CHAPTER 9

SEQUENTIAL SAMPLING

(Version 5, 15 March 2013)

Page

Ecologists usually sample populations and communities with the classical statistical approach outlined in the last chapter. Sample sizes are thus fixed in advance by the dictates of logistics and money, or by some forward planning as outlined in Chapter 7. Classical statistical analysis is then performed on these data, and the decision is made whether to accept or reject the null hypothesis. All of this is very familiar to ecologists as the classical problem of statistical inference.

But there is another way. *Sequential sampling* is a statistical procedure whose characteristic feature is that *sample size is not fixed in advance*. Instead, you make

observations or measurements one at a time, and after each observation you ask the accumulated data whether or not a conclusion can be reached. Sample size is thus minimized, and in some cases only half the number of observations required with classical sampling are needed for sequential sampling (Mace 1964). The focus of sequential sampling is thus decision making, and as such it is most useful in ecological situations which demand a decision – should I spray this crop or not, should I classify this stream as polluted or not, should I sample this population for another night or not? If you must make these kinds of decisions, you should know about sequential sampling. The focus of sequential sampling is to minimize work, and for ecologists it can be a useful tool for some field studies.

The critical differences between classical and sequential sampling are:

	Sample size	Statistical inference
Classical statistical analysis	Fixed in advance	Two possibilities Fail to reject null hypothesis Reject null hypothesis
Sequential analysis	Not fixed	Three possibilities Fail to reject null hypothesis Reject null hypothesis Uncertainty (take another sample)

The chief advantage of sequential sampling is that it minimizes sample size and thus saves time and money. It has been applied in ecological situations in which sampling is done serially, so that the results of one sample are completed before the next one is analyzed. For example, the number of pink bollworms per cotton plant may be counted, and after each plant is counted, a decision can be made whether the population is dense (= spray pesticide) or sparse (do not spray). If you do not sample serially, or do not have an ecological situation in which sampling can be terminated on the basis of prior samples, then you must use the fixed sample approach outlined in Chapter 7. For example, plankton samples obtained on oceanographic cruises are not usually analyzed immediately, and the cost of

sampling is so much larger than the costs of counting samples on shore later, that a prior decision of a fixed sample size is the best strategy.

There is a good discussion of sequential sampling in Dixon and Massey (1983) and Mace (1964), and a more theoretical discussion in Wetherill and Glazebrook (1986). Morris (1954) describes one of the first applications of sequential sampling to insect surveys, and Nyrop and Binns (1991) provide an overview for insect pest management.

9.1 TWO ALTERNATIVE HYPOTHESES

We shall consider first the simplest type of sequential sampling in which there are two alternative hypotheses, so that the statistical world is black or white. For example, we may need to know if insect density is *above or below* 10 individuals/leaf. Or whether the sex ratio is *more* than 40% males or *less* than 40% males. These are called one-sided alternative hypotheses in statistical jargon because the truth is either A-or B, and if it is not A it must be B.

9.1.1 Means From A Normal Distribution

To illustrate the general principles of sequential sampling, I will describe first the application of sequential methods to the case of variables that have a normal, bell-shaped distribution. As an example, suppose that you have measured the survival time of rainbow trout fry exposed to the effluent from a coal-processing plant. If the plant is operating correctly, you know from previous laboratory toxicity studies that mean survival time should not be less than 36 hours. To design a sequential sampling plan for this situation, proceed as follows:

1. *Set up the alternatives you need to distinguish*. These must always be phrased as *either-or* and are stated statistically as two hypotheses; for example,

H_1: mean survival time $\leq$ 36 hours

H_2: mean survival time $\geq$ 40 hours

Note that these two alternatives must not be the same, although they could be very close (e.g. 36 and 36.1 hours instead of 36 and 40 hours).

The alternatives to be tested must be based on prior biological information. For example, toxicity tests could have established that trout fry do not survive on average longer than 36 hours when pollution levels exceed the legal maximum. The alternatives selected must be carefully chosen in keeping with the ecological decisions that will flow from accepting H_1 or H_2. If the two alternatives are very close, larger sample sizes will be needed on average to discriminate between them.

2. *Decide on the acceptable risks of error* α *and* β. These probabilities are defined in the usual way: α is the chance of rejecting H_1 (and accepting H_2) when in fact H_1 is correct, and β is the chance of rejecting H_2 (and accepting H_1) when H_2 is correct. Often $\alpha = \beta = 0.05$ but this should not be decided automatically since it depends on the risks you wish to take. In the rainbow trout example, when legal action might occur, you might assign $\alpha = 0.01$ to reduce Type I errors and be less concerned about Type II errors and assign $\beta = 0.10$.*

3. *Estimate the statistical parameters needed*. In the case of means, you must know the standard deviation to be expected from the particular measurements you are taking. You may know, for example, that for rainbow trout survival time, $s = 16.4$ hours, from previous experiments. If you do not have an estimate of the standard deviation, you can conduct a pilot study to estimate it (see page 000).

All sequential sampling plans are characterized graphically by one or more sets of parallel lines, illustrated in Figure 9.1. The equations for these two lines are:

$$\text{Lower line: } Y = bn + h_1 \qquad (9.1)$$

$$\text{Upper line: } Y = bn + h_2 \qquad (9.2)$$

where:

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* The usual situation in statistics is that α and β are related through properties of the test statistic and thus cannot be set independently. The reason that this is not the case here is that the two alternative hypotheses have been set independently.

b = Slope of lines
h_1 = y-intercept of lower line
h_2 = y-intercept of upper line
n = Sample size
Y = Measured variable

The slope of these lines for means from a normal distribution is estimated from:

$$b = \frac{\mu_1 + \mu_2}{2} \qquad (9.3)$$

where:

b = Slope of the sequential sampling lines
μ_1 = Mean value postulated in H_1
μ_2 = Mean value postulated in H_2

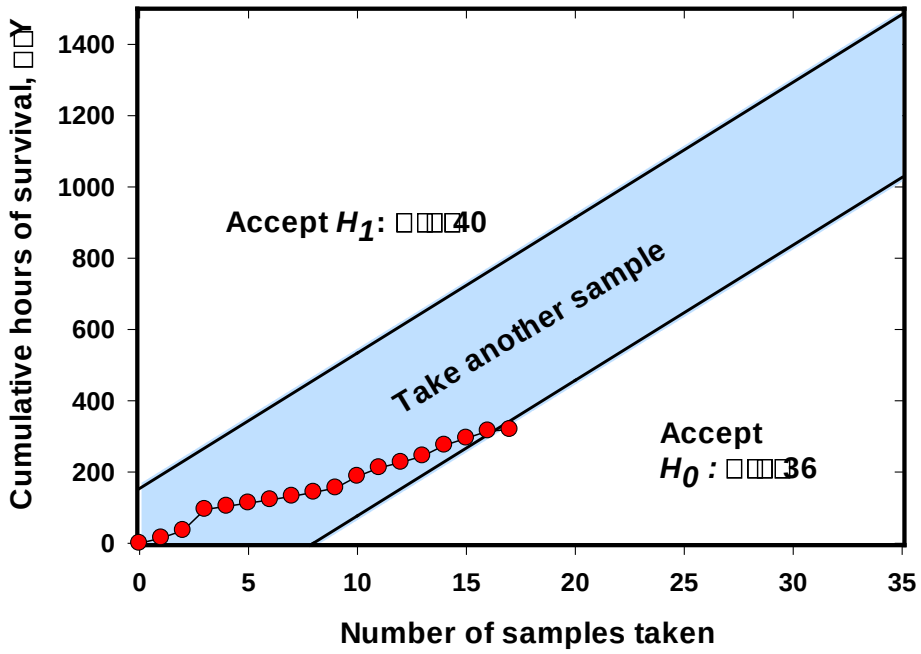

Figure 9.1 Sequential sampling plan for rainbow trout survival experiment discussed in the text. The hypothesis H_1 that mean survival time is less than or equal to 36 hours is tested against the alternative hypothesis H_2 that survival time is 40 hours or more. In the shaded area of statistical uncertainty, continue taking samples. An example is graphed in which the decision to accept H_1 is taken after n = 17. Survival time is assumed to follow a normal distribution.

For our rainbow trout example above, H_1 is that μ_1 = 36, and H_2 is that μ_2 = 40 so:

$$b = \frac{36 + 40}{2} = 38$$

The *y*-intercepts are estimated from the equations:

$$h_1 = \frac{Bs^2}{\mu_1 - \mu_2} \tag{9.4}$$

$$h_2 = \frac{As^2}{\mu_2 - \mu_1} \tag{9.5}$$

where:

h_0 = *y*-intercept of lower line
h_1 = *y*-intercept of upper line
$A = \log_e \left(\frac{1-\alpha}{\beta} \right)$
$B = \log_e \left(\frac{1-\beta}{\alpha} \right)$
μ_1 = Mean value postulated in H_1
μ_2 = Mean value postulated in H_2

Note that when $\alpha = \beta$ these two equations are identical so that $h_1 = -h_2$. For the rainbow trout example above, α = 0.01 and β = 0.10 so:

$$A = \log_e \left(\frac{1 - 0.01}{0.10} \right) = 2.29253$$
$$B = \log_e \left(\frac{1 - 0.10}{0.01} \right) = 4.49981$$

and thus from equations (9.4) and (9.5) with the standard deviation estimated to be 16.4 from previous work:

$$h_1 = \frac{4.49981(16.4)^2}{36 - 40} = -302.6$$
$$h_2 = \frac{2.29253(16.4)^2}{40 - 36} = +154.1$$

The two sequential lines thus become:

$$Y = 38n - 302.6$$

$$Y = 38n + 154.1$$

These lines are graphed in Figure 9.1. Note that the *y*-axis of this graph is the accumulated sum of the observations (e.g. sum of survival times) for the *n* samples plotted on the *x*-axis. You can plot a graph like Figure 9.1 by calculating 3 points on the lines (one as a check!); for this example -

Lower line		Upper line	
If n =	$\sum Y$ =	If n =	$\sum Y$
0	-302.6	0	15401
10	77.4	10	534.1
20	457.4	20	914.1

This graph can be used directly in the field to plot sequential samples with the simple decision rule to *stop sampling as soon as you leave the zone of uncertainty*. If you wish to use computed decision rules for this example, they are:

1. Accept H_1 if $\sum Y$ is less than ($38n - 302.6$)

2. Accept H_2 if $\sum Y$ is more than ($38n + 154.1$)

3. Otherwise, take another sample and go back to (1).

You would expect to take relatively few samples, if the true mean is much lower than that postulated in H_1, and also to sample relatively little if the true mean is much larger than that postulated in H_2. It is possible to calculate the sample size you may *expect* to need before a decision is reached in sequential sampling. For means from a normal distribution, the expected sample sizes for the three points μ_1, μ_2 and ($\mu_1 + \mu_2/2$) are:

1. For μ_1:

$$n_1 = 2\left[\frac{h_2 + (1-\alpha)(h_1 - h_2)}{\mu_1 \ \mu_2}\right] \qquad (9.6)$$

2. For μ_2:

$$n_2 = 2\left[\frac{h_2 + \beta(h_1 - h_2)}{\mu_2 - \mu_1}\right] \qquad (9.7)$$

3. For $(\mu_1 + \mu_2)/2$:

$$n_M = \frac{-h_1 h_2}{s^2} \tag{9.8}$$

where:

n_1 = Expected sample size required when mean = μ_1
n_2 = Expected sample size required when mean = μ_2
n_M = Expected sample size required when mean = $(\mu_1 + \mu_2)/2$
h_2 = *y*-intercept of upper line (equation 9.5)
h_1 = *y*-intercept of lower line (equation 9.4)
s^2 = Variance of measured variable

For example, in the rainbow trout example above:

For μ_1 = 36:

$$n_1 = 2\left[\frac{154.1 + (1-0.01)(-302..6-154.1)}{36-40}\right] = 149.0$$

For μ_2 = 40:

$$n_2 = 2\left[\frac{154.1 + 0.10(-302.6-154.1)}{40-36}\right] = 54.2$$

For μ = 38:

$$n_M = \frac{-(-302.6)(154.1)}{(16.4)^2} = 173.4$$

These values are plotted in Figure 9.2. Note that the expected sample size curve will be symmetric if $\alpha = \beta$, which is not the case in this example shown in Figure 9.2.

