HYDROPERIOD OF WETLANDS AND REPRODUCTION IN WOOD FROGS (RANA SYLVATICA) AND SPOTTED SALAMANDERS (AMBYSTOMA MACULATUM)

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Chapter 3

AN EVALUATION OF POND SAMPLING TECHNIQUES FOR LARVAL AMPHIBIANS IN WETLANDS

ABSTRACT

A common objective of many aquatic amphibian studies involves documenting the presence or species richness of larval amphibians at breeding pools. Although several techniques are commonly employed for sampling larval amphibians, a side-by-side comparison of their efficiency at documenting species presence or in capturing individuals is not often assessed. I used 4 larval sampling techniques (dip nets, pipe samplers, funnel traps, and bottle traps) to sample amphibian larvae in 30 wetlands in July and August 1999. For the 4 focal species (i.e., spotted salamanders [*Ambystoma maculatum*], eastern newts [*Notophthalmus viridescens*], green frogs [*Rana clamitans*], and bull frogs [*Rana catesbeiana*]), funnel traps had the highest probability of detection for a given level of effort (i.e., number of stations). For spotted salamanders and bull frogs, dip nets were the least effective; for eastern newts, bottle traps performed the poorest; and for green frogs, pipe samplers and bottle traps had the lowest probability of detection for a given level of effort. Green frog larvae had the highest probability of detection and spotted salamanders had the lowest for all 4 techniques. The mean number of person hours per station was lowest for dip nets and was similar for pipe samplers, funnel traps, and bottle traps. In terms of numbers of individuals captured, funnel traps generally captured the most spotted salamanders and bull frogs. For eastern newts, pipe samplers captured the most individuals whereas for green frogs, pipe samplers and funnel traps typically captured the most individuals. Dip nets and bottle traps typically yielded the lowest numbers of individuals across species.

INTRODUCTION

For amphibians that rely on wetlands for breeding, a question that is often asked is whether a target species actually breeds in a given wetland? Call surveys are frequently used to document anuran breeding, but this technique can be misleading because anurans often vocalize at sites in which they are known not to breed (personal observation) or in which reproduction is unsuccessful and some species have such a short breeding period that vocalization could be missed. Larval surveys take more time to implement than call surveys, but they 1) document actual breeding of species, 2) can provide an index of reproductive success at some point of larval development, and 3) document non-calling amphibians such as salamanders.

Several techniques are commonly used to sample larvae (see reviews of methods by Olson et al. 1997, Shaffer et al. 1994), but their efficiency is rarely investigated (e.g., Buech and Egeland 2002a and 2002b). Local and regional differences in the assemblage of larval amphibians or the physical characteristics of the wetlands may also warrant the use of different techniques. For example, in wetlands with clear water or minimal vegetation, active sampling techniques that use visual cues (e.g., visual counts, snorkeling) may be warranted, whereas these techniques would not be suitable for vegetation-choked or murky waters.

The purpose of this study was to compare 4 larval sampling techniques in terms of detecting species presence, determining species richness, and capturing numbers of individuals of a given species. I sampled larval amphibians in 30 wetlands in Acadia National Park, Maine, USA in July and August 1999. I examined the probability of capture of common species for the 4 techniques and used that information to determine the time required to implement each technique, as well as its effectiveness in determining species richness, for a given level of effort. I calculated the minimum number of stations required to document the presence of individual species, with a probability of 90%. I also examined the mean numbers of individuals captured per station for each of the techniques across all wetlands.

METHODS

Study Area

The study area is located along the mid-coast of Maine, USA, on the Mount Desert Island portion of Acadia National Park, Hancock County (44^0 13' – 44^0 27' North, 68^0 10' – 68^0 26' West). The terrain of Mount Desert Island consists of north-south oriented ridges separated by deep U-shaped valleys (Patterson et al 1983). The highest elevation (466 m) is on the northeast portion of the island at the summit of Cadillac Mountain. Mount Desert Island is situated at the southern limit of the spruce-fir northern hardwoods zone (Westfeld et al. 1956). Uplands are dominated by thin, granitic soils (Gilman et al. 1988; Chapman 1970) with organic soils common in wetlands (Calhoun et al. 1994). Six percent of the island contains palustrine wetlands, with most concentrated in the eastern half of the island. Ponds and lakes cover 4% of the island, 25 of which are greater than 3 ha in area. For the 40 km^2 of palustrine wetlands in Acadia National Park and vicinity (i.e., Mount Desert Island, Schoodic Penninsula, and the surrounding islands): <1% are aquatic bed, 9% are emergent, 48% are forested, 38% are scrub-shrub, and 5% are unconsolidated bottom (Calhoun et al. 1994). Beaver-created wetlands, both occupied and abandoned, are common, particularly on the east side of the island. Wetland conditions vary from oligotrophic sites with gravel substrates and sparse vegetation to eutrophic sites with muck substrates and abundant vegetation. Most wetlands are dystrophic, with tannin-stained waters, and thus the visibility in the water column is generally low.

