

## Double-observer approach to estimating egg mass abundance of pool-breeding amphibians

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### Abstract

Interest in seasonally flooded pools, and the status of associated amphibian populations, has initiated programs in the northeastern United States to document and monitor these habitats. Counting egg masses is an effective way to determine the population size of pool-breeding amphibians, such as wood frogs (*Rana sylvatica*) and spotted salamanders (*Ambystoma maculatum*). However, bias is associated with counts if egg masses are missed. Counts unadjusted for the proportion missed (i.e., without adjustment for detection probability) could lead to false assessments of population trends. We used a dependent double-observer method in 2002–2003 to estimate numbers of wood frog and spotted salamander egg masses at seasonal forest pools in 13 National Wildlife Refuges, 1 National Park, 1 National Seashore, and 1 State Park in the northeastern United States. We calculated detection probabilities for egg masses and examined whether detection probabilities varied by species, observers, pools, and in relation to pool characteristics (pool area, pool maximum depth, within-pool vegetation). For the 2 years, model selection indicated that no consistent set of variables explained the variation in data sets from individual Refuges and Parks. Because our results indicated that egg mass detection probabilities vary spatially and temporally, we conclude that it is essential to use estimation procedures, such as double-observer methods with egg mass surveys, to determine population sizes and trends of these species.

### Introduction

Concern over the alteration and loss of isolated, seasonally-flooded wetlands and their surrounding upland habitats has spurred several northeastern states to document, monitor and attempt to protect these wetlands and their associated wildlife (Kenney 1995; Colburn 1997; Tappan 1997; Preisser et al. 2000; Burne 2001; Calhoun and Klemens 2002; Calhoun 2003). Documenting the presence or abundance of egg masses in seasonal

pools has been recommended as an effective way to monitor pool-breeding amphibians, such as wood frogs (*Rana sylvatica* LeConte) and spotted salamanders (*Ambystoma maculatum* Shaw) (Windmiller 1996; Crouch and Paton 2000). While breeding activity of wood frogs and spotted salamanders in seasonal pools can be inferred from calling activity or presence of spermatophores, egg masses or larvae, counting egg masses is potentially more useful for determining breeding population size and persistence of these species than

surveys of larvae or adults alone. Crouch and Paton (2000) recommended the use of egg mass counts over calling surveys and drift fence arrays to provide indices of population size at considerably less effort and cost. Wood frog females lay one egg mass per year, so egg mass censuses for this species may provide a 1:1 relationship to the female breeding population at a seasonal pool (Corn and Livo 1989; Crouch and Paton 2000; but see Davis and Folkerts 1986). Spotted salamanders lay 2–4 egg masses per year (Petranka 1998), so greater error is associated with estimating female spotted salamander breeding population sizes from egg mass numbers. Despite this, population trends from spotted salamander egg mass counts could still yield important information, provided the ratio of egg masses to females at a site is fairly constant over the study period.

Wood frogs and spotted salamanders lay conspicuous egg masses attached to vegetation and woody debris within pools. Wood frog egg masses are typically aggregated in large rafts at the water's surface, often with over 100 egg masses laid communally, although approximately 16% may be distributed away from the main raft areas (Crouch and Paton 2000). Wood frog egg masses consist of up to 1500 eggs, viable embryos are black, and the egg mass surface appears bumpy (Kenney and Burne 2000). Spotted salamanders lay egg masses individually or in aggregated clusters and tend to lay eggs deeper in the water column, occasionally on the bottom of pools (Windmiller 1996; Petranka 1998). Spotted salamander egg masses are quite distinct from those of wood frogs in that each complement of 30–250 eggs in the mass is surrounded by a firm smooth gelatinous matrix that can be clear, opaque or green due to the presence of certain proteins or symbiotic algae in the jelly matrix (Kenney and Burne 2000; Petranka 1998). Opaque and green spotted salamander egg masses may be more easily detected than clear ones, particularly in sediment-clouded or tannic acid stained waters of some seasonal pools. Because of these factors, a complete census of egg masses in breeding pools is logistically impossible.

In any wildlife survey designed to estimate the abundance of animals, researchers must take into account two important sampling issues: spatial variability and detectability (Williams et al. 2002). This paper focuses on the estimation of detectability and incorporation of detection probabilities

in the estimation of egg mass numbers from survey data. In this paper, the term 'population' refers to the total number of egg masses, while the term 'individual' refers to each egg mass. Detectability refers to the fact that counts of animals (or egg masses) are typically incomplete and represent only a proportion of the actual population present. The probability that observers detect all individuals in a survey area such as a seasonal pool is likely very small (Lancia et al. 1994; Windmiller 1996; Crouch and Paton 2000; Williams et al. 2002). The relationship between counts and the actual population size can be expressed as  $E(C) = Np$ , where  $N$  is the true or actual population size,  $C$  is the count collected during the survey,  $E$  denotes statistical expectation, and  $p$  is the detection probability, or the probability that a member of  $N$  appears in  $C$ . If raw counts are used as an index for true population abundance across space and time, researchers are assuming that: (1) the relationship between the count ( $C$ ) and the actual population ( $N$ ) is approximately linear, and (2) the detection probability ( $p$ ), which can range from 0 (none detected) to 1 (all detected, i.e., census), is constant across space and time. Sometimes this assumption can be relaxed if one can assume that the average detection probability does not vary over time or space. If these assumptions are violated, unadjusted counts will not provide robust estimates of change in population size, and the estimation of detection probability becomes critical to inferences about population size and change. Accordingly, if detection probabilities are  $< 1$  and vary over time and/or space, population estimation procedures such as capture-recapture, removal, or double-observer techniques must be employed when conducting egg mass surveys (Williams et al. 2002).

Several factors may influence detection probabilities. Observer error, due to inexperience or differences in visual ability, or other factors may produce variation in detection probability (Hayek 1994). Density has been shown to influence differences in observer detection probabilities from bird point counts (Bart and Schoultz 1984). Detection probabilities may also vary among species and individuals of the same species because of differences in habitats, egg mass deposition sites, or season of observation (Diehl 1981). Additionally, environmental factors such as weather and time of day may influence the

probability of detecting animals or egg masses (Cook and Jacobson 1979).

One technique that has been employed to account for observer differences and to estimate detection probabilities and population sizes is the incorporation of a second observer. Double-observer estimates have been used for surveys of birds (Nichols et al. 2000), musk oxen (Aastrup and Mosbech 1993), moose and whitetail deer (Cook and Jacobson 1979), and amphibian larvae (Jung et al. 2002). In a dependent double-observer protocol, two observers survey a pool together with observers trading off between primary and secondary roles (see Methods; Nichols et al. 2000). From resulting statistics, one can compute detection probability estimates for egg masses and adjust counts based on these probabilities to obtain unbiased estimates of the total number of egg masses.

