

Puget Sound Submerged Vegetation Monitoring Program

2010-2013 Report

February 27, 2015



**PUGET SOUND ECOSYSTEM
MONITORING PROGRAM**



**WASHINGTON STATE DEPARTMENT OF
Natural Resources**

Peter Goldmark - Commissioner of Public Lands

The Submerged Vegetation Monitoring Project is a component of the Puget Sound Ecosystem Monitoring Program (PSEMP) (<http://sites.google.com/site/pugetsoundmonitoring/>).

Cover Photo: Eelgrass bed west of Dumas Bay, King County. Helen Berry, DNR.

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Nearshore Habitat Program
Aquatic Resources Division



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The Nearshore Habitat Program is part of the Washington State Department of Natural Resources' (DNR) Aquatic Resources Division, the steward for state-owned aquatic lands. Program funding is provided through the Aquatic Lands Enhancement Act. The Nearshore Habitat Program monitors and evaluates the status and trends of marine vegetation for DNR and the Puget Sound Partnership.

The following document fulfills DNR's Eelgrass Monitoring performance measure. It also fulfills tasks in the Puget Sound Partnership's Action Agenda by providing information on the status and trends of one of the selected indicators of environmental health.

The principal authors of this report include Bart Christiaen, Pete Dowty, Lisa Ferrier, Helen Berry, Mike Hannam and Jeff Gaeckle. Fred Short managed the monitoring program during a portion of the period covered in this report, and contributed significantly to data interpretation. Several people played a critical role in the video data collection and post-processing for the work summarized in this report including Kiri Kreamer, Dolores Sare, Andrew Ryan, Jessica Stowe and Rose Whitson. John Van Sickle developed the use of the permutation test for assessing long-term trends in Soundwide Eelgrass Area (Appendix 1).

The Nearshore Habitat Program would like to give special recognition to Ian Fraser and Jim Norris of Marine Resources Consultants who continue to play a significant role in the success of the project. Marine Resources Consultants showed great dedication and logged many hours of sea time collecting data for the project.

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Copies of this report may be obtained from:

http://www.dnr.wa.gov/ResearchScience/Topics/AquaticHabitats/Pages/aqr_nrsh_eelgrass_monitoring.aspx

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Executive Summary

The Washington State Department of Natural Resources (DNR) manages 2.6 million acres of state-owned aquatic lands for the benefit of current and future citizens of Washington State. DNR's stewardship responsibilities include protection of native seagrasses such as eelgrass (*Zostera marina*), an important nearshore habitat in greater Puget Sound. DNR monitors the status and trends of native seagrass abundance and depth distribution throughout greater Puget Sound using underwater videography. Monitoring was initiated in 2000. The monitoring results are used by the Puget Sound Partnership as one of 21 vital signs to track restoration progress (PSP 2014).

Key Findings:

1. **Soundwide native seagrass area has been stable over the monitoring record.** There is no significant long-term linear trend in soundwide native seagrass area (permutation test, $p=0.63$). It is possible that small variations in soundwide native seagrass area occurred below the detection limits of the SVMP program, but seagrass in Puget Sound has not experienced a major decline.
2. **Current native seagrass conditions have not yet met the Puget Sound Partnership's target for a 20% increase in area by 2020.** Statistical tests show that current soundwide native seagrass area is less than the target defined by the Puget Sound Partnership. It is too early to tell if the trend in seagrass area is on a trajectory to meet the target by 2020. Test results are equivocal on whether current conditions have progressed from the baseline conditions.
3. **Most of the 347 individual sites that were analyzed for change were stable throughout the entire monitoring record.** Twenty-five sites decreased in native seagrass area, 17 sites increased in native seagrass area, 209 sites experienced no detectable change, and 60 sites did not have seagrass beds present. Thirty-six sites had insufficient data for trend analysis (sampled only 1 year). Many of the sites with long-term decreases in native seagrass area were located near Hood Canal, Southern Puget Sound and the San Juan Islands (Figure A).
4. **Seagrass conditions improved in the recent 2-3 years.** Analysis of individual site data in recent years ($n=156$) shows that there are more sites with increasing ($n=25$) than decreasing ($n=5$) native seagrass area between 2010 and 2013. The reason is unknown; it could be a short-term anomaly or part of a longer-term pattern (Figure B).
5. **Native seagrass area increased at two river deltas following major restoration projects:** the Skokomish River delta in lower Hood Canal and the Nisqually River delta in southern Puget Sound.

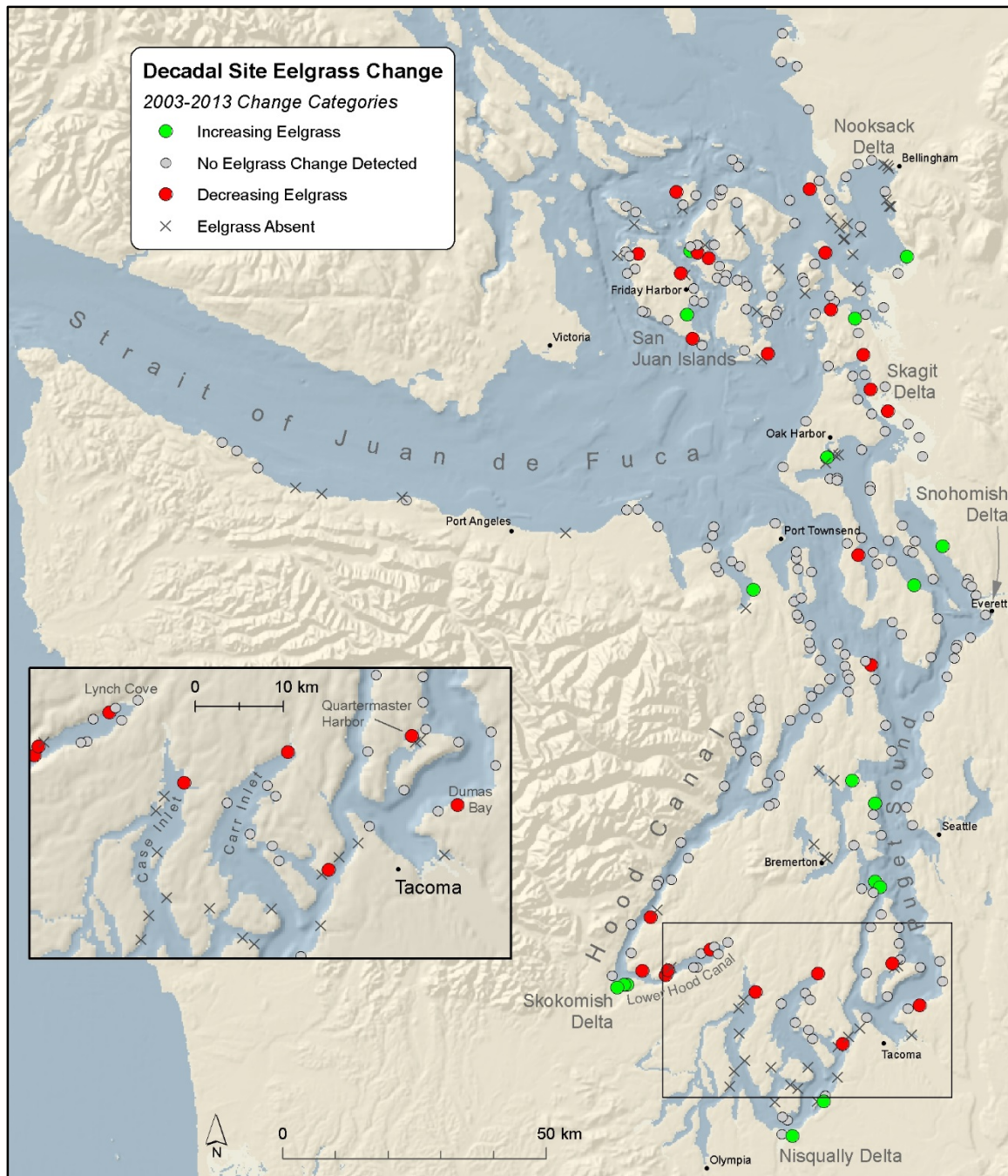


Figure A. Increases and decreases in site native seagrass area based on all available data for each site between 2003 and 2013.

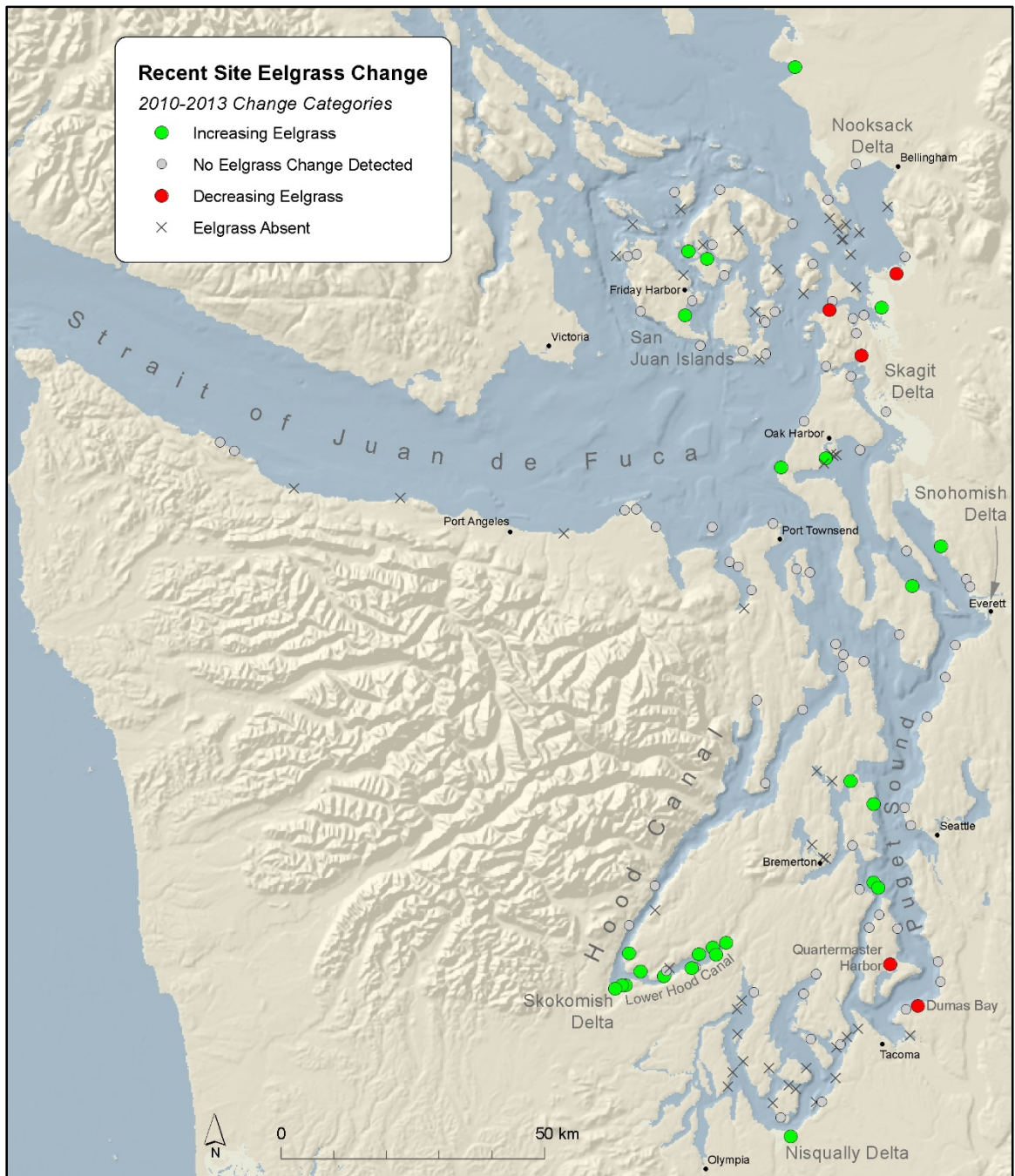


Figure B. Recent increases and decreases in site native seagrass area based on all available data for each site between 2010 and 2013.

Priorities:

1. Continue to monitor the status and trends in native seagrasses throughout Puget Sound to meet goals defined by DNR and the Puget Sound Partnership. Improve the monitoring program's ability to detect long-term trends in seagrass area by revising the soundwide site rotation protocol. Continue to evaluate transect placement protocols at sites for potential improvements to status and trends estimates.
2. Provide technical support and data to scientists and managers on the status and trends in native seagrass, and on sites and regions of concern in Puget Sound.
3. Collaborate with other researchers to further assess changes in sites of particular interest, including those listed in the 2014 Puget Sound Eelgrass Recovery Strategy (Goehring et al. in prep). Initial focus will be on sites:
 - a. near river deltas in response to restoration;
 - b. in lower Hood Canal where recent increases in eelgrass contrast with long-term declines.



1 Introduction

1.1 The SVMP Program

Eelgrass (*Zostera marina*) provides a wide range of important ecosystem services. In Puget Sound, eelgrass offers spawning grounds for Pacific herring (*Clupea harengus pallasii*), out-migrating corridors for juvenile salmon (*Oncorhynchus* spp.) (Phillips 1984, Simenstad 1994), and important feeding and foraging habitats for waterbirds such as the black brant (*Branta bernicla*) (Wilson and Atkinson 1995) and great blue heron (*Ardea herodias*) (Butler 1995). In addition, eelgrass provides valued hunting grounds and ceremonial foods for Native Americans and First Nation People in the Pacific Northwest (Suttles 1951, Felger and Moser 1973, Kuhnlein and Turner 1991, Wyllie-Echeverria and Ackerman 2003). Eelgrass responds quickly to anthropogenic stressors such as physical disturbance, and reduction in sediment and water quality due to excessive input of nutrients and organic matter. This makes it an effective indicator of habitat condition (Dennison et al. 1993, Short and Burdick 1996, Lee et al. 2004, Kenworthy et al. 2006, Orth et al. 2006). Research has generated an abundance of peer-reviewed literature and brought significant ecological and political attention to the species (e.g., Phillips 1984, Orth and Moore 1988, Krause-Jensen et al. 2003, Kemp et al. 1983, 2004, Moore and Short 2006, Waycott et al., 2009).

The Washington State Department of Natural Resources (DNR) stewards 2.6 million acres of state-owned aquatic land. As part of its stewardship responsibilities, DNR monitors the native seagrass population (predominantly eelgrass, *Zostera marina*) across the nearshore of greater Puget Sound. The monitoring data is used to characterize the status of native seagrass and is one of 21 vital signs used by the Puget Sound Partnership to track progress in the restoration and recovery of Puget Sound (PSP 2014). Earlier ecosystem indicator efforts in Puget Sound also included results from the seagrass monitoring data (PSP 2013, 2010; PSAT 2007, 2005, 2002).

In February 2011, the Partnership adopted a restoration target for native seagrass that reflects a 20% gain in area by 2020 (PSP 2011) compared to a 2000-2008 baseline. In order to identify approaches to reach the target, the Puget Sound Partnership and DNR facilitated development of a multi-agency strategy for protection and restoration of eelgrass in 2014 (Goehring et al, in press). DNR's seagrass monitoring is conducted on an annual basis by the Submerged Vegetation Monitoring Program (SVMP) – a component of the Nearshore Habitat Program in DNR's Aquatic Resources Division. The SVMP is one component of the broader Puget Sound Ecosystem Monitoring Program (PSEMP), a multi-agency monitoring program coordinated by the Puget Sound Partnership.

Since eelgrass is the dominant native seagrass, the name is often used to represent the entire native seagrass population in Puget Sound. However, there are two less abundant species of surfgrass that are native to Puget Sound and also targeted by the SVMP: *Phyllospadix scouleri* and *P. serrulatus*. Observations of the seagrass *Zostera japonica* are also recorded as part of monitoring but these are excluded from SVMP area estimates because this species is non-native and has a number of resource management issues (Bando 2006, Mach et al. 2010, Shafer et al. 2014, Hannam and Wyllie-Echeverria 2014). Observations of all of these seagrasses are included in the eelgrass monitoring dataset that is available online (see section 1.2)

Other Washington State agencies also recognize the value of native seagrasses as an aquatic resource. The Washington State Department of Fish and Wildlife designated areas of eelgrass as habitats of special concern (WAC 220-110-250) under its statutory authority over hydraulic projects (RCW 77.55.021). Similarly, the Washington State Department of Ecology designated eelgrass areas as critical habitat (WAC 173-26-221) under its statutory authority to implement the state's Shoreline Management Act (RCW 90.58).

This report summarizes the methods and key results from the latest SVMP analysis. This analysis is based on the most recent version of the monitoring dataset that spans 14 years (2000-2013) and includes data from over 22,000 transects and over 7 million points where the vegetation on the nearshore has been classified.

1.2 Data Access

The SVMP monitoring database and a User Manual are available through the DNR GIS data download web page. The data is also accessible through an online data viewer. The User Manual (NHP 2014) includes a more detailed description of project methods than are included in this report.

http://www.dnr.wa.gov/BusinessPermits/Topics/Data/Pages/gis_data_center.aspx.

http://www.dnr.wa.gov/ResearchScience/Topics/AquaticHabitats/Pages/aqr_ps_eelgrass_dataviewer.aspx



2 Methods

A comprehensive presentation of SVMP methods is available in the User Manual distributed with the digital dataset (see section 1.2 on p.6). Here, a brief overview of methods is presented and recent developments are highlighted.

