-method, CDT combination ($\chi^2 = 3.84$, $df = 1$, $P < 0.05$). The efficacy of both D and T surveys was improved when combined as DT ($\chi^2 = 6.77$ and 7.66 , $df = 1$, $P < 0.01$) (Fig. 4b). In contrast, efficacies of the two-method ED, ET, or DT survey sets for documenting wood frog eggs or larvae were similar.

During the post-metamorphosis survey, recently metamorphosed wood frogs were observed in 57 ponds (data not shown), including nine ponds in which there were no eggs or larvae documented by other methods.

The number of amphibian species recorded in ponds varied both among years and among methods (Fig. 5). In 1998, the C, D, and T surveys recorded a maximum richness of four species (Fig. 5a). Although the efficacy of these methods differed, the relative distribution of species richness among ponds did not differ ($\chi^2 = 15.35$, $df = 10$, $P < 0.12$). In 1999 (Fig. 5b), C and D surveys identified a maximum species richness of only three species in ponds, whereas T surveys identified four species. Although the overall efficacy of the C, D, and T surveys was similar, the relative distribution of species richness among ponds was different ($\chi^2 = 39.10$, $df = 8$, $P < 0.001$); D surveys documented fewer species in ponds relative to other methods. Results for the three methods in combination, were similar among years (Fig. 5). However, CD and CT surveys did a better job of documenting species richness in ponds than did DT surveys in 1998 ($\chi^2 = 11.96$ and 14.18 , $df = 5$, $P < 0.035$). The CD and CT survey combinations identified one more species (gray treefrog) in ponds compared to the DT survey combination (in both years, only the C survey detected gray treefrogs).

Discussion.—Efficacy of the three primary methods was variable among species and years. Life history, reproductive phase, logistical factors, and environmental variation might account for much of this variation. For example, C surveys identified *Pseudacris* species in a much higher proportion of ponds than did D or T surveys. These species call often and over a relatively long period so they are likely to be noted when present. In contrast, C surveys identified wood frogs in ponds about as well as D or T surveys in one year but not the other. Wood frogs breed early in the season, but for only a short period, and breeding can be postponed by cold weather (Mossman et al. 1998). Consequently, it is difficult to time surveys to encompass consistently their breeding season, especially if there are large-program logistical constraints, such as the need to survey many wetlands over several days under variable weather conditions.

