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QUALITY ASSURANCE PROJECT PLAN

for

Evaluating and Refining the Estuarine Habitat Assessment Protocol on Puget Sound and Pacific Northwest Reference Sites

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Signature Approval for Implementation

of

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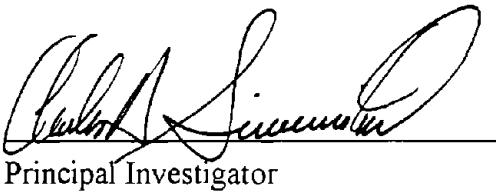

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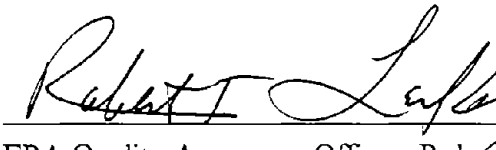

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QUALITY ASSURANCE PROJECT PLAN

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Evaluating and Refining the Estuarine Habitat Assessment Protocol on Puget Sound and Pacific Northwest Reference Sites

4.0 INTRODUCTION

Many estuarine wetland scientists and managers are of the opinion that present wetland habitat assessment procedures are inadequate for application to specific geographic regions and may be too subjective to provide consistent results. This is particularly the case in Pacific Northwest estuaries, where wetlands are structurally and functionally different from southeast and Gulf coast estuaries, for which most assessment approaches were originally developed. To increase the effectiveness of our management and conservation of estuarine habitats, we need assessment and monitoring procedures that: (1) are based explicitly on habitat function; (2) are specific to the region of application; (3) use methods that are standardized, consistent and comparable; (4) generate quantitative data rather than qualitative indices; (5) are designed to be thoroughly objective among different users and sites; (6) will be adaptive in terms of building on prior results; and (7) are structured in a flexible form, wherein both the biotic community and target (e.g., species) resources can be addressed.

The *Estuarine Habitat Assessment Protocol* (Simenstad *et al.*, 1991; hereafter referred to as the *Protocol*) represents such an approach to assessing the function of estuarine habitats for fish and wildlife, and specifically for the Pacific Northwest region. Fish and wildlife support functions of estuarine habitats were the chosen focus of the *Protocol* because they have historically been the "forcing functions" behind resource agency requirements for compensatory mitigation. Other important habitat functions, such as maintenance of water quality or flood desynchronization, should be assessed with similar rigor. The *Protocol* is intended to address the need for a systematic procedure that can be applied uniformly across a variety of wetland and associated nearshore habitats using objective, scientific methods. The approach is directly applicable for the study of natural wetland systems and in evaluating compensatory mitigation projects in estuarine habitats. The *Protocol* also has the potential to facilitate the development of design criteria for estuarine habitat restoration.

The general purposes of this project are:

1) to evaluate the applicability of a subset of *Protocol* attributes to a regional developed from experience in Puget Sound. We will assess whether the selected

subset of attributes applies across a broader geographical range (the Pacific Northwest coast) than was originally encompassed in the development of the *Protocol*. To assess the geographical applicability of these attributes, we will sample for selected attributes in appropriate strata;

2) to refine the sampling designs, procedures/methods, and parameters for these attributes by evaluating the monitoring strategies and approaches recommended in the *Protocol* and determining the most statistically valid sampling designs achievable considering the costs of field and laboratory efforts.

   To address sampling designs we will:

   a) delineate major strata (e.g. low marsh, high marsh, mudflat) at each site
   b) conduct preliminary investigations of spatial scale for all attributes

   To address procedures and methods, we will:

   a) pay particular attention to the details of each sampling method in order to further refine instructions in the *Protocol*
   b) compare different methods of assessing percent cover of emergent marsh vegetation and pore water salinity

   To address parameters (see Table 1), we will evaluate the relative precision and costs (field, processing, destruction to habitat) of assessing emergent marsh vegetation using above ground biomass, below-ground biomass, and percent cover, using the methods of Bros and Colwell (1987)

3) to sample certain physical parameters (elevation, pore water salinity, sediment redox potential) to establish correlations with abundances of biological attributes.

   To assess physical parameters, we will:

   a) establish elevation transects at each site
   b) sample pore water salinity along these transects and within other sampling areas
   c) sample sediment redox potential where samples are taken

4) to develop QA/QC procedures to be added to the *Protocol* since the original *Protocol* does not make specific recommendations about data quality assurance or control.

The data produced by this project will be used to evaluate the appropriateness of selected attributes from the *Protocol*, to further refine estuarine wetland sampling designs, and to establish criteria and methodologies that will optimize the precision, accuracy, representativeness and comparability of data collected given the limitations of time and funding. In the future, we hope to use the data gathered to assess levels of variability to be expected in natural wetlands and to cull from our experience in this project a general

process of sampling and evaluation to be used in future investigations of *Protocol* attributes and methods. It is hoped that the final procedural recommendations to the *Protocol* will constitute a statistically evaluated suite of procedures for assessing fish and wildlife support functions for estuarine wetlands. Such procedures could then be incorporated into regulatory and other rule-making processes, such as evaluation of compensatory mitigation under CWA[1]. Although not necessarily intended as a tool in planning wetland management programs, the concept of the *Protocol* and its accompanying Quality Assurance Project Plan may also contribute to consistency and scientific validity incorporated into any wetland assessments associated with the planning process.

This QA Plan is a first step in the development of a final QA Plan that will accompany the *Protocol.* Some of the QA/QC procedures used to gather data this field season are not final, but are being tested and evaluated. Due to the more limited scope of the sampling effort and size of the work force during this field season, not all QA/QC procedures will be fully developed (for example, data tracking). Development of procedures will proceed according to the needs of the current effort; more full blown procedures will be enumerated in final recommendations to the *Protocol.*

5.0 PROJECT DESCRIPTION

In order to provide a more empirically-based *Protocol*, we will focus especially on investigating and specifying (1) *sampling designs*, i.e., how sampling effort should be distributed to achieve optimal statistical representation with minimal cost; (2) *sampling parameters*, whether the parameters designated by the *Protocol* are representative as assessments of wetland attributes; and, (3) *sampling methodologies*, what sampling methods and dimensions (e.g., sampling units) are the most effective statistically and in terms of costs. These three levels are interdependent; their relationships are based on the spatial distributions (scale of spatial variation) of the attributes. To improve our understanding of the distribution of wetland attributes, to assess the efficiency of *Protocol* methods for different attributes, and to provide information for decisions about how to allocate future sampling efforts, spatial sampling schemes and independent tests of *Protocol* methods were included in the sampling program. Because, for most attributes, we currently have no means for assessing the accuracy of the methods (the "truth" in the field is not possible to measure with our time and means) methods will be compared in terms of their relative precisions and biases. Tests of accuracy may be developed in the

1 Clean Water Act

future.