The study described herein is one component of the US Geological Survey's (USGS) Amphibian Research and Monitoring Initiative (ARMI), designed as a long-term monitoring survey of amphibians on federal lands in the northeastern United States. The primary goal of this study is to draw inferences about changes in wood frog and spotted salamander egg mass abundance in seasonal pools over time (allowing inference about population trends) and space (allowing inference of the relationship between egg mass abundance and landscape and habitat variables). Because of possible variation in detection probabilities over time and space, estimation of detection probabilities may be critical for inferring population trend and relative abundance. In this paper, we present the results of dependent double-observer egg mass surveys and test whether detection probabilities differ among species and observers or are influenced by factors such as pool area, pool maximum depth, or within-pool vegetation. We examine whether survey- and site- specific covariates affect detection probabilities across Refuges and Parks over a two-year period (2002–2003), and address strengths and weaknesses of the double-observer protocol.

Regarding our *a priori* hypotheses, we predicted moderate variation in the factors that influence detection probabilities. The study areas covered a large region, leading to the expectation that sources of variation in detection probability would likely vary. Observer variation is common to most animal survey methods and was expected to be

important. We predicted that observers trained by primary study investigators might have higher detection probabilities than observers receiving secondary training from other personnel. Egg masses of both amphibian species are readily detectable, though we hypothesized that the egg mass deposition behavior of wood frogs would result in higher detection probabilities. We expected detection probability to be negatively related to water depth, wetland area, and vegetation, with egg masses more difficult to find in larger, deeper, and more vegetated pools.

## Methods

Wood frog and spotted salamander egg mass double-observer surveys were conducted at 13 National Wildlife Refuges, 1 National Park, 1 National Seashore and 1 State Park in the northeastern US from March through June, 2002–2003 (Table 1). Data from Aroostook National Wildlife Refuge could not be included in analyses for 2003 because single rather than double observers conducted surveys. Refuge and Park biologists identified pools by examining USGS color infrared digital ortho-photo quarter quadrangle (DOQQ) maps (1:24,000 scale) using Arc View (Version 3.2, Environmental Systems Research Institute), and by ground-truthing in the field. Pools that appeared likely to harbor breeding amphibians were chosen so that we could conduct double-observer estimation techniques and monitor population trends. Observers in the study included USGS, US Fish and Wildlife Service, National Park Service and State Park lead biologists trained by REJ (primary training in double-observer technique) and technicians and volunteers trained by lead biologists (secondary training).

Each pool was visited at least twice during the peak-breeding season, which varied depending on geographic location. In the northeastern US, which extends from Virginia to Maine and west to West Virginia, wood frogs typically deposit their eggs from late February to May (Wright and Wright 1949) and spotted salamanders from March to June (Petranka 1998). Wood frogs often breed earlier than spotted salamanders, but presence of both species' egg masses overlapped in most pools during at least one survey. Breeding phenologies of both species can vary widely from year to year

Table 1. Participating National Wildlife Refuges (NWR), National Parks (NP), National Seashore (NS) and State Park, their state locations and the number of pools and observers and survey dates and years in which double-observer egg mass surveys were conducted.

Location (Park or Refuge)	State	2002			2003		
		# pools	# observers	Survey dates	# pools	# observers	Survey dates
Acadia	Maine	4	3	Apr 18–May 5	4	2	Apr 29–May 20
Aroostook	Maine	3	3	May 6–25 Jun	–	–	–
Canaan Valley NWR	West Virginia	4	2	Mar 19–May 1	4	2	Mar 27–May 13
Canaan Valley SP	West Virginia	3	2	Mar 18–May 11	4	4	Mar 25–Apr 30
Cape Cod	Massachusetts	6	3	Apr 12–May 17	4	3	Apr 14–May 2
E. Massachusetts <sup>a</sup>	Massachusetts	2	2	Apr 12–May 9	4	6	Apr 7–May 9
Erie	Pennsylvania	4	4	Apr 5–Apr 26	4	3	Apr 3–Apr 25
Great Bay	New Hampshire	3	2	Apr 8–Apr 24	4	5	Apr 11–Apr 25
Great Swamp	New Jersey	4	5	Apr 4–Apr 25	3	3	Mar 21–Apr 29
Iroquois	New York	4	3	Apr 11–May 17	3	2	Apr 4–May 16
Lake Umbagog	New Hampshire	4	4	May 1–Jun 5	4	4	Apr 28–May 20
Mississquoi	Vermont	4	2	Apr 11–Apr 27	4	2	Apr 17–May 16
Moosehorn	Maine	4	2	Apr 30–May 16	4	3	May 3–May 22
Patuxent Research Refuge <sup>b</sup>	Maryland	13	8	Mar 6–Apr 3	3	6	Mar 22–Apr 8
Rachel Carson	Maine	4	2	Apr 5–May 21	4	2	Apr 17–May 20
Wallkill River	New Jersey	4	2	Mar 27–Apr 24	5	2	Mar 31–May 7

<sup>a</sup>Eastern Massachusetts includes Assabet River NWR, Oxbow NWR, and Sudbury State Forest.

<sup>b</sup>Patuxent Research Refuge (PRR) also includes pools located on the US Department of Agriculture Beltsville Agricultural Research Station.

depending on the weather and can also differ by a week or two among pools located within the same county (Crouch and Paton 2000). The sampling window for egg mass surveys is constrained by the developmental rate of the embryos. Wood frog egg masses last from 1 to 4 weeks prior to hatching, and spotted salamanders from 4 to 7 weeks. By surveying pools repeatedly over the breeding season, we were able to obtain counts when the maximum number of egg masses for each species was present. We constructed models and estimated detection probabilities for the survey date in which we recorded the maximum number of egg masses for each species.

Observers surveyed the pools during the day (0900–1500 h), recorded pool locations using a global positioning system (GPS) receiver, measured pool maximum depth (cm), pool maximum length (m), and maximum width (m), and summed estimated categories of percent of the pool covered with submerged aquatic vegetation, emergent vegetation, shrubs, and trees (0=0%, 1=1–10%, 2=11–25%, 3=26–50%, 4=51–75%, 5=≥76%), resulting in a variable describing within-pool vegetation with a cover class scale ranging from 0 to 10. Pool area (m<sup>2</sup>) was estimated by multiplying pool maximum width by maximum length. For

each survey, observers sketched a map of the site including landmarks such as trees and shrubs to use in recording the location of egg masses. Observers wore polarized sunglasses to reduce solar glare reflected off the water's surface. All areas within pools < 1.5 m in depth were surveyed.

