

# Evaluation of seven aquatic sampling methods for amphibians and other aquatic fauna

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**Abstract.** To design effective and efficient research and monitoring programs researchers must have a thorough understanding of the capabilities and limitations of their sampling methods. Few direct comparative studies exist for aquatic sampling methods for amphibians. The objective of this study was to simultaneously employ seven aquatic sampling methods in 10 wetlands to compare amphibian species richness and number of individuals detected with each method. Four sampling methods allowed counts of individuals (metal dipnet, D-frame dipnet, box trap, crayfish trap), whereas the other three methods allowed detection of species (visual encounter, aural, and froglogger). Amphibian species richness was greatest with froglogger, box trap, and aural samples. For anuran species, the sampling methods by which each life stage was detected was related to relative length of larval and breeding periods and tadpole size. Detection probability of amphibians varied across sampling methods. Box trap sampling resulted in the most precise amphibian count, but the precision of all four count-based methods was low (coefficient of variation > 145 for all methods). The efficacy of the four count sampling methods at sampling fish and aquatic invertebrates was also analyzed because these predatory taxa are known to be important predictors of amphibian habitat distribution. Species richness and counts were similar for fish with the four methods, whereas invertebrate species richness and counts were greatest in box traps. An effective wetland amphibian monitoring program in the southeastern United States should include multiple sampling methods to obtain the most accurate assessment of species community composition at each site. The combined use of frogloggers, crayfish traps, and dipnets may be the most efficient and effective amphibian monitoring protocol.

**Key words:** Anuran; aural; box trap; caudate; detection; dipnet; froglogger; funnel trap; inventory; monitoring; survey; visual encounter.

## Introduction

In order to conduct ecological research, inventories, and monitoring programs, effective sampling methods must be employed. Concern about the status of amphibian populations has led to a refinement in the development and implementation of am-

phibian monitoring programs (Collins and Storfer, 2003; Corn et al., 2005; Muths et al., 2005; Dodd et al., in press). Designing a standard sampling protocol for amphibians is complicated because many amphibian species have a biphasic life cycle with terrestrial and aquatic forms (Heyer et al., 1994). To address this challenge, a wide variety of sampling techniques has been developed for amphibians in terrestrial and aquatic habitats (Heyer et al., 1994; Olson et al., 1997). Evaluation of sampling methods should include the effectiveness in terms of the number of species and individuals collected, as well as the relative efficiency in terms of labor and cost. A comparison of multiple methods at replicated sites is the best way to evaluate sampling methods (Turner and Trexler, 1997; Corn et al., 2000).

Amphibian research often focuses on aquatic habitats because many amphibians spend at least a portion of their life cycle in these habitats, and detectability of many amphibians is higher in aquatic than terrestrial habitats (Heyer et al., 1994). Some aquatic sampling methods also allow greater flexibility in sampling design than terrestrial sampling methods such as drift fences, coverboards, and PVC pipe refugia which require long-term effort in installation and continual monitoring. Aquatic-based techniques for detecting amphibians include anuran call surveys, visual encounter surveys, seining, dipnetting, quantitative enclosure (box or throw trap) sampling, litterbags, and funnel traps (Heyer et al., 1994; Olson et al., 1997; Dodd, 2003; Willson et al., 2005).

Quantitative comparisons of aquatic sampling methods have been conducted for several freshwater taxa, including fish (Chick et al., 1992; Layman and Smith, 2001), invertebrates (Turner and Trexler, 1997), and to a lesser extent, amphibians (Smith et al., 2006). For fish, active sampling (seining) may yield very different capture rates and species composition than passive sampling (funnel traps) (Layman and Smith, 2001). For sampling invertebrates in South Florida marshes, a combination of funnel trap, dipnet, and box trap sampling is recommended (Turner and Trexler, 1997). However, there are few quantitative, side-by-side comparisons of aquatic sampling methods for amphibians. Comparison of several techniques for estimating tadpole population estimates revealed that dipnetting and mark-recapture methods provided better estimates of population size than visual encounter sampling (Jung et al., 2002). A study of amphibians at Okefenokee National Wildlife Refuge in southern Georgia using nine aquatic and terrestrial methods suggested that anuran call surveys, traps, and dipnetting yielded the best estimates of species richness (Smith et al., 2006).

The objective of this study was to compare species richness and abundance estimates using seven aquatic amphibian sampling techniques. Although the focus of this study was lentic wetland habitats in the southeastern United States, the results may be applied to other habitats and amphibian communities. The specific objectives of this study were to determine which amphibian species were collected by each method and to evaluate the precision of individual counts for selected methods, with the goal of determining what combination of methods should be used to provide the best estimates of community richness.

## Materials and Methods

This study was conducted at the Ordway Swisher Biological Station in Putnam County, Florida, on 2-11 May 2005. A limited temporal sampling interval was selected for this study to increase the likelihood that differences in amphibian species detected were due to sampling method and not to changes in amphibian species present at sites over time. I selected 10 localities for sampling that included small sandhill ponds and large clear and dark water lakes (North Anderson Que Lake, Lake Barco, Blue Pond, Clear Pond, Fox Pond, Goose Lake, One Shot Pond, Piney Lodge Pond, Smith Lake, Lake Suggs) (Franz and Hall, 1991). Most localities had dense submergent and emergent vegetation, eliminating the possibility of evaluating seining as an aquatic sampling method.

