Using Egg Mass Surveys to Monitor Productivity and Estimate Population Sizes of Three Pool-breeding Amphibians at Marsh-Billings-Rockefeller National Historical Park

FINAL TECHNICAL REPORT TO THE NATIONAL PARK SERVICE, WOODSTOCK, VT

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Executive Summary

The Marsh-Billings-Rockefeller National Historical Park was established to interpret conservation history, the evolving nature of land stewardship, and to continue careful management of the park's historic 555-acre Mount Tom forest, the oldest professionally-managed forest in the United States. To improve upland habitat for pool-breeding amphibians, the park's forest management plan called for selective thinning of two conifer plantations in order to slowly convert them to native hardwood cover. A three-year study to monitor amphibian productivity via egg mass counts, estimate population sizes of Wood Frog (*Rana sylvatica*), Spotted Salamander (*Ambystoma maculatum*), and Jefferson Salamander (*Ambystoma jeffersonianum*), and provide baseline data with which to assess the effects of forestry operations on these species, was conducted at MABI from 2009–2011.

Dependent double-observer egg mass counts were conducted twice annually at seven vernal pools. This technique allowed for the estimation of detection probabilities and removal of observer bias, resulting in more robust estimates of the total number of eggs present. For each species, detection probabilities were modeled as a function of four covariates hypothesized to affect the probability of detecting egg masses of each species. The population size of each species was estimated for three distinct breeding populations deemed independent based on their distance from other pools in the study area. Hydrology (pool depth and size) and water chemistry data (pH, dissolved O_2, conductivity, tannic acid, and water temperature) were also collected at each pool during egg mass surveys.

Maximum annual egg mass surveys resulted in a total of 1,498 Jefferson Salamander egg masses, 971 Wood Frog egg masses, and 846 Spotted Salamander egg masses over three years. The largest breeding pool in the study area consistently supported the highest counts of egg masses for all three species, with an average of 280.3 Jefferson Salamander egg masses, 144.7 Spotted Salamander egg masses, and 136.3 Wood Frog egg masses per year. Although egg masses of all three species were detected in all seven pools, only Spotted Salamander eggs were detected in all pools every year. Estimated detection probabilities were highest for Wood Frog (91%), followed by Jefferson Salamander (83%), and Spotted Salamander (80%). There was considerable variation in detection probabilities among different observers, underscoring the need to account for observer bias when estimating population trends over time. Annual egg mass counts, and therefore population estimates showed considerable variation between years. For Jefferson Salamander the estimated number of breeding adults ranged from 265 in 2009, to a high of 751 in 2010; for Spotted Salamander estimates ranged from 438 individuals in 2010 to 891 in 2011; while Wood Frog numbers were slightly more stable, ranging from 906 in 2011 to 1,206 in 2010. The cluster of four pools just north of the Pogue supported 90% of the study area's Wood Frog population, 81% of the Spotted Salamander population, and 58% of the Jefferson Salamander population.

Water chemistry and hydrology measurements were similar between pools. The number of eggs present at MABI pools was positively correlated to pool size for all three species. Currently, acidity does not appear to be a limiting factor for amphibian populations at MABI. However, the Saddle Pool, near the Mt. Tom overlook, had the lowest ph (mean = 6.24), and further acidification due to acid precipitation could drop its pH below 5.5, which can reduce larval survival of Jefferson Salamanders.

Although these data were insufficient to evaluate the effects of forestry operations on pool-breeding amphibians at MABI, they established valuable baseline data on estimated population sizes for all three pool-breeding species, and demonstrated that dramatic annual variations in reproductive effort occur. Recommendations for future monitoring to assess the effectiveness of forest management activities on pool-breeding amphibians are discussed.

Introduction

The Marsh-Billings-Rockefeller National Historical Park (MABI) was established, in part, to interpret conservation history and land stewardship in America. As such, the park continues to actively manage the historic 555-acre Mount Tom forest, guided by a detailed forest management plan (NPS 2005) that balances sustainable forestry goals with preserving ecological integrity. To help guide the development of the park's forest management plan, several natural resource inventories were conducted in order to assess biological diversity, natural communities, and identify critical habitats (Faccio 2001, 2003a, Gawler and Engstrom 2011, Hughes and Cass 1997).

The MABI forest management plan follows vernal pool protection guidelines established by Calhoun and deMaynadier (2004), supplemented with local data from Faccio (2003b). Under these guidelines, vernal pool depressions are protected from forestry disturbance, while timber harvests retain at least 75% canopy cover within 31 m of the pool, and at least 50% canopy cover–with openings of no more than 1 acre–from 31-200 m of the pool. All harvesting is done on frozen or dry ground, and abundant coarse woody debris is either retained or augmented.

Following the recommendations of Faccio (2001), the park selectively thinned portions of two stands in order to slowly convert them to native hardwood cover and improve upland habitat for pool-breeding amphibians. The affected stands were the French red pine plantation, just north and within the life zone (200 m) of vernal pool KFEA, and a stand adjacent to the POPO (see Fig. 1). The stands were marked and thinned to about a 33% reduction in basal area overall, and while only a portion of the life zones fell within the thinned forest stands, canopy cover was retained at well over 50%. Snags and coarse woody debris were both retained during forestry operations. The French red pine stand, thinned in 2007, is scheduled to be thinned again in 2012, but with a basal area target that will again result in a minimal reduction in canopy closure.

Through a Cooperative Agreement between the NPS and the Vermont Center for Ecostudies, a three-year study to monitor amphibian productivity via egg mass counts and provide baseline data with which to assess the effects of these forestry operations on vernal pool-breeding amphibians, was conducted at MABI from 2009–2011. Specific objectives of the project were to:

1. Conduct twice-annual egg mass counts of three amphibian species (Jefferson Salamander [*Ambystoma jeffersonianum*], Spotted Salamander [*Ambystoma maculatum*], and Wood Frog [*Rana sylvatica*]) at seven vernal pools within or adjacent to MABI (Fig. 1);
2. Sample basic hydrology (water depth) and water chemistry (temperature, conductivity, pH, tannic acid, dissolved oxygen) at each pool, and;
3. Estimate population sizes of the Wood Frog (WOFR), Spotted Salamander (SPSA), and Jefferson Salamander (JESA) (a Vermont-listed species of special concern and one of the primary species of management concern for the park).

Methods

Double-observer egg mass counts for all three species were conducted at seven vernal pools at MABI (Fig. 1) from April 2009 through May 2011. Each pool was visited twice annually during the peak breeding season (April-May). Timing of initial surveys each year depended on ice-out and suitable weather conditions for amphibian movement, and follow-up surveys were conducted from 5-9 days after the initial visit.