We used a dependent double-observer technique (Cook and Jacobson 1979; Nichols et al. 2000), in which observer 1 points to and counts out egg masses to observer 2 (Figure 1). Observer 2 records what observer 1 reports, but also records in a separate column any additional egg masses that observer 1 missed (withholding any comments to observer 1). Halfway through the survey of the pool, the observers switch roles, with observer 2 now initiating the counts and observer 1 recording the number of egg masses detected by observer 2 and any additional egg masses that observer 2 may have missed. We analyzed data from the survey date(s) with the greatest number of detected egg masses of wood frogs or spotted salamanders at each pool.

Two methods were used to ask questions about the detection probabilities of spotted salamander and wood frog egg masses in northeastern pools. As noted above, we modeled the detection probability,  $p$ , as a function of several covariates



*Figure 1.* Illustration of the dependent double-observer technique. The first observer (at left in the photograph) points out egg masses to the second observer (standing), who records the first observer's count and any additional egg masses he sees. The observers survey the pool together, with the second observer following slightly behind the first. Midway through the survey, the observers switch roles, and the first observer follows the second observer around the pond, recording egg masses seen and missed.

hypothesized to affect detection probabilities of these species (Table 2). For most inferences we used an information-theoretic approach (Burnham and Anderson 1998) to select the most appropriate model for each data set. However, one question was whether variation in detection probability varied between observers who received primary versus secondary training. This question could not

be addressed via the use of general competing models, so we used point estimates of detection probabilities to address the question within a hypothesis-testing framework.

#### *Model development and selection*

We used the program SURVIV (<http://www.mbr-pwrc.usgs.gov/software.html>; White 1992 with revisions by Hines 1996) to fit the various models (Table 2). Models were developed for sets of pools from each Refuge and Park separately. The most general model,  $p(\text{observer, pool, species})$ , assumes that detection probabilities were dependent on observer, pool, and species, while the most specific model,  $p(\cdot)$ , assumes detection probabilities are constant and not dependent on observer, pool, or species. A series of other models incorporated parameter constraints on the general model [e.g.  $p(\text{observer})$ ,  $p(\text{species})$ ,  $p(\text{pool})$ ]; see Appendix 1 for an example of the SURVIV code]. Still other models incorporated linear-logistic relationships between detection probability and different habitat variables (pool area and maximum depth, within-pool vegetation; see Appendix 2 for an example of the SURVIV code). We initially tried to model all habitat variables simultaneously with observer and species, but the data did not support this generality in many cases. Thus, we addressed questions about habitat variables using general models that included observer, species, and single habitat variables [e.g.,  $p(\text{observer, species, depth})$ ] as well as simple models with only habitat variables [e.g.,  $p(\text{depth})$ ]. For example, model [ $p(\text{observer, species,}$

*Table 2.* Eleven models tested for the double-observer methods in 2002–2003 to explore assumptions regarding egg mass detection probabilities and constraints of each.

Model	Constraints
$p(\cdot)$	Detection probabilities independent of observer, pool, and species.
$p(\text{species})$	Detection probabilities dependent on species, independent of pool and observers.
$p(\text{pool})$	Detection probabilities dependent on pool, independent of observers and species.
$p(\text{obs})$	Detection probability dependent on individual observers, independent of species and pool.
$p(\text{obs, species, pool})$	Most parameterized model; detection dependent on observers, species, and pool.
$p(\text{area})$	Detection probabilities a function of pond area ( $\text{m}^2$ ).
$p(\text{obs, species, area})$	Detection probabilities a function of observer, species and pond area ( $\text{m}^2$ ).
$p(\text{depth})$	Detection probabilities a function of pond depth (m).
$p(\text{obs, species, depth})$	Detection probabilities a function of observer, species and pond depth (m).
$p(\text{veg})$	Detection probabilities a function of overall pond vegetation (cover class scale of 0–10).
$p(\text{obs, species, veg})$	Detection probabilities a function of observer, species and overall pond vegetation (cover class scale of 0–10).

depth)] was based on the following linear-logistic relationship for detection probability:

$$p = \frac{e^{(\beta_1 O + \beta_2 S + \beta_3 D)}}{1 + e^{(\beta_1 O + \beta_2 S + \beta_3 D)}}$$

where  $O$  denotes an indicator variable for observer,  $S$  is an indicator variable for species (0 = wood frog, 1 = spotted salamander),  $D$  is the measurement for the depth covariate, and  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are the associated model coefficients to be estimated.

Goodness-of-fit tests for our most general model [ $p$ (observer, pool, species)] indicated that the model fit the dataset for each Park or Refuge well (Table 3(a), (b)). Therefore, we used the second-order Akaike's Information Criterion differences ( $\Delta AIC_c$ ) to compare models and select the model that most parsimoniously explained the variation in the data using the smallest number of parameters (Burnham and Anderson 1998). Small  $\Delta AIC_c$  values (<2) indicated models similar in their ability to fit the structure of the data (Burnham and Anderson 1998; Nichols et al. 2000).

#### *Hypothesis testing*

As noted, the hypothesis about observer training could not be placed in a model selection framework. To test whether primary training resulted in larger observer detection probabilities, we used a nonparametric Wilcoxon two-sample rank test. This test was conducted using SAS version 8, and results were considered significant at  $\alpha < 0.05$ .

### **Results and discussion**

A total of 73 pools were surveyed in 2002 and 59 pools were surveyed in 2003, with 45 pools surveyed in both 2002 and 2003. The number of observers in a season per Refuge or Park ranged from 2 to 8 (Table 1). A total of 67 observers participated in the egg mass surveys in 2002–2003, with 21 observers participating in both survey years.

Development of models using the program SURVIV allowed us to evaluate whether observers, amphibian species, and/or habitat covariates influenced detection probabilities of individual egg

masses (Table 3). We were particularly interested in assessing: (1) whether detection probabilities differed among species, observers, pools, or habitat variables, and (2) whether specific models were selected at a large proportion of the Refuges and Parks (i.e., whether there was a consistent pattern in the variables that affected detection probabilities among the Refuges and Parks).

The analyses provided evidence of the importance of most of the identified potential sources of variation in detection probability for at least some study locations in some years (Table 3). No specific model was consistently selected as 'best' across the study locations during the survey period. Observer, species, and habitat variables were all found to be important determinants of detection probability throughout the analyses.