Seven sampling methods were evaluated in this study. I collected 10 samples at each locality for the four methods which allowed for counts of individuals (count samplers: box trap, metal dipnet, D-frame dipnet, and crayfish trap). An exception was at Fox Pond, where only four samples using these methods were collected due to its extremely small size. For each sample, I sorted the contents in the field and recorded identity and number of all macrofauna. I identified amphibians and fish to the species level and aquatic invertebrates to order or family. I recorded the amount of time required to complete each of these four sampling methods. I also used three methods that allowed only collection of detected/not detected data at each locality (non-count samplers: visual encounter, froglogger, and aural). The pH of each locality was recorded using a Hydrolab Quanta water-quality meter.

### *Box trap*

The box trap was a 0.5 m<sup>2</sup> aluminum box with an open top and bottom (Chick et al., 1992, Gunzburger and Travis, 2004, Mullins et al., 2004). Ten box trap samples were taken at each site, and each box trap sample was separated by at least 3 m. For each sample, the box trap was gently thrown in the littoral zone and pushed firmly into the substrate. Macrofauna were removed from the trap by 10 sweeps with a D-frame dipnet (1 mm mesh). Contents of each trap were sorted using small aquarium nets, identified, and counted.

### *Dipnets*

I evaluated two different types of dipnets, a D-frame sweep net (wooden handle, metal net frame, mesh size = 1 mm, mouth area 35 × 28 cm, Wards Natural Science) and a metal dipnet (metal handle and net frame, mesh size = 3 mm, mouth area 52 × 41.5 cm, Memphis Net and Twine). These two nets span the size range of dipnets used most often by aquatic ecologists in terms of mesh size and mouth area. I standardized each sweep to 1 m in length by using a floating meter stick for reference, and all net sweeps were performed by a single individual. Sweeps were separated by at least 1 m from the previous sweep, and I alternated between the two

net types for a total of 10 sweeps of each net type. Most net sweeps were in shallow water, where the frame of the net was scraped along the substrate through aquatic vegetation. The contents of each sweep were sorted and counted prior to taking the next sweep.

### *Crayfish trap*

Crayfish traps, designed for the commercial capture of crayfish, are large pyramid-shaped mesh traps with three funnels at the bottom and a neck on top that allows captured animals to surface and breathe air. The crayfish traps in this study were purchased with a lining of smaller mesh size (mesh size = 5 mm) than standard commercial traps. The use of these traps has become an established technique for sampling amphibians, particularly large aquatic salamanders, that may be difficult to detect using other methods (Johnson and Barichivich, 2004; Sorensen, 2004). I placed 10 unbaited crayfish traps at least 3 m apart at each locality. The bottom of each trap was pressed firmly into the substrate, and traps were held upright by a PVC pipe pushed into the substrate of the pond. Crayfish traps were set in the afternoon and checked in the morning at each locality, with an approximate deployment time of 14 h. All captured animals were removed from each trap, identified, and counted. The amount of time to check all 10 traps was recorded.

### *Froglogger*

A froglogger is an automated recording device that records sounds over a specified interval of time (Peterson and Dorcas, 1992; Peterson and Dorcas, 1994; Penman et al., 2005). The frogloggers in this study consisted of a tape recorder, microphone, timer with voice-time stamp, and 12 V battery (Dodd, 2003). At each locality, one froglogger was set for one night (either 2 May 2005 or 9 May 2005) and recordings were conducted for one minute every hour between 1800 and 0800 h. To reduce the chance of incidentally recording anuran calls from areas other than the locality being sampled, the microphone was pointed directly into the center of each wetland, and a plastic cone around the microphone served to reduce sound from surrounding areas. However, it is possible that some anurans recorded on the tapes were calling from adjacent wetland or upland habitats. The identity of each species of anuran calling on each tape was recorded by two separate listeners and any discrepancies were arbitrated by a third listener.

### *Visual encounter*

For this study, visual encounter sampling consisted of recording amphibians observed incidentally at a site while performing the other sampling methods. Visual encounter surveys are usually either time or area constrained (Heyer et al., 1994), but in this study, the amount of time for visual encounter sampling was not standardized, but rather was dependent on the amount of time spent completing the other sampling methods at each site.

### *Aural*

For this study, aural sampling consisted of recording a list of all species of anurans heard calling diurnally while performing the other sampling methods. Aural surveys for anurans are typically conducted by observing nocturnal breeding choruses for a constrained period of time (Heyer et al., 1994), but many species of anurans call during the day, either periodic territorial or “rain” calls, or in full breeding choruses (Mount, 1975). Similar to visual encounter sampling in this study, the amount of time available for aural sampling at each site was dependent on the amount of time spent completing the other sampling methods.

### *Data analysis*

I used one-way ANOVA with post-hoc means comparisons using Bonferonni correction to test the null hypothesis that the number of amphibian species detected did not differ among sampling methods. I used linear regression to determine if (1) there was a correlation between the number of localities at which a species was detected and the number of methods with which a species was detected, and (2) there was a relationship between the number of anuran species detected as adults and as larvae among the 10 sites. For the count methods (with multiple samples of each method at each site), I constructed species accumulation curves for each sampling method to determine the number of samples necessary to yield the maximum number of species for each method.