5.1 Selection of Attributes, Methods, and Sampling Sites

Attributes, parameters, and most sampling methods used during this project were selected during the development of the *Protocol*. The history of the *Protocol* development process is described in the Introduction to the *Protocol* and a list of attributes and parameters used in this project is found in Table 1. Research sites and possible strata within those sites were selected during a workshop conducted on 17 December 1990 at the Padilla Bay National Estuarine Research Center, Mt. Vernon, Washington. Two technical representatives each from resource management agencies, academic scientists, tribes and environmental consultants from the Pacific Northwest region[2] were invited to attend and provide input toward defining reference site selection criteria and recommending specific sites in Puget Sound.

The following criteria were adopted as priorities in the selection of reference sites: (1) the site contains a broad diversity of estuarine wetland habitats representative of the (Columbian province) geographic region; (2) the site is available for long-term monitoring, has comparatively easy access and there is some assurance that the integrity of the wetlands will be protected from disturbance in the future; (3) there is minimal or no direct disturbance to the existing natural wetland in the estuary (e.g., industrial or heavy recreational or aquaculture usage) and indirect disruption of natural processes (e.g., major regulation of riverine inflow; extensive logging in watershed) is minimal; and, (4) there is a relatively short, undisrupted continuum of wetland habitats along the estuarine gradient, from euhaline (e.g., eelgrass/mudflat) to freshwater tidal (e.g., Sitka spruce-Western red cedar swamp).

In addition, the following criteria were considered desirable if found associated with the site: (1) a dedicated local, state or Federal government site (e.g., National Estuarine Research Reserve [NERR], National Park, Washington Department of Natural Resources [WDNR] Preserve) dedicated to or encouraging research rather than multiple use or exploitation; (2) a historic or on-going research or monitoring history, including US Geological Survey (USGS) gauging stations, National Oceanographic and Atmospheric Administration-National Ocean Survey (NOAA-NOS) stations, meteorological stations, etc.; (3) proximity to education facilities; and (4) proximity to past or current wetland restoration sites.

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[2] Washington State departments of Fisheries, Game and Ecology; U.S. Environmental Protection Agency-Region 10, Wetlands Program and Office of Coastal Waters; U.S. Army Corps of Engineers, Seattle District; U.S. National Oceanographic and Atmospheric Administration, HAZMAT; Suquamish Tribe; University of Washington's School of Ocean and Fishery Sciences, Department of Zoology, and Center for Urban Horticulture; consultants: Shapiro & Assoc., G. L. Williams & Assoc.

Based on these criteria, the Workshop participants ranked a number of potential sites in Puget Sound and the Washington coast as to their priority as reference sites. At this time, the following sites have been established for testing the *Protocol* under the US Environmental Protection Agency's Regional Applied Research Effort [RARE] grant:

Puget Sound:	Kennedy Creek, Toten Inlet
Washington Coast:	Elk River estuary, Grays Harbor
Oregon Coast:	South Slough, Coos Bay (South Slough National Estuarine Research Reserve)

All these sites meet the primary criteria to a large degree and many of the secondary criteria. In particular, two out of the three sites are in close proximity to existing (Elk River estuary) or proposed (South Slough) estuarine wetland mitigation sites, which potentially broadens the potential test of the *Protocol* with supplemental sampling at the mitigation sites (see Figure 1). In addition, the proposed reference site monitoring at South Slough would intermesh extensively with an estuarine and upland habitat inventory and monitoring activity recently initiated or proposed by the SSNERR[3]. We have received permission to conduct sampling in both of these estuaries. Due to delays in acquiring access for researchers to the Kennedy Creek property, which is on private property, and to funding constrains involved in both research and QA/QC development at the other two sites, a Puget Sound site was not included in this project. However, support is being sought to initiate reference site monitoring within the next year at Kennedy Creek or another site in Puget Sound or Hood Canal, and for this additional monitoring site we will adopt modifications to the *Protocol* that have emerged or become resolved as a result of this project.

The stratification of habitat in estuaries (see *Protocol*, pp. 15-18) enforces a stratified sampling design for most *Protocol* attributes. For each estuary, large-scale reference material (e.g., National Wetland Inventory [NWI] maps, aerial photographs, USGS topo. maps) and ground surveys were used to aid selection of sites. Three locations (herein termed "gradient site") along the estuarine gradient were chosen in each estuary. In each estuary, a site at the mouth of the river (most saline, site number 1), a "mid-estuary" (mesohaline, site number 2), and up-river (freshwater, site number 3), were selected. In late summer/early fall 1991, the Elk River and South Slough sites were visited (see Figure 2). Potential sites were visually surveyed, temporary transects were established, and test sampling was conducted. During the summer of 1992, habitat strata within the sites were delineated. Strata were selected as representative (spatially

[3] South Slough National Estuarine Research Reserve

prominent) in the estuary, common to all sites and fitting into broad categories of the estuarine wetland vegetation assemblages designated in the *Protocol* (see Representativeness, above). In 1992, it was possible to delineate each site into three strata: (1) a high marsh stratum characterized by the presence of emergent vascular plants, including *Dechampsia caespitosa*, (2) a low marsh stratum characterized by emergent vegetation, where *D. caespitosa* was not prominent; and (3) a mudflat stratum with no emergent vegetation. Microhabitats (e.g., tidal channels) and divergent plant assemblages that vary over the habitat's tidal elevations, surface topography and exposure were embedded in each stratum.

