Statistical Framework for Monitoring Basal Area Coverage of Eelgrass in Puget Sound

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1.0 Introduction

The purpose of this report is to describe the statistical methods used to estimate basal area coverage (BAC) of eelgrass within sites and across Puget Sound based on survey sampling data. This report describes the calculation of variance estimates for within-site sampling error as well as Puget Sound-wide sampling error. Rotational sampling designs will be used to estimate BAC and updated annual estimates in year i using data collected in year $i+1$. Annual change in BAC will be calculated and methods for determining a five-year trend described.

The sampling in Puget Sound for a particular year can be conceptualized as a stratified sampling program. The four strata correspond to four mutually exclusive and exhaustive categories as follows:

Stratum 1: Core areas selected nonprobabilistically.
Stratum 2: Embayment areas encompassing an eelgrass meadow on two or more sides of the shoreline (i.e., flats).
Stratum 3: Shoreline strips with moderate eelgrass abundance (i.e., regular fringe).
Stratum 4: Shoreline strips with high eelgrass abundance (i.e., regular fringe).

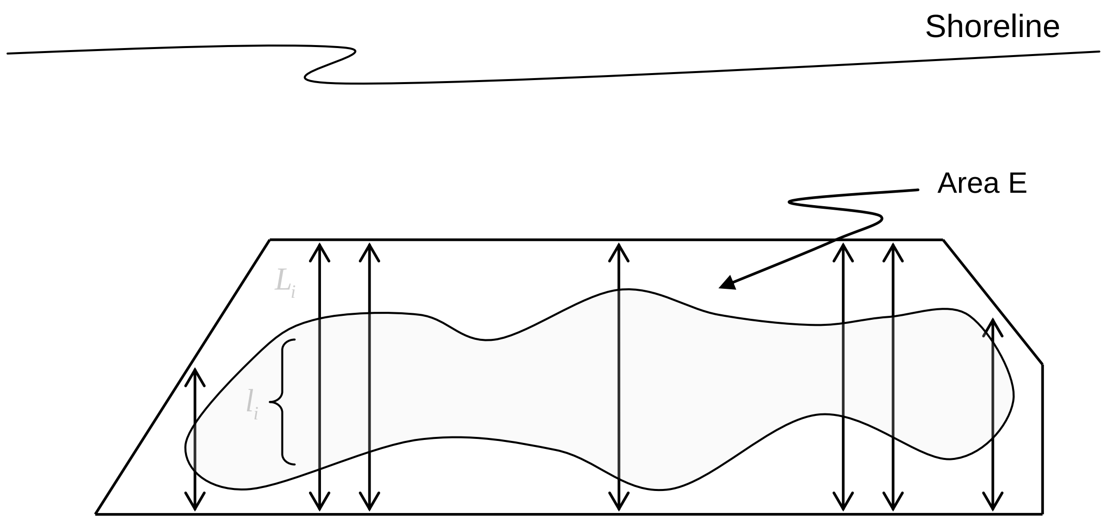
Within embayment and fringe strata, site selection will be conducted using simple random sampling (SRS).

Over years, rotational sampling will be conducted independently within the three probabilistically sampled strata. The fractional rotation of sampling units in and out of strata will be approximately 20%.

2.0 Within-Site Estimation of BAC

Within a sampling unit, eelgrass abundance (i.e., BAC) will be estimated in a two-step process of (1) delineating the area of the bed, (2) conducting line-intercept transect sampling to estimate the percent cover. Figure 1 illustrates conceptually the sampling process. The estimator of eelgrass BAC can then be expressed as

Figure 1. Schematic of sampling an eelgrass bed for basal area coverage. Perimeter of bed based on minimum convex polygon and percent cover estimated from replicate line-intercept transects.



$$\hat{X} = E \cdot \hat{\bar{p}} \tag{1}$$

where

E = maximum outward size of the eelgrass bed based on a minimum convex polygon,

$\hat{\bar{p}}$ = estimated average percent cover along a transect through the eelgrass bed.

The estimate of average percent cover $\left(\hat{\bar{p}}\right)$ will be based on a ratio estimator of the form

$$\hat{\bar{p}} = \frac{\sum_{i=1}^{m} l_i}{\sum_{i=1}^{m} L_i}$$

where

l_i = length of the ith transect $(i = 1, \ldots, m)$ that contains eelgrass,

L_i = actual total length of the ith transect $(i = 1, \ldots, m)$.

This ratio estimator has an approximate variance of

$$V\hat{a}r\left(\hat{\bar{p}}\right) = \frac{\sum_{i=1}^{m}\left(l_i - \hat{\bar{p}} L_i\right)^2}{(m-1)m\bar{L}^2}$$

where

$$\bar{L} = \frac{\sum_{i=1}^{m} L_i}{m}.$$

Should all the transects be of equal length (i.e., $L_i = L \ \forall_i$), then the variance estimate for $\hat{\bar{p}}$ simplifies to

$$Var\left(\hat{\bar{p}}\right) = \frac{\sum_{i=1}^{m}\left(p_i - \hat{\bar{p}}\right)^2}{(m-1)m}$$

where

$$p_i = \frac{l_i}{L_i}.$$

The variance of the estimate of BAC for the site is then

$$Var\left(\hat{X}\right) = E^2 Var\left(\hat{\bar{p}}\right). \tag{2}$$

Estimator (1) and its variance are based on the following assumptions:

1. Area E is known without error.
2. The transect lines are randomly distributed within the area E.
3. The transect lines are infinitely narrow.
4. The fraction of the lines intercepting eelgrass is measured accurately.

3.0 Estimating Regional Abundance in Year i

Within any year i, the monitoring program is a stratified random sampling scheme within Puget Sound. Define

X_{ij} = BAC of eelgrass in the *j*th sample location $\left(j = 1,\ldots,m_i\right)$ for the *i*th strata $(i = 1,\ldots,4)$;

$\hat{X}_{ij}$ = estimated BAC of eelgrass in the *j*th sample location $\left(j = 1,\ldots,m_i\right)$ in the *i*th stratum $(i = 1,\ldots,4)$;

N_i = number of sample locations in the *i*th stratum;

n_i = actual number of sample locations drawn in the *i*th stratum;

$Var\left(\hat{X}_{ij} \middle| X_{ij}\right)$ = sampling variance associated with estimating eelgrass BAC X_{ij} by $\hat{X}_{ij}$ at the *j*th sample location $\left(j = 1, \ldots, n_i\right)$ for the *i*th stratum $(i = 1, \ldots, 4)$.