2.1 Overview of SVMP Methods

The SVMP is a regional monitoring program, initiated in 2000, where a sample of sites within the greater Puget Sound study area is annually selected for study. Details on the two sampling frames (flats and fringe), the stratification (core, persistent flats, rotational flats, narrow fringe, wide fringe), and site selection within strata are described in the User Manual. For each site selected for study in a given year, detailed protocols for site surveying are followed as described in the User Manual. Boat-deployed underwater video is collected along transects that are oriented perpendicular to shore and span the area where native seagrasses grow at the site. The video is later reviewed and each transect segment of nominal one-meter length (and one-meter width) is classified with respect to the presence of eelgrass, surfgrass and *Z. japonica*. Survey results include native seagrass area, minimum depth, maximum depth, and other parameters such as species distribution and patchiness.

Regional estimates, produced by the "soundwide" study, are based primarily on a probabilistic design. The majority of the sites in the soundwide study (between 70 and 80 depending on the sample year) are randomly selected, sampled for 5 years, and then rotate out of the sampling pool, to be replaced by new randomly selected sites. Two types of sites are sampled in perpetuity: 6 core sites which were hand-picked to represent a range of conditions and sites of scientific and management interest, and 3 additional sites which were identified as having a large influence on Puget Sound estimates (see User Manual for a map of these sites). Analysis of the soundwide data produces regional estimates that characterize native seagrasses across the entire study area, as well as site estimates for the individual sites sampled. Analysis methods are summarized in section 2.2. Additional details are provided in the User Manual.

The same SVMP site survey methods have been applied to sites selected as part of special studies. Results from these site surveys are outside the regional design and do not contribute to soundwide estimates of native seagrass area. From 2004 to 2012, supplemental sites were sampled each year in one of five sub-regions of the study area in order to produce estimates at the sub-region, or focus area, scale with a return every five years to the same focus area. This work is referred to as the “focus area study”. In 2013, new site survey methods were tested at a subset of sites to evaluate techniques to improve the precision of site results (described in section 2.2.6). In addition to special studies implemented by the program, the SVMP frequently completes surveys as part of local eelgrass characterizations, often in collaboration with other research, resource management, and citizen groups.

2.2 Recent Methodological Developments

2.2.1 Vegetation Classification

The target population of the monitoring program has been refined to include the entire population of native seagrasses of greater Puget Sound. Previously, the eelgrass indicator included *Z. marina* and *P. serrulatus*, which are the two most common native seagrass species, and are difficult to distinguish in underwater videography. The surfgrass *P. scouleri* is now included. This change aligns with ecological and management interests in native species. While *P. scouleri* is an important component of the population at some exposed sites, this change has little impact on the large area estimates since *P. scouleri* is a minor component of the overall native seagrass population. Populations of *P. scouleri* located on intertidal rocks tend to be under-sampled due to navigational issues. Presence of the non-native species *Z. japonica* is recorded but is not included in estimates of areal extent.

The underwater video imagery is currently classified for the presence of the following vegetation categories:

1. Eelgrass (*Z. marina*)
2. Surfgrass (*Phyllospadix* spp.)
3. *Z. japonica*
4. Undifferentiated native seagrass (where surfgrass and eelgrass cannot be confidently distinguished).

For analysis and reporting purposes, the eelgrass, surfgrass and undifferentiated categories are combined into a single native seagrass category. As part of this adjustment in vegetation categories, the underwater video was reviewed and reclassified for all sites that had any surfgrass observations in the SVMP dataset (back to 2000). Also, over the last several years in working with the SVMP dataset, some cases were identified where abrupt changes in the location of mixed *Z. marina* - *Z. japonica* zones between years could potentially be explained by species misclassification. The video from many of these cases has been reviewed and revised. This is an ongoing data maintenance activity.

2.2.2 Site Trend Detection

For sites that have been sampled for three or more years, a linear trend analysis is used to detect significant site trends. The methods for this analysis have been updated because it is more appropriate to conduct regressions on individual observations rather than means (Freund 1971). For SVMP site analysis, this means conducting weighted regressions on site seagrass area estimates based on each individual transect rather than using a mean value from all the transects collected in a single sampling occasion as was done previously. Input values were weighted based on transect length. The site trend analyses were restricted to data from the years 2003-2013 to focus on the most recent decade and to avoid loss of precision from including data from the early monitoring years (2000-2001) when site sampling was less intensive and used slightly different protocols.

2.2.3 Site Change Classification

A new approach was implemented to classify sites that have been sampled on multiple occasions to characterize change in native seagrass area over time. These sites were classified as “increasing”, “decreasing” or “no change detected” using the following steps:

- All sites with total loss in native seagrass were put in the decreasing category. The total loss category includes all sites where native seagrass was observed on one sampling occasion but not found on subsequent sampling occasions.
- All sites with a significant linear regression slope ($\alpha = 0.05$) in native seagrass area were placed in increasing or decreasing categories. A linear regression is calculated for each site sampled on three or more occasions and with native seagrass present. Violations of the homogeneity of variance assumption for regression were not assessed.
- All sites with only two occasions were categorized as increasing, decreasing or no change detected based on the result of a 2-year change test ($\alpha = 0.01$).

Further, all sites were reviewed by inspection of the spatial patterns in the transect data and the result obtained from the steps above was overruled in specific cases. These cases include when a significant change can be attributed to differences in the random placement of transects between sampling occasions and when inspection of spatially coincident transects give clear evidence of change that was not detected in the statistical testing. The frequency of overruling the algorithm classification was low (<2% of all sites with multiple years of data).

2.2.4 Calculation of Baseline and 2020 Target Values

The Puget Sound Partnership adopted the total area of eelgrass (native seagrass for the purpose of this report) in greater Puget Sound as the Eelgrass Vital Sign. The 2020 restoration target is a 20% increase relative to the 2000-2008 baseline. The SVMP protocol has undergone several changes since the start of the monitoring project. Most notable is the change in stratification in 2004, which has significantly reduced the variance around soundwide native seagrass area estimates (Dowty, 2005). Another, more subtle, change is the different method for selection of the native seagrass polygon at site level (see the User Manual) starting in 2002. Data prior to 2002 are likely to be less accurate compared to later years.

We therefore calculated the baseline and the 2020 target using two different timeframes (2000-2008 and 2004-2008), and calculated variance around the baseline and current estimates using different approaches (Appendix 2).

2.2.5 Soundwide Native Seagrass Area and Trend Significance Estimation

Annual estimates of soundwide native seagrass area are based on statistical extrapolation of site-level estimates within each sampled stratum and a sum of estimates within censused strata (Skalski 2003, Dowty et al. 2005). Year to year differences in soundwide native seagrass area were calculated by extrapolating the relative change in sites sampled in each of two consecutive years (Skalski 2003).

We used a permutation test to assess the significance of the linear trend in annual estimates of soundwide native seagrass area (Appendix 1). We eliminated data from 2000 and 2001 for calculating the linear trend, because the differences between 2000, 2001 and later years are likely influenced by methodological changes that are difficult to separate from change in seagrass area. During permutation testing, the slope of the variance weighted linear trend from 2002-2013 is compared to a null-hypothesis distribution of slopes. In contrast to conventional trend tests, the null distribution of trend slopes is not obtained from an assumed theoretical, parametric distribution. Instead, the null distribution is obtained by repeatedly permuting the sample data itself, in a manner that assumes that the null hypothesis is true. The advantage of the permutation test over testing with the regression statistics (i.e., standard error on the slope) is that the permutation test does not require independent data across sampling occasions.

Table 1. Formulas for calculating mean and variance of soundwide native seagrass area for the 2000-2008 and 2011-2013 periods. Subscripts represent individual years.

<p>Unweighted</p>	<p>Mean</p> $\bar{X} = \frac{\sum_{i=1}^n X_i}{n}$ <p>Variance</p> $Var(\bar{X}) = \frac{\sum_i (X_i - \bar{X})^2}{n-1}$ <p>Variance with error propagation, correlation considered</p> $Var(\bar{X}) = \frac{1}{n^2} \left[\sum_i Var(X_i) + 2 \sum_i \sum_{j \neq i} \rho_{ij} \sqrt{Var(X_i) Var(X_j)} \right]$
<p>Weighted</p>	<p>Weighted mean</p> $\bar{X} = \frac{\sum_i w_i X_i}{\sum_i w_i}, \quad w_i = \frac{1}{Var(X_i)}$ <p>Variance with error propagation, correlation neglected</p> $Var(\bar{X}) = \frac{1}{\sum_i \left(\frac{1}{Var(X_i)} \right)}$

Three tests were conducted to compare current native seagrass area to the 2000-2008 baseline value and the recovery target described by the Puget Sound Partnership. First, the mean soundwide native seagrass area from 2011-2013 was compared to the 2000-2008 baseline and the 2020 target by calculating the variance using yearly values as replicates. In a second test, variance was calculated based on error propagation from the site estimates (Table 1). We assumed 80% correlation between samples 1 year apart, 60% correlation between samples 2 years apart, etc. (since there is a 20% site rotation). Finally, we calculated a weighted mean and used error propagation, without considering correlation. For more detail and alternative calculation methods, see Appendix 2.

2.2.6 New Site Survey Methods at Demonstration Sites

New site survey methods were developed for sampling at 40 demonstration sites in 2013 with the intent of assessing these methods for eventual incorporation into the main soundwide monitoring study. The impetus for developing the new methods is the possibility of strongly improving the power to detect site-level seagrass changes as demonstrated in Schultz (2008, 2011). There are two main components to the new site survey methods in development:

1. The use of a fixed set of randomly selected transects that would be sampled repeatedly over time. This contrasts with the current SVMP practice of taking a new draw of randomly selected transects on each sampling occasion.
2. The use of a restricted random transect selection approach that spatially distributes transects across the site and avoids the possibility of transect clumping that can occur with simple random transect selection.

For the initial implementation, transects from previous years were selected for resampling. New transects were set and sampled with the intent that they would be resampled in the future, or a previously sampled set of simple random transects were repeated in 2013. These data provide an opportunity to compare methods. The analysis of data from the 40 2013 demonstration sites is ongoing, and will not be described in this report.

2.2.7 Database Development

The new site survey methods implemented at the 2013 demonstration sites generate more complex data streams than the methods of the main soundwide study. In particular, transects can now be selected under different methods (simple random, restricted random) and can have different types of pairing relationships with other transect data. This necessitated adjustments to the SVMP database design. These adjustments will be reflected in the distributed data.

3 Results

3.1 Field Effort Summary

The number of sites sampled for the SVMP studies are shown in Table 2. In 2013, the SVMP regional focus study was suspended and this effort was reallocated to sampling at demonstration sites using developmental site survey methods (described in section 2.2.6).

Table 2. Number of SVMP sites sampled and the allocation over different studies from in 2000 to 2013. The number of sites visited but not sampled due to obstruction are listed in the last column.

Year	Number of Sites Sampled				Sites Visited but Obstructed
	Soundwide Study	Focus Study	Special Studies	2013 Demonstration Sites	
2000	62	0	0	0	5
2001	72	0	0	0	4
2002	73	0	0	0	3
2003	76	0	7	0	0
2004	79	28	4	0	0
2005	78	32	0	0	1
2006	79	24	3	0	0
2007	79	32	5	0	0
2008	76	29	32	0	3
2009	80	28	17	0	0
2010	78	30	40	0	2
2011	77	24	1	0	2
2012	77	32	27	0	2
2013	79	0	23	39	1

3.2 Soundwide Native Seagrass: Area Estimates and Changes over Time

Figure 1 shows annual soundwide native seagrass area from 2009-2013, relative to the 2000-2008 baseline, and the 20% increase target. Table 3 shows native seagrass area per stratum and soundwide. The long-term (2000-2013) average soundwide native seagrass area is 22,000 ha.

Table 3. Soundwide and stratum native seagrass area estimates and standard errors. Early in the monitoring project, the stratification of sites changed. Consequently, stratum estimates from the early monitoring years are not directly comparable to estimates from later years in the altered strata. Values with an * indicate early years where stratification was different from the later years. The core and flats strata listed represent distinct strata that differed in 2000, 2001-2003 and 2004-2013. "frn" is high abundance fringe in 2000 and narrow fringe thereafter. "frw" is low abundance fringe in 2000 and wide fringe thereafter.

	Total	std err	core	std err	flats	std err	frn	std err	frw	std err
2000	18,812	7,227	1,343*	61	11,257*	7,061	5,499*	1,457	713*	500
2001	22,246	6,407	3,722*	110	9,342*	6,241	3,958	745	5,224	1,236
2002	21,666	5,860	3,958*	156	8,461*	5,723	4,460	770	4,787	986
2003	21,323	5,607	3,534*	208	7,760*	5,469	5,402	828	4,628	895
2004	21,555	1,544	6,260	212	3,695	875	6,603	984	4,997	777
2005	20,567	1,684	6,271	223	3,859	1,087	6,817	1,087	3,621	651
2006	22,179	1,875	6,178	189	4,583	961	8,378	1,523	3,041	489
2007	21,564	1,893	5,631	276	4,887	735	8,880	1,652	2,165	488
2008	22,809	2,299	6,395	185	5,971	1,399	8,526	1,726	1,917	561
2009	22,263	1,778	5,896	239	7,710	815	7,311	1,502	1,346	430
2010	23,803	2,026	6,020	280	8,858	1,105	7,102	1,463	1,822	814
2011	22,440	1,807	5,864	176	8,793	1,192	5,813	1,051	1,970	841
2012	24,201	1,901	6,503	174	8,266	1,153	5,916	1,118	3,515	1,002
2013	22,610	2,166	6,559	203	6,179	1,517	6,401	1,140	3,470	1,025

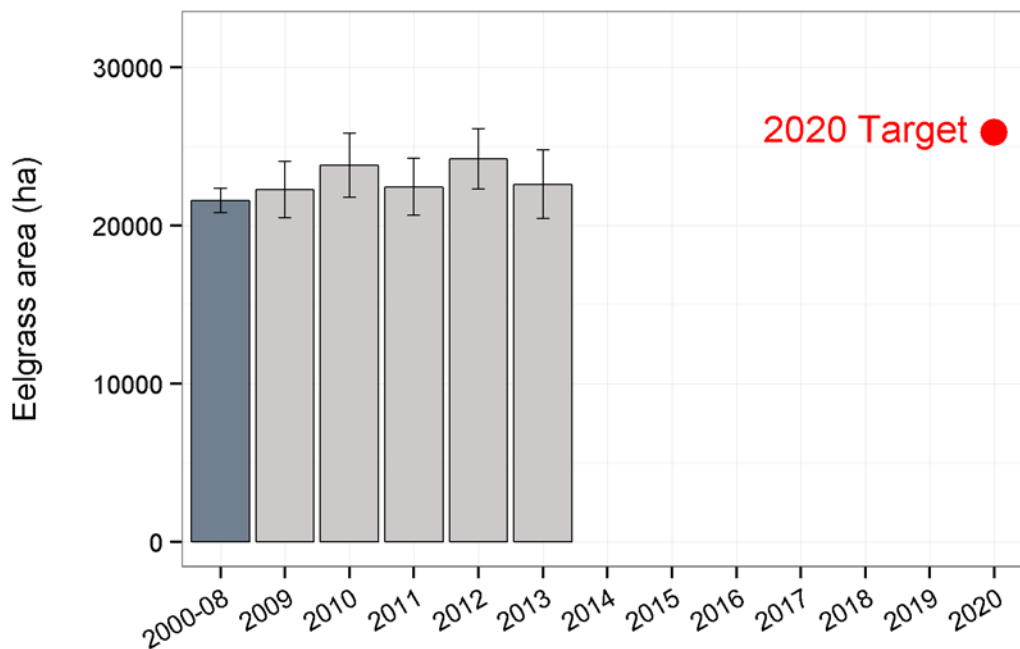


Figure 1. Soundwide native seagrass area estimates for 2009-2013, based on methods presented by Skalski (2003). The baseline value represents the mean seagrass abundance from 2000-2008. The red dot represents the 2020 target of a 20% increase. Error bars represent standard error.

3.2.1 Native Seagrass Relative to 2020 Target

Three different significance tests compared the 2011-2013 mean area of native seagrass in Puget Sound to the 2020 target: one based on the annual averages treated as point estimates, one based on a weighted average with error propagation of all annual estimates, and one based on propagation of error including the covariance term. The 2020 target is calculated as a 20% increase compared to a 2000-2008 baseline. If the variance is calculated from point estimates, the mean soundwide native seagrass area from 2011 to 2013 is significantly different from both the 2020 target value ($p < 0.001$) and the baseline ($p = 0.007$). When estimating variance based on propagation of errors around the weighted mean, the 2011-2013 native seagrass area is different from the 2020 target ($p = 0.006$), and from the baseline at a α -value of 0.2 ($p = 0.132$). Estimating variance based on propagation of errors using covariance gives the largest uncertainty around the mean (Figure 2). The 2011-2013 native seagrass area is still significantly different from the 2020 target value, but only at a α -value of 0.2 ($p = 0.127$). The 2011-2013 native seagrass area is not significantly different from the 2000-2008 baseline ($p = 0.346$).