At each site, three permanent, elevation sampling transects were established perpendicular to the tidal elevation, from the upper margin of the intertidal (irregularly flooded) zone, at the upland transition, to the shallow subtidal (irregularly exposed) zone. The endpoints of the transects were marked with metal survey stakes; the rest of the transect stations were marked with PVC pipe. Transects were surveyed back to established survey datum (USGS/NOAA benchmarks) for exact elevation profiles.

5.2 Sampling Design

The sampling design for the 1992 season was developed to address the fact that plants and animals in wetlands are heterogeneously distributed in patches of variable sizes with variable taxonomic compositions and abundances. Traditionally, "marsh wide" averages, with concomitant, enormous standard deviations, were calculated from samples distributed randomly throughout an entire marsh. Such data is not precise or local enough to provide usable information for time series monitoring of changes. During the summer of 1992, one of our highest priorities was to sample in ways that would allow us to investigate the scale of spatial variation of as many of the attributes as we could, so that future sampling efforts could be more efficiently distributed to provide more precise estimates of species abundances. For attributes whose distribution are not visually accessible (epibenthos, meiofauna, microbiota), we sampled systematically along transects to see if such sampling revealed any information about scale or pattern for these organisms. For emergent vegetation, the patchiness of which is visually more apparent, we considered the usefulness of further stratifying the high and low marsh areas and of "mapping" patches by 1) marking their borders and following their changes in size and shape over time as well as 2) following changes in cover or standing stock within a patch over time. To develop marsh-wide averages, a rotating subset of patches could be monitored for changes in percent cover or biomass (and size). It is hoped that the added

precision of within-patch estimates of patches whose locations are known would be high enough to provide more meaningful indicators of change over time. Our work in August was aimed to test methods for sampling for within-patch cover and standing stock.

During the 1992 field season, sampling for most attributes was conducted at measured locations along constant elevation, horizontal transects or within delineated, constant elevation 25 x 10 m areas. Epibenthic plankters, benthic meiofauna, and benthic microbiota were sampled along a horizontal transect through the mudflat at the two most saline sites in each estuary. Emergent vegetation was sampled within 25 x 10 m areas at each site and, at one site, independent tests included sampling at different resolutions and with different methodologies. Above-ground and below-ground standing stock were sampled within these delineated areas as well as along the three elevation transects at each site.

Table 1 lists the attributes selected for investigation in 1992, the parameters measured, the units of measurement and the precision of measurement. The sampling design is summarized in Table 2 and explained in more detail in Section 8.0, "Sampling Procedures, Description of Methods".

5.3 Schedule

1988-1990	*	Development of Protocol: Selection of appropriate attributes, respective parameters, compilation of best known sampling methods, sample size recommendations
12/17/90	*	Site selection: Selection of appropriate criteria for reference sites, selection of estuaries fitting criteria, selection of sites along estuarine gradient in each estuary.
Summer 1991	*	Preliminary investigation of Estuaries: Investigation of gradient sites within estuary, temporary transects, test sampling
Summer 1992	*	Full Field Effort: Delineation of strata, permanent elevation transects, sampling for selected *Protocol* attributes
2/15-4/15	*	preliminary evaluation of sample unit, replication, precision, and accuracy estimated from 1991 data
	*	field survey preparation: equipment assembly, map preparation, training of field personnel, contact with local personnel
4/15-4/22	*	establish permanent transects at sites in Elk River and South Slough, record site characteristics (location, elevation, other notes)

4/22-5/30	*	finalize sampling design
	*	draw up data sheets, labels, inventory sheets
	*	gather containers, prepare storage areas
5/30-6/6	*	sample epibenthic plankters
6/27-7/5	*	sample benthic microbiota, sedentary infauna
8/25-9/2	*	sample rooted vascular plants, pore water
9/2 ->	*	analyze data, develop final sampling and QA recommendations

6.0 PROJECT QA ORGANIZATION AND RESPONSIBILITIES

All project personnel participate in field activities and data analysis in order to obtain maximum continuity between field collection of data, laboratory sample analyses, and data evaluation. The Principal Investigator and Project Leader have primary responsibility for developing appropriate QA/QC methods and ensuring that the research tasks and procedures are followed according to methods listed in the *Protocol* and QA/QC requirements in this report. The Project Leader, in conjunction with the Statistical Coordinator, is specifically responsible for the QC aspect of the project, including monitoring all plant and invertebrate sampling processing, generating the standards, conducting the internal quality control checks and developing correction procedures. Principal QA/QC responsibilities are as follows:

6.1 QA Responsibilities

Responsibilities under the Quality Assurance portion of the Plan are to:

(1) lead the development of the QA plan;

(2) ensure that all project participants follow both the *Protocol* procedures and the emerging QA plan;

(3) interact with the project officer and ERL-C QA staff to evaluate both on-site and in-lab QA procedures;

(4) verify that QC activities are performed and data quality is determined as required in the QA project plan; and,

(5) document QC outputs.

6.2 QC Responsibilities

Responsibilities under the Quality Control components of the Plan are to:

(1) ensure that *Protocol* procedures are followed;

(2) follow instrument manufacturer's specifications;
(3) perform and document preventive maintenance;
(4) maintain up-to-date field and laboratory notebooks;
(5) track data acquisition and verification;
(6) conduct analytical and data quality determinations; and,
(7) report all problems and corrective actions to the Principal Investigator.

6.3 Field/Lab Team

The Wetlands Ecosystem Team is composed of the following investigators, with their associated responsibilities:

Charles Simenstad	*Principal Investigator*; team leader; responsible for overall sampling program
Ronald Thom	*Consultant*; co-team leader; responsible for guidance and quality control on rooted vascular plant, benthic macroalgae, and benthic microbiota sampling
Jeffery Cordell	*Project Leader*; responsible for field logistics and for guidance and quality control on sedentary infauna and epibenthic plankters sampling
Lucinda Tear	*Statistical Coordinator*; responsible for development of field sampling design, organization, preparation, and conductance of sampling, tracking of sample custody and archiving, and statistical processing and evaluation of data
Laurie Weitkamp	*Fisheries Biologist*; field assistant, with particular responsibility for benthic infauna and epibenthos collections and laboratory processing, collection of above and below ground vegetation samples
W. Gregory Hood	*Research Assistant*; field assistant, with particular responsibility for rooted vascular plant taxonomy, guidance and quality control on tidal elevation surveying
David Shreffler	*Consultant*
Craig Cornu	*Field Assistant* (South Slough), plant identification, porewater (South Slough)

All members of the field team cooperated for establishing and surveying site transects. Weitkamp, Cordell, Simenstad, and Tear participated in the first two sampling trips. Cornu and Hood joined for sampling emergent vegetation.

