A HABITAT BASED DESIGN FOR SAMPLING AND MONITORING STREAM AMPHIBIANS WITH AN ILLUSTRATION FROM REDWOOD NATIONAL PARK

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ABSTRACT-We present a sampling method for use in small to medium-sized streams that is rigorous and repeatable, and contains the necessary flexibility to be species- or habitat-specific. Our habitat-based method generates large sample sizes for reliable statistical analyses yet remains cost effective. Streams are mapped, "habitat typed", and cross-stream sampling units (belts) are placed to systematically sample each stream based on habitat composition. Biotic and abiotic attributes of the habitat associated with each belt and animal capture are measured or estimated. We employed this sampling design to compare amphibian densities within streams in an old-growth coast redwood *(Sequoia sempervirens)* ecosystem in northwestern California. Our method targets stream-dwelling larval amphibians, but is also effective for adults and neotenes that share lotic habitats with larvae. This method provides baseline data on amphibian presence and abundance, as well as habitat-specific density estimates. It can also yield highly accurate population estimates for streams where sample sizes are sufficiently large.

"The first signs of environmental stress usually occur at the population level, affecting especially sensitive species" (Odum 1992, p 542). Amphibians are potentially excellent bioindicators of both qualitative and quantitative changes in environmental conditions because of their high densities, long life, site fidelity, and marked physiological sensitivity (Baringa 1990; Blaustein and Wake 1990; Vitt et al. 1990; Wake and Morowitz 1990; Wyman 1990; Wake 1991; Blaustein 1994) (but see Pechmann and Wilbur 1994). However, rigorous, repeatable sampling methods are needed to permit investigators to test and exploit the intrinsic advantages of amphibians as bioindicators.

Stream amphibians are challenging to sample because they are unevenly distributed, cryptically colored, and often hidden within the substrate. Corn and Bury (1989) used an area-constrained search technique for sampling stream amphibians that involved intensive searching of all substrates in a single, 10-m long plot located in a "typical" stretch of stream. They first walked a few hundred meters of the stream and then selected 1 site that appeared representative of the area they had viewed (purposive selection). However, a single purposively selected sample unit per stream does not allow assessment of variability within streams, and does not allow valid statistical analyses. Welsh (1987) modified Corn and Bury's (1989) method to include 3 systematically spaced, 5-m long sample units per stream, which provided a minimum estimate of variation and allowed a greater choice of analytic approaches. Bury and Corn (1991) later concurred in recommending a minimum of 3 samples per stream. None of these approaches, however, adequately addressed the problem of differential use of stream habitats by amphibian species, and the resulting potential for poor sample estimates.

The 2-stage designs proposed by Hankin (1984, 1986) for surveys of stream-dwelling fishes supposed that some unbiased method of estimation could be used to estimate numbers of fish present in natural stream habitat units. In his example applications, Hankin assumed that the within unit method of estimation relied on electrofishing and removal method estimation. This method is not effective for amphibians, however, because amphibians are often not in the water column or on the surface of the substrate (Corn and Bury 1989). Substrate-dwelling amphibians often remain under cover after being stunned, resulting in abundance estimates that are biased low. Hankin's method also required sampling the entire habitat unit for fish. In our experience, we have found that most habitat units are too large to allow use of the thorough sampling approach required for amphibian sampling.

Our proposed method for estimating amphibian abundance within selected habitat units is similar to that proposed by Shaffer and others (1994). It employs cross-stream belts which are essentially modified transects, patches (Jaeger 1994a,b), or quadrats (Jaeger and Inger 1994). However, we estimate population size and densities by habitat type instead of averaging across types (Shaffer and others 1994) because amphibians are often habitat-specific. Using our habitat-based method, streams are first stratified for sampling by habitat type as recommended by Hankin (1984, 1986), Shaffer and others (1994), Jaeger (1994a), Hayek (1994), Scott and Woodward (1994), Scott (1994), and Crump and Scott (1994). Habitat types are then sampled in proportion to their occurrence and size in order to sample a minimum amount of all available habitats (Hankin 1984, 1986). While Heyer and others (1994) offered many sampling strategies that can be habitat-specific (for example, Shaffer and others 1994; Jaeger 1994a; Jaeger and Inger 1994; Hayek 1994; Scott and Woodward 1994), there was little emphasis on sampling habitat relative to availability. Here, we articulate such an approach for lotic systems.

Actual monitoring of amphibian populations usually requires a methodology that can estimate population size and detect changes therein (Donnelly and Guyer 1994). Such methods should be considered only when absolutely necessary to address a specific research question because they are time-consuming and labor-intensive, requiring extensive data collection and analysis (Southwood 1978). Donnelly and Guyer (1994) present 2 such approaches using mark-recapture or removal sampling. Here we present a population estimator, based on our stratified habitat-based sampling method, that does not require multiple samplings to derive an initial population estimate.

Our objectives for this paper were to develop and test a sampling method for stream amphibians that is flexible, cost effective, and provides samples of sufficient size for reliable statistical analyses; and to devise a population estimator for use with this habitat-based sampling method.

HABITAT-BASED SAMPLING METHOD

Description of the Case Study

Construction of the Prairie Creek State Park Highway Bypass by Caltrans (California Department of Transportation) resulted in a large infusion of fine sediments into pristine streams in Prairie Creek Redwoods State Park (a state-owned adjunct to Redwood National Park) during an October 1989 storm. We used our habitat-based sampling to assess the impacts of these sediments on the densities of common aquatic amphibians in 5 sediment-impacted streams by comparing them with 5 nearby unimpacted streams (Fig. 1). All 10 streams were similar in physical attributes and adjacent forest vegetation. Our study objectives were to determine if there were differences in amphibian abundance between the 2 sets of streams, and to quantify the habitat associations of these amphibians to discern possible habitat-specific responses to sedimentation between the sets of streams (results to be published elsewhere). All sampling was completed by a 2-person crew from 13 June to 21 August 1990.