Study Site Selection

Potential study sites were identified from palustrine wetlands, less than 15 ha in area, on National Wetland Inventory (NWI) maps and from among smaller, unmapped wetlands that were known to exist or that were encountered during preliminary surveys as part of a larger study (See Chapters 1 and 2). Wetland study sites were initially selected randomly, stratified on: 1) size of wetland (0.01 – 15.00 ha), 2) dominant wetland class (based on the Cowardin classification system [1979]), 3) hydrogeomorphic setting (i.e., isolated versus connected to a stream), and 4)

presence/absence of beaver. Seventy-two wetlands were monitored in 1999 as part of a larger study; the sites ranged in duration of flooding from seasonal wetlands of short duration to permanent wetlands. Thirty sites still contained water in July and August 1999 and were sampled for this study. The sites were generally limited to larger wetlands, with some semi-permanent or permanent smaller wetlands; size range for sites during basin-full, spring conditions was 0.05 – 15.00 ha. Twenty-five sites were influenced by an active or abandoned beaver dam and channels.

Sampling Methods

The 30 wetlands were separated into 5 groups with 4 – 8 sites per group; sites were grouped by location, so that travel time between sites would be minimized. Each group was sampled for 6 consecutive days between 12 July and 10 August 1999. Potential larval habitat was considered all areas of standing water that were less than 1 meter depth, yet deep enough to allow sampling equipment to be fully submerged. On the first sampling day for each group, sampling stations were established throughout potential larval habitat. For large wetlands that had similar vegetation on two sides, sampling stations were established only on one side to reduce sampling time. In large sites with broad areas of potential larval habitat along the edge, stations were staggered alternately, with one near-edge station, followed by one far-from-edge station. The number of stations sampled was based on the the approximate area of potential larval habitat at a particular wetland to keep sampling effort in proportion to area.

Four techniques were used at each station at each wetland: 2 active sampling techniques (dip nets and pipe samplers) and 2 passive sampling techniques (funnel traps and bottle traps). A consecutive 6-day sampling period consisted of: 1 day dip-net sampling, 1 day pipe sampling, 4 days simultaneously funnel trapping and bottle trapping (1st day consisted of setting the traps, and then 3 days of checking the traps). The order of the sampling days varied across the sites (although funnel and bottle trapping were always conducted on consecutive days). At each station 3 pipe and 2 dip net samples were taken; fewer dip nets were taken in an effort to sample similar volumes of water. At each station, both a funnel and bottle trap were established. I also

recorded the number of person hours it took to sample each site with each of the four techniques; this included set up time as well as actual sampling time.

Dip Nets. Dip nets were 'D" shape, 30.5 cm diameter (i.e., maximum width) and depth 20.1 cm; sides of net were made of heavy cotton and nylon canvas with a 500 micron mesh bottom. Two dip net sweeps, approximately 3 meters apart, were taken at each station. Each sweep began at the top of the water column in front and slightly to the side of the observer; the net was then pulled quickly down (towards the bottom) and backwards (towards, and then behind the observer), then out of the water column covering a path of 1 meter. Any larval amphibians captured were identified to species and counted, then released at the station.

Pipe Samplers. Three pipe samples were taken at each station approximately 3 meters apart to prevent disturbance. Pipe samplers were constructed from 30 cm diameter culvert pipe, 1 meter in height. Handles were attached near the top of the pipe sampler. Pipe samples were taken by projecting the pipe sampler roughly 1 meter in front of the observer (while hanging onto the handles), then pushing it forcibly straight down through the water column. Once the pipe sampler was situated, a 25.4 cm x 15.2 cm aquarium dip net was then used to sample larvae from within the tube. Dip nets were extended flush with the top of the substrate then, with a circular motion the dip net was moved to "sweep" the entire bottom of the sampler in a 360^0 rapid sweep; then quickly drawn up through the tube. After each dip net, any larvae captured were identified to species and counted. Dip net samples were taken repeatedly, until 5 consecutive sweeps yielded no larval amphibians. This method is similar to sampling used by Skelly (1992) in Michigan.