7.0 OBJECTIVES FOR MEASUREMENT

7.1 Accuracy

7.1.1 *Field*

Accuracy of quantitative data collected in the field, such as percent cover of vegetation, is difficult to assess because ground-truthing is either impossible or very costly. It is not possible to know how accurate estimates of cover, standing stock, or densities are without censusing the population. Therefore, in this project, we focus on relative bias and precision of quantitative techniques through analysis of data gathered using different techniques or approaches to the same measurement and bootstrap analyses (see Precision below). Estimates of accuracy/bias of the methods will require an experimental approach and mapping at some future time. Accuracy is enhanced, although it can not be assessed, by use of consistent methods. Field team members who are not familiar with a given field technique receive training through classroom education or demonstration in the field. All field sampling methods are reviewed in the field by the team to assure that all members use consistent methods. Training and consistent methods also contribute to precision of estimates and representativeness of individual samples

Accuracy of species identification of vegetation in the field is guaranteed by field assistants with experience with local wetland plants, taking voucher specimens when necessary, and using local field keys and herbaria collections to identify species whose identities are uncertain.

7.1.2 *Laboratory*

Accuracy in the laboratory pertains to the weighing, measuring, counting, sorting, and identification of species collected in various types of samples. For weighing and measuring, accuracy will depend on the accuracy of equipment used and calibration of scales before each weight is taken. All equipment is maintained and serviced on a regular basis as part of University of Washington servicing Contracts or other standard procedures (see Calibration Procedures and Frequency). For counting and sorting, and species identification a careful system of repeated measurement and cross checking, described under "Precision" below is carried out for epibenthic plankters and benthic meiofauna, and above ground and below-ground biomass. Training is important in assuring good accuracy in counting, sorting, and species identification. Training for each task is carried out by the person responsible for the quality control of the procedure.

7.2 Precision

7.2.1 *Field*

Epibenthic plankters and sedentary infauna--Consistent methods are important in assuring precision of estimators. The methods for collecting benthic fauna described in detail under "Sampling Procedures, Description of Methods" were used in the collection of all benthic fauna in this project. In epibenthic sampling, it is important to sample down current of the sampling locations to prevent sediment plumes from footsteps or previous sample taking from contaminating samples. We are always careful to determine the current direction, and when possible, sample from a boat to minimize the number of people in the water. It is also important to take all epibenthic samples on an incoming tide, when the water is at a consistent depth.

Emergent marsh vegetation, above ground percent cover-For visual estimation of percent cover, precision of individual measurements will be tested by having individuals repeat measurements of the same quadrats several times. Effects of quadrat size, plant form, and individual on the consistency of these measurements will be tested using ANOVA techniques. Independent tests of methods to sample for percent cover are conducted in the same areas to investigate comparability of estimators, and the data will be used to assess the relative precision and/or relative bias of the methods for a given number of samples or a given time. Confining these tests to the same spatial areas insures that variability caused by spatial heterogeneity will not be confounded with differences between methods. We are not able to test for individual measurement error or sampling error related to point placement with the point quadrat method, but will be able to test precision using points sampled by bootstrapping data to investigate effects of sample size on precision.

Emergent marsh vegetation, above ground standing stock, Emergent marsh vegetation, below ground standing stock, Microbiota--Collection methods for these and all above data are standardized as much as possible and when tests for effects of individual measurement error are not possible, effects will be controlled to the extent possible through training, cross checking by another observer, using consistent methods, and limiting the number of people involved in data collection. Specific methods to assure precision are detailed in the "Sampling Procedures" section.

7.2.2 *Laboratory*

Epibenthic plankters and sedentary infauna--Sorted samples are checked by a second person until remains are less than 5%. 10% of samples are rechecked by the Project Leader for proper species identifications. If similarity is less than 90%, all samples are rechecked by the Project Leader.

Emergent marsh above-ground standing stock (biomass per unit area)--Ten percent of samples are reweighed by a second technician, and if disagreement is greater than 10%, then sources of error are investigated and, ultimately, all samples may be reweighed.

Emergent marsh below-ground standing stock determination--Cores taken for below-ground standing stock are kept frozen until analysis to prevent any deterioration of organic matter. Protocols for separation of living and dead matter have been suggested by Hsieh and Yang (1992). Some of our below-ground biomass samples have been processed; one person made decisions about living vs. dead categories. New criteria for making these decisions will be evaluated based on the above article, used in processing the remaining samples, and written into revisions of the *Protocol*.

Microbiota--The fluorometer is zeroed daily with 90% acetone and calibrated by running a sample of known chlorophyll content (known dilution of a species that does not produce phaeopigments) the chlorophyll content of which was previously measured using a spectrophotometer.

7.3 Completeness

Completeness is measured as the number of samples processed or analyzed using consistent methods vs. the number taken. Estimated sample sizes required for each parameter were determined using preliminary pilot data and bootstrap analyses to investigate the necessary number of samples required to detect a difference equal to the mean of the samples or one half the mean. Because natural variability is high and sampling and processing costly, minimum detectable differences must be set quite high to be achieved with 90-95% confidence (with beta = .1). In all our sampling, we tried to gather more data than pilot studies indicated would be required and used a hierarchical sampling design discussed below to assure collection of adequate groups of data. The field methods we used to gain information about the spatial variability of sites and differences between sampling methods were also useful in assuring that it would be possible to

analyze whatever data was collected and insure desired levels of confidence.