It is worth noting that the within-site eelgrass abundance X_{ij} will be actually estimated by $\hat{X}_{ij}$ which will be assumed to be an unbiased estimator , i.e.,

$$E\left(\hat{X}_{ij}\right) = X_{ij}$$

with an unbiased variance estimator

$$E\left[V\hat{a}r\left(\hat{X}_{ij} \middle| X_{ij}\right)\right] = Var\left(\hat{X}_{ij} \middle| X_{ij}\right).$$

The total BAC (B_T) of eelgrass in Puget Sound will be expressed as

$$B_T = B_1 + B_2 + B_3 + B_4$$

where B_i is the BAC in stratum i $(i = 1, \ldots, 4)$ and estimated by

$$\hat{B}_T = \sum_{i=1}^{4} \hat{B}_i \tag{3}$$

with associated variance

$$Var\left(\hat{B}_T\right) = \sum_{i=1}^{4} Var\left(\hat{B}_i \middle| B_i\right).$$

and estimated variance

$$V\hat{a}r\left(\hat{B}_T\right) = \sum_{i=1}^{4} V\hat{a}r\left(\hat{B}_i \middle| B_i\right). \tag{4}$$

3.1 Estimation Within Core Stratum

In this stratum, all N_1 of N_1 sites will be sampled, in which case

$$\hat{B}_1 = \sum_{j=1}^{N_1} \hat{X}_{ij} \tag{5}$$

with associated variance estimator

$$Var\left(\hat{B}_1 \middle| B_1\right) = \sum_{j=1}^{N_1} Var\left(\hat{X}_{ij} \middle| X_{ij}\right) \tag{6}$$

the sum of the within-site measurement errors.

3.2 Estimation Within Fringe Strata

The shoreline strata (i.e., regular fringe and wide fringe) were subdivided into N_i segments of equal length (i.e., 1000 m). A simple random sample of n_i of the shoreline segments were was selected for measurement. However, the shoreline could not be subdivided evenly into 1000-m segments in all cases. There were instances where smaller segments of beach were left over because the beaches were not exact multiples of 1000 m. In order to correctly extrapolate the sample observations to the entire stratum, the sample observations have to be expanded by the multiplier

$$\frac{L_T}{L_N}$$

where

L_T = total linear length of a fringe stratum,

$L_N = N_i \cdot 1000 \text{ m}$ = total linear length of the sampling frame for a fringe stratum.

The estimate of total basal area coverage for a fringe stratum is then calculated as follows:

$$\hat{B}_3 = \left(\frac{L_T}{L_N} \right) \left[\frac{N_3}{n_3} \sum_{j=1}^{n_3} \hat{X}_{ij} \right] \tag{7}$$

with associated estimated sampling variance

$$Var\left(\hat{A}_3 \middle| A_3 \right) = \left(\frac{L_T}{L_N} \right)^2 \left[\frac{N_3^2 \left(1 - \frac{n_3}{N_3} \right) s_{\hat{X}_{ij}}^2}{n_3} + \frac{N_3}{n_3} \sum_{j=1}^{n_3} Var\left(\hat{X}_{ij} \middle| X_{ij} \right) \right] \tag{8}$$

and where

N_3 = number of regular fringe sites in Puget Sound,
n_3 = number of sites actually surveyed,

$$s_{\hat{X}_{ij}}^2 = \frac{\sum_{j=1}^{n_3} \left(\hat{X}_{ij} - \hat{\bar{X}}_{ij} \right)^2}{\left(n_3 - 1 \right)},$$

$$\hat{\bar{X}}_{ij} = \frac{\sum_{j=1}^{n_3} \hat{X}_{ij}}{n_3}.$$

The estimates of $\hat{B}_4$ and $Var\left(\hat{B}_4 \middle| B_4 \right)$ are analogous to Equations (7) and (8), respectively.

3.3 Estimation Within Embayment Stratum

In this stratum, the sampling units are of dramatically different sizes. A simple random sample of embayments/flats will be performed and BAC estimated using a ratio estimator (Cochran 1977: p. 151) of the form

$$\hat{B}_2 = \left[\frac{\sum_{j=1}^{n_2}\hat{X}_{2j}}{\sum_{j=1}^{n_2}a_{2j}}\right]\cdot\sum_{j=1}^{N_2}a_{2j} = \left[\frac{\sum_{j=1}^{n_2}\hat{X}_{2j}}{\sum_{j=1}^{n_2}a_{2j}}\right]\cdot A_2 \tag{9}$$

where

a_{2j} = area of the *j*th embayment $\left(j=1,\ldots,n_2\right)$ in the second stratum,

$A_2 = \sum_{j=1}^{N_2}a_{2j}$ = the total areal extent of embayment sites within stratum 2.

The estimator and associated variance assume the areas $a_{2j}\ \left(j=1,\ldots,L_2\right)$ are measured without error. The variance for $\hat{B}_2$ can be expressed (Appendix B) as

$$Var\left(\hat{B}_2\right) = N_2^2\left(1-\frac{n_2}{N_2}\right)\frac{\sum_{j=1}^{N_2}\left(X_{2j}-a_{2j}R\right)^2}{n_2\left(N_2-1\right)} + \frac{N_2}{n_2}\sum_{j=1}^{N_2}Var\left(\hat{X}_{2j}\middle|X_{2j}\right) \tag{10}$$

and where

$$R = \frac{\sum_{j=1}^{N_2}X_{2j}}{\sum_{j=1}^{N_2}a_{2j}}.$$

In turn, this variance can be estimated by

$$Var\left(\hat{B}_2\right) = N_2^2\left(1-\frac{n_2}{N_2}\right)\frac{\sum_{j=1}^{n_2}\left(\hat{X}_{2j}-a_{2j}\hat{R}\right)^2}{n_2\left(n_2-1\right)} + \frac{N_2\sum_{j=1}^{n_2}Var\left(\hat{X}_{2j}\middle|X_{2j}\right)}{n_2} \tag{11}$$

where

$$\hat{R} = \frac{\sum_{j=1}^{n_2} \hat{X}_{2j}}{\sum_{j=1}^{n_2} a_{2j}}.$$

4.0 Retrospective Adjustment of BAC in Year i Using Year $i+1$ Data

During the monitoring program, rotational sampling will be conducted at strata 2-4 where probabilistic sampling occurs. At those strata, some fraction f_i of the sampling sites in the previous year will be replaced with new locations selected at random. In the core area stratum, the same reference sites will be sampled each year. The current year's estimate of eelgrass BAC will be based on the same estimators presented in Section 3.0.

However, because of the positive correlation between eelgrass measurements in consecutive years, the estimate of abundance in the past year can be updated with an anticipated improvement in precision. The estimate of the updated total eelgrass BAC will be computed as

$$\tilde{B}_T = \hat{B}_1 + \tilde{B}_2 + \tilde{B}_3 + \tilde{B}_4 \tag{12}$$

for a previous year where $\tilde{B}_2$, $\tilde{B}_3$, and $\tilde{B}_4$ are updated estimates of BAC in strata 2-4 using information from both years i and $i+1$. The retrospective adjustment for total BAC will be done on a stratum-by-stratum basis. The goal of the rotational design is to improve upon the initial estimate taking into account data collected in year $i+1$. The variance for the updated estimate of total BAC for Puget Sound will be calculated as follows:

$$Var\left(\tilde{B}_T\right) = Var\left(\hat{B}_1\right) + Var\left(\tilde{B}_2\right) + Var\left(\tilde{B}_3\right) + Var\left(\tilde{B}_4\right) \tag{13}$$

based on the stratified sampling scheme.

