

Effectiveness of amphibian monitoring techniques in a Taiwanese subtropical forest

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ABSTRACT

We compared the effectiveness of three techniques for surveying frogs in four different habitats in a subtropical area of Taiwan. We conducted surveys biweekly from July 2000 to July 2001 employing three sampling techniques (nocturnal line-transects, automated recording systems, and side-flap pail-traps) concurrently in each of four habitats (temporary pond, permanent pond, ephemeral stream, and permanent marsh). We detected 22 species of anurans from five families, representing 76% of the anuran species found in Taiwan. Line-transect sampling and automated recorders detected 22 and 20 species respectively, with an average of 12.3 ± 3.2 (mean \pm SD) and 10.4 ± 3.5 species per survey. In contrast, traps only captured 11 species, with an average of 2.1 ± 1.5 species per survey. Automated recorders were most effective at detecting hylids, rhacophorids, and microhylids that have loud calls and/or prolonged periods of calling. Recorders were less effective at detecting ranid and bufonid species that have relatively quiet calls. Traps were good at capturing most of the ranids, species that were usually missed with automated recorders. The combination of recorders and traps was equivalent to line-transect sampling, suggesting that these two techniques are a good alternative to nocturnal line-transect sampling, a technique that is difficult to use in remote areas and/or habitats that are inaccessible at night.

INTRODUCTION

Recent concerns about amphibian declines (Barinaga, 1990; Fellers & Drost, 1993; Drost & Fellers, 1996; Berger *et al.*, 1998; Lips, 1999; Davidson *et al.*, 2001, 2002) have encouraged the development and standardization of surveying and monitoring methods for amphibians (Heyer *et al.*, 1994). The

standardization of sampling techniques allows researchers and conservationists worldwide to implement effective and accurate surveying programs. Sampling techniques vary with respect to cost, time investment, personnel requirements, and effectiveness. Thus, it is critical for researchers to test and implement methods that are most appropriate for their region (Fellers & Freel, 1995; Parris, 1999; Corn *et al.*, 2000). Numerous studies have compared the effectiveness and suitability of amphibian sampling techniques. Some of this work has suggested that nocturnal line-transect sampling is particularly effective (Pearman *et al.*, 1995; Parris, 1999; Parris *et al.*, 1999). One major drawback is that line transects cannot be used in areas that are inaccessible to researchers at night, a situation that is often the case in tropical and subtropical forests. Therefore, it is important to evaluate alternative sampling techniques.

Automated recording systems have been used for a number of years, but the technique has been evaluated only in a limited variety of habitats. An automated recorder has the advantage of allowing researchers to collect data for an extended period without disturbance to the study animals, and it can be used in areas that are difficult to access at night. Parris *et al.* (1999) compared automated recorders with other sampling techniques and found that recorders detected 71% of the species in a study in southeast Queensland, Australia, while line-transect sampling accounted for 93% of the species.

Our study was designed to compare three amphibian-sampling techniques in a subtropical forest in central Taiwan. We used nocturnal line-transect sampling, automated tape recorders, and side-flap pail-traps. Side-flap pitfall traps are good for sampling ground-dwelling frogs, but are ineffective for some species, especially arboreal frogs such as hylids

(Nadoronzny & Barr, 1997). One advantage of the trap is that it can be used in areas where it can be deployed and maintained during the day. Our work was designed to determine if the combination of automated recorders and traps was as effective at detecting anurans as nocturnal line transects across a variety of habitats. Most research on sampling techniques has been conducted in North and South America, and in Australia (Heyer *et al.*, 1994; Parris *et al.*, 1999; Bridges & Dorcas, 2000; Corn *et al.*, 2000); much less attention has been given to other areas, especially tropical and subtropical regions. The anuran community, as well as other biotic components, and the physical environment vary among regions. Thus, it is necessary to evaluate the effectiveness of sampling techniques in distinctly different settings.