7.3.1 *Field*

To insure the collection of adequate and useful groups of data, the sampling design was created as a hierarchy of components or modules; the completion of each component would allow statistical analysis, the completion the entire program would be ideal. For example, if sampling occurs along transects, sampling is completed along one transect before beginning another. Sites are surveyed in their entirety before they are linked to benchmarks. Percent cover measurements in one area are completed before beginning data gathering in another area. All independent tests of methodological differences are conducted in one area and completed before beginning sampling other areas. Time estimates from past sampling, careful scheduling of personnel, and attention to fatigue and tides were considered in advance in order to minimize the chances that time would unexpectedly curtail sampling.

7.3.2 *Laboratory*

Samples are stored in groups corresponding to the groupings in which they were gathered, so processing may occur under the same hierarchical program as was used for sampling. When feasible, samples are restored after laboratory processing so that any errors discovered at a later date, such as species misidentifications or possible misweighings or miscountings (outliers discovered during data analysis), can be reevaluated. Data will also be analyzed in groups.

7.4 Representativeness

As explained in "Project Description", sampling sites and strata were chosen using criteria for representativeness that had been determined by local experts. The estuaries sampled have different hydrologic regimes, geographic locations, and geological forms, and fall well within the continuum of estuary types found in the Pacific Northwest. Strata within sites represent the range of habitat types that can be found within estuarine wetland sites in the Pacific Northwest. In addition, the attributes in the *Protocol* have been carefully chosen, through the Protocol development process, to enable development of representative, reliable indices of wetland functions. The attributes selected for focus this summer were also chosen, after careful consideration, to provide a representative sample of *Protocol* attributes to ensure that Protocol methods will be adequately tested.

The units of measurement used for most parameters are direct; to whatever degree sampling methods allow, they are measurements of the parameter of interest. For

example, cover, biomass, density, and elevation are all direct measures of the parameter of interest. We are still in the preliminary stages of learning how to sample certain physical characteristics, but we will be measuring, for example, salinity and redox directly, and not with indirect or secondary parameters.

Consistent methods are an important aspect of representativeness in that they assure that each sample is an equivalent sampling unit; that it "represents" the population in the same way.

7.5 Comparability

The purpose of the *Protocol* is to develop standardized procedures that can be used by all wetland scientists to insure comparability among data sets. The parameters and methods in the Protocol are a compilation of methods commonly used by estuarine biologists. Parameters such as plant cover or biomass, animal abundance or standing stock are parameters often measured by wetland scientists as well as by ecologists in other habitat types. Use of consistent methods insures that estimates of abundance from different areas can be compared.

8.0 SAMPLING PROCEDURES

Aspects of the sampling design and procedures that occurred before the 1992 field season are described under "Project Description". Procedures and methods pertinent to field and laboratory efforts of the 1992 season are described below. Types of equipment and containers used, transport, and storage aspects of methods are described in Tables 3 and 4; Appendix 1 provides examples of field book and laboratory data collection formats.

8.1 Elevation

8.1.1 *Field - May 1992*

In May 1992, the Wetland Ecosystems Team:

1) Delineated three estuarine habitat strata (high marsh, low marsh, and mudflat) at
   each of the three sites in each estuary according to the criteria listed under "Project Description;" the up-river site in each estuary (site number 3) did not contain a mudflat stratum.
2) Laid out three permanent, baseline transects, 50-100 m apart, across habitat strata,
   from upland to mudflat, parallel to the elevation gradient; the upland end of

each transect was marked with an iron rod, the transects themselves were marked by PVC pipes placed 10-20 m apart depending on width and slope of site. (See Figure 1)

3) "Surveyed" each transect using a Leitz Total Station to find the relative elevations
of each of the transect markers and to tie the sites in to local USGS benchmarks. In some cases, tying to benchmarks may need to be repeated. Time, weather, the distances to be covered by boat, the difficulty in finding stable ground for the total station, and the difficulty in estimating the range of siting prevented completion of a few tie in's.

Surveying transects involves the following steps at each site:

1. Set a "control point" at each site (marked with an iron rod) from which the elevations of the "topographic" points (transect markers along the gradient) are sited.
2. Place the total station is placed at the control point.
3. Use Total Station to site mirror on rod held by second person at each "topo" point.
4. Use Total Station to calculate vertical angle, horizontal angle, difference in elevation, and distance to each topo point.
5. Record data electronically in data recorder using Sokkia software.
6. Hand copy data into field books to prevent loss of data.

The Leitz system is extremely accurate. Difficulty in interpreting the very "accurate" data arises because wetlands are highly channelized and irregular. In the future, several points around marked topo points could be measured to show whether the more local topography around each point and whether the point is on a hummock or in a channel.

8.1.2 *Lab - Fall 1992-Winter 1993*

Electronically recorded data files were checked with hand copied data, back up copies were made, and ASCII files were extracted to generate site maps.

8.2 Epibenthos

8.2.1 *Field - May 1992*

Epibenthic fauna were sampled every 2 meters along a 40-meter horizontal (constant elevation) transect in the mudflat stratum at ELK1 and SS1 (most saline sites) and along an 80 m transect at ELK2 and SS2 (mid-estuary sites). It is hoped that this

sampling effort coincided with epibenthic blooms in each of the areas. Epibenthos were collected using an 0.018-m^2 epibenthic pump (see *Protocol* for description). To collect epibenthic organisms:

1. Place pump head lightly on the mud surface disturbing surface layer as little as possible.
2. Run pump for 20 seconds to suction a constant volume and take up epibenthic fauna at each sampling location.
3. Sieve suctioned water through a 130-μm mesh screen.
4. Wash screen contents into 8-16 oz. plastic jars.
5. Add pre-made label and close jar.
6. On return to shore, reopen each jar, add 10% buffered formaldehyde, close, and store.

8.3 Benthic Meiofauna

8.3.1 *Field - June 1992*

Benthic meiofauna were collected at the same sites and using the same spatial sampling scheme used for epibenthos (above) using the following method:

1. Insert 1 1/2 inch diameter PVC core approximately 10 inches into the mud.
2. Place stopper in core, remove from sediment.
3. Place core contents into 16 oz plastic jars (use plunger if necessary) with pre-made
   label and close.
4. On shore, reopen jars, add 10% buffered formaldehyde, shake well to insure all particles are separated and preserved.