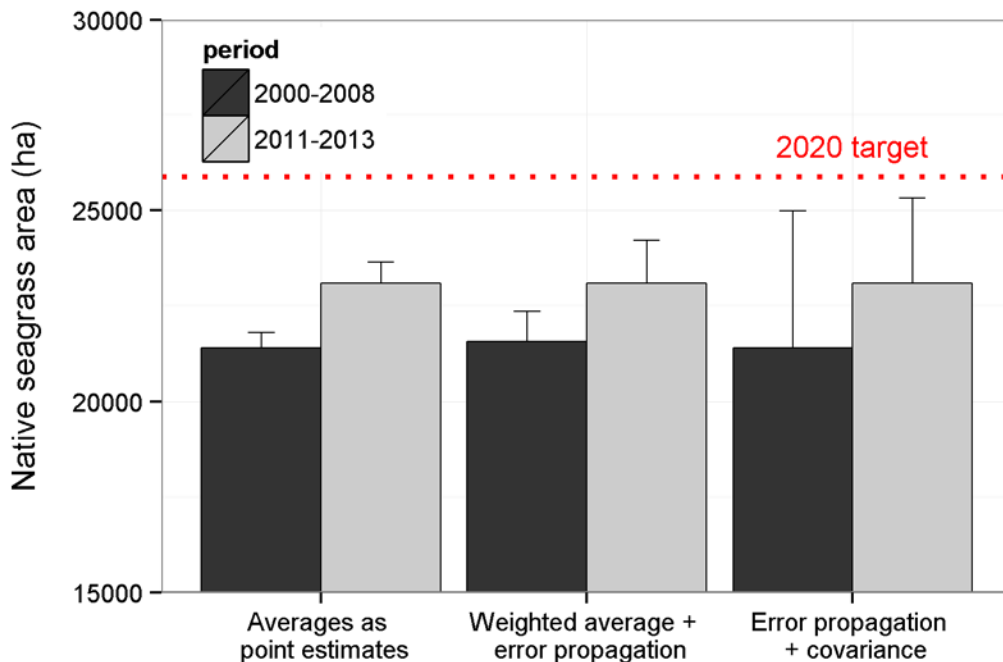


Figure 2. Mean soundwide native seagrass area from 2011-2013 compared to the 2000-2008 baseline. The red line indicates the 2020 restoration target. Error bars represent standard error, calculated using 3 different approaches (see Appendix 2).

A Monte Carlo simulation of variances calculated for a simulated population indicates that the best estimate for uncertainty is larger than the value based on treating the annual estimates as replicates, and smaller than the one estimated from propagation of error using the covariance term (Appendix 2). The weighted average with error propagation provides the best estimate for uncertainty. The results of this analysis suggests that while soundwide native seagrass area may have slightly increased, the long-term goal has not been met.

3.2.2 Long-term Trends in Soundwide Area

The annual estimates appear to increase slightly over time, but these differences are within the uncertainty around the slope of the trend line. The variance-weighted linear trend (Figure 3) has a positive slope of approximately 260 ha/yr. This slope is well within the distribution of null-hypothesis slopes, obtained by repeatedly permuting the sample data itself, in a manner that assumes that the null hypothesis is true ($p=0.63$). This indicates that there is no significant trend over time in soundwide native seagrass area (Figure 4).

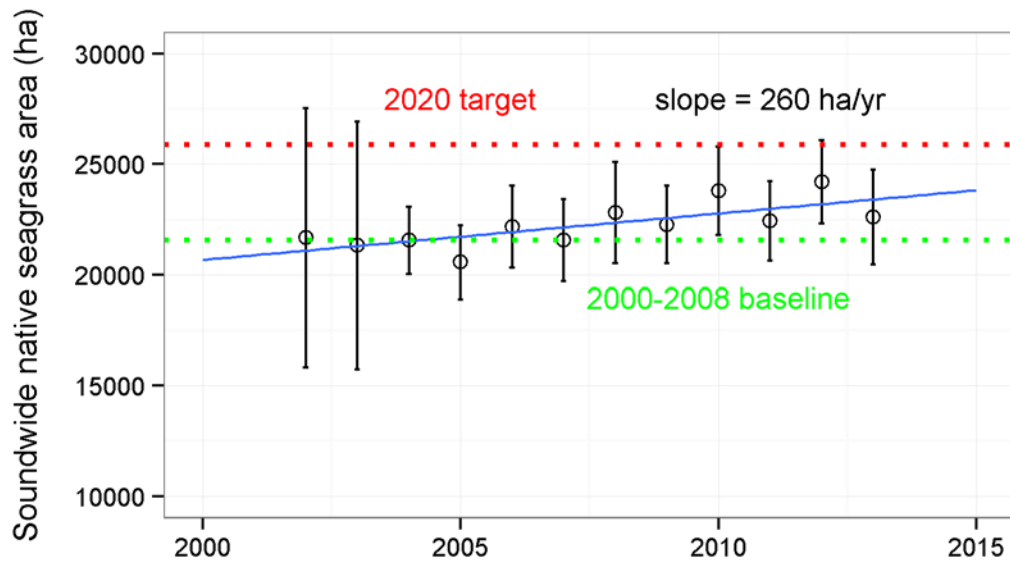


Figure 3: Long-term trend in mean soundwide native seagrass area (ha) compared to the baseline and the 2020 target. The blue line is based on a variance weighted linear regression. The years 2000 and 2001 were excluded from trend analysis because of differences in stratification and selection of the native seagrass polygon. Error bars are standard error.

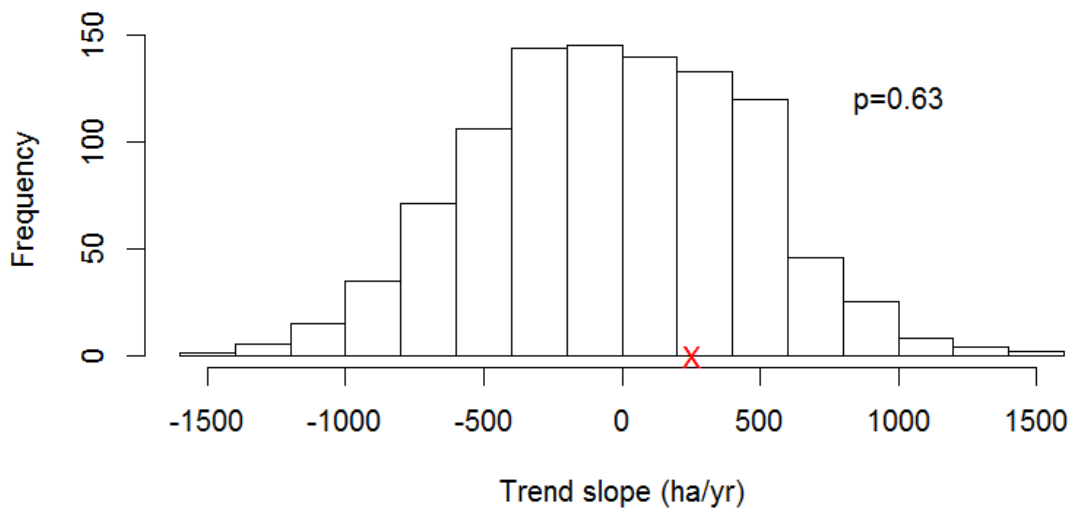


Figure 4: Slope of the variance weighted linear regression line (red X) compared to a distribution of null-hypothesis slopes, obtained by repeatedly permuting the sample data itself, in a manner that assumes that the null hypothesis is true. This illustrates that the observed slope could easily have been obtained by chance if the true trend was zero ($p=0.63$).

The differences among annual estimates can be attributed, in part, to rotation of randomly selected sites in and out of the sample pool. Figure 5 shows the annual estimates of native seagrass area in the narrow and wide fringe strata, compared to the percent of influential sites (sites with native seagrass area higher than the 90th percentile of all sites in that stratum) sampled during that particular year. Influential sites can have a big impact because of the skewed distribution of site native seagrass area. Effects of site rotation will be described further in the discussion (section 4.1.2).

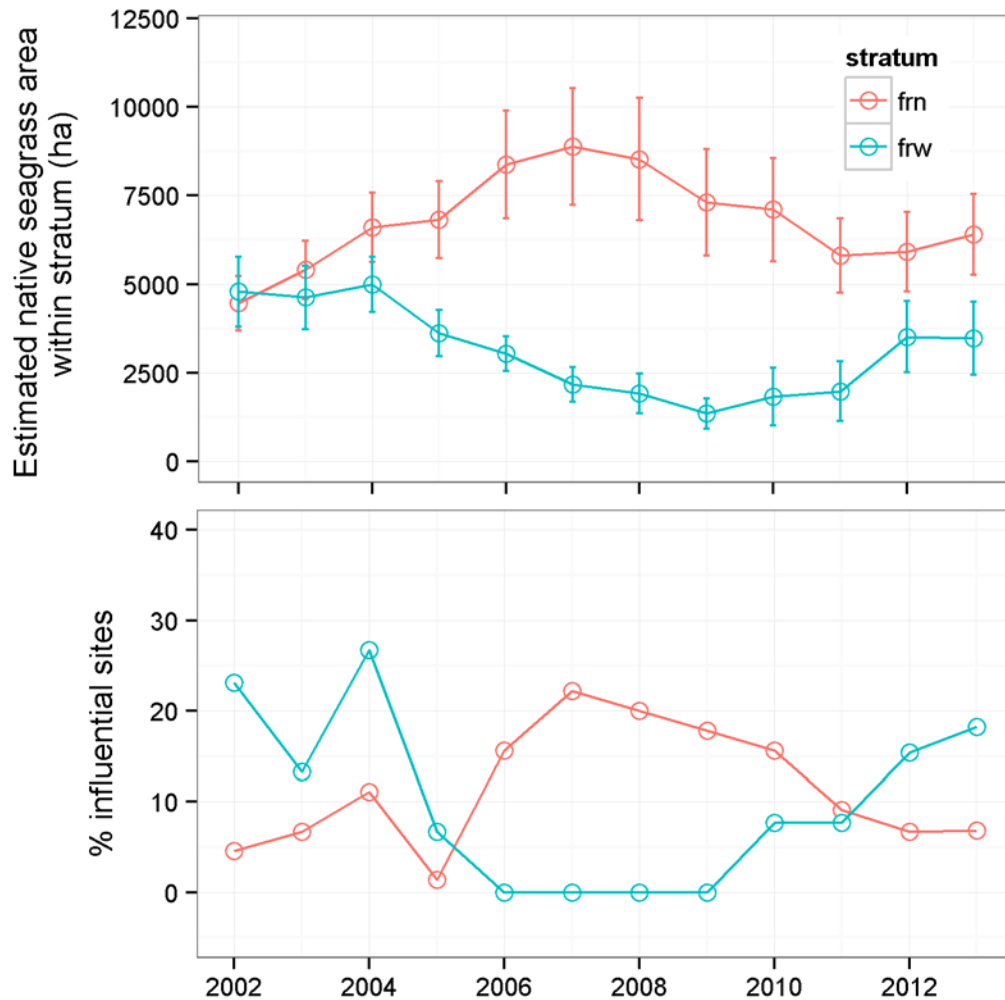


Figure 5: Top: Long-term trends in native seagrass area in the narrow (red) and wide (blue) fringe strata (error bars = standard error). Bottom: % influential sites during each year for both of the strata. Influential sites are those with a native seagrass area that is higher than the 90th percentile of all sites sampled within that stratum between 2002 and 2013. These plots illustrate that the trends in native seagrass area in both strata are influenced by the number of “influential sites” that are sampled during particular years.

3.2.3 Relative Change Estimate

Figure 6 estimates annual relative change in soundwide native seagrass area between 2000 and 2013 based on data from sites sampled in each of two consecutive years (Skalski 2003). The error bars represent 80% and 95% confidence intervals, derived from Monte Carlo simulations. No annual changes are significant at $\alpha=0.05$. However, there are two intervals with significant increases at $\alpha=0.2$ (2003-04, 2012-13). Similar to the extrapolation of site results to stratum and soundwide level, the relative change statistic is susceptible to the effect of site rotation. When a relatively large site rotates in, it can have a substantial impact on the estimates of slope for the entire stratum, and subsequently on the soundwide estimates for that particular set of years.

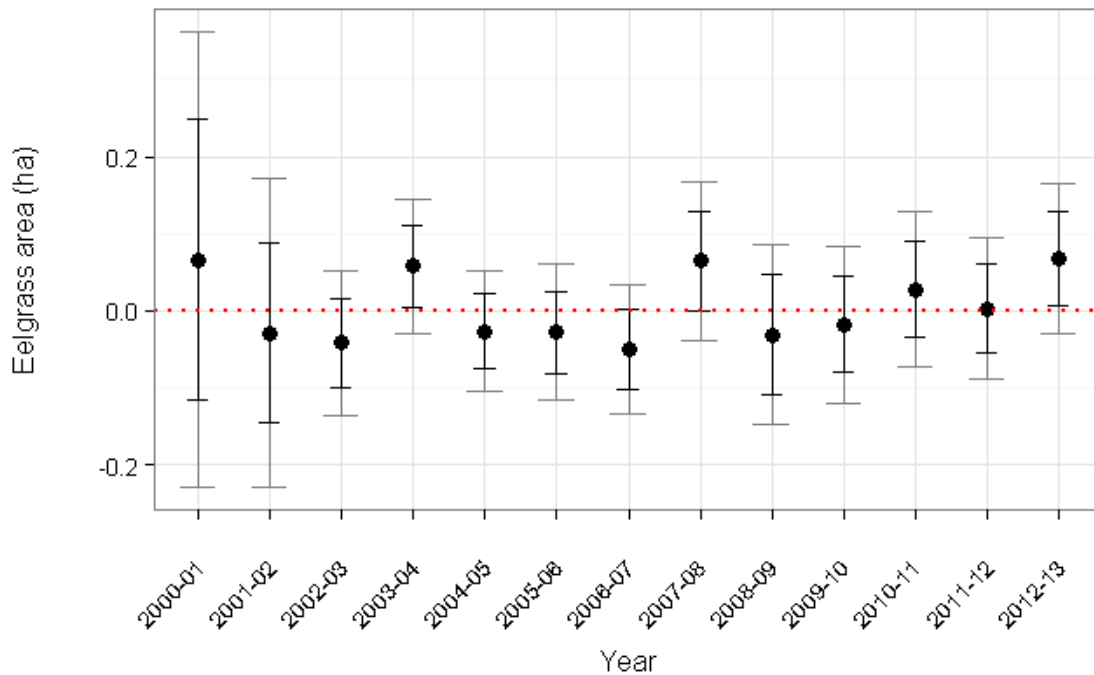


Figure 6. Annual relative change in soundwide native seagrass area. The error bars represent 80% (black) and 95% (grey) confidence intervals derived from Monte Carlo simulations.

It is interesting to note that the different methods of assessing soundwide native seagrass area (or changes in soundwide native seagrass area) show different patterns on short temporal scales, despite the fact that they are based on the same input data. This illustrates that different analysis methods have different weaknesses and strengths, which will be described in more detail in the discussion.

3.3 Site Level Change in Native Seagrass Area

3.3.1 Overview of Site Level Change

In total, 347 sites were classified according to change in native seagrass area from 2003 to 2013 (section 2.2.3), based on a statistical analysis followed by expert review. This includes 25 sites with decreasing native seagrass area, 17 sites with increasing area, 60 sites without native seagrass present, and 209 sites with no change detected (Table 4). Thirty-six (36) sites have insufficient data for trend analysis (sampled only 1 year). Data collected between 2010 and 2013 were analyzed to assess short-term trends in native seagrass area at the site-level. Of the 156 sites with sufficient data for trend analysis, there were 75 sites with no change detected, 51 sites where native seagrass was absent, 5 sites with decreasing seagrass area and 25 sites where native seagrass area was increasing.

3.3.2 Spatial Patterns in Site Level Change

The spatial patterns of sites with increasing or decreasing native seagrass area depict regional patterns that are not captured by the statistical results when the sites are pooled. A visual assessment of long-term trends from 2003 to 2013, reveals several clusters of decreasing sites (Figure 7). In the north, there are two clusters - one in the San Juan Islands and another in the northern part of Skagit Bay. To the south there is a cluster in lower Hood Canal and another within southern Puget Sound. These clusters of sites with decreasing native seagrass area are not exclusive. There are two increasing sites mixed in with the decreasing sites in the San Juan Islands. In lower Hood Canal there are increases at the Skokomish River delta. The clusters of sites with decreasing seagrass area do not correspond to human population density, a factor known to be a stressor on seagrass beds, in the Puget Sound basin. This is emphasized by the fact that the area of highest population density, central Puget Sound, has a cluster of a few sites with increasing native seagrass area.

In reviewing the site-level data, it became clear that many recent patterns of change were counter to long-term patterns in the dataset. Whereas the overall dataset revealed clusters of decreasing sites, the recent data (2010-2013) revealed mostly increasing native seagrass (Figure 8). This contrasting response in recent years is most obvious in lower Hood Canal. However, this result is partly due to the high density of sampled sites at that location. The magnitude of the recent increases is small relative to the magnitude of loss over the long-term data record, but the coherence of the recent increases in native seagrass area suggests conditions have been good for seagrass growth in recent years.