Funnel Traps. One plastic funnel trap was placed at each station. The funnel trap was a standard-sized, near cylindrical minnow trap, 43.2 cm long and 22.9 cm maximum diameter (at center of trap). Mesh size varied along the trap and ranged from 2 x 4 mm (at the 2 ends) to 4 x 7 mm (at the center). Dimensions of each funnel were: 15.5 cm wide, 11.4 cm deep, with a 2.4 cm diameter entrance hole. Rocks were placed in the trap to prevent it from floating. Traps were checked every 24 hours for 3 days. Any amphibian larvae captured were identified to species and counted.

Bottle Traps. One plastic bottle trap was placed at each station and checked every 24 hours for 3 days (same time as checking the funnel traps). A small rock was placed in the bottle to prevent it from floating. The bottle traps were constructed from two 2-litre plastic soda bottles. The tapered part of one bottle (top) was cut off and inverted and attached to the base of the bottle with clear plastic waterproof tape. The tapered part of a second bottle was also inverted to act as a funnel on the other end of the trap. The neck of each of the funnels was cut so that the opening was 2.4 cm and width and depth of the funnel was 10.5 cm and 6.5 cm, respectively. Small slits or holes were made in the trap to help keep the trap submerged. The bottle traps were similar to those used by Smith and Rettig (1996), Richter (1995), and Griffiths (1985), but had 2 funnel openings (instead of 1) and a larger entrance hole.

Data Analysis

Presence of a species as well as overall species richness at a site was determined by using the results of all sampling methods combined. Probability of capture of 4 common species: *Amybstoma maculatum* (spotted salamander), *Notophthalmus viridescens* (eastern newt), *Rana clamitans* (green frog), and *Rana catesbeiana* (bull frog) was determined for each of the 4 techniques. For each site at which a species was documented, the proportion of stations that the species was recorded was calculated for each technique. For example, imagine that spotted salamanders were documented at site A and site A had 20 stations. If spotted salamanders had been recorded at 10 stations using dip nets, 5 using pipe samplers, and 0 using funnel traps and bottle traps, then the proportion of stations would be: $p = 0.50$ for dip nets, $p = 0.25$ for pipe samplers, and $p = 0.00$ for both funnel and bottle traps. For the 4 common species, I averaged the proportion of stations at which the species was detected for each technique across all sites at which the species was recorded. The mean value and standard error were then used to determine a binomial distribution indicating the probability of detection of a species per unit of effort (i.e., number of stations) for each technique, as well as the standard error of that distribution. Based on this analysis, I determined the minimum number of stations required to

document the presence of each of the 4 common species, with a probability of 90%, given that the species is actually present.

I calculated the amount of time to implement a technique by averaging the number of person hours per station across all sites for each of the techniques; this was done for the number of sampling stations used for a typical medium and large wetland: 12 and 24, respectively. Similarly, I calculated the mean species richness for a medium and large wetland, given that the 4 key species were present, for each of the techniques. I also calculated the mean numbers of individuals of a given species captured per station for each technique, averaged across all sites in which they were documented.

RESULTS

Green frog larvae were documented at all 30 sites; eastern newt at 26 sites; spotted salamander at 22 sites; and bull frog at 7 sites (Appendix A). Spring peeper (*Pseudacris crucifer*) and pickerel frog (*Rana palustris*) larvae were captured at 6 and 12 sites, respectively; these 2 species were not analyzed further because they were captured in low numbers and many larvae had already reached metamorphosis prior to sampling. When considering all sampling techniques combined, overall species richness of the sites ranged from 1 – 5.

For all 4 focal species (i.e., spotted salamanders, eastern newts, green frogs, and bull frogs), funnel traps had the highest probability of detection for a given level of effort (i.e., number of stations) (Figures 3.1 – 3.4; Table 3.1). For spotted salamanders and bull frogs, dip nets were the least effective; for eastern newts, bottle traps performed the poorest; and for green frogs, pipe samplers and bottle traps had the lowest probability of detection for a given level of effort. Green frog larvae had the highest probability of detection and spotted salamanders had the lowest for all 4 techniques. Minimum number of stations required to detect the presence of each species of amphibian larvae showed similar relative differences between the species and techniques as for overall probability of detection (Table 3.2).