8.3.2 *Laboratory - June 1992-October 1993*

Benthic fauna samples are processed according to the following procedures:

1. Samples are sorted at 25X magnification (key organisms removed from sediment).
2. All samples are rechecked for remains by another person until error is reduced to 5%.
3. Samples are filtered through a 153 μm screen and scanned to determine if subsampling is necessary.
4. If subsampling is necessary (more than 100 organisms per sample), samples are split in a Fulsom™ plankton splitter or a known volume is taken up in a Hensen's-Stempel™ pipette (Hensen 1895) (Wildco, 301 Cass Street, Saginaw,

Michigan 48602) until the total count for the most numerous species exceeds 100.

5. Samples are taxonomically enumerated by technician, the predominant forms are identified to species. 10% of those samples are rechecked by the Project Leader. If similarity is less than 90%, all samples are rechecked by the Project Leader, who is an acknowledged taxonomic expert on Harpacticoida and other epibenthic crustaceans in the Pacific Northwest. (See also Puget Sound Protocols [Tetra Tech, Inc. 1986] and Cordell *et al.* 1992).
6. Abundances are calculated to m^{-3} for each attribute
7. Species identifications and abundances are recorded on data sheets using NODC

   codes and entered into a relational database by the data processing department

8.4 Benthic Microflora (Chlorophyll *a*)

8.4.1 *Field - June 1992*

Microflora samples were taken at every fourth meiofauna sample site (above) to test correlation of meiofauna abundance with epibenthic primary productivity using the following procedure:

1). Insert .5 '" diameter syringe (narrow end removed) 3 cm into sediment
2. Pull up plunger to suction in surface scum and sediment
3. Push plunger down to eject all but 2 cm of sediment
4. Push remaining sample into black plastic jar with pre-made label and close
5. Store samples on ice in field to slow photosynthesis
6. Freeze samples on shore until processing time.

8.4.2 *Lab - 1992*

Procedures used for processing microbiota samples follow Strickland and Parsons (1977) IV.3.IV. Fluourometric Determination of Chlorophylls. These procedures are well accepted in the oceanographic community, and have been modified as follows to accommodate benthic sampling regimes.

1. Samples are frozen in the field and kept frozen and in the dark until processing. (The freezing/thawing process aides in breaking down the cells and facilitating the extraction of all pigments.)
2. Thaw samples, add measured amounts of magnesium carbonate and acetone to stabilize sample and extract chlorophyll. Eelgrass fragments are removed, and

diluted samples are ground with a mortar and pestle for several minutes to further crush cells and release pigments.

3. Refrigerate samples in the dark for 8 hours.
4. Remove from refrigeration, stir to equalize suspension, spin in centrifuge at 2000
   rpm for 10 minutes to separate organic matter and sediments.
5. Process supernatant in a Turner 111 fluorometer
6. Take readings of:
   a) (F_O): measure excitation levels for all pigments
   b) (F_a): measure emission due to phaeopigments.
7. Perform calculations in accordance with the above reference; chlorophyll measurements are standardized to g/m^2.

8.5 Emergent Vegetation

In August, sampling of emergent marsh vegetation was conducted in the high and low marsh strata. As stated, sampling of emergent vegetation was designed to investigate methods for sampling within-patch abundances. Three parameters, percent cover, above- and below-ground standing stock, were investigated, and several methods were used. Figure 1 summarizes the overall sampling design.

In the *Protocol*, the benthic quadrat is the recommended sampling unit for percent cover and visual estimation is one of the recommended sampling methods. Since species are distributed at different scales, percent cover estimations for many species will be related to the size of the quadrat and the most appropriate quadrat size, i.e. the size that will give the "truest" value for the entire area, will vary from species to species. In addition, the statistics of the visual estimation technique are not known; within and between observer variability and bias has not been quantified and appears to vary unpredictably in different situations. Therefore, we tested the visual estimation within quadrats method against a method that minimizes visual estimation errors, eliminates the problems of scale associated with the benthic quadrat, and allows estimation of scale for each species. Point quadrat (pq) sampling was chosen as the common strategy for determining percent cover at all sites and against which to test the visual estimation methods. In point quadrat sampling, the size of the quadrat is decreased to the size of a point, and visual estimation is reduced to a frequency determination of contact or no contact with species at that point. Frequently, point quadrats are clustered 50 to 100 within 1 x 1 or .5 x .5 m quadrats. Since one would assume that many points in a small

area would carry a certain amount of redundancy because points close together would be correlated, Goodall (1951) recommends distributing point quadrats randomly within the area of interest, rather than clustering them in quadrats as a more efficient sampling design. This version of pq sampling was chosen as the common strategy for percent cover estimations at all sites. (Dethier 1990) found high within quadrat variability using 50 points within benthic quadrats. This variability is likely a function of both sampling error and small scale variability in a plants form and distribution. Both of these complicate estimation percent cover for the larger area of interest.)

If the locations of the x,y coordinates used are known, the correlation structure of a species can be investigated and the distances required for sampling points to be considered independent can be calculated. Variance estimates from the sampling effort can be combined with distances required for sampling points to be independent to generate density and total number of points required for a desired precision. This information can be used to refine pq sampling within areas to create the most efficient designs (no redundancy through sampling correlated points). We hope to use t his data to create a systematic sampling grid that will obviate the need to precisely locate each new point, facilitate relocating sampling points from minimal markers, and preserve independence and sampling precision. While the size of the grid and total area required will vary from species to species, the spatial information from this or similar studies will allow grids and sampling designs to be tailored to meet the sampling needs of a given project at a given site (e.g. to choose to sample for rare or abundant species in different sized patches.) These methods will be elaborated on in future papers.

