MONITORING OF POND BREEDING AMPHIBIANS AT

CAPE COD NATIONAL SEASHORE, 2003

Prepared By:

Robert P. Cook
Kelly Boland

National Park Service
Cape Cod National Seashore
99 Marconi Site Road
Wellfleet, Massachusetts 02667

March, 2004

Cape Cod Prototype Monitoring Program
National Park Service, Department of Interior

TABLE OF CONTENTS

TABLE OF CONTENTS…….……...…ii
APPENDICES…….ii
LIST OF FIGURES……..……...ii
LIST OF TABLES……..……...……iii
EXECUTIVE SUMMARY……....iv
INTRODUCTION……...1
VERNAL POND EGG MASS COUNTS……...1
Introduction……...……...1
Methods……....2
Results……..6
Spotted salamander egg mass counts……...6
Wood frog egg mass counts… …….…….……..6
Environmental conditions……9
Habitat parameters and spotted salamander EMC…….……10
Discussion……..12
Temporal trends in spotted salamander egg mass counts……..12
Spatial variation in spotted salamander egg mass counts……..12
ANURAN CALL COUNTS……..14
Methods……..14
Results……18
Discussion……..23
RECOMMENDATIONS AND FUTURE PLANS……...24
LITERATURE CITED……..……25
APPENDICES
1. Comparison of egg mass count methods……...27
2. Historical and current egg mass counts at CACO…….30
3. Water quality data from surveyed ponds……...32
4. Adjacent habitat attributes of egg mass ponds…….34
5. Program PRESENCE model comparison statistics for anuran call counts…..35
LIST OF FIGURES
1. Egg mass count sites in Eastham…….……3
2. Egg mass count sites in Truro and Wellfleet……...4
3. Egg mass count sites in Provincetown…….…5
4. Anuran call survey route 1…….15
5. Anuran call survey route 2…….16
6. Anuran call survey route 3…….17
7. Seasonal variation of calling anurans by species……...……22

LIST OF TABLES

1. Spotted salamander egg mass maximum count and percent change, 2002 to 2003……7
2. Trend analysis of spotted salamander from 2001 to 2003……...7
3. Spotted salamander counts from 2002…….8
4. Spotted salamander counts from 2003…….8
5. Wood frog counts from 2002 and 2003……...9
6. Water temperatures at egg mass ponds over time in 2002……...9
7. Water temperatures at egg mass ponds over time in 2003…….10
8. Maximum and mean pond depth for 2002 and 2003…….11
9. Anuran occurrence summary by pond and species……19
10. Detectability statistics for anuran call counts….……...……..20
11. Coefficients for anuran call count parameters…….…21

EXECUTIVE SUMMARY

Given the abundance and ecological significance of freshwater wetlands at Cape Cod National Seashore (CACO), the important role that amphibians play in them, and concerns that both global/regional (e.g. air pollutants, acid rain, diseases) and local factors (e.g. development, road kill, ground water withdrawal) may alter the abundance, distribution, and structure of amphibian communities, long term monitoring of pond breeding amphibians was initiated in 2003. It is part of the park's long term ecological monitoring program, and consists of two components. Vernal pond egg mass counts monitor the abundance and distribution of spotted salamander (*Ambystoma maculatum*) and wood frogs (*Rana sylvatica*). Anuran call counts monitor abundance, distribution, and habitat association of the park's anurans (frogs and toads). In addition, data on each pond's physical and chemical attributes and vegetation are collected.

In early spring 2003, three counts of egg masses were conducted in 20 vernal ponds. Based on the highest or "maximum" count for each pond, a total of 5450 masses of spotted salamander and 61 masses of wood frog were present. Spotted salamanders occurred from Eastham to Truro (to the limit of glacial deposits at High Head) whereas wood frogs only breed in Eastham. For a small number of ponds, data collected during protocol development in 2001 and 2002 was combined with that from 2003 to compare counts across two and three consecutive years. There were no significant differences in egg mass counts between years, nor was there any relationship between egg mass counts and rainfall during the breeding migration season. Given the lack of long term data, the lack of significant trends is not surprising. Analysis of landscape and within pond factors potentially related to egg mass abundance found only one factor, amount of submerged aquatic vegetation (SAV) to be significant. We believe this reflects pond hydroperiod (length of time when water is present) and suggests that the largest breeding populations of spotted salamanders occur at vernal ponds that hold water longest. Such a conclusion is consistent with research conducted elsewhere.

Anuran call counts were conducted weekly at 30 freshwater wetland and pond sites for 14 consecutive weeks, from mid-April to late July. Counts consisted of visiting ponds after dark, listening for five minutes, and recording the abundance of species heard as an index value ranging from 0 to 3. A total of eight species of frog and toads, every species known to occur at CACO, was recorded at least once. In descending order, the most widespread species were spring peeper (*Pseudacris crucifer*), green frog (*Rana clamitans*), Fowler's toad (*Bufo fowleri*), bullfrog (*Rana catesbiana*), pickerel frog (*Rana palustris*), eastern spadefoot toad (*Scaphiopus h. holbrooki*), grey treefrog (*Hyla versicolor*), and wood frog. Generally, abundance of species was correlated with how widespread a species was, except for spadefoot toads, which were limited in occurrence but abundant where they did occur. Analysis of species occurrence and site features, as well as seasonal patterns, indicate that the species here are similar to other anuran communities in the Northeastern U.S. in their habitat use and breeding season chronology. This first season's data showed that grey treefrog, a species first recorded at CACO in 2001, is more widespread than previously thought, and will provide an excellent starting point for monitoring the abundance and distribution of the park's frogs and toads.

INTRODUCTION

Cape Cod National Seashore (CACO) supports a great abundance and diversity of freshwater wetlands. Few landscapes in the region contain such a wealth of wetlands, which in turn support many regionally uncommon species of wetland-dependent flora and fauna. Among these, amphibians play a significant role in the energy flow, biomass, and community structure of freshwater wetlands, and contribute significantly to terrestrial ecosystems as well. Consequently, monitoring of pond breeding amphibians was initiated in 2003 as a component of freshwater wetland monitoring in the Cape Cod National Seashore prototype monitoring program (Roman and Barrett 1999). Specific rationale for the program includes concerns for individual habitats and species, as well as questions related to changes in abundance, distribution, and structure of the park's amphibian communities in the face of potential impacts from acid deposition, road mortality, groundwater borne and air borne contaminants, habitat changes, and groundwater withdrawal (Paton et al. 2003).

Pond breeding amphibian monitoring at CACO consists of two components; monitoring occurrence and abundance of the vernal pond breeding species spotted salamander (*Ambystoma maculatum*) and wood frog (*Rana sylvatica*) through egg mass counts, and monitoring occurrence and relative abundance of the breeding anuran community park wide, in a range of wetland types, through the use of anuran call counts. Since these components entail distinct methods, target organisms, and sample sites, each will be reported on separately.

VERNAL POND EGG MASS COUNTS

Introduction

Prior to this long term monitoring program, spotted salamander egg masses were counted from 1986 to 1996 for a study of relationships between pond water chemistry and embryonic mortality (Portnoy 1990) and more recently (1998, 1999, 2001) in the course of monitoring protocol development (Colburn et al. 2000, Paton et al. 2003). In 2002, egg masses were counted at 16 ponds as part of the USGS Amphibian and Reptile Monitoring Initiative (ARMI) Program (Jung 2002). While participation in the ARMI program provides an important contribution to this regional monitoring program, sampling sites were not sufficiently CACO-wide to meet CACO needs. However, since data collected for ARMI can be incorporated into CACO's program, in 2003 (and in future years), we continued to participate in the ARMI Program, while at the same time collecting data to meet CACO-specific needs. Only data relevant to CACO are presented here. With 2003 being the inaugural year of the current program, we continued to work on resolving questions of methodology (Appendix 1), and will attempt, to the extent possible, to incorporate historic data (Appendix 2) into the present analysis.

Methods

Counts of spotted salamander and wood frog egg masses were conducted in 16 vernal ponds in 2002 and 20 in 2003. The data collected in 2002 were solely for the ARMI program; the CACO monitoring program was still in development then. Two ponds from 2002 were dropped in 2003 because they were no longer needed for the ARMI program, and six new ponds were added in 2003, selected randomly to increase the number and geographic scope of CACO sample ponds. The 20 sites sampled in 2003 will continue to be monitored in the future. Ponds ranged geographically from Eastham to Provincetown and include most of the Eastham vernal pool complex (figs. 1-3).

Four counts were conducted in 2002 between 4/4/02 and 5/17/02. Three counts were conducted in 2003, between 3/30/03 and 5/5/03. For each species at given pond in a given year, the highest or maximum count was used as the measure of abundance (see Appendix 1). In conjunction with each count, maximum water depth (at a marked point determined to be deepest point in pond), air and water temperature were recorded (Paton et al. 2003). In 2003, maximum pond length and width (Jung 2002) were measured at each count, and the maximum values used to calculate pond size. In 2003, analysis of a suite of water quality parameters was conducted, based on water samples collected in April. Analysis was conducted at the North Atlantic Coastal Lab, North Truro, MA using methods described in Boland and Cook (2004).

Analysis of between year (2002 *vs* 2003) differences in maximum egg mass counts was conducted by a paired t-test. In addition, data for spotted salamanders from 2001 (Paton et al. 2003) were used to augment our own and provide three year's consecutive data for seven ponds. Trends in these data were analyzed using linear regression, as recommended by Paton et al. (2003). In addition, since there is a significant positive correlation between annual breeding effort in *Ambystoma* salamanders and rainfall during the breeding migration season (Semlitsch 1987), the effects of rainfall-related variation in total egg mass counts were removed using Kendall's partial rank correlation (Pechmann et al. 1991). Since spotted salamanders in Massachusetts migrate to breeding ponds in March and April, migration season rainfall is total rainfall for these two months, recorded at a Cape Cod National Seashore rain gauge in Eastham, MA.