You can compare these sample sizes with that expected in a single sampling design of fixed *n* determined as in Chapter 7. Mace (1964 p. 134) gives the expected sample size in fixed sampling for the same level of precision to be:

$$n_s = \left[\frac{(z_\alpha + z_\beta)s}{\mu_1 - \mu_0}\right]^2 \tag{9.9}$$

where z_α and z_β are estimated from the standard normal distribution (i.e. $z_{.05}$ = 1.96). For this trout example, with α = .01, β = .10 and s = 16.4:

$$n_s = \left[\frac{(2.576 + 1.645)(16.4)}{40 - 36}\right]^2 = 299.5 \text{ samples}$$

which is more than twice the number of observations expected to be taken under sequential sampling.

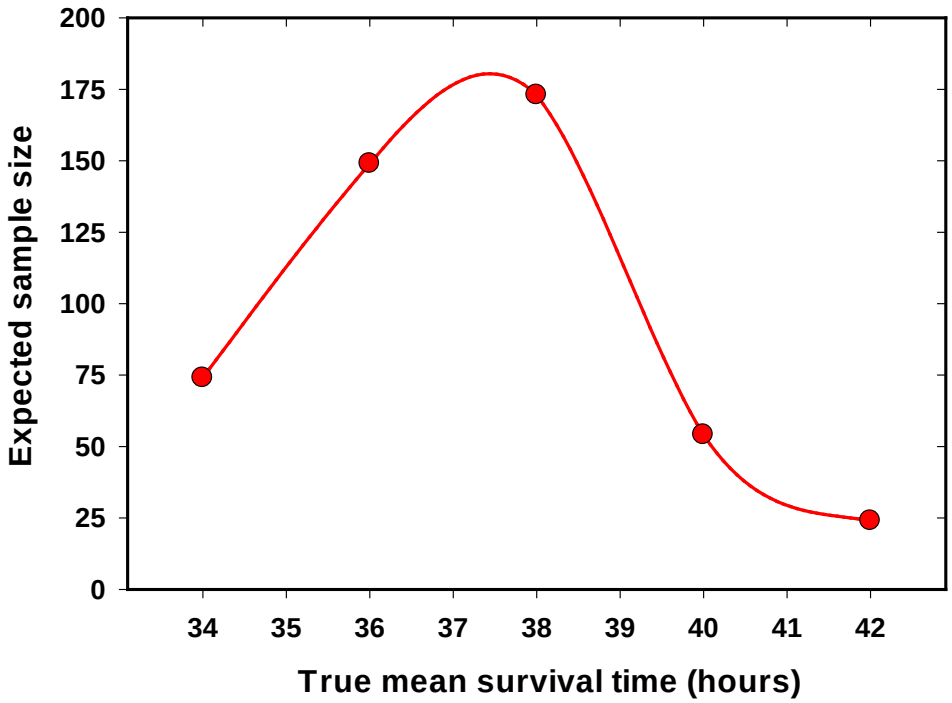

Figure 9.2 The theoretical curve for expected sample sizes in relation to the true mean of the population for the rainbow trout example in the text. Given α = .01 and β = .10, with s = 16.4, this is the sample size you would expect to have to take before you could make a sequential sampling decision about the mean. In any particular study you would not know the true mean survival time, and you would get an estimate of this mean only after doing the sampling. These theoretical expected sample size curves are useful however in planning sampling work since they show you how much work you might have to do under different circumstances.

This sequential sampling test assumes that you have an accurate estimate of the standard deviation of the measurement for your population. Since this is never the case in practice, you should view this as an approximate procedure.

9.1.2 Variances From A Normal Distribution

In some cases an ecologist wishes to test a hypothesis that the variability of a measurement is above or below a specified level. There is an array of possible tests available if sample sizes are fixed in advance (Van Valen 1978), but the problem can also be attacked by sequential sampling. We consider the two hypotheses:

$$H_1: \quad s^2 \leq \sigma_1^2$$
$$H_2: \quad s^2 \geq \sigma_2^2$$

We assume that the variable being measured is normally distributed. For example, you might be evaluating a new type of chemical method for analyzing nitrogen in moose feces. You know the old method produces replicate analyses that have a variance of 0.009. You do not want to buy the new equipment needed unless you are certain that the new method is about 10% better than the old. You thus can express the problem as follows:

$$H_1: \quad s^2 \leq 0.008 \quad \text{(and thus the new method is better)}$$
$$H_2: \quad s^2 \geq 0.009 \quad \text{(and thus the new method is not better)}$$

Let α = 0.01 and β = 0.05. These values are specified according to the risks you wish to take of rejecting H_0 or H_1 falsely, as explained above.

To carry out this test, calculate the two lines (Dixon and Massey 1983):

$$Y = bn + h_1$$
$$Y = bn + h_2$$

The slope of these lines is:

$$b = \frac{\log_e\left(\sigma_2^2 / \sigma_1^2\right)}{1/\sigma_1^2 - 1/\sigma_2^2} \tag{9.10}$$

The *y*-intercepts are given by:

$$h_1 = \frac{-2B}{1/\sigma_1^2 - 1/\sigma_2^2} \tag{9.11}$$

$$h_2 = \frac{2A}{1/\sigma_1^2 - 1/\sigma_2^2} \tag{9.12}$$

where:

h_1 = y-intercept of lower line
h_2 = y-intercept of upper line
$A = \log_e \left[(1 - \alpha)/\beta\right]$
$B = \log_e \left[(1 - \beta)/\alpha\right]$
σ_1^2 = Postulated variance for H_1
σ_2^2 = Postulated variance for H_2

When $\alpha = \beta$, $h_1 = -h_0$ and the lines are symmetric about the origin. For the nitrogen analysis problem above:

$$b = \frac{\log_e (0.009/0.008)}{1/0.008 - 1/0.009} = \frac{0.11778}{13.8889} = 0.0084802$$

$$h_1 = \frac{-2\log_e \left[(1 - 0.05)/0.01\right]}{1/0.08 - 1/0.09} = \frac{-9.10775}{13.8889} = -0.65576$$

$$h_2 = \frac{2\log_e \left[(1 - 0.05)/0.01\right]}{1/0.08 - 1/0.009} = \frac{5.97136}{13.8889} = 0.429938$$

These lines are plotted in Figure 9.3. Note that the *Y*-axis in this graph is the *sums of squares* of *X* measured by:

$\sum (x - \mu)^2$ = Sum of squares of the measurements x

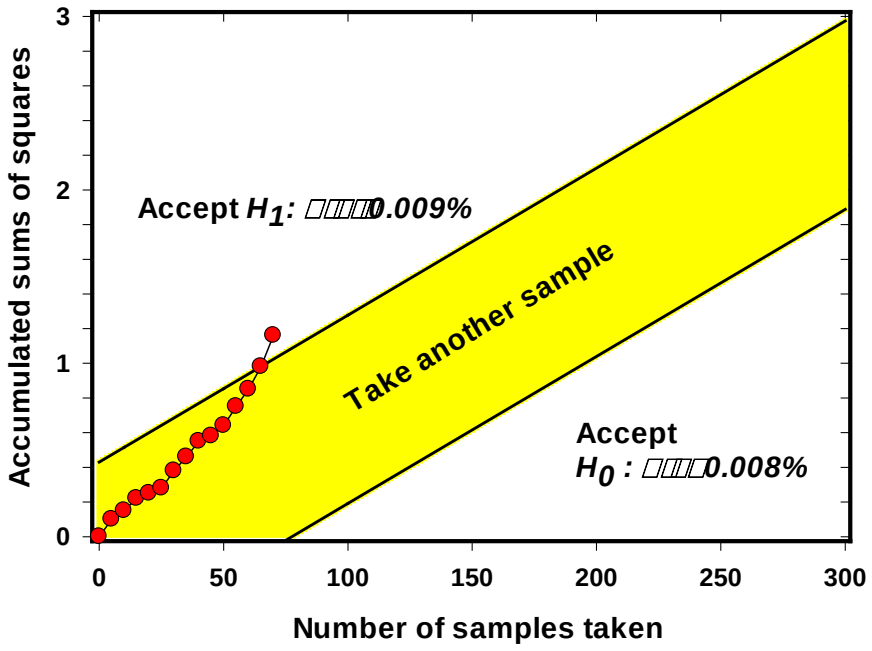

Figure 9.3 Sequential sampling plan for variances from a normal distribution, illustrated for the nitrogen analysis experiment discussed in the text. The hypothesis H_1 that the variance of replicate samples is less than or equal to 0.008 is tested against the alternative hypothesis H_2 that the variance is 0.009 or more. A sample data run in which a decision is made after n = 70 is shown for illustration.

If you know the true mean of the population this sum is easy to compute as an accumulated sum. But if you do not know the true mean, you must use the observed mean $\bar{x}$ as an estimate of μ and recompute the sums of squares at each sample, and plot these values against (n - 1) rather than n.

The expected sample sizes can be calculated from these equations (Dixon and Massey 1983):

1. For σ_1^2:

$$n_1 = \frac{(1-\alpha)h_1 + \alpha h_2}{\sigma_1^2 - b} \tag{9.13}$$

2. For σ_2^2:

$$n_2 = \frac{\beta h_1 + (1-\beta)h_2}{b - \sigma_1^2} \tag{9.14}$$

3. For $\sigma^2 = b$:

$$n_M = \frac{-h_1 h_2}{2b^2} \tag{9.15}$$

where:

n_0 = Expected sample size required when $s^2 = \sigma_0^2$
n_1 = Expected sample size required when $s^2 = \sigma_1^2$
n_M = Expected sample size required when $s^2 = b$
h_1 = y-intercept of upper line (equation 9.12)
h_0 = y-intercept of lower line (equation 9.11)
b = Slope of the sequential lines (equation 9.10)

These sample sizes can be plotted as in Figure 9.2.