We had no expectation of a single model being generally applicable to all data sets. The spatial and temporal heterogeneity associated with our data sets, spanning a distance of nearly 1500 km in the northeast region, may explain some of the differences in importance of covariates across the data sets. Differences among data sets and years in pools, observers, precipitation, vegetation patterns, canopy closure, water chemistry, and other factors may contribute to the variation at the scale of Park or Refuge among years. This identification of the potential importance of several variables as sources of variation in detection probability, rather than a single dominant factor, underscores the need to adjust egg mass counts to infer population sizes and trends.

#### *Variation in detection probability associated with species*

We detected wood frog egg masses in 63/73 pools in 2002 and 51/59 pools in 2003, and spotted salamander egg masses in 50/73 pools in 2002 and 44/59 pools in 2003. Although model selection results suggested the importance of species as a source of variation in detection probability (Table 3), the difference between the species did not appear to be consistent over time or area. The differences between point estimates from the same locations differed in sign from one location to another as did the estimated species coefficients in the habitat models (Table 4). Point estimates from the

Table 3.  $\Delta AIC_c$  values for the models of detection probability fit to each data set for 2002 (a) and 2003 (b).

<i>n</i>	<i>p</i> (.)	<i>p</i> (species)	<i>p</i> (pool)	<i>p</i> (obs)	<i>p</i> (obs, species, pool)	<i>p</i> (depth)	<i>p</i> (obs, species, depth)	<i>p</i> (area)	<i>p</i> (obs, species, area)	<i>p</i> (obs, species, veg)
<b>(a) 2002 Location (data set)</b>										
Acadia	4	15.6	14.3	20.2	<b>1.0</b> (0.20)	2.2 (0.11)	3.0 (0.07)	—	<b>0.0</b> (0.34)	—
Aroostook	3	7.4 (0.02)	3.7 (0.10)	9.7	<b>1.9</b> (0.24)	<b>0.0</b> (0.62)	—	6.1 (0.03)	—	<b>0.4</b> (0.27)
Cape Cod	6	32.6	34.5	32.3	19.4	15.9	7.9 (0.02)	3.0 (0.18)	—	<b>0.0</b> (0.80)
Canaan Valley SP	3	3.0 (0.07)	<b>0.0</b> (0.32)	7.0 (0.01)	4.3 (0.04)	7.7 (0.01)	4.2 (0.04)	<b>0.5</b> (0.25)	—	—
Canaan Valley NWR	4	37.9	38.9	26.6	39.6	<b>0.0</b> (1.00)	31.6	40.9	—	38.9
E. Massachusetts	2	12.6	11.1	12.7	4.2 (0.02)	3.0 (0.05)	<b>0.0</b> (0.20)	<b>0.5</b> (0.16)	<b>0.2</b> (0.18)	<b>0.2</b> (0.18)
Erie	4	<b>0.0</b> (0.29)	<b>0.2</b> (0.26)	2.8 (0.07)	<b>0.0</b> (0.29)	2.4	—	—	—	—
Great Bay	3	15.8	15.8	11.1	5.9 (0.03)	<b>0.0</b> (0.48)	<b>0.9</b> (0.30)	—	—	<b>1.9</b> (0.19)
Great Swamp	4	4.9 (0.03)	4.9 (0.03)	<b>0.5</b>	<b>0.0</b> (0.36)	6.6 (0.01)	—	2.7 (0.09)	—	<b>1.2</b> (0.20)
Iroquois	4	47.0	47.0	<b>1.5</b> (0.31)	<b>0.0</b> (0.67)	7.7 (0.01)	—	—	—	—
Lake Umbagog	4	51.9	7.1 (0.02)	30.9	25.7	8.5 (0.01)	13.9	<b>0.0</b> (0.84)	26.4	30.4
Missisquoi	4	25.9	25.9	26.6	11.5	31.4	<b>0.0</b> (0.98)	7.9 (0.02)	5.0	5.7
Moosehorn	4	4.8 (0.04)	5.5 (0.03)	4.8 (0.04)	6.2 (0.02)	7.0 (0.01)	—	2.2 (0.16)	<b>0.0</b> (0.49)	4.5 (0.05)
PRR	13	99.1	92.1	43.9	39.2	32.4	57.7	62.8	<b>0.0</b> (1.00)	93.1
Rachel Carson	4	90.5	65.5	87.0	37.4	<b>1.5</b> (0.32)	—	—	—	<b>0.0</b> (0.68)
Walkill River	4	6.5 (0.03)	4.1 (0.10)	<b>0.0</b> (0.80)	5.5 (0.05)	11.7	—	8.9 (0.01)	—	9.6 (0.01)
<b>(b) 2003 Location (data set)</b>										
Acadia	4	<b>1.5</b> (0.20)	<b>0.5</b> (0.32)	3.9 (0.06)	<b>0.0</b> (0.42)	9.4	—	—	—	—
Cape Cod	4	4.8 (0.05)	6.4 (0.02)	<b>1.3</b> (0.29)	5.5 (0.04)	5.7 (0.03)	<b>0.0</b> (0.56)	745.3	—	—
Canaan Valley SP	4	9.0 (0.01)	<b>0.0</b> (0.89)	14.0	4.8 (0.08)	13.4	9.1 (0.01)	—	—	9.8 (0.01)
Canaan Valley NWR	4	15.9	13.3	10.1 (0.01)	28.1	17.0	11.9	<b>0.0</b> (0.97)	24.8	11.3
E. Massachusetts	4	28.9	17.8	15.1	17.0	23.1	17.8	<b>0.0</b> (0.98)	10.7	10.5
Erie	4	22.2	13.2	<b>0.0</b> (0.51)	12.6	<b>0.2</b> (0.46)	21.8	9.6	11.5	13.1
Great Bay	4	16.4	18.4	15.0	2.1 (0.23)	<b>0.0</b> (0.66)	—	—	—	3.6 (0.11)
Great Swamp	3	14.1	14.1	<b>0.0</b> (0.88)	13.3	4.3 (0.10)	—	—	—	8.9 (0.01)
Iroquois	2	2.1 (0.11)	2.1 (0.11)	<b>0.0</b> (0.32)	2.1 (0.11)	<b>0.0</b> (0.32)	—	—	—	5.9 (0.02)
Lake Umbagog	4	357.8	101.6	220.3	<b>0.0</b> (0.99)	12.3	262.1	15.0	216.9	285.1
Missisquoi	4	<b>0.6</b> (0.22)	<b>0.6</b> (0.22)	4.8 (0.03)	<b>0.0</b> (0.30)	10.4	2.1 (0.11)	2.0 (0.11)	—	—
Moosehorn	4	3.8 (0.07)	<b>0.9</b> (0.28)	6.5 (0.02)	2.0 (0.16)	7.2 (0.01)	—	<b>0.0</b> (0.44)	5.4 (0.03)	—
PRR	3	8.0 (0.01)	6.1 (0.04)	10.6	<b>0.0</b> (0.76)	5.1 (0.06)	4.7 (0.07)	9.1 (0.01)	9.2 (0.01)	7.4 (0.02)
Rachel Carson	4	7.1 (0.02)	4.1 (0.10)	4.0 (0.01)	8.6 (0.01)	20.6	12.7	—	—	12.2
Walkill River	4	<b>1.2</b> (0.28)	<b>0.0</b> (0.52)	6.5 (0.02)	2.9 (0.12)	4.5 (0.05)	—	—	—	<b>0.0</b> (0.76)