Several authors have suggested use of a combination of meth-

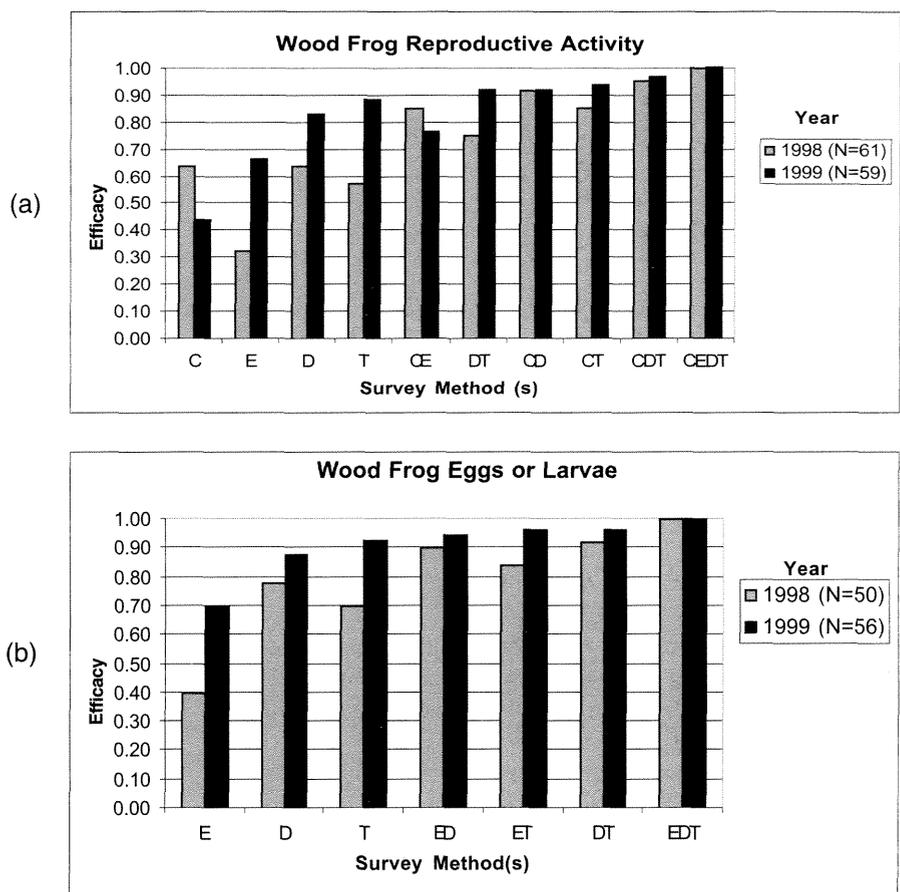


FIG. 4. A comparison of the efficacy of an expanded set of survey methods for measuring (a) reproductive activity of wood frogs and (b) presence of wood frog eggs or larvae in 76 seasonal forest ponds in north central Minnesota in 1998 and 1999. Wood frog reproductive activity was declared as occurring in a pond if it was recorded present during breeding call (C), egg mass (E), dip net (D), or funnel trap (T) surveys in that year. Wood frog eggs or larvae were declared present in a pond if they were recorded during E, D, or T surveys in that year. The measure of efficacy was taken as the pond count for a method or combination of methods expressed as a proportion of the number of ponds in which wood frogs were recorded by all methods combined. The sample size listed for 1998 and 1999 is the total number of ponds in which a) reproductive activity was recorded by any of the four methods, and b) eggs or larvae were recorded by any of the three methods.

ods to meet objectives of documenting presence and relative abundance of species in amphibian communities (Heyer et al. 1994; Olson and Leonard 1997). Our results support this suggestion. A combination of a C survey with either D or T surveys documented the occurrence of calling species in more ponds than any one method alone. This is probably because of the similarity in results between D and T surveys, and the contrast between these methods and the C survey. Despite similarities between D and T surveys, there were instances in which results improved substantially by conducting both of these surveys. However, although results often improved by using multiple types of surveys, it is unknown how much of the improvement was because of adding another survey method per se, or simply because of an additional visit to the site or spending more time conducting surveys at the site.

Multiple visits to a pond clearly contributed to the efficacy of funnel trap surveys. Although a single day of trapping identified nearly all ponds known to contain wood frog larvae, this species

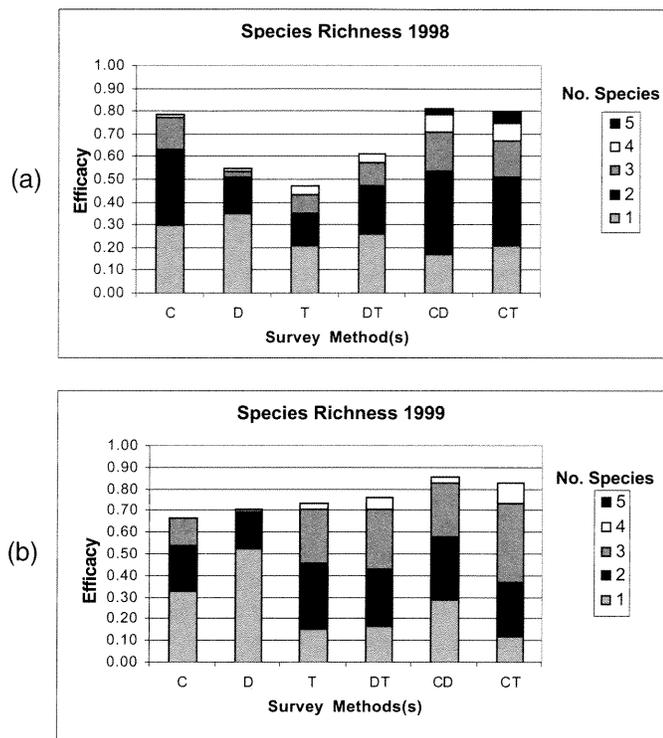


FIG. 5. A comparison of the efficacy of three survey methods for documenting the richness of amphibian species (wood frogs, spring peepers, western chorus frogs, gray treefrogs, and blue-spotted salamanders) breeding in (a) 1998 and (b) 1999 in a collection of 76 seasonal forest ponds in north central Minnesota. Methods used were breeding call (C), dip net (D), and funnel trap (T) surveys, which were analyzed singly and in combination. The measure of efficacy was taken as the pond count for a method or combination of methods expressed as a proportion of the number of ponds in which a species was recorded by all methods combined.

was very abundant. In contrast, two additional days of trapping continued to yield improvements in detection of less abundant amphibian species whose abundance was, on average, three to four percent of wood frogs. For chorus frogs, additional days of trapping might have increased the number of ponds in which they were detected. Overall, it seems that three days of trapping, as recommended by Adams et al. (1997), did a reasonable job of identifying the species composition of amphibians in small seasonal forest ponds.