Sampler efficacy was evaluated by comparing the number of individuals collected and the precision of abundance estimates for the four count sampling methods. I used linear regression to determine if there was a relationship between the number of amphibian species at a site and the total count of amphibians at a site. I used linear regression to evaluate the relationship between the total count of each species in the box trap (for the nine species detected with this method) with the number of sites at which each species was detected. I used one-way ANOVA to determine if the count of amphibians varied among the four sampling methods. For this analysis, I calculated the  $\bar{x}$  number of amphibians per sample method at each site. I evaluated variation in abundance estimates for each sampling method by calculating a coefficient of variation for each sampling method at each site ( $CV = \text{standard deviation}/\text{mean} \times 100$ ). I then averaged this CV across the 10 sites to provide an overall mean CV for each method. I used one-way ANOVA to evaluate whether the count samplers varied in the amount of time per sample. I conducted linear regression for each of the two dipnet types to determine if the count using dipnets was correlated with the count in the box trap.

Proportion of area occupied, calculated using multiple visits to replicate sites, is increasingly used as a metric for amphibian monitoring programs (Corn et al., 2005). In order to evaluate the influence of sampling method on estimates of site occupancy and detection probability, I selected the five amphibian species collected at highest abundances (*A. gryllus*, *H. femoralis*, *H. gratiosa*, *R. sphenoccephala*, and

*N. perstriatus*) to compare occupancy estimates and detection probability for three methods: box trap, crayfish trap, and metal dipnet. I considered the 10 separate samples of each method as separate “visits” to each locality. Fox Pond was excluded from this analysis because only four samples were taken, leaving nine localities. Detection probability ( $p$ ) was set as a constant for each method across localities for these models, with the assumption that detection does not change over the course of the 10 samples for each method. Site covariates included in each model were presence of fish and pH. I calculated occupancy and detection using PRESENCE 2 software.

I evaluated the effectiveness of the box trap, dipnets, and crayfish trap in detecting fish and aquatic invertebrates with one-way ANOVAs of species (or taxa for invertebrates) richness and total count of each of these taxa among the four count sampling methods. I evaluated the CV for each method for fish and invertebrates as described above for amphibians. I also constructed accumulation curves for both fish species and invertebrate taxa for each count sampling method. All data analyses, except occupancy and detection analyses, were performed using SYSTAT 11 software.

## Results

### *Amphibian species richness*

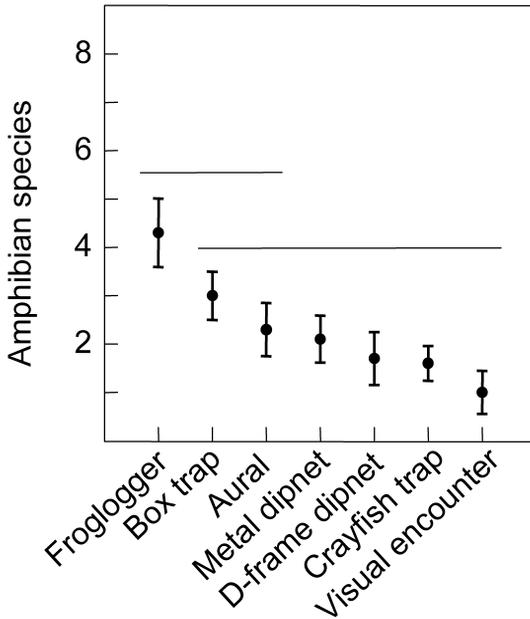
A total of 11 species of anurans (*Acris gryllus*, *Bufo terrestris*, *Gastrophryne carolinensis*, *Hyla cinerea*, *H. femoralis*, *H. gratiosa*, *Pseudacris ocularis*, *Rana capito*, *R. catesbeiana*, *R. gryllio*, *R. sphenoccephala*) and two species of caudates (*Siren lacertina* and *Notophthalmus perstriatus*) was detected. Six species were detected by three or fewer sampling methods (*S. lacertina*, *G. carolinensis*, *H. cinerea*, *P. ocularis*, *R. capito* and *R. catesbeiana*). Two species were detected with six or seven methods (*A. gryllus* and *R. sphenoccephala*). Two anuran species were detected only with frogloggers or aural sampling (*H. cinerea* and *G. carolinensis*). No species was detected by only one sampling method.

Detection of anurans varied by life stage and species, and appeared to be related to breeding season and larval period length (table 1). Three species were usually detected at both tadpole and adult stages; all of these species have relatively long breeding seasons and, *R. sphenoccephala* also has a long larval period. Four species were usually detected as adults, but were sometimes detected at both stages or as larvae. Three species were only detected as adults, and all of these species breed over a medium or long season (*H. cinerea* continually, *P. ocularis* and *G. carolinensis* generally in response to rain), and all have relatively short larval periods (table 1). One species, *R. capito*, was detected only as larvae. This species has a short breeding season and a prolonged larval period.