MATERIALS AND METHODS

Study area

Field work was conducted from July 2000 to July 2001 at the Taiwan Forestry Research Institute experimental forest at Lien Hua Chih station (23°55' N, 120° 52'E), Nantou County, in central Taiwan. The station encompasses a 461-ha watershed, half of which is covered by undisturbed lowland primary forest. The site is characterized by low topography, with elevations ranging from 576 to 975 m. The mean annual air temperature is 21.1° C, and ranges from 9.9° C in January to 30.0° C in July. The area receives approximately 220 cm of rain annually. Although rain falls in all months, the wet season begins in May and ends in October. Heavy rains are most often associated with the "plum rains" or "Mei rains" in spring (April - June) and the typhoon season in summer (July - September). The "plum rain" season is characterized by continuous light rain for many days, with occasional

heavy thunderstorms. Typhoons usually bring heavy rains that can cause flash floods and damage to forests.

Survey sites

We chose one study site in each of four different habitats: a temporary pond, permanent pond, ephemeral stream, and permanent marsh. The temporary pond was filled with water in the spring and summer (March to September), but dried up in the fall and winter (October to February). The pond was surrounded by elephant grass (*Pennisetum purpureum*) and bamboo stands (*Phyllostachys* sp.). It covered approximately 400 m² during the wet season. The permanent pond was a 450-m² abandoned paddy field that held water all year, even though the water level dropped during the dry season. The ephemeral stream was 1-3 m wide and 20-30 cm deep during the wet season, with water passing through riffles and pools. During the dry season, reduced flow resulted in the formation of intermittent pools. The stream bank and bottom consisted of sand, gravel, boulders, and bedrock. The stream was completely shaded by broadleaf trees: *Michelia formosana*, *Adenantha microsperma*, *Pellionia radicans*, and *Entada phaseoloides*. The shallow, permanent marsh was 25 m² in area and fed by a spring. The dominant vegetation was *Juncus effusus*, *Cynodon dactylon*, and *Dicranopteris linearis*. The marsh was located at the edge of a primary forest consisting of *Michelia formosana*, *Gordonia axillaries*, *Cyathea metteniana*, and *Prunus campanulata*.

Sampling techniques

We surveyed biweekly from July 2000 to July 2001, employing three

techniques concurrently at each site: nocturnal line transects, automated recording systems, and side-flap pail-traps. In nocturnal line-transect sampling, two people with headlights walked slowly along a 100-m transect, spotlighting for frogs within 2 m of the transect line. All four transects were surveyed on the same night, beginning at 1900 h. Each transect took 30-45 min; all four were finished in 3-4 h. The sequence of transects was randomly selected each night. We counted the number of frogs of each species that were seen or heard.

We used automated tape recorders to record frog calls over a 12-h period that coincided with the trapping and line transects. The system consisted of a AIWA TP-VS480 tape recorder (with AE-120 TDK tapes), a 12 V rechargeable battery, a timer (CEC, Type-CTW) housed in a waterproof box, and an AIWA stereo external microphone secured to a tree approximately 2 m above the ground. Each recorder began recording at 1900 h, recorded for 1 min, and then turned off for 12 min; this cycle was repeated until 0700 h the next morning. In theory, we would obtain a total of 60 min of recording each night, but we only obtained about 57-58 min each night due to the delay of the timer. We put one recorder at each end of the line transect, and the results from these two stations were pooled for each night. Tape recordings were played back in the laboratory. We identified each vocalization to species.