Table 4. Individual sites with long-term trends (2003-2013) at individual sites in Puget Sound

Site code	Region	Site name	Years sampled	03-13 trend	
core006		Burley Spit, Henderson Bay	13	decreasing	
cps1967		Sunshine Beach, Vaughn	6	decreasing	
cps2068		NE of Point Fosdick, Gig Harbor	4	decreasing	
cps2221		Point No Point Lighthouse South	6	decreasing	
core005	Central Puget Sound	Dumas Bay, Federal Way	13	decreasing	
flats33		Quartermaster Harbor, Vashon	2	decreasing	
cps1821		Eastward, Steilacoom	5	increasing	
flats35		Nisqually Delta East	9	increasing	
cps1054		Agate Passage SE, Bainbridge	5	increasing	
cps1066		Rolling Bay, Bainbridge	2	increasing	
cps1108		Blake Isl. West	2	increasing	
cps1114		Blake Isl. South	2	increasing	
hdc2345		Hood Canal	SE of Jiggs Lake, Tahuya	4	decreasing
hdc2323			N of Dewatto Bay	2	decreasing
hdc2355	Stimson Creek, Belfair		2	decreasing	
hdc2338	S of Wildberry Lake, Tahuya		8	decreasing	
hdc2344	East of Wheeler Lake, Tahuya		7	decreasing	
hdc2383	Indian Hole, Anna's Bay		5	increasing	
hdc2380	Skokomish Flats East		2	increasing	
hdc2381	Skokomish Flats West		2	increasing	
nps0059	North Puget Sound	Sinclair Island	7	decreasing	
nps1363		S Village Point, Lummi Isl.	6	decreasing	
nps1487		Loveric's, Anacortes	5	decreasing	
flats11		Samish Bay N	12	increasing	
flats15		Fidalgo Bay North	5	increasing	
sjs0635	San Juan Straits	Watumough Bay, Lopez Isl.	6	decreasing	
sjs0351		North Bay S, Waldron Isl.	5	decreasing	
flats53		Westcott Bay, San Juan Isl.	6	decreasing	
sjs0205		American Camp East, San Juan Isl.	5	decreasing	
sjs0154		SW of Neck Point, Lopez Isl.	2	decreasing	
sjs0557		North Coon Isl.	2	decreasing	
sjs0081		Broken Point, Shaw Isl.	7	decreasing	
sjs2628		Adelma Beach Rd S, Port Discovery	4	increasing	
sjs0544		West Reef Isl.	5	increasing	
sjs0133		Merrifield Cove, San Juan Isl.	4	increasing	
flats20		Saratoga Whidbey Basin	Skagit Bay N	13	decreasing
flats19			La Conner	6	decreasing
swh0922			Greenbank, SE Whitbey	2	decreasing
flats18	Similk Bay		9	decreasing	
swh0955	West Langley, SE Whitbey		6	increasing	
swh0885	Blower's Bluff North, Whidbey		2	increasing	
swh1615	Sunny Shores N, Tulalip		2	increasing	

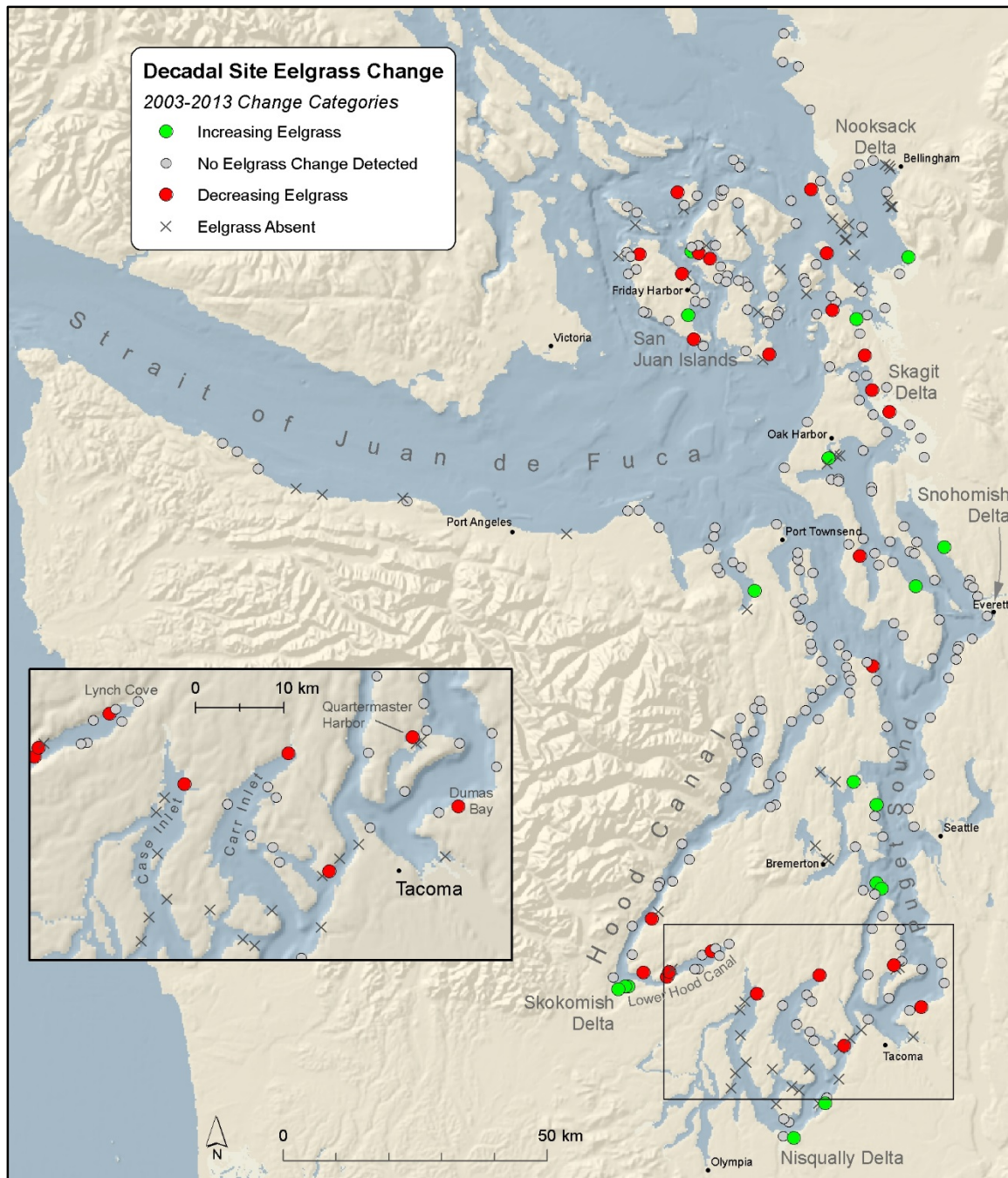


Figure 7. Increases and decreases in native seagrass area at SVMP sites sampled between 2003 and 2013. This includes sites subject to linear trend analysis where multiple years of data are available and sites subject to two-year change analysis where only two years of data are available. For some sites no recent sampling was conducted so the results represent conditions from earlier in the data record.

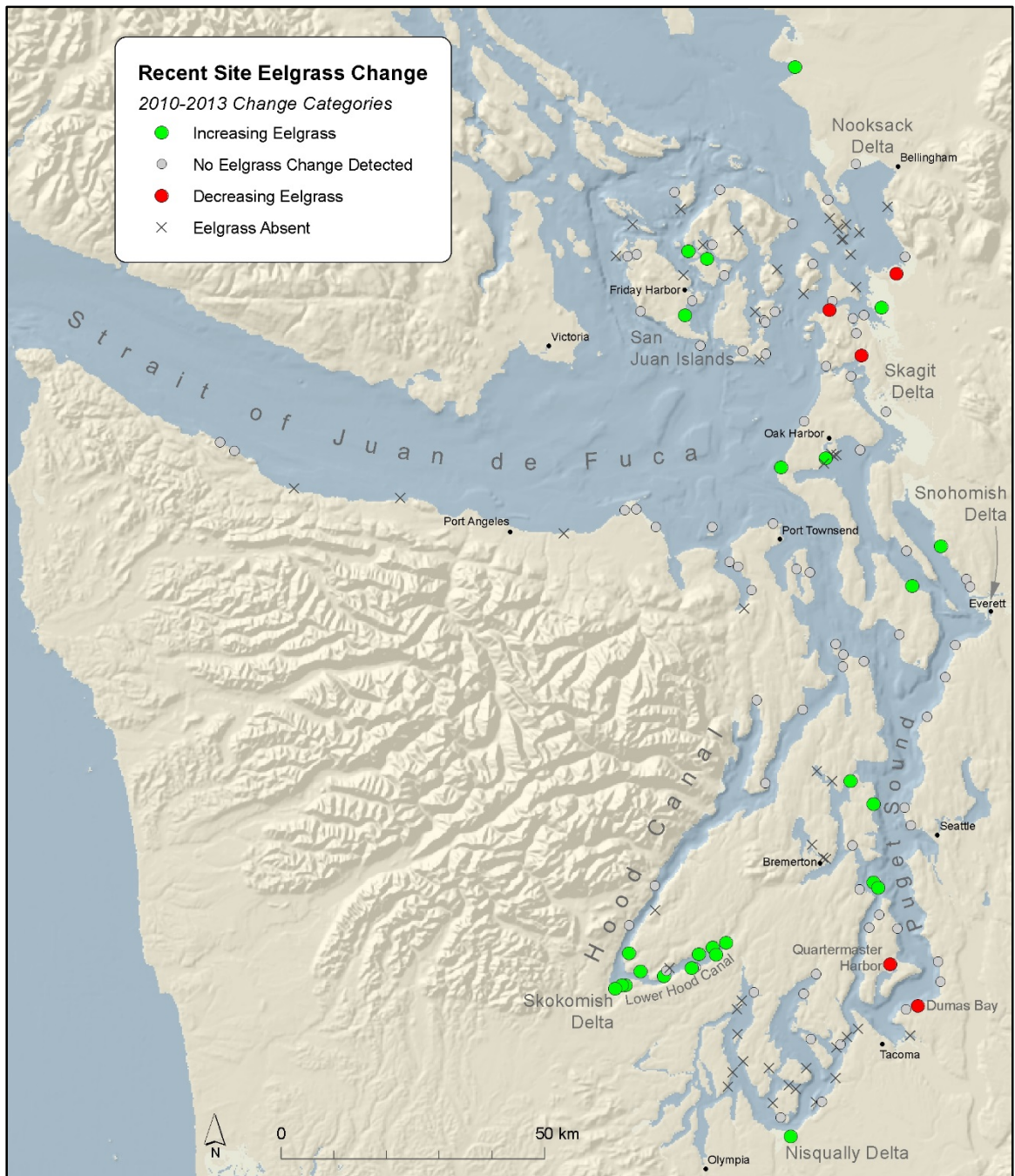


Figure 8. Recent (2010-2013) increases and decreases in site native seagrass area. This map includes sites subject to linear trend analysis where multiple years of data are available and sites subject to two-year change analysis where only two years are available.

3.4 Native Seagrass Depth Distribution

When all native seagrass observations for the years 2003-2013 are reduced to the absolute maximum and minimum depth where seagrass was observed, clear patterns emerge. The pattern of maximum native seagrass depth shows a close association with proximity to the oceanic waters of the Strait of Juan de Fuca (Figure 9).

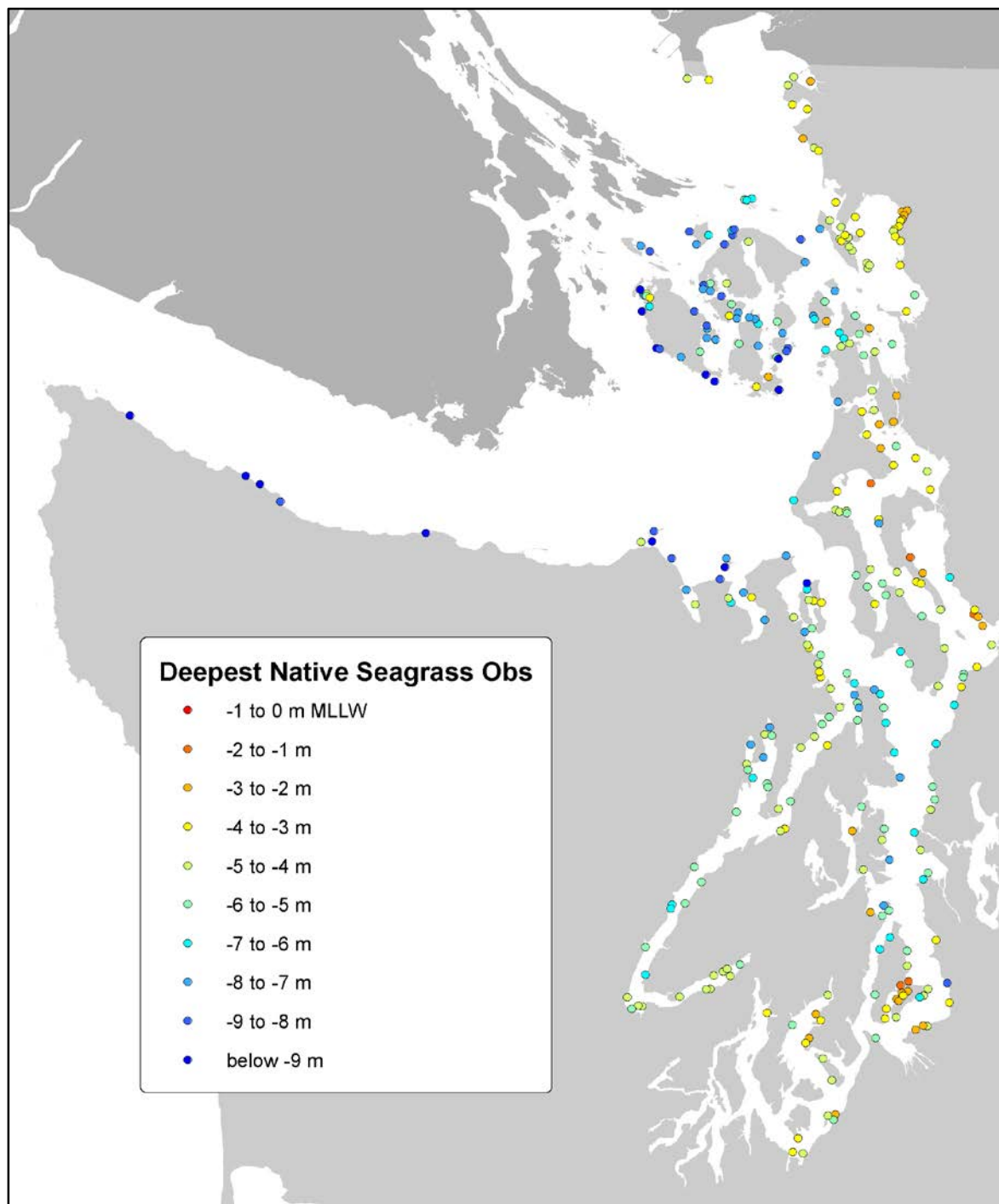


Figure 9. The maximum depth (m, MLLW) where native seagrass was observed between 2003 and 2013.

The pattern of minimum depth also shows an association with proximity to the Strait of Juan de Fuca but this association is somewhat weaker than that of maximum depth (Figure 10).

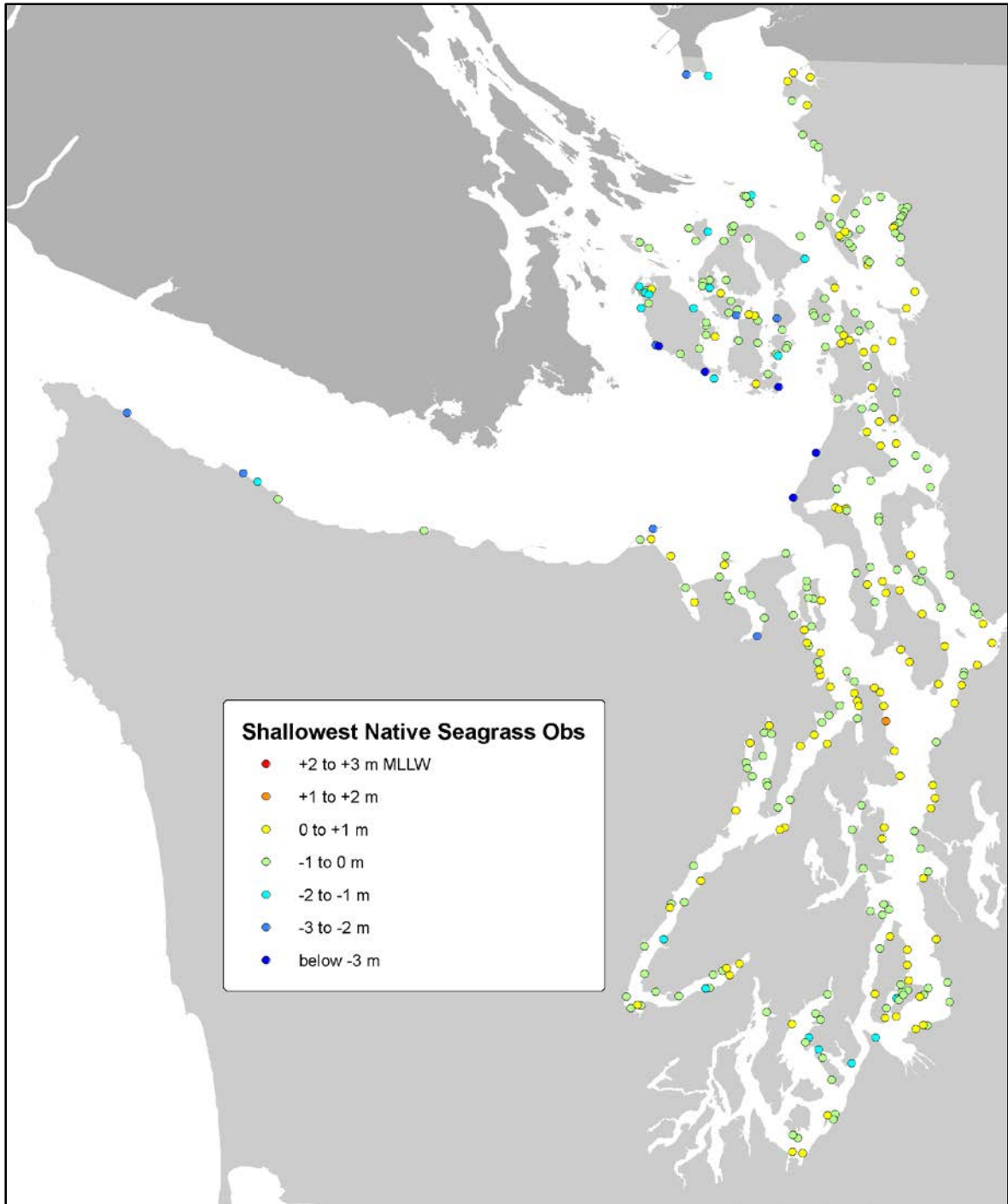


Figure 10. The minimum depth where native seagrass was observed, 2003-2013.