With the exception of South Slough Site mid-estuary site (SS2), where two areas were sampled (SS2B and SS2D), each site was represented by only one plot or habitat type. In this way, then, we have used pq estimation in seven different habitat types or patches. Although it would have been desirable to have replicates within a habitat type, time did not permit this given the program requirements to sample at all six sites in order to test the applicability of the attributes. Such replication can be carried out at a future date in order to investigate the effects of sampling error and differences between patches. The data from 1992 sampling will serve as an excellent pilot study for future, more complete, investigations. More complete characterization a site will involve mapping and sampling representative patches as discussed in the "Project Description."

We used the plots at site SS2 (SS2B), to test the relative efficiency of the density of pq estimates used at all sites against estimates derived from three other methods. Since time did not permit such testing at every site, testing can not be said to be complete in that methods may have different efficiencies in different community types. The methods tested

include:

1. higher intensity of sampling (increased the number of pq's);
2. visual estimations of percent cover, controlling for individual measurement errors; and,
3. systematic pq sampling along a transect.

At plot SS2D we investigated:

1. ability of pq sampling to distinguish between visually different communities at the same site: PQ sampling at a second plot at site SS2 (SS2D); and,
2. within- and between-observer variability and effects of quadrat size using visual estimation technique: repeated visual estimations by different observers in
   different sized quadrats (SS2D).

The sections below describe the field methods used to set up the point quadrat sampling areas (plots), and to conduct the tests described above.

8.5.1 *Random Point Quadrat Method at All Sites*

Field

1. Delineate a 25 x 10 m area within a relatively "homogeneous", high marsh area. An area was judged homogeneous if there was no obvious elevational change within the area (wide, deep channel or unusual hummock) and species composition was visually consistent. We chose areas with a range of plant forms and coverage.
2. Circumscribe area using meter tapes.
3. Insert wooden stakes or flags every 5 m around the perimeter.
4. Lay meter tape down the middle of the plot (The tapes and stakes served as visual measurement aids for the sampler who noted species at 104 randomly generated x,y coordinates within the plot)
5. At each x,y coordinate chosen, point a thin metal rod vertically at the ground.
6. Record all species touched by the rod in field books. Recordings are made by a second person who also calls out the coordinates to the sampler. If a species is intercepted more than once at a given point, it is recorded only once.
7. Record any obvious clumps or species not sampled by the end of the sampling.

Individual measurement error in the random point quadrat method is directly related to the size of the pin used, whether readings are taken at fixed pins, or observers place pins anew (Goodall 1951), and observer "bias" about what constitutes a "hit". We did not test for these two effects, but tried to control for them by using the same, very small diameter, pin in all trials the same two people taking readings. These two observers agreed on a protocol and occasionally validation by the other observer was sought. To truly test variability in pq estimates due to individual measurement error, repeat measures by the same and different observers should be performed both on the same pins and placing the pins anew at each reading. To estimate sampling error, repeated samplings of individual plots, using the same and different points would need to be carried out. We will not be able to assess these levels of error this field season.

Sampling error in this method relates to the precision with which points can be located and the number of points deployed. The methods used last summer have been refined to allow much easier and more precise point location. By presorting sampling points in the field book so that the sampler can move up and down rows in the area with out trampling the area extensively and having the x and y axis measuring aids always within one meter, points can be easily and precisely located. These methods involve:

1. Generate two columns of numbers of desired n, uniform zero to the distance desired there 0-10 and 0-25).
2. Sort these numbers first by the first column (x coordinate).
3. Stack the numbers in groups of one meter intervals (e.g., 0-1, 1-2, 2-3...9-10).
4. Sort each "stack" again, now by the second column. Alternate sorting such that the first column is sorted in ascending order, the next in descending order, the next in ascending, etc.
5. Delimit the desired area using meter tapes.
6. Place a stake at every meter mark along two opposite ends.
7. Lay a tape between the second two meter markers as the y axis.
8. Use a meter stick to measure the distance along the x axis.
9. Have a reader read the x,y coordinates and record species touched as the observer proceeds up the first row and down the second row.
10. Move the y axis tape to the 4th meter mark.
11. Continue recording points up and down successive rows until the area is completely sampled.

Laboratory

Data will be entered into a Microsoft Excel for Windows™ database, checked, and three copies of all files were made. Percent cover is calculated as the number of intercepts of a species per total number of points (random point quadrats or rpq's) investigated (104). Binomial confidence intervals for each species percent cover estimations will be calculated.

8.5.2 *Above -ground Standing Stock at All Sites*

Field

Above-ground standing stock samples were taken within the 25 x 10 m plots for estimates of local variability of individual species and along two to three of the elevational transects at each site for estimates of cross gradient total above-ground biomass. Within the plots, samples were taken at each fourth random point (until a maximum n of 24). Along the elevation transects samples were taken at a random number of paces between each topo transect marker. The methods for collecting the samples were:

1. Clip above-ground standing stock of vegetation rooted within a 0.25 x 0.25 m quadrat to ground level
2. Place vegetation from each quadrat in a separate plastic bag with pre-made label, tie bag.
3. Keep samples cool in ice chests until return to shore.

Laboratory

Above-ground Standing Stock:

For samples gathered within 25 x 10 m plots:

1. Sort plant matter in each sample bag by species. The team worked together, and any identification questions were resolved by the team.
2. For each sample, wrap each species loosely in aluminum foil, with label including site, date, sample #, and species name. Label aluminum foil with same information.
3. Keep samples cool until drying.
4. Place samples loosely in drying oven and dry at 150°C for two to three days.
5. Check samples periodically to assure even drying.
6. Weigh each sample, remove sample contents and weigh aluminum foil.
7. Subtract weight of aluminum foil from total weight.

For samples gathered along elevation transects (total biomass):

1. Wrap each sample loosely in aluminum foil and label.
2. Complete steps 3,4,5,6,7 above.

8.5.3 *Below -ground Standing Stock at All Sites*

Field

At each site, below-ground standing stock was collected at the same locations as above ground samples along the elevation transects. At SS2B, below-ground biomass was also collected at the same locations as each above ground sample within the delineated plot. One team of two people was responsible for collection of all above- and below-ground standing stock samples. Methods for collecting below-ground biomass include:

1. Pound a 3.18-cm diameter PVC tube approximately 30-cm into the ground.
2. Place a stopper on top of the tube, twist core, pull to extract.
3. Place each core in a separate bag with label.
4. Place each bag on ice.
5. On return to shore, freeze samples.