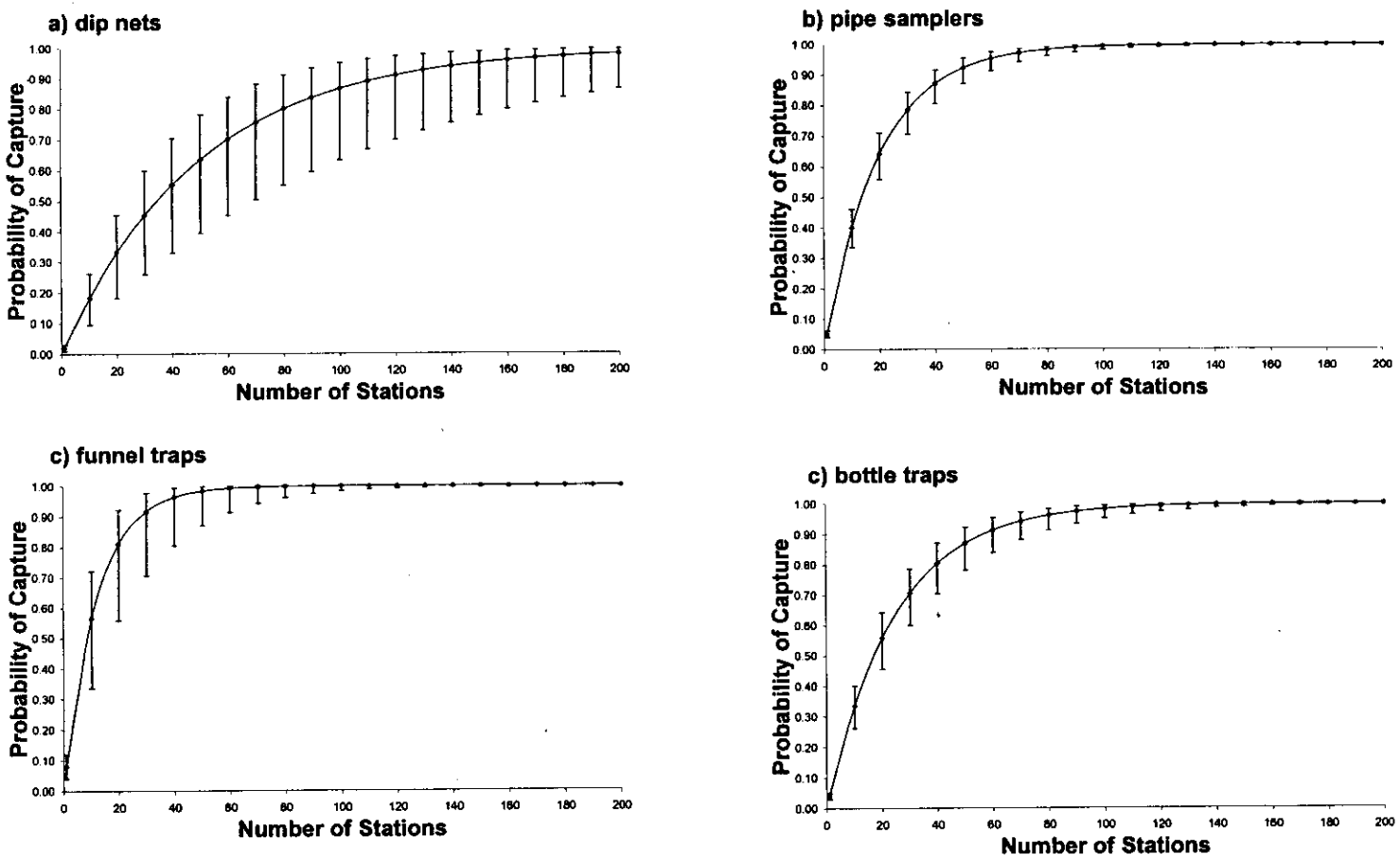


Figure 3.1. Probability of capture of *Ambystoma maculatum* larvae (mean + standard error) per number of sampling stations for 4 sampling techqniques in wetlands (n = 22).