3.5 Seagrass Expansion near the Skokomish and Nisqually River Deltas

Three sites near the delta of the Skokomish (hdc2380, hdc2381 and hdc2383) in lower Hood Canal show a marked increase in native seagrass area between 2005 and 2013 (Figure 11). The increase was especially clear at site hdc2380 on the eastern side of the delta (Figure 12). At this point, there is insufficient data to assess if there was an increase in other sites near the delta. There was also a recent increase in native seagrass area at two sites near the Nisqually delta (flats 34 and flats35) between 2007 and 2012. This increase was lower in magnitude compared to the increase near the Skokomish delta (Figure 13).

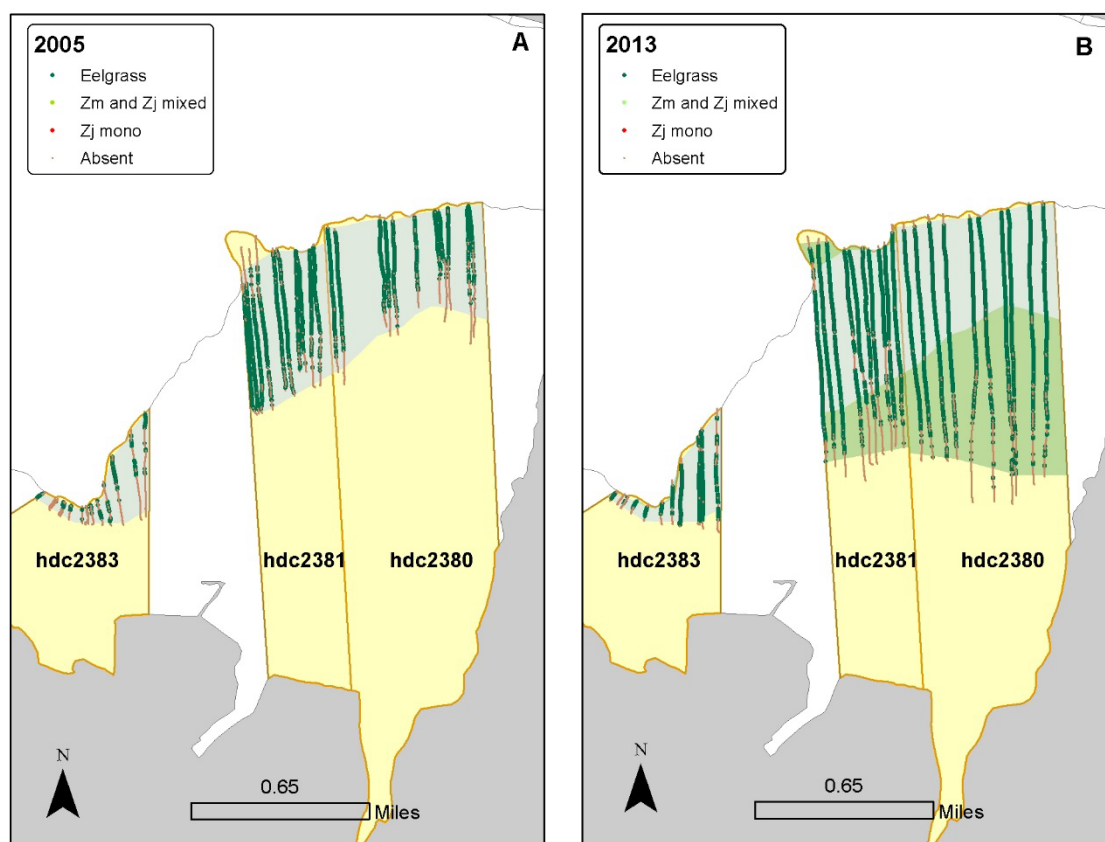


Figure 11. Increase in seagrass area at three sites located in the Skokomish delta from 2005 to 2013. The yellow color represents the area of potential eelgrass habitat based on the bathymetry of the sites. The light green (2005) and dark green (2013) polygons represent the vegetated area at each site. Transects illustrate where eelgrass was measured during 2005 and 2013. Both the size of the seagrass polygons and transect lengths are significantly larger in 2013. Different vegetation categories include monospecific stands of *Zostera marina* (Eelgrass), mixed stands of eelgrass and *Zostera japonica* (Zm and Zj mixed), monospecific stands of *Zostera japonica* (Zj mono) and no vegetation (Absent).

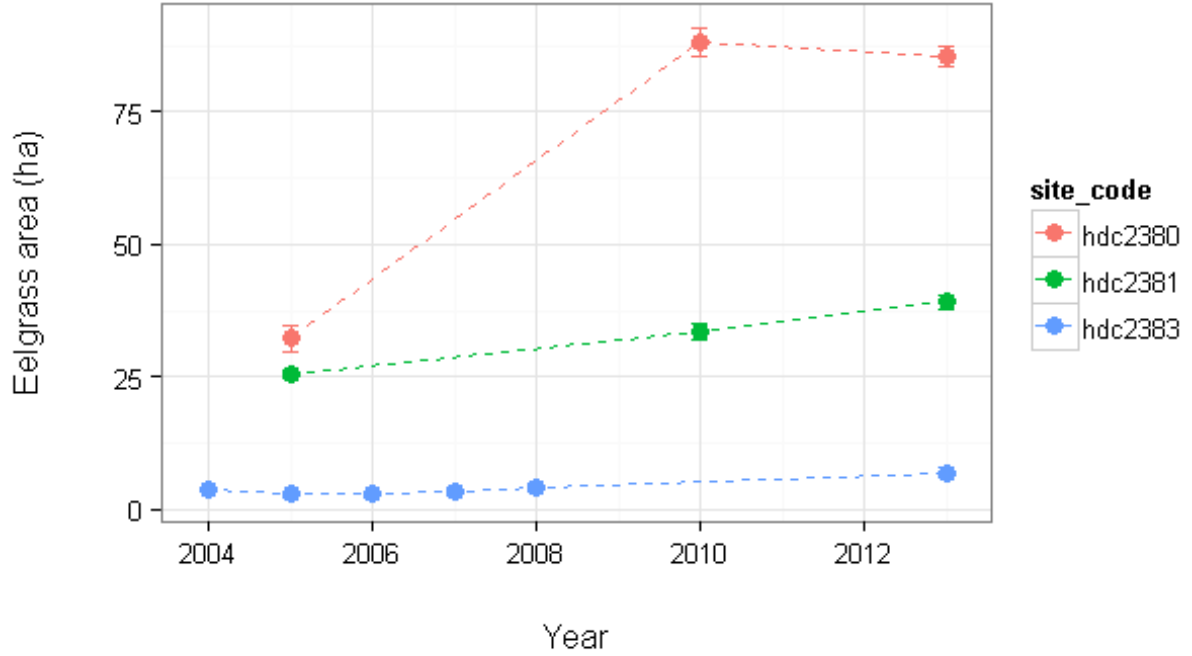


Figure 12: Increase in native seagrass area at three sites located in the Skokomish delta from 2005 to 2013. Error bars are standard error.

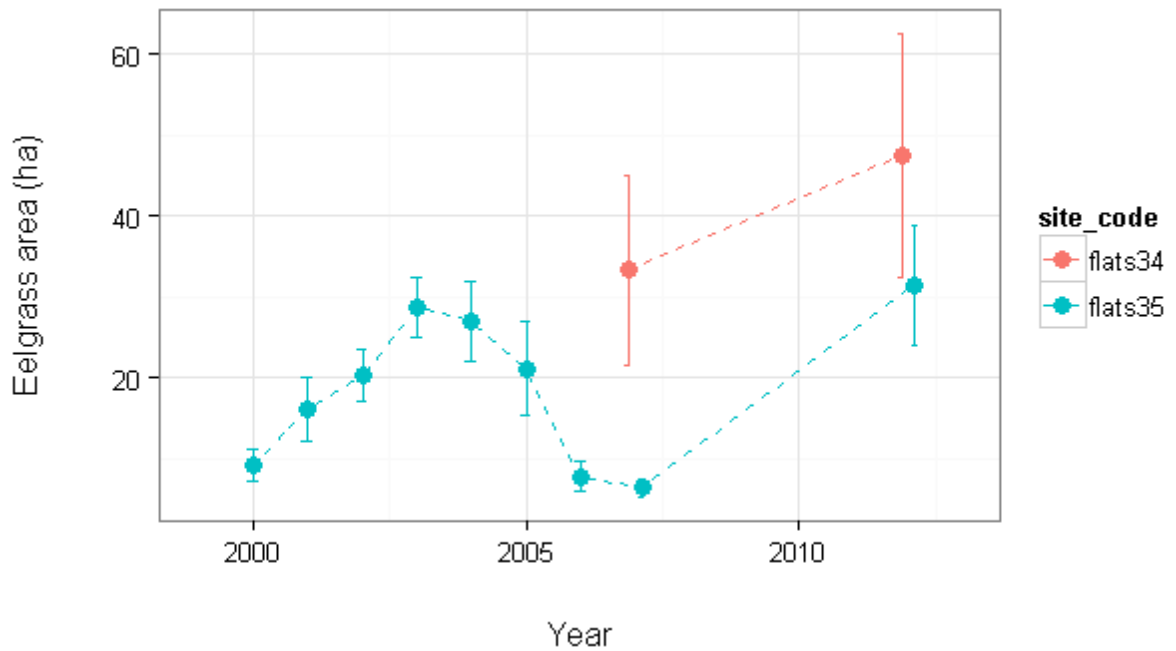


Figure 13: Increase in native seagrass area two sites located in the Nisqually delta from 2007 to 2012. Note the inter-annual variability in eelgrass area between 2000 and 2006. Error bars are standard error.



4 Discussion

4.1 *Spatial and Temporal Scales for Monitoring in Puget Sound*

Seagrass ecosystems exhibit variability over multiple spatial and temporal scales. They are impacted by seasonal, inter-annual and long-term oscillations in environmental drivers (Duarte 1989, Rasheed and Unsworth 2011) and vary on multiple spatial scales depending on the physical characteristics of the environment, the degree of anthropogenic disturbance, and biological processes such as grazing and disease (Lee et al., 2007, Heck and Valentine 2007, Burkholder et al. 2007). In Puget Sound, several physical processes have the potential to create large scale patterns in seagrass distribution, such as the gradient in tidal amplitude, wave/current energy, and the differences in water residence time. Other factors, such as river discharge, log-rafting, and point sources of nutrient pollution have the potential to impact seagrass beds on a local scale. Some stressors that generally act on the local scale can sometimes occur over large areas. One example is the slime mold *Labyrinthula*, which is associated with major seagrass die-offs in the Northwest Atlantic Ocean and Wadden Sea (Short et al. 1988). Examples of processes that function on large temporal scales are the El Nino Southern Oscillation (ENSO), changes in tidal range in association with tidal epochs, and long term changes in population density on the shores of Puget Sound. Processes that function on shorter timescales are seasonal variability in temperature and precipitation, sewer overflows after heavy rainfall, and physical disruption of seagrass beds by anchoring or prop scarring.

In all monitoring programs, there is a trade-off between the ability to detect changes in seagrass distribution over spatial and temporal scales. Monitoring over large spatial scales usually involves taking a representative sample from the entire area of interest and extrapolating results over a large spatial scale. This implies that there will be a certain degree of uncertainty around the results, which makes it difficult to detect changes over short periods of time. Monitoring on small spatial scales allows for detection of local trends in seagrass area, but these trends may not translate well to larger spatial scales. The SVMP program processes information on different temporal and spatial scales using a hybrid approach. Changes in soundwide native seagrass area are assessed in the framework of a long term target (20% increase in soundwide seagrass area by 2020), while local patterns in seagrass distribution (the site level) are examined on a shorter timeframe (inter-annual variability).

4.2 Soundwide Area Estimates

4.2.1 Soundwide Trends in Native Seagrass Area

Puget Sound has undergone a transformation over the last 150 years. The increase in human population from approximately 50000 to over 4 million people has brought profound changes to nearshore ecosystems. River deltas have experienced a large loss in area and shoreline, tidal wetlands decreased by 56%, several small embayments have been eliminated and many beaches and bluffs have been modified as a result of shoreline armoring (Simenstad et al. 2011, Fresh et al. 2011). It is not clear if there has been a long-term loss in native seagrass area resulting from the increased human population in Puget Sound, because there are no comprehensive records of the extent of seagrass meadows from before the major influx of humans in the late 1800s (Thom and Hallum 1990). However, there is evidence of increases and decreases in seagrass cover in Padilla Bay and Bellingham Bay, and anecdotic observations suggest that eelgrass has decreased at several locations in Central Puget Sound (Thom and Hallum 1990) during the last century.

The results of the SVMP program indicate that soundwide native seagrass area has been stable over the 2002-2013 monitoring record. There are no significant 11-year trends, although there is some evidence of increasing eelgrass area between 2010 and 2013. With respect to the Puget Sound Partnership eelgrass indicator, the results indicate that we have not achieved the 20% increase target. It is not possible to predict whether soundwide native seagrass area will meet the target, although recent increases provide reason for cautious optimism regarding future gains.

4.2.2 Methodological Considerations related to the Soundwide Estimate

Soundwide native seagrass area was calculated based on site surveys at a sample of sites in the study area (Skalski 2003), and the current status was examined by comparing the mean soundwide area from 2011-2013 with the baseline and the target values. The long-term trend in soundwide native seagrass area was tested using a permutation test (Appendix 1). Year to year changes were examined by looking at relative change in soundwide native seagrass area at paired sites and by comparing the number of individual sites with increasing or decreasing native seagrass area.

We used three different approaches to compare the current soundwide native seagrass area to the baseline and the target defined by the Puget Sound Partnership. The more conservative test (error propagation with covariance) suggests that it is difficult to distinguish the 2011-2013 soundwide area from the baseline, but that it can be distinguished from the 2020 target. The less conservative test (yearly values as replicates) suggests that current native seagrass area is significantly greater than the baseline, and significantly smaller than the target (Figure 2). Modeling efforts suggest that the truth lies between these two extremes (Appendix 2). The best estimate for uncertainty appears to be the calculation of variance around the weighted mean using error propagation.

When using an inverse variance weighted regression and permutation test to assess significance, no trend was measured in soundwide area (Figures 3 and 4). The strength of this metric is that it draws from the population through randomly selected, rotating sites. The associated weakness is that random site rotation triggers changes in the soundwide estimate when outlier sites of varying seagrass area enter or leave the sampling pool

(Figure 5). This is discussed further in the next section. The relative change estimate (Figure 6) showed no changes throughout most of the monitoring record, except a significant increase in native seagrass area in 2003-2004 and 2012-2013 ($\alpha=0.2$). Minor differences between these results are expected because the first metric considers multiyear trends, while the second metric looks at change on a year-to year basis.

It is important to realize that soundwide estimates of change in total seagrass area are heavily influenced by a few large sites that contain a disproportionately large amount of native seagrasses, such as Padilla Bay, Jamestown, and Samish Bay. Because of this, the combined areal change estimate may be relatively insensitive to deteriorating conditions affecting large areas of Puget Sound if those areas do not host any of the large seagrass beds. An alternative is to look at the number of site increases and site declines in the SVMP dataset (see section 4.3: Site Specific Trends in Native Seagrass Area). Individual site assessments show a much greater number of recent increases (Figure 8) than over the longer term record (Figure 7).