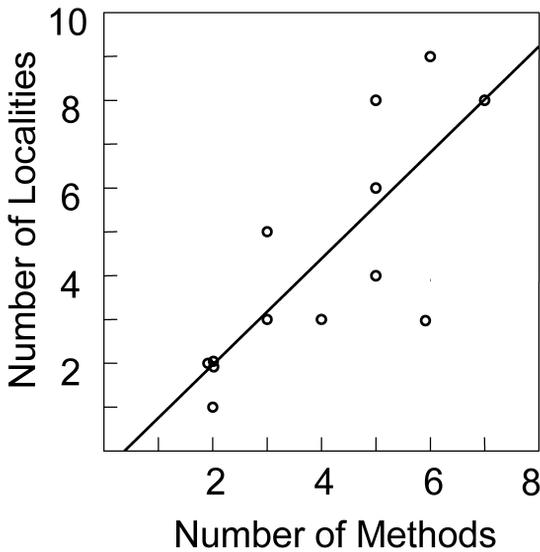
Amphibian species richness varied among sampling methods (ANOVA  $F_{6,63} = 4.832$ ,  $P < 0.001$ ; fig. 1). Froglogger sampling resulted in the highest species

**Table 1.** Number of localities ( $N = 10$ ) at which 11 anuran species were detected by life stage. Breeding season and larval period length for each species are defined as: short (<1 month), med (1–4 months) and long (>4 months) (Wright and Wright, 1995; Mount, 1975).

| Species                          | Number of sites detected |       |      | Length          |               |
|----------------------------------|--------------------------|-------|------|-----------------|---------------|
|                                  | Larvae                   | Adult | Both | Breeding season | Larval period |
| Detected usually at both stages  |                          |       |      |                 |               |
| <i>Acris gryllus</i>             | 0                        | 2     | 7    | long            | med           |
| <i>Hyla gratiosa</i>             | 0                        | 1     | 3    | med             | med           |
| <i>Rana sphenoccephala</i>       | 0                        | 2     | 6    | long            | long          |
| Detected usually as adult        |                          |       |      |                 |               |
| <i>Hyla femoralis</i>            | 0                        | 6     | 2    | med             | med           |
| <i>Rana catesbeiana</i>          | 1                        | 2     | 0    | long            | long          |
| <i>Rana grylio</i>               | 0                        | 4     | 2    | long            | long          |
| <i>Bufo terrestris</i>           | 0                        | 2     | 1    | long            | short         |
| Detected only as adult           |                          |       |      |                 |               |
| <i>Gastrophryne carolinensis</i> | 0                        | 2     | 0    | med             | short         |
| <i>Hyla cinerea</i>              | 0                        | 2     | 0    | long            | short         |
| <i>Pseudacris ocularis</i>       | 0                        | 5     | 0    | med             | short         |
| Detected only as larvae          |                          |       |      |                 |               |
| <i>Rana capito</i>               | 2                        | 0     | 0    | short           | long          |



**Figure 1.** Average  $\pm$  standard error amphibian species richness among the seven sampling methods. Horizontal lines join methods not significantly different in an ANOVA.