Mesohabitat Typing and Mapping of Streams

Our method was derived from fish population sampling methods developed by Hankin (1984, 1986) and Hankin and Reeves (1988), and stream habitat classifications developed by Bisson and others (1982), and McCain and others (1990). We modified Hankin's design to subsample within natural habitat units by randomly placing bank-to-bank belts for area-constrained searches (ACS; see Welsh 1987; Bury and Corn 1991; see also quadrat sampling Jaeger and Inger 1994; Shaffer and others 1994).

Before sampling for amphibians, each stream was mapped from mouth to headwaters. This mapping can include subdivision and classification of each stream at the level of geomorphological reach type (alluvial, braided, or confined) and channel type (Rosgen 1994), if streams vary among these categories. We simultaneously mapped (Fig. 2) and classified each stream habitat type (for example, low gradient riffle, lateral scour pool, run, glide; Bisson and others 1982; McCain and others 1990). These stream habitat types were defined as mesohabitats. There

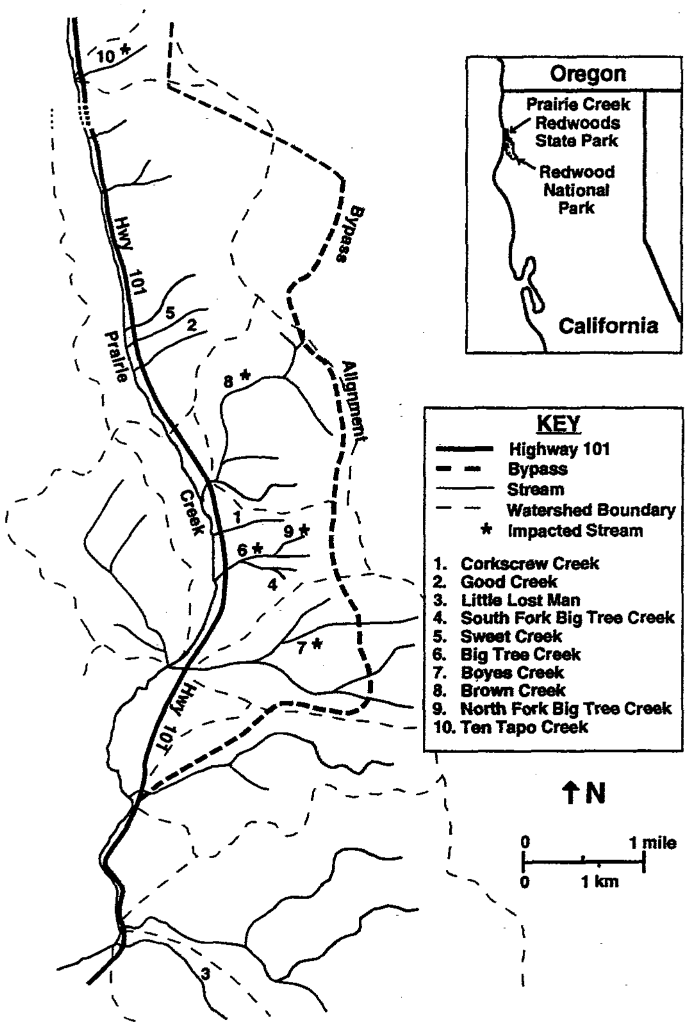

FIGURE 1. Geographic relationships of streams sampled in Prairie Creek Redwoods State Park and Redwood National Park, Humboldt County, California. Sampling occurred in summer, 1990. Stars indicate sediment impacted streams (see text for explanation).

may be up to 24 different mesohabitat types in an inventory, but it is unusual for any 1 stream to contain all types. Hawkins and others (1993) presented a simpler hierarchical system of stream habitat classification that may be better suited to a stratified sampling design; see Discussion.

Depending upon study objectives various physical variables can be measured during the inventory and mapping to describe each mesohabitat unit encountered (Appendix). We inventoried habitat-forming structures within the stream channel (for example, logs by species, position within the channel, and size), measured pool sediment (Lisle and Hilton 1992), and recorded a number of other variables.

Selection of Units for Amphibian Sampling

After streams were mapped, we selected quasi-systematic samples of individual mesohabitat units from within each mesohabitat type. For each mesohabitat type, we sampled the 1st mesohabitat unit located E of Highway 101 (Fig. 1). We then selected a random integer between 1 and 5 to locate the next unit to sample and we selected every 5th unit (k=5) thereafter until all mesohabitat units of a given type were exhausted. This method of selection allowed valid statistical comparisons between different habitat types by ensuring selection of $\geq$ 1 unit from each identified mesohabitat type, approximately equal sampling effort within each mesohabitat type, good

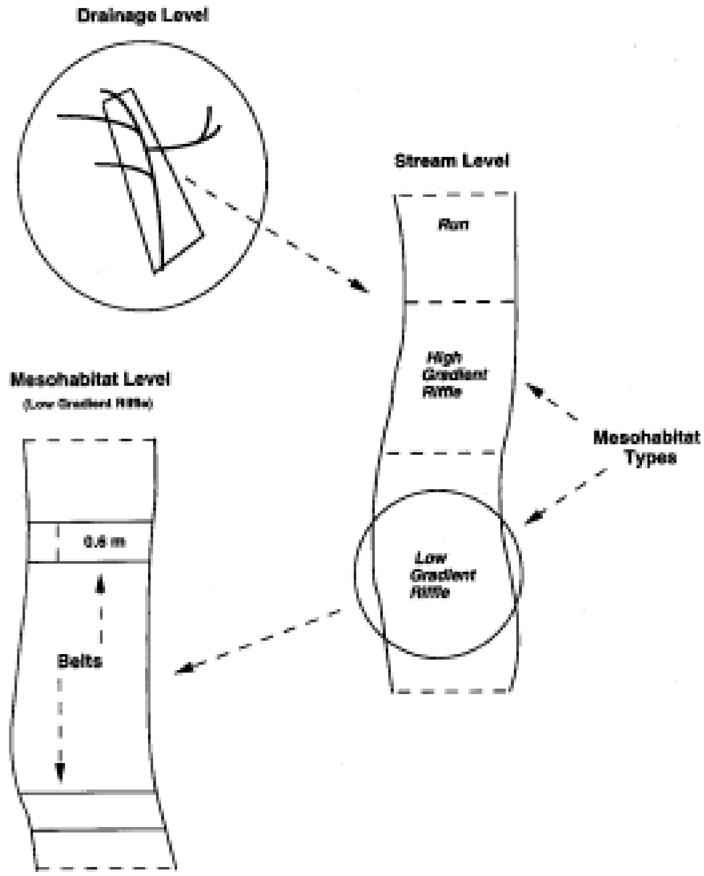

FIGURE 2. Schematic representation of random-systematic sampling design based on habitat structure of streams.