We trapped amphibians using modified side-flap pail-traps modeled after Nadorozny & Barr (1997). The trap was 60 x 30 x 35 cm (length x width x depth). An 8 x 8 cm entrance opening was cut in the side of the box, 8 cm up from the bottom, and a 9 x 9 cm piece of thin Styrofoam was hung on the inside of the opening with tape. The flap was cut larger than the opening and acted as a one-way entrance. We made a 10 x 10 cm screen window on the

opposite side of the entrance for ventilation. A wet sponge was placed in the bottom of the container to minimize dehydration of trapped animals. Water and stones were also placed in each trap to provide refuges for animals and to stabilize the trap. A drift fence 60 cm high and 5 m long was constructed from nylon fabric. The bottom of the fence was buried 5 cm in the ground, and the fence was supported with stakes. A single trap was placed at each end of the fence. Each trap was buried 8 cm in the ground so the bottom of the entrance was flush with the ground. A wire mesh funnel was placed in front of the entrance to direct frogs from the drift fence into the trap. On each survey night, the trap was opened at 1900 h and closed at 0700 h the next morning, at which time trapped animals were identified and released.

Statistical analyses

We performed statistical analyses using SAS (SAS Institute Inc., 1996). We used Pearson correlation coefficients to assess the relationship between the total number of species detected on a night of survey and weather variables. Data for all four sites were combined to give the total number of species detected on a night of survey, and data were log transformed to meet the parametric assumption of normality. In addition, a stepwise regression to determine the relative importance of each meteorological variable in predicting total number of species detected. We used one-way ANOVA to compare the effectiveness between line transect sampling and other methods (automated recording system and trap methods combined) by testing the number of anuran species detected in each survey. We used the Chi-Square test to examine the effectiveness of sampling methods among habitats.

RESULTS

Sensitivity of the sampling techniques

We conducted 27 fortnightly surveys at each site from July 2000 to July 2001 (Table 1). We completed all 108 line-transect surveys (27 days X 4 habitats) without significant problems or interruption. The automated recorders had a failure rate of 6% (13/216), whereas traps failed only 1% of the time (1/108).

We detected 22 frog species belonging to five families (Table 1). Except for *Hyla chinensis*, *Buergeria japonica*, and *Microhyla ornate*, we detected other species at least 6 times during the 27 surveys. *Rana kuhlii*, *Rana latouchi*, and *Rana swinhoana* were found during every survey.

We detected 22 species of frogs using nocturnal line transects, with an average of 12.3 ± 3.2 (range 7-17) species per survey. Line-transect sampling was more effective at detecting bufonids, microhylids, and ranids than other methods (Table 1; Fig. 1).

We obtained recordings from 11,481 one-minute sampling intervals, and identified 21,503 frogs or groups of frogs. The results for *Microhyla heymonsi* and *M. ornata* were combined because it was difficult to distinguish their calls reliably. The automated recorders detected 20 species, with an average of 10.4 ± 3.5 (range 1-12) species per survey. *Chirixalus eiffingeri*, *Rana adenoplura*, *R. latouchi*, and *R. swinhoana* were detected during every survey. The recorders did not pick up *Rana plancyi*, a species with a weak call, even though this frog was abundant at our study sites. Automated recorders were marginally more effective at detecting hylids and rhacophorids than line transects. The traps did not capture frogs from either of these two families (Table 1).

The traps captured only 11 frog species, with an average of 2.1 ± 1.5 (range 0-3) species per survey. This technique was the least effective of all. Aside from *R. latouchi*, no anurans were detected more than 10 times during the 27 surveys, and the traps did not detect any arboreal species (e.g., rhacophorids and *H. chinensis*; Table 1; Fig. 1).

Each survey technique detected at least 60% of its cumulative total species during the first survey, and reached 90% after only 7-10 surveys (Fig. 2). The rate at which species accumulated was influenced by both season and weather. All three sampling methods recorded the most species between April and August, and the fewest between October and January. Weather had a significant influence on the number of species detected with each sampling technique (Table 3). Pearson correlation analyses revealed that cumulative rainfall for the four days prior to survey and air temperature one day post-survey were correlated significantly to the number of species detected for all three methods. Results of stepwise regressions revealed that the total number of species detected was influenced significantly only by cumulative rainfall for the four days prior to survey in line-transect sampling and side-flap pail-trap methods (Table 3), whereas the respective variables was influenced significantly only by air temperature in automated recording system.