The most parsimonious model that describes variation in the data set is indicated by a value of  $\Delta AIC_c = 0.0$  (in bold). Values in italicized bold have  $\Delta AIC_c < 2.0$ . Non-zero normalized model weights are in parentheses. Models with a  $< ->$  could not be fit to the data.

SURVIV model  $p(\text{species})$  were averaged over all areas for each year and replication-based SE's computed. These estimates were high for both species for both years (wood frogs in 2002:  $0.96 \pm 0.02$ , 2003:  $0.96 \pm 0.01$ ; spotted salamanders in 2002:  $0.95 \pm 0.01$ , 2003:  $0.93 \pm 0.32$ ).

Despite the differences in breeding phenology and egg mass deposition behavior (aggregated in wood frogs and separate deposition of egg masses in spotted salamanders), the double-observer method appears to be effective at detecting both species.

#### Variation in detection probability associated with observers

Model selection results provided strong evidence of variation between observers in detection probability. Models with separate parameters for different observers typically had high model weights, emphasizing the need to deal with observer differences in egg mass surveys.

Previous egg mass count studies have also found differences between observer counts. Windmiller (1996) used an independent single-

Table 4. Estimated detection probabilities for *Rana sylvatica* and *Ambystoma maculatum* in (a) 2002 and (b) 2003 from the  $p(\text{species})$  model, and estimates of the species coefficient in the covariate model  $p(\text{observer, species, depth})$ .

	Estimated detection probability ( $p$ ) for <i>Rana sylvatica</i>	Estimated detection probability ( $p$ ) for <i>Ambystoma maculatum</i>	Estimated coefficient
(a) 2002 Location (data set)			
Acadia	1.00 (–)	0.96 (0.01)	–
Aroostook	0.99 (0.01)	0.95 (0.02)	–
Cape Cod	1.00 (–)	0.96 (0.01)	–
Canaan Valley SP	1.00 (–)	0.98 (0.00)	0.07 (0.30)
CanaanValley NWR	0.94 (0.13)	0.96 (0.11)	–5.15 (5.41)
E. Massachusetts	0.78 (0.12)	0.93 (0.01)	10.00 (–)
Erie	0.98 (0.02)	0.94 (0.02)	–
Great Bay	0.94 (0.04)	–	–
Great Swamp	0.94 (0.02)	–	–
Iroquois	1.00 (–)	–	–
Lake Umbagog	1.00 (–)	0.88 (0.03)	–3.55 (3.29)
Mississquoi	0.98 (0.01)	–	–
Moosehorn	0.83 (0.09)	0.94 (0.01)	–
PRR	0.97 (0.01)	0.99 (0.00)	–
Rachel Carson	1.00 (–)	0.84 (0.04)	–
Wallkill River	0.99 (0.01)	1.00 (–)	–
(b) 2003 Location (data set)			
Acadia	0.98 (0.01)	1.00 (–)	–
Cape Cod	1.00 (–)	0.95 (0.09)	–
Canaan Valley SP	1.00 (–)	0.97 (0.01)	–
CanaanValley NWR	0.96 (0.02)	0.99 (0.01)	1.30 (1.07)
E. Massachusetts	0.98 (0.01)	0.89 (0.02)	–2.20 (2.04)
Erie	0.94 (0.03)	0.63 (0.14)	10.00 (–)
Great Bay	0.99 (0.01)	1.00 (–)	–
Great Swamp	0.89 (0.03)	–	–
Iroquois	0.99 (0.02)	–	–
Lake Umbagog	1.00 (–)	–	–1.01 (3.05)
Mississquoi	0.98 (0.01)	–	–
Moosehorn	0.88 (0.09)	0.99 (0.01)	4.14 (5.13)
PRR	0.85 (0.03)	0.92 (0.02)	–0.44 (2.47)
Rachel Carson	1.00 (–)	0.95 (0.03)	–
Wallkill River	1.00 (–)	0.96 (0.03)	–

Standard errors follow the estimates in parentheses. Standard errors could not be estimated [denoted as (–)] when parameter estimates were on the boundary of the parameter space (e.g., 1.00). In the last column, a negative coefficient indicates that the detection probability was higher for *Rana sylvatica*, while a positive coefficient indicates that the detection probability was higher for *Ambystoma maculatum*. A (–) indicates that the model could not be fit to the data.



observer count – recount of spotted salamander egg masses in four seasonal pools in Massachusetts and found <10% difference between counts. Crouch and Paton (2000) found that counts of wood frog egg masses in the same pools by two independent observers varied by 12%. Egan (unpubl. data) found that independent double-observer counts of spotted salamander egg masses varied by 25%, while wood frog egg mass counts varied by 11%. Though double-observer estimation techniques were not used in these studies, the percent difference in raw counts among observers indicates that some observers failed to detect all egg masses.

We used point estimates of detection probabilities from the SURVIV model  $p(\text{observer})$  to test whether observers who received primary training exhibited increased detection probabilities when compared to observers who received secondary training in the double-observer egg mass detection method. We averaged the detection probabilities for 21 observers who participated in both survey years, with a total of 67 observers included in the analysis. Observers who received primary training ( $n = 23$ ) had a mean detection probability of  $0.93 \pm 0.02$  (replication-based SE), while observers who received secondary training ( $n = 44$ ) had a mean detection probability of  $0.88 \pm 0.03$ , though the difference was not significant (Wilcoxon  $Z = -0.53$ ,  $p = 0.60$ ). Observers with secondary training nearly always conducted surveys with an observer who had received primary training, likely reducing the effects of a naïve observer included in the counts. Estimating detection probabilities for each observer participating in a survey allows realistic approximations of actual population sizes irrespective of variation in the relative ability of each observer to detect egg masses.