spatial coverage of selected units from among all those units of a given type, and independence of sampling among identified mesohabitat types. Sampling rate can be adjusted by increasing or decreasing the systematic selection interval. We discovered that selecting, with certainty, the 1st unit encountered does not conform to systematic sampling. We now recommend selecting the starting unit by randomly selecting an integer between 1 and k. Then, locate subsequent samples as described above.

Individual selected mesohabitat units were generally too long to allow complete enumeration of amphibian presence so that subsampling within the selected units was necessary. When complete enumeration of a unit was impossible, we counted numbers of amphibians within ≥ one, 0.6-m wide belt running bank to bank, perpendicular to stream flow (Fig. 3). In mesohabitat units < 10m long, we located a single belt, centered in the unit. In units > 10 m, we located a 1st belt at a random distance between 0 and 10 m from the downstream edge of the unit. Additional belts were placed every 10 m thereafter until the end of the unit was reached (Fig. 3).

ENUMERATION OF AMPHIBIANS

Belts were thoroughly searched for all amphibians using ACS. We first scan d the area for visible animals and then all cover objects were moved systematically, working upstream and across until the entire belt was searched. Animals were spotted using a glass or plexiglass bottomed box held at the water surface, and then captured with a metal mesh net (for example, a kitchen strainer) or a fabric aquarium net held downstream of the animal. Individual amphibians were identified, sexed, measured, and released. We replaced all cover objects after sampling. Belt width and area searched were recorded for later use in calculation of amphibian densities. We assumed that

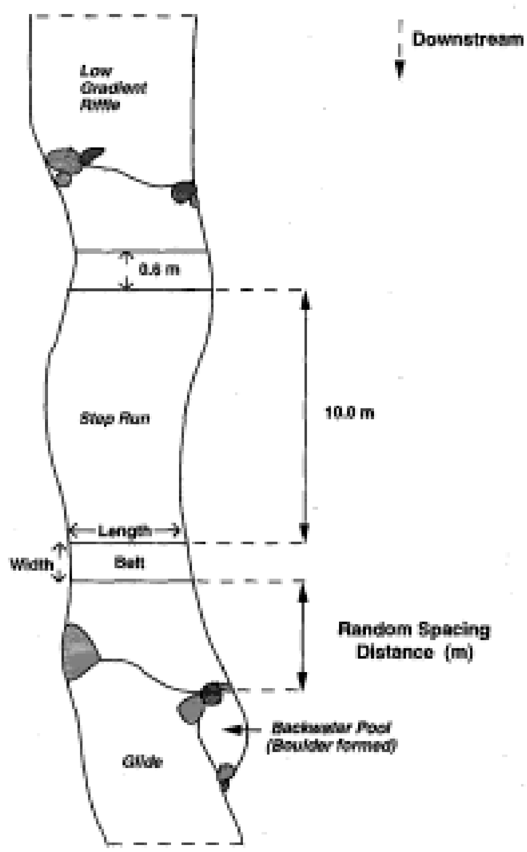

FIGURE 3. Schematic representation of random-systematic belt placement within a selected mesohabitat.

observed captures in a given belt were equal to the true number of amphibians present in that belt. The chance of missing an amphibian during a thorough search is believed slight (Bury and Corn 1989). It is possible that some animals may escape notice, however. Thus density and population estimates derived should be considered minimum values.

The formulas that we present below are generally consistent with the 2-stage formulas presented by Hankin (1984, 1986) and are appropriate for estimation of the total number of amphibians (of a given species) within a given mesohabitat type. Because each mesohabitat type is independently sampled, an estimate of the total number of amphibians present in an entire stream can be calculated by simply summing the estimates across individual mesohabitat types (strata). An estimate of sampling variance for the entire stream can be similarly calculated by summing estimated sampling variances across individual mesohabitat types. In the formulas presented below, we consider estimation for a single mesohabitat type (stratum). Definitions are provided in Table 1. Carats ("^") above symbols distinguish estimators (or estimated values) from true values.

Estimation Within a Selected Mesohabitat Unit

Because our original method of selecting belts within mesohabitat units does not exactly coincide with conventional methods for selecting random samples (see Discussion), we first estimate the approximate number of 0.6-m belts present within a given mesohabitat unit:

$$\hat{N}_i = \frac{L_i}{0.6m}. \qquad (1)$$

An estimate of the total number of amphibians present in unit *i* is based on expansion of the mean number of amphibians found in the n_i belts sampled in unit *i*:

$$\hat{Y}_i = \hat{N}_i \frac{\sum^{n_i} y_{ij}}{n_i} = \hat{N}_i \bar{y}_i, \qquad (2)$$

where $\bar{y}_i$ = mean number counted per belt in mesohabitat unit *i*. An approximate estimate of sampling variance (for $n_i > 1$) for the estimated number of amphibians present in mesohabitat unit *i* can be calculated as:

$$\hat{V}(\hat{Y}_i) = \hat{N}_i^2 \frac{(\hat{N}_i - n_i)}{\hat{N}_i n_i} \frac{\sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2}{(n_i - 1)}$$

$$= \hat{N}_i (\hat{N}_i - n_i) \frac{s_i^2}{n_i}, \qquad (3)$$

where

$$s_i^2 = \frac{\sum_{j=1}^{n_i} (y_{ij} - \bar{y}_i)^2}{n_i - 1}.$$

Note that when only 1 belt is counted within a selected mesohabitat unit (that is, $n_i = 1$), it is impossible to use equation 3 to calculate error of estimation.