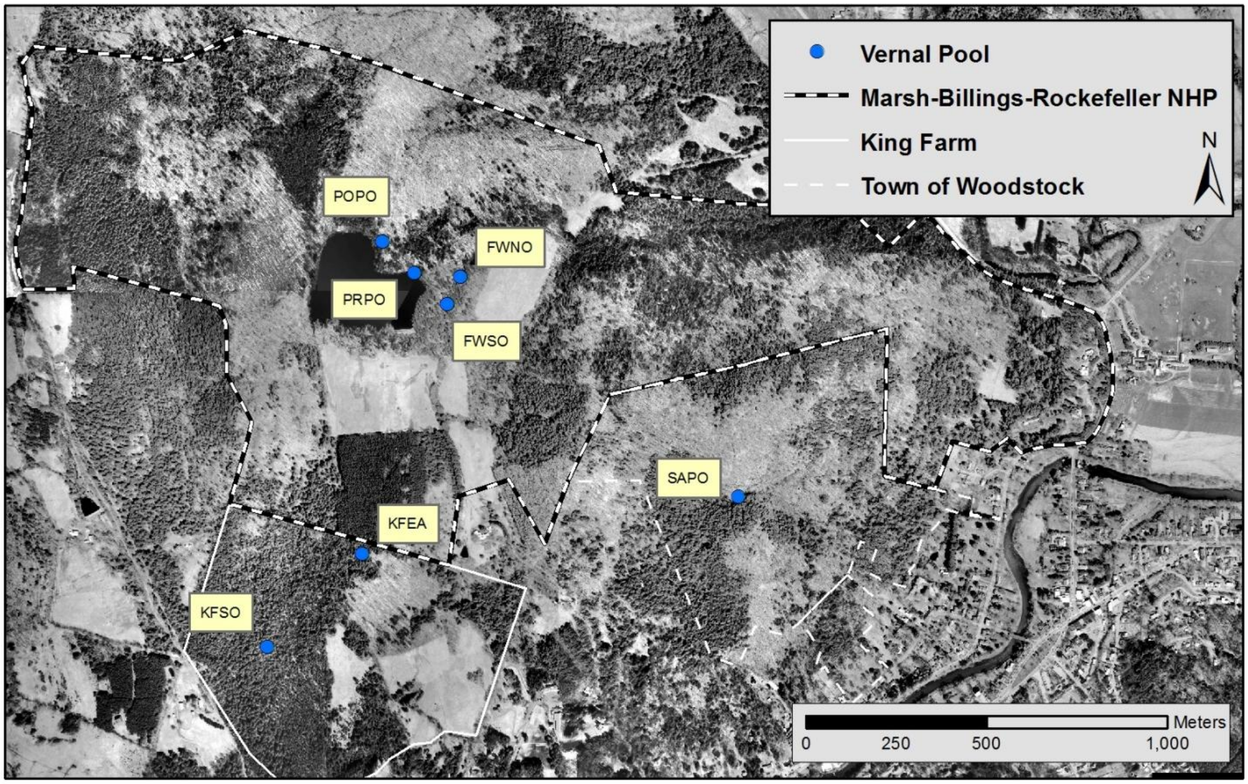

Figure 1. Location of seven vernal pools where egg mass surveys were conducted at Marsh-Billings-Rockefeller NHP and adjacent lands, 2009-2011.

Wood Frogs are synchronized breeders, with each female depositing a single egg mass, often in large communal aggregates, within a 7-10 day period (Crouch and Paton 2000). Adults congregate at breeding ponds during periods of warm weather, but rainfall is not necessary to stimulate movement. In contrast, Spotted and Jefferson salamanders usually have 2 or 3 major breeding bouts between mid-April to mid-May that are initiated during rainfall events. However, some JESAs will migrate on nights when melting snow creates damp, humid conditions at ground level. Female salamanders deposit 1-4 egg masses each, often attached to sticks, grasses, or other supporting structures (Pentranka 1998). Distinguishing between the egg masses of these three species is relatively easy due to morphology. Spotted Salamander egg masses are typically globular and surrounded with a dense, firm outer jelly matrix, while those of JESA tend to be smaller and sausage-shaped, with a thin, soft jelly matrix (Brodman 2002, Petranka 1998). Wood Frog egg masses are globular and lack an outer jelly matrix (Fig. 2).

The survey sampling window each year was constrained by the developmental rate of embryos. Salamander egg masses typically persist for 4-7 weeks prior to hatching, while WOFR eggs may begin hatching in 1 to 4 weeks. However, WOFR egg masses that are laid in large communal rafts begin to fuse together about a week after being deposited, making it impossible to distinguish and count individual masses. Therefore, an effort was made to conduct sampling immediately after eggs were laid, but in a few instances, egg rafts fused prior to initial surveys and could not be counted, reducing WOFR counts.

Figure 2. From left; Egg masses of Jefferson Salamander, Spotted Salamander, and Wood Frog.

Double Observer Protocol

A dependent double-observer technique (Grant et al. 2005) for counting egg masses was used that allows for the estimation of detection probabilities and removal of observer bias, resulting in more robust estimates of the total number of eggs present by adjusting population estimates for the probability that both observers missed egg masses. In this protocol, Observer 1 identified, counted and pointed out egg masses to Observer 2. Observer 2 recorded what Observer 1 reported, but also wrote down in a separate column any additional egg masses that Observer 1 missed or over-counted (without making any comments to Observer 1). Approximately halfway around the pool, observers switched roles so that Observer 1 recorded the egg masses detected by Observer 2, noting any over- or under-counts.

Estimated Population Size

The population size of each species was estimated for three breeding populations deemed independent based on the maximum dispersal distances reported for WOFR (~350 m; Baldwin et al. 2006), and for SPSA and JESA (~400 m; Faccio 2003b). Independent breeding populations were identified as SAPO, which was isolated from all other pools in the study area by $\geq$1 km; the Pogue Cluster, which consisted of four pools separated by <250 m in the area north of the Pogue; and the King Farm Cluster, consisting of two pools separated by ~325 m (Fig. 1). The distance between the Pogue and King Farm clusters was $\geq$750 m.

Estimated population size for each species and pool/cluster was calculated from annual egg mass counts adjusted for detection probability (see *Data Analysis* below), the reported average number of egg masses laid per female, and the reported sex ratios of each species. I first estimated the number of breeding females from the adjusted egg mass count based on the reported average number of egg masses laid per female (WOFR = 1 [Berven 1988]; both salamanders = 2 [Petranka 1998]). I then estimated the number of males based on the sex ratios as determined through drift fence captures at MABI during 1999-2000 (sex ratios for WOFR = 2.0 male:1 female; JESA = 1.4 female:1 male; SPSA = 2.6 male:1 female)

(Faccio 2001). The estimated number of males and females were then summed to estimate the population size for each year and pool/cluster.