Data from 2003 were analyzed to explore relationships between spotted salamander egg mass counts and physical, chemical, and ecological attributes of ponds and their adjacent areas. Many water quality parameters (Appendix 3) were highly significantly correlated (e.g. pH and alkalinity ($r=0.91$, $p<0.001$), conductivity and chloride ($r=0.97$, $p<0.000$), absorption coefficient 440 and visual color ($r=0.92$, $p<0.000$), and absorption coefficient 440 and tannin lignin ($r=0.87$, $p<0.000$)). To remove these redundant variables and simplify analysis, only pH, conductivity, and color (Absorbance Coefficient at 440 nanometers (AbsCo440)) were retained for use in analysis. Ecological attributes of ponds and adjacent areas are based on the ARMI program (Jung 2002). Adjacent landscape parameters were distance to nearest paved road, number of vernal ponds within 250 meters, and percent of woodland, paved road, field, wetland, and residential within 50 meters. Within pond-parameters were area, depth, pH, conductivity, absorbance, and

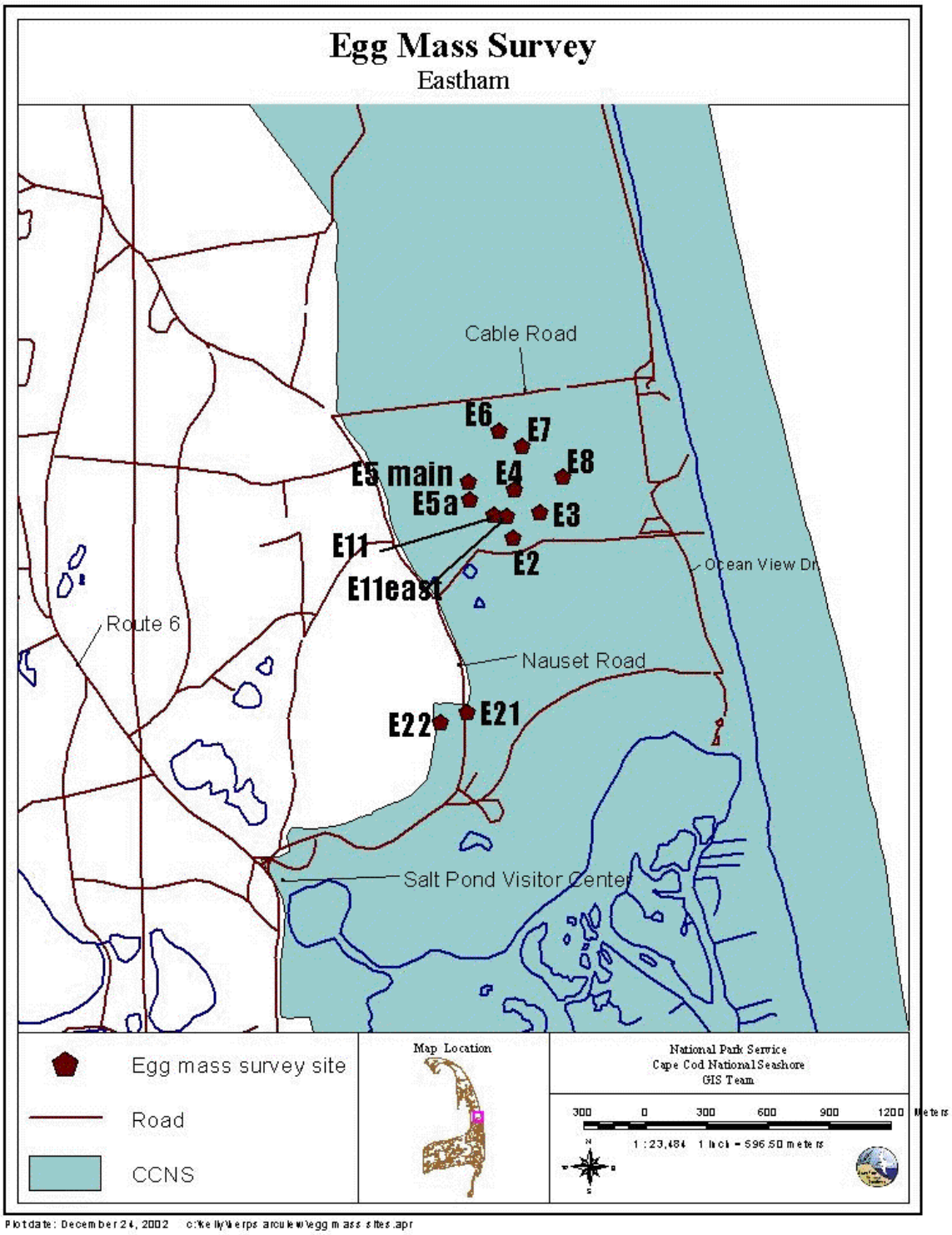

Figure 1. Vernal pond egg mass count sites in Eastham.

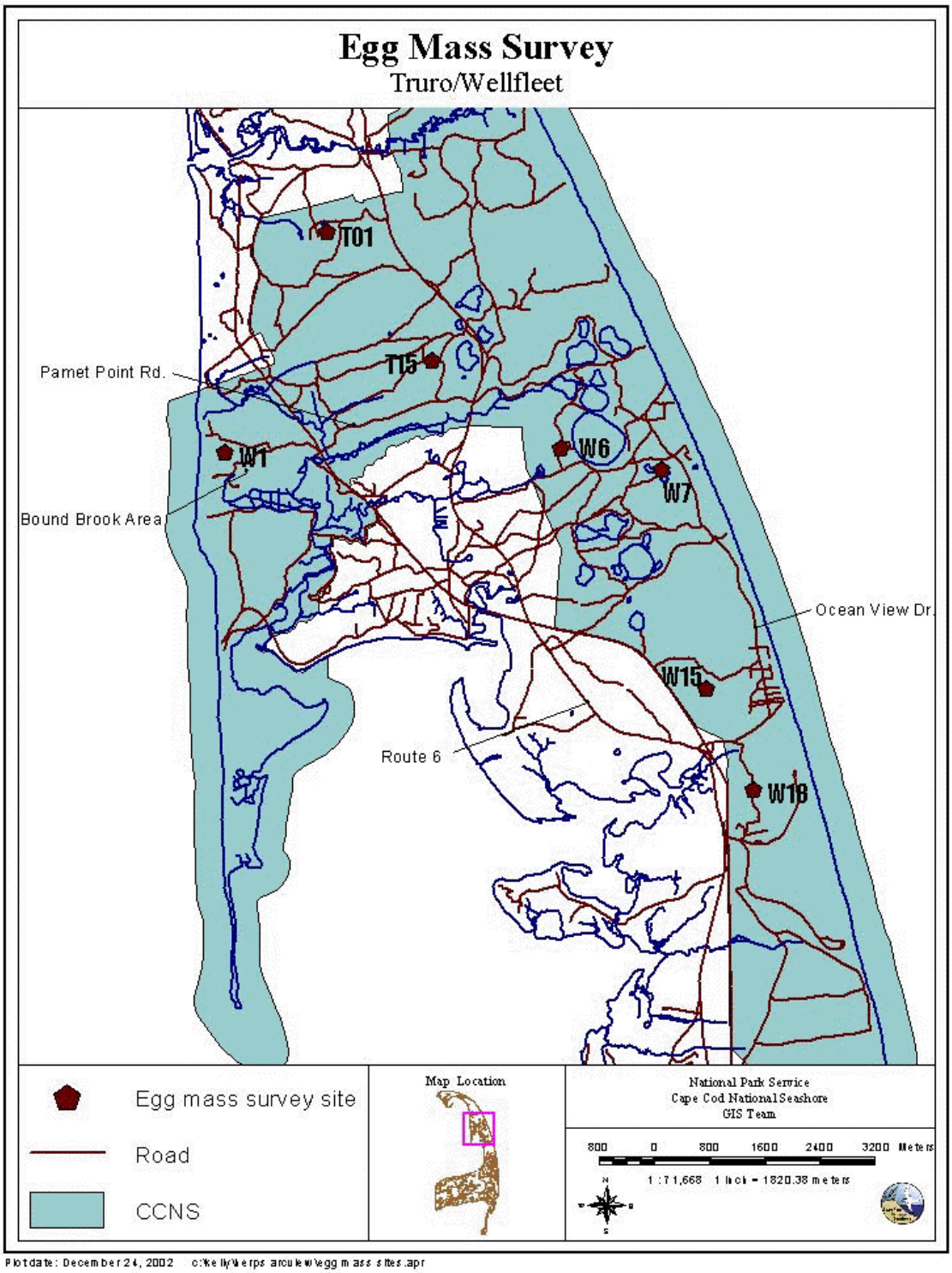

Figure 2. Vernal pond egg mass count sites in Truro/Wellfleet.

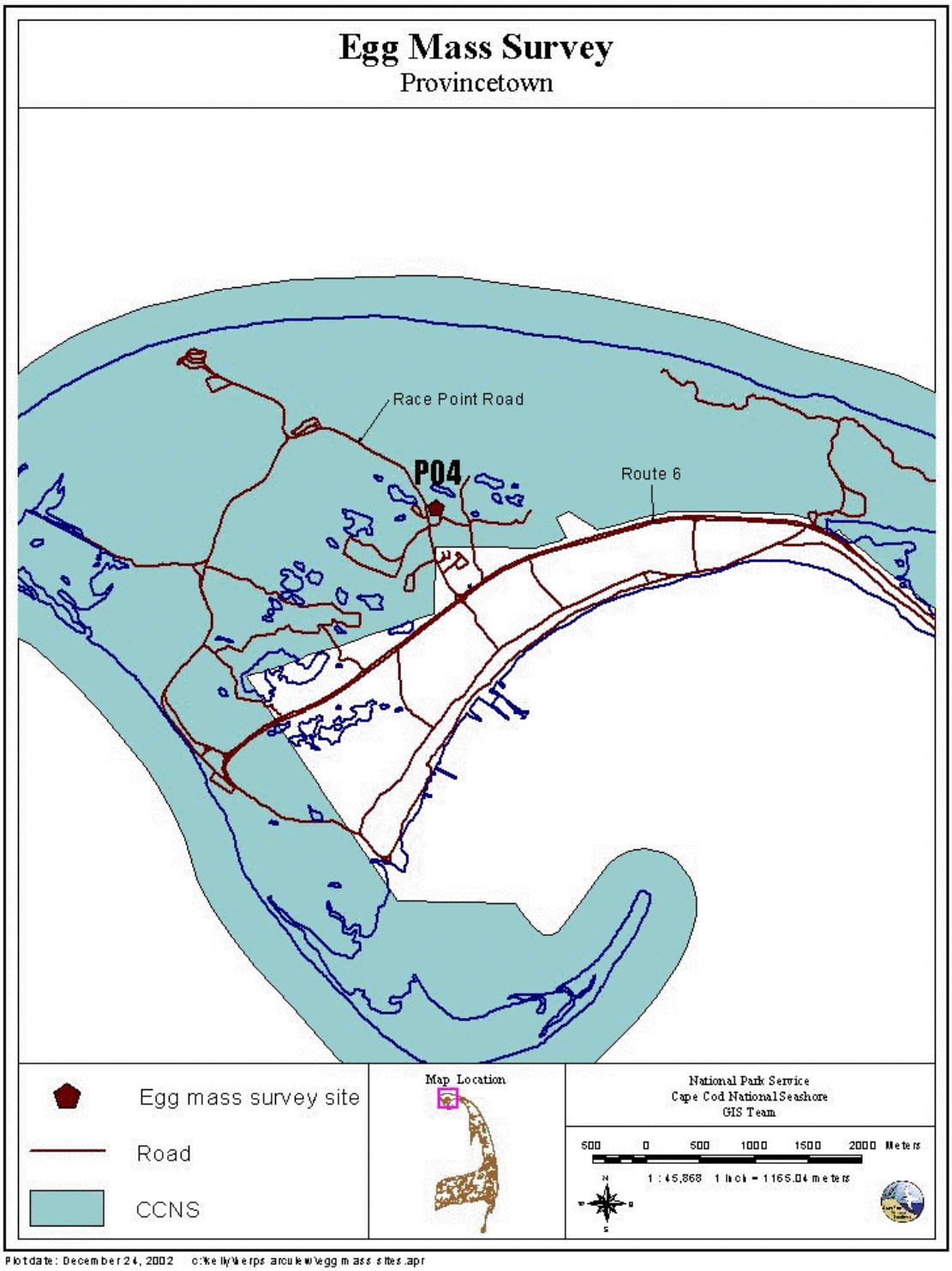

Figure 3. Vernal pond egg mass count sites in Provincetown.

percent of pond occupied by leaf litter, woody debris, submerged aquatic vegetation (SAV), moss, emergent, shrubs, and trees.

The relationship between spotted salamander egg mass counts and habitat parameters was analyzed using forward stepwise multiple regression, with variables entered and removed at critical values of p = 0.05 and p = 0.10, respectively (Egan 2001). Percentage data were arcsine transformed prior to analysis. Remaining habitat variable data (Appendix 4) were tested for normality using the Shapiro-Wilks test of program STATISTICA (Statsoft 2000). Those not meeting assumptions of normality were transformed to best meet assumptions of normality using either the square root or log transformation procedures detailed in Zar (1996). Analysis was performed separately on within-pond and adjacent landscape variables. Pond P04 was excluded, since it lies outside the known range of spotted salamander at CACO.