4.2.3 Uncertainty Associated with Soundwide Estimates

The SVMP design estimates native seagrass area in sampled sites, extrapolates these results per stratum, and sums the stratum estimates for each year. This method suffers from the effects of site rotation resulting from the annual replacement of 20% of sites in strata subject to random sampling. The effects of rotation are seen because the underlying distribution of site native seagrass area is highly skewed rather than approximating a normal distribution. Most sites have small seagrass beds but there are a small number that have very large beds. When sites with large native seagrass beds rotate in, or sites with small native seagrass beds rotate out of the sample set, the estimated native seagrass area per stratum will increase. This increase is solely due to random site selection, and does not represent an actual increase of seagrass area in Puget Sound. As a consequence, it is not possible to interpret small increases or decreases as an actual trend in the dataset, as these represent random noise introduced by site rotation. Actual trends in native seagrass area can be distinguished from rotation effects by looking at the data in a longer temporal framework. In concept, very large sample sizes could overcome the effects of the skewed distribution (Cochran 1977, pp.39-44) but that is beyond the current resources of the monitoring program.

By calculating inter-annual change in soundwide native seagrass area based on data from sites sampled in each of two consecutive years, one can gain a better understanding of short term changes in seagrass area. This method is less sensitive to rotation effects on shorter timeframes, but the potential effects of site rotation are not eliminated. The random introduction of new sites can influence the slope of the regression lines, used to calculate inter-annual change per stratum. Because of this, it is not effective to use these data to construct time-series representing cumulative change over a sequence of years, since this would propagate potential errors over time, resulting in increased uncertainty around soundwide seagrass area estimates.

The observed weaknesses of 20% site rotation in both the soundwide seagrass area estimates and the year-to-year change estimates outweigh the intended benefits of rotation (i.e. more closely representing actual Puget Sound conditions by measuring a larger

portion of the population over time). As a result, the SVMP program is evaluating alternative rotation designs.

4.3 Site Specific Trends in Native Seagrass Area

When comparing data from 2003 through 2013, there are clusters of sites with significant declines or absence of seagrass. These declines are not correlated with the major population centers – a point that is highlighted by native seagrass increases seen in Central Puget Sound. In recent years (2010-2013) there has been a general pattern of more sites with measurable increases in native seagrass area (Figure 8). These increases do not compensate for the losses seen at many sites over the last decade, but nonetheless reflect a recent reversal in the direction of change. Local conditions likely explain the diverse responses among individual sites. The following sections discuss hypotheses related to local factors that could explain observed trends near large river deltas, in lower Hood Canal, in southern Central Puget Sound, and near the San Juan Islands.

4.3.1 Large River Deltas

Notable increases in native seagrass area occurred at two river deltas following major restoration projects: the Skokomish River delta in lower Hood Canal and the Nisqually River delta in southern Puget Sound. Seagrass gains at these deltas contrast with long-term trends at nearby sites (Figure 7).

Along the Skokomish River delta, three sites have gained more than 80 hectares of native seagrass, some of the largest site-level increases measured by the SVMP since program inception. The seagrass increases were first noted in 2010, following restoration work that was initiated in 2006 to remove dikes and restore tidal channels. Subsequent increases in native seagrass area were observed in 2013. At the eastern portion of the Nisqually River delta (flats 35), native seagrass area decreased between 2004 and 2007, followed by a major increase observed in 2012 that remained stable through 2013. These increases followed the largest dike removal effort in the Pacific Northwest in 2009 (<http://nisquallydeltarestoration.org>).

In contrast to the observed increases at these two river deltas, monitoring results show a decade-long decline in native seagrass at the northern part of the Skagit River delta, which has been identified as a priority for future restoration. Research has shown that most of the fluvial sediment delivered to the delta is currently exported offshore by channelized dike complexes. This has led to fragmentation of the native seagrass beds and degradation of other valued nearshore components (Grossman, 2013). The North Fork of the Skagit has recently experienced a significant change in outflow patterns through the delta. In late 2014 an avulsion in the channel rerouted the majority of the flow through a new cut in the wetlands. This site is currently being monitored by DNR Aquatic Resources Division in cooperation with USGS, to assess potential changes to local seagrass beds.

The observed trends in native seagrass area at deltas suggest a link between river delta restoration and seagrass recovery. Planned and ongoing projects at major deltas throughout Puget Sound provide an opportunity to understand the mechanisms related to changes in seagrass condition. A better understanding of these mechanisms could in turn lead to

improved design of future restoration projects with goals of improving nearshore vegetation. This may result in important synergistic benefits to both native seagrasses and juvenile salmon, which are known to rely on delta habitat.

4.3.2 Lower Hood Canal

Lower Hood Canal and Lynch Cove are located on the southern edge of Hood Canal, a narrow, fjord-like body of water that is characterized by naturally occurring low dissolved oxygen concentrations (Cope and Roberts, 2013). Several sites along lower Hood Canal show a decrease in native seagrass area between 2003 and 2013, indicating that local conditions were suboptimal for seagrass survival. Several SVMP monitoring sites in this region show loss of seagrass at the deep edge of the bed, which suggests stress due to light limitation (Short 2014). A possible mechanism is shading by phytoplankton in the water column, or overgrowth with nuisance algae due to high nutrient loading.

Puget Sound receives the vast majority of its nitrogen load from marine sources, but nutrient fluxes from rivers, runoff from watersheds, wastewater treatment plants and outfalls represent a potential pollution threat to Puget Sound water quality and ecological health. This is especially true of the poorly flushed bays and inlets in the southern ends of Puget Sound where surface nitrates may be depleted in the summer with high levels of algae, and DO often reaches critical levels near the seabed (Khangaonkar et al. 2012). Areas with restricted water exchange such as lower Hood Canal and South Puget Sound could be vulnerable to increases in nutrient loading (Albertson et al. 2002; Newton et al. 2007).

In 2007, the Hood Canal Coordinating Council completed work on 13 septic systems (Dahlen and Swanson 2009), and in 2012 the Belfair Wastewater and Water Reclamation Facility replaced approximately 200 septic systems. During this timeframe, sites with local declines or previously stable seagrass levels showed an increase in seagrass area. Since seagrasses grow very close to shore, they are particularly vulnerable to nutrients from terrestrial sources. Seagrass beds that grow near outfalls, or in locations with restricted circulation, may be impacted by local nutrient sources, even if the total load from that source is small compared to the marine load to the entire basin. As such, it is possible that there is a connection between local trends in seagrass area and nitrogen loading from human sources at some locations in lower Hood Canal. More research is needed to evaluate this hypothesis.

4.3.3 Southern Central Puget Sound

Many sites in southern central Puget Sound have declines in seagrass area or lack seagrass altogether (Figure 7). It is interesting to note that in contrast with lower Hood Canal, sites in southern Central Puget Sound do not show a clear reversal in trend between 2010 and 2013. Four sites of concern are discussed below.

Quartermaster Harbor is a shallow embayment on Vashon Island. It is surrounded by low intensity residential development, and heavily utilized by recreational boaters. Historical data show that Quartermaster Harbor used to support a near continuous band of eelgrass along the entire shoreline of the embayment. Data from herring spawn surveys show that this eelgrass bed gradually declined between 1980 and 2011 (Department of Natural

Resources, 2013). By 2013, only a fraction of a hectare remained near Burton Cove at the head of the inner harbor. Quartermaster Harbor has been selected as a geographic focus area in the 2014 Puget Sound Eelgrass Recovery Strategy (Goehring et al. in prep).

Dumas Bay is a 16 ha embayment near Federal Way. This site shows a long-term decline in native seagrass area from 2000 to 2013. The loss in seagrass area is most pronounced near the center of the bay. Several blooms of macro-algae have been documented during this period of time (Nelson and Melton 2011). It is unclear if these blooms impacted native seagrass abundance in Dumas Bay.

Sites at the heads of Carr and Case Inlets have showed decadal declines in native seagrass area (Burley Spit and Sunshine Beach, respectively). Sites at the head of embayments are generally of greater water quality concern due to restricted flushing, however nearshore water quality data is lacking for both areas. A broader modeling study by the Washington State Department of Ecology (Ahmed et al 2014) concluded that in the deeper waters adjacent to the declining sites, anthropogenic nutrient loads from South Puget Sound and Central Puget Sound likely caused dissolved oxygen declines that violate the regulatory threshold.

4.3.4 San Juan Islands

The San Juan Islands have distinct environmental characteristics compared to the poorly flushed inlets in areas of lower Hood Canal and Central Puget Sound. They are located near the Strait of Juan de Fuca, which has a low residence time compared to other areas in Puget Sound (Khangonkar et al., 2012), but are impacted by the outflow of the Fraser River, British Columbia (Banas et al. 2014), which has a higher annual sediment load than all rivers in Puget Sound combined (Czuba et al., 2011). The higher maximum seagrass depth in the Strait of Juan de Fuca and the San Juan Islands is probably related to differences in water clarity between these locations and southern Puget Sound.

Seagrass loss has been documented both by the SVMP and the Seagrass Lab, Friday Harbor Laboratories (FHL), University of Washington (Table 5). Washington DNR and FHL have sampled different sites over different time periods and different spatial scales using different methodologies (random video transects throughout the entire bed vs. fixed transects parallel to shore on the shallow edge of the bed), so not all declines have been captured by both groups. It is interesting to note that the SVMP did not document sites within the San Juan Islands with loss of native seagrass beds between 2010 and 2013. As of yet, there is not enough data to assess if this apparent pattern reflects an actual trend.

Losses have been observed at the head of shallow embayments. While the cause of these declines is not yet known, one hypothesis is that increased sediment sulfide concentrations diminish seedling survival at these locations (Dooley et al. 2013). Another potential cause for seagrass decline is the occurrence of infections with *Labyrinthula zosterae* (wasting disease). Wasting disease is present in multiple sites in the San Juan Islands (Groner et al. 2014).

Table 5: Overview of sites with significant declines in seagrass area based on data from the SVMP and the Seagrass Lab, Friday Harbor Laboratories (FHL).

Site	Habitat type	Source	Notes
Westcott Bay	embayment	SVMP, FHL	Decline from 2000-2003, eelgrass absent since 2004 (except patches near Bell Point and the mouth of bay)
Garrison Bay	embayment	SVMP, FHL	Decline between 1992 and 2003, no significant change after 2003
False Bay	embayment	FHL	Decline observed at the shallow edge in 2007, 2011 and 2014
Shallow Bay	embayment	FHL	Recent eelgrass loss at shallow edge
Fisherman Bay	embayment	FHL	High prevalence of wasting disease
Blind Bay	embayment	FHL	Decline in 2003, no recovery since
Picnic Cove	embayment	SVMP, FHL, Groner et al. 2014	No trend in SVMP data. Decline at the shallow edge (FHL). High prevalence of wasting disease
Watmough Bay	embayment	SVMP	Documented decline 2003-2010
North Bay South	Open coastline	SVMP	Documented decline 2000-2010
SW of Neck Point	Open coastline	SVMP	Documented decline 2004-2009
American Camp E	Open coastline	SVMP	Documented decline 2005-2009
North Coon Island	Open coastline	SVMP	Documented decline 2004-2009
Broken Point	Open coastline	SVMP	Documented decline 2000-2010

4.4 Puget Sound Partnership Interim Targets

The Puget Sound Partnership’s Leadership Council has adopted several ecosystem recovery targets for Puget Sound. These targets are policy statements that reflect the region’s commitment to and expectations for recovery of the Puget Sound ecosystem by 2020. One of these targets is to expand the Puget Sound native seagrass population by 20% by the year 2020 relative to a 2000-2008 baseline. The Leadership Council also introduced a number of interim targets, to provide shorter timeframes for measuring progress towards the 2020 target (Table 6). These interim targets can provide input for adaptive management of seagrasses in Puget Sound.

The 2014 interim targets are scheduled for formal assessment using the 2014 monitoring results (the sampling year subsequent to this report). Given the broad improvement in native seagrass conditions in the 2010-2013 timeframe, the 2014 interim targets appear to have been generally achieved during the 2013 timeframe. It is unclear if the improvement is associated with management actions or synoptic climatic conditions, and this is a priority for further research. The 2014 interim targets are more conservative than the subsequent targets, with a greater emphasis on stability, and lower thresholds for proportions of sites or regions.

It is important to note that, like other large area status and trends indicators, soundwide native seagrass area estimates may not detect small changes over short periods of time

(Orians et al 2012). There is uncertainty associated with estimating changes from a sample of sites. Using highly precise quantitative interim targets to measure progress to the 2020 target may not be possible given the uncertainties associated with the SVMP yearly estimates. As a result, the interim target assessment may rely heavily on qualitative interpretation of overall findings. The interim targets will be assessed in the next iteration of the SVMP report (2014). Currently, the Nearshore Habitat Program is updating information on depth distribution to meet the interim target *Depth Distribution Identified*. The Puget Sound Partnership and DNR also convened a multi-agency workgroup to develop an eelgrass strategy. The final report is undergoing review.

Table 6: Interim targets.

	2014	2016	2018	2020
Progress Milestones and 2020 Target	<p>Overall soundwide eelgrass area increasing or stable relative to 2000-2008</p> <p>Two or more of the 5 regions show eelgrass area stability or improvement</p> <p>Within each region, fewer sites show eelgrass declines compared to 2011</p> <p>Depth distribution Identified</p>	<p>Overall soundwide eelgrass area increasing 5% relative to 2000-2008 baseline</p> <p>Three or more of the 5 regions show eelgrass area stability or improvement</p> <p>Ratio of increasing to decreasing sites improves in all regions</p> <p>Depth distribution of eelgrass stabilized, relative to 2014</p>	<p>Overall soundwide eelgrass area increasing 10% relative to 2000-2008 baseline</p> <p>At least 4 of the 5 regions show eelgrass area stability or improvement</p> <p>More increasing than decreasing sites in all regions</p> <p>Depth distributions of eelgrass increasing, relative to 2016.</p>	<p>Eelgrass extent in 2020 is 120 percent of area measured in the 2000-2008 baseline period.</p>
Outputs	Eelgrass recovery target strategy developed	Implement coordinated strategy to achieve the 2020 eelgrass recovery target	Continue to implement a coordinated strategy to achieve the 2020 eelgrass recovery target	

4.5 Research and Monitoring Priorities

In addition to completing ongoing monitoring, the SVMP will continue to improve long-term monitoring methods in order to most effectively and efficiently address scientific and management priorities for Puget Sound. We have identified the following priorities to guide our future efforts:

1. Continue soundwide monitoring and special studies to increase our knowledge of current seagrass distribution.
2. Evaluate improvements to the current design for estimation of soundwide eelgrass area, including:
 - Complete the paired transect analysis of data from the 2013 and 2014 demonstration sites to assess the possible benefits of repeat transects for detecting trends at sites.
 - Re-sample the fixed transects at the 2013 and 2014 demonstration sites during 2015, to assess the use of this methodology for multi-year trend detection.
 - Assess the use of alternative statistical analysis to better capture trends in soundwide native seagrass area (including mixed model regression).
 - Assess the strengths and weaknesses of different site rotation designs and potential scenarios for implementing gradual changes to the sample schedule starting in 2015.
3. Continue monitoring native seagrass area in Lower Hood Canal to assess the recent increases in seagrass at sites with long-term declines.
4. As resources permit, expand monitoring of seagrass beds at sites near river delta restoration projects, including the deltas of the Skokomish, Nisqually, Elwha River and Skagit River.



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6 Appendix 1: Permutation test

6.1 Introduction

In April 2012, DNR contracted statistical consultant John Van Sickle to develop a more robust framework for assessing long-term trends in soundwide eelgrass area. Up to this point, long-term trends were tested by fitting a linear regression in which each year's soundwide eelgrass area was inversely weighed by its variance estimate. However, there was a concern that the statistical significance of the slope based on the slope standard error was inaccurate, as the regression assumption of independent data points is violated by the SVMP design. The consultant suggested that a permutation approach would be more appropriate in this case because it does not rely on an assumption of independence.

6.2 Methods

6.2.1 Conventional Trend Test

The conventional significance test for a (variance-weighted) linear trend uses a null hypothesis of “zero trend”. Assuming the null hypothesis is true, statistical theory defines the parametric distribution of trend slopes that would be seen if the slopes were repeatedly estimated from a very large number of replicated random samplings. If the observed slope is extremely large (positively or negatively), relative to this null-hypothesis distribution, then that observed slope was very unlikely to have been generated by a random sample from a population with zero trend. Hence, we would reject the null hypothesis and conclude that the observed trend must have been generated by sampling eelgrass areas that had a non-zero true trend. The p-value of the conventional test is the theoretical probability of obtaining, by chance, a slope from the null distribution that is as extreme as the observed slope. The null-hypothesis distribution of trend slopes, and hence the P-value, of the conventional trend test, assumes a) normally-distributed variation around the trend line, and b) year-to-year independence of annual estimates. However, the instability and skewness of site-level eelgrass areas, coupled with the sequential dependence of annual samples that are dominated by a few, rotated-in sites, may violate these assumptions. Permutation testing (aka randomization testing) does not require these assumptions, and thus is better suited to the eelgrass area data.

6.2.2 Permutation Testing for Trend – General Strategy

In permutation testing, the null distribution of trend slopes is not obtained from an assumed theoretical, parametric distribution. Instead, the null distribution is obtained by repeatedly permuting the sample data itself, in a manner that assumes that the null hypothesis is true (Manly 1991, Good 1994). Suppose we generate a new sample data set from the original (site x year) data, by randomly selecting the year in which each observation was sampled. All other values in the data set (Site ID, eelgrass area and its variance) are left unchanged. Because sampling years have been assigned at random, this new data set can be viewed as data that might have been obtained if there were in fact no trend, that is, if the null hypothesis were true.