Estimation For All Units in a Given Mesohabitat Type

Equations 1 and 2 allow estimation of the total number of amphibians present within a selected

TABLE 1. Definitions of terms used to calculate mean-per-unit and 2-stage ratio population estimators (based on Hankin 1984, 1986).

$i = 1, 2, 3, \ldots, N$ = unit label in mesohabitat type (stratum)

n = number of mesohabitat units selected for sampling = first stage sample size

n_i = number 0.6 m belts selected from the N_i such belts present within mesohabitat unit i; second stage sample size within mesohabitat unit i

N_i = number of 0.6 m belts possible in mesohabitat unit i

L_i = length of mesohabitat unit i

l_{ij} = length of belt j in mesohabitat unit i

y_{ij} = number of amphibians present in the jth 0.6 m belt within mesohabitat unit i, $j = 1,2,3,\ldots,N_i$

$$\bar{y}_i = \frac{\sum_{j=1}^{n_i} y_{ij}}{n_i} = \text{mean belt captures in mesohabitat unit } i$$

$$Y_i = \sum_{j=1}^{N_i} y_{ij} = \text{total number of amphibians present in mesohabitat unit } i$$

$$Y = \sum_{i=1}^{N} Y_i = \text{total number of amphibians present in all units}$$

$$W_i = \text{mean width of mesohabitat unit } i$$

$$M_i = L_i W_i = \text{area of mesohabitat unit } i$$

$$M_0 = \sum_{i=1}^{N} M_i = \text{total area of all mesohabitat units (of a given type)}$$

$$\bar{Y}_i = \frac{\hat{Y}_i}{M_i} = \text{mean number present per unit area in mesohabitat unit } i$$

$$\bar{\bar{Y}} = \frac{Y}{M_0} = \text{mean number of amphibians present per unit area in all mesohabitat units}$$

$$s_l^2 = \frac{\sum_{j=1}^{n_i} \left(y_{ij} - \bar{y}_i\right)^2}{\left(n_i - 1\right)} = \text{among belt variation in amphibians present within mesohabitat unit } i$$

$$\hat{V}\left(\hat{Y}_i\right) = \left(\frac{N_l^2\left(N_i - n_i\right)}{N_i}\right)\frac{s_l^2}{n_i} = \text{estimated second stage variance within mesohabitat unit } i$$

mesohabitat unit, and equation 3 allows calculation of errors of estimation within this same selected mesohabitat unit. Below we present 2 alternative methods for estimation of the total number of amphibians present in all units of a given mesohabitat type. The 1st method is a 2-stage "mean-per-unit" estimator, whereas the 2nd method is a 2-stage ratio estimator that accounts for the area surveyed as compared to the total area present. The mean-per-unit estimator is unbiased but may have high sampling variance when true unit counts are highly correlated with unit areas. Although the ratio estimator is not unbiased, bias is normally small and sampling variance may be reduced if unit counts are highly correlated with unit areas. For both approaches, overall errors of estimation (estimated sampling variances) have 2 basic components: errors that originate from uncertainty in numbers of amphibians present within selected mesohabitat units (2nd-stage errors) and errors that originate from extrapolation from the small number of sampled mesohabitat units, n, to the total number of mesohabitat units, N (1st-stage errors). See Hankin (1984, 1986) and Cochran (1977) for further discussion.

Mean Per Unit Expansion.-In the formulas presented below, we treat our quasi-systematic sample of size *n* as if it were a true systematic sample and we use formulas appropriate for simple random sampling to estimate sampling variance. However, use of simple random sampling formulas usually results in overestimation of true sampling variance when systematic sampling is used to select units (Hankin 1984, 1986; Cochran 1977). Formulas presented below are thus "conservative" in the sense that, on average, they are likely to overestimate (rather than underestimate) true errors of estimation that may arise by the methods that we propose.

By this method, the total number of amphibians present in all mesohabitat units is estimated by simple expansion using:

$$\hat{Y}_{mpu} = N \sum_{i=1}^{n} \frac{\hat{Y}_i}{n}. \tag{4}$$

An estimate of sampling variance for this estimator may be calculated as:

$$\hat{V}\left(\hat{Y}_{mpu}\right) = N^2 \frac{(N-n)}{Nn} \frac{\sum^{n}\left(\hat{Y}_i - \hat{\bar{Y}}\right)^2}{(n-1)} + N \frac{\sum^{n} \hat{V}\left(\hat{Y}_i\right)}{n}, \tag{5}$$

where

$$\hat{\bar{Y}} = \frac{\sum^{n} \hat{Y}_i}{n}$$

= estimated mean per mesohabitat unit.

Ratio Estimator.-By this procedure:

$$\hat{Y}_R = M_0 \frac{\sum^{n} \hat{Y}_i}{\sum^{n} M_i} \tag{6}$$

An estimate of sampling variance for equation 6 can be calculated using:

$$\hat{V}\left(\hat{Y}_R\right) = N^2 \frac{(N-n)}{Nn} \frac{\sum^{n} M_i^2 \left(\hat{\bar{Y}}_i - \hat{\bar{\bar{Y}}}^2\right)}{(n-1)} + N \frac{\sum \hat{V}\left(\hat{Y}_i\right)}{n}, \tag{7}$$

where

$$\hat{\bar{Y}}_i = \frac{\hat{Y}_i}{M_i}; \hat{\bar{\bar{Y}}} = \frac{\hat{Y}_R}{M_0}.$$

Equation 7 may tend to underestimate true sampling variance for sample sizes < 12 (Cochran 1977), whereas equation *5* provides an unbiased estimate of sampling variance for all sample sizes.