The sample size required for a single sampling plan of comparable discriminating power, according to the fixed sample size approach of Chapter 7 is given by Mace (1964 p. 138) as:

$$n_s \cong \frac{3}{2} + \frac{1}{2}\left(\frac{z_\alpha + R z_\beta}{R-1}\right)^2 \qquad (9.16)$$

where:

n_s = Expected sample size under fixed sampling
z_α, z_β = Standard normal deviates (e.g., $z_{.05}$ = 1.96)
R = Ratio of the two standard deviations
$= \frac{\sigma_1}{\sigma_2} = \sqrt{\frac{\text{Larger variance}}{\text{Smaller variance}}}$

9.1.3 Proportions from a Binomial Distribution

Sequential sampling can also be applied to attributes like sex ratios (proportion of males) or incidence of disease studies, as long as sampling is conducted serially. It is possible to examine samples in groups in all sequential sampling plans, and little efficiency is lost as long as group sizes are reasonable. For example, you might take samples of 10 fish and inspect each for external parasites, and then plot the results, rather than proceeding one fish at a time.

For proportions, we consider the two hypotheses:

$$H_1: \quad p \le \pi_1$$
$$H_2: \quad p \ge \pi_2$$

where:

p = Observed proportions
π_1 = Expected lower estimate of the population proportion
π_2 = Expected upper estimate of the population proportion

For example, you might hypothesize that if more than 10% of fish are parasitized, treatment is required; but if less than 5%, no treatment is needed. Thus:

$H_1: \quad p \le 0.05$ (no treatment of fish needed)
$H_2: \quad p \ge 0.10$ (treatment is required)

You must decide α and β, and for this example assume $\alpha = \beta = 0.05$.

The two lines to be calculated are:

$Y = bn + h_1$

$Y = bn + h_2$

where Y = Number of individual *x*-types in sample of size *n*

The slope of the sequential lines for proportions is given by:

$$b = \frac{\log_e (q_1 / q_2)}{\log_e (p_2 q_1 / p_1 q_2)} \tag{9.17}$$

where b = Slope of the sequential sampling lines (cf. Figure 9.4).

and the other terms are defined below.

$$h_1 = \frac{-A}{\log_e (p_2 q_1 / p_1 q_2)} \tag{9.18}$$

$$h_2 = \frac{B}{\log_e (p_2 q_1 / p_1 q_2)} \tag{9.19}$$

where:

h_1 = y-intercept of lower line
h_2 = y-intercept of upper line
$A = \log_e \left[(1 - \alpha) / \beta \right]$
$B = \log_e \left[(1 - \beta) / \alpha \right]$
$p_1 = \pi_1$ = Expected proportion under H_1
$p_2 = \pi_2$ = Expected proportion under H_2
$q_1 = 1 - p_1$
$q_2 = 1 - p_2$

Note that the denominator is the same in all these three formulas (Mace 1964, p. 141).

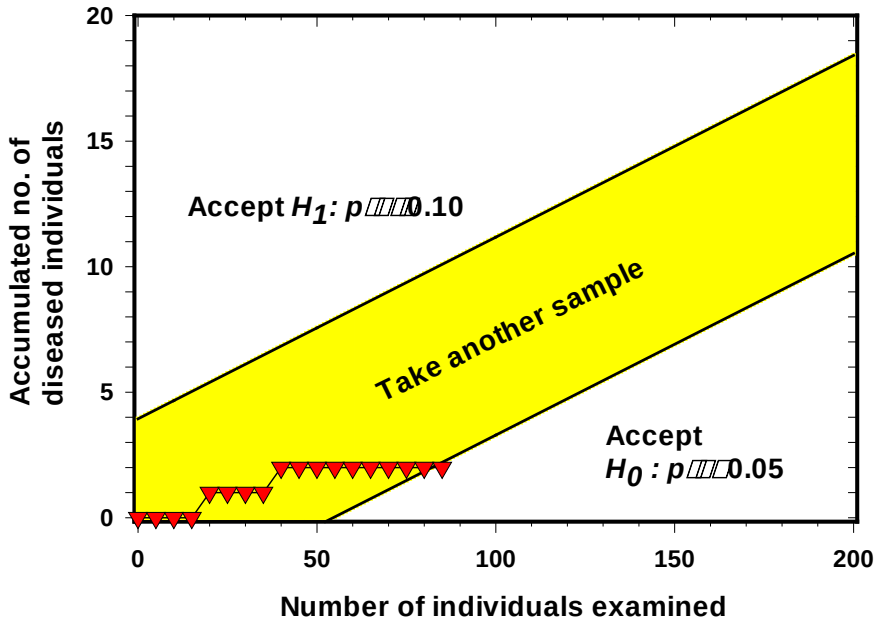

Figure 9.4 Sequential sampling plan for proportions from a binomial distribution, illustrated for the fish disease example discussed in the text. The hypothesis H_1 that the fraction of diseased fish is less than or equal to 0.05 is tested against the alternative hypothesis H_2 that the fraction is 0.10 or more. A sample data run in which a decision is reached after n = 85 is shown for illustration.

For the fish disease example, if $\alpha = \beta = 0.05$ and $p_1 = 0.05$ and $p_2 = 0.10$, then:

$$b = \frac{\log_e\left(0.95/0.90\right)}{\log_e\left[0.10(0.95)/0.05(0.90)\right]} = \frac{0.054067}{0.747214} = 0.07236$$

$$h_1 = \frac{-\log_e\left[(1 - 0.05)/0.05\right]}{0.747214} = -3.94$$

$$h_2 = \frac{\log_e\left[(1 - 0.05)/0.05\right]}{0.747214} = 3.94$$

These lines are illustrated in Figure 9.4 for this disease example. Note that the *Y*-axis of this graph is the accumulated number of diseased individuals in the total sample of *n* individuals.

You can calculate the expected sample size curve (like Fig. 9.2 page 000) for the proportion in the population from the points:

1. For p_1:

$$n_1 = \frac{-A(1-\alpha) + \alpha B}{p_1\left[\log_e\left(p_2/p_1\right)\right] + q_1\left[\log_e\left(q_2/q_1\right)\right]} \tag{9.20}$$

2. For p_2:

$$n_2 = \frac{-A\beta + (1-\beta)B}{p_2\left[\log_e\left(p_2/p_1\right)\right] + q_2\left[\log_e\left(q_2/q_1\right)\right]} \tag{9.21}$$

3. For $p = 1.0$:

$$n_U = \frac{B}{\log_e\left(p_2/p_1\right)} \tag{9.22}$$

4. For $p = 0.0$:

$$n_l = \frac{-A}{\log_e\left(q_2/q_1\right)} \tag{9.23}$$

5. For $p = \frac{p_1 + p_2}{2}$:

$$n_M = \frac{-A(B)}{\log_e\left(p_2 / p_1\right)\,\log_e\left(q_2 / q_1\right)} \qquad (9.24)$$

where all terms are as defined above. The maximum expected sample size will occur around the midpoint between p_1 and p_2, as defined in equation (9.24).

The sample size required for a fixed sample of comparative statistical precision as determined by the methods outlined in Chapter 7 is given by Mace (1964, p. 142) as:

$$n_s = \left(\frac{z_\alpha + z_\beta}{2 \arcsin \sqrt{p_2} - 2 \arcsin \sqrt{p_1}} \right)^2 \qquad (9.25)$$

where:

z_α, z_β = Standard normal deviates (e.g., $z_{.05} = 1.96$)
p_1 = Expected proportion under H_1
p_2 = Expected proportion under H_2

and the arcsines are expressed in radians (*not* in degrees).

It is sometimes convenient to specify a sequential sampling plan in tabular form instead of in a graph like Figure 9.4. Table 9.1 illustrates one type of table that could be used by field workers to classify a population based on proportion of plants infested.

9.1.4 Counts From A Negative Binomial Distribution

In many sampling programs, organisms are aggregated so that quadrat counts will often be best described by a negative binomial distribution (Chapter 4, page 000). A sequential sampling scheme for negative binomial counts can be designed if you know the exponent *k* of the variable being measured.

For example, counts of the green peach aphid (*Myzus persicae*) on sugar beet plants follow the negative binomial distribution with an average *k*-value of 0.8 (Sylvester and Cox 1961). You wish to test two hypotheses:

H_1: Mean aphid abundance $\leq$ 10 per leaf
H_2: Mean aphid abundance $\geq$ 20 per leaf

Assume that $\alpha = \beta = 0.05$ for this example. Note that for a negative binomial distribution,

$$\mu = kc$$

and so we define

$$c_1 = \frac{\text{Mean value postulated under } H_1}{k}$$
$$c_2 = \frac{\text{Mean value postulated under } H_2}{k}$$

For this particular example, c_1 = 10/0.8 = 12.5 and c_2 = 20/0.8 = 25.

To carry out this statistical test, calculate the slope and y-intercepts of the two sequential sampling lines:

$$Y = bn + h_1$$
$$Y = bn + h_2$$

The *y*-axis of this graph is the cumulative number of individuals counted (c.f. Figure 9.5).

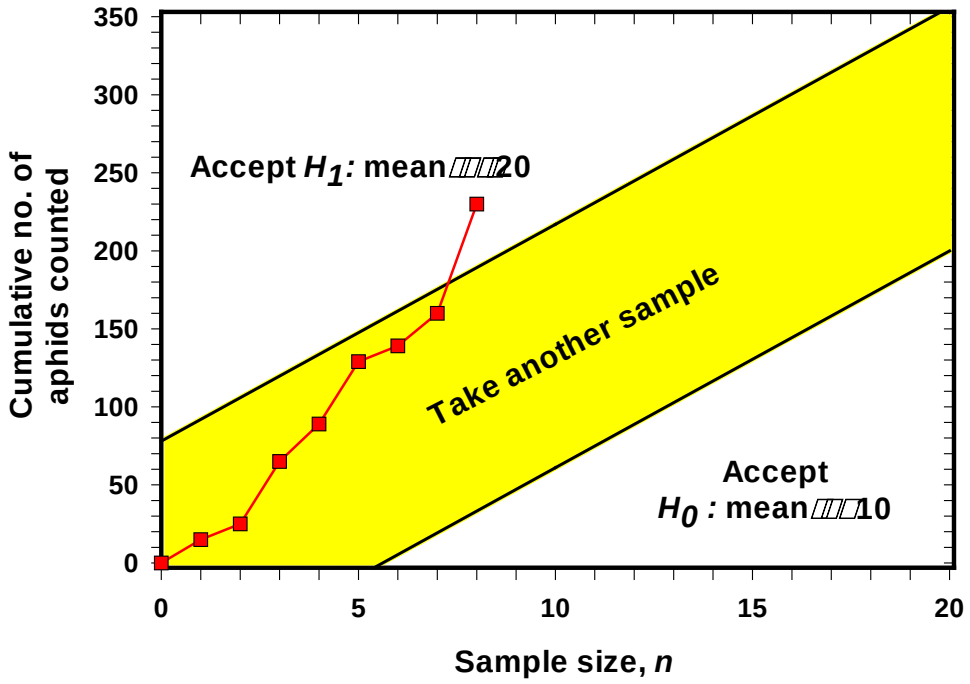

Figure 9.5 Sequential sampling plan for counts from a negative binomial distribution, illustrated for the green peach aphid data in Box 9.1. The hypothesis H_1 that mean aphid abundance is less than or equal to 10 aphids per leaf is tested against the alternative hypothesis H_2 that the mean is more than 20 per leaf.