Based on this idea, the following permutation testing strategy can be used:

- Generate a large number (e.g., 1000) of new data sets that are identical to the original (site x year) data set, except that the sampling years of all observations have been randomly permuted. These data sets are all consistent with a zero-trend null hypothesis.
- For each of the new data sets, first estimate the soundwide, total eelgrass area (and its variance) in each year, by summing over the stratum-specific estimates derived by Skalski (2003). Then fit a variance-weighted linear regression model to the soundwide estimates to estimate the trend slope.
- Steps 1 and 2 generate a distribution of 1000 trend slope estimates that each could have resulted from sampling under a null hypothesis of zero trend. The permutation test is then performed by comparing the observed trend slope with this null-hypothesis distribution of slopes. The two-sided p-value of the test is equal to the proportion of null-hypothesis trends that are at least as large in magnitude as the observed trend slope.

Under this strategy, the original eelgrass measurements for each site, and their estimation variances, are not altered in any way. Thus, each permuted data set has the same skewness properties as the original data. In addition, the complete set of yearly observations for each site, and their corresponding variances, remain attached to that site – only the sampling years are altered.

6.2.3 Permutation Strategy Details

In this section, the permutation test is demonstrated for an example dataset, consisting of SVMP data from 2000 to 2011. In the permuted data sets, it was assumed that the permuted sampling years would continue to specify the estimation stratum. Thus, for example, site “flats42” from 2007 was included in the rotational flats stratum estimate (flr), in the original design. In a permuted data set, the measured area from 2007 at this site might be reassigned to year 2002, in which case it would be included in the flats (2001-2003) (fl2001) stratum estimate. This general strategy did not always make sense and required 2 modifications:

Because fringe strata were redefined between 2000 and 2001, there was no clear way to reassign a wide-fringe site sampled in 2008, for example, to either the low or high abundance stratum of 2000. In addition, the flats strata were also altered between 2000 and 2001. Thus, it was assumed that all of the year 2000 data (n=62 observations) was held fixed in all permuted data sets. Only the 851 observations taken between 2001 and 2011

had their sampling years permuted. Given this decision, the remaining issue of stratum change was the splitting of the flats stratum into rotational and persistent substrata in 2004. Sites flats11 and flats20 were sampled under the fl2001 stratum before 2004, and in the persistent flats (flp) stratum in 2004 and later. Thus, it was assumed their sampling years could be permuted during the full 2001-2011 period, the same as with the flats42 example above. However, the flats12 site entered the design as flp only in 2004 and this site would not obviously have been a legitimate sample from the fl2001 stratum. As such, permuted sampling years for flats12 were restricted to 2004 or later.

A second permutation issue arose in dealing with the rotation structure of noncore sites. In the SVMP design, rotational fringe and flats sites were sampled for 5 consecutive years after rotating in, after which they were dropped (rotated out). Because there was uncertainty about whether to preserve this rotation structure during permutations, the permutation test was performed twice, with and without rotation:

- Without-rotation test – Permuted years were chosen at random from 2001-2011, with no restrictions on their sequencing. For example, flats42 was originally sampled in 2006, 2007, 2008, 2009, and 2010. In a permuted data set, these 5 years might be permuted to years 2008, 2001, 2003, 2011, and 2005, in that order. As mentioned earlier, the eelgrass values assigned to 2001 and 2003 in the permuted data would be included in the fl2001 stratum estimates for those 2 years, and the other 3 values would be included in the flr stratum estimates for those 3 years.
- With-rotation test – In this version, the 5-year sequence of sampling was preserved for each rotational site, while permuting the rotating-in year and also permuting the years during the rotated-in period. For example, flats42, originally sampled in 2006-2010, might be assigned a new rotating-in year of 2003 in a permuted data set. The sampling years 2006-2010 might then be permuted to be years 2007,2003,2005,2004, and 2006, in that order. Thus flats42 retained a five-year sequence of eelgrass estimates in the permuted data set. With this strategy, a permuted starting year had to occur early enough to accommodate the full sequence of data from a rotated-in site. For example, starting years for flats42 had to be 2007 or earlier, to include all 5 years of its data up through 2011 at the latest. Because core sites were sampled continuously from 2000 to 2011, their sampling years from 2001-2011 were permuted freely, in both the with- and without-rotation tests.

6.3 Results

Figure 2 shows that the observed trend slope was well within the distribution of null-hypothesis slopes obtained from 1000 permuted data sets, under the “without-rotation” permutation approach. Specifically, 370 of the null-hypothesis slopes were as large, or larger, in magnitude than the observed slope (219 ha/yr). The corresponding two-sided **P-value** for the test is $370/1001 = \mathbf{0.370}$. (The observed case ($n=1$) is added to the number of null-hypothesis cases ($n=1000$), when counting the total number of cases in the P-value’s denominator).

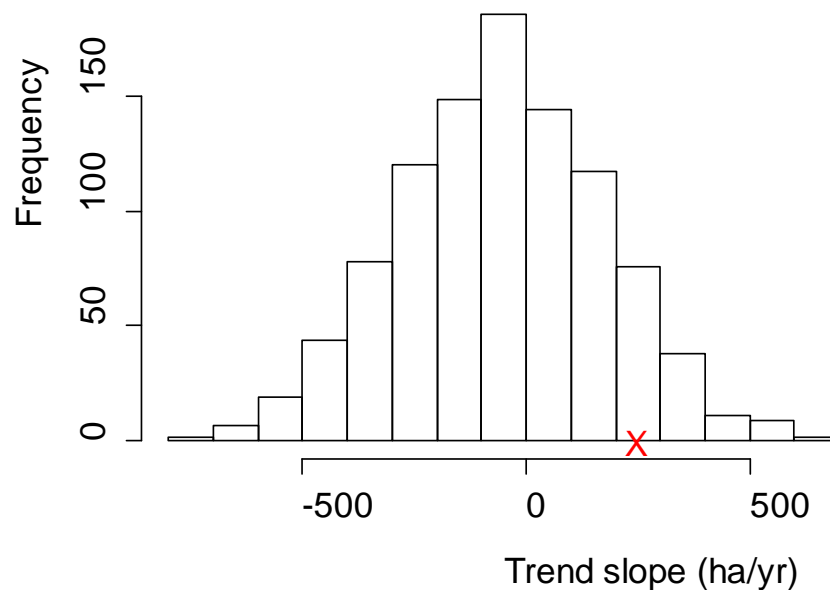


Figure 1. Histogram of 1000 trend slope estimates from 1000 data sets containing random permutations of sampling years at each site, for the period 2001-2011. Red X denotes the observed slope estimate from the original sample data. Permutations were done without rotation.

When the permutation test was re-run under the “**with rotation**” approach, the null distribution of trend slopes (Figure 2) was even broader than seen in Figure 1, resulting in a **P-value = 0.683**. Regardless of whether the permutations were done with or without rotation, Figures 1 and 2, and the corresponding permutation test P-values, indicate that the observed slope could easily have been obtained by chance if the true trend was zero. Thus, the tests fail to reject the null hypothesis, and the observed linear trend during 2000-2011 is not significant.

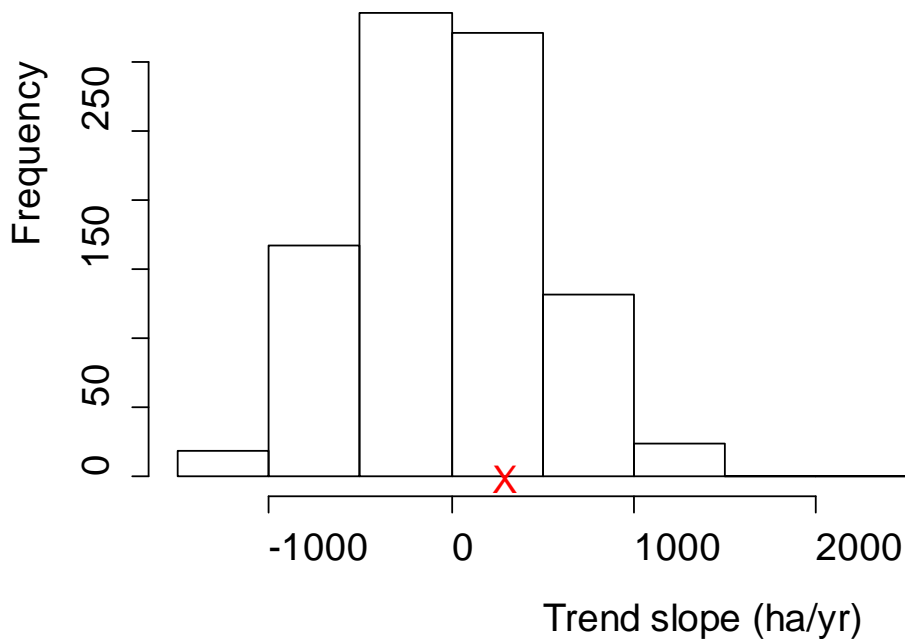


Figure 2. Histogram of 1000 trend slope estimates from 1000 data sets containing random permutations of sampling years at each site, for the period 2001-2011. Red X denotes the slope estimate from the original sample data. Permutations were done with rotation.

6.4 Discussion and Recommendations

The variance-weighted linear trend regression is a sensible model for the soundwide trend, because it down-weights annual estimates that have greater uncertainty. However, the permutation test results imply that the regression's conventional trend test greatly overstates the statistical significance of the observed trend (P-value = 0.017), because the test made unrealistic distributional and independence assumptions.

Apparently, the skewness of site-level data creates greater potential variability in soundwide estimates, under the null hypothesis, than is indicated by the variability seen in Figure 1. The ultimate effects of this greater variability on the distribution of null-hypothesis slopes is shown in Figure 2 distribution, a distribution that ignores the rotating nature of the flats and fringe sampling design. When, in addition, sites with large eelgrass areas are assumed to “rotate in” during a random year, and then yield 5 consecutive years of data, the potential variability of soundwide estimates and trend slopes under the null hypothesis is even greater yet, as suggested by Figure 3.


In future estimates of soundwide trend, the permutation test is recommended, rather than the conventional parametric test for trend, in the context of a variance-weighted (or un-weighted) linear regression. The permutation method would also apply to any nonparametric trend test, such as the Cox and Stuart test (Conover 1999).

Figures 1 and 2 suggest that a soundwide trend would need to be quite large in magnitude, (~ 500 to 1000 ha/yr), in order to be declared significant by a permutation test. This is a consequence of the current rotating design, in which very few sites are monitored for more than 5 years, coupled with the highly patchy nature of eelgrass beds in the sound. The power of the permutation test will increase over time, as the time series gets longer.

Changes in the SVMP design might improve trend detection. For example, WA DNR might identify the largest beds, those that dominate the soundwide totals, and then add them to the core stratum. This was the apparent goal of the flats stratum changes in 2003-4. This suggestion is based on the general principle that the most powerful design for trend detection is to revisit the same sites again and again in the future. Another option is to set up a serially alternating panel design, in which a static set (“panel”) of sites is sampled every 5 years, in years 1, 6, 11, ..., while a second panel is sampled in years 2,7,12..., and so forth (Urquhart et al. 1998). The tradeoff, of course, is that a relatively small number of consistently revisited sites will not adequately represent the soundwide population of eelgrass. These ideas might be explored by setting up scenarios of future trend, based on the data accumulated so far, and then synthetically sampling those scenarios, to evaluate the trend detection power of various designs.

6.5 References

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- Good, P. 1994. *Permutation tests*. Springer-Verlag, New York.
- Manly, B.F.J. 1991. *Randomization and Monte Carlo methods in biology*. Chapman and Hall, London.
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7 Appendix 2: SVMP Multiyear Estimates

7.1 Comparison of Multi-Year Averages of Soundwide Eelgrass Area

Several alternative estimates of multi-year means were made to represent baseline and current conditions. For the purposes of testing for differences, the 2020 target value associated with each baseline estimate was treated as a fixed value with no uncertainty. The alternative multi-year mean estimates were generated by varying several aspects of the calculations:

- Annual estimates contributing to the mean were either weighted inversely with their variance or un-weighted.
- Variance of the mean was based on propagation of error from annual estimates to the mean or on variability in the annual estimates treated as replicates.
- For estimates based on propagation of error, covariance between the annual estimates was either neglected or explicitly considered.
- The baseline period was either 2000-08 or 2004-08.
- The mean was based either on averaging soundwide total area for each year (“soundwide totals”) or on a total of averages for each stratum over the same years (“stratum means”).

This approach produced many point and interval estimates for baseline and current (2011-2013) eelgrass area. It also produced many test results evaluating differences between these estimates and the target values. Each result was based on its own set of approximations. Tests for differences between multi-year means were based on z-statistics.

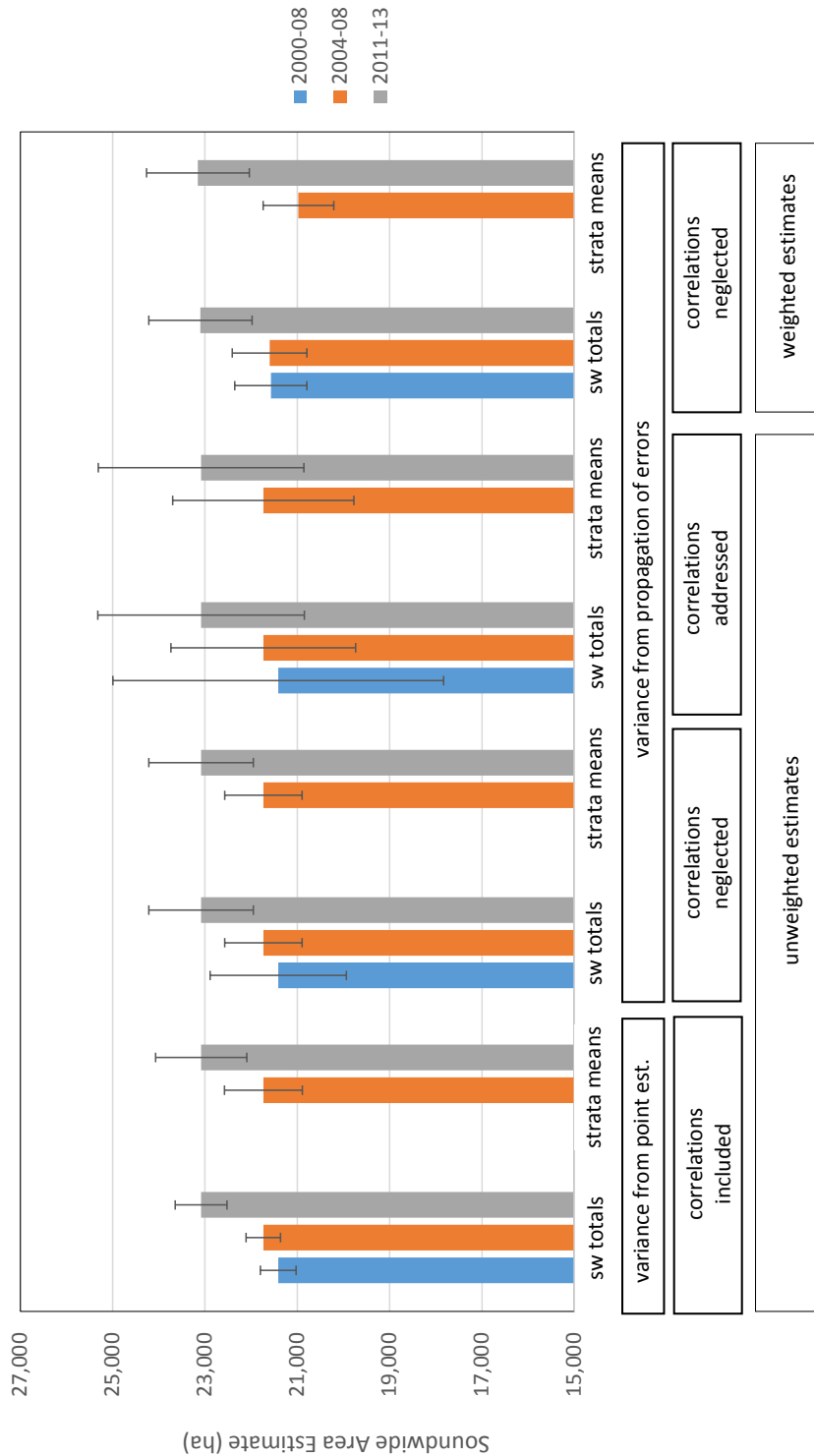


Figure 1. All estimates made for 2000-08, 2004-08 and 2011-13 means of soundwide native seagrass area. The labels along the x-axis indicate whether the estimates are weighted or un-weighted means; whether correlations were considered when propagating error; whether variance was calculated directly from the annual point estimates or by propagation of error; and whether the estimate is a mean of annual soundwide totals (sw totals) or a total of multi-year stratum means (strata means). Error bars are standard errors.

Table 1. Values displayed in In the case of the un-weighted results that include covariance terms on the mean of soundwide totals (estimates #4-6), the violation of independence is listed as “no (bias)”. This indicates that the analysis explicitly accommodates the data dependence but the correction is slightly biased since it is applied across all strata including core where the estimates are not correlated.