When only a single belt was counted in a selected mesohabitat unit, we calculated the second terms in equations *5* and 7 using an "effective" sample size equal to the number of mesohabitat units for which $n_i > 1$. For the 1st terms in equations 5 and 7, we used the full sample size, *n* (number of mesohabitat units sampled).

RESULTS

Stream Typing, Mapping and Amphibian Sampling

We present summaries of the distances surveyed and time required to map and habitat type all 10 streams, along with the number of mesohabitat types encountered (Table 2). We also provide a breakdown of the belt sampling by stream, including: total belts searched, belts with captures, mean area of the belts, person-hours expended, and percent of total stream habitat area sampled (Table 2). We sampled approximately 2% of the available mesohabitat area of each stream using the spacing and belt size described above. Additionally, we present capture summaries by habitat type for the 3 most abundant species: the tailed frog, *Ascaphus truei*, the Pacific giant salamander, *Dicamptodon tenebrosus*, and the southern torrent salamander, *Rhyacotriton variegatus* (Table 3).

Abundance Estimates

To illustrate calculations at the various steps in estimating amphibian abundance, we present the set of step pool habitat units typed and sampled in Little Lost Man Creek in Redwood National Park, Humboldt County, California in 1990 (Table 4). Measurements reported are in meters. During habitat typing, 13 step pool habitat units were encountered (Table 4). Thus, N, the total number of mesohabitat units in the sampling universe was 13.

Four of the habitat units were selected by our systematic sampling design for amphibian sampling: units 1, 3, 8, and 13 (Table 4). Thus, *n*, the total number of mesohabitat units in the sample was 4. The belts and their corresponding measurements are then listed. For belt 1 in

TABLE 2. Time requirements and yield of habitat-based sampling on 10 streams in Prairie Creek Redwoods State Park and Redwood National Park in summer 1990. Both stages of sampling, mesohabitat typing (Bisson et al. 1982; McCain et al. 1990) and area-constrained sampling (belts) are shown.

	Mesohabitat typing			Belt sampling				
Stream	Km surveyed	Person-hours	Meso-habitat types surveyed	Belts	Belts with captures	Mean belt area	Person-hours	Proportion habitat sampled
Big Tree	1.104	27.50	12	39	30	1.26	59.50	0.02
Boyes	0.499	4.66	7	17	6	1.34	6.50	0.02
Brown	0.987	14.00	13	42	32	1.68	28.66	0.03
Corkscrew	0.505	13.00	8	17	13	0.68	11.50	0.03
Good	0.910	33.50	10	37	26	1.05	42.00	0.02
Little Lost Man	0.993	12.00	10	37	29	1.98	26.00	0.02
N. Fork Big Tree	0.832	10.66	10	25	13	0.98	9.00	0.02
S. Fork Big Tree	0.069	5.50	7	7	6	0.57	6.66	0.08
Sweet	1.013	13.00	10	32	22	0.73	14.00	0.02
Ten Tapo	0.528	4.66	5	14	8	0.77	6.00	0.02
Total	7.440	138.32	20	267	184	1.23	209.82	0.02

step pool 1 these values are: $l_{1,1}$ = 0.6, $y_{1,1}$ = 5 captures.

Data are then summarized to the mesohabitat level beginning with sample size. There were 3 belts sampled in step pool 1 (n_1). This is followed by determining the total number of belts possible in step pool 1 (N_1) using equation 1:

$$\hat{N}_1 = \frac{32.0}{0.6} = 53.33.$$

The mean number of amphibians present per belt in mesohabitat 1 is then estimated using equation 2:

$$\bar{y}_1 = \frac{(5+0+0)}{3} - 1.67.$$

TABLE 3. Total numbers captured of 3 larval aquatic amphibian species sampled in 10 streams in Prairie Creek Redwoods State Park and Redwood National Park in summer 1990. Numbers of adults shown in parentheses.

Mesohabitat type	Units surveyed	Units sampled	*Ascaphus truei*	*Dicamptodon tenebrosus*	*Rhyacotriton variegates*
Low-gradient riffle	101	26	25 (1)	36	10
High-gradient riffle	80	20	37 (1)	27	8
Cascade	4	1	0	0	0
Run	29	12	4	13	0
Glide	6	3	0	1	0
Step run	83	26	95 (2)	77	9
Pocket water	1	1	0	0	0
Step pool	98	31	32 (1)	72 (3)	12
Channel confluence pool	1	1	0	3	0
Dammed pool	10	4	0	7	0
Plunge pool	22	10	4	8	0
Lateral scour pool bedrock	20	9	1	8 (1)	0
Lateral scour pool boulder	4	3	0	2	0
Lateral scour pool log	47	15	3 (1)	29 (1)	0
Lateral scour pool rootwad	9	3	0	1	0
Mid-channel pool	3	2	1	0	0
Corner pool	6	4	3	6	0
Secondary channel pool	4	3	0	5	0
Backwater pool boulder	2	2	0	0	0
Backwater pool log	4	3	0	1	0
Total	534	179	205 (6)	296 (5)	39

TABLE 4. Data for this example calculation of population size using a two-stage estimator (Hankin 1984, 1986) were collected in Little Lost Man Creek, Redwood National Park, in 1990. Step pool mesohabitat, belt, and Pacific giant salamander (*Dicamptodon tenebrosus*) capture data are reported. Units mapped but not selected by our stratified sampling design for amphibian sampling are indicated by a dash (-).