Hydrology and Water Chemistry Sampling

Basic hydrology (pool depth and size) and water chemistry data (pH, dissolved O_2, conductivity, tannin/lignin, and water temperature) were also collected at each pool during egg mass surveys. Upon arriving at each pool, water chemistry instruments were calibrated and deployed, and all measurements were recorded immediately following surveys. In 2009, an Oakton Con100 Series meter was used to measure conductivity, an Oakton pH meter was used to measure pH and temperature, while a LaMotte DO400 meter was used to measure dissolved oxygen. In 2010 and 2011, an YSI 600XL water quality sonde was used to measure all variables except tannin/lignin, which was evaluated in all years using a LaMotte tannin test kit, model TL, which measures tannic acid in ppm.

Data Analyses

In preparing data for analyses, the annual survey with the highest egg mass count of each species at each pool was used. Detection probabilities (*p*) were modeled as a function of four covariates hypothesized to affect the probability of detecting egg masses of each species. The four covariates were; (1) *pool visibility* (a binary category depending on whether or not visibility was impaired by surface film, pollen, vegetation, etc.), (2) *observer experience* (binary, depending on whether or not an observer had experience conducting egg mass counts), (3) *observer ID*, and (4) *pool difficulty* (a linear variable with values of 1 [*easy*], 2 [*moderate*], or 3 [*difficult*], depending on how complex the pool was to survey; e.g. small pools with well-defined shorelines and no vegetation were easy to survey accurately, while large pools with irregular shaped shorelines and dense vegetation were difficult).

For each species, 12 models were evaluated using Huggins closed capture models in Program MARK (White and Burnham 1999) (Table 1). For all models, we assumed that different observer skill levels would affect *p*, so all models included *observer experience*, *observer ID*, or both, but never neither. Akaike's Information Criterion differences (ΔAIC_c) were used to evaluate the relative strength of models and select the model that most parsimoniously explained the variation in the data (Burnham and Anderson 2002). Models that had ΔAICc values of <2 were considered to have substantial support, after evaluating for potential misleading parameters (Burnham and Anderson 2002). Misleading parameters occur when a parameter makes no contribution to improving the model, but is simply within two AIC units of another model because of the two unit penalty for adding a parameter. Therefore, if two models differed by one parameter and the deviance of the models was within 1 unit, the additional parameter was considered unimportant and models with that parameter were removed. Model averaging was used for estimating detection probabilities and 90% confidence intervals for JESA and SPSA, while for WOFR inference was made on the best model because the second place model was greater than 10 ΔAICc units away and model averaging was not needed. Model averaging was conducted using a free spreadsheet tool (Mitchell 2008).

Table 1. Twelve models evaluated for each species to estimate egg mass detection probabilities (p).

Model Set	**Description**
Observer ID + observer experience + pool difficulty + pool visibility	Global model; p is a function of observer ID, experience, pool difficulty and visibility.
Observer ID + observer experience + pool difficulty	p not affected by pool visibility
Observer ID + observer experience + pool visibility	p not affected by pool difficulty
Observer ID + pool difficulty + pool visibility	p not affected by observer experience
Observer experience + pool difficulty + pool visibility	p not affected by observer ID
Observer ID + observer experience	p is a function of observer ID and experience
Observer ID + pool visibility	p is a function of observer ID and pool visibility
Observer ID + pool difficulty	p is a function of observer ID and pool difficulty
Observer experience + pool visibility	p is a function of observer experience and pool visibility
Observer experience + pool difficulty	p is a function of observer experience and pool difficulty
Observer ID	p is a function of observer ID
Observer experience	p is a function of observer experience

Results and Discussion

Egg Mass Surveys

A total of four observers in three teams of two, conducted egg mass counts during the 3-year study. All surveys in a given year were conducted by a single team of observers, thereby avoiding observer changes within survey-years. In addition, each team of two consisted of an experienced observer paired with an inexperienced observer. Visibility was good to excellent during most surveys, resulting in a high degree of confidence that few egg masses were missed. However, visibility issues at two pools, FWNO and KFEA, are worth noting. FWNO is a relatively large (~97 x 24 m), shallow, shrub-dominated wetland with emergent vegetation, a complex shoreline, and several deeper (~41 cm) embedded pools. Visibility was impaired during all surveys around shrub thickets and emergent vegetation. KFEA is a small (~9 x 11 m), relatively deep (~40 cm) vernal pool with well-defined boundaries. Visibility was impaired somewhat during one survey on 13 May, 2011 due to a surface film, along with pollen and beech bud scales floating on the water.

Using maximum yearly raw counts, a total of 1,498 JESA egg masses were detected over the three years, followed by 971 WOFR eggs, and 846 SPSA eggs (Table 2). FWNO consistently had the highest counts for all three species, with a mean of 280.3 JESA eggs per year, 144.7 SPSA eggs, and 136.3 WOFR eggs. For Jefferson Salamander, KFSO and SAPO also supported relatively large populations (mean = 88.0 and 108.0, respectively). With the exception of KFEA, Spotted Salamander and WOFR eggs were more evenly distributed among the remaining 5 pools. Although egg masses of all three species were detected in all seven pools, only SPSA eggs were detected in all pools every year (Table 2, Fig. 3). Jefferson Salamander eggs were not detected in FWSO in 2011, and were only found in POPO in 2009, while Wood Frog eggs were not found in KFEA during 2009 surveys.

Table 2. Estimated detection probabilities (*p*), maximum raw egg mass counts, adjusted egg mass counts, and 90% confidence intervals for Jefferson Salamander, Spotted Salamander, and Wood Frog by pool and year.