The slope of these lines is, for the negative binomial case:

$$b = \frac{(k)\log_e\left[(c_2 + 1)/(c_1 + 1)\right]}{\log_e\left[(c_2 + c_1c_2)/(c_1 + c_1c_2)\right]} \quad (9.26)$$

where:

b = Slope of sequential sampling lines
k = Negative binomial exponent
c_1 = (Mean value postulated under H_1) / k
c_2 = (Mean value postulated under H_2) / k

The *y*-intercepts are calculated as follows:

$$h_1 = \frac{-A}{\log_e\left[(c_2 + c_1c_2)/(c_1 + c_1c_2)\right]} \quad (9.27)$$

$$h_2 = \frac{B}{\log_e\left[(c_2 + c_1c_2)/(c_1 + c_1c_2)\right]} \quad (9.28)$$

where:

h_0 = *y*-intercept of lower line
h_1 = *y*-intercept of upper line
$A = \log_e\left[(1 - \alpha)/\beta\right]$
$B = \log_e\left[(1 - \beta)/\alpha\right]$
c_1 = (Mean value postulated under H_0) / k
c_2 = (Mean value postulated under H_1) / k
k = Negative binomial exponent

Box 9.1 illustrates the use of these formulae for negative binomial data.

Box 9.1 SEQUENTIAL SAMPLING FOR COUNTS FROM A NEGATIVE BINOMIAL DISTRIBUTION

An insect ecologist wishes to test the abundance of green peach aphids on sugar beets to distinguish two hypotheses:

H_1: mean aphid abundance $\leq$ 10 per leaf

H_2 : mean aphid abundance $\geq$ 20 per leaf

She decides to set $\alpha = \beta = 0.05$, and she knows from previous work that the counts fit a negative binomial distribution with exponent k = 0.8 and that k is reasonably constant over all aphid densities.

1. Calculate c_1 and c_2:

$$c_1 = \frac{\text{mean value under } H_1}{k} = \frac{10}{0.8} = 12.5$$

$$c_2 = \frac{\text{mean value under } H_2}{k} = \frac{20}{0.8} = 25.0$$

2. Calculate the slope of the sequential lines using equation (9.26):

$$b = \frac{(k)\log_e\left[(c_2 + 1)/(c_1 + 1)\right]}{\log_e\left[(c_2 + c_1c_2)/(c_1 + c_1c_2)\right]}$$

$$b = \frac{0.8\log_e\left(26.0/13.5\right)}{\log_e\left(337.5/325\right)} = \frac{0.52432}{0.03774} = 13.893$$

3. Calculate the *y* intercepts using equations (9.27) and (9.28); note that the denominator of these equations has already been calculated in step (2) above.

$$h_1 = \frac{-\log_e\left[(1 - \alpha)/\beta\right]}{\log_e\left[(c_2 + c_1c_2)/(c_1 + c_1c_2)\right]}$$

$$= \frac{-\log_e\left(0.95/0.05\right)}{0.03774} = -78.02$$

$$h_2 = \frac{\log_e\left[(1 - \beta)/\alpha\right]}{\log_e\left[(c_2 + c_1c_2)/(c_1 + c_1c_2)\right]}$$

$$= \frac{\log_e\left(0.95/0.05\right)}{0.03774} = 78.02$$

Note that if $\alpha = \beta$, these two intercepts are equal in absolute value.

4. Calculate three points on the lines:

y_0=13.9n - 78.0

y_1=13.9n + 78.0

Lower line		Upper line	
n	$\sum Y$	n	$\sum Y$
0	-78	0	78
10	61	10	217
20	200	20	256

These are plotted in Figure 9.5 for this example.

5. Suppose you were field sampling and obtained these results

	Sample							
	1	2	3	4	5	6	7	8
No. of aphids	20	19	39	10	15	48	45	41
Accumulated no. of aphids	20	39	78	88	103	151	196	237

You would be able to stop sampling after sample 7 and conclude that the study zone had an aphid density above 20/leaf. These points are plotted in Figure 9.5 for illustration.

You may then use these counts in the usual way to get a mean density and 95% confidence interval for counts from the negative binomial distribution, as described in Chapter 4. For these 8 samples the mean is 29.6 and the 95% confidence limits are 16.3 to 41.3.

Program SEQUENTIAL (Appendix 2, page 000) can do these calculations.

The expected sample size curve (c.f. Fig. 9.2, page 000) for negative binomial counts can be estimated from the general equation:

$$\left\{\begin{array}{l}\text{Expected sample size}\\ \text{to reach a decision if}\\ \text{the true mean is } \mu\end{array}\right\} = \frac{h_2 + (h_1 - h_2)L(c)}{\mu - b} \qquad (9.29)$$

where:

h_1 and h_2 = y-intercepts (equations 9.27 and 9.28)
μ = Population mean (defined in equation 9.31)
b = Slope of sequential sampling lines (equation 9.26)
$L(c)$ = Probability of accepting H_0 if true mean is μ (equation 9.30)

This equation is solved by obtaining pairs of values of μ and $L(c)$ from the paired equations:

$$L(c) = \frac{A^h - 1}{A^h - B^h} \qquad (\text{for } h \neq 0) \qquad (9.30)$$

$$\mu = k\left[\frac{1 - (q_1/q_2)^h}{(c_2q_1/c_1q_2)^h - 1}\right] \qquad (\text{for } h \neq 0) \qquad (9.31)$$

where:

$$A = (1-\beta)/\alpha$$
$$B = \beta/(1-\alpha)$$
$$q_1 = c_1 + 1$$
$$q_2 = c_2 + 1$$

and other variables are defined as above. The variable *h* is a dummy variable and is varied over a range of (say) -6 to +6 to generate pairs of μ and *L*(*c*) values (Allen *et al*. 1972), which are then used in equation (9.29).

9.2 THREE ALTERNATIVE HYPOTHESES

Not all decisions can be cast in the form of *either-or*, and there are many ecological situations in which a choice of three or more courses of action is needed. For example, spruce budworm larvae in conifer forests damage trees by their feeding activities, and budworm infestations need to be classified as light, moderate, or severe (Waters 1955). The simplest way to approach three alternatives is to construct two separate sequential sampling plans, one between each pair of neighboring hypotheses. For example, suppose in the budworm case:

H_1: Mean density $\leq$ 1 larvae/branch (light infestation)
H_2: Mean density $\geq$ 5 larvae/branch but $\leq$ 10 larvae/branch
H_3: Mean density $\geq$ 20 larvae/branch (severe infestation)

Using the procedures just discussed, you can calculate a sequential sampling plan for the alternatives H_1 and H_2, and a second sampling plan for the alternatives H_2 and H_3. For example:

Plan A	Plan B
H_1: $x \leq 1$	H_2: $x \leq 10$
H_2: $x \geq 5$	H_3: $x \geq 20$

The result is a graph like that in Figure 9.6. In some cases the results of combining two separate sequential sampling plans may lead to anomalies that must be decided somewhat arbitrarily by continued sampling (Wetherill and Glazebrook 1986).

Note that in constructing Figure 9.6 we have used only two of the three possible sequential sampling plans. For example, for the spruce budworm:

Plan A	Plan B	Plan C
H_1: $x \leq 1$	H_2: $x \leq 10$	H_1: $x \leq 1$
H_2: $x \geq 5$	H_3: $x \geq 20$	H_3: $x \geq 20$

We did not include Plan C because the alternatives are covered in Plans A and B. Armitage (1950), however, suggested that all three sequential plans be computed. One advantage of using all 3 plans is that some possible sampling anomalies are avoided (Wetherill and Glazebrook 1986), but in practice these may rarely occur anyway.

There is at present no way to calculate the expected sample sizes needed for three alternative hypotheses (Wetherill and Glazebrook 1986). The only approach available is to calculate them separately for each pair of sequential lines and use these as an approximate guideline for field work.

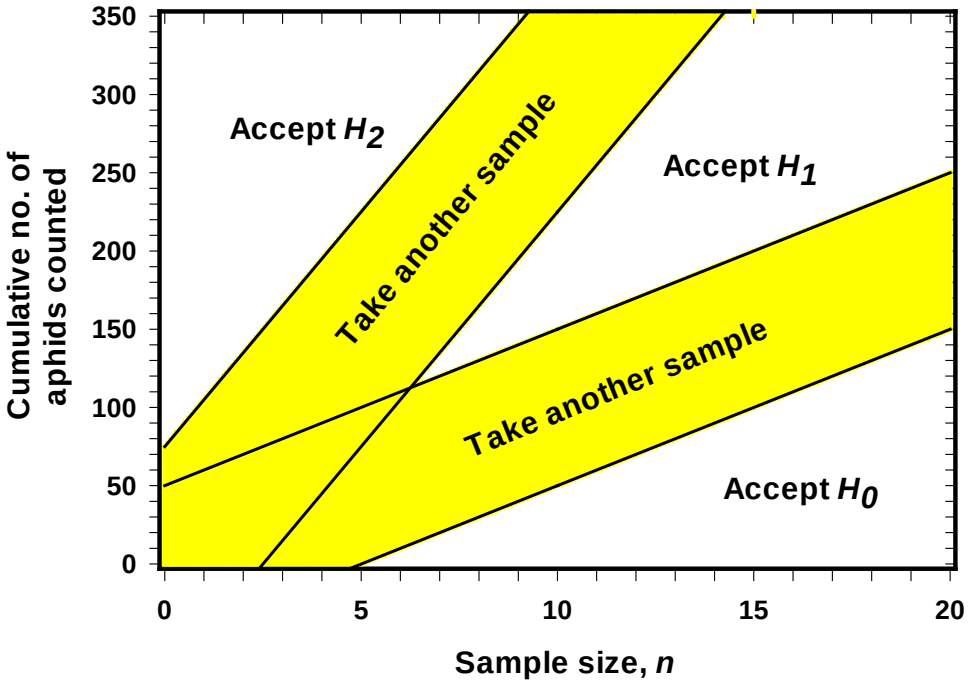

Figure 9.6 Hypothetical example of a sequential sampling plan to distinguish three hypotheses. Two separate plans like that in Figure 9.1 are superimposed, defining two regions of uncertainty between the three hypotheses H_1, H_2, and H_3. A plan like this, for example, could be set up to distinguish low, moderate, and severe insect infestations.