These results, as well as double-observer data unadjusted for differences in detection probability from Windmiller (1996); Crouch and Paton (2000), and Egan (2001) indicate that observer differences in counts between two observers are inherent in egg mass surveys. Estimation of detection probabilities using a double-observer method, such as those outlined in this study, allow for the removal of this bias from the estimation of population size by adjusting population estimates for the probability that both observers missed egg masses.

#### *Variation in detection probability associated with habitat variables*

Models with detection probabilities modeled as functions of habitat variables sometimes had high model weights (Table 3), but the estimated relationships showed no real consistency (Table 5). Estimated coefficients relating detection probabilities and habitat variables varied in magnitude and direction across the different data sets. The prediction of negative association between detection probability and depth, area, and vegetation seemed to hold in some cases, but was not supported overall (Table 5). Over the range of variable values encountered in this study, these variables were not good predictors of detection probabilities.

Windmiller (1996) reported that, in a Massachusetts pool lacking vegetation, spotted salamanders deposited 91% of egg masses at depths within 36–56 cm, with 49% of egg masses deposited at depths between 40 and 50 cm. Wood frog egg masses are typically aggregated on the water surface, and thus, pool depth likely has little influence on the detection of egg masses of this species. Pool maximum depth is an indicator of pool hydroperiod (Brooks and Hayashi 2002) and therefore an indicator of larval survival to metamorphosis (Rowe and Dunson 1995), yet may not be necessarily associated with the detection probability of egg masses. A more meaningful measurement may be the slope of the pool bottom, which would provide a greater area for egg deposition. A combination of pool maximum area and the slope of the pool bottom may better explain the variation in spotted salamander detection probabilities than a single maximum depth or area measurement alone. We are surprised that the percentage of vegetation located within a pool did not show high model weights more consistently. Submerged and emergent vegetation are typically located on the margins of seasonal pools, and therefore, we expected *a priori* that the percentage of vegetation would influence the ability of observers to detect egg masses. Perhaps, because observers were aware that wood frogs and spotted salamanders are likely to attach egg masses to vegetation (Kenney and Burne 2000), observers searched the vegetation more thoroughly, and reduced the influence of vegetation on detection probability.

Table 5. Estimates of the slope coefficients (estimable standard errors in parentheses) relating detection probability and habitat covariates in different covariate models in (a) 2002 and (b) 2003.

	$p(\text{depth})$	$p(\text{observer, species, depth})$	$p(\text{area})$	$p(\text{observer, species, area})$	$p(\text{veg})$	$p(\text{observer, species, veg})$
<i>(a) 2002 Location data set</i>						
Acadia NP	-0.16 (1.46)	-	0.17 (0.65)	-	0.09 (1.50)	-
Aroostook NWR	-	-	-	0.59 (5.66)	-	-
Cape Cod NS	1.27 (1.00)	-	-0.62 (0.38)	-	-3.56 (3.61)	-
Canaan Valley SP	-0.002 (0.30)	0.07 (0.30)	-0.09 (0.77)	-0.08 (0.77)	-0.13 (1.82)	-
CanaanValley NWR	-2.93 (4.04)	-5.15 (5.41)	-0.28 (0.09)	-1.32 (1.93)	1.98 (2.25)	3.89 (2.52)
E.Mass NWR	3.5 (1.33)	10.00 (-)	0.73 (0.34)	0.77 (0.66)	5.28 (2.49)	4.24 (4.34)
Erie NWR	-	-	-	-	-	-
Great Bay NWR	-9.37 (3.95)	-	-	-	-8.21 (4.89)	-
Great Swamp NWR	-	-	10.00 (0.36)	-	10.00 (-)	-
Iroquois NWR	-	-	-	-	-	-
Lake Umbagog NWR	-5.83 (9.40)	-3.55 (3.29)	1.65 (0.57)	-	3.17 (1.22)	2.23 (1.06)
Mississquoi NWR	10.00 (-)	-	-1.56 (3.61)	1.08 (0.48)	3.43 (3.49)	4.70 (3.39)
Moosehorn NWR	-	-	2.70 (1.52)	3.06 (1.51)	4.97 (3.59)	-
PWRC-USDA RR	2.36 (0.63)	-	10.00 (-)	10.00 (-)	0.18 (0.81)	-0.17(0.70)
Rachel Carson NWR	-	-	-	-	-10.00 (-)	-
Wallkill River NWR	-	-	2.83 (3.49)	-	-0.27 (4.22)	-
<i>(b) 2003 Location data set</i>						
Acadia NP	-	-	-	-	-	-
Cape Cod NS	-1.30 (2.28)	-	-	-	-0.33 (0.61)	-
Canaan Valley SP	-0.31 (0.69)	-	1.79 (1.99)	-	0.70 (0.77)	-
CanaanValley NWR	1.35 (1.07)	1.30 (1.07)	6.99 (4.46)	-	-	6.57 (4.55)
E.Mass NWR	-2.61 (2.28)	-2.20 (2.04)	-8.75 (8.47)	-0.84 (0.73)	-1.04 (0.68)	-7.15 (8.94)
Erie NWR	1.79 (2.48)	10.00 (-)	7.48 (1.49)	9.74 (0.83)	9.60 (0.85)	10.00 (-)
Great Bay NWR	-	-	10.00 (-)	-	-	-
Great Swamp NWR	-	-	10.00 (-)	-	-	-
Iroquois NWR	-	-	10.00 (-)	-	-	-
Lake Umbagog NWR	-4.36 (2.14)	-1.01 (3.05)	2.33 (0.49)	2.24 (3.00)	2.75 (0.31)	1.24 (3.26)
Mississquoi NWR	0.22 (6.34)	-	-	-	0.29 (1.36)	-
Moosehorn NWR	-	4.14 (5.13)	-	-	-0.91 (3.58)	-
PWRC-USDA RR	-1.09 (2.73)	-0.44 (2.47)	0.21 (1.77)	0.22 (0.60)	0.12 (0.60)	-1.16 (3.38)
Rachel Carson NWR	0.66 (5.98)	-	-2.70 (7.49)	-	-	-9.78 (7.32)
Wallkill River NWR	-	-	-	-	-	-

A negative coefficient indicates that increases in the habitat covariate (e.g., increased depth, area, or vegetation) reduced the detection probability for both species. A (-) indicates that the model could not be fit to the data. Standard errors could not be estimated [denoted as (-)] when parameter estimates were on the boundary of the parameter space (e.g., 10.00). For each covariate, the first model expresses detection probability as a function of the covariate of interest only. Under the second model of each pair, detection probability is modeled as a function of observer and species, as well as the habitat covariate.