Comparison of sampling techniques

Detection of frogs was dependent on sampling technique (Fig. 2). The difference between line-transect sampling and automated recorders and traps combined was not significant (ANOVA, $F_{1,52} = 0.4$, $P = 0.528$). Line-transect sampling recorded the largest number of species at the temporary pond,

ephemeral stream, and permanent marsh. Automated recorders detected the most species at the permanent pond. Traps were the least effective, capturing only one-third of species found in each site (Table 2). Despite these differences, there were no statistically significant differences in sampling techniques among habitats ($\chi^2 = 0.50$, $df = 6$, $P = 0.99$).

DISCUSSION

Taiwan contains 29 species of anurans belonging to five families (Zhao & Alder, 1993; Lue *et al.*, 1999). Our surveys detected 22 species at our study area in Lien Hua Chih, which is seven more species than were found during an earlier study (Lu & Lin, 1995). In addition, this is the largest number of anuran species ever recorded at a single locality in Taiwan. Our success in detecting a wide range of species in an area with such high diversity indicates that our techniques are well suited for monitoring programs in Taiwan, and are likely to be useful in other tropical or subtropical regions as well.

Nocturnal line-transect sampling was the most effective method for detecting anurans in our study area, consistent with previous studies (Berrill *et al.*, 1992; Pearman *et al.*, 1995; Parris, 1999; Parris *et al.*, 1999). Past research has also shown that amphibian surveys are most effective during the warm, wet season, when most anurans are active (Aichinger, 1987; Bertoluci, 1998). In our study, the line-transect method was particularly effective during the wet season (March to June), when we detected 91% of the species at our study site. Some species can easily be missed, however. Because each survey took < 1 h at each site, species with small populations or with brief periods of activity could be overlooked. In Taiwan, this is particularly true for *H. chinensis*, *Buer. japonica*, *Buergeria robustus*, and *Rana rugulosa*.

Automated recorders were most effective at detecting species with loud calls. Nine of the most commonly detected species fit this description (*H. chinensis*, *Buer. japonica*, *Buer. robustus*, *C. eiffingeri*, *Chirixalus idiotocus*, *Polypedates megacephalus*, *R. adenopleura*, *Rana psaltes*, and *R. rugulosa*). Recorders worked well at detecting *Microhyla* due to their loud, continuous choruses, but it was not possible to distinguish reliably between *M. ornata* and *M. heymonsi* due to the similarity of their calls. We did not record species that produce weak calls, even when the species was abundant (e.g., *R. plancyi*). In habitats with high levels of ambient noise, such as running water or nearby traffic, it would be more difficult to detect the calls of species with quiet vocalizations (e.g., *Bufo bankorensis*). Despite these weaknesses, the use of automated recorders was the second most effective technique, detecting 95% of the species at our study site. In Australia, Parris *et al.* (1999) used a similar technique and detected only 71% of the known species. The lower level of detection was probably due to differences in call characteristics in the local anuran communities.

In some situations, automated recorders had a distinct advantage over nocturnal line transects. Only automated recorders detected some species at the ephemeral stream (*Buer. japonica*), the temporary pond (*Rhacophorus moltrechti*), and the permanent pond (*H. chinensis*, *Rana guentheri* and *Rhaco. moltrechti*, Table 1). This is probably because calls were recorded throughout the night and species were detected that call only late at night, are secretive, or are relatively rare. In contrast, nocturnal line transects are typically conducted shortly after dark and seldom run throughout the night (Aichinger, 1987; Vandewalle *et al.*, 1996; Garcia-Rutledge & Narins, 2001). This might be important because Bridges & Dorcas (2000) reported that *Rana*

sphenocephala began to call after midnight; thus, nocturnal line transects would probably miss or underestimate the population size of this species or others with similar calling patterns.