9.3 STOPPING RULES

Sequential sampling plans provide for a quick resolution of two or more competing hypotheses in all cases where the population mean differs from the mean value postulated in the alternative hypotheses. But problems will arise whenever the population mean is near the critical limits postulated in H_1 or H_2. For example, suppose that the true mean is 38.0 in the example shown in Figure 9.1 (page 000). If this is the case, you are likely to remain forever in the central zone of uncertainty and take an infinite number of samples. This is clearly undesirable, and hence there has been an attempt to specify closed boundaries for sample size, so that there is a maximum sample size at which sampling stops, even if one is still in the zone of uncertainty. Wetherill and Glazebrook (1986, Chapter 6) discusses some methods that have been developed to specify closed boundaries. None of them seem particularly easy to apply in ecological field situations. The simplest practical approach is to calculate the sample size expected in a single sampling scheme of fixed sample size from formulas like equation (9.9), and to use this value of n as an upper limit for sample size. If no decision has been make by the time you have sampled this many quadrats, quit.

An alternative approach to specifying an upper limit to permissible sample size was devised by Kuno (1969, 1972), and Green (1970) suggested a modified version of this stopping rule. Both approaches are similar to those discussed in Chapter 7 in which a specified precision of the mean is selected, but are applied to the sequential case in which sample size is not fixed in advance. These stopping rules are useful to field ecologists because they allow us to minimize sampling effort and yet achieve a level of precision decided in advance. A common problem in estimating abundance, for example, is to take too many samples when a species is abundant and too few samples when it is rare. Stopping rules can be useful in telling you when to quit sampling.

9.3.1 Kuno's Stopping Rule

Kuno (1969) suggested a fixed precision stopping rule based on obtaining an estimate of the mean with a fixed confidence belt. To determine the stop line from this method proceed as follows:

1. Fit the quadratic equation to data previously obtained for this population:

$$s^2 = a_1 \bar{x} + a_2 \bar{x}^2 + b \quad (9.32)$$

where:

s^2 = Observed variance of a series of measurements or counts
$\bar{x}$ = Observed mean of a servies of measurements or counts
a_1, a_2 = Regression coefficients for the quadratic equation
b = y-intercept

Standard statistical textbooks provide the techniques for fitting a quadratic equation (e.g. Steel and Torrie, 1980, p. 338, or Sokal and Rohlf, 1995, p. 665). If there is not a quadratic relationship between the mean and the variance in your samples you cannot use this technique. Note that many different samples are needed to calculate this relationship, each sample being one point in the regression.

2. Specify the size of the standard error of the mean density that you wish to obtain. Note that this will be about one-half of the width of the 95% confidence interval you will obtain.

3. From previous knowledge, estimate the mean density of your population, and decide on the level of precision you wish to achieve:

$$D = \frac{s_{\bar{x}}}{\bar{x}} = \frac{\text{Desired standard error}}{\text{Estimated mean}} \quad (9.33)$$

D is sometimes called the coefficient of variation of the mean and will be approximately one-half the width of the 95% confidence belt.

4. For a range of sample sizes (n) from 1 to (say) 200, solve the equation:

$$\sum_{i=1}^{n} Y_i = \frac{a_1}{D^2 - a^2 / n} \quad (9.34)$$

where:

$\sum Y_i$ = Total accumulated count in n quadrats
a_1, a_2 = Slope parameters of quadratic equation (9.32)
D = Desired level of precision as defined in equation (9.33)

Equation (9.34) has been called the "stop line" by Kuno (1969) since it gives the accumulated count needed in *n* samples to give the desired precision of the mean. The sampler can stop once the $\sum Y_i$ is exceeded, and this will in effect set an upper boundary on sample size. Allen *et al*. (1972) illustrate the use of this stop rule on field populations of the cotton bollworm in California.

Program SEQUENTIAL (Appendix 2, page 000) can calculate the stop line from these equations.

9.3.2 Green's Stopping Rule

If you have counts from quadrats and your sampling universe can be described by Taylor's Power Law, you can use Green's (1970) method as an alternative to Kuno's approach.

To calculate the stop line for this sequential sampling plan, you must have estimates of the two parameters of Taylor's Power Law (*a*, *b*, see below, page 000), which specifies that the log of the means and the log of the variances are related linearly (instead of the quadratic assumption made by Kuno above). You must decide on the level of precision you would like. Green (1970) defines precision as a fixed ratio in the same manner as Kuno (1969):

$$D = \frac{s_{\bar{x}}}{\bar{X}} = \frac{\text{standard error}}{\text{mean}} \tag{9.35}$$

Note that *D* is expressed as one standard error; approximate 95% confidence levels would be 2*D* in width.

The stop line is defined by the log-log regression:

$$\log\left(\sum_{i=1}^{n} Y_i\right) = \frac{\log\left(D^2 \diagup a\right)}{b-2} + \left[\frac{b-1}{b-2}\right]\log(n) \tag{9.36}$$

where

$\sum_{i=1}^{n} Y_i$ = Cumulative number of organisms counted in n samples
D = Fixed level of precision (defined above in eq. 9.35)
a = y-intercept of Taylor's Power Law regression
b = Slope of Taylor's Power Law regression
n = Number of samples counted

Figure 9.7 illustrates the stop line for a sampling program to estimate density of the European red mite to a fixed precision of 10% (D = 0.10).

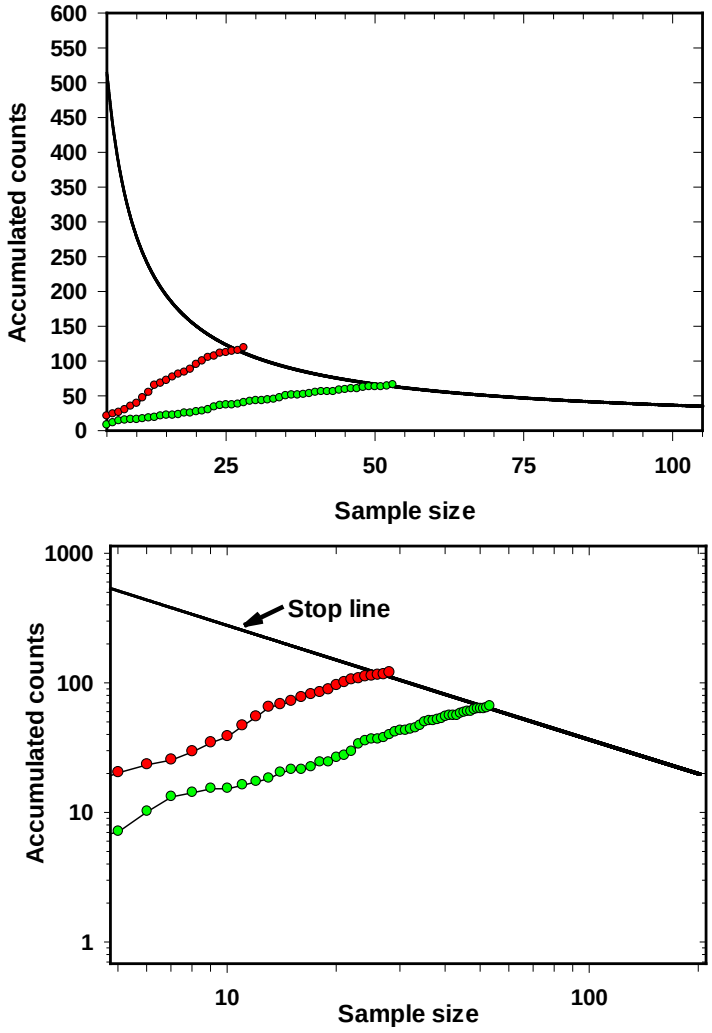

Figure 9.7 Green's fixed precision sampling plan for counts of the intertidal snail *Littorina sitkana*. For this population Taylor's Power Law has a slope of 1.47 and a y-intercept of 1.31. The desired precision is 0.15 (S.E./mean). The linear and the log-log plot are shown to illustrate that the stop line is linear in log-log space. Two samples are shown for one population with a mean of 4.83 (red, stopping after 28 samples) and a second with a mean of 1.21 (green, stopping after 53 samples).

Green's stopping rule can be used effectively to reduce the amount of sampling effort needed to estimate abundance in organisms sampled by quadrats. It is limited to sampling situations in which enough data has accumulated to have prior estimates of Taylor's Power Law which specifies the relationship between means and variances for the organism being studied.

9.4 ECOLOGICAL MEASUREMENTS

In all the cases discussed so far, we have used sequential sampling as a method of testing statistical hypotheses. An alternative use of sequential sampling is to estimate population parameters. For example, you may wish to estimate the abundance of European rabbits in an area (as opposed to trying to classify the density as low, moderate or high). There is a great deal of statistical controversy over the use of standard formulas to calculate means and confidence intervals for data obtained by sequential sampling (Wetherill and Glazebrook 1986, Chapter 8). The simplest advice is to use the conventional formulas and to assume that the stopping rule as defined sequentially does not bias estimates of means and confidence intervals obtained in the usual ways.

There are special sequential sampling designs for abundance estimates based on mark-recapture and on quadrat counts.

9.4.1 Sequential Schnabel Estimation of Population Size

In a Schnabel census, we sample the marked population on several successive days (or weeks) and record the fraction of marked animals at each recapture (c.f. pp. 000-000). An obvious extension of this method is to sample sequentially until a predetermined number of recaptures have been caught. Sample size thus becomes a variable. The procedure is to stop the Schnabel sampling at the completion of sample *s*, with *s* being defined such that:

$$\sum_{t=1}^{s-1} R_t < L \qquad \text{but} \qquad \sum_{t=1}^{s} R_t \geq L$$

where

$\sum R_t$ = Cumulative number of recaptures to time t
L = Predetermined number of recaptures

L can be set either from prior information on the number of recaptures desired, or from equation 7.18 (page 000) in which L is fixed once you specify the coefficient of variation you desire in your population estimate (see Box 9.2, page 000).

There are two models available for sequential Schnabel estimation (Seber 1982, p. 188).

Goodman's Model This model is appropriate only for large sample sizes from a large population. The population estimate derived by Goodman (1953) is:

$$\hat{N}_G = \frac{\left(\sum_{i=1}^{t} Y_i\right)^2}{2L} \tag{9.37}$$

where:

$\hat{N}_G$ = Goodman's estimate of population size for a sequential Schnabel experiment
Y_i = Total number of individuals caught at time i
L = Predetermined stopping rule value for $\sum R_t$ as defined above

The large-sample variance of Goodman's estimate of population size is:

$$\text{Variance of } \tilde{N}_G = \frac{N_G^2}{L} \tag{9.38}$$

If the total number of recaptures exceeds 30, an approximate 95% confidence interval for $\hat{N}_G$ is given by this equation (Seber 1982 p. 188):

$$\frac{2\left(\sum Y_i\right)^2}{\left(1.96 + \sqrt{4L - 1}\right)^2} < \hat{N}_G < \frac{2\left(\sum Y_i\right)^2}{\left(-1.96 + \sqrt{4L - 1}\right)^2} \tag{9.39}$$

where all terms are defined above. For the simple case when approximately the same number of individuals are caught in each sample (Y_i = a constant), you can calculate the expected number of samples you will need to take from:

$$\left\{\begin{matrix}\text{Expected number}\\ \text{of samples to reach}\\ L \text{ recaptures}\end{matrix}\right\} \cong \frac{\sqrt{2\hat{N}L}}{Y_i} \qquad (9.40)$$

where:

$\hat{N}$ = Approximate guess of population size
L = Predetermined stopping rule for $\sum R_t$
Y_i = Number of individuals caught in each sample (a constant)

Thus if you can guess, for example, that you have a population of 2000 fish and L should be 180, and you catch 100 fish each day, you can calculate:

$$\left\{\begin{matrix}\text{Expected number}\\ \text{of samples to reach}\\ 180 \text{ recaptures}\end{matrix}\right\} \cong \frac{\sqrt{2(2000)(180)}}{100} = 8.5 \text{ samples}$$

This is only an approximate guideline to help in designing your sampling study.