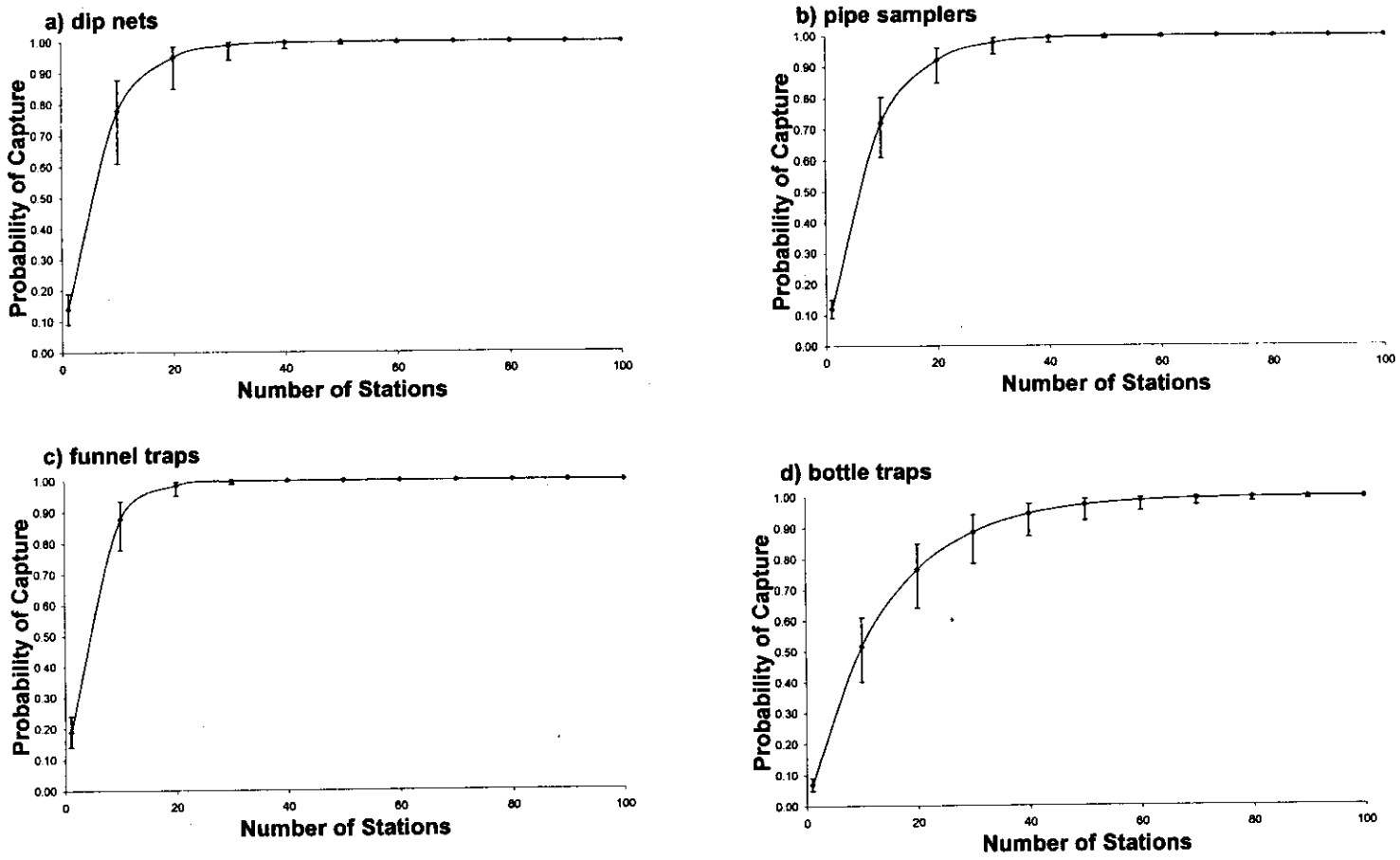


Figure 3.2. Probability of capture of *Notophthalmus viridescens* larvae (mean + standard error) per number of sampling stations for 4 sampling techqniques in wetlands (n = 26).