Step pool habitat unit (i)	Mesohabitat: Length (L_i)	Mesohabitat: Width (W_i)	Mesohabitat: Area (M_i)	Belt: Belt number (j)	Belt: Length (l_{ij})	Belt: Salamander captures (y_{ij})	Belt: # Belts sampled (n_i)	Belt: Mean belt captures ($\bar{y}_i$)	Belt: Variance among belts (s_i^2)	Total # salamanders present ($\hat{Y}_i$)	Mean salamander density ($\hat{\bar{Y}}_i$)	Total # belts possible (N_i)	Second stage variance ($\hat{V}(\hat{Y}_i)$)
1	32.0	3.8	121.60	1	0.6	5	3	1.67	8.33	89.06	0.73	53.33	7452.85
				2	0.6	0							
				3	0.6	0							
2	15.3	3.3	50.49	-		-							
3	38.8	4.0	155.20	1	0.6	2	4	1.25	2.25	80.84	0.52	64.67	2206.99
				2	0.6	0							
				3	0.6	0							
				4	0.6	3							
4	52.8	3.7	195.36	-		-							
5	41.2	3.9	160.68	-		-							
6	20.5	3.7	75.85	-		-							
7	74.1	4.0	296.40	-		-							
8	6.4	4.0	25.60	1	0.6	1	1	1.00	–	10.67	0.42	10.67	–
9	39.3	3.0	117.90	-		-							
10	18.3	3.5	64.05	-		-							
11	19.8	5.5	108.90	-		-							
12	85.6	3.7	316.72	-		-							
13	56.4	2.0	112.80	1	0.6	2	5	1.00	0.50	94.00	0.83	94.00	836.60
				2	0.6	1							
				3	0.6	1							
				4	0.6	0							
				5	0.6	1							
		M_0 =	1796.02										

TABLE 5. Population estimates derived using two-stage mean-per-unit and ratio estimators (Hankin 1984, 1986). Data are from two streams sampled in Prairie Creek Redwoods State Park and Redwood National Park in summer 1990. Estimates are of Pacific giant salamander (*Dicamptodon tenebrosus*) population size in step pool and step run habitat types.

		Mean-per-unit estimator			Ratio estimator		
Stream/ habitat type	N	Total # salamanders present ($\hat{Y}_{mpu}$)	Estimated variance ($\hat{V}(\hat{Y}_{mpu})$)	95% confidence interval (CI)	Total # salamanders present ($\hat{Y}_R$)	Estimated variance ($\hat{V}(\hat{Y}_R)$)	95% confidence interval (CI)
Little Lost Man							
Step pools	4	892.35	90,036.88	892.35 ± 954.80 (0-1847.15)	1187.70	54,747.34	1187.70 ± 744.53 (443.17-1932.23)
Step runs	3	2405.76	387,447.00	2405.76 ± 2678.41 (0-5084.17)	1538.33	847,168.37	1538.33 ± 3960.56 (0-5498.89)
Brown Creek							
Step pools	4	682.53	91,596.60	682.53 ± 963.03 (0-1645.56)	468.63	37,374.62	468.63 ± 615.16 (0-1083.79)
Step runs	3	781.92	31,974.71	781.92 ± 769.44 (12.48-1551.36)	510.19	37,554.69	510.19 ± 833.88 (0-1344.07)

This value is then used in calculation of the among belts variation (s_1^2) and total number of amphibians present in step pool 1 ($\hat{Y}_i$, equation 2). These are estimated as:

$$s_1^2 = \frac{\left[(5-1.67)^2 + (0-1.67)^2 + (0-1.67)^2\right]}{(3-1)}$$

$$= 8.33;$$

$$\hat{Y}_1 = (53.33)1.67 = 89.06.$$

These estimates are then followed by deriving an approximate estimate of sampling variance (a 2nd-stage variance) for the estimated numbers of amphibians present in step pool 1. This is calculated using equation 3 as follows:

$$\hat{V}(\hat{Y}_1) = 53.33(53.33-3)\frac{8.33}{3} = 7452.85.$$

These base estimates are then used to calculate either of the 2-stage population estimates and their corresponding estimates of sampling variance (Hankin 1984, 1986). Results from these estimators when applied to 2 habitat types (step pools and step runs) within 2 streams in Prairie Creek Redwoods State Park and Redwood National Park are shown in table 5. We continue with the step pool example from Little Lost Man Creek to illustrate the 2 estimators.

Mean-per-unit estimator.-Using equations 4 and 5 we calculate the mean-per-unit estimate as follows (equation 4):

$$\hat{Y}_{mpu} = 13\frac{(89.06+80.84+10.67+94.00)}{4}$$

$$= 892.35.$$

Calculation of the estimate of sampling variance (equation 5) requires the calculation of the estimated mean number of amphibians per step pool unit,

$$\hat{\bar{Y}} = \frac{(89.06+80.84+10.67+94.00)}{4} = 68.64,$$

followed by the calculation of estimated sampling variance:

$$\hat{V}(\hat{Y}_{mpu})$$

$$= (13)^2 \frac{(13-4)}{(13)(4)}$$

$$\cdot \{[(89.06 - 68.64)^2 + (80.84 - 68.64)^2 + (10.67 - 68.64)^2 + (94.00 - 68.64)^2] / (4 - 1)\}$$

$$+ 13 \frac{(7452.85 + 2206.99 + 0 + 836.60)}{3}$$

$$= 90036.88.$$

Ratio estimator.-Using equations 6 and 7 we calculate the estimated total and estimated sampling variance as follows:

$$\hat{Y}_R =$$

$$1796.02\frac{(89.06+80.84+10.67+94.00)}{(121.60+155.20+25.60+112.80)}$$

$$= 1187.70.$$

Calculation of the estimate of sampling variance requires calculation of the estimated mean number of amphibians per step pool unit (table 4) and the mean number of amphibians present per unit area (m^2) in all the step pool units,

$$\hat{\bar{\bar{Y}}} = \frac{1187.70}{1796.02} = 0.66,$$

followed by the calculation of the estimated sampling variance:

$$\hat{V}(\hat{Y}_R)$$

$$= 13^2\frac{(13-4)}{(13)(4)}$$

$$\{[121.6^2(0.73 - 0.66)^2$$
$$+ 155.2^2(0.52 - 0.66)^2$$
$$+ 25.6^2(0.42 - 0.66)^2$$
$$+ 112.8^2(0.83 - 0.66)^2]/(4 - 1)\}$$
$$+13\frac{(7452.85+2206.99+0+836.60)}{3}$$