4.1 Core Area Stratum

Rotational sampling is not conducted within the core stratum. Hence, no further update is possible using the $(i+1)$ th year data. As such, for the core stratum $\tilde{B}_1 = \hat{B}_1$, and the estimate remains unchanged with regard to the $(i+1)$ data update.

4.2 Fringe Strata

For the fringe strata under rotational sampling, the initial estimator $\hat{B}_i$ is composed of an estimate based on matched sites (sampled both years i and $i+1$) and nonmatched sites (sampled year i but not in year $i+1$).

An updated estimator for $\hat{B}_i$ [Equation (13)] using the sample data in year $(i+1)$ is

$$\tilde{B}_i = \left(\frac{L_T}{L_N}\right) N \left[W \hat{\bar{X}}'_{U1} + (1-W)\, \hat{\bar{X}}'_{M1} \right] \tag{14}$$

where

$\hat{\bar{X}}'_{U1} = \frac{1}{u}\sum_{j=1}^{u} \hat{X}_{j1}$ estimate of the mean based on unmatched (u) sites surveyed in year i;

$\hat{\bar{X}}'_{M1}$ = revised estimate of the mean in year i based on regression of matched values in year i and $i+1$, where

$$\begin{aligned} \hat{\bar{X}}'_{M1} &= \hat{\bar{X}}_{M1} + \hat{\beta}\left(\hat{\bar{X}}_2 - \hat{\bar{X}}_{2M}\right) \\ &= \alpha + \beta\left(\hat{\bar{X}}_2\right); \end{aligned}$$

and where

$\hat{\bar{X}}_{M1}$ = estimated mean based on matched sites measured in year i;

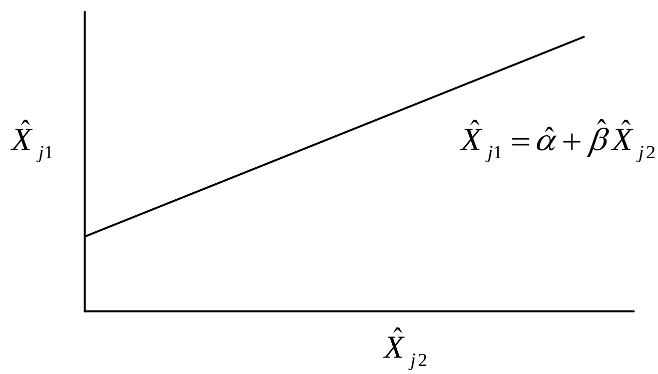
$\hat{\bar{X}}_{2M}$ = estimated mean based on matched sites measured in year $i+1$;

$\hat{\bar{X}}_{2}$ = estimated mean based on all sites measured in year $i+1$.

To estimate $\hat{\bar{X}}'_{M1}$, calculate the regression relationship

$$\hat{X}_{j1} = \hat{\alpha} + \hat{\beta}\hat{X}_{j2}$$

of the form



using the m matched samples collected in year i $(\hat{X}_{j1};\ j=1,\ldots,m)$ and your $i+1$ $(\hat{X}_{j2};\ j=1,\ldots,m)$.

The weights used in Equation (14) are of the form

$$W = \frac{\dfrac{1}{Var\left(\hat{\bar{X}}'_{U1}\right)}}{\dfrac{1}{Var\left(\hat{\bar{X}}'_{U1}\right)} + \dfrac{1}{Var\left(\hat{\bar{X}}'_{M1}\right)}} = \frac{Var\left(\hat{\bar{X}}'_{M1}\right)}{Var\left(\hat{\bar{X}}'_{U1}\right) + Var\left(\hat{\bar{X}}'_{M1}\right)}. \tag{15}$$

In turn,

$$Var\left(\hat{\bar{X}}'_{U1}\right)=\frac{s^2_{\hat{X}_{j1}}\left(1-\frac{u}{N}\right)}{u} \tag{16}$$

where

$$s^2_{\hat{X}_{j1}}=\frac{\sum_{j=1}^{u}\left(\hat{X}_{1j}-\hat{\bar{X}}_{U1}\right)^2}{(u-1)}.$$

The variance of $\hat{\bar{X}}'_{M1}$ is based on double sampling (Cochran 1977: p. 339), in which case

$$Var\left(\hat{\bar{X}}'_{M1}\right)=\frac{s^2_{\hat{X}_{j1}\cdot\hat{X}_{j2}}}{m}+\frac{s^2_{\hat{X}_{j1}}-s^2_{\hat{X}_{j1}\cdot\hat{X}_{j2}}}{n}-\frac{s^2_{\hat{X}_{j1}}}{N} \tag{17}$$

and where

$$s^2_{\hat{X}_{j1}}=\frac{\sum_{j=1}^{m}\left(\hat{X}_{j1}-\hat{\bar{X}}_{m1}\right)^2}{m-1}, \tag{18}$$

$$s^2_{\hat{X}_{j1}\cdot\hat{X}_{j2}}=\frac{1}{m-2}\left[\sum_{j=1}^{m}\left(\hat{X}_{j1}-\hat{\bar{X}}_{m1}\right)^2-\hat{B}^2\sum_{j=1}^{m}\left(\hat{X}_{j2}-\hat{\bar{X}}_{m2}\right)^2\right] \tag{19}$$

$$=\frac{\text{SSE}}{m-2}= \text{ MSE from the ANOVA for the regression analysis.}$$

The weighted estimator [Equation (13)] is composed of two independent estimators, in which case

$$Var\left(\tilde{B}_i\right)=\left(\frac{L_T}{L_N}\right)^2 N^2\left[W^2 Var\left(\hat{\bar{X}}'_{U1}\right)+(1-W)^2 Var\left(\hat{\bar{X}}'_{M1}\right)\right]$$

which simplifies to

$$Var\left(\tilde{B}_i\right)=\left(\frac{L_T}{L_N}\right)^2 N^2\left[\frac{1}{\dfrac{1}{Var\left(\hat{\bar{X}}'_{U1}\right)}+\dfrac{1}{Var\left(\hat{\bar{X}}'_{M1}\right)}}\right]$$

$$=\left(\frac{L_T}{L_N}\right)^2 N^2\left[\frac{Var\left(\hat{\bar{X}}'_{U1}\right)\cdot Var\left(\hat{\bar{X}}'_{M1}\right)}{Var\left(\hat{\bar{X}}'_{U1}\right)+Var\left(\hat{\bar{X}}'_{M1}\right)}\right]. \tag{20}$$

Cochran (1977: pp. 346-347) shows the variance estimator has the expected value of

$$Var\left(\tilde{B}_i\right)=\left(\frac{L_T}{L_N}\right)^2\frac{N^2\left(1-\dfrac{n}{N}\right)S_1^2\left(n-u\rho^2\right)}{\left(n^2-u^2\rho^2\right)}. \tag{21}$$

Optimal fraction (P_{OPT}) of n that should be matched one year to the next is

$$P_{OPT}=\frac{\sqrt{1-\rho^2}}{1+\sqrt{1-\rho^2}}$$

where ρ is the correlation coefficient from year i to year $i+1$.