Results

Spotted Salamander Egg Mass Counts

Maximum egg mass counts were generally lower in 2003 than 2002. For the 14 ponds with two year's data, the total number of egg masses declined from 5444 to 4013. Counts increased at six ponds and decreased at eight (table 1). Mean increase was 46 egg masses, whereas mean decline was 213. Collectively, differences in egg mass counts between years were not significant (t=1.79, df=13, p=0.096). For the seven ponds with three year's data, the trend in combined egg mass counts was positive (slope=0.72) but not significantly different from zero (p=0.49). Six of the seven ponds also had positive slopes. Only E3 had a negative slope. None of these slopes deviated significantly from zero (table 2). Trends in egg mass count after correcting for rainfall were also non significant (Kendall's tau=0.000) as was correlation between egg mass count and migration season rainfall (r = -976, p=0.14).

Egg deposition occurred later in the year in 2003 compared to 2002. For a given pond, maximum counts in 2002 occurred primarily in replicate one and two (table 3), whereas in 2003 they occurred in replicates two and three (table 4).

Wood Frog Egg Mass Counts

Wood frog egg masses were only recorded from ponds in Eastham. In 2002, 13 ponds were surveyed in Eastham. Six ponds contained a total of 52 egg masses. In 2003, 12 ponds were surveyed in Eastham. Nine ponds contained a total of 61egg masses (table 5). From 2002 to 2003, egg mass counts increased at six ponds and decreased at three. For the 12 ponds in Eastham counted in both 2002 and 2003, differences in egg mass counts between years were not significant (t=-0.317, df=11, p=0.76).

Table 1. Maximum counts for spotted salamander in 2002 and 2003.

Pond	2002 MC	2003 MC	Change	%Change
E02	30	50	20	67%
E03	25	38	13	52%
E04	1227	633	-594	-48%
E05a	677	315	-362	-53%
E05main	596	767	171	29%
E06	599	575	-24	-4%
E07	226	269	43	19%
E08	243	250	7	3%
E11	359	254	-105	-29%
E11e	29	24	-5	-17%
E21	434	261	-173	-40%
E22	910	486	-424	-47%
W06	8	27	19	238%
W15	81	64	-17	-21%
Sum	5444	4013	-1431	-26%

Table 2. Trends analysis of spotted salamander egg mass counts at 7 CACO vernal ponds from 2001 through 2003.

Pond	2001 MC	2002 MC	2003 MC	Slope	R^2	p
E03	48	25	38	-.43	.19	.71
E04	503	1227	633	.17	.03	.89
E05main	174	596	767	.97	.94	.15
E06	168	599	575	.84	.71	.36
E07	92	226	269	.96	.92	.18
E11	101	359	254	.58	.35	.60
E11e	0	29	24	.77	.60	.44
All	1086	3061	2560	.72	.52	.49

Table 3. Totals of spotted salamander egg mass counts by replicate for 2002. Bold indicates the maximum count for each site.

	Rep 1	Rep 2	Rep 3	Rep 4
Pond	**4/4-4/12**	**4/18-4/19**	**4/24-5/3**	**5/10-5/17**
E02	**30**	29	22	19
E03	23	**25**	7	7
E04	**1227**	1062	1153	680
E05a	**677**	594	396	445
E05main	483	**596**	405	414
E06	397	**599**	404	427
E07	197	223	224	**226**
E08	**243**	197	228	223
E11	**359**	334	285	237
E11e	**29**	**29**	24	20
E18	4	**6**	4	0
E21	238	347	**434**	254
E22	**910**	475	873	343
W03	0	0	0	0
W06	6	**8**	0	0
W15	81	**79**	75	44

Table 4. Totals of spotted salamander egg mass counts by replicate for 2003. Bold indicates the maximum count for each site.

	Rep 1	Rep 2	Rep 3
Pond	**3/30-4/7**	**4/14-4/21**	**4/28-5/5**
E02	2	44	**50**
E03	23	**38**	30
E04	326	**633**	514
E05a	129	**315**	260
E05main	335	**767**	481
E06	258	**575**	353
E07	119	**269**	203
E08	136	**250**	243
E11	71	234	**254**
E11east	6	**24**	16
E21	47	**261**	129
E22	103	**486**	385
P04	0	0	0
T01	135	**544**	261
T15	20	**22**	20
W01	248	**489**	467
W06	0	26	**27**
W07	101	**338**	170
W15	9	**64**	61
W18	9	31	**44**

Table 5. Maximum counts for wood frog in 2002 and 2003.

Pond	2002 MC	2003 MC
E02	0	0
E03	0	1
E04	2	1
E05a	9	15
E05main	0	16
E06	8	16
E07	3	7
E08	0	0
E11	0	1
E11east	0	0
E21	11	2
E22	19	2
W06	0	0
W15	0	0
W07	0	0
W18	***	0
P04	***	0
T01	***	0
T15	***	0
W01	***	0
E18	0	***
W03	0	***

***denotes ponds that were not sampled in the specified year

Environmental Conditions

Pond water temperatures in 2002 (table 6) were higher than in 2003 (table 7). For the four ponds with water temperature data for 2002 and 2003 (E21, E4, W6, W15), the mean water temperature was 14.93 °C in 2002 and 6.88 °C in 2003. Differences in water temperature were not significant between ponds ($F_{3,15}$=0.3438, p=0.79) but were between years ($F_{1,15}$=17.81, p=0.0007).

Table 6 .Water temperature (°C) of the six ponds where egg mass counts were conducted in 2002 as part of the ARMI program.

Pond	rep1	rep2	rep3	rep4
E04	20.0	dry	20.0	dry
E18	11.5	14.5	16.5	15.0
E21	6.0	13.0	18.5	12.5
W03	15.5	17.0	9.5	17.0
W06	12.0	14.5	12.2	16.4
W15	11.0	21.0	11.0	15.5

Table 7. Water temperature (^{o}C) data for ponds where egg mass counts were conducted in 2003.

Pond	rep1	rep2	rep3
E11	6.0	10.0	13.0
E11east	7.5	9.5	11.0
E2	4.0	12.0	10.5
E21	7.0	5.0	13.5
E22	7.5	9.0	17.5
E3	7.0	9.0	16.0
E4	2.0	5.0	9.0
E5a	9.0	16.0	18.0
E5main	10.0	13.0	11.0
E6	8.0	12.0	16.0
E7	6.0	7.0	16.0
E8	6.0	10.0	13.0
P4	1.0	8.0	11.0
T1	6.0	12.0	15.0
T15	4.0	10.0	12.0
W1	3.0	9.5	10.0
W15	4.0	4.5	10.0
W18	4.0	4.0	7.0
W6	2.0	6.5	14.0
W7	6.5	7.0	9.0

Water depth in 2002 was less than in 2003 (table 8). For the 13 ponds measured in 2002 and 2003, the mean "Maximum Depth" in 2002 (16.45 cm) was significantly less than in 2003 (71.04 cm) ($t=-14.8738$, $df=12$, $p=0.0000001$). Total rainfall during the breeding migration season was 32.8 cm in 2001, 26.5 cm in 2002, and 26.7 cm in 2003.

Pond maximum depth ranged from 50 to 106 cm, with a mean of 76.2 cm and a standard deviation of 16.5. Pond area ranged from 228 to 34,320 m^2, with a mean of 3361 and a standard deviation of 7630. All ponds were acidic: pH ranged from 3.9 to 5.78, with a mean of 4.753 and a standard deviation of 0.388. Conductivity ranged from 30.2 to 90.2 µS/cm, with a mean of 51.3 and standard deviation of 14.5. The absorption coefficient at 440 nanometers (absco 440) ranged from 0.00080 to 0.00440, with a mean of 0.00189 and standard deviation of 0.0009. Physical parameters of individual ponds where egg mass counts were conducted are in Appendix 4.

Habitat Parameters and Spotted Salamander Egg Mass Counts

Woodland habitat comprised from 90 to 100% of pond adjacent habitat. Only a few ponds had any roads, field, wetland, or residential use within 50 m, and in these instances, those habitat/land use categories only accounted for from 5 to 10% of the adjacent zone (Appendix 4). Of the adjacent landscape parameters, none were significant enough to be entered into the regression model. Within pond, the dominant substrate was leaf litter, which covered from 5 to 95% of pond bottoms (mean 56%). Sticks only comprised from 0 to 15% of pond substrate (mean 6%). Within-pond vegetation tended

Table 8. Maximum depths recorded during egg mass counts in 2002 and 2003. Mean represents the mean value of the maximum depth recorded during four replicates in 2002 and three replicates in 2003.

Pond	Maximum Depth 2002 (cm)	Maximum Depth 2003 (cm)	Mean Depth 2002 (cm)	Mean Depth 2003 (cm)
E02	15.24	59.00	13.15	51.75
E03	12.70	60.50	4.32	52.63
E04	5.08	71.00	1.91	63.00
E05a	10.16	78.00	6.83	66.50
E05main	8.13	80.00	3.62	69.00
E06	20.32	87.00	17.40	79.00
E07	16.51	50.00	13.65	46.25
E08	17.78	65.00	16.70	61.50
E11	22.86	67.00	17.65	57.00
E11e	1.27	58.00	0.32	49.50
E18	45.72	***	39.69	***
E21	45.72	106.00	28.89	100.50
E22	***	102.00	over head	96.25 @ end of dock
W03	20.32	***	15.56	***
W06	20.32	56.00	9.91	53.00
W15	17.78	86.00	7.49	70.75
P04	***	77.00	***	75.00
T01	***	82.00	***	74.00
T15	***	90.00	***	82.00
W01	***	74.00	***	62.50
W07	***	102.00	***	94.00
W18	***	74.00	***	66.00

***denotes sites that were not sampled in specified year

to be a mix of both shrubby and emergent. Emergent vegetation comprised from 0 to 80% of a pond (mean 40%) and shrubby from 0 to 80% (mean 22%). Ponds heavily dominated by shrubs tended to lack emergent vegetation, and vice versa (Appendix 4). Of the within-pond parameters, only SAV was entered into the regression model ($R = 0.774$, $F_{1,18} = 25.329$, $p = 0.000$).

Discussion

Temporal Trends in Spotted Salamander Egg Mass Counts

Annual variation in reproductive effort of *Ambystoma* salamanders is well documented. Numbers of egg masses deposited in a pond in a given year reflect both the size of the adult population and the proportion of that population that bred. Breeding populations vary more than adult populations, and long term data show that much of the annual variation in breeding populations (reproductive effort) is highly correlated with rainfall during the breeding migration season (Semlitsch 1987, Pechmann et al. 1991). Yet, data on spotted salamander collected by Shoop (1974) over a five year period in eastern Massachusetts do not show this correlation.

The data collected to date at CACO are very short term. The degree of annual variability in both egg mass counts and migration season rainfall recorded so far is relatively small compared to that reported by others over longer time periods (Pechmann et al. 1991, Semlitsch 1987, Shoop 1974). Given the limited data, several years of additional data will be necessary before meaningful trend analysis can begin.