#### *Alternatives to the dependent double-observer method*

Besides the dependent double-observer technique employed in this study, other survey options might include single-observer capture-recapture or "independent" double-observer methods. A single observer could survey a pond multiple times in a capture-recapture framework. Because the same observer would be more likely to see (and miss) the same egg masses, a behavioral response model (e.g.,  $M(b)$  or  $M(bh)$ , Williams et al. 2002) is likely

to be most useful for such sampling. The time interval between sampling occasions should also be kept short, in order not to violate the closure assumption (i.e., all egg masses must be available to be detected at each occasion).

In an independent double-observer method, a different observer conducts a second count separately without sharing information until after the survey has been completed. We used this approach in 2001 (unpubl. data), but had difficulties matching observer counts (for each identified egg mass, it is important to note whether one or both observers

detected it) and found that the dependent double-observer method was superior for interpretation of the data. Combining capture-recapture (i.e., marking egg masses in some fashion; Windmiller 1996; Hels and Buchwald 2001) with independent observer methods may increase precision, reduce bias, and allow estimation of detection probabilities.

The study objectives and the resources available should determine the selection of egg mass survey technique. One possible compromise between the need for precision in estimates and limited resources for monitoring is to employ the dependent double-observer approach only periodically or on a subset of pools, for example, where detection probabilities of egg masses may be especially low because of habitat parameters, such as deep water or dense vegetation. The results of this study caution against this approach because of the wide variety of factors that were determined to be important in the estimation of detection probabilities.

## Conclusions

This study tested a procedure designed to provide more accurate estimates of population trends of wood frog and spotted salamanders. Our results indicate that the factors that affected detection probabilities were not consistent among years and Park or Refuge data sets, and no consistent set of covariates adequately explained variation in detection of wood frog and spotted salamander egg masses. Based on these results, we conclude that the estimation of detection probabilities using a double-observer sampling procedure or some other capture-recapture method is essential when the goal of the study is to draw inferences about population change in wood frog and spotted salamanders based on numbers of egg masses.

Alford and Richards (1999) and more recently Schmidt (2003) indicated that the amphibian

decline literature makes inferences about population trends largely without estimation of the detection probability of different species or of the detection probabilities associated with individual survey locations or observers. Indices derived from unadjusted count data may have bias associated with sampling variation, whereas the parameter of interest is population size and the change in a population over time. In long-term monitoring programs, in which detection probabilities are likely to vary due to differences in observers and other variables, there is a need to estimate population sizes in a manner that is not confounded with the variation in detection probabilities in order to make reasonable inference about population change. The dependent double-observer method allows for the assessment of variables that may influence the detection of biologically important parameters, such as the number of egg masses in a seasonal pool.

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*Appendix 1.* Example SURVIV code as modified for this study.

---

```
PROC TITLE 'SURVIV code for E. Massachusetts NWR detection probability analysis';
PROC MODEL NPAR = 12 addcell;
/* Pool 'A' */
COHORT = 1 /* # of AMAC egg masses seen by both observers */;
1:S(1)/(S(1) + S(2) - S(1)*S(2))/*# of AMAC egg masses seen by observer 1 only*/;
COHORT = 0 /*# of AMAC masses seen by both observers when observer 2 acted as first observer */;
0:(1.-S(2))*S(1)/(S(1)+S(2)-S(1)*S(2)) /*# seen by observer 1 when acting as second observer */;
```

---

## Appendix 1. Continued.

---

```

/* Pool 'B' */
COHORT = 0 /*# of AMAC egg masses seen by both observers at */;
0:S(3)/(S(3) + S(4) - S(3)*S(4)) /*# of AMAC masses seen by observer 1 only*/;
COHORT = 1 /*# of AMAC masses seen by both observers when observer 2 acted as the first observer */;
0:(1-S(4))*S(3)/(S(3)+S(4)-S(3)*S(4)) /* number seen by observer 1 when acting as second observer */;
COHORT = 1 /*# of RSYL egg masses seen by both observers */;
1:S(5)/(S(5) + S(6) - S(5)*S(6)) /*# of RSYL masses seen by observer 1 only*/;
COHORT = 19 /*# of RSYL egg masses seen by both observers when observer 2 acted as first observer */;
4:(1-S(6))*S(5)/(S(5) + S(6) - S(5)*S(6)) /* number seen by observer 1 when acting as second observer */;

/* Pool 'C' */
COHORT = 138 /*# of AMAC egg masses seen by both observers */;
121:S(7)/(S(7) + S(8) - S(7)*S(8)) /*# of AMAC masses seen by observer 1 only */;
COHORT = 297 /*# of AMAC egg masses seen by both observers when observer 2 acted as first observer */;
9:(1-S(8))*S(7)/(S(7) + S(8) - S(7)*S(8)) /* number seen by observer 1 when acting as second observer */;

/* Pool 'D' */
COHORT = 1 /*# of AMAC egg masses seen by both observers */;
0:S(9)/(S(9) + S(10) - S(9)*S(10)) /*# of AMAC masses seen by observer 1 only */;
COHORT = 0 /*# of AMAC egg masses seen by both observers when observer 2 acted as first observer */;
0:(1-S(10))*S(9)/(S(9) + S(10) - S(9)*S(10)) /* number seen by observer 1 when acting as second observer */;
COHORT = 2 /*# of RSYL egg masses seen by both observers */;
2:S(11)/(S(11) + S(12) - S(11)*S(12)) /*# of RSYL masses seen by observer 1 only */;
COHORT = 0 /*# of RSYL egg masses seen by both observers when observer 2 acted as first observer */;
0:(1-S(12))*S(11)/(S(11) + S(12) - S(11)*S(12)) /* number seen by observer 1 when acting as second observer */;

LABELS;
S(1) = p1(pool 'A' AMAC);
S(2) = p2(pool 'A' AMAC);
S(3) = p1(pool 'B' AMAC);
S(4) = p2(pool 'B' AMAC);
S(5) = p1(pool 'B' RSYL);
S(6) = p2(pool 'B' RSYL);
S(7) = p1(pool 'C' AMAC);
S(8) = p2(pool 'C' AMAC);
S(9) = p1(pool 'D' AMAC);
S(10) = p2(pool 'D' AMAC);
S(11) = p1(pool 'D' RSYL);
S(12) = p2(pool 'D' RSYL);

proc estimate novar maxfn = 32000 name = p(.);
constraints; /* sets expected cell probabilities equal */
S(2) = S(1);
S(3) = S(1);
S(4) = S(1);
S(5) = S(1);
S(6) = S(1);
S(7) = S(1);
S(8) = S(1);
S(9) = S(1);
S(10) = S(1);
S(11) = S(1);
S(12) = S(1);

proc estimate novar maxfn = 32000 name = p(species);
constraints; /* sets expected cell probabilities which describe the same species equal */
S(2) = S(1);
S(3) = S(1);
S(4) = S(1);