4.2.1 Simple Illustration for Calculating an Adjusted Fringe Stratum Total

Consider the following dataset collected in years i and $i+1$ for a population of size N = 40, and where $L_N = L_T$,

	Year 1	Year 2	
	7		
$u_1 = 4$	10		
$\hat{\bar{X}}'_{u1} = 9.75$	8		
	14		
	9	12	
	15	21	
$m = 7$	14	17	$m = 7$
$\hat{\bar{X}}'_{m1} = 10.571428$	10	14	$\hat{\bar{X}}_{m2} = 14.428571$
	7	10	
	8	13	
	11	14	
		15	
		17	$u_2 = 4$
		14	$\hat{\bar{X}}_{u2} = 16.25$
		19	
	$\hat{\bar{X}}_{n_1} = 10.27\overline{27}$	$\hat{\bar{X}}_{n_2} = 15.09\overline{09}$	

The stratum total for year 1 is estimated to be

$$\hat{B}_1 = 40(10.2727) = 410.9091.$$

For year 2, the stratum of total is estimated to be

$$\hat{B}_2 = 40\left(15.09\overline{09}\right) = 603.6364.$$

Using the $n = 7$ matched samples, the following regression model is constructed

$$\hat{X}_{j1} = -0.806985 + 0.788603\,\hat{X}_{j2}\ .$$

Then the updated estimate of the sample mean at time 1 is computed as

$$\hat{\bar{X}}'_{M1} = -0.806985 + 0.788603(15.0909)$$
$$= 11.09375.$$

There are now two estimates of $\hat{\bar{X}}_1$, $\hat{\bar{X}}'_{U1}$ = 9.750 based on the unmatched samples in year 1, and $\hat{\bar{X}}'_{M1}$ = 11.094 based on the regression model. The best adjusted estimate is their weighted average

$$\tilde{B} = W(9.750) + (1-W)(11.094).$$

The variance of $\hat{\bar{X}}'_{U1}$ is computed to be

$$Var\left(\hat{\bar{X}}'_{U1}\right) = \frac{\left(1-\frac{4}{40}\right)(9.5833)}{4} = 2.15625$$

where

$$s^2_{\hat{X}_1} = 9.5833.$$

The variance of $\hat{\bar{X}}'_{M1}$ is computed to be

$$Var\left(\hat{\bar{X}}'_{M1}\right) = \frac{1.0768}{7} + \frac{8.9524 - 1.0768}{11} - \frac{8.9524}{40}$$
$$= 0.64598$$

where

$$s^2_{\hat{X}_{j1}} = \frac{\sum_{j=1}^{7}\left(\hat{X}_{j1} - 10.5714\right)^2}{(7-1)} = 8.9524$$

$$s^2_{\hat{X}_{j1}\cdot\hat{X}_{j2}} = \frac{1}{(7-2)}\left[\sum_{j=1}^{7}\left(\hat{X}_{j1} - 10.5714\right)^2 - 0.7886^2\sum_{j=1}^{7}\left(\hat{X}_{j2} - 14.4286\right)^2\right]$$
$$= \frac{5.3842}{5} = 1.0768.$$

The subsequent weight W is computed as

$$W = \frac{0.64598}{2.15625 + 0.64598} = 0.23052.$$

The adjusted average $\tilde{X}_1$ is then estimated to be

$$\begin{aligned}\tilde{X}_1 &= 0.23052(9.750) + 0.76948(11.094) \\ &= 10.7840\end{aligned}$$

and the adjusted total $\tilde{B}_1$ = 40 (10.7840) = 431.36. The estimated variance $\tilde{X}_1$ is then

$$Var\left(\tilde{X}_1\right) = \frac{1}{\frac{1}{2.15625} + \frac{1}{0.64598}} = 0.49707$$

and the variance of $\tilde{B}$ is

$$Var\left(\tilde{B}_1\right) = 40^2\left(0.49707\right) = 795.309$$

or

$$SE\left(\tilde{B}_1\right) = 28.201.$$

Note in year 1, the original sample had a mean of $\hat{\hat{X}}'_{M1} = 10.\overline{27}$ with a variance estimate of

$$Var\left(\hat{\hat{X}}_{M1}\right) = \frac{\left(1 - \frac{11}{40}\right)\left(8.41\overline{81}\right)}{11} = 0.5548.$$

This translates to a total of $\hat{B}_1$ = 410.9091 and a standard error of $SE(\hat{B}_1)$ = 29.7949. In this artificial example, with r =0.9486, the variance decreased by 10.4% and the standard error by 5.3% using the rotational adjustment.

4.3 Flats Stratum

The estimate of total BAC in the flats stratum is calculated as follows:

$$\hat{B}_2 = \left[\frac{\sum_{j=1}^{n_2} \hat{X}_{2j}}{\sum_{j=1}^{n_2} a_{2j}} \right] \cdot A_2 \tag{22}$$

where

$\hat{X}_{2j}$ = estimate of BAC in the *j*th embayment ($j = 1, \ldots, N_2$) in the flats stratum;

a_{2j} = area of the *j*th embayment ($j = 1, \ldots, N_2$) in the flats stratum;

$A_2 = \sum_{j=1}^{N_2} a_{2j}$ = total area in flats stratum.

It is assumed the a_{2j} are measured without error and represents the geographic area of an embayment that does not change over time.

An adjusted estimator of BAC in year 1, $\hat{B}_i$, using the data collected in both years i and $i+1$, can be written as

$$\tilde{B} = W \cdot \hat{B}'_{U1} + \left(1 - W\right) \hat{B}'_{M1} \tag{23}$$

where

$$\hat{B}'_{U1} = \frac{\sum_{j=1}^{u_1} \hat{X}_{2jU_1}}{\sum_{j=1}^{u_1} a_{2jU_1}} \cdot A_2 \tag{24}$$

= estimate of BAC using only the unmatched sites sampled in year 1.