Chapman's Model This model is more useful in smaller populations but should not be used if a high fraction (>10%) of the population is marked. The population estimate derived by Chapman (1954) is:

$$\hat{N}_C = \frac{\sum_{i=1}^{t} Y_i M_i}{L} \qquad (9.41)$$

where:

$\hat{N}_C$ = Chapman's estimate of population size for a sequential Schnabel experiment
Y_t = Number of individuals caught in sample t (t = 1, 2,…, s)
M_t = Number of marked individuals in the population at the instant before sample t is taken
L = Predetermined stopping rule for $\sum R_t$ as defined above

The variance of this population estimate is the same as that for Goodman's estimate (equation 9.36). For a 95% confidence interval when the total number of recaptures exceeds 30, Chapman (1954) recommends:

$$\frac{4B}{\left(1.96 + \sqrt{4L - 1}\right)^2} < \hat{N}_C < \frac{4B}{\left(-1.96 + \sqrt{4L - 1}\right)^2} \qquad (9.42)$$

where $B = \sum Y_t M_t$ (for all samples)

Box 9.2 works out an example of sequential Schnabel estimation for a rabbit population.

Box 9.2 SEQUENTIAL ESTIMATION OF POPULATION SIZE BY THE SCHNABEL METHOD

A wildlife officer carries out a population census of European rabbits. He wishes to continue sampling until the coefficient of variation of population density is less than 8%. From equation (7.18):

$$\mathrm{CV}\left(\hat{N}\right) \cong \frac{1}{\sqrt{\sum R_t}} = 0.08$$

Thus:

$$\sqrt{\sum R_t} = \frac{1}{0.08} \quad \text{or} \quad \sum R_t = 156.25$$

Solving for $\sum R_t$, he decides that he needs to sample until the total number of recaptures is 157 (L). He obtains these data:

Day	Total caught, Y_t	No. marked, R_t	Cumulative no. marked caught, $\sum R_t$	No. of accidental deaths	No. marked at large, M_t
1	35	0	0	0	0
2	48	3	3	2	35
3	27	8	11	0	78
4	39	14	25	1	97
5	28	9	34	2	121
6	41	12	46	1	138
7	32	15	61	0	166
8	19	9	69	0	183
9	56	26	95	1	194
10	42	18	113	0	223
11	39	22	135	1	247
12	23	12	147	1	263
13	29	16	163	0	273

At sample 13 he exceeds the predetermined total of recaptures and so he stops sampling. At the end of day 13 the number of actual recaptures L is 163, in excess of the predetermined number of 156.3.

Goodman's Method

Since more than 10% of the rabbits are marked we use Goodman's method. From equation (9.37):

$$\hat{N}_G = \frac{\left(\sum Y_t\right)^2}{2L} = \frac{(458)^2}{2(163)} = 643.4 \text{ rabbits}$$

The 95% confidence limits from equation (9.39) are

$$\frac{2\left(\sum Y_t\right)^2}{\left(1.96 + \sqrt{4L-1}\right)^2} < \hat{N}_G < \frac{2\left(\sum Y_t\right)^2}{\left(-1.96 + \sqrt{4L-1}\right)^2}$$

$$\frac{2(458)^2}{(1.96 + 25.51)^2} < \hat{N}_G < \frac{2(458)^2}{(-1.96 + 25.51)^2}$$

$$555.8 < \hat{N}_G < 756.1$$

For the same rabbit data, the Schnabel estimate of population size (equation 2.9) is 419 rabbits with 95% confidence limits of 358 to 505 rabbits. The Goodman sequential estimate is nearly 50% higher than the Schnabel one and the confidence limits do not overlap. This is likely caused by unequal catchability among the individual rabbits with marked animals avoiding the traps. This type of unequal catchability reduces the number of marked individuals recaptured, increases the Goodman estimate, and reduces the Schnabel estimate. You would be advised in this situation to use a more robust closed estimator that can compensate for heterogeneity of trap responses.

Program SEQUENTIAL (Appendix 2, page 000) can do these calculations.

In all the practical applications of sequential Schnabel sampling, the only stopping rule used has been that based on a predetermined number of recaptures. Samuel (1969) discusses several alternative stopping rules. It is possible, for example, to stop sampling once the ratio of marked to unmarked animals exceeds some constant like 1.0. Samuel (1969) discusses formulas appropriate to Schnabel estimation by this stopping rule (Rule C in her terminology).

9.4.2 Sampling Plans for Count Data

Some but not all count data are adequately described by the negative binomial distribution (Kuno 1972, Taylor *et al.* 1979, Binns and Nyrop 1992). If we wish to develop sequential sampling plans for count data, we will have to have available the

negative binomial model as well as other more general models to describe count data. There are three models that can be applied to count data and one of these will fit most ecological populations:

(1) ***Negative binomial model***: if the parameter *k* (exponent of the negative binomial) is constant and independent of population density, we can use the sampling plan developed above (page 393) for these populations. For negative binomial populations the variance is related to the mean as:

$$s^2 = \mu + \frac{\mu^2}{k} \tag{9.43}$$

Unfortunately populations are not always so simple (Figure 9.8). If negative binomial *k* is not constant, you may have a sampling universe in which *k* is linearly related to mean density, and you will have to use the next approach to develop a sampling plan.

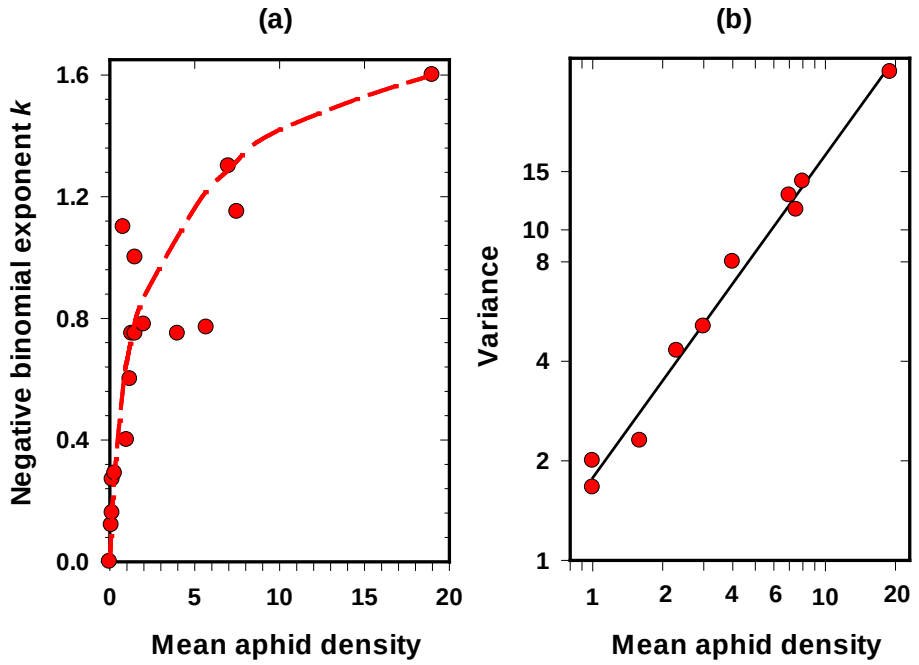

Figure 9.8 Counts of the numbers of green peach aphids (*Myzus persicae*) on sugar beet plants in California. (a) The negative binomial exponent *k* varies with density through the season, so a constant *k* does not exist for this system. (b) Taylor's Power Law regression for this species is linear for the whole range of densities encountered over the season. The regression is: variance = 3.82 (mean$^{1.17}$) (Data from Iwao, 1975.)

(2) ***Taylor's Power Law*:** Taylor (1961) observed that many quadrat counts for insects could be summarized relatively simply because the variance of the counts was related to the mean. High density populations had high variances, and low density populations had low variances. Taylor (1961) pointed out that the most common relationship was a power curve:

$$s^2 = a\,\bar{x}^b \tag{9.44}$$

where

s^2 = Observed variance of a series of population counts
$\bar{x}$ = Observed mean of a series of population counts
a = Constant
b = Power exponent

By taking logarithms, this power relationship can be converted to a straight line:

$$\log\left(s^2\right) = \log(a) + b\log\left(\bar{x}\right) \tag{9.45}$$

where the power exponent b is the slope of the straight line.

To fit Taylor's Power Law to a particular population, you need to have a series of samples (counts) of organisms in quadrats. Each series of counts will have a mean and a variance and will be represented by one point on the graph (Figure 9.9). A set of samples must be taken over a whole range of densities from low to high to span the range, as illustrated in Figure 9.9. If the organisms being counted have a random spatial pattern, for each series of samples the variance will equal the mean, and the slope of Taylor's Power Law will be 1.0. But most populations are aggregated, and the slope (b) will usually be greater than 1.0.

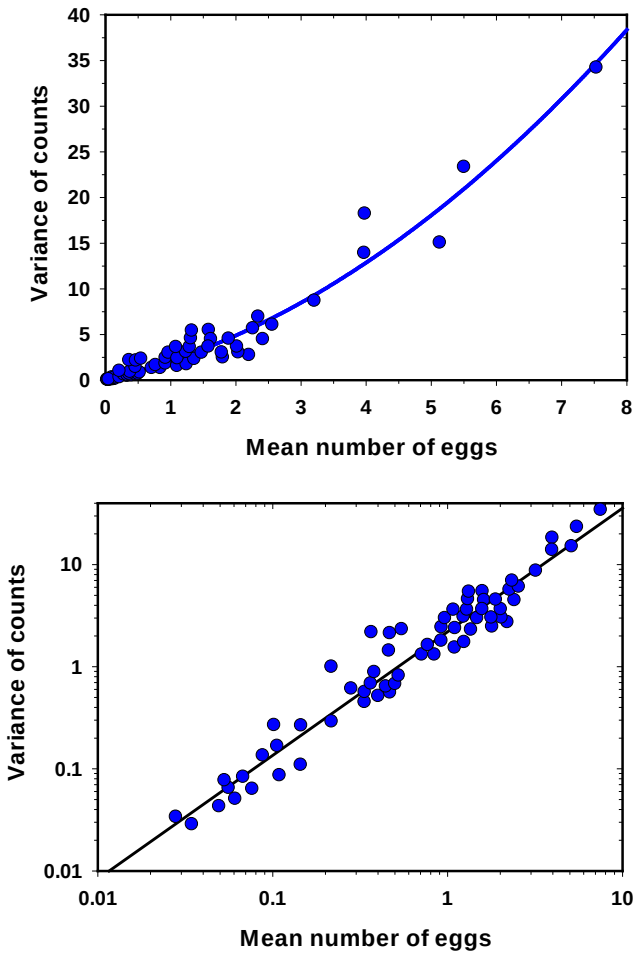

Figure 9.9 The relationship between the mean density of eggs of the gypsy moth *Lymantria dispar* and the variance of these counts. The top graph is a arithmetic plot and illustrates that the mean and variance are not linearly related. The lower graph is Taylor's Power Law for these same data, showing the good fit of these data to the equation $s^2 = 2.24\ m^{1.25}$, which is a straight line in a log-log plot. (Data from Taylor 1984, p. 329)

Taylor's Power Law is a useful way of summarizing the structure of a sampling universe (Taylor 1984, Nyrop and Binns 1991, Binns and Nyrop 1992). Once it is validated for a particular ecosystem, you can predict the variance of a set of counts once you know the mean density, and design a sampling plan to obtain estimates of specified precision, as will be shown in the next section. There is a large literature on the parameters of Taylor's Power Law for insect populations (Taylor 1984) and it is clear that for many populations of the same insect in different geographical regions, the estimated slopes and intercepts are similar but not identical (Trumble *et al.*

1987). It is safest to calibrate Taylor's Power Law for your local population rather than relying on some universal constants for your particular species.