		Jefferson Salamander				**Spotted Salamander**				**Wood Frog**			
Pool	**Year**	*p*	Raw Count	Adjusted Count	90% CI	*p*	Raw Count	Adjusted Count	90% CI	*p*	Raw Count	Adjusted Count	90% CI
FWNO	2009	0.928	113	124.18	119.6-131.9	0.858	124	135.57	131.0-143.2	0.996	112	112.46	112.2-113.0
	2010	0.715	397	519.90	470.3-603.3	0.858	86	97.94	93.2-105.9	0.996	153	153.57	153.3-154.2
	2011	0.927	331	362.24	352.5-376.9	0.924	224	239.17	232.5-251.1	0.980	144	146.68	145.3-149.6
FWSO	2009	0.912	9	9.71	9.4-10.3	0.677	24	34.15	29.5-42.8	0.952	89	92.82	91.0-96.2
	2010	0.652	1	1.08	1.0-1.1	0.797	5	6.25	6.0-6.6	0.804	57	68.71	64.5-75.2
	2011		0	0		0.797	33	36.89	35.9-38.4	0.804	49	60.64	57.1-65.8
KFEA	2009	0.894	8	8.96	8.6-9.6	0.797	2	2.12	2.1-2.2	0.804	3	3.69	3.5-4.0
	2010	0.652	6	14.34	11.7-18.1	0.797	5	7.69	7.2-8.2	0.804	3	3.78	3.6-4.1
	2011	0.913	4	4.58	4.3-5.0	0.797	2	2.60	2.5-2.7		0	0	
KFSO	2009	0.903	78	84.76	82.4-88.5	0.872	21	25.53	24.1-27.6	0.935	15	16.23	15.7-17.2
	2010	0.667	53	66.76	62.2-73.7	0.872	43	54.92	51.2-60.3	0.935	22	23.76	23.0-25.1
	2011	0.921	133	147.96	143.3-154.9	0.872	26	28.13	27.4-29.3	0.935	15	15.88	15.5-16.4
POPO	2009	0.912	5	5.45	5.2-5.8	0.677	12	15.56	13.9-18.6	0.952	33	34.93	34.1-36.4
	2010		0	0		0.797	11	16.43	15.5-17.6	0.804	47	60.13	56.8-64.6
	2011		0	0		0.677	35	71.81	54.9-103.1	0.952	30	31.70	31.0-33.0
PRPO	2009	0.894	8	9.05	8.6-9.8	0.677	28	36.64	32.7-44.0	0.952	58	61.37	59.9-64.0
	2010	0.652	17	37.22	30.9-46.4	0.797	44	52.37	50.6-54.8	0.804	70	86.01	80.9-93.6
	2011	0.913	11	12.49	11.9-13.5	0.797	66	73.30	71.3-76.2	0.804	26	31.55	29.6-34.5
SAPO	2009	0.921	62	66.68	64.9-69.6	0.778	18	21.41	20.0-23.8	0.986	24	24.32	24.2-24.6
	2010	0.667	115	237.32	194.9-302.3	0.872	7	7.74	7.5-8.1	0.934	6	6.42	6.2-6.7
	2011	0.935	147	156.66	152.4-164.4	0.778	30	42.96	37.6-52.2	0.986	15	15.19	15.1-15.3
Mean *p* (± SE)		0.832 (0.028)				0.799 (0.016)				0.906 (0.018)			

Model Selection and Detection Probabilities

Overall, no specific model was consistently selected as "best." Model selection results indicated there was variation between observers in detecting egg masses, but the data set for one observer was too sparse to use *observer ID* in inferences for WOFR and SPSA (Table 3). As a result, all the models for those two species that included *observer ID* had estimation problems and were therefore discarded. For WOFR, that left a single "best" model consisting of *observer experience* + *pool difficulty* + *pool visibility* for estimating detection probability (Table 3). For SPSA, four models that contained the variables *observer experience*, *pool difficulty*, and *pool visibility* were used to estimate detection probabilities with model averaging. For JESA the top four models, all of which contained *observer ID* and *observer experience* with various combinations of the other two covariates, fit the data best and were used with model averaging to estimate *p*.

Estimated detection probabilities were highest for WOFR, (mean = 0.906 ±0.018 SE; range = 0.804-0.996), followed by JESA (mean = 0.832 ±0.028 SE; range = 0.652-0.928), and SPSA (mean = 0.799 ±0.016 SE; range = 0.677-0.872) (Table 2). Detection probabilities varied within species by pool and year (observer).

These results are similar to what was expected based on egg mass morphology and deposition behavior. Wood Frogs, because of their large, communally deposited egg masses, were easier to detect compared to the singularly deposited eggs of both salamanders. However, I expected *p* for JESA to be lower than that for SPSA due to JESA's smaller, less conspicuous egg masses. This disparity may have been due to misidentification of *Ambystomid* egg masses by inexperienced observers, rather than missing them entirely. Using the same double-observer methods Grant et al. (2005) surveyed dozens of pools across the northeastern U.S. and reported slightly higher mean detection probabilities for WOFR (0.96) and SPSA (0.94) compared to results in this study. Crouch and Paton (2000) found that independent double-observer counts of WOFR eggs varied by 12%, while Eagan (unpublished data) found counts of SPSA eggs by two independent observers varied by 25%.

Population Assessment

Across the entire study area, annual population estimates fluctuated considerably. For JESA the total number of breeding adults ranged from 265 in 2009, to a high of 751 in 2010; for SPSA estimates ranged from 438 individuals in 2010 to 891 in 2011; while WOFR numbers appeared slightly more stable, ranging from 906 in 2011 to 1,206 in 2010 (Table 4). For all three species, the largest breeding populations occurred around the Pogue cluster of pools, with most breeding in the FWNO (Tables 3 and 4; Figs 3 and 4). Over the three years, this important cluster of pools supported 90% of the study area's breeding WOFR population (mean = 941.41 ±86.20 SE), 81% of the SPSA population (mean = 498.70 ±136.59 SE), and 58% of the JESA population (mean = 308.91 ±101.69 SE). In contrast the King Farm pools supported just 6% of the study area's WOFR population, 12% of the SPSAs, and 17% of the JESA population, while the SAPO supported 4% of the WOFRs, 7% of the SPSAs, and 25% of JESAs.

These population estimates are likely conservative since they do not include juveniles and non-breeding adults, and in some cases WOFR egg masses could not be counted because rafts of eggs had fused together. Downs (1989) reported that among JESAs, males typically breed every year while females often skip one or more years before returning to breed. Data for SPSA are not as clear, with some studies indicating that most adults breed every year (Whitford and Vinegar 1966; Douglas and Monroe 1981), while Phillips and Sexton (1989) found that 38% of females and 30% of males returned to breed every year. It is unknown if this behavior alone accounted for the wide annual variation that was observed in egg mass counts at MABI (Fig. 3), and hence population estimates. Although Vasconselos and Calhoun (2004) reported that SPSAs were highly philopatric, others found they will readily switch to nearby breeding pools (Pentranka et al. 2004) or colonize newly established pools (Patrick et al. 2008), which could explain some of the annual variation in this study (Fig. 3). However, when demographically independent groups of pools were clustered into isolates, annual variation remained pronounced (Fig. 4), indicating that if between-pond shifting occurred, it was insufficient to explain the annual variation. It is also possible for weather to play a role in annual breeding effort. A particularly prolonged dry spell in late-April and May could delay or reduce the number of amphibians that are able to reach breeding pools.

In a 21-year study of Wood Frogs in Michigan, Berven (2009) demonstrated that annual variation in adult WOFR population size was largely due to variation in juvenile recruitment. Most male and female WOFRs breed for the first time at one and two years of age, respectively, so that years of high larval and juvenile survival are followed by years of high adult populations. That study also revealed that while the majority of adult frogs bred only once in their life, in years of low adult population sizes, both males and females lived longer and reproduced multiple times.