```

---

## Appendix 1. Continued.

---

```

S(6) = S(5);
S(7) = S(1);
S(8) = S(1);
S(9) = S(1);
S(10) = S(1);
S(11) = S(5);
S(12) = S(5);

proc estimate novar maxfn = 32000 name = p(pool);
constraints; /* sets expected cell probabilities which describe the same pool equal */
S(2) = S(1);
S(4) = S(3);
S(5) = S(3);
S(6) = S(3);
S(8) = S(7);
S(10) = S(9);
S(11) = S(9);
S(12) = S(9);

proc estimate novar maxfn = 32000 name = p(observer);
constraints; /* sets expected cell probabilities which explain the same observer equal */
S(3) = S(1);
S(4) = S(2);
S(5) = S(1);
S(6) = S(2);
S(7) = S(1);
S(8) = S(2);
S(9) = S(1);
S(10) = S(2);
S(11) = S(1);
S(12) = S(2);

proc estimate novar maxfn = 32000 name = p(observer, species, pool);
initial; /* sets the values for S(I) back to the original values */
retain = p(observer); /* obtains initial estimates from output of p(observer) model */
constraints;
S(2) = 1;
S(3) = 1;
S(10) = 1;
S(12) = 1;
proc test;
proc stop;

```

---

See the SURVIV user's manual for explanation of the basic command syntax (<http://www.mbr-pwrc.usgs.gov/software/doc/surviv.html>). A group of COHORT statements followed by user-specified algebraic expressions allow the calculation of detection probability estimates. User-specified modeling constraints can be used to create multiple models based on the same general model structure. Counts of egg masses of each species are included in separate sets of COHORT statements to allow calculation of individual species' detection probabilities in the  $p(\text{species})$  model. Each <proc estimate> statement is followed by the name of the model, and a group of constraint statements that alter the individual cell probabilities [S(I)] and allow the calculation of detection probability estimates for each model. In the most general model (largest number of parameters), [ $p(\text{observer, pool, species})$ ], the code constrains a parameter equal to 1 when the COHORT = 0 [i.e., the parameters S(2), S(3), S(10) and S(12) are constrained to '1' in the final <proc estimate> constraints line], which allows the fitting of a general model to sparse data. The code (below, in bold) is then entered into the SURVIV program (<http://www.mbr-pwrc.usgs.gov/software/surviv.html>). Text enclosed between /\* and \*/ symbols describes each line of code.

*Appendix 2.* Example SURVIV code including a covariate, <max area>, which is the maximum pool area (as defined in the text). The explanations of the COHORT statements are identical to those described above, but the syntax is altered to allow inclusion of the logit functions specified in the <inline> statements. The individual cell probabilities [S(J)] are estimated when the measured covariate is entered for each pool surveyed [e.g.,  $V1 = \text{EXP}(S(1) + S(2) * (\text{max area})) / (1 + \text{EXP}(S(1) + S(2) * (\text{max area})))$ ]. The constraint statements are provided to bound the estimate between -10 and 10, and to further restrict the model for the detection probabilities. The code (below, in bold) is then entered into the SURVIV program (<http://www.mbr-pwrc.usgs.gov/software/surviv.html>). The p(slopes equal) model returns the point estimates and AIC values to use in model comparison. Values for other covariates can be entered to determine the most suitable model to explain the variation in detection.

---

```

PROC TITLE 'E. Massachusetts NWR detection probability analysis: Dependent double-observer method, including a covariate
<max area >';
PROC MODEL NPAR = 4 addcell;
/* Pool 'A' */
inline V1 = EXP(S(1) + S(2)*0.145)/(1 + EXP(S(1) + S(2)*0.145));
inline V2 = EXP(S(3) + S(4)*0.145)/(1 + EXP(S(3) + S(4)*0.145));
/* Pool 'B' */
inline V3 = EXP(S(1) + S(2)*0.304)/(1 + EXP(S(1) + S(2)*0.304));
inline V4 = EXP(S(3) + S(4)*0.304)/(1 + EXP(S(3) + S(4)*0.304));
/* Pool 'C' */
inline V5 = EXP(S(1) + S(2)*2.78)/(1 + EXP(S(1) + S(2)*2.78));
inline V6 = EXP(S(3) + S(4)*2.78)/(1 + EXP(S(3) + S(4)*2.78));
/* Pool 'D' */;
inline V7 = EXP(S(1) + S(2)*0.140)/(1 + EXP(S(1) + S(2)*0.140));
inline V8 = EXP(S(3) + S(4)*0.140)/(1 + EXP(S(3) + S(4)*0.140));
/* Pool 'A' */
COHORT = 1;
1:V1/(V1 + V2 - V1*V2);
COHORT = 0;
0:(1.-V2)*V1/(V1 + V2 - V1*V2);
/* Pool 'B' */
COHORT = 0;
0:V3/(V3 + V4 - V3*V4);
COHORT = 1;
0:(1.-V4)*V3/(V3 + V4-V3*V4);
COHORT = 1;
1:V3/(V3 + V4 - V3*V4);
COHORT = 19;
4:(1.-V4)*V3/(V3 + V4 - V3*V4);
/* Pool 'C' */
COHORT = 138;
121:V5/(V5 + V6 - V5*V6);
COHORT = 297;
9:(1.-V6)*V5/(V5 + V6 - V5*V6);
/* Pool 'D' */
COHORT = 1;
0:V7/(V7 + V8 - V7*V8);
COHORT = 0;
0:(1.-V8)*V7/(V7 + V8 - V7*V8);
COHORT = 2;
2:V7/(V7 + V8 - V7*V8);
COHORT = 0;
0:(1.-V8)*V7/(V7 + V8 - V7*V8);
labels;
S(1) = intercept observer_1;
S(2) = slope observer_1;
S(3) = intercept observer_2;
S(4) = slope observer_2;
proc estimate novar maxfn = 32000 name = p(slopes equal);
constraints;
-10 < S(1) < 10;
-10 < S(2) < 10;

```

---

## Appendix 2. Continued.

---

```

-10 < S(3) < 10;
S(4) = S(2);
initial;
S(2) = -1;
S(4) = -1;
proc estimate novar maxfn = 32000 name = p(area);
constraints;
-10 < S(1) < 10;
-10 < S(2) < 10;
-10 < S(3) < 10;
-10 < S(4) < 10;
initial;
retain = p(slopes equal);
proc test;
proc stop;

```

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