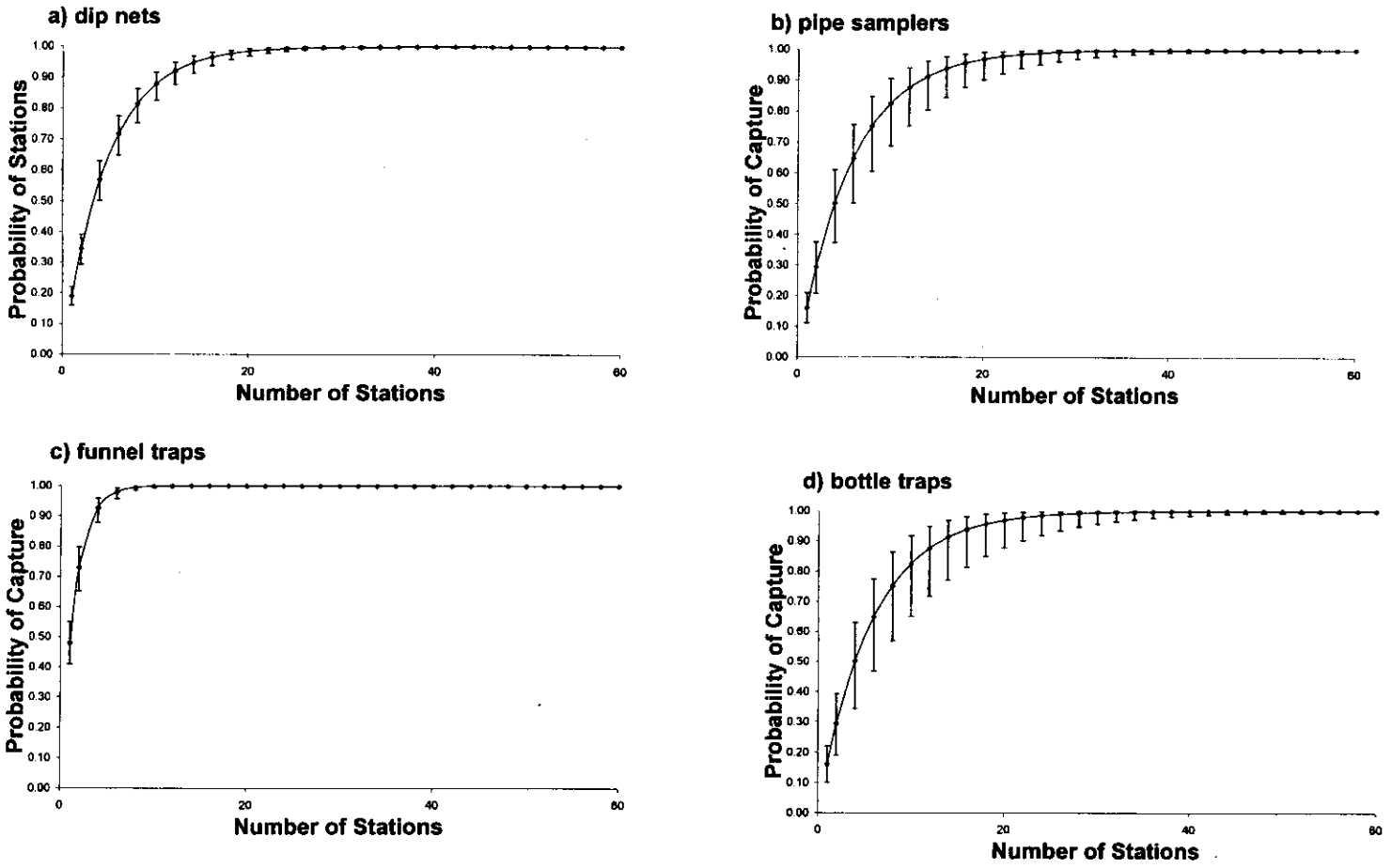


Figure 3.3. Probability of capture of *Rana clamitans* larvae (mean + standard error) per number of sampling stations for 4 sampling techqniques in wetlands (n = 30).