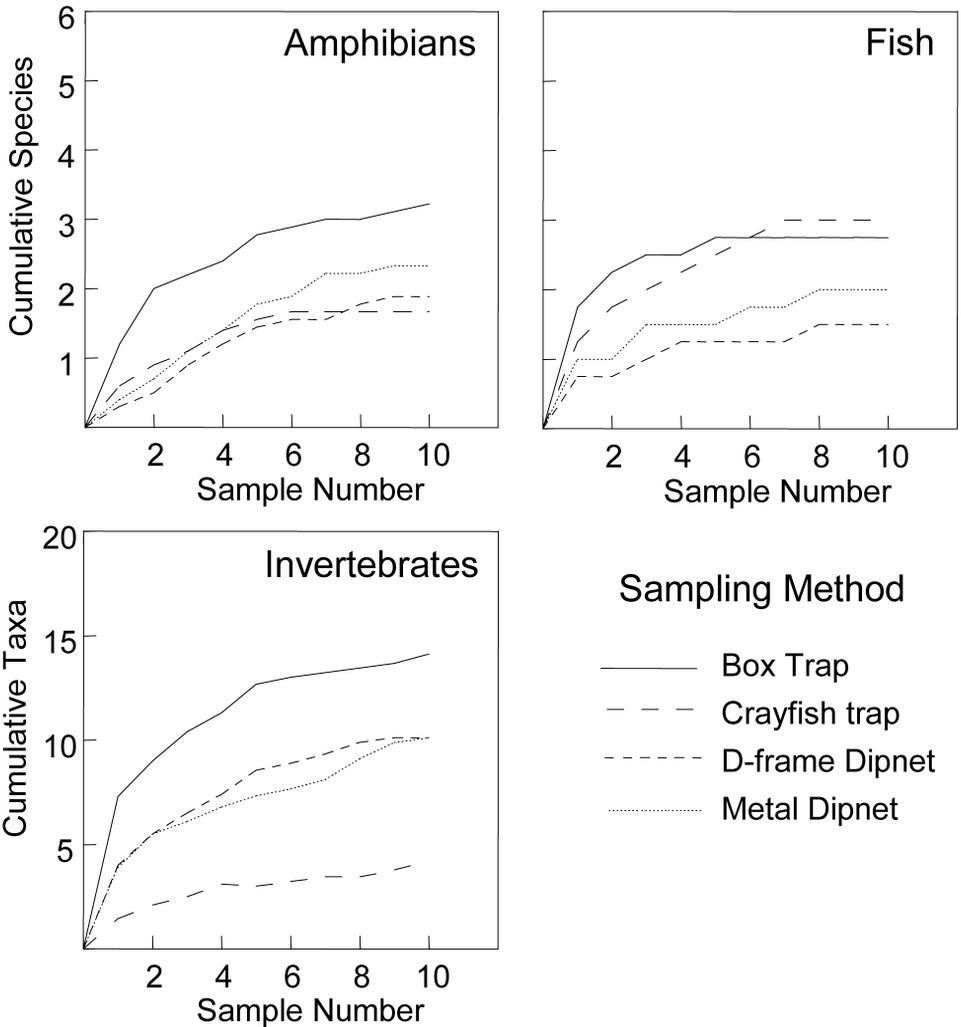


**Figure 2.** Relationship between the number of methods (1-7) by which a species was detected with the number of localities (1-10) at which a species was detected.

richness, followed by box trapping, aural sampling, dipnets, and crayfish traps, whereas visual encounter sampling resulted in the lowest species richness. Species richness did not differ between the two dipnet types. The number of localities an amphibian species was found at increased with the number of methods by which it was detected ( $r^2 = 0.601$ ,  $F_{1,11} = 16.57$ ,  $P = 0.002$ ; fig. 2). There was no relationship between the number of anuran species detected as adults (by froglogger, visual, aural sampling) and the number of anuran species detected as larvae (crayfish trap, dipnet, box trap) at a site ( $r^2 = 0.295$ ,  $F_{1,8} = 3.35$ ,  $P = 0.105$ ). Species accumulation curves suggested that 10 samples of dipnets and crayfish traps is probably sufficient to detect most amphibian species, but more than 10 box trap samples may be necessary (fig. 3).

### *Amphibian abundance*

There were significant differences in the number of amphibian individuals captured by the four count methods. Smaller tadpoles (hylids and bufonids) were collected in greater numbers in box traps and dipnets, whereas larger tadpoles (ranids) were collected in greater numbers in crayfish traps (table 2). This difference is unlikely due to small tadpoles escaping from any of the four samplers, because mesh size of the lined crayfish traps is similar to that of the dipnets. *Rana sphenoccephala* was an exception to this pattern and was collected in relatively large numbers using all methods (table 2). *Notophthalmus perstriatus* was collected in greatest numbers using the box trap but was also detected using the other three methods. *Siren lacertina* was detected very rarely, and only by crayfish traps and dipnets.



**Figure 3.** Accumulation curves for amphibian species, fish species, and invertebrate taxa for the four count sampling methods. Each line is the average across 10 localities, except for fishes, in which the line is the average across the four localities where fishes were detected.

The total number of individuals collected at a site was positively correlated with the number of amphibian species detected at that site by all methods ( $r^2 = 0.584$ ,  $F_{1,8} = 11.25$ ,  $P = 0.01$ ). However, there was no correlation between the total count of any species and the number of sites at which that species was detected. More amphibian individuals were collected with the box trap than by using the other three methods (ANOVA,  $F_{3,36} = 4.25$ ,  $P = 0.011$ ). Precision for the four count sampling methods was relatively low and similar for the four methods (table 3).

Time per trap varied across the four count sampling methods; box trap sampling took significantly longer than dipnets or crayfish traps (ANOVA,  $F_{3,34} = 73.57$ ,

**Table 2.** Total number of amphibian individuals collected at 10 localities ( $N = 94$  samples of each method) using four aquatic sampling methods that allow counts of individuals.

| Species                                    | Box trap | Crayfish trap | D-frame dipnet | Metal dipnet |
|--|----------|---------------|----------------|--------------|
| <b>Anurans</b>                             |          |               |                |              |
| Detected usually with box trap and dipnets |          |               |                |              |
| <i>Acris gryllus</i>                       | 32       | 0             | 16             | 21           |
| <i>Bufo terrestris</i>                     | 1        | 0             | 4              | 7            |
| <i>Hyla femoralis</i>                      | 40       | 0             | 10             | 17           |
| <i>Hyla gratiosa</i>                       | 69       | 3             | 28             | 40           |
| Detected usually with crayfish trap        |          |               |                |              |
| <i>Rana capito</i>                         | 3        | 13            | 0              | 0            |
| <i>Rana catesbeiana</i>                    | 0        | 2             | 0              | 0            |
| <i>Rana grylio</i>                         | 8        | 3             | 0              | 1            |
| <i>Siren lacertina</i>                     | 1        | 1             | 0              | 0            |
| Detected with all methods                  |          |               |                |              |
| <i>Rana sphenoccephala</i>                 | 64       | 114           | 7              | 16           |
| <i>Notophthalmus perstriatus</i>           | 169      | 11            | 35             | 42           |