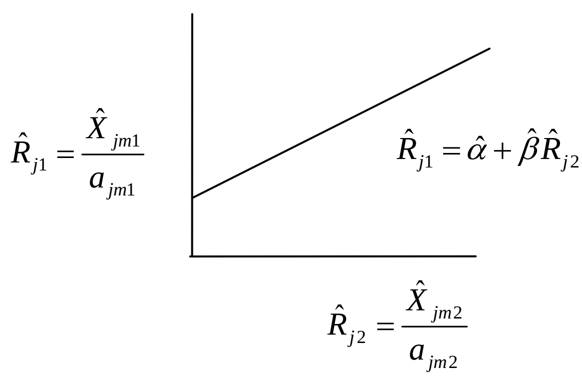
The variance of $\hat{B}'_{U1}$ is estimated using Equation (11) based on the u_1 unmatched sites only in year i; in other words

$$Var\left(\hat{B}'_{U1}\right) = N^2\left(1-\frac{u_1}{N}\right)\frac{\sum_{j=1}^{u_1}\left(\hat{X}_{j1} - a_{j1}\hat{R}_{U1}\right)^2}{u_1\left(u_1-1\right)} + \frac{N\sum_{j=1}^{u_1}\left(\hat{X}_{j1}\middle| X_{j1}\right)}{u_1} \qquad (25)$$

where

$$\hat{R}_{U1} = \frac{\sum_{j=1}^{u_1}\hat{X}_{j1}}{\sum_{j=1}^{u_1}a_{j1}}.$$

The estimator $\hat{B}'_{M1}$ is calculated from a regression relationship of the form



which is a straight-line relationship between the site ratios (i.e., density $\hat{X}_{jm1}/a_{jm1}$) measured in year 1 against site ratios measured in year 2 for the m matched sites. The estimate of $\hat{B}'_{M1}$ is then calculated as

$$\hat{B}'_{M1} = \hat{\alpha} + \hat{\beta}\left(\frac{\sum_{j=1}^{n}\hat{X}_{j2}}{\sum_{j=1}^{n}a_{j2}}\right). \qquad (26)$$

The quotient $\sum_{j=1}^{n} \hat{X}_{j2} \Big/ \sum_{j=1}^{n} a_{j2}$ is the ratio estimator using all n sites measured in year 2.

The variance of $\hat{B}'_{M1}$ is estimated by the expression

$$Var\left(\hat{B}'_{M1}\right) = A_2^2\left[\frac{s^2_{\hat{R}_{j1}\hat{R}_{j2}}}{m} + \frac{s^2_{\hat{R}_{j1}} - s^2_{\hat{R}_{j1}\hat{R}_{j2}}}{n} - \frac{s^2_{\hat{R}_{j1}}}{N}\right] \tag{27}$$

where

$$s^2_{\hat{R}_{j1}} = \frac{\sum_{j=1}^{m}\left(\hat{R}_{j1} - \hat{\bar{R}}_1\right)^2}{m-1}$$

where

$$\hat{\bar{R}}_1 = \frac{\sum_{j=1}^{m}\hat{R}_{j1}}{m}$$

$$\hat{R}_{j1} = \frac{\hat{X}_{j1}}{a_{j1}} \text{ for } j = 1,\ldots,m$$

and where

$s^2_{\hat{R}_{j1}\hat{R}_{j2}}$ = MSE from the ANOVA for the regression analysis.

The weight (W) used in Equation (23) is calculated as follows

$$W = \frac{Var\left(\hat{B}'_{M1}\right)}{Var\left(\hat{B}'_{U1}\right) + Var\left(\hat{B}'_{M1}\right)}\,. \tag{28}$$

The adjusted estimator [Equation (23)] is composed of two independent estimators, in which case

$$Var\left(\tilde{B}\right)=W^2 Var\left(\hat{B}'_{U1}\right)+\left(1-W\right)^2 Var\left(\hat{B}'_{M1}\right) \tag{29}$$

which simplifies to

$$Var\left(\tilde{B}\right)=\frac{Var\left(\hat{B}'_{U1}\right)\cdot Var\left(\hat{B}'_{M1}\right)}{Var\left(\hat{B}'_{U1}\right)+Var\left(\hat{B}'_{M1}\right)} \tag{30}$$

analogous to Equation (20) for fringe sites.

4.3.1 Simple Illustration for Calculating an Adjusted Flats Stratum Total

Consider the following dataset collected in years i and $i+1$ for a population of size N = 20, with total area A_2 = 1705, and where

	Year 1		Year 2		
	X	a	X	a	
u_1 = 5	12	53			
	6	37			
	19	101			
	5	21			
	13	72			
m = 5	27	133	31	133	m = 5
	18	97	20	97	
	31	165	35	165	
	8	36	10	36	
	14	74	16	74	
			15	81	u_2 = 5
			24	111	
			6	37	
			11	60	
			26	151	
Totals	153	789	194	945	
	$\hat{R}_1$ = 0.19392		$\hat{R}_2$ = 0.20529		

For this simple example, measure error will be ignored.

In year 1, the estimate of BAC would be computed to be

$$\hat{B} = \frac{153}{789} \cdot 1705 = 330.6274$$

with associated variance estimator

$$Var\left(\hat{B}\right) = 20^2\left(1 - \frac{10}{20}\right)\frac{\sum_{j=1}^{10}\left(X_j - a_j\left(\frac{153}{789}\right)\right)^2}{10(10-1)} = 23.8828.$$

An updated estimator using the data in year 2 is computed in two steps. First, using the unmatched data in year 1

$$\hat{B}'_{U1} = \frac{55}{284} \cdot 1705 = 330.1937$$

with an associated variance estimate of

$$Var\left(\hat{B}'_{U1}\right) = 20^2\left(1 - \frac{5}{20}\right)\frac{\sum_{j=1}^{5}\left(X_{j1} - a_j\left(\frac{55}{284}\right)\right)^2}{5(5-1)} = 96.6958.$$

Next, fitting a linear regression model for the site ratios (i.e., $\hat{X}_j / a_j$) in year 1 against year 2 for the matched sites yields

$$R_{1j} = 0.07712 + 0.5258\, R_{2j}$$

with r = 0.98389 and MSE = 0.000005077.

The estimate of $\hat{B}'_{M1}$ is then calculated to be

$$\hat{B}'_{M1} = \left[0.07712 + 0.5258\left(\frac{194}{945}\right)\right]1705$$
$$= 0.18506(1705)$$
$$= 315.5375$$

with associated variance estimator

$$Var\left(\hat{B}'_{M1}\right) = (1705)^2 \left[\frac{0.000005077}{5} + \frac{0.00023638 - 0.000005077}{10} - \frac{0.00023638}{20}\right]$$
$$= (1705)^2 (0.000012327)$$
$$= 35.8344$$

where

$s^2_{\hat{R}_{j1}}$ = 0.00023638

$s^2_{\hat{R}_{j1}\hat{R}_{j2}}$ = MSE = 0.000005077.