Traps were the least effective of the three methods we tested, detecting only 50% of the species. Traps also had a relatively low capture rate of 31 animals/100 trap nights. Most trapping methods, including our side-flap pail-traps (Bury & Corn, 1987; Dodd, 1991; Greenberg *et al.*, 1994) captured mostly ground-dwelling species, even though funnel traps along drift fences have proven effective at catching arboreal anurans (Enge, 2001). In this study, the traps were particularly effective for ranids, capturing 9 of the 10 local species. Nadoronzny & Barr (1997) described the side-flap pail-trap method that we used, and they reported good success in capturing species that are strong jumpers (e.g., *Rana clamitans*, *R. pipiens*, and *R. sylvatica*). This result agrees with ours. Importantly, this type of trap was effective at capturing frogs of a wide range of sizes, from *M. heymonsi* (SVL 25 mm) to *R. guentheri* and *R. plancyi* (up to 80 mm SVL). Our study suggests that side-flap pail-traps can be much more effective than standard pitfall traps because the flap acts as a one-way door and prevents escapes. We tested this idea prior to our surveys by placing eight species of frogs in traps and leaving them for three days, and no frogs escaped. In contrast, Parris *et al.* (1999) used standard pitfall traps and reported 0.56 animals/100 trap nights, and they detected only 14% of the total species found during the study. Most of the species studied by Parris *et al.* (1999) were treefrogs, species that could readily escape from standard pitfall traps.

The combined effectiveness of automated recorders and traps was equivalent to that of the line-transect sampling technique in detecting frogs;

furthermore, even though the macro- and microenvironments of each habitat were different, our sampling methods worked consistently across all types of habitats. Thus, we believe that recorders and traps are a good substitute for nocturnal line transects. We recommend that automated recorders be considered as a viable sampling technique, especially in tropical or subtropical regions where nocturnal work is often dangerous, difficult, or remote. Even though automated recorders can be expensive initially (Corn *et al.*, 2000), the technique provides results that may not be obtainable otherwise, and allows for sampling over a longer time period than line transects. The combination of automated recorders and traps can document a high percentage of anurans in different community types. The availability of these two methods should encourage researchers to conduct amphibian monitoring even in remote areas that might otherwise be difficult to survey.

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Table 1. The frequency of anuran species detected in 27 surveys by nocturnal line-transect sampling (L), automated recording system (R), and side-flap pail-trap (T) methods in a temporary pond, permanent pond, ephemeral stream, and permanent marsh in Taiwan.

	Temporary pond			Permanent pond			Ephemeral stream			Permanent marsh			Total Species Detected		
	L	R	T	L	R	T	L	R	T	L	R	T	L	R	T
Bufonidae															
<i>Bufo bankorensis</i>	11			4			11	1	1	23		3	24	1	4
<i>Bufo melanostictus</i>	5	1								1	1		6	2	0
Hylidae															
<i>Hyla chinensis</i>	1	1			1								1	2	0
Rhacophoridae															
<i>Buergeria japonica</i>								1		1	2		1	3	0
<i>Buergeria robustus</i>	2	11		1	4		4	4		2			8	12	0
<i>Chirixalus eiffingeri</i>	6	9		8	16		17	25		15	24		23	27	0
<i>Chirixalus idiotocus</i>	18	19		9	15		7	5		14	18		18	22	0
<i>Polypedates</i>															
<i>megacephalus</i>	11	15		12	15					1	3		14	15	0