Taylor's Power Law is probably the most common model that describes count data from natural populations, and is the basis of the general sequential sampling procedure for counts given below (page 412).

(3) ***Empirical models***: If neither of the previous models is an adequate description of your sampling universe, you can develop a completely empirical model. Nyrop and Binns (1992) suggest, for example, a simple model:

$$\ln\left(-\ln\left(p_0\right)\right) = a + b\ln\left(\bar{x}\right) \tag{9.46}$$

where

$p_0 =$ Proportion of samples with no organisms
$\bar{x} =$ Mean population density
$a =$ y-intercept of regression
$b =$ Slope of linear regression

They provide a computer program to implement a sequential sampling plan based on this empirical relationship. If you need to develop an empirical model for your population, it may be best to consult a professional statistician to determine the best model and the best methods for estimating the parameters of the model.

9.4.3 General Models for Two Alternative Hypotheses from Quadrat Counts

Sequential decision making from count data can often not fit the simple models described above for deciding between two alternative hypotheses. Iwao (1975) suggested an alternative approach which is more general because it will adequately describe counts from the binomial, Poisson, or negative binomial distributions, as well as a variety of clumped patterns that do not fit the usual distributions.

Iwao's original method assumes that the spacing pattern of the individuals being counted can be adequately described by the linear regression of mean crowding on mean density (Krebs 1989, p. 260). Taylor (1984) and Nyrop and Binns (1991) pointed out that Iwao's original method makes assumptions that could lead to

errors, and they recommended a more general method based on Taylor's Power Law.

To determine a sequential sampling plan from Taylor's Power Law using Iwao's method, proceed as follows:

1. Calculate the *slope* (*b*) and *y-intercept* (*a*) of the log-log regression for Taylor's Power Law (equation 9.45) in the usual way (e.g. Sokal and Rohlf, 2012, page 466).

2. Determine the critical density level μ_0 to set up the two-sided alternative:

H_0 : Mean density $= \mu_0$
H_1 : Mean density $< \mu_0$ (lower density)
H_2 : Mean density $> \mu_0$ (higher density)

3. Calculate the upper and lower limits of the cumulative number of individuals counted on *n* quadrats from the equations:

$$\begin{aligned} &\text{Upper limit:} \quad U_n = n\mu_0 + 1.96A \\ &\text{Lower limit:} \quad L_n = n\mu_0 - 1.96A \end{aligned} \qquad (9.47)$$

where:

U_n = Upper limit of cumulative counts for *n* quadrats at $\alpha = 0.05$
L_n = Lower limit of cumulative counts for *n* quadrats for $\alpha = 0.05$
μ_0 = Postulated critical density (mean per quadrat)
$A = \sqrt{n\left(\text{var}\left(\mu_0\right)\right)}$
$\text{var}(\mu_0)$ = variance of the critical density level μ_0

Calculate these two limits for a range of sample sizes from *n* = 1 up to (say) 100. If you wish to work at a 99% confidence level, use 2.576 instead of 1.96 in the equations (9.47) and if you wish to work at a 90% confidence level use 1.645 instead of 1.96.

4. Plot the limits U_n and L_n (equation 9.47) against sample size (*n*) to produce a graph like Figure 9.10. Note that the lines in this sequential sampling graph will *not* be straight lines, as has always been the case so far, but in fact diverge.

5. If the true density μ is close to the critical density μ_0, sampling may continue indefinitely (e.g. Fig. 9.7). This is a weak point in all sequential sampling plans, and we need a stopping rule. If we decide in advance that we will quit when the confidence interval is $\pm d$ $(\text{at } \mu = \mu_0)$, we can determine the maximum sample size from this equation (Iwao 1975):

$$n_M \cong \frac{4}{d^2}\left[\text{var}(\mu_0)\right] \tag{9.48}$$

where:

n_M = Maximum sample size to be taken
d = Absolute half-width of the confidence interval for 95% level
μ_0 = Critical density
$\text{var}(\mu_0)$ = variance expected from Taylor's Power Law for the critical density

If you wish to use 99% confidence belts, replace 4 with 6.64 in this equation, and if you wish 90% confidence belts, replace 4 with 2.71.

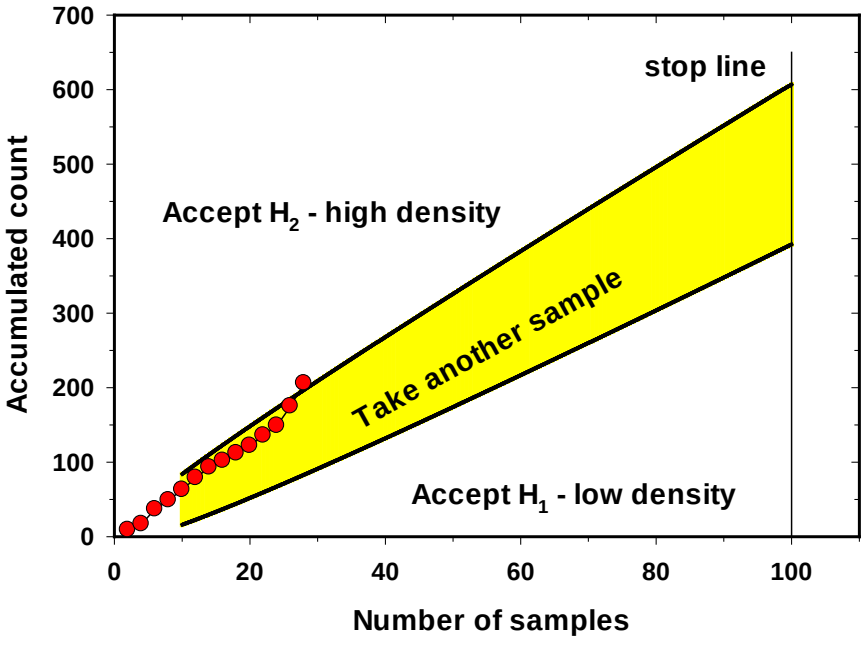

Figure 9.10 Sequential sampling plan for the European red mite in apple orchards. Iwao's (1975) method was used to develop this sampling plan, based on Taylor's Power Law specifying the relationship between the mean and the variance for a series of sample counts. For this population the slope of the Power Law was 1.42 and the intercept 4.32. At any given density, mite counts fit a negative binomial distribution, but *k* is not a constant so we cannot use the methods in section 9.1.4. The critical density for biological control is 5.0

mites per quadrat, and 10% levels were used for α and β. A maximum of 100 samples specifies the stop line shown. One sampling session is illustrated in which sampling stopped at *n* = 28 samples for a high density population with a mean infestation of 7.6 mites per sample. (Data from Nyrop and Binns, 1992).

The method of Iwao (1975) can be easily extended to cover 3 or more alternative hypotheses (e.g. Fig. 9.6) just by computing two or more sets of limits defined in equation (9.47) above. This method can also be readily extended to two-stage sampling, and Iwao (1975) gives the necessary formulas.

The major assumption is that, for the population being sampled, there is a linear regression between the log of the variance and the log of the mean density (Taylor's Power Law). A considerable amount of background data is required before this can be assumed, and consequently you could not use Iwao's method to sample a new species or situation in which the relationship between density and dispersion was unknown.

Box 9.3 illustrates the application of the Iwao method for green peach aphids.

Program SEQUENTIAL (Appendix 2, page 000) can calculate the sequential sampling lines for all of the types of statistical distributions discussed in this chapter.

Box 9.3 IWAO'S METHOD FOR SEQUENTIAL SAMPLING TO CLASSIFY POPULATION DENSITY ESTIMATED BY QUADRAT COUNTS

The first requirement is to describe the relationship between the variance and the mean with Taylor's Power Law. For the green peach aphid, prior work (Figure 9.8) has shown that the variance and the mean density are related linearly by

$$\log\left(s^2\right) = \log(4.32) + 1.42 \log\left(\bar{x}\right)$$

in which density is expressed as aphids per sugar beet plant and the sampling unit (quadrat) is a single plant (Sylvester and Cox, 1961). Thus *a* = 4.32 and *b* = 1.42 for equation (9.45).

The critical density for beginning insecticide treatment was 5.0 aphids/plant, so μ = 5.0. The expected variance of density at a mean of 5.0 from Taylor's Power Law above is:

$$\log\left(s^2\right) = \log(4.32) + 1.42 \log\left(\bar{x}\right) = 0.63548 + 1.42 \left(\log(5.0)\right) = 1.62802$$
$$s^2 = \text{antilog}\left(1.62802\right) = 10^{1.62802} = 42.464$$

The upper and lower limits are calculated from equation (9.47) for a series of sample sizes (*n*) from 1 to 200. To illustrate this, for *n* = 10 plants and

$\alpha = \beta = 0.10$:

$$U_n = n\mu_0 + 1.64A$$
$$U_{10} = 10(5.0) + 1.64\ A_{10}$$

where:

$$A = \sqrt{n\left(\text{var}\left(\mu_0\right)\right)}$$

where $\text{var}(\mu_0)$ = variance of the critical density level μ_0

$$A_{10} = \sqrt{10\left[42.464\right]} = 20.607$$

and thus:

$$U_{10} = 10(5.0) + 1.64(20.607) = 83.8 \text{ aphids}$$
$$L_n = n\mu_0 - 1.64A$$
$$L_{10} = 10(5.0) - 1.64(20.607) = 16.2 \text{ aphids}$$

Similarly calculate:

n	Lower limit	Upper limit
20	51.9	148.1
30	91.1	208.9
40	132.0	268.0
50	174.0	326.0
60	216.7	383.3

Note that these limits are in units of *cumulative* total number of aphids counted on all *n* plants. These limits can now be used to plot the sequential lines as in Figure 9.1 or set out in a table that lists the decision points for sequential sampling (e.g., Table 9.1).