The weight is computed from the variance estimates to be

$$W = \frac{35.8344}{96.6958 + 35.8344} = 0.27039.$$

The adjusted BAC estimate for year 1 is then computed to be

$$\tilde{B} = 330.1937(0.27039) + 315.5307(1 - 0.27039)$$
$$= 319.5003.$$

The variance of $\tilde{B}$, in turn, is calculated to be

$$Var\left(\tilde{B}\right) = \frac{96.6958(35.8344)}{96.6958 + 35.8344}$$
$$= 26.1453.$$

In this example, the variance of the adjusted BAC actually increased over the original estimate.

5.0 Estimating the Change in BAC Between Years i and $i+1$

5.1 Relative Change Within a Stratum

The best and easiest way of estimating the fractional change (RC) in BAC defined as

$$RC = \frac{B_{i+1} - B_i}{B_i} = \frac{B_{i+1}}{B_i} - 1 \tag{31}$$

is to perform a regression analysis. Fit a straight-line regression through the origin of the form

$$\hat{X}_{i+1,j} = \hat{X}_{i+1,j}\beta + \varepsilon_j \tag{32}$$

where

$\hat{X}_{ij}$ = estimated BAC at the *j*th location in year i,

$\hat{X}_{i+1,j}$ = estimated BAC at the *j*th location in year $i+1$,

β = regression coefficient;

ε_i = random error term $\sim N(0, \sigma^2)$.

Equation (32) describes a straight-line regression through the origin. Then it is easy to see

$$\frac{\hat{X}_{i+1,j}}{\hat{X}_{ij}} = \beta + \varepsilon_j \ .$$

Hence, we can estimate the fractional change by

$$RC = \hat{\beta} - 1 \tag{33}$$

and where

$$Var\left(RC\right) = Var\left(\hat{\beta} - 1\right) \\ = Var\left(\hat{\beta}\right). \quad (34)$$

The analysis should be conducted on the *m*-matched sites surveyed during both years i and $i+1$ in a stratum. Separate analyses should be performed for each stratum.

5.2 Relative Change in Puget Sound

The estimate of relative change between years i and $i+1$ across Puget Sound is then estimated by the quantity

$$RC_T = \frac{\sum_{j=1}^{4} \hat{B}_{ij} RC_j}{\sum_{j=1}^{4} \hat{B}_{ij}} \quad (35)$$

where

$\hat{B}_{ij}$ = estimated BAC in the *j*th stratum in year i,

RC_j = estimated relative change in the BAC in the *j*th stratum between years i and $i+1$.

The variance of RC_T for Puget Sound can be approximated using the delta method (Seber 1982: p. 7) where

$$Var\left(RC_T\right) = \sum_{j=1}^{4} \left[Var\left(RC_j\right) \left(\frac{\hat{B}_{ij}}{\sum_{j=1}^{4} \hat{B}_{ij}} \right)^2 \right] + \sum_{j=1}^{4} \left[Var\left(\hat{B}_{ij}\right) \left(\frac{RC_j \sum_{j=1}^{4} \hat{B}_{ij} - \sum_{j=1}^{4} \hat{B}_{ij} RC_j}{\left(\sum_{j=1}^{4} \hat{B}_{ij} \right)^2} \right)^2 \right]. \quad (36)$$

5.3 Areal Change Within a Stratum

For the *j*th stratum $(j = 1, \ldots, 4)$, the areal change (AC_j) in BAC between years i and $i+1$ can be estimated by the quantity

$$AC_j = \hat{B}_{ij} \cdot RC_j \tag{37}$$

with estimated variance

$$Var\left(AC_i\right) = Var\left(\hat{B}_{ij}\right) \cdot RC_j^2 + Var\left(RC_j\right) \cdot \hat{B}_{ij}^2 - Var\left(\hat{B}_{ij}\right) \cdot Var\left(RC_j\right). \tag{38}$$

5.4 Areal Change in Puget Sound

For the entire Puget Sound, areal change would be estimated by the quantity

$$AC_T = \sum_{j=1}^{4} AC_j \tag{39}$$

with associated variance estimator

$$Var\left(AC_T\right) = \sum_{j=1}^{4} Var\left(AC_j\right). \tag{40}$$

5.5 Relative Change Within a Site

The percent relative change (RC) in basal area coverage (B) from one year (i.e., B_i) to the next year (B_{i+1}) at a site can be estimated by the quantity

$$\begin{aligned} RC &= \left(\frac{\hat{B}_{i+1} - \hat{B}_i}{\hat{B}_i} \right) \cdot 100\% \\ &= \left(\frac{\hat{B}_{i+1}}{\hat{B}_i} - 1 \right) \cdot 100\%. \end{aligned} \tag{41}$$

The RC estimates the percent increase or decrease in BAC from year i to year $i+1$.

The variance of RC is expressed as

$$Var\left(RC\right)=\left(\frac{\hat{B}_{i+1}}{\hat{B}_i}\right)^2\left[\frac{Var\left(\hat{B}_i\right)}{\hat{B}_i^2}+\frac{Var\left(\hat{B}_{i+1}\right)}{\hat{B}_{i+1}^2}\right]\cdot\left(100\%\right)^2. \qquad (42)$$

The standard error is expressed as

$$SE\left(RC\right)=\left(\frac{\hat{B}_{i+1}}{\hat{B}_i}\right)\cdot 100\%\sqrt{\frac{Var\left(\hat{B}_i\right)}{\hat{B}_i^2}+\frac{Var\left(\hat{B}_{i+1}\right)}{\hat{B}_{i+1}^2}}\,. \qquad (43)$$

Finally, an asymptotic normal confidence interval is then calculated as

$$RC\pm Z_{1-\frac{\alpha}{2}}\cdot SE\left(RC\right) \qquad (44)$$

where for a 95% CI, $Z_{1-\frac{\alpha}{2}}$ = 1.96 or for a 90% CI, $Z_{1-\frac{\alpha}{2}}$ = 1.645.

6.0 Test for a Five-Year Regional Trend

6.1 Test of Slope

Using a straight-line regression of annual response versus year (i.e., t = 0, 1, 2, 3, 4), the null hypothesis of no decline can be written as

$$\mathrm{H_o}:\quad \beta\geq 0 \qquad (45)$$

vs.

$$\mathrm{H_a}:\quad \beta<0$$

where β is the slope of the regression model $\hat{B}_t=\alpha+\beta t$. The null hypothesis can be tested using the t-statistic

$$t_{m-2} = \frac{\left|\hat{\beta} - 0\right|}{\sqrt{\frac{\text{MSE}}{\sum_{i=1}^{m}\left(t_i - \overline{t}\right)^2}}}. \tag{46}$$

6.2 Power Calculations

In the special case of a five-year test of trend

a. $\sum_{i=1}^{m}\left(t_i - \overline{t}\right)^2 = 10$ for $t_i = (0,1,2,3,4)$

b. $E(\text{MSE}) = \sigma_N^2 + \overline{\text{Var}\left(\hat{B}_T \middle| B_T\right)}$

where

σ_N^2 = natural variation in response,

$\text{Var}\left(\hat{B}_T \middle| B_T\right)$ = variance in the annual estimate of Puget-Sound-wide BAC.

c. $\beta = B_0\Delta$ for a linear change in response $B_i = B_0(1 - i\Delta)$

and where

Δ = annual fractional reduction in response,

B_0 = regional BAC in the first year.