Table 3. Comparison and ranking of 12 models (based on AICc) used to estimate the detection probabilities of egg mass counts for Wood Frog, Spotted Salamander, and Jefferson Salamander.

Model	**AICc**	**ΔAICc**	**Weight**	***K***	**Deviance**	**Rank**
a) Wood Frog (N = 971)						
Observer ID, observer experience, difficulty, visibility	286.50	0.00	0.51	5	276.43	1
Observer ID, observer experience, difficulty	287.89	1.39	0.26	4	279.84	2
Observer ID, observer experience, visibility	288.12	1.62	0.23	4	280.07	3
Observer ID, difficulty, visibility	298.95	12.45	0.00	4	290.91	4
Observer ID, observer experience	300.36	13.86	0.00	3	294.33	5
Observer ID, difficulty	305.53	19.03	0.00	3	299.50	6
Observer ID, visibility	320.86	34.37	0.00	3	314.84	7
Observer experience, difficulty, visibility *	322.16	35.66	0.00	3	316.14	8
Observer experience, difficulty	341.68	55.18	0.00	2	337.67	9
Observer experience, visibility	393.89	107.39	0.00	2	389.88	10
Observer ID	476.83	190.33	0.00	2	472.82	11
Observer experience	515.88	229.38	0.00	1	513.87	12
b) Spotted Salamander (N = 846)						
Observer ID, observer experience, difficulty, visibility	400.37	0.00	0.51	5	390.30	1
Observer ID, observer experience, difficulty	401.01	0.63	0.37	5	390.94	2
Observer ID, observer experience, visibility	404.02	3.64	0.08	5	393.95	3
Observer ID, observer experience	406.09	5.71	0.03	4	398.04	4
Observer ID, difficulty, visibility	429.69	29.32	0.00	5	419.62	5
Observer ID, difficulty	432.43	32.06	0.00	4	424.39	6
Observer ID, visibility	456.62	56.25	0.00	4	448.58	7
Observer experience, difficulty, visibility *	460.61	60.24	0.00	3	454.58	8
Observer experience, difficulty *	464.51	64.14	0.00	2	460.50	9
Observer experience, visibility *	493.58	93.20	0.00	2	489.56	10
Observer experience *	494.47	94.10	0.00	1	492.47	11
Observer ID	543.28	142.91	0.00	3	537.26	12
c) Jefferson Salamander (N = 1,498)						
Observer ID, observer experience, visibility *	897.39	0.00	0.39	5	887.35	1
Observer ID, observer experience, difficulty *	897.91	0.52	0.30	5	887.87	2
Observer ID, observer experience, difficulty, visibility *	898.74	1.35	0.20	6	886.69	3
Observer ID, observer experience *	899.79	2.40	0.12	4	891.77	4
Observer ID, difficulty, visibility	972.69	75.30	0.00	5	962.65	5
Observer ID, difficulty	976.32	78.93	0.00	4	968.29	6
Observer experience, difficulty	983.64	86.25	0.00	2	979.63	7
Observer experience, difficulty, visibility	985.22	87.83	0.00	3	979.20	8
Observer experience, visibility	1021.15	123.76	0.00	2	1017.14	9
Observer experience	1060.06	162.67	0.00	1	1058.06	10
Observer ID, visibility	1122.07	224.68	0.00	4	1114.05	11
Observer ID	1190.65	293.26	0.00	3	1184.63	12

AICc is Akaike Information Criteria, adjusted for small sample size. The difference between the model with the lowest AICc and each other candidate model (ΔAICc) is reported as a measure of comparison. Weight is the probability that the model is the best model in the set. *K* is the number of parameters in a model. Deviance is a measure of the model's ability to explain the data, compared with a saturated model that fits the data perfectly by design. * denotes models used in model averaging to estimate detection probabilities.

Table 4. Estimated breeding population size based on adjusted annual egg mass counts of Jefferson Salamander, Spotted Salamander and Wood Frog at three independent pool/pool clusters.

Pool/Pool Cluster	Year	Adjusted Annual Count[1]	Estimated Number of Females[2]	Estimated Number of Males[3]	Estimated Population Size[4]
		Jefferson Salamander			
SAPO	2009	67.00	33.50	23.93	57.43
	2010	237.32	118.66	84.76	203.42
	2011	157.00	78.50	56.07	134.57
	Mean (±SE)	153.77	76.89	54.92	131.81 (42.17)
Pogue Cluster	2009	148.00	74.00	52.86	126.86
	2010	558.19	279.10	199.35	478.45
	2011	375.00	187.50	133.93	321.43
	Mean (±SE)	360.40	180.20	128.70	308.91 (101.69)
King Farm Cluster	2009	94.00	47.00	33.57	80.57
	2010	81.00	40.50	28.93	69.43
	2011	152.53	76.27	54.48	130.74
	Mean (±SE)	109.18	54.59	38.99	93.58 (18.86)
		Spotted Salamander			
SAPO	2009	21.00	10.50	27.30	37.80
	2010	8.00	4.00	10.40	14.40
	2011	42.96	21.48	55.85	77.33
	Mean (±SE)	23.99	11.99	31.18	43.18 (18.36)
Pogue Cluster	2009	222.00	111.00	288.60	399.60
	2010	173.00	86.50	224.90	311.40
	2011	421.16	210.58	547.51	758.09
	Mean (±SE)	272.05	136.03	353.67	489.70 (136.59)
King Farm Cluster	2009	28.00	14.00	36.40	50.40
	2010	62.61	31.31	81.39	112.70
	2011	31.00	15.50	40.30	55.80
	Mean (±SE)	40.54	20.27	52.70	72.97 (19.93)
		Wood Frog			
SAPO	2009	24.32	24.32	48.64	72.96
	2010	6.00	6.00	12.00	18.00
	2011	15.00	15.00	30.00	45.00
	Mean (±SE)	15.11	15.11	30.21	45.32 (15.87)
Pogue Cluster	2009	302.00	302.00	604.00	906.00
	2010	368.41	368.41	736.82	1,105.23
	2011	271.00	271.00	542.00	813.00
	Mean (±SE)	313.80	313.80	627.61	941.41 (86.20)
King Farm Cluster	2009	20.00	20.00	40.00	60.00
	2010	27.53	27.53	55.06	82.59
	2011	16.00	16.00	32.00	48.00
	Mean (±SE)	21.18	21.18	42.35	63.53 (10.14)

[1] Annual egg mass count adjusted for detection probability (from Table 2)
[2] Adjusted annual egg mass count/average number of egg masses laid per female
[3] Number of females adjusted by sex ratio of each species (Faccio 2001)
[4] Number of females + number of males

Synchrony in Annual Population Change

Synchrony in annual population change was weak among individual pools (Fig. 3), but was higher for groups of pools deemed demographically independent (Fig. 4). The strongest synchrony was among the

SAPO and the Pogue pool cluster, although WOFR showed good synchrony between the Pogue and King Farm clusters. Pentranka et al. (2004) found higher synchrony for SPSA than for WOFR, and suggested that it was due to WOFRs shifting breeding pools more frequently between years. A number of studies indicate that amphibians perceive local pools as habitat patches rather than entire habitats, and assess the quality of the pool prior to egg-laying in order to avoid pools that offer low-quality habitat to developing larvae (Kats and Sih 1992; Hopey and Petranka 1994).