Spatial Variation in Spotted Salamander Egg Mass Counts

The influence of within-pond and adjacent landscape attributes on numbers of spotted salamander egg masses has been moderately well studied. In Pennsylvania, number of eggs present in ponds was positively correlated with pH and pond size, and negatively correlated with total cations and silica (Rowe and Dunson 1993). In Ontario, number of eggs in ponds was positively correlated with alkalinity (Clark 1986, cited in Petranka 1998). In Rhode Island, spotted salamander occurrence was associated with presence of woodland habitat (Egan 2001) and number of eggs in ponds was negatively correlated with road density. Beyond those landscape features, large numbers of egg masses were more likely to be deposited in larger ponds with greater canopy closure, extensive shrub cover and persistent non-woody vegetation, and relatively longer hydroperiod (Egan and Paton 2004). Similarly, in eastern Massachusetts, viable populations of spotted salamanders were associated with relatively large (>1000 m^2), deep (>1 m), fishless, permanent or semi-permanent ponds with relatively open canopies in a well drained, topographically varied, unfragmented forested landscape (Windmiller 1996).

The ponds monitored at CACO are fewer than the numbers sampled in the above works and were chosen for monitoring based, in part, on their known use by spotted salamander. In addition, they are inside the park, in a relatively uniform forested landscape. Thus, the ponds monitored here at CACO probably represent a much narrower range of conditions

than would be found in a random sample of vernal ponds from a larger geographic area. Consequently, the parameters that differentiate between ponds in a broad scale analysis may not be informative at the park scale. For example, whereas Windmiller (1996) and Egan (2001) found that landscapes with low road density and woodland habitat were correlated with larger populations of spotted salamanders, all of the ponds monitored at CACO meet this description. The lack of any significant relationship between egg mass counts and adjacent habitat features is due to the fact that all ponds were essentially in woodlands, with from 90 to 100% of their adjacent area occupied by woodland (Appendix 4).

The analysis of the relationship of egg mass counts to within-pond features only found a significant relationship to percent SAV. Since the presence of SAV in vernal ponds is indicative of ponds with longer hydroperiods, it would appear that percent SAV is a correlate of hydro-period. As such, the strong positive relationship between egg mass counts and SAV (hydroperiod) is consistent with findings from Rhode Island (Egan and Paton 2004), eastern Massachusetts (Windmiller 1996), and coastal Maine (Baldwin and Vasconcelos 2003). Also, given the positive relationship between hydroperiod and reproductive success in other *Ambystoma* species at a single pond over time (Semlitsch 1987, Pechmann et al. 1991), and the well established philopatry of spotted salamanders, it seems logical that among a group of vernal ponds, those with longer hydroperiods would tend to support larger populations. The lack of any other significant variables being detected may again be a case of the study ponds being comparatively more similar than dissimilar. For example, Egan and Paton (2004) found that ponds with a mix of shrubby and emergent vegetation tended to support larger populations. At CACO, most of the ponds monitored contain such a mix. Thus, among the woodland vernal ponds being monitored here at CACO, it appears that percent SAV, as a correlate of hydroperiod, is the only significant predictor of egg mass numbers.

Based on landscape analyses of spotted salamander abundance in Rhode Island and eastern Masachusetts (Windmiller 1996, Egan 2001, Egan and Paton 2004), the ideal landscape for spotted salamanders is a non-urbanized, non-fragmented, roadless, forested landscape with well drained soils and moderately hilly topography, occupied by long hydroperiod vernal ponds. This describes much of the CACO landscape, particularly that associated with the sample ponds in Eastham. This complex of many ponds, each supporting large numbers of spotted salamanders, appears exceptional. Whereas Windmiller (1996) found only 12 of 94 ponds occupied by spotted salamanders in the largely urbanized landscape in eastern Massachusetts had “viable populations” (egg mass counts >104 egg masses), nine of the 12 ponds sampled in Eastham in 2003 did.

While CACO currently has what appears to be an ideal landscape for supporting viable populations of spotted salamanders, urbanization, road construction, increased traffic volume, and habitat fragmentation all have the potential to reduce spotted salamander abundance. These stressors will likely have their greatest impacts outside of CACO, suggesting that CACO will become increasingly more important regionally for maintaining viable populations. However, considering that the negative effects of forest

habitat alteration and road impacts can extend up to 300 meters (Windmiller 1996), there is also potential for these impacts to extend into the park.

ANURAN CALL COUNTS

Methods

Anuran call counts were conducted at a total of 30 sites (figs. 4-6). The sites had been selected through a stratified random process designed to sample across the range of freshwater wetlands present at CACO, as well as along the length of the park's long axis from Eastham to Provincetown (Paton et al. 2003). Each site was sampled once/weekly, for 14 consecutive weeks, beginning on 4/15/03 and extending until 7/17/03. The thirty sites were divided into three groups of 10 (survey routes 1, 2, and 3). Within a given week, one survey route was sampled each night, such that a complete sampling of all 30 ponds occurred over the course of three nights, nearly always Tuesday, Wednesday, and Thursday.

Nightly sampling occurred from 30 minutes after sunset until ca. midnight – 0100 hours, and consisted of listening for and identifying anuran vocalizations. Vocalizations were scored according to an index value that ranged from 0 to 3 (Mossman et al. 1998). In addition, data on air and water temperature, sky, wind, and precipitation conditions were recorded. See Paton et al. (2003) for further details of sampling procedure. Water samples from the 30 call count ponds were collected and analyzed in conjunction with those collected from ponds where egg mass counts were conducted.

Call count data provided a measure of distribution, based on number of sites recorded, and a measure of abundance, based on the calling index. For each species, the maximum index value recorded at each site over the course of sampling was determined. As a measure of a species' abundance at sites where it was present, the mean of these maxima was calculated (based only on sites where the species was present).

For each species recorded over the course of the season, program PRESENCE (MacKenzie et al. 2002) was used to estimate probability of detection (probability of detecting a species at a site on a given sampling occasion, given it is actually present at the site) and determine the role of sampling covariates (air and water temperature) in detectability. The data set was reduced by only including data from the first to last week (inclusive) when a given species was recorded. PRESENCE was also used to estimate site occupancy rates (proportion of sites that species is estimated to occur at) for each species detected, and the relationship of each species occurrence to site covariates. One group of site covariates was based on pond hydro-period (temporary, semi-permanent, or permanent) and a second group of site covariates related to water chemistry (pH, conductivity, and color (AbsCo440) (table 10). Temporary ponds were defined as ponds that dry out every, or nearly every year. Conversely, semi-permanent ponds were defined as ponds that retain water in most years, but dry out infrequently. Permanent ponds retain standing water even during droughts.

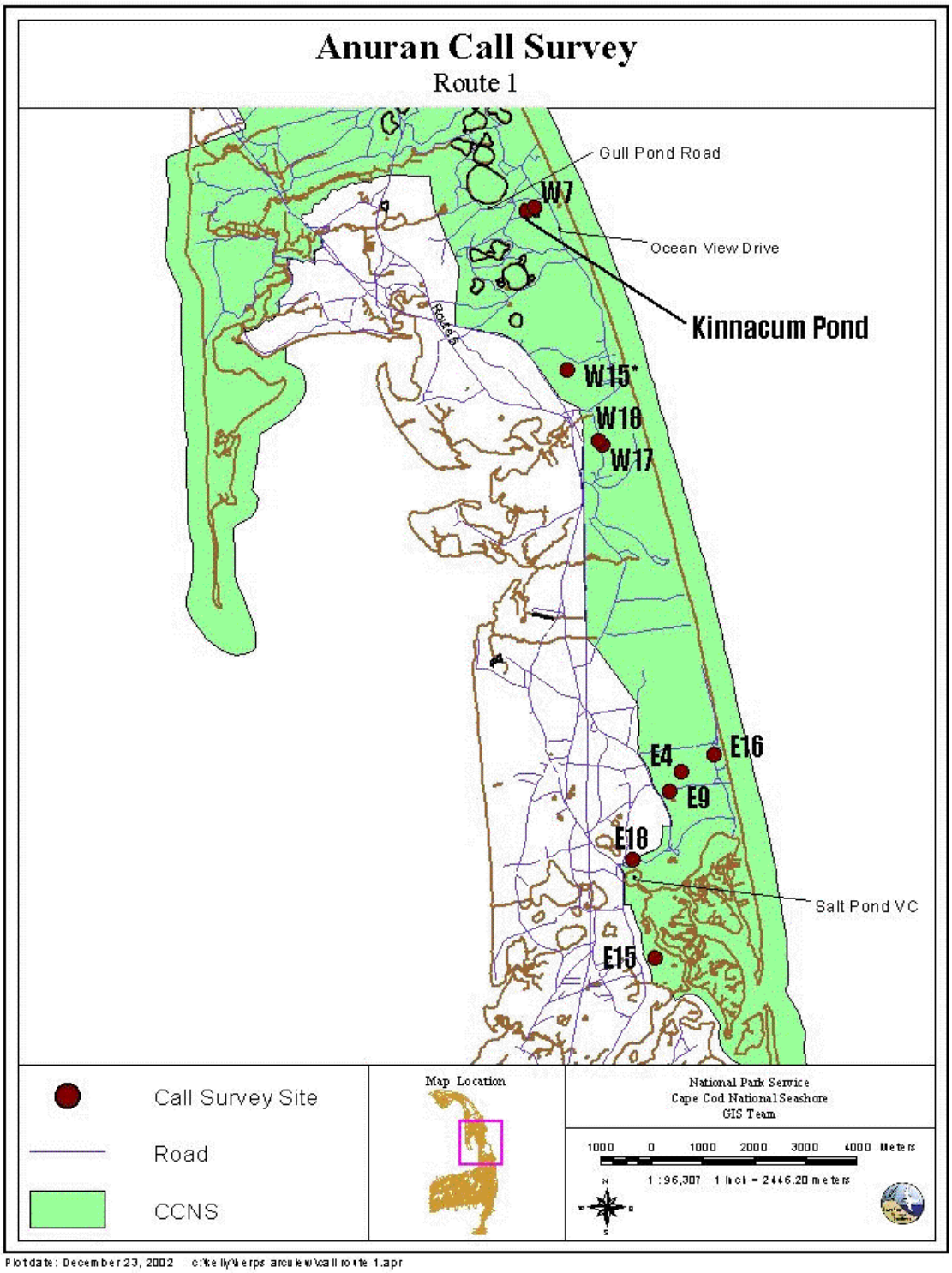

Figure 4. Anuran call survey Route 1.