If the true density is nearly equal to 5.0, you could continue sampling very long. It is best to fix an upper limit from equation (9.48). Assume you wish to achieve a confidence interval of $\pm$ 20% of the mean with 90% confidence (if the true mean is 5.0 aphid/plant). This means for μ_0 = 5.0, $d = \pm 1.0$ aphids per plant and thus from equation (9.45):

$$n_M \cong \frac{2.71}{d^2}\left[\text{var}\left(\mu_0\right)\right]$$
$$= \frac{2.71}{1.0^2}\left[42.464\right] = 115$$

and about 115 plants should be counted as a maximum.

Program SEQUENTIAL (Appendix 2, page 000) can do these calculations.

9.5 VALIDATING SEQUENTIAL SAMPLING PLANS

Sequential sampling plans are now in wide use for insect pest population monitoring, and this has led to an important series of developments in sequential sampling. Nyrop and Simmons (1984) were the first to recognize that although ecologists set α-levels for sequential sampling at typical levels (often 5%), the actual Type I error rates can be much larger than this. This is because in sequential sampling you never know ahead of time what the mean will be, and since variances are not constant (and are often related to the mean in some way) and count data are not normally distributed, the resulting error rates are not easy to determine.

To determine actual error rates for field sampling situations, we must rely on computer simulation. Two approaches have been adopted. You can assume a statistical model (like the negative binomial) and use a computer to draw random samples from this statistical distribution at a variety of mean densities. This approach is discussed by Nyrop and Binns (1992), who have developed an elegant set of computer programs to do these calculations. The results of computer simulations can be summarized in two useful graphs illustrated in Figure 9.11. The operating characteristic curve (OC) estimates the probability of accepting the null hypothesis when in fact the null hypothesis is true. Statistical power, as discussed in Chapter 6, is simply (1.0 - OC). For sequential sampling we typically have two hypotheses (low density, high density), and the OC curve will typically show high values when mean density is low and low values when mean density is high. The critical point for the field ecologist is where the steep part of the curve lies. The second curve is the average sample number curve (ASN) which we have already seen in Figure 9.2 (page 000). By doing 500 or more simulations in a computer we can determine on average how many samples we will have to take to make a decision.

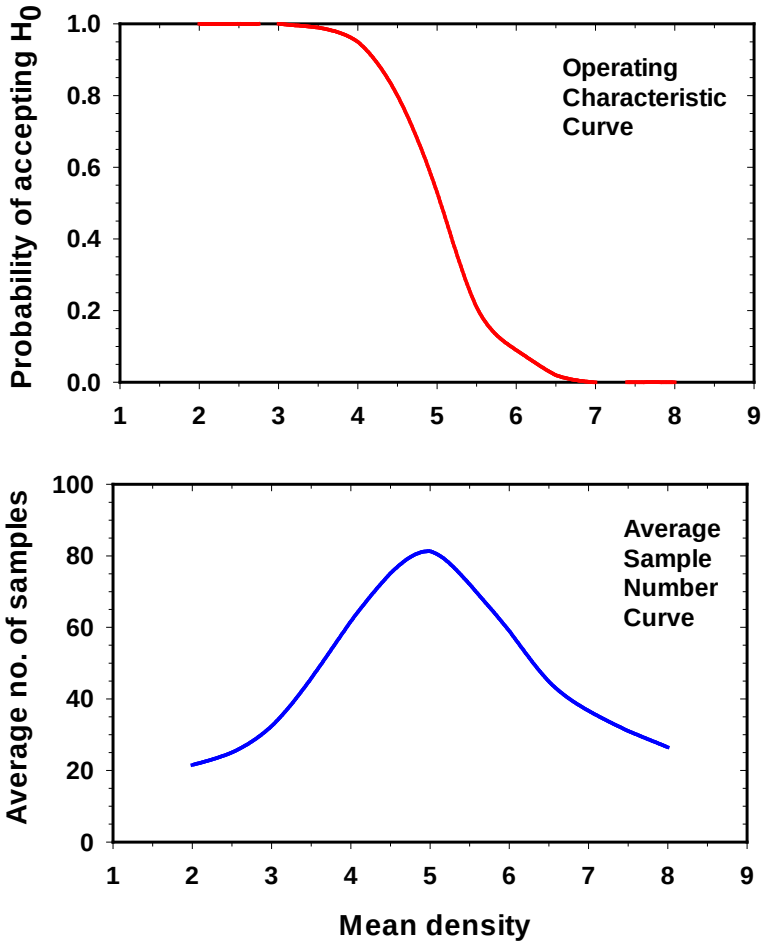

Figure 9.11 Sequential sampling scheme for the European red mite on apple trees. The critical density is 5.0 mites per leaf. Simulations at each density were run 500 times, given Taylor's Power Law for this population (a = 4.32, b = 1.42) and assuming a negative binomial distribution of counts and ($\alpha = \beta = 0.10$). The Operating Characteristic Curve gives the probability of accepting the hypothesis of low mite density (H_1) when the true density is given on the x-axis, and is essentially a statistical power curve. The average number of samples curve gives the expected intensity of effort needed to reach a decision about high or low mite density for each level of the true mean density. (After Nyrop and Binns 1992).

A second approach to determining actual error rates for field sampling programs is to ignore all theoretical models like the negative binomial and to use resampling methods to estimate the operating characteristic curve and the expected sample size curve. Naranjo and Hutchison (1994) have developed a computer program RVSP (*Resampling Validation of Sampling Plans*) to do these calculations. Resampling methods (see Chapter 15, page 000) use a set of observations as the basic data and grab random samples from this set to generate data for analysis.

Resampling methods are completely empirical, and make no assumptions about statistical distributions, although they require prior information on the mean-variance relationship in the population being studied (e.g. Taylor's Power Law). There is at present no clear indication of whether the more formal statistical models or the more empirical resampling models give better insight for field sampling. In practice the two methods often give similar pictures of the possible errors and the effort required for sequential sampling schemes. Field ecologists will be more at ease with the empirical resampling schemes for designing field programs.

9.6 SUMMARY

Sequential sampling differs from classical statistical methods because *sample size is not fixed in advance*. Instead, you continue taking samples, one at a time, and after each sample ask if you have enough information to make a decision. Sample size is minimized in all sequential sampling schemes. Sequential methods are useful only for measurements that can be made in sequential order, so that one can stop sampling at any time. They have been used in ecology principally in resource surveys and in insect pest control problems.

Many sequential sampling schemes are designed to test between two alternative hypotheses, such as whether a pest infestation is light or severe. You must know in advance how the variable being measured is distributed (e.g. normal, binomial, or negative binomial). Formulas are given for specifying the slope and y-intercepts of sequential sampling lines for variables from these different statistical distributions. The expected sample size can be calculated for any specific hypothesis in order to judge in advance how much work may be required.

If there are three or more alternative hypotheses that you are trying to distinguish by sequential sampling, you simply repeat the sequence for two-alternatives a number of times.

One weakness of sequential sampling is that if the true mean is close to the critical threshold mean, you may continue sampling indefinitely. To prevent this from occurring, various arbitrary stopping rules have been suggested so that sample size never exceeds some upper limit.

Sequential sampling schemes have been designed for two common ecological situations. The Schnabel method of population estimation lends itself readily to sequential methods. Quadrat counts for estimating density to a fixed level of precision can be specified to minimize sampling effort.

Sequential decision making has become important in practical problems of insect pest control. To test between two alternative hypotheses Iwao developed a general method for quadrat counts which are not adequately described by the negative binomial distribution. If the relationship between the mean and the variance can be described by Taylor's Power Law, Iwao's method can be used to design a sampling plan for sequential decision making from quadrat data.

Field sampling plans for deciding whether populations are at low density or high density should be tested by computer simulation because the nominal α level in sequential decisions may differ greatly from the actual error rate. Statistical power curves and expected sample size curves can be generated by computer simulation to illustrate how a specified sampling protocol will perform in field usage.

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QUESTIONS AND PROBLEMS

9.1 Construct a sequential sampling plan for the cotton fleahopper. Pest control officers need to distinguish fields in which less than 35% of the cotton plants are infested from those in which 50% or more of the plants are infested. They wish to use $\alpha = \beta = 0.10$.

(a) Calculate the sequential sampling lines and plot them.
(b) Calculate the expected sample size curve for various levels of infestation.
(c) Compare you results with those of Pieters and Sterling (1974).

9.2 Calculate a sequential sampling plan for the data in Box 9.1 (page 000) under the false assumption that these counts are normally distributed with variance = 520. How does this sequential sampling plan differ from that calculated in Box 9.1?

9.3 Construct a stopping rule for a sequential Schnabel population estimate for the data in Box 2.2 (page 000) for coefficients of variation of population size estimates of 60%, 50%, 25% and 10%.

9.4 Calculate a sequential sampling plan that will allow you to classify a virus disease attack on small spruce plantations into three classes: <10% attacked, 15-25% attacked and >30% attacked. Assume that it is very expensive to classify stands as >30% attacked if in fact they are less than this value.
(a) Calculate the expected sample size curves for each of the sequential lines.
(b) How large a sample would give you the same level of precision if you used a fixed-sample-size approach?

9.5 Calculate a sequential sampling plan for the cotton bollworm in which Taylor's Power Law describes the variance-mean regression with a slope (*b*) of 1.44 and a *y*-intercept (*a*) of 0.22, and the critical threshold density is 0.2 worms/plant. Assume $\alpha = \beta$ = 0.05.

(a) What stopping rule would you specify for this problem?
(b) What would be the general consequence of defining the sample unit not as

1 cotton plant but as 3 plants or 5 plants? See Allen *et al*. (1972 p. 775) for a discussion of this problem.

9.6 Construct a sequential sampling scheme to estimate population size by the Schnabel Method using the data in Table 2.2 (page 000). Assume that you wish to sample until the coefficient of variation of population size is about 25%. What population estimate does this provide? How does this compare with the Schnabel estimate for the entire data set of Table 2.2?

9.7. Beall (1940) counted beet webworm larvae on 325 plots in each of five areas with these results:

No. of larvae	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
0	117	205	162	227	55
1	87	84	88	70	72
2	50	30	45	21	61
3	38	4	23	6	54
4	21	2	5	1	12
5	7		2		18
6	2				21
7	2				16
8	0				14
9	1				2

(a) Fit a negative binomial distribution to each of these data sets and determine what spatial pattern these insects show.

(b) Design a sequential sampling scheme for these insects, based on the need to detect low infestations (<0.8 larvae per plot) and high infestations (>1.0 larvae per plot).

(c) Determine a stopping rule for this sequential scheme and plot the resulting sequential sampling scheme.

9.8 Sequential sampling methods are used in a relatively low fraction of ecological studies. Survey the recent literature in your particular field of interest and discuss why sequential methods are not applied more widely.