A comparison of our egg survey results to those of Crouch and Paton (2000) also demonstrates the value of multiple visits. We obtained poor results from a single visit to survey wood frog eggs in 76 ponds. In 1998 and 1999, respectively, our E surveys identified only 40 and 70% of ponds known to contain wood frog eggs or larvae. In contrast, Crouch and Paton (2000) visited 15 ponds at 3 to 6 day intervals from early March to mid-April. They observed that $\geq 85\%$ of wood frog egg deposition was completed in < 8 days, but it took an average of $17.4 (\pm 4.8)$ days for all egg masses to be deposited. Thus, they recommended that egg surveys be conducted over a three-week period and concluded that egg surveys can provide accurate information for monitoring abundance of wood frogs. Taken together, our results and those of Crouch and Paton (2000) suggest that while egg surveys can provide accurate information on wood frog reproduction, under our

protocols, a single visit was not adequate to document presence. Two likely reasons for the false negatives that we recorded are failing to see communal egg masses that were present and surveying ponds before egg masses were laid. We believe the latter explanation is especially appropriate to our 1998 results; our timing was too early for a single-visit survey.

Methods differed in regard to effort required to conduct surveys under the protocols we used. E and D surveys were easiest to conduct because they only required a single visit each year and the survey itself required about 10–20 and 30–60 min at each pond, respectively. C surveys required relatively more effort. Although it took less time to conduct the survey at the site, two visits (each about 5–10 min) were required each year during nocturnal hours to assess the amphibian species that use seasonal forest ponds. T surveys required the most effort, primarily because our protocols stipulated that each wetland be surveyed once each year, and the survey itself took about 15 to 60 min for each of 4 days. Cost of equipment was zero for the C and E surveys, about US \$70 for a dip net, and about US \$8 and \$20 for one funnel trap (depending on mesh size).

As others have reported (Heyer et al. 1994; Mossman et al. 1998), methods also differed in their sensitivity to weather conditions. For one, it can be difficult to schedule surveys to cover a large number of study sites to accommodate seasonal and daily weather effects on amphibian activity. For example, we experienced difficulty in timing C and E surveys of a large number of ponds to consistently survey an explosive breeder like wood frogs. In contrast, scheduling D and T surveys is less sensitive to short-term effects of weather.

Surveying amphibians in seasonal forest ponds adds another dimension. We encountered instances of water levels that were too low to conduct surveys, concentrations of larvae in small residual puddles, ponds that prematurely dried up, and ponds that dried up and subsequently rehydrated. Such vagaries in the hydroperiods of seasonal ponds can induce daily and annual variation in data. In most cases, the consequence was inability to conduct a survey because of inadequate water. Although we tried to maintain a constant effort based on pond surface area, we suspect that high water might have diluted, and low water might have concentrated captures of amphibian larvae.

Finally, it is important to choose parameters and methods of measuring them that are congruent with objectives (Heyer et al. 1994). Although we observed differences in efficacy among methods, the fact that C, E, D, and T surveys document occurrence of species at different stages in ontogeny might affect choice of survey method. This was especially true of metamorph surveys. Metamorph surveys detected wood frogs at ponds in which no eggs or larvae were found, suggesting those surveys might have detected dispersers from other ponds. Thus, we do not recommend metamorph surveys as a tool for inventorying specific ponds.

Overall, our results suggest that while single surveys might provide representative presence-absence data for some species, we encountered biases across species and years. Use of multiple survey types improved the detection of species reproductive activity in ponds and produced less variation among years. Thus, depending on survey objectives and resources, our results support the use of multiple types of surveys (e.g., toolbox approach of Olson et al. 1997). Our results also suggest that efficacy of a survey might

improve by extending its duration or repeating it more frequently. The protocols that we used seemed appropriate for small seasonal forest wetlands in our region. If we had more resources available or fewer ponds to monitor, we would have chosen to conduct breeding call surveys for a longer period than 3 minutes, and visit ponds more frequently than once to conduct wood frog egg surveys. For seasonal forest wetlands of the upper Great lakes region, we generally recommend a combination of breeding call followed by either dip net or funnel trap (3 x 3 mm mesh) surveys conducted prior to metamorphosis.

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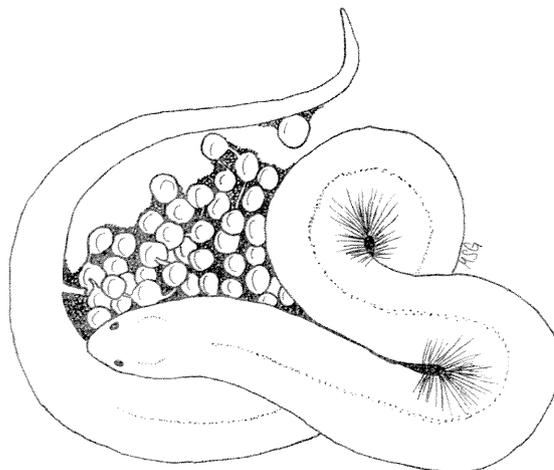
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Amphiuma means (Two-toed Amphiuma). USA: Florida, Leon Co., Lake Iamonia. Adult with eggs. Illustration by Margaret Gunzburger.