$$= 54747.34.$$

DISCUSSION

We believe that our proposed habitat-based 2-stage survey designs represent important advancements over previous single- and 3-sample methods (Welsh 1987; Corn and Bury 1989; Bury and Corn 1991). The most important advancement in our proposed designs is that they allow calculation of errors of estimation. Alternative procedures rely on ill-specified and/or purposive selection of sample reaches from which it is impossible to calculate statistically valid errors of estimation (for example, methods used or proposed by Welsh 1987; Corn and Bury 1989; Bury and Corn 1991). Our proposed primary survey units are also natural stream mesohabitat units. Streams should be sampled as a collection of habitat units so that structural variability among sample units along a study reach is incorporated into the sampling design (Hankin 1984, 1986; Hawkins and others 1993). As previously noted by Hankin (1984, 1986), stratification by mesohabitat type should result in considerable reduction in overall sampling variance and should also produce much greater understanding of the relationships between distribution, abundance, and habitat. In previous amphibian survey methods, which essentially ignore the underlying variation in habitat quality within fixed survey reaches, it is impossible to compare amphibian densities among mesohabitat types. For our proposed survey designs, confidence intervals for estimated population size can be constructed for each identified mesohabitat type and densities of amphibians may be validly compared among mesohabitat types. An effective sampling technique should address differential use of stream habitats by amphibian species.

The greater problem with the habitat-based stratified design as implemented in this study, is inconsistency in identification of the large number of habitat types required by Bisson and others (1982) and McCain and others (1990). This difficulty can be somewhat overcome with a thorough course in habitat type identification prior to sampling. The more complex mesohabitat typing proposed by McCain and others (1990) may lead to improved understanding of fine-scale habitat features through analysis of amphibian abundance. The flexibility of this sampling design permits application to small- or large-scale questions about aquatic amphibians and may be used to gather habitat- or species-specific data or both. Sampling intensity is easily adjusted depending on the needs of the investigator or resource limits. We caution, however, that when employing this method for estimation of population size, > 2 belts per habitat unit are essential to derive an accurate estimate of variance.

As an alternative to using the 24 types proposed by McCain and others (1990), mesohabitat mapping could be performed using habitat composites as described by Hawkins and others (1993) and Hankin and Reeves (1988). The advantages of using the simpler systems advocated by the latter authors are a reduction in ambiguity of classification, and an increase in ease of repeatability in unit classification among observers. Achieving large sample sizes sufficient for population estimation may also be easier and more cost effective to attain employing the habitat composites.

Our design is effective for sampling larval amphibians and aquatic or neotenic adult amphibians in a lotic environment. It will generate baseline information (inventories), data on species' habitat associations, and population estimates. This method provides a framework for repeated sampling; such as that required for monitoring. Because the method can be species- or habitat-specific, sampling of breeding sites as described by Scott and Woodward (1994) is also possible.

Two-person crews can accomplish the sampling with efficiency. We recommend the use of plexi-glass bottomed boxes to maintain a high detection rate and reduce the potential for differences between observers in capture success. However, this method should not be used when the water is cloudy, too deep for movement of cover objects, or if the majority of cover objects are too large to move. Our method may be inappropriate in large streams (> 10-m wide), which tend to have large, deep pools that may be difficult to search without snorkeling.

During mesohabitat mapping and typing, sample units should be marked carefully for easy relocation and monitoring. Lengths (0.5 m) of 1 cm steel concrete reinforcement bar (rebar), embedded bankside with attached aluminum tags, are inexpensive, unobtrusive, permanent markers. However, repeated intensive sampling of the same areas over brief periods of time can result in habitat degradation and reduced capture success (Welsh 1987).

In studies of amphibians it is essential to know the behavior and reproductive cycles of the sampled species (Scott and Seigel 1992; McDiarmid 1994). Such knowledge permits timing of sampling so breeding pulses or migrations of adults out of streams during winter-spring flows do not bias the data. Additional sampling techniques may be needed for highly mobile species or those with dual life stages that may not be adequately sampled by a strictly aquatic technique (Welsh 1987; Corn and Bury 1990; Heyer and others 1994).

Methods presented in this paper represent our initial attempt to adapt the stream survey designs suggested by Hankin (1984, 1986) to surveys of amphibians in small streams. In future applications, we propose to adopt several minor changes that should make our approaches better conform with conventional sampling theory. In particular, we recommend the following modifications of the methods reported in this paper:

(1) Strict systematic samples should be selected rather than the quasi-systematic samples used in our preliminary applications. When preexisting maps show that some mesohabitat types are represented by relatively few units, set the systematic selection interval, k, so as to ensure that ≥ 2 units of every mesohabitat type will be selected.
(2) Within selected mesohabitat units, selection methods should guarantee that ≥ 2 belts are selected (to allow estimated errors of estimation within individual units), and selection methods should produce an integer number of belts within each selected mesohabitat unit.
(3) We propose adoption of some simpler stratification of mesohabitat types than the 24-type classification proposed by Bisson and others (1982) and McCain and others (1990), thus simplifying unit classifications and increasing sample sizes within mesohabitat types.

In future work, we plan to carry out formal comparisons of the performance of our proposed designs with those advocated by other researchers. Based on data we have thus far collected using methods described in this paper, we should be able to construct a reasonable hypothetical representation of the distribution and configuration of habitat units and representative amphibians in an entire small stream. Given such a hypothetical sampling universe, it then becomes possible to explore the performance of alternative sampling designs (see Hankin 1984). From such comparisons, we hope to provide additional evidence in support of the superiority of our proposed procedures and we hope also to provide better guidance regarding choice and tradeoffs among belt width, numbers of belts sampled per unit, and number of mesohabitat units surveyed.

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APPENDIX

Sampling procedures and variables measured or estimated in association with amphibian searches of quasi-systematically selected mesohabitat units in streams of the Prairie Creek drainage (Prairie Creek Redwoods State Park and Redwood National Park), northwestern California. See Fig. 2 for sampling design. Mesohabitat typing after Bisson et al. (1982) and McCain et al. (1990).