Taking into account factors a-c, the noncentrality parameter associated with the noncentral F-distribution under H_a can be written as

$$\Phi_{1,3} = \frac{1}{\sqrt{2}} \cdot \frac{\left|B_0\Delta\right|}{\sqrt{\frac{\sigma_N^2 + \overline{\text{Var}\left(\hat{B}\middle|B\right)}}{10}}}. \tag{47}$$

Currently, based on observations for 2000-2002, we would estimate $\sigma_N^2 = 0$. Therefore, if we assume the magnitude of the natural variation is near zero (i.e., $\sigma_N^2 = 0$), then the noncentrality parameter can be rewritten as

$$\Phi_{1,3} = \sqrt{5} \cdot \frac{|\Delta|}{\sqrt{\mathrm{CV}^2}} \tag{48}$$

where

$$\mathrm{CV}^2 = \frac{\overline{Var\left(\hat{B} \middle| B\right)}}{B_0^2}.$$

6.2.1 Example: Power Calculations for Detecting a Five-Year Decline

For the sound-wide estimates of BAC, the average CV for the years 2000-2002 was 0.256 based on unadjusted annual estimates. However, for the one year (i.e., 2001) for which we have a rotational-design, adjusted estimate, the CV = 0.070. Consider, first, the case where CV = 0.256 and Δ = -0.0625 [i.e., -0.25 = (-0.0625) 4 changes in five years], then

$$\Phi_{1,3} = \sqrt{5} \cdot \frac{|-0.0625|}{\sqrt{(0.256)^2}} = 0.5459.$$

Reading for the noncentral table, statistical power is $1-\beta \approx 0.30$ (Skalski and Robson 1992) at α = 0.10, one-tailed. In the second case where future CVs are anticipated to be approximately 0.070, the power to detect a 25% decline in five years is

$$\Phi_{1,3} = \sqrt{5} \cdot \frac{|-0.0625|}{\sqrt{(0.07)^2}} = 1.9965 ,$$

corresponding to a statistical power of $1-\beta \approx 0.8666$ at α = 0.10, one-tailed.

6.3 Detecting a 10-Year Decline

The noncentrality parameter for a 10-year test of a linear trend is

$$\Phi_{1,8} = \frac{1}{\sqrt{2}} \cdot \frac{|B_0 \Delta|}{\sqrt{\frac{\text{Var}\left(\hat{B} \middle| B\right)}{82.5}}}$$

or

$$\Phi_{1,8} = \sqrt{41.25} \cdot \frac{|\Delta|}{\sqrt{\text{CV}^2}} . \quad (49)$$

Using Equation (49), the power to detect a 25% reduction in regional eelgrass within 10 years can be calculated where Δ = 0.02778 [i.e., -0.02778 (9) = -0.25].

$$\Phi_{1,8} = \sqrt{41.25} \cdot \frac{|-0.02778|}{\sqrt{(0.07)^2}} = 2.5489 .$$

Reading for the noncentral F-table, $1-\beta \approx 0.9460$ at α = 0.10, one-tailed. This power calculation is based on the assumption that the average CV for the future rotational adjusted estimates of sound-wide BAC will be 0.070.

7.0 Literature Cited

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Appendix A: Derivation of Variance for SRS with Measurement Error

The variance of $\hat{B}_3$ can be found in stages as follows:

$$Var\left(\hat{B}_3\right) = Var\left(\frac{N_3}{n_3}\sum_{j=1}^{n_3}\hat{X}_{ij}\right) = Var_2\left[E_1\left(\frac{N_3}{n_3}\sum_{j=1}^{n_3}\left(\hat{X}_{ij}\middle|2\right)\right)\right] + E_2\left[Var_1\left(\frac{N_3}{n_3}\sum_{j=1}^{n_3}\left(\hat{X}_{ij}\middle|2\right)\right)\right]$$

where

1 denotes selection of sampling units within a stratum,

2 denotes sampling of eelgrass abundance within a sampling unit.

Then

$$Var\left(\hat{B}_3\middle|B_3\right) = Var_2\left[\left(\frac{N_3}{n_3}\sum_{j=1}^{n_3}X_{ij}\right)\right] + E_2\left[\frac{N_3^2}{n_3^2}\sum_{j=1}^{n_3}Var\left(\hat{X}_{ij}\middle|X_{ij}\right)\right]$$

$$= \frac{N_3^2\left(1-\frac{n_3}{N_3}\right)S_{x_i}^2}{n_3} + \frac{N_3}{n_3}\sum_{j=1}^{n_3}Var\left(\hat{X}_{ij}\middle|X_{ij}\right). \qquad \text{(A1)}$$

The second term of Equation (A1) can be unbiasedly estimated by

$$\left(\frac{N_3}{n_3}\right)^2\sum_{j=1}^{n_3}V\hat{a}r\left(\hat{X}_{ij}\middle|X_{ij}\right). \qquad \text{(A2)}$$

However, substituting $s_{\hat{X}_i}^2$ into the first term of Equation (A1) results in an expected value of

$$E\left[\frac{N_3^2\left(1-\frac{n_3}{N_3}\right)s_{\hat{X}_{ij}}^2}{n_3}\right] = \frac{N_3^2\left(1-\frac{n_3}{N_3}\right)S_{X_i}^2}{n_3} + \frac{N_3^2\left(1-\frac{n_3}{N_3}\right)}{n_3}\cdot\frac{1}{N_3}\sum_{j=1}^{N_3}Var\left(\hat{X}_{ij}\middle|X_{ij}\right). \qquad \text{(A3)}$$

Hence, there is a positive bias of

$$\frac{N_3\left(1-\frac{n_3}{N_3}\right)}{n_3}\sum_{j=1}^{N_3}Var\left(\hat{X}_{ij}\middle|X_{ij}\right). \qquad \text{(A4)}$$