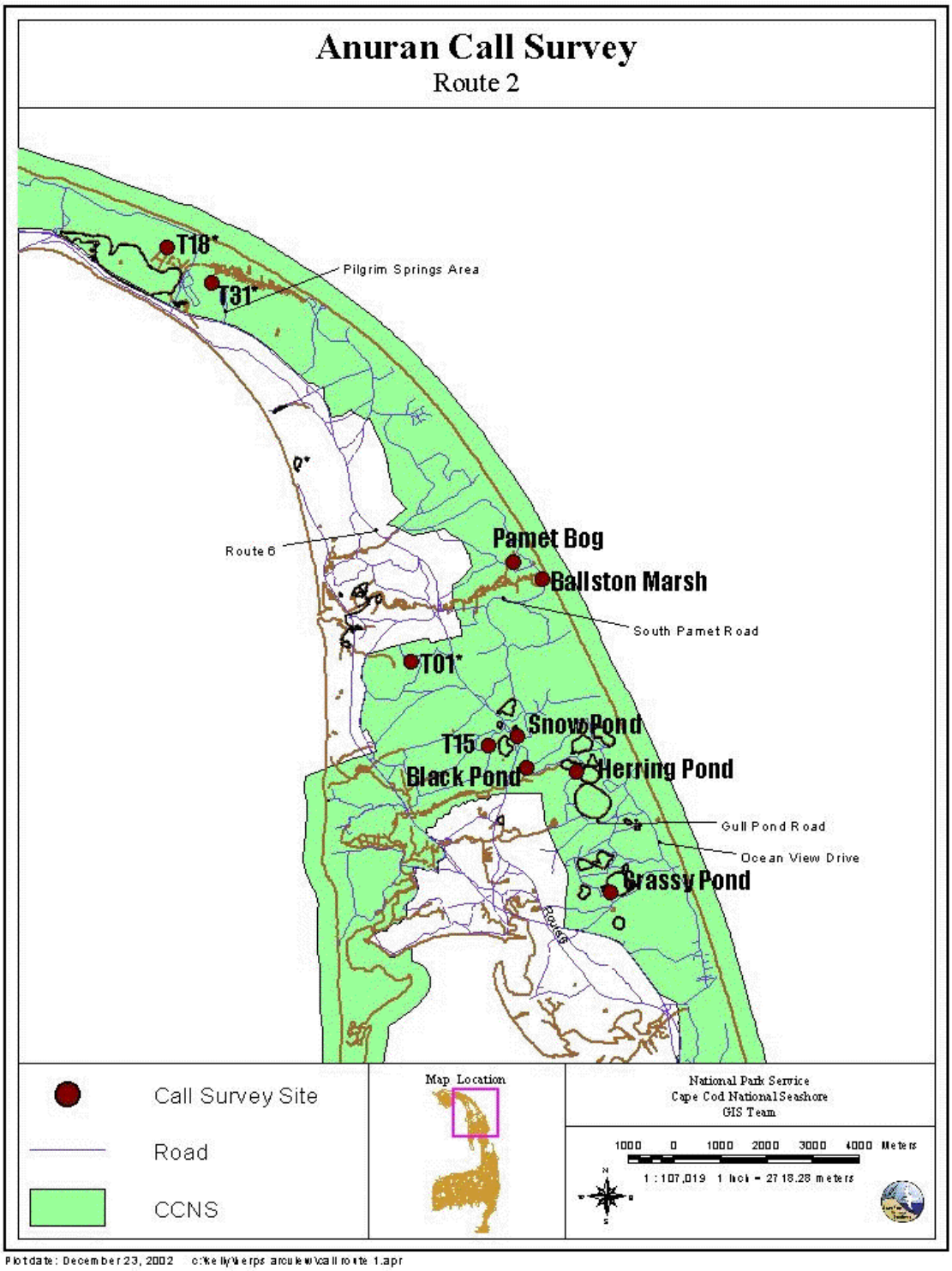

Figure 5. Anuran call survey Route 2.

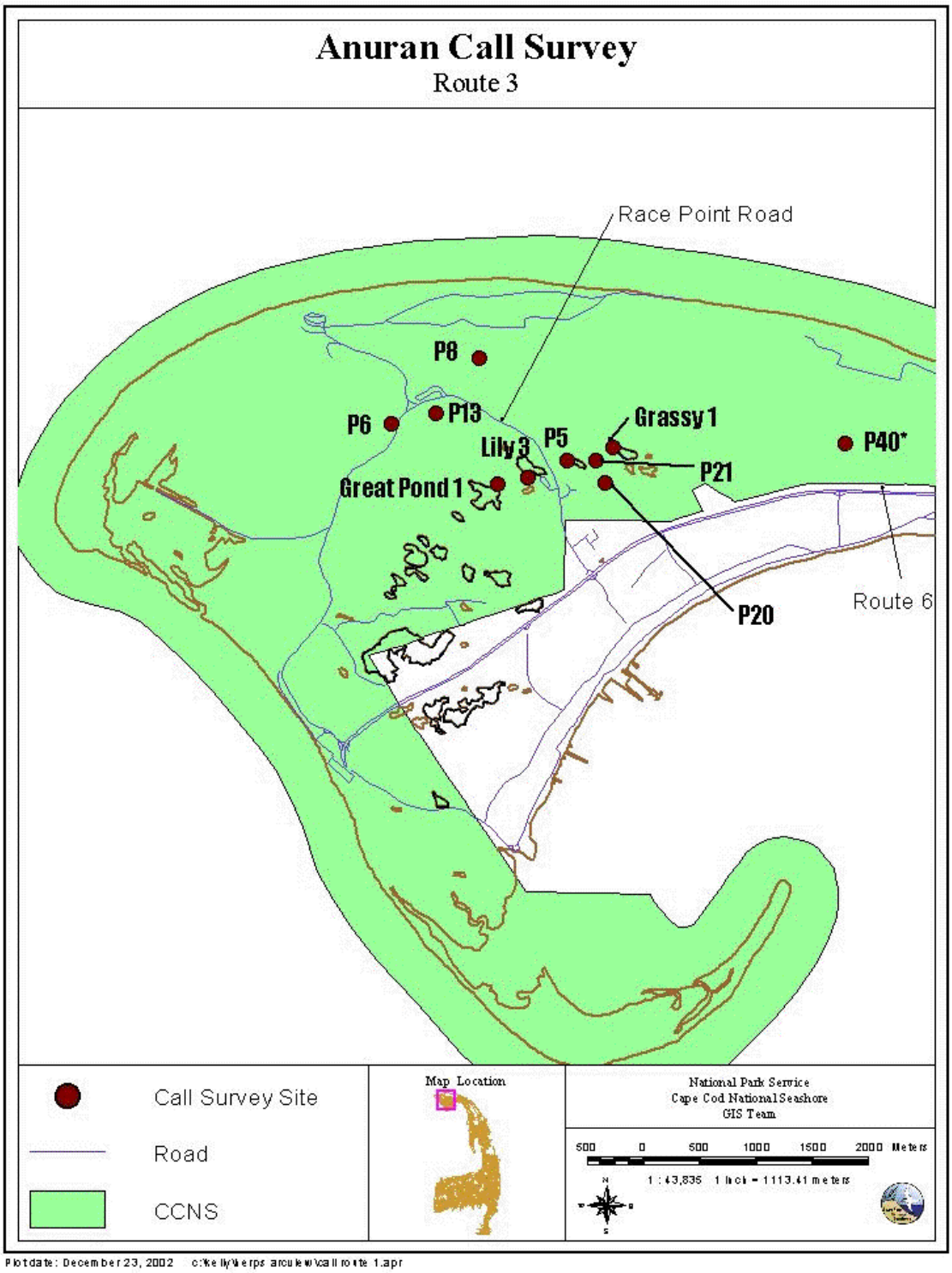

Figure 6. Anuran Call Survey Route 3.

The process of constructing and selecting models to explain detectability and occurrence with PRESENCE involved first determining the best model for detectability. Pre-defined models for constant ($p(.)$) and time dependent ($p(t)$) probability of detection were run, and compared to custom models of detectability based on air and water temperatures recorded during sampling events. PRESENCE calculated the Akaike Information Criterion (AIC) for each model and, based on differences in AIC and a model weighting procedure detailed in Cooch and White (2001), the best model for explaining detectability was selected. Additional models testing the role of hydro-period and water chemistry covariates in explaining occurrence (ψ) were built upon the best detectability model. AIC weighting was used to determine the most informative hydroperiod and water chemistry covariates and a final model, containing both of these two covariates (and the detectability covariate) was constructed. These four models, plus a null model (constant occurrence, constant detectability) were compared based on AIC weighting, and the best overall model determined.

Results

A total of eight species were recorded. Spring peeper (*Pseudacris crucifer,* PSCR) was the most widespread. It was detected at 27 sites and, at those sites, had a mean maximum index value of 2.56. Wood frog (*Rana sylvatica*, RASY) and grey treefrog (*Hyla versicolor,* HYVE) were least widespread, detected at two and three sites respectively, as well as tied for least abundant, with a mean maximum index value of 1.0. In contrast, eastern spadefoot toad (*Scaphiopus holbrooki,* SCHO) was also limited in distribution (only recorded at three sites), but abundant where detected (mean maximum index value of 3.0) (table 9).

In terms of seasonal chronology, spring peeper, wood frog, and pickerel frog (*Rana palustris,* RAPA) began calling earliest, at week one (4/15/03). As the season progressed, Fowler's toad (*Bufo fowleri,* BUFO), green frog (*Rana clamitans,* RACL), and spadefoot toad were first recorded in week three (4/29/03), bullfrogs (*Rana catesbiana,* RACA*)* in week eight (6/3/03), and grey treefrog in week nine (6/10/03). Breeding season duration (number of weeks from first to last records, inclusive) was shortest for wood frog (three weeks) and longest for Fowler's toad and green frog (12 weeks) (fig. 7).

Best models for explaining detection and occurrence varied by species. For spadefoot toad and wood frog, detectability was positively associated with air temperature, whereas for grey treefrog, pickerel frog, and green frog it was positively associated with water temperature. Detectability of Fowler's toad, spring peeper, and green frog varied by sampling occasion, but was not related to either temperature parameter (table 10, 11).

Table 9. Anuran call count maximum index values and site covariates. Mean maximum index represents the mean of maximum index values for a species, based only on sites where species was recorded.

Route	Site	RASY	RACL	RACA	PSCR	RAPA	SCHO	BUFO	HYVE	#Species	Wetland Type	HydroPer	pH	Cond	AbsCo440
1	E15	1	0	0	0	0	0	0	0	1	Swamp-red maple	Temp	4.07	140.2	0.0046
1	E18	0	1	0	3	0	0	1	0	3	Vernal Pool	Temp	4.84	49.9	0.0012
1	E9	0	1	0	3	0	0	0	1	3	Vernal Pool	Temp	4.55	80.1	0.0011
1	E4	0	1	0	3	0	0	0	0	2	Vernal Pool	Temp	4.83	41.2	0.0012
1	E16	1	1	0	3	0	0	0	0	3	Vernal Pool	Temp	4.57	242.1	0.0037
1	W18	0	1	0	2	0	0	0	0	2	Vernal Pool	Temp	4.58	90.2	0.0044
1	W17	0	0	0	1	0	0	0	0	1	Swamp-white cedar	Temp	4.08	116.5	0.0024
1	W15*	0	0	0	3	0	0	0	0	1	Vernal Pool	Temp	4.55	57.3	0.0018
1	Kinnacum	0	1	1	2	0	0	2	0	4	Kettle Pond	Perm	4.78	69.9	0.00005
1	W7	0	1	0	3	0	0	0	0	2	Vernal Pool	Temp	5.05	55.4	0.0029
2	Grassy Pond	0	1	0	3	0	0	0	0	2	Kettle-shallow	Semi	4.5	78.1	0.0017
2	Herring Pond	0	1	1	2	3	0	2	0	5	Kettle Pond	Perm	6.63	133.3	0.0001
2	Black Pond	0	2	0	0	1	0	0	0	2	Riparian Marsh	Perm	6.18	125.6	0.00005
2	Snow Pond	0	1	1	3	2	0	1	0	5	Kettle Pond	Perm	5.41	82.4	0.0001
2	T15	0	0	0	1	0	0	0	0	1	Vernal Pool	Temp	4.65	52.9	0.0028
2	T01*	0	3	0	3	2	0	0	0	3	Vernal Pool	Semi	4.68	30.2	0.0008
2	Ballston	0	1	0	1	0	0	2	0	3	Riparian Marsh	Perm	6.9	3620	0.0001
2	Pamet Bog	0	1	3	2	1	0	0	0	4	Bog	Perm	6.15	506.1	0.0009
2	T31*	0	2	0	3	0	0	0	0	2	Vernal Pool	Temp	4.62	69.4	0.0018
2	T18*	0	0	0	3	0	0	2	0	2	Dune Slack	Temp	5.73	60.3	0.0014
3	P40*	0	0	0	1	0	0	1	0	2	Dune Slack	Temp	5.3	59.6	0.0004
3	P20*	0	2	0	3	0	0	1	0	3	Interdune pond	Perm	4.66	82.1	0.0015
3	P21*	0	0	0	0	0	0	0	0	0	Vernal Pool	Temp	4.25	81.5	0.0032
3	Grassy 1*	0	2	0	3	0	0	2	0	3	Interdune pond	Perm	4.79	87	0.0016
3	P5	0	1	0	3	0	0	3	1	4	Dune Slack	Semi	4.57	93.7	0.0027
3	Lily Pond 3	0	2	1	3	0	0	1	0	4	Interdune pond	Semi	5	67.4	0.0017
3	Great Pond 1	0	2	1	3	0	0	2	1	5	Interdune pond	Perm	6.02	64.4	0.0013
3	P13	0	2	0	3	0	3	3	0	4	Dune Slack	Temp	4.82	81.2	0.0008
3	P8	0	0	0	3	0	3	2	0	3	Dune Slack	Temp	5.19	91.9	0.0009
3	P6	0	0	0	3	0	3	3	0	3	Dune Slack	Temp	5.45	108.3	0.0006
Mean Maximum Index		**1.00**	**1.43**	**1.33**	**2.56**	**1.80**	**3.00**	**1.87**	**1.00**						
Total # Ponds		**2**	**21**	**6**	**27**	**5**	**3**	**15**	**3**						

Table 10. Results of analysis of anuran call count data by program PRESENCE. Best model explaining detectability (*p*) and occurrence (ψ), naive occupancy rate (frequency of occurrence), estimated site occupancy rate (ψ), and average probability of detection (*p*) for each species. Average probability of detection was obtained from the constant probability of detection model (p(.)). *PRESENCE could not estimate ψ.