<i>Rhacophorus</i>															
<i>moltrechti</i>	3		2		8	8		22	20		23	19	0		
<i>Rhacophorus</i>															
<i>taipeianus</i>	8	10	10	11	2	8		10	11		12	12	0		
Microhylidae															
<i>Microhyla heymonsi</i>	13	13*	7	13	14*	1	5	5*		13	13*	2	15	14*	8
<i>Microhyla ornata</i>	1			2									3	0	0
Ranidae															
<i>Rana adenopleura</i>	15	20		24	26	2	19	20	2	17	24	3	25	27	6
<i>Rana guentheri</i>	12	10	3		1								12	11	3
<i>Rana kuhlii</i>	2	2		5	8		25	12		27	17	7	27	18	7
<i>Rana latouchi</i>	22	18	1	22	21	8	25	20	11	26	16	2	27	27	15
<i>Rana limnocharis</i>	7	8	3	3	6	1				14	10		19	13	4
<i>Rana plancyi</i>	18		4	1									19	0	4
<i>Rana psaltes</i>					1					12	13	1	12	13	1
<i>Rana rugulosa</i>	4	6		3	6								6	9	0
<i>Rana sauteri</i>				2		1	8	5	1	2			9	5	2
<i>Rana swinhoana</i>							27	27	4	6	18		27	27	4

Total	22	20	11
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* We could not differentiate between calls of *M. heymonsi* and *M. ornata*. We assumed most calls recorded were by *M. heymonsi*, because line-transect sampling revealed that *M. heymonsi* outnumbered *M. ornata* by 973 to 3.

Table 2. The number of frog species detected in four different habitats in Taiwan using three different sampling methods.

	Sampling sites			
	Temporary pond	Permanent pond	Ephemeral stream	Permanent marsh
Line-transect sampling	17	15	12	17
Automated recording system	15	15	13	14
Side-flap pail-trap	5	5	5	6

Table 3. Pearson correlation coefficients (r) of correlation analyses and partial correlation (r^2) of stepwise regressions between the number of species detected by three sampling methods and meteorological variables.

	Weather variables	Pearson (r)	Partial r^2
<u>Line-transect sampling method</u>			
	Air temperature	0.43*	
	Maximum air temperature	0.27	
	Minimum air temperature	0.35	
	Mean air temperature - four days pre-survey	0.34	
	Air temperature - one day post-survey	0.47**	
	Relative humidity	-0.41*	0.10*
	Rainfall	0.29	
	Total rainfall - four days pre-survey	0.62***	0.39***
	Rainfall - one day post-survey	0.27	
		$P=0.0005$	
<u>Automated recording system</u>			
	Air temperature	0.35	
	Maximum air temperature	0.30	
	Minimum air temperature	0.24	
	Mean air temperature - four days pre-survey	0.27	

Air temperature - one day post-survey	0.42*	
Relative humidity	-0.36	0.07
Rainfall	0.26	
Total rainfall - four days pre-survey	0.63***	0.39***
Rainfall - one day post-survey	0.17	0.04
	$P = 0.0004$	

Side-flap pail-trap method

Air temperature	0.51**	0.26**
Maximum air temperature	0.41*	
Minimum air temperature	0.46*	
Mean air temperature - four days pre-survey	0.33	0.06
Air temperature - one day post-survey	0.49*	
Relative humidity	-0.19	
Rainfall	0.19	
Total rainfall - four days pre-survey	0.46*	0.10
Rainfall - one day post-survey	0.10	0.09
	$P = 0.0004$	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure legends:

Figure 1. The number of anuran species by families detected by line-transect sampling, automated recording system, and side-flap pail-traps methods in Taiwan.

Figure 2. Cumulative species detected by three sampling methods individually, the sum of three sampling methods, and the sum of automated recording system and side-flap pail-traps as a function of the number of survey. The number of survey corresponds to following date: 1=2000/7/5; 2=7/19; 3=8/2; 4=8/16; 5=8/31; 6=9/15; 7=9/30; 8=10/14; 9=10/28; 10=11/11; 11=11/25; 12=12/9;13=12/21; 14=2001/1/7; 15=1/19;16=2/1; 17=2/17; 18=3/3; 19=3/17; 20=3/31; 21=4/14; 22=4/28; 23=5/12; 24=5/26; 25=6/9; 26=6/23; 27=2001/7/7