**Table 3.** Average count and overall average coefficient of variation (CV) (average of all CVs for each method at each locality) for amphibians, fishes, and invertebrates using four sampling methods.

| Sampling method | Amphibians     |     | Fishes         |     | Invertebrates  |     |
|-----------------|----------------|-----|----------------|-----|----------------|-----|
|                 | $\bar{x}$ (SD) | CV  | $\bar{x}$ (SD) | CV  | $\bar{x}$ (SD) | CV  |
| Box trap        | 5.09 (4.61)    | 141 | 2.25 (4.16)    | 94  | 48.7 (25.66)   | 39  |
| Crayfish trap   | 1.65 (2.21)    | 142 | 1.42 (2.39)    | 102 | 1.51 (1.07)    | 122 |
| D-frame dipnet  | 1.03 (1.56)    | 169 | 0.58 (0.87)    | 116 | 10.73 (11.51)  | 64  |
| Metal dipnet    | 1.52 (1.99)    | 173 | 0.54 (0.99)    | 137 | 11.19 (9.57)   | 67  |

$P < 0.001$ ; table 4). In order to compare time-constrained dipnetting to dipnetting for a constrained number of sweeps, I calculated the number of sweeps that would have been completed at each site if the total amount of time spent dipnetting was set at 30 min per site. The average number of dipnet sweeps in 30 min was 11.5, with a range of four to 24 sweeps. The count of amphibian individuals in dipnets was positively correlated with the count in the box trap (D-frame dipnet  $r^2 = 0.411$ ,  $F_{1,8} = 5.576$ ,  $P = 0.046$ ; metal dipnet  $r^2 = 0.422$ ,  $F_{1,8} = 5.85$ ,  $P = 0.042$ ), but the correlation coefficient was low.

### Occupancy and detection

Occupancy and detection varied for the five amphibian species tested across three sampling methods (table 5). The widest variation in detection probability was for *H. gratiosa*, which ranged from a detection probability of 0.09 with crayfish traps to 0.52 with the box trap. Occupancy varied most strongly for *N. perstriatus*, which ranged from 0.11 with crayfish traps to 0.33 with box traps. Detection and occupancy did not vary in the same way for the five species tested. For

**Table 4.** Comparison of time and cost among the seven sampling methods. Time per sample includes the total time necessary to complete data collection for each sampling method (e.g., time spent setting traps, sorting and counting trap contents, and listening to froglogger tapes, but not the running time of the froglogger).

| Sampling method  | Time per sample (min) | Cost per sampler (US\$) | Supplier                 |
|------------------|-----------------------|-------------------------|--------------------------|
| Box trap         | 14                    | \$300                   | custom fabricator        |
| D-frame dipnet   | 3.3                   | \$40                    | Wards Natural Science    |
| Metal dipnet     | 3.3                   | \$100                   | Memphis Net & Twine      |
| Crayfish trap    | 2                     | \$45                    | Lee Fisher International |
| Froglogger       | 45                    | \$340                   | see Dodd, 2003           |
| Aural            | variable              | \$0                     | NA                       |
| Visual encounter | variable              | \$0                     | NA                       |

**Table 5.** Occupancy (naïve and estimated  $\Psi$ ) and detection probabilities ( $p$ ) for five species of amphibians at nine localities using three different sampling methods (10 repeat samples for each method are considered separate visits in the analysis). A dash indicates the species was not detected with that method. Two site covariates included in the analysis were pH and presence of fish.

|                                  | Box trap |        |      | Crayfish trap |        |      | Metal dipnet |        |      |
|----------------------------------|----------|--------|------|---------------|--------|------|--------------|--------|------|
|                                  | Naïve    | $\Psi$ | $p$  | Naïve         | $\Psi$ | $p$  | Naïve        | $\Psi$ | $p$  |
| <i>Acris gryllus</i>             | 0.78     | 0.79   | 0.37 | –             | –      | –    | 0.78         | 0.89   | 0.19 |
| <i>Hyla femoralis</i>            | 0.22     | 0.22   | 0.55 | –             | –      | –    | 0.22         | 0.23   | 0.29 |
| <i>Hyla gratiosa</i>             | 0.44     | 0.44   | 0.52 | 0.22          | 0.35   | 0.09 | 0.33         | 0.34   | 0.33 |
| <i>Rana sphenocephala</i>        | 0.67     | 0.67   | 0.40 | 0.67          | 0.67   | 0.70 | 0.56         | 0.63   | 0.19 |
| <i>Notophthalmus perstriatus</i> | 0.33     | 0.33   | 0.77 | 0.11          | 0.11   | 0.50 | 0.22         | 0.22   | 0.70 |

*R. sphenocephala*, the highest detection was with crayfish traps, whereas for the other four species, the highest detection was with box trap sampling. Box trap sampling yielded consistently higher detection than dipnet sampling for all species.