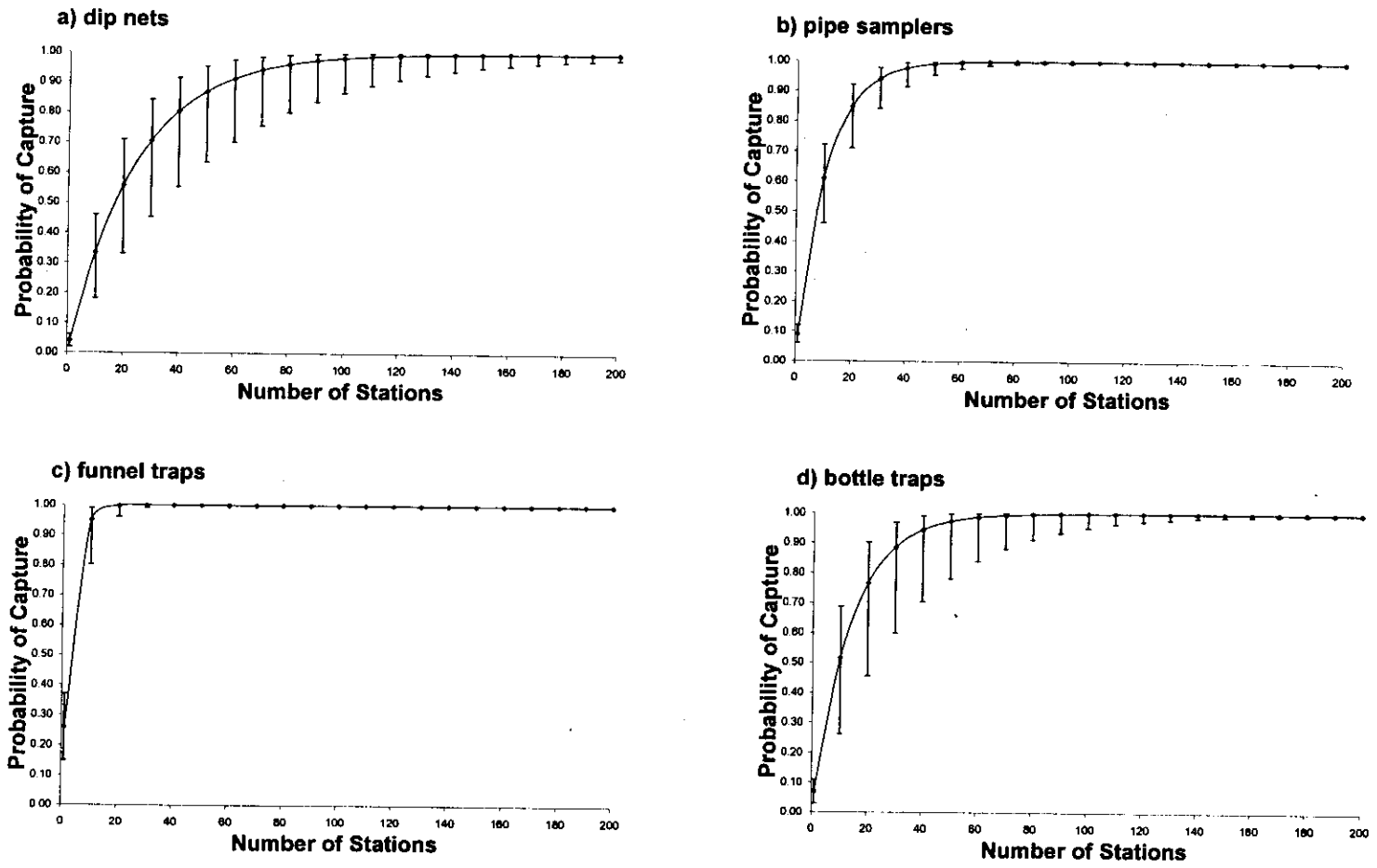


Figure 3.4. Probability of capture of *Rana catesbeiana* larvae (mean + standard error) per number of sampling stations for 4 sampling techqniques in wetlands (n = 7).

Table 3.1. Proportion of stations (mean ± standard error) at which each species of amphibian larvae was detected for all sites at which the species was recorded, for each of 4 sampling techniques.

	DIP NET	PIPE SAMPLER	FUNNEL TRAP	BOTTLE TRAP
Ambystoma maculatum (n = 22)	0.02 ± 0.01	0.05 ± 0.01	0.08 ± 0.04	0.04 ± 0.01
Notophthalmus viridescens (n = 26)	0.14 ± 0.05	0.12 ± 0.03	0.19 ± 0.05	0.07 ± 0.02
Rana clamitans (n = 30)	0.19 ± 0.03	0.16 ± 0.05	0.48 ± 0.07	0.16 ± 0.06
Rana catesbeiana (n = 7)	0.04 ± 0.02	0.09 ± 0.03	0.26 ± 0.11	0.07 ± 0.04

Table 3.2. Minimum number of stations required to detect each species of amphibian larvae for each of 4 sampling techniques, based on sampling in 30 wetlands in Acadia National Park, 1999.

	DIP NET	PIPE SAMPLER	FUNNEL TRAP	BOTTLE TRAP
Ambystoma maculatum (n = 22)	112	44	28	56
Notophthalmus viridescens (n = 26)	15	18	11	33
Rana clamitans (n = 30)	11	13	4	13
Rana catesbeiana (n = 7)	56	24	8	32

The mean estimate of number of person hours per station (averaged across all 30 sites) was lowest for dip nets and was similar for pipe samplers, funnel traps, and bottle traps (Table 3.3). Given the calculated probability of detection for each of the focal species and techniques, mean species richness was highest for funnel traps and lowest for dip nets (Table 3.4). In terms of numbers of individuals captured (Table 3.5), funnel traps generally captured the most spotted salamanders and bull frogs. For eastern newts, pipe samplers captured the most individuals whereas for green frogs, either pipe samplers or funnel traps typically captured the most individuals. Dip nets generally yielded the lowest numbers of individuals across species.

Cost of the equipment to implement each technique varied: one dip net (purchased from BIOEQUIP) was roughly $50.00; one pipe sampler (custom made at a local metalworking factory) was about $25.00; 1 plastic funnel (minnow) trap (purchased from Plasti-Lite Corporation; now available from Nylon Net Company) was $6.27; 1 plastic bottle trap cost approximately $0.50, plus labor for construction. Because number of sites sampled and number of funnel or bottle traps used will vary from study to study, cost of this equipment was not analyzed further.