Each stream was habitat typed to mesohabitat level from mouth to headwaters. The 1st unit of every type east of Highway 101 was sampled, then a random unit between 2 and 6, and every 5th unit thereafter. Each mesohabitat type was sampled relative to availability. The mesohabitat mapping yielded 20 types.

I. Measurements and estimates made at every mesohabitat as the stream was mapped and typed prior to amphibian sampling. These variables were taken as part of the mapping and were used to characterize each unit and quantify the total amount of each mesohabitat type on each stream.
   A. Mean water depth: average of depths taken at 3 to 5 points across the unit at the top, middle, and bottom of each mesohabitat (cm).
   B. Mean channel width: average of 3 measurements taken at the top, middle, and bottom of the mesohabitat unit (m).
   C. Mesohabitat length: measured along the mid-channel line of each unit (m).
   D. Canopy open: % estimated at center of mesohabitat.
   E. Slope: % measured in the center of channel from mesohabitat bottom facing upstream (clinometer).
   F. Aspect: (degrees 0-360) taken facing downstream in the top center of the mesohabitat unit. Values were converted to one of 9 directions for analysis (1 = north, 9 = south).

G. Pools:
1. Maximum mesohabitat depth (cm).
2. Pool tail embeddedness: visual estimate (%-see below).
3. Depth at pool tail crest (cm).
4. Sediment depth in the pool bowl (measured at the top, middle, and bottom)(cm). Measured along the center line of the pool bowl.

II. Downed logs were counted during the mesohabitat inventory. Logs were classified (irrespective of log length) into conifer or hardwood in three DBH classes (DBH = diameter at breast height; classes used were: 13 to 52 cm, 53 to 127 cm, and ≥ 128 cm).

III. Vegetation plots were placed at every 5th habitat sampled for amphibians. Each vegetation sample consisted of two, 10 X 30 m rectangular blocks placed at the center of the mesohabitat sampled and reached 30 m up each bank. Measurement for plot placement started at the bank edge.
A. Tree counts: by DBH class and species within each block.
B. Logs, stumps, and snags were counted within each plot (assessment of potential woody debris recruitment into the stream).
C. Aspect (in degrees) of each plot (direction slope faces) (compass).
D. Slope of each plot (%) (clinometer).
E. Width of stream (nearest 0.1 m).
F. Width of bank (m, measured from water edge to upslope edge of riparian vegetation).
G. Width of vegetation overhanging the stream (nearest 0.1 m, measured from the bank to the edge of the vegetation).

IV Belt samples for amphibians generated captures/ mz. Belts were bank to bank and 0.6 m wide. They were placed randomly from the downstream edge of the mesohabitat unit (0 to 10 m) and then every 10 m thereafter. Belts were placed in the middle of habitats less than 10 m in length; we later discovered that this practice prevented calculation of a within-habitat type variance and now recommend the approach described in the text.
A. The following estimates and measurements of the physical habitat were taken in conjunction with these searches:
1. Climatic data:
-weather at time of search in 4 categories (cloud cover, precipitation, temperature, and wind).
-air temperature (°C).
-relative humidity (%) (sling psychrometer).
2. Water temperature °C.
3. Algal cover: visual estimate (%) of belt substrate covered by filamentous and non-filamentous algae.
4. Cemented: estimate (%) of belt substrate that was immovable.
5. Embedded: estimate (%) of belt substrate buried in fine sediments (silt or sand).
6. Canopy open: (%) measured at center of belt (spherical densiometer).
7. Belt length (m).
8. Instream cover of belt (%) (visual estimate):
-undercut banks.
-small vegetative debris (diameter <13 cm, included leaves and twigs).
-large woody debris (diameter >13 cm).
-terrestrial vegetation (height <30 cm from water surface).
-aquatic vegetation (rooted in water and hanging within 30 cm of water surface).
-white water: bubble cloud on water surface caused by turbulence.
-boulders (area available as cover under edges).
-bedrock ledges (underwater area available under edges).
-area without instream cover (sum of above categories subtracted from 100%).
9. Substrate composition of belt, visual estimates (%) based on Platts et al. (1983):

Fine sediment = <0.06 mm.
Sand = 0.06 to 2.0 mm.
Gravel = 2.0 to 32.0 mm.
Pebble = 32.0 to 64.0 m, m.
Cobble = 64.0 to 256.0 mm.
Boulder = >256.0 mm.
Bedrock.
Small vegetative debris.
Large woody debris.

10. Channel flow composition: visual estimate (%) thalweg, intermediate, and margin.
11. Flow rates at 3 channel positions (thalweg, intermediate, and margin) were measured with a flow meter (cm / sec).

B. The following data were recorded with each amphibian capture:
1. Species.
2. Channel position (margin, intermediate, or thalweg).
3. Microhabitat (characterize habitat immediately around the capture as pool, riffle, run, cascade, etc.).
4. Water depth (cm).
5. Position of capture (on or in) interpreted relative to substrate.
6. Substrate (see IV A-11).
7. Cover (see IV A-10 plus root, bark, log, shrub, and fern).
8. Sex and stage (if possible).
9. Animal length (cm, total and snout-vent).
10. Tail autotomy (none, nicked, tail lost, tail bud).

V. Sediment samples were collected from above each sample belt, removed with a small shovel, and placed in sediment collection bags. Sediments were dried and sieved to determine dry weight of each particle size in 10 categories (Platts et al. 1983). The > 32 mm size category was deducted from the total weight of each sample because this size category (pebbles and larger) was not consistently sampled by this method. The 10 categories were combined into 5 for the analysis:

Sediment_silt	=	<0.063 mm.
Sediment_fine sand	=	0.063 - 0.50 mm.
Sediment_coarse sand	=	0.50 to 2.00 mm.
Sediment_fine gravel	=	2.0 to 16.0 mm.
Sediment_coarse gravel	=	16.0 to 32.0 mm.