Hydrology and Water Chemistry Sampling

Water chemistry and hydrology metrics are summarized in Table 5. No significant differences were found among individual metrics between pools (ANOVA). Mean pH was lowest for SAPO (6.24 ±0.16 SE) and PRPO (6.66 ±0.15 SE) and highest for KFEA (7.15 ±0.07 SE) and FWSO (7.12 ±0.10 SE). However, the highest pH measured during the study was at FWNO (7.70) on 4 May 2009, while the lowest was at SAPO (5.96) on 6 May 2011. There was no relationship between pH and number of egg masses laid per pool for all species combined ($r^2 = 0.044$), or for individual species. Rowe and Dunson (1993) found a positive correlation between pH and the number of egg masses for both JESA and SPSA. In some amphibian populations, a pH between 4.5 and 5.5 can reduce hatching success, and larval growth and development (Brodman 1993; Clark 1986). Studies indicate that JESA is the least tolerant to low pH, followed by SPSA and WOFR, resulting in reduced larval survival and lower recruitment rates (Rowe et al. 1992; Sadinski and Dunson 1992). At MABI, acidity does not appear to be a limiting factor for amphibian populations. However, pH at the SAPO was consistently lower than all other pools, and further acidification due to acid precipitation could drop the pH below the 5.5 threshold, which may negatively affect JESA productivity. Anecdotally, a higher proportion of dead embryos among JESA eggs were noted in the SAPO compared to other pools sampled, but this was not quantified.

The number of eggs present at MABI pools was positively correlated to pool area for all three species (Fig. 5.). Although the FWNO had a large influence on these relationships, correlations were strongest for SPSA ($r^2 = 0.952$, $P<0.0001$) and WOFR ($r^2 = 0.803$, $P = 0.007$). Other studies have found that pool size was positively correlated with number of egg masses of WOFR and SPSA (Rowe and Dunson 1993; Skidds et al. 2007). Larger pools have several advantages over smaller pools, including the potential to support a greater number of larvae before density-dependent competition and predation have an effect. In addition, larger pools often have longer hydroperiods providing larvae more time to metamorphose at a larger size, which is associated with increased fitness and higher survival (Semlitsch et al. 1988; Berven 1990).

Conductivity measurements were generally low at MABI pools (median = 130.2, range = 4.7-227.0) (Table 5), reflecting low concentrations of strong electrolytes and weakly conductive organic solutes. Measurements of tannic acid were low (2 ppm) and consistent at all pools (Table 5). In contrast, Portnoy (1990) found tannic acid concentrations ranging from 2 to 7.6 ppm in vernal pools sampled on Cape Cod, MA, where low pH and relatively high amounts of organic acids are common due to the predominance of coniferous forests.

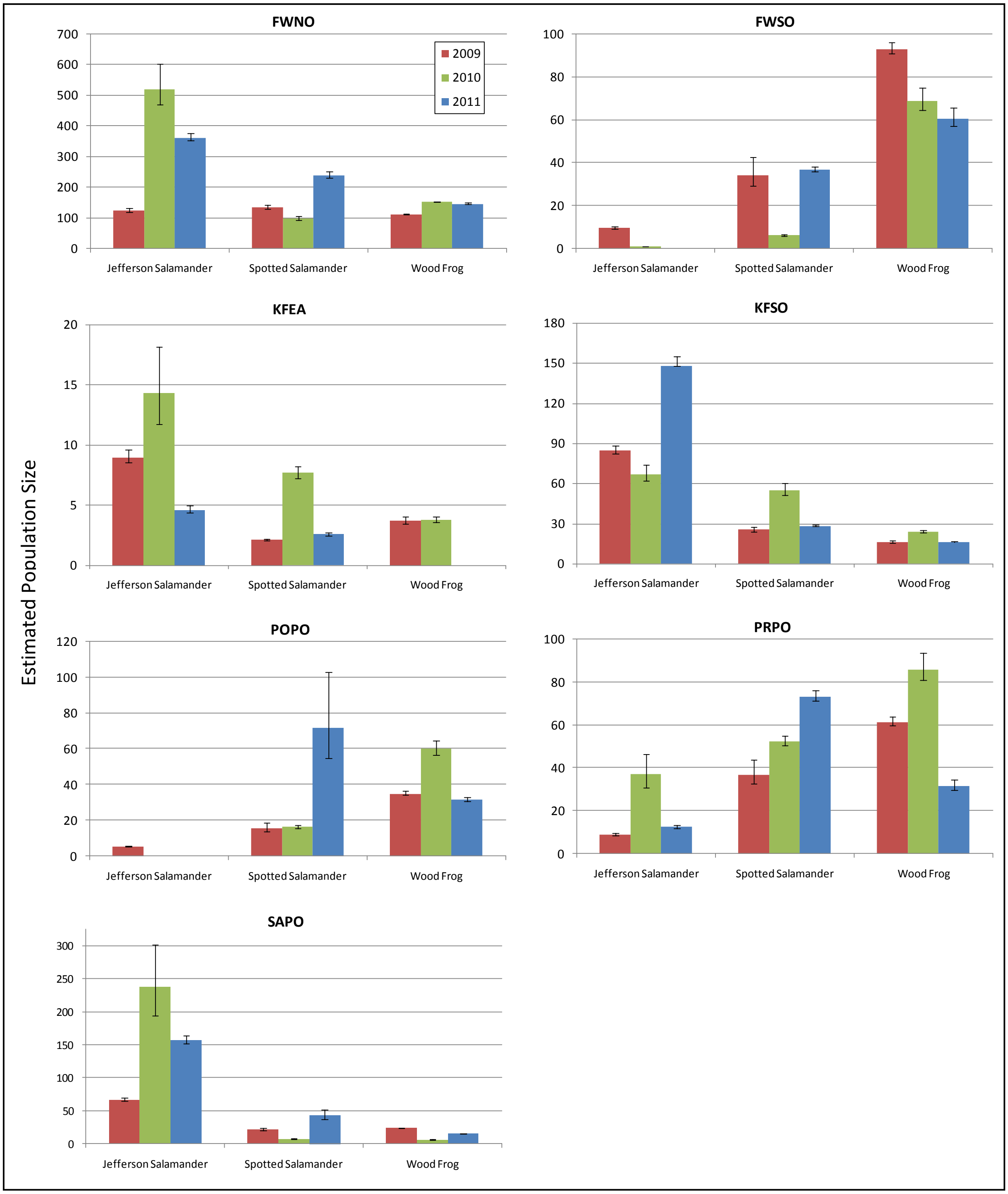

Figure 3. Maximum annual egg mass counts (adjusted for detection probabilities) of three amphibian species at seven breeding pools, 2009-2011. Label above each panel indicates pool. Note different scales on vertical axes.