DISCUSSION

The choice of a technique for sampling amphibian larvae will vary, depending on the particular question being asked. However, a few generalizations can be made. Funnel traps were consistently superior to the other methods tested in terms of probability of capture of the 4 focal species of larvae, as well as for determining species richness. Funnel traps also generally captured high numbers of individuals for each of the key species. The number of person hours required for sampling was similar for funnel traps, pipe samplers, and bottle traps; however, because it took 4 days to operate funnel traps (1 day for setting traps and 3 days of checking), travel time to and from study sites will be greater for these methods. I did not consider this issue in my calculations because it is site specific. Funnel traps are recommended as a sampling method for larval (and adult) amphibians in a variety of lentic habitats in the Pacific Northwest, particularly where visibility or maneuverability in the water column hinders other sampling

Table 3.3. Number of person hours (mean ± standard error) to sample at one station, a medium wetland (i.e., 12 sampling stations), and a large wetland (i.e., 24 sampling stations) for 4 larval sampling techniques.

	DIP NET	PIPE SAMPLER	FUNNEL TRAP	BOTTLE TRAP
Time for 1 station	0:12 ± 0:01	0:17 ± 0:01	0:17 ± 0:01	0:17 ± 0:01
Medium wetland (12 stations)	2:24 ± 0:12	3:24 ± 0:12	3:24 ± 0:12	3:24 ± 0:12
Large wetland (24 stations)	4:48 ± 0:24	6:48 ± 0:24	6:48 ± 0:24	6:48 ± 0:24

Table 3.4. Mean species richness of larval amphibians detected for a medium wetland (i.e., 12 sampling stations) and a large wetland (i.e., 24 sampling stations), given that *Ambystoma maculatum*, *Notophthalmus viridescens*, *Rana clamitans*, and *Rana catesbeiana* larvae are present and total species richness is 4, for 4 larval sampling techniques.

	DIP NET	PIPE SAMPLER	FUNNEL TRAP	BOTTLE TRAP
Medium wetland (12 stations)	2.37	2.80	3.52	2.43
Large wetland (24 stations)	2.96	3.54	3.85	3.24

Table 3.5. Number of amphibian larvae (mean ± standard error) captured per sampling station for a subset of 30 wetlands for which the particular species was recorded as present.

	DIP NET	PIPE SAMPLER	FUNNEL TRAP	BOTTLE TRAP
Ambystoma maculatum (n = 22)	0.28 ± 0.18	0.27 ± 0.11	0.70 ± 0.25	0.10 ± 0.04
Notophthalmus viridescens (n = 26)	0.42 ± 0.17	0.83 ± 0.51	0.44 ± 0.13	0.23 ± 0.11
Rana clamitans (n = 30)	0.70 ± 0.27	2.73 ± 0.99	2.68 ± 0.50	0.85 ± 0.32
Rana catesbeiana (n = 7)	0.03 ± 0.02	0.24 ± 0.13	0.42 ± 0.19	0.08 ± 0.04

methods (see Adams et al. 1997). In addition, because the skill and experience of the field worker has little impact on capture success, funnel trapping enables the development of trapping protocols that can be consistently repeated across sites (Adams et al. 1997).

Bottle traps typically yielded the lowest probability of capture for a given species as well as captured the fewest individuals (Figures 3.1 – 3.4, Tables 3.1 and 3.5), and, thus, their use is not supported by this study. In contrast, studies in the Pacific Northwest had favorable results with bottle traps of a slightly different design (Richter 1995). However, species assemblages differ between the regions; e.g., in the northeast, bull frog tadpoles take 2 years to develop and grow to such a large size that the entrance hole for Richter's bottle trap is too small for them.

Pipe samplers and dip nets performed similarly, although pipe samplers tended to have greater success in terms of both probability of capture as well as in abundance of individuals. However, the number of person hours per station was much lower for dip nets than for pipe samplers (as well as the other techniques) and, thus, for a given level of effort, more stations could be added using dip nets, thereby increasing the likelihood of detecting uncommon species.

In some situations, high numbers of individuals may be desired (e.g., sampling to document the presence or abundance of deformities). However, to generate reliable population estimates for a species at a given wetland, consistency of captures within and among stations would be necessary. In this study, overall consistency of captures within and between stations for all of the techniques was low. Specifically, most samples yielded no larval amphibians, presumably these wetlands were fairly large and had low densities of amphibian larvae. This suggests that some questions cannot be addressed with these methods. For example, density estimates or population indices will have high standard errors. However, it may be possible to use these techniques for other types of wetlands (e.g., woodland pools or smaller wetlands) if the likelihood of capture of individuals was greater.

In terms of overall performance, the use of funnel traps was best supported by this study. Pipe samplers and dip nets also showed promise. Because dip nets took less time to sample per station as compared to the other methods, number of samples taken could be increased to

increase numbers of captures without a substantial increase in effort. The use of bottle traps was not supported by this study.

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