Combing the results of Equations (A1- A4), the estimated variance of $\hat{B}_3$ can be written as

$$Var\left(\hat{B}_3\middle|B_3\right)=\frac{N_3^2\left(1-\frac{n_3}{N_3}\right)s_{\hat{X}_i}^2}{n_3}-\frac{N_3\left(1-\frac{n_3}{N_3}\right)}{n_3}\frac{N_3}{n_3}\sum_{j=1}^{n_3}Var\left(\hat{X}_{ij}\middle|X_{ij}\right)+\left(\frac{N_3}{n_3}\right)^2\sum_{j=1}^{n_3}Var\left(\hat{X}_{ij}\middle|X_{ij}\right)$$

which simplifies to

$$Var\left(\hat{B}_3\middle|B_3\right)=\frac{N_3^2\left(1-\frac{n_3}{N_3}\right)s_{\hat{X}_i}^2}{n_3}+\frac{N_3}{n_3}\sum_{j=1}^{n_3}V\hat{a}r\left(\hat{X}_{ij}\middle|X_{ij}\right). \qquad \text{(A5)}$$

Appendix B: Variance for Ratio Estimator with Sampling Error

$$Var\left(\hat{B}_2\right) = Var\left(A_2 \frac{\sum_{j=1}^{n_2} \hat{X}_{2j}}{\sum_{j=1}^{n_2} a_{2j}} \right) = Var_1\left[E_2\left(A_2 \frac{\sum_{j=1}^{n_2} \hat{X}_{2j}}{\sum_{j=1}^{n_2} a_{2j}} \middle| 1 \right)\right] + E_1\left[Var_2\left(A_2 \frac{\sum_{j=1}^{n_2} \hat{X}_{2j}}{\sum_{j=1}^{n_2} a_{2j}} \middle| 1 \right)\right]$$

where

1 denotes stage one sampling of n_2 of N_2 sites,

2 denotes stage two sampling within a site.

Then

$$Var\left(\hat{B}_2\right) = Var_1\left[A_2 \frac{\sum_{j=1}^{n_2} X_{2j}}{\sum_{j=1}^{n_2} a_{2j}} \right] + E_1\left[\left(\frac{A_2}{\sum_{j=1}^{n_2} a_{2j}} \right)^2 \sum_{j=1}^{n_2} Var\left(\hat{X}_{2j} \middle| X_{2j} \right) \right]$$

$$= A_2^2 \left(1 - \frac{n_2}{N_2}\right) \frac{\sum_{j=1}^{N_2} \left(x_{2j} - a_{2j} R \right)^2}{\bar{A}^2 n_2 \left(N_2 - 1 \right)} + \left(\frac{A_2}{\frac{n_2}{N_2} \sum_{j=1}^{N_2} a_{2j}} \right)^2 \frac{n_2}{N_2} \sum_{j=1}^{N_2} Var\left(\hat{X}_{2j} \middle| X_{2j} \right)$$

$$= N_2^2 \left(1 - \frac{n_2}{N_2}\right) \frac{\sum_{j=1}^{N_2} \left(X_{2j} - a_{2j} R \right)^2}{n_2 \left(N_2 - 1 \right)} + \frac{N_2}{n_2} \sum_{j=1}^{N_2} Var\left(\hat{X}_{2j} \middle| X_{2j} \right) \qquad \text{(B1)}$$

$$Var\left(\hat{B}_2\right) = \frac{N_2^2 \left[\left(1 - \frac{n_2}{N_2}\right) \frac{\sum_{j=1}^{N_2} \left(\hat{X}_{2j} - a_{2j} R \right)^2}{\left(N_2 - 1 \right)} + \overline{Var\left(\hat{X}_{2j} \middle| X_{2j} \right)} \right]}{n_2} \qquad \text{(B2)}$$

Deriving an Estimated Variance for $\hat{B}_2$

The second term in Equation (B2) can be unbiasedly estimated by

$$\overline{Var\left(\hat{X}_{2j} \middle| X_{2j}\right)} = \frac{1}{n_2}\sum_{j=1}^{n_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right).$$

The term $\dfrac{\sum_{j=1}^{N_2}\left(X_{2j} - a_{2j}R\right)^2}{\left(N_2 - 1\right)}$ can be estimated by the expression

$$\frac{\sum_{j=1}^{n_2}\left(\hat{X}_{2j} - a_{2j}\hat{R}\right)^2}{\left(n_2 - 1\right)}$$

but its expected value is approximately

$$E\left[\frac{\sum_{j=1}^{n_2}\left(\hat{X}_{2j} - a_{2j}\hat{R}\right)^2}{n_2 - 1}\right]$$

$$= E\left[\frac{\sum_{j=1}^{n_2}\left(\left(\hat{X}_{2j} - X_{2j}\right) + \left(X_{2j} - a_{2j}\hat{R}\right)\right)^2}{\left(n_2 - 1\right)}\right]$$

$$= E\left[\frac{\sum_{j=1}^{n_2}\left(\hat{X}_{2j} - X_{2j}\right)^2}{\left(n_2 - 1\right)} + \frac{\sum_{j=1}^{n_2}\left(X_{2j} - a_{2j}\hat{R}\right)^2}{\left(n_2 - 1\right)} + \frac{2\sum_{j=1}^{n}\left(X_{2j} - a_{2j}\hat{R}\right)\left(\hat{X}_{2j} - X_{2j}\right)}{\left(n_2 - 1\right)}\right]$$

$$= \frac{\frac{n_2}{N_2}\sum_{j=1}^{N_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right)}{\left(n_2 - 1\right)} + \frac{\sum_{j=1}^{N_2}\left(X_{2j} - a_{2j}R\right)^2}{\left(N_2 - 1\right)}. \qquad \text{(B4)}$$

Hence, (B4) has a positive bias of

$$\frac{\frac{n_2}{N_2}\sum_{j=1}^{N_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right)}{\left(n_2-1\right)}. \tag{B5}$$

This bias can be estimated by

$$\frac{\sum_{j=1}^{n_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right)}{\left(n_2-1\right)}. \tag{B6}$$

Combining terms (B2, B3, B4, and B6), a variance estimator for $\hat{B}_2$ can be expressed as

$$Var\left(\hat{B}_2\right) = \frac{N_2^2\left[\left(1-\frac{n_2}{N_2}\right)\left[\frac{\sum_{j=1}^{n_2}\left(\hat{X}_{2j}-a_{2j}\hat{R}\right)^2}{\left(n_2-1\right)} - \frac{\sum_{j=1}^{n_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right)}{\left(n_2-1\right)}\right] + \frac{\sum_{j=1}^{n_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right)}{n_2}\right]}{n_2}$$

which simplifies to

$$Var\left(\hat{B}_2\right) = N_2^2\left(1-\frac{n_2}{N_2}\right)\frac{\sum_{j=1}^{n_2}\left(\hat{X}_{2j}-a_{2j}\hat{R}\right)^2}{n_2\left(n_2-1\right)} + \frac{N_2\sum_{j=1}^{n_2} Var\left(\hat{X}_{2j} \middle| X_{2j}\right)}{n_2}. \tag{B7}$$