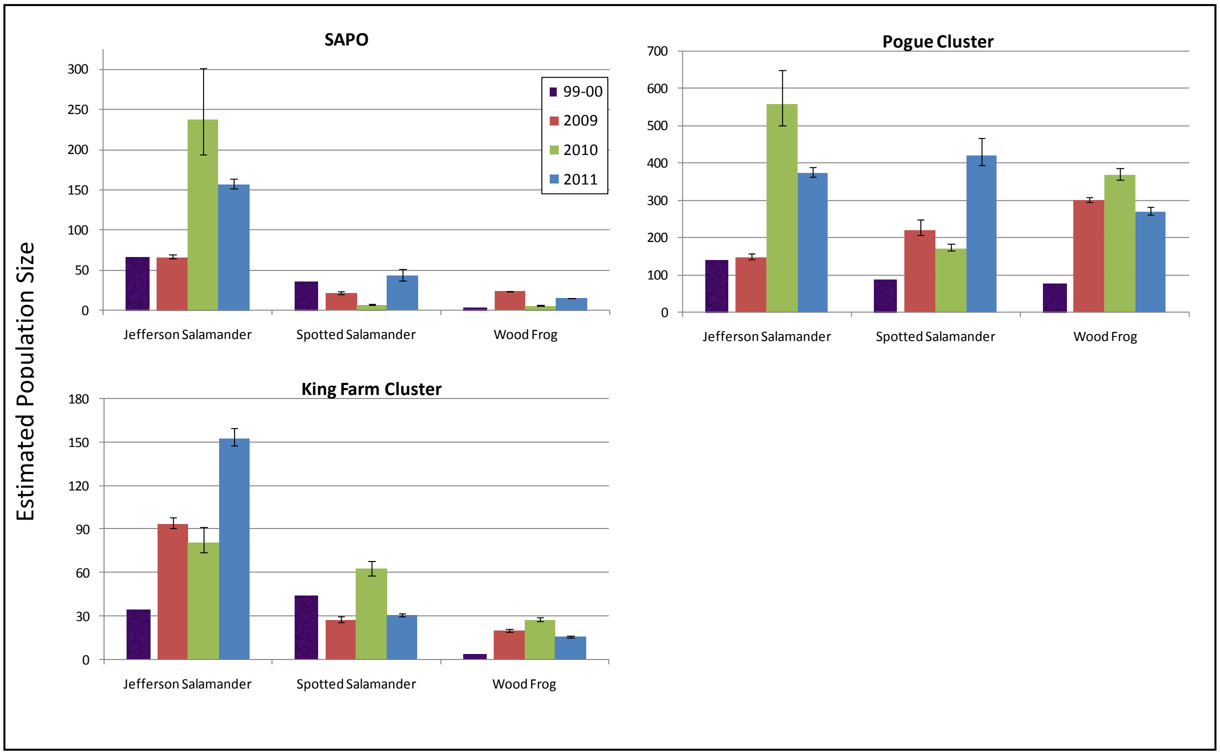

Figure 4. Estimated breeding population sizes (adjusted for detection probabilities) of three amphibian species based on double observer egg mass counts at one pool and two groups of pools deemed independent populations, 2009-2011. Data presented for 1999-2000 represent maximum raw counts of single-observer egg mass surveys conducted during 2-year amphibian inventory, and are presented for relative comparison (see Faccio 2001). Note different scales on vertical axes.

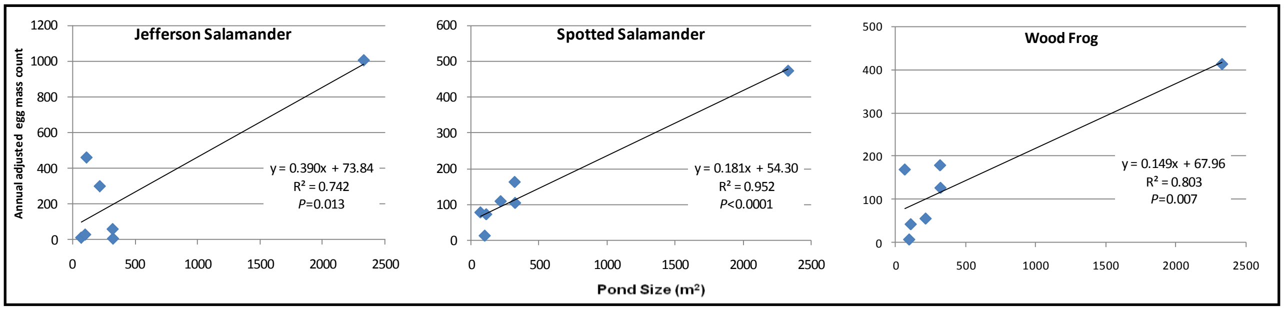

Figure 5. Relationship between pool size and the number of egg masses counted (adjusted for detection probabilities) for Jefferson Salamander, Spotted Salamander, and Wood Frog at seven vernal pools at MABI, 2009-2011.

Table 5. Water chemistry and hydrology metrics for seven vernal pools sampled at MABI, 2009-2011.