Species	Best Model	naïve	ψ	*p*
SCHO	ψ (perm, AbsCo), p(air)	0.100	*	0.258
BUFO	ψ (AbsCo), p(t)	0.500	0.502	0.319
PSCR	ψ (AbsCo), p(t)	0.900	0.900	0.650
HYVE	ψ (AbsCo), p(water)	0.100	0.333	0.140
RASY	ψ (cond), p(air)	0.067	*	0.022
RAPA	ψ (perm, semi, AbsCo), p(water)	0.170	0.170	0.420
RACL	ψ (perm, semi), p(t)	0.700	0.700	0.560
RACA	ψ (perm, semi), p(water)	0.267	0.272	0.439

The most important parameters influencing species occurrence were hydroperiod and water color (AbsCo440). Their influence varied by species (table 10, 11). For spadefoot toads, the negative association with permanent ponds and water color indicates a positive association with temporary and semi-permanent ponds with clear, as opposed to stained water. Fowler's toad was not associated with any particular hydro-period, but the negative coefficient for color indicates an association with clear water sites. This same is true for spring peeper, though the negative association with color is not as strong. Grey treefrog was not associated with any particular hydro-period, and the positive coefficient for color indicates an association with darker water sites. Wood frogs showed a slight positive association with conductivity. For pickerel frogs, the positive coefficient for permanent and semi-permanent hydro-period and negative coefficient with color indicates an association with clear, permanent water bodies. Both green frog and bullfrog, with positive coefficients for permanent and semi-permanent hydro-period, were associated with relatively permanent water bodies.

Site occupancy rates estimated by PRESENCE ranged from 0.17 for pickerel frog to 0.900 for spring peeper, and generally were very similar if not identical to a species' naïve rate (percentage of sites a species is recorded from). For the two species with the lowest naïve rate (spadefoot toad and wood frog), PRESENCE was unable to estimate site occupancy rate (table 10). Spring peepers were the most detectable (probability of detection=0.65) and wood frogs the least detectable (p=0.022) (table 10).

Table 11. Coefficients for parameters included in “best” model for each species by Program PRESENCE.

Species	Parameter	Coefficient
SCHO	air temperature	1.393
	perm	-28.728
	AbsCo	-953.340
BUFO	AbsCo	-1168.224
PSCR	AbsCo	-650.01
HYVE	water temperature	1.452
	AbsCo	1898.929
RASY	air temperature	0.489
	cond	2.419
RAPA	water temperature	0.316
	perm	53.349
	semi	52.424
	AbsCo	-2671.669
RACL	perm	26.442
	semi	28.337
RACA	water temperature	.367
	perm	39.083
	semi	36.656

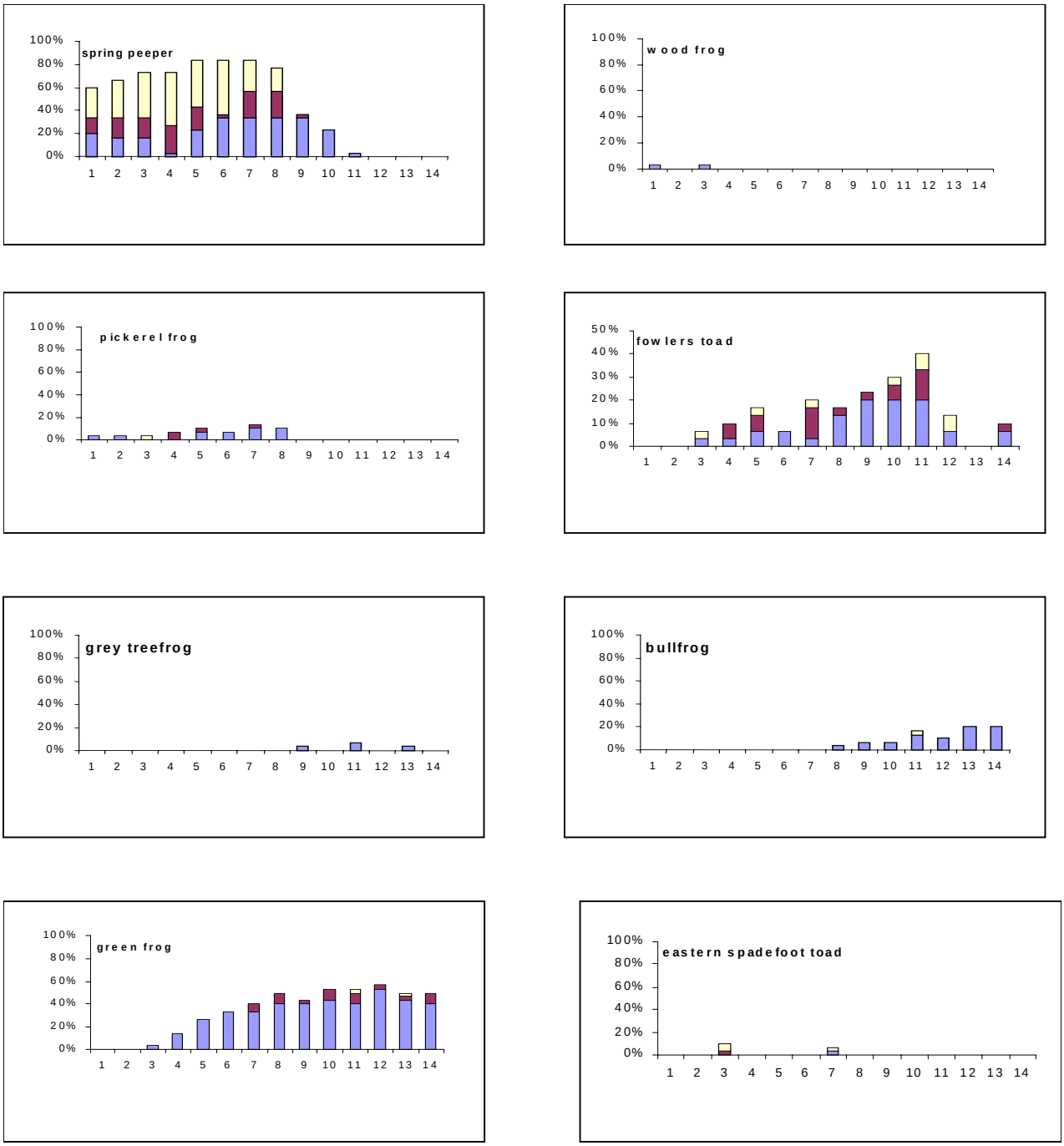

Figure 7. Seasonal variation in calling index values over course of sampling for each encountered species. White bars = index value 3, dark gray = index value 2, light gray = index value 1.

Discussion

While it is not possible to make any year to year comparisons based on this first year of anuran call count data, they do provide some insight into the occurrence, distribution, abundance, and habitat affinity of the anuran species here. In general, they confirm our previously held impressions, and conform to known habitat affinities for these species (Lazell and Michener 1976, Klemens 1993).

All of the eight species recorded in 2003 had been previously recorded at CACO, though the grey treefrog had only been recorded once previously, in Eastham in 2001. Thus, the observations recorded in 2003 increase the number of sites from which this species has been recorded, and extend its known range up to Provincetown. However, the lack of observations from Wellfleet and Truro, and the low site occupancy rate, given the abundance of suitable habitat is puzzling. Breeding wood frogs continue to be restricted to vernal ponds in Eastham, in spite of the presence of seemingly suitable breeding ponds and incidental records of adults from Wellfleet. Spadefoot toads, though known to occur throughout the park, were only recorded in Provincetown, where they are known to be abundant. Pickerel frogs were only recorded at a small number of sites from northern Wellfleet and southern Truro and seemingly correspond to the distribution of suitable habitat, permanent, clearwater ponds. Similarly, the remaining species are fairly widespread and have a distribution that essentially reflects the distribution of their preferred habitats.

Site occupancy rates of CACO anurans show both similarities and differences compared to other areas sampled with anuran call counts. Spring peeper was most widespread at CACO (90% of CACO sites), as well as in Southern Rhode Island (68%)(Crouch and Paton 2002) and Prince Edward Island, Canada (90%)(Stevens et al. 2002). However, while both Couch and Paton (2002) and Stevens et al (2002) found wood frog to be the second most widespread species (65% and 83% occupancy rates, respectively), wood frogs were the most restricted of the eight species recorded here at CACO, with a 6.7% naïve occupancy rate. This difference is likely due to two factors. While woodland vernal pond habitat is widespread at CACO, wood frogs have only been recorded from vernal ponds in Eastham. Moreover, since wood frogs typically breed in small vernal ponds, some of this disparity reflects the fact that ponds sampled in those studies tended to be smaller than those sampled at CACO, and thus more likely to be used by wood frog. The second most widespread species at CACO, green frog, a species of permanent water bodies, had an occupancy rate here of 70%, but only 32% in Rhode Island. This difference too, is likely due to differences between studies in the size and permanence of sample sites. For the remaining species, occupancy rates were fairly similar to those reported by Crouch and Paton (2002). In addition, patterns of seasonal chronology and breeding season duration were also similar to those reported from southern Rhode Island (Crouch and Paton 2002), though the season on Cape Cod generally is a few weeks later in the year.

Overall, the results of this first year of anuran call counts indicate that this protocol is relatively easy and problem-free in its implementation, and that the results obtained

confirm and conform to generally accepted patterns of species-habitat association, calling season chronology, and species occurrence in the park. However, it has also provided data demonstrating the geographic range of some species is much more extensive than previously thought. Over the long term, these data should be very useful in monitoring the distribution, abundance, and composition of the park's anuran community.

RECOMMENDATIONS AND FUTURE PLANS

Monitoring will continue into the 2004 field season and annually thereafter. While we have attempted some trends analysis with the limited data currently available, a more in-depth analysis should be conducted after five years. In addition to trends, analysis should look at annual variability, power, and sampling frequency and determine if protocol modifications are called for.