### *Fish and invertebrates*

Twelve species of fish and 25 taxa of aquatic invertebrates were detected using the four count sampling methods. The number of fish species detected did not vary among these sampling methods (ANOVA,  $F_{3,36} = 0.39$ ,  $P = 0.76$ ). This result did not change if the six localities without fish were excluded from the analysis. A similar number of fish was collected among all methods (ANOVA,  $F_{3,36} = 1.06$ ,  $P = 0.38$ ). However, comparison of the two most frequently captured fish species indicates there may be significant variation among fish species in terms of the most effective sampling method. Large predatory fishes (*Lepomis gulosus*) were captured almost exclusively with crayfish traps, whereas small fishes (*Gambusia holbrooki*) were sampled more evenly by all methods (table 6). Sampler precision was low and similar among all four methods for fish counts (table 3). Continued sampling beyond 10 samples of these four methods appears unlikely to result in detection of additional fish species (fig. 3).

**Table 6.** Total number of individuals of selected fishes and invertebrates (Aquatic Insects) collected at 10 localities ( $N = 94$  samples of each method) using four aquatic sampling methods that allow counts of individuals.

| Taxa                      | Box trap | Crayfish trap | D-frame dipnet | Metal dipnet |
|---------------------------|----------|---------------|----------------|--------------|
| Fish                      |          |               |                |              |
| <i>Gambusia holbrooki</i> | 166      | 42            | 44             | 33           |
| <i>Lepomis gulosus</i>    | 0        | 87            | 0              | 1            |
| Aquatic Insects           |          |               |                |              |
| Coleoptera                | 691      | 35            | 100            | 102          |
| Odonata                   |          |               |                |              |
| Aeshnidae                 | 53       | 20            | 12             | 28           |
| Libellulidae              | 2376     | 26            | 204            | 414          |
| Zygoptera                 | 762      | 3             | 344            | 305          |
| Naucoridae                | 116      | 2             | 37             | 50           |
| Notonectidae              | 95       | 4             | 60             | 57           |

The number of invertebrate taxa detected was greatest using the box trap, then dipnets, then crayfish traps (ANOVA,  $F_{3,36} = 16.61$ ,  $P < 0.001$ ). Significantly more individuals were collected using the box trap than by using the other three count methods (ANOVA,  $F_{3,36} = 19.83$ ,  $P < 0.001$ ). Counts of the six most frequently encountered invertebrate taxa were much higher in the box trap relative to dipnets, which were in turn much higher than counts in crayfish traps (table 6). For invertebrates, box trap and dipnets had higher precision (lower CV) than crayfish traps (table 3). Additional box trap and dipnet sampling beyond 10 samples may yield additional invertebrate taxa (fig. 3).

## Discussion

This study demonstrated significant variation among the seven sampling methods tested in terms of species richness and counts of amphibians. In general, the methods that required the most time (froglogger and box trap) also yielded the greatest species richness. Caudates were consistently detected with fewer methods than anurans because they are impossible to detect aurally and are unlikely to be observed during visual encounter surveys, thus are only likely to be detected by four of the seven methods. The number of localities at which a species was detected increased with the number of sampling methods by which that species was detected. This could indicate that species that are habitat generalists are easier to detect, and species that are detected by fewer methods are actually more rare. However, it could be that species that were detected by fewer methods were actually present at the same number of sites but were less likely to be detected by the methods used in this study. The relationship between abundance and detection probably varies among species, and in this study, species detected at more sites were not more abundant at those sites than species found at fewer sites. Sites with higher counts of amphibians also had higher amphibian species richness. These results emphasize the

importance of estimating detection probabilities for monitoring data (MacKenzie and Kendall, 2002; MacKenzie et al., 2002). However, the accuracy of models incorporating detection probabilities will be highly dependent on the sampling methods employed and the relationship between abundance and detectability for the focal species (table 5). Thus, comparisons of site occupancy by an amphibian species or community across studies with different sampling methods may be invalid.

The variation in effectiveness of these seven methods is due in part to variation in behavior and life history of the different amphibian species. For detection of calling male frogs to be used as an indicator of successful breeding, it must be correlated with the presence of larvae of that species. I found no correlation between the number of species detected as adults and the number of species detected as larvae at a site, and three species of anurans were only detected as adults. Using methods that allow detection of both adults and larvae increases the total number of species detected, because species with short or sporadic breeding seasons but long larval periods are likely to be detected as larvae even if adults are not detected. Species with short breeding seasons and larval periods are least likely to be detected, unless sampling is targeted during appropriate breeding conditions. The short time frame of this study (11 days) biased detection towards species with long breeding or larval seasons and reduced the chance of detecting explosive breeders with short larval periods. However, many amphibian inventory and monitoring programs require rapid assessment techniques similar to this study. Monitoring programs may be based on surveys of species presence or abundance. For pond-breeding amphibians, counts of larval stages may not result in an accurate indicator of changes in population size over time; however, these counts may be used to refine detection estimates for monitoring programs (Dodd and Dorazio, 2004). In addition, quantification of percent composition of larval amphibian communities may be important in studies of community ecology (Gunzburger, 2005).

Detection of larvae was correlated with tadpole size and activity level, with larger, more active tadpoles (ranids) captured more often using passive crayfish traps, and smaller tadpoles (hylids) more often detected using active sampling methods. An exception is *R. sphenoccephala*, which was caught by all sampling methods, but the highest capture rate was with crayfish traps. Although not explicitly evaluated in this study, the timing of activity (diurnal or nocturnal) may also have an effect on larval capture rates. Dipnetting and box trap sampling were conducted during the day, whereas the crayfish traps were deployed in late afternoon and checked in the early morning, so the most likely species to be captured would be those active at night (Anderson and Graham, 1967).