		Vernal Pool						
Variable	**Date**	**FWNO**	**FWSO**	**KFEA**	**KFSO**	**POPO**	**PRPO**	**SAPO**
pH	29-Apr-09	7.07	7.53	7.30	7.37	7.27	6.78	6.50
	4-May-09	7.70	7.23	7.31	7.50	7.43	7.34	6.93
	13-Apr-10	6.71	6.93	7.26	6.74	7.04	6.42	6.03
	22-Apr-10	6.70	7.06	6.99	7.01	6.96	6.52	6.00
	6-May-11	6.63	7.13	7.06	7.11	6.96	6.42	5.96
	13-May-11	6.52	6.85	6.96	6.80	6.93	6.47	5.99
Mean pH (±SE)		6.89 (0.18)	7.12 (0.10)	7.15 (0.07)	7.09 (0.12)	7.10 (0.08)	6.66 (0.15)	6.24 (0.16)
Dissolved O_2 (mg/l)	29-Apr-09	2.50	6.84	6.68	6.00	3.80	2.75	9.65
	4-May-09	10.22	6.23	6.41	7.65	4.25	5.51	7.64
	13-Apr-10	2.66	3.01	6.52	3.39	3.62	2.54	2.38
	22-Apr-10	2.39	5.17	4.68	5.74	1.52	2.40	1.23
	6-May-11	2.23	5.76	6.50	8.16	5.56	6.55	4.83
	13-May-11	1.05	2.08	1.97	3.37	1.18	2.26	1.77
Mean Dissolved O_2 (±SE)		3.51 (1.36)	4.85 (0.77)	5.46 (0.76)	5.72 (0.83)	3.32 (0.68)	3.67 (0.76)	4.58 (1.40)
Water Temp. (°C)	29-Apr-09	6.90	2.50	4.70	7.60	7.60	12.50	5.60
	4-May-09	8.80	7.20	5.90	3.70	9.30	13.00	4.60
	13-Apr-10	6.69	7.03	5.19	4.34	7.78	9.98	3.78
	22-Apr-10	9.90	12.40	8.35	6.52	9.92	10.53	5.31
	6-May-11	9.95	13.17	7.65	8.84	6.43	7.76	4.75
	13-May-11	15.23	18.06	9.95	8.33	10.18	12.72	11.40
Mean Temp. (±SE)		9.58 (1.27)	10.06 (2.26)	6.96 (0.83)	6.56 (0.87)	8.54 (0.61)	11.08 (0.84)	5.91 (1.13)
Conductivity ($\mu S/cm^3$)	29-Apr-09	80.90	122.10	163.00	138.30	138.90	60.40	4.70
	4-May-09	84.00	139.60	163.80	119.20	145.70	55.60	47.40
	13-Apr-10	91.00	160.00	217.00	164.00	167.00	56.00	49.00
	22-Apr-10	94.00	90.00	227.00	171.00	183.00	64.00	55.00
	6-May-11	76.00	146.00	193.00	142.00	145.00	47.00	45.00
	13-May-11	80.00	154.00	209.00	155.00	190.00	71.00	48.00
Mean Conductivity (±SE)		84.32 (2.82)	135.28 (10.52)	195.47 (11.11)	148.25 (7.73)	161.60 (8.83)	59.00 (3.34)	41.52 (7.49)
Tannic Acid (ppm)	29-Apr-09	2	2	2	2	2	2	2
	13-Apr-10	2	2	2	2	2	2	2
	6-May-11	2	2	2	2	2	2	2
Pool Depth (cm)	29-Apr-09	45.0	24.0	32.0	30.0	46.0	28.0	39.0
	4-May-09	45.0	24.0	28.0	28.0	40.0	18.0	38.0
	13-Apr-10	45.0	48.0	45.0	23.5	54.0	38.0	54.0
	22-Apr-10		41.0	55.0	32.0	53.0	40.0	48.0
	6-May-11	27.3	24.1	44.5	27.9	60.9	53.3	43.2
	13-May-11	45.7	20.3	35.6	25.4	45.7	30.5	43.1
Mean Depth (±SE)		41.60 (3.58)	30.23 (4.64)	40.02 (4.07)	27.80 (1.25)	49.93 (3.05)	34.63 (4.92)	44.22 (2.44)
Max. Pool Size (m)		97 x 24	9 x 7.5	11 x 9	24 x 9	23 x 14	29 x 11	11 x 10
Approximate Pool Area (m^2)		2,328.0	67.5	99.0	216.0	322.0	319.0	110.0

Conclusions

Three years of egg mass surveys at MABI have established valuable baseline data on estimated population sizes for all three pool-breeding species, and demonstrated that dramatic annual variations in reproductive effort occur. Among salamanders, which are relatively long-lived, at least some of this annual variation is probably due to the fact that all females do not breed every year, while among short-lived WOFRs it is likely due to variations in larval and juvenile survival and recruitment. This study also confirmed that FWNO supports the largest breeding populations of all three species in the park, while SAPO and KFSO support smaller but significant breeding populations of JESA. The four other pools support considerably smaller populations of these amphibians. However, given the duration of the study, methodologies, and annual variations in breeding effort observed, these data were insufficient to evaluate the effects of forestry operations on pool-breeding amphibians.

The dependent double-observer method used for conducting egg mass counts in this study produced relatively high detection probabilities for egg masses of all species, ranging from 80% for SPSA to 90% for WOFR. There was considerable variation in detection probabilities among different observers, but due to relatively small sample sizes, not all models could utilize observer variance in making inferences about the data. These results underscore the need to account for differences in observers and other variables when estimating population trends over time. Otherwise, indices derived from unadjusted counts may have bias associated with sampling variation.

Recommendations for Future Monitoring

MABI supports significant breeding populations of pool-breeding amphibians, including substantial populations of JESA, a species whose status in Vermont is listed as "Special Concern (rare; status should be watched)," and whose state rank is "S2 (rare; at high risk of extinction or extirpation due to very restricted range, very few populations [often 20 or fewer], steep declines, or other factors)" (VFWD 2011). Therefore, MABI has high responsibility to monitor these populations to ensure that silvicultural activities or other factors do not negatively impact this important piece of the park's biodiversity. Using the double-observer sampling procedure utilized in this study provides a cost-effective method that allows for the calculation of detection probabilities, and therefore unbiased estimates of population size based on number of egg masses.

I recommend that egg mass surveys be continued in order to monitor population trends over time, and to assess the effectiveness of forest management activities on pool-breeding amphibians. Annual double-observer counts would provide data with the most power to detect population change. However, it may also be possible to conduct egg mass surveys periodically, perhaps every 3 to 5 years, and still meet the park's monitoring objectives, but a power analysis should be conducted using data from this study to be sure that any monitoring protocol would be sufficient to detect the desired change in population. Given the annual variance in egg mass numbers found in this study, periodic monitoring would probably need to consist of several consecutive years of surveys followed by gaps of several years without surveys.

In addition to egg mass surveys, the hydroperiod (length of time that a pool holds water) should be monitored at all pools during the amphibian egg-laying and larval stages (April thru August). A pool's hydroperiod is one of the most important factors in determining its suitability as breeding habitat for all three amphibian species present at MABI (Skidds and Golet 2005). These data may reveal pools that routinely dry too quickly for metamorphosis to occur, acting as population "sinks," and may also provide insights that help explain the annual variance in the size of adult breeding populations by correlating them with larval survival or failure in previous years.

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