Beginning in 2004, for the vernal pond egg mass count protocol, we recommend that the total count or locus method be discontinued and the maximum count method be used. As detailed above, the latter provides essentially the same data for considerably less effort. For both vernal pond egg mass counts and anuran call counts, we recommend that further research and consideration be given to identifying, defining, measuring, and analyzing pond and landscape parameters and their relationship to the distribution and abundance of target species.

LITERATURE CITED

Baldwin, R.F. and D. Vasconcelos. 2003. *Ambystoma maculatum* (Spotted Salamander) and *Rana sylvatica* (Wood Frog). Habitat. Herp. Review 34(4):353-354.

Boland, K. and R.P. Cook. 2004. Amphibian water quality monitoring protocol. Unpublished protocol, Cape Cod National Seashore, Wellfleet, MA.

Clark, K.L. 1986. Responses of *Amybstoma maculatum* populations in central Ontario to habitat acidity. The Canadian Field Naturalist. 100:463-469.

Clarke, K.R. and R.N. Gorley 2001 PRIMER v5: User Manual/Tutorial. PRIMER-E: Plymouth Marine Laboratory, UK.

Colburn, E.A., J. Milam, and T. Tyning. 2000. Inventory and monitoring of amphibians of the Cape Cod National Seashore. Unpubl. Report to Cape Cod National seashore, Wellfleet, MA.

Crouch, W. B., and P.W.C. Paton. 2002. Assessing the use of call surveys to monitor breeding anurans in Rhode Island. J. Herpetology, 36(2):185-2002.

Egan, R.S. 2001. Within-pond and landscape –level factors influencing the breeding effort of Rana sylvatica and Ambystoma maculatum. M. S. Thesis. University of Rhode Island, Kingston, RI, USA.

Egan, R.S. and P.W. Paton. 2004. Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. Wetlands 24: 1-13.

Jung, R E. 2002. Wood frog and spotted salamander egg mass counts and percent vernal pools occupied by amphibian species on DOI lands in the northeastern United States. US Geological Survey Amphibian Research and Monitoring Initiative (ARMI) in the Northeast Region, USGS Patuxent Wildlife Research Center, Laurel, Maryland.

Klemens, M.W. 1993. Amphibians and Reptiles of Connecticut and Adjacent Regions. New York: State Geological and Natural History Survey of Connecticut. Bulletin No. 112. ISBN No. 0-942081-04-8.

Lazell, J.D. and M.C. Michener. 1976. This Broken Archipelago. New York: Demeter Press. 260 pp.

MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S.Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83(8): 2248-2255.

Mossman, M.J., L.M. Hartman, R.Hay, J.R. Sauer, and B.J. Dhuey. 1998. Monitoring long-term trends in Wisconsin frog and toad populations. p. 169-205 *In* M.J. Lannoo, Status and Conservation of Midwestern Amphibians. University of Iowa Press, Iowa City, USA.

Paton P.W., B. Timm and T. Tupper. 2003. Monitoring pond breeding amphibians. A protocol for the long-term ecosystem monitoring program at Cape Cod National Seashore. 113 pp.

Pechmann, J.H., D.E. Scott, R.D. Semlitsch, J.P. Caldwell, L.J. Vitt, J.W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. Science 253:892-895.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Instititution Press, Washington, DC. 576 pp.

Portnoy, J.W. 1990. Breeding biology of the spotted salamander *Ambystoma maculatum* (Shaw) in acidic temporary ponds at Cape Cod, USA. Biological Conservation 53:61-75.

Roman, C.T. and N.E. Barrett. 1999. Conceptual framework for the development of long-term monitoring protocols at Cape Cod National Seashore. USGS Patuxent Wildlife Research Center, Cooperative National Park Studies Unit, Univ. of Rhode Island, Narragansett, RI 02882.

Rowe, C.L. and W.A. Dunson. 1993. Relationships among biotic parameters and breeding effort by three amphibians in temporary wetlands of central Pennsylvania. Wetlands 13:237-246.

Semlitsch, R.D. 1987. Relationship of pond drying to the reproductive success of the salamander *Ambystoma talpoideum*. Copeia 1:61-69.

Shoop, R.C. 1974. Yearly variation in larval survival of *Ambystoma maculatum.* Ecology 55:440-444.

Statsoft. 2000. STATISTICA for Windows. Vols.1-3. Statsoft Inc. Tulsa, OK.

Stevens, C.E., A.W. Diamond and T.S. Gabor. 2002. Anuran call surveys on small wetlands in Prince Edward Island, Canada restored by dredging of sediments. Wetlands 22(1): 90-99.

Windmiller, B. 1996. The pond, the forest, and the city: spotted salamander ecology and conservation in a human-dominated landscape. Ph.D. Dissertation, Tufts University, Boston, Massachusetts.

Zar, J.H. 1996. Biostatistical Analysis. (3rd. Ed.) Upper Saddle River, NJ: Prentice Hall.

Appendix 1. Comparison of egg mass counting methods

Introduction

In 2003, we compared two methods for counting egg masses in breeding ponds. The maximum count method involves carefully counting all the egg masses that can be found in a breeding pond on a series of dates in early spring, and selecting the largest count as the measure of egg mass abundance for that pond that year. A second approach, the total count or locus method involves counting the numbers of egg masses in groups of egg masses or loci in a pond, and identifying and marking each locus with flagging (Paton et al. 2003). Each locus and the number of masses it contains is recorded. On a subsequent visit, each locus is recounted and number of masses present recorded. Differences in the number of egg masses present in a locus from one count to the next may be due to the loss of egg masses to predation or dislodging, the laying of additional egg masses, or a mass that was present on both counts only being counted on one. New (unmarked) loci encountered are also marked, counted, and recorded. These new loci may also represent egg masses deposited after the previous visit, or egg masses that were present but overlooked. After the repeat visits, the maximum count for each individual locus is selected and all loci in a pond summed, providing a total count of the eggs masses deposited in that pond.

The advantage of the total count method is that, by tracking the number of egg masses in a locus over sampling visits, egg masses once counted but then lost to predation or dislodged during counting are still counted, as are newly deposited egg masses. This provides a count of the total number of egg masses deposited. In contrast, maximum counts do not allow for tracking the disappearance of older or appearance of newer egg masses, and result in a measure of abundance that could be less than the total number of egg masses laid.

Methods

In spring 2003 we conducted egg mass counts using the total count method at 18 ponds in Cape Cod National Seashore. The first count did not employ the total count method, and was conducted between 30 March 2003 and 7 April 2003. Counts two and three used the total count method, and occurred between 14 April 2003 and 21 April 2003, and 28 April 2003 and 5 May 2003 respectively. Since data collected on a given day as part of the total count method can also be treated as a daily count, we used these data to compare the results obtained by the two different methods. For each pond, the highest of the three daily counts (which essentially was either count 2 or 3) was the maximum count, whereas total count was obtained by taking the highest of two counts (counts 2 and 3) of each locus and summing for all the loci in a pond.

Results

The results obtained by these two different methods are very similar, with the maximum count method underestimating from 0% to 12% relative to the total count (appendix table

1). The results obtained by the two methods were highly significantly correlated ($r=0.9996$, $p<0.000000$). Thus, the maximum count method provided results similar to those provided by the total count method.

Table 1. Comparison of maximum count (MC) versus total count (locus method) values for spotted salamander egg masses in 2003.

Pond	2003 MC	2003 Total Count	Difference	%Deviation
E02	50	51	1	2%
E03	38	41	3	7%
E04	633	639	6	1%
E05a	315	321	6	2%
E05main	767	790	23	3%
E06	575	601	26	4%
E07	269	273	4	1%
E08	250	263	13	5%
E11	254	261	7	3%
E11east	24	26	2	8%
E21	261	264	3	1%
T01	544	549	5	1%
T15	22	25	3	12%
W01	489	521	32	6%
W06	27	29	2	7%
W07	338	338	0	0%
W15	64	66	2	3%
W18	44	46	2	4%

Discussion

Implementation of the total count method was problematic. Determining what constituted a “locus” was often difficult. While many egg masses form discrete loci, many egg masses are also laid singly or in small groups, often spread over a large area rather than concentrated in a small one. When single egg masses were near a larger group of masses, we had to decide whether to consider it a separate locus or group it with the larger locus. Moreover, since egg mass deposition takes place over the course of a few weeks, seemingly distinct loci early in the season become less so as the space in between them fills in with additional egg masses. To deal with this, when a locus was identified, different colored flags were placed around the entire locus and labeled with the same locus identifier. Using this method, we could be sure we were counting the same locus as in the prior sample and could determine whether egg masses were added or lost. Thus each locus had to be labeled and its boundary delineated with flagging, a very time consuming process. The mean field time for data collection using the maximum count method was 34 minutes/pond, whereas, it was 137 minutes/pond using the total count

(locus) method. In addition, the maximum count method took less staff time to enter and tabulate data.

These results indicate that egg mass monitoring based on the maximum count method provides essentially identical data regarding abundance as the total count (locus) method), but in a more time efficient and economical fashion. Therefore our preliminary recommendation (subject to peer review) is to use the maximum count method, though we caution that counts must be conducted in a careful, methodical fashion, dividing the pond into quadrants and walking parallel transects 2 meters wide. Therefore, in this report, egg mass data presented will be maximum count data.

Appendix 2. Maximum *Ambystoma maculatum* egg mass counts by year. Data from 1985 through 1999 are based on a single count in late April. From 2001 onward, data represent the maximum of multiple counts conducted from late March to early May.

Pond	1985	1986	1990	1991	1992	1993	1996	1998	1999	2001	2002	2003
E01		30	49	dry	dry	1	2		17	11		
E02											30	50
E03	0	1						28+	16	48	25	38
E04	11	11						11+	180+	503	1227	633
E05 (main pool)	41	23								174	596	767
E05A (small-south)											677	315
E06	18	56		221				57	181	168	599	575
E07				11				30+	34+	92	226	269
E08								10+	9		243	250
E8east								54+	92			
E09	5	12						7+	5	469		
E10									83	333		
E11	10	22						20		101	359	254
E11east								13+		0	29	24
E14	20	1										
E16				4								
E18											6	
E21											434	261
E22 (Turtle Pond)											910	486
P04										0		0
P05										0		
P09										0		
P10										0		
P15										0		
P16										0		
T01 (Holsberry Rd)	107	104	225	344	513	103	169					544

Name	1985	1986	1990	1991	1992	1993	1996	1998	1999	2001	2002	2003
T02	54	51	65									
T12	6	1										
T13	28	50										
T14	26	7	3	4	4	11	8			3		
T15(fairy shrimp)	70	52	75		91	58	82		36	42		22
T21	14	7										
T22	7	0										
TSP						194	45					
W01	101	213	412	130	255	367	325					489
W02	0	11		28								
W03											0	
W04	dry	7										
W06	dry	6									8	27
W07	29	30	324	7	505	deep	148			488		338
W08	dry	24										
W10	dry	4										
W12	dry	2										
W13	dry	5										
W14	dry	10										
W15	41	51	39	26	3	20	48				81	64
W16	0	2								2		
W17 (White Cedar Swamp)										0		
W18		45			34	83	47			79		44

Appendix 3. Water quality parameters for all amphibian monitoring sites.