Dipnetting is probably the most commonly used aquatic survey method for amphibians, and attempts to standardize this sampling method typically include either dipnetting for a specified number of dipnet sweeps or for a specified period of time (Heyer et al., 1994; Baber et al., 2004; Bishop et al., 2006). Sweep-constrained dipnetting allows greater standardization for projects involving multiple surveyors

(Bishop et al., 2006). This study demonstrated that time-constrained dipnetting results in highly variable effort across localities due to variation in the amount of time needed to sort through net contents. The objectives of some amphibian research or monitoring projects may require accurate density estimates of amphibians. The extent to which amphibian counts in the four count sampling methods evaluated in this study are correlated to the actual density of amphibians at each site is unknown. For crayfish, the density of individuals in a large field enclosure was highly correlated with the density estimate from box traps (Dorn et al., 2005). In this study, sampling using the box and crayfish traps yielded the least variable amphibian count estimate of the four count methods (table 3). Box trap sampling is the only method tested in this study that may allow estimation of density, because a known area of habitat is sampled with each trap. However, box trap sampling took much longer to complete (140 min) than dipnetting (33 min) for 10 samples. Therefore, box trap sampling may not be feasible for monitoring programs that have a goal of sampling many sites in a short period of time. Dipnet counts have been used as indices of abundance for amphibians (Babbitt et al., 2003). The count of amphibian individuals in dipnets was only weakly correlated with box trap counts, and the use of dipnet counts as density indices for amphibians should be the subject of further study.

The amphibian species detected during this study represent about half the total number of amphibian species documented to occur on the Ordway Swisher Biological Station. Thirteen species of amphibians were detected at 10 sites during this study, whereas 27 species of amphibians have been detected on the Ordway Swisher Biological Station during more than two decades of extensive ecological research (Franz, 1995). Most of the species not detected in this study are either rare on the Ordway Swisher Biological Station or not likely to be present at the localities sampled during the time frame of this study. Several of the species are either not typically present in wetlands during the time period of the study (*Pseudacris crucifer*, *Eurycea quadridigitata*), are typically not found in lake and pond habitats that were the focus of the sampling (*Plethodon grobmani*, *Eleutherodactylus planirostris*), or are considered rare on the Ordway Swisher Biological Station (*Pseudobranchius striatus*, *Notophthalmus viridescens*, *Hyla chrysoscelis*, *Rana heckscheri*) (Franz, 1995). Two species (*Bufo quercicus*, *Scaphiopus holbrookii*) breed explosively in response to heavy rainfall and have short larval periods. The remaining four species (*Amphiuma means*, *Siren intermedia*, *Hyla squirella*, *Rana clamitans*) are considered common on the Ordway Swisher Biological Station and are typically present in aquatic habitats for extended periods of time, so the reason they were not detected in this study is unclear. Repeated sampling of wetlands over time would likely have increased the number of species detected.

Although the focus of this study was amphibian sampling, the methods evaluated can also be used for fish and aquatic invertebrates. Many species of fish and aquatic invertebrates are predators of amphibian eggs and larvae, and thus may be important

correlates of amphibian habitat distribution (Wellborn et al., 1996; Babbitt et al., 2003; Gunzburger and Travis, 2004). Collecting data on the occurrence of fish and aquatic invertebrates may be integral to a successful amphibian monitoring program. Aquatic invertebrates were collected with all methods, but greatest counts were with the box trap and lowest with the crayfish trap. This pattern may be related to size and activity level, inasmuch as most invertebrates are relatively small and many (especially insects) are inactive relative to fish and amphibian larvae. An exception is crayfish, which are large relative to most other invertebrates and highly mobile, especially at night, although crayfish were only captured at three sites during this study and never in high abundance. Large, highly mobile predatory fish are detected best with the crayfish trap, whereas smaller fish will likely be detected with all methods. This difference in capture rates is important for projects that seek to understand amphibian population dynamics and habitat distribution, because the presence of large predatory fish is one of the primary factors affecting amphibian community structure (Wellborn et al., 1996).

The choice of amphibian sampling methods will depend on the objectives of the research or monitoring program. For species inventories, the widest possible range of sampling techniques should be employed, including methods that detect adult and larval stages of both anurans and caudates. A combination of frogloggers, crayfish traps, and dipnetting is a cost and time efficient sampling protocol for inventory and monitoring. However, the use of frogloggers and crayfish traps require visits to each site on two days in order to set and retrieve the froglogger and traps. If two visits to each site are not possible given time or cost constraints, a combination of dipnetting, aural, and visual encounter sampling would allow single visits to many sites in a relatively short time period. This study has demonstrated the importance of recording incidental, and virtually cost-free, observations including visual encounter and aural sampling, because these techniques together detected all but two of the nine species of anurans found in this study. If the objective of the study is to compare density of amphibians, then box trap sampling should be considered. Researchers should consider evaluating their sampling methods prior to and during monitoring and research projects in order to evaluate the capabilities and limitations of each method.

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