Weighting	Variance	SW or stratum	Years	Violation of homoscedasticity	Violation of independence	Mean	Standard Error		
no	var from obs	sw	00-08	major	major	1	21,413	388	
			04-08	minor	major	2	21,735	373	
			11-13	minor	major	3	23,084	561	
		stratum	04-08	minor	major	4	21,735	844	
			11-13	minor	major	5	23,083	989	
			00-08	major	major	6	21,413	1,477	
	prop of error - correlations neglected	sw	04-08	minor	major	7	21,735	839	
			11-13	minor	major	8	23,084	1,134	
			04-08	minor	major	9	21,735	839	
		stratum	11-13	minor	major	10	23,083	1,134	
			00-08	major	no (bias)	11	21,413	3,584	
			04-08	minor	no (bias)	12	21,735	2,000	
	prop of error correlations included	sw	11-13	minor	no (bias)	13	23,084	2,239	
			stratum	04-08	minor	no	14	21,735	1,963
				11-13	minor	no	15	23,083	2,228
yes	prop of error - correlations neglected	sw	00-08	no	major	16	21,573	783	
			04-08	no	major	17	21,600	810	
			11-13	no	major	18	23,098	1,121	
		stratum	04-08	no	major	19	20,976	764	
			11-13	no	major	20	23,152	1,114	

Table 2. z-values for tests of difference between means and between means and values for the 2020 target.

	2000-08 and 2011-13	2004-08 and 2011-13	2011-13 and 00-08 target	2011-13 and 04-08 target	2000-08 and target	2004-08 and target
sw totals var from pt obs	2.4498	2.0024	4.6553	5.3440	11.0376	11.6542
strata means var from pt obs		1.0368		3.0324		5.1505
sw totals prop. of err, corr neglected	0.8974	0.9563	2.3030	2.6437	2.8995	5.1812
strata means prop. of err, corr neglected		0.9556		2.6446		5.1812
sw totals prop of error, corr included	0.3954	0.4493	1.1664	1.3390	1.1949	2.1735
strata means prop of error, corr included		0.4540		1.3461		2.2145
weighted sw totals prop of err, corr neglected	1.1153	1.0831	2.4885	2.5174	5.5103	5.3333
weighted strata means prop of err, corr neglected		1.6109		1.8126		5.4911

Table 3. *p*-values for difference tests. Significant values ($p < 0.1$) are bolded.

	2000-08 and 2011-13	2004-08 and 2011-13	2011-13 and 00-08 target	2011-13 and 04-08 target	2000-08 and target	2004-08 and target
sw totals var from pt obs	0.0071	0.0226	0.000	0.000	0.000	0.000
strata means var from pt obs		0.1499		0.0012		0.000
sw totals prop. of err, corr neglected	0.1848	0.1695	0.0106	0.0041	0.0019	0.000
strata means prop. of err, corr neglected		0.1696		0.0041		0.000
sw totals prop of error, corr included	0.3463	0.3266	0.1217	0.0903	0.1161	0.0149
strata means prop of error, corr included		0.3249		0.0891		0.0134
weighted sw totals prop of err, corr neglected	0.1324	0.1394	0.0064	0.0059	0.000	0.000
weighted strata means prop of err, corr neglected		0.0536		0.0349		0.000

7.2 Simulation Exercise

A simulation exercise was conducted to gain insight on the relative reliability of two contrasting test results comparing current eelgrass area estimates to 2020 targets. The two test results each indicate that current conditions (2011-13, 3-yr mean) can be distinguished from the 2020 target values (based on a 2000-2008 baseline) but with strongly differing significance levels ($p = 0.000$ and $p = 0.16$; variance from treating samples as replicates and variance from propagation of error with correlation, respectively). We could simply report a significance of $p < 0.2$. While this would be consistent with our results it may strongly underestimate the true significance of the result. The purpose of this simulation exercise was to see if there are grounds to improve our reporting of significance.

Monte Carlo simulations were used to compare the two different confidence interval estimators for a mean of three sample mean estimates of site eelgrass area. The premise here was that the calculation method that had the more accurate confidence interval estimator would also have the most accurate significance level in the test for difference.

This exercise was simplified in that it compared estimates for a single stratum only. It was assumed that the comparison would also reflect the relative performance of the estimators at the aggregated soundwide level.

A complete model of the SVMP narrow fringe stratum was used for this exercise. This model has the same number of sites as the actual stratum ($n = 1965$). The eelgrass area values assigned to each site were randomly drawn from a Weibull distribution fit to the existing SVMP narrow fringe data (described in more detail elsewhere). The fit was done manually to capture the gross characteristics of the distribution and resulted in a Weibull function given by

$$f(x) = \left(\frac{k}{\lambda}\right) \left(\frac{x}{\lambda}\right)^{(k-1)} e^{-\left(\frac{x}{\lambda}\right)^k}$$

with parameters $k = 0.8$ and $\lambda = 3.5$.

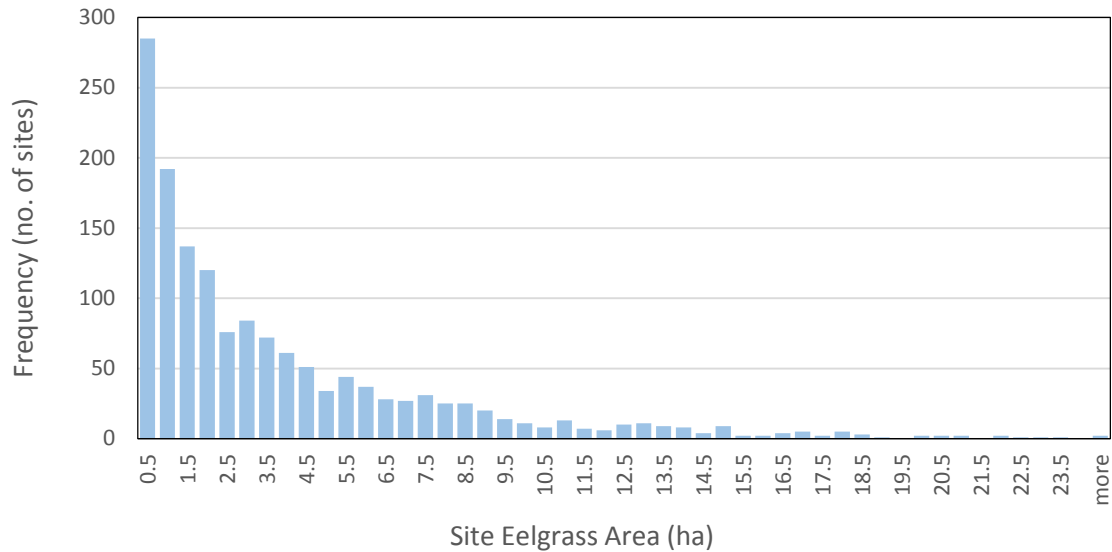


Figure 2. Frequency histogram of site eelgrass area in the model narrow fringe stratum (n= 1965). The model sites with zero eelgrass area are not shown (n= 469).

Table 4. Population parameters associated with the model narrow fringe population depicted in Figure 2.

population parameter	value
total eelgrass area (ha)	5,401
mean site area (ha)	2.75
median site area (ha)	1.10
variance of site area (ha ²)	15.2
standard deviation of site area (ha)	3.90

A single simulation consists of the following steps:

- a) Construct three samples from the population of size $n = 20$ to represent sampling over three consecutive years with 20% rotation of sites between samples.
- b) For each sample calculate the sample mean site area, the sample variance s^2 , and the variance on the mean $\frac{s^2}{n}$.
- c) Calculate the average of the three sample means (“three-sample average”). This average is analogous to the current estimates (2011-13) based on a three-year average. In this exercise the model site data are static and do not change between samples so we are isolating the precision and accuracy of estimation approaches (i.e. eliminating variability associated with real change in the population).
- d) Calculate the variance of the three-sample average of mean site area values by treating the three samples as replicates, and also by propagating the sample mean variances with correlation terms.

- e) Calculate the 80% confidence intervals associated with the three-sample averages based on each of the variance estimates from (d). The confidence interval calculations relied on a z-statistic and were given by

$$\left[\bar{X} - z_{1-\alpha/2} \sqrt{\text{Var}(\bar{X})}, \bar{X} + z_{1-\alpha/2} \sqrt{\text{Var}(\bar{X})} \right].$$

The single simulation described in steps (a) through (e) above was repeated 1000 times. The 1000 three-sample averages estimated from these simulations are summarized in Figure 3. This figure also includes the true 80% confidence interval determined by coverage of the 80% of the estimates (no dependence on variance estimation).

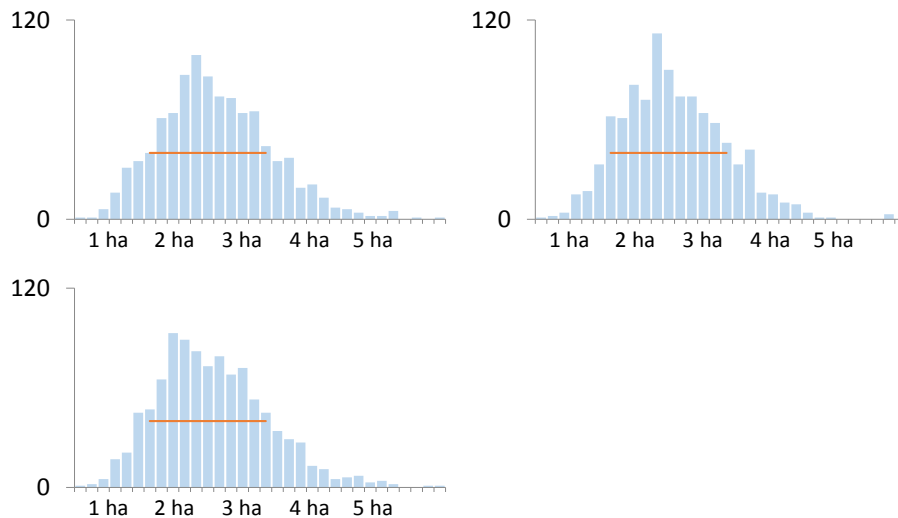


Figure 3. Frequency distribution of three-sample averages from step (c) above. Each graph summarizes a different set of 1000 simulations. The means of these distributions are (from the left) 2.76 ha, 2.76ha and 2.78 ha. The horizontal line represents the true 80% confidence interval that encompasses 80% of the simulations (half-width = 1.0 ha).

The two alternative confidence intervals for the three-sample average calculated for the 1000 simulations are summarized in Figure 4. The two calculation methods produce very different distributions of interval estimates.

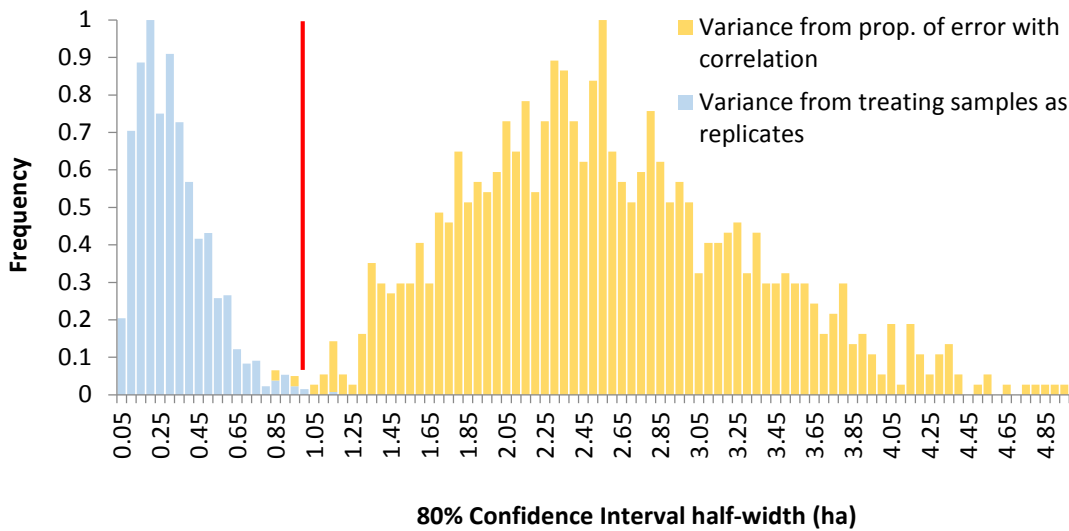
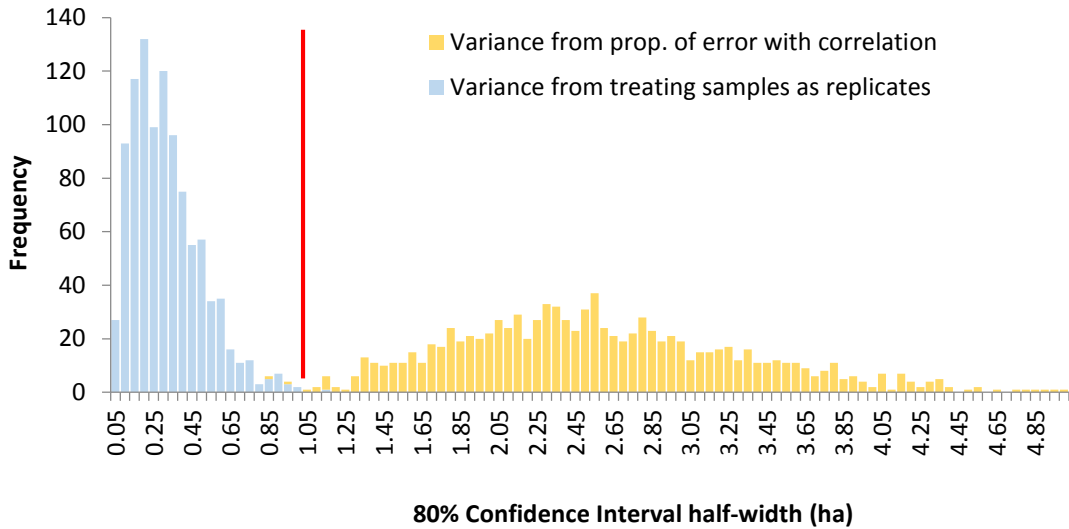


Figure 4. Frequency histogram of 80% confidence interval estimates from step (e) above based on variance calculated in two different ways. The graph summarizes 1000 simulations with the two different variance calculations applied to each simulation. TOP: absolute frequency (counts of interval estimates); BOTTOM: relative frequency where each distribution is scaled to its mode. The mean confidence interval half-width for blue data (three samples treated as replicates) is 0.29 ha. The mean for the orange data (propagation of error with correlation) is 2.52 ha. The red line represents the true confidence interval half width (1 ha).

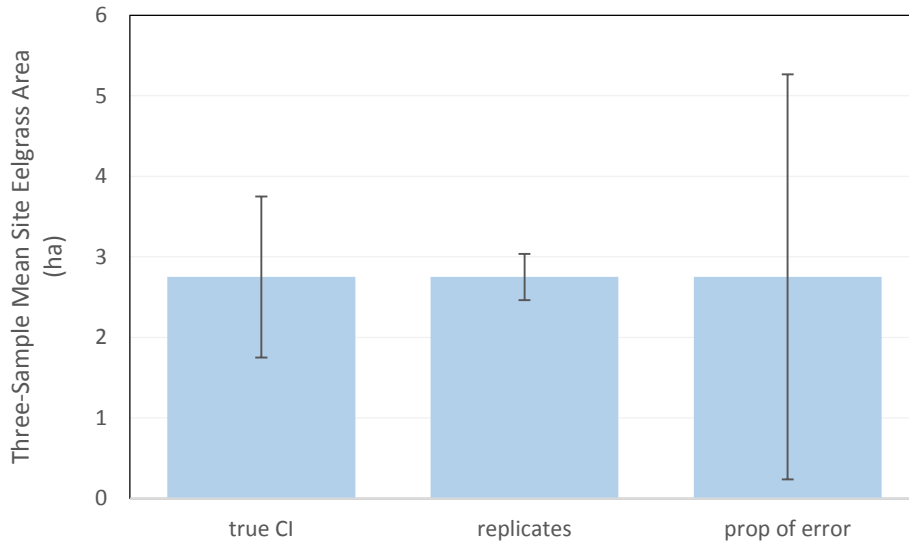


Figure 5. Comparison of 80% confidence intervals about the estimates of the three-sample average: “true CI” is based on coverage of 80% of the estimates from the 1000 simulations; “replicates” is based on the variance estimate that treats the three sample means as replicates – this confidence interval is the mean of the blue distribution in 4; “prop of error” is based on the variance estimate from the propagation of errors with correlations considered – this confidence interval is the mean of the orange distribution in 4.

The confidence intervals based on a variance estimate that treats the three samples as replicates underestimates the true confidence interval width. The confidence intervals based on a variance estimate from propagation of error with correlations strongly overestimates the confidence interval width. This is clearly shown in a comparison of the true confidence interval with the means of each confidence interval distribution (Figure 5).

If we assume that the relative performance in confidence interval estimation is indicative of relative performance of associated tests for difference of the three-sample average compared to a fixed value, then this is directly relevant to the SVMP results presented in The simulation results suggest that variance estimates (and associated confidence interval estimates) based on treating annual estimates as replicates underestimate the true variance. In contrast, variance estimates based on the propagation of error with explicit consideration of correlations strongly overestimate variance.