Site ID	Collection Date	pH	Alkalinity ($mgCaCO_3/L$)	Conductivity (µS/cm)	Visual Color (nessler units)	AbsCo 440	Spec Color (slope)	Chloride (mL)	Tannin/Lignin (mg/L tannic acid)
E15	04/23/03	4.07	-5.8	140.2	500	.0046	-0.018	70	6.7
E18	04/23/03	4.84	-0.4	49.9	167	.0012	-0.015	23	3
E09	04/23/03	4.55	-1.8	80.1	111	.0011	-0.0159	33	3.4
E04	04/23/03	4.83	-0.7	41.2	175	.0012	-0.015	24	3.7
E16	04/23/03	4.57	-1.8	242.1	667	.0037	-0.0125	90	4.7
W18	04/24/03	4.58	-2.3	90.2	500	.0044	-0.0118	43	5.1
W17	04/24/03	4.08	-5.9	116.5	292	.0024	-0.0132	38	5.4
W15	04/24/03	4.55	-2.3	57.3	200	.0018	-0.014	33	3.4
Kinnacum	04/14/03	4.78	-0.65	69.9	10	.00005	-0.0224	38	0
W7	04/24/03	5.05	0.1	55.4	300	.0029	-0.0131	33	4.4
Grassy_Well	04/24/03	4.5	-2.4	78.1	208	.0017	-0.0142	28	3.5
Herring	04/14/03	6.63	3.65	133.3	5	.0001	-0.0211	50	0.7
Black Pond	04/24/03	6.18	4	125.6	15	.00005	-0.0218	44	1
Snow	04/10/03	5.41	0.25	82.4	250	.0001	-0.0214	50	0.9
T15	04/24/03	4.65	-3.3	52.9	333	.0028	-0.0131	25	4.7
T01	04/22/03	4.68	-0.65	30.2	100	.0008	-0.0161	18	2.1
Ballston Marsh	04/22/03	6.9	41.85	3620	10	.0001	-0.0208	14990	No data
Pamet Bog	04/22/03	6.15	0.9	506.1	88	.0009	-0.016	163	1.7
T31	04/24/03	4.62	-1.6	69.4	167	.0018	-0.0141	31	4.2
T18	04/24/03	5.73	1.3	60.3	156	.0014	-0.0148	29	2.6
P40	04/25/03	5.3	0.2	59.6	50	.0004	-0.0177	27	1.4
P20	04/25/03	4.66	-1.2	82.1	200	.0015	-0.0146	31	2.9
P21	04/25/03	4.25	-3.6	81.5	417	.0032	-0.0128	29	5

Site ID	Collection Date	pH	Alkalinity	Conductivity	Visual Color	AbsCo 440	Spec Color	Chloride	Tannin/Lignin
Grassy 1_prov	04/25/03	4.79	-0.8	87	214	.0016	-0.0146	34	3.1
P05	04/25/03	4.57	-1.7	93.7	333	.0027	-0.0133	35	4
Lily 3	04/25/03	5	-0.1	67.4	278	.0017	-0.0144	27	2.6
Great 1	04/25/03	6.02	2.3	64.4	156	.0013	-0.015	31	2.6
P13	04/25/03	4.82	-0.6	81.2	100	.0008	-0.0164	31	2.3
P08	04/25/03	5.19	0.3	91.9	120	.0009	-0.0159	37	2.1
P06	04/25/03	5.45	1.1	108.3	75	.0006	-0.0169	45	1.6
E02	04/23/03	4.63	-1.4	38.6	125	.0012	-0.0148	25	3.7
E03	04/23/03	4.72	-1.1	47.5	250	.0017	-0.0141	27	4.2
E05a	04/23/03	5.78	4.2	44.2	250	.0017	-0.0141	24	4.9
E05main	04/23/03	4.77	-0.7	49.2	179	.0019	-0.0139	34	5
E06	04/23/03	4.74	-0.9	50.5	179	.0019	-0.0139	28	4.6
E07	04/23/03	4.8	-0.8	37.4	139	.0018	-0.0143	26	5.1
E08	04/23/03	4.51	-2.2	36.7	125	.0011	-0.0153	26	2.9
E11	04/23/03	4.54	-1.6	43	125	.0012	-0.0151	26	3.9
E11east	04/23/03	4.76	-1.0	68.6	300	.0025	-0.0132	42	5.3
E21	04/23/03	5.27	0.8	54	100	.0009	-0.0158	28	2.3
E22	04/23/03	3.9	-7.3	57.8	167	.0015	-0.0145	31	3.5
P04	04/25/03	4.26	-4.2	85.3	500	.0043	-0.012	44	7.1
W01	04/24/03	5.2	0.7	45.3	200	.0016	-0.0145	32	4.6
W06	04/24/03	4.35	-3.8	75.4	375	.0031	-0.0127	33	6.2

Appendix 4. Maximum *Ambystoma maculatum* egg mass counts (MC), within-pond variables (columns 2-6, 9-15) and adjacent habitat variables (columns 7-8, 16-20). Columns 9-20 are % cover.

Site	MC	depth	area	pH	condct	absco	adjpool	rddist	leaflit	sticks	sav	moss	emerg	shrub	tree	woods	road	field	wetlnd	rsdnt
E02	50	59	3193	4.63	38.6	0.0012	6	530	75	0	0	75	12	80	0	100	0	0	0	0
E03	38	60.5	1134	4.72	47.5	0.0017	6	565	25	0	0	40	75	0	0	95	0	5	0	0
E04	633	71	1802	4.83	41.2	0.0012	10	454	30	5	10	50	50	0	0	100	0	0	0	0
E05a	315	78	598	5.78	44.2	0.0017	4	414	25	0	10	0	50	0	0	95	0	0	5	0
E05main	767	80	2130	4.77	49.2	0.0019	6	355	30	5	20	5	65	0	0	95	0	0	5	0
E06	575	87	1792	4.74	50.5	0.0019	3	185	30	0	10	5	60	0	0	95	0	5	0	0
E07	269	50	1056	4.8	37.4	0.0018	5	258	90	5	5	5	75	5	0	100	0	0	0	0
E08	250	65	6100	4.51	36.7	0.0011	5	397	5	0	0	90	35	25	0	95	0	0	5	0
E11	254	67	1276	4.54	43	0.0012	6	506	75	5	10	5	80	0	0	100	0	0	0	0
E11east	24	58	300	4.76	68.6	0.0025	6	569	70	15	0	5	15	10	0	100	0	0	0	0
E21	261	106	992	5.27	54	0.0009	1	26	30	5	10	0	70	10	0	95	5	0	0	0
E22	486	102	34320	3.9	57.8	0.0015	1	72	60	5	5	5	45	65	0	90	0	0	0	10
T01	544	82	3010	4.68	30.2	0.0008	1	277	10	0	30	35	70	15	0	100	0	0	0	0
T15	22	90	460	4.65	52.9	0.0028	1	557	95	5	0	5	0	35	10	100	0	0	0	0
W01	489	74	600	5.2	45.3	0.0016	1	1262	95	5	0	5	0	80	5	90	0	10	0	0
W06	27	56	1620	4.35	75.4	0.0031	0	45	95	15	0	5	0	35	15	95	5	0	0	0
W07	338	102	2891	5.05	55.4	0.0029	0	247	70	15	15	30	25	30	10	100	0	0	0	0
W15	64	86	360	4.55	57.3	0.0018	3	827	65	15	0	5	10	10	0	100	0	0	0	0
W18	44	74	228	4.58	90.2	0.0044	1	729	95	5	0	5	20	20	5	95	0	0	5	0

*P04 not included, no reasonable expectation of spotted salamander presence

Appendix 5. Program PRESENCE model comparison, by species. AIC is the Akaike Information Criterion, w_i is the model weight, ψ is the site occupancy rate, naïve is the naïve detection rate, and *p* detection is the average probability of detection. *PRESENCE could not estimate ψ.

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
SCHO	ψ (perm, AbsCo) p(air)	5	28.278	0.000	0.832	*	0.100	
SCHO	ψ (perm) p(air)	4	33.494	5.216	0.061	0.101	0.100	
SCHO	ψ (.) p(air temp)	3	33.766	5.488	0.053	0.101	0.100	
SCHO	ψ (AbsCo440) p(air)	4	33.799	5.521	0.053	0.101	0.100	
SCHO	ψ (.) p(.)	2	41.470	13.192	0.001	0.130	0.100	0.258

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
BUFO	ψ (AbsCo440) p(t)	14	245.620	0.000	0.636	0.502	0.500	
BUFO	ψ (perm,AbsCo440), p(t)	15	247.160	1.540	0.294	0.502	0.500	
BUFO	ψ (perm) p(t)	14	250.620	5.000	0.052	0.502	0.500	
BUFO	ψ (.) p(t)	13	252.760	7.140	0.018	0.502	0.500	
BUFO	ψ (.) p(.)	2	271.570	25.950	0.000	0.505	0.500	0.319

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
PSCR	ψ (AbsCo440) p(t)	13	279.529	0.000	0.322	0.900	0.900	
PSCR	ψ (.) p(t)	12	279.565	0.037	0.316	0.900	0.900	
PSCR	ψ (semi) p(t)	13	280.657	1.128	0.183	0.900	0.900	
PSCR	ψ (semi, AbsCo440) p(t)	14	280.697	1.168	0.179	0.900	0.900	
PSCR	ψ (.) p(.)	2	408.150	128.600	0.000	0.900	0.900	0.630

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
HYVE	ψ (AbsCo440) p(water)	4	31.405	0.000	0.396	0.333	0.100	
HYVE	ψ (.) p(water)	3	31.970	0.565	0.299	0.164	0.100	
HYVE	ψ (semi, AbsCo440) p(water)	5	33.302	1.897	0.154	0.350	0.100	
HYVE	ψ (semi) p(water)	4	33.473	2.068	0.141	0.156	0.100	
HYVE	ψ (.) p(.)	2	38.740	7.335	0.010	0.185	0.100	0.140

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
RASY	ψ (cond) p(air)	4	14.724	0.000	0.662	*	0.067	
RASY	ψ (perm, cond) p(air)	5	16.516	1.791	0.270	0.133	0.067	
RASY	ψ (.) p(air temp)	3	20.334	5.610	0.040	1.000	0.067	
RASY	ψ (perm) p(air)	4	21.857	7.132	0.019	0.700	0.067	
RASY	ψ (.) p(.)	2	23.182	8.458	0.010	1.000	0.067	0.022

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
RAPA	ψ (perm,semi, AbsCo440), p(water temp)	6	74.130	0.000	0.440	0.170	0.170	
RAPA	ψ (AbsCo440), p(water temp)	4	74.380	0.250	0.389	0.180	0.170	
RAPA	ψ (perm,semi), p(water temp)	5	76.140	2.010	0.161	0.170	0.170	
RAPA	ψ (.) p(water temp)	3	82.030	7.900	0.008	0.170	0.170	
RAPA	ψ (.) p(.)	2	85.460	11.330	0.002	0.170	0.170	0.420

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
RACL	ψ (perm,semi) p(t)	15	337.500	0.000	0.709	0.700	0.700	
RACL	ψ (perm,semi, pH) p(t)	16	339.370	1.870	0.278	0.700	0.700	
RACL	ψ (.) p(t)	13	346.650	9.150	0.007	0.700	0.700	
RACL	ψ (pH) p(t)	14	347.030	9.530	0.006	0.700	0.700	
RACL	ψ (.) p(.)	2	385.920	48.420	0.000	0.700	0.700	0.560

Species	Model	# param	AIC	Δ AIC	w_i	ψ	naïve	*p* detection
RACA	ψ (perm,semi) p(water)	5	83.090	0.000	0.607	0.272	0.267	
RACA	ψ (perm, semi,AbsCo440) p(water)	6	83.997	0.906	0.386	0.273	0.267	
RACA	ψ (AbsCo440) p(water)	4	92.065	8.974	0.007	0.273	0.267	
RACA	ψ (.) p(water temp)	3	98.456	15.366	0.000	0.299	0.267	
RACA	ψ (.) p(.)	2	115.514	32.423	0.000	0.271	0.267	0.439