Laboratory

1) Store samples frozen until analysis.
2) Thaw cores.
3) Wash away sediment and remove organic matter.
4) Separate dead and live matter, wrap labeled samples loosely methods of Hsieh and Yang (1992) may be used to distinguish live from dead matter.
5) Dry samples in drying oven and weigh as above for above-ground biomass.

8.5.4 *Porewater Salinity and Redox*

Field

The *Protocol* recommends measuring physicochemical parameters to see if relationships with biological attributes can be found. To complement the elevation data gathered, we attempted to take porewater salinity and sediment redox at each of our sample locations. Redox probes were quickly and unretrievably clogged by mud and water and took a long time to equilibrate so that multiple readings with one probe were very time consuming. In addition, the solid ground in the high marsh area required that holes be dug in order to insert the probes. Therefore, subsequent "pore water" samples were taken by inserting the probe of a salinometer into the water that seeped into the

holes left after the removal of the below- ground standing stock samples taken along the elevational transects. Holes were drilled in a line around 50.8 cm-long, 3.18-cm diameter PVC pipes approximately six inches from one end. These pipes were inserted into the holes left by the below-ground standing stock cores, stoppered, and later returned to for salinity readings. In most pipes, water accumulated within an hour, in other pipes another tidal cycle was required, in others still, water did not accumulate during our field stay. Considerable controversy ensued about exactly what water was being sampled by this technique and how much interchange of water there would be between water in the pipe and water in the soil outside the pipe. It is not clear whether what we sampled was, indeed, pore water, or rather ground water. Data from these wells will be compared to readings taken by squeezing water from a syringe full of sediment onto a refractometer. This test is described under "Independent Tests".

8.5.5 *Independent Tests of Percent Cover and Scale (Site SS2)*

At South Slough Site Number 2 (SS2), the following independent methods of estimating percent cover and scale of spatial variability were carried out.

Random point quadrat, second plot within same site (SS2D)

A second 25 x 10 m plot at SS2 (SS2D), was sampled using the same techniques described above under "RPQ Methods, All Sites". This test was designed to assure that the rpq method would detect differences between plots at the same site (SS2D had a very different species composition than the first plot (SS2B)) and to provide alternate estimates for the repeated (by individuals) measures test which was conducted in the same area.

Random point quadrat, higher intensity in a smaller area (SS2A)

While it seems likely, and has been shown in previous studies, that relatively abundant species will be tend to be well estimated by a variety of techniques and intensities of sampling, rarer species are more difficult to quantify precisely. To see if rarer species could be more precisely estimated by higher resolution rpq sampling (increasing "n"), we sampled at a higher intensity than in the larger area SS2B by assessing 67 additional random point quadrats for all species, and 220 additional random point quadrats for the rarer species (*Triglochin maritima*, *Atriplex patula*, and *Glaux maritima*) in a 3 x 5-m area within SS2B called SS2A. These data will also be used to investigate spatial scale (see Horizontal transect, below).

RPQ vs. Visual Estimation Technique (SS2B)

This test was designed to compare estimates of percent cover produced by the visual estimation technique with estimates from the random point quadrat method described above. Because visual estimations of percent cover can be highly variable, even within observer, to allow this comparison we tried to control individual estimation error by creating teams to perform the estimations, by giving the teams visual aids to assist them is their estimations, and by conducting several pre-experiment group estimations when all observers discussed their reasons for making the estimations they did. Four people trained in plant identification were used to create 6 teams of two people. At every fourth random point (up to n=24) in the first 25 x 10-m area (SS2B), a 0.5 x 0.5 m quadrat was laid down. Each team was randomly assigned four quadrats and given manila cards cut to equal 1%, 2%, 5%, 10%, 15%, and 20% of the 0.25-m^2 encompassed by each quadrat. These cards were held over the quadrat to aid in estimating the total area covered by dispersed clumps of plants or irregularly shaped clumps, and to assure that all teams were operating with the same mental/visual "scales". Percent cover of all species in each quadrat was estimated by each team reaching consensus about each estimation. Collecting the data in this way controlled for individual measurement error as much as possible, and will allow for testing of a team effect (One Way ANOVA) in data analysis.

Visual estimation - Individual Measurement Error (SS2D)

In this test, we investigated the distribution of an individual's visual estimations in quadrats of different sizes. In the second 25 x 10 m area (SS2D), we randomly selected and marked five of the 104 rpq coordinates. At each point, we conducted three sets of estimates. Each set was composed of three to four "rounds" at a given quadrat size. During each round, each observer recorded his/her visual estimations of percent cover of each species observed in each of the five quadrats. To do this, we laid a 0.5 x 0.5-m quadrat at each of the five marked points. Each observer silently recorded his/her estimations at each quadrat. After completing one "round" of estimations and taking a break, each person then began again, until, for each quadrat, each person had recorded four visual estimations. We all found that we did not remember estimates from past rounds. When everyone was finished, we replaced the 0.5 x 0.5-m quadrats with 1 x 1-m quadrats and each person completed three rounds of estimations. The third set of three estimations was conducted used 0.25 x 0.25-m quadrats place within the 0.5 x 0.5 m area.

This "experiment" will allow us to see how individuals differ from one another in their estimations, how consistent or inconsistent different individuals are, and whether this individual consistency is related to the size of the area being estimated. It is well known

that fatigue effects visual acumen in this technique. We did not try to control for or sort out the effects of fatigue, but rather, because fatigue would play a role in any large scale monitoring project, allowed it to have its effect. We do know the order in which estimations were made on a quadrat by quadrat level if this information should seem relevant later. The following types of comparisons will be made:

1. Variability within observers:
   a) calculate variance and standard deviation of each observer's estimates for each species and each quadrat;
   b) record maximum difference between any two estimates for a species in a quadrat for each observer; and,
   c) compare mean differences and standard deviations for different species, different quadrat sizes.
2. Differences in between observer variability:
   a) compare variability estimates from (a); and,
   b) assess number of times an observer was high or low relative to other observers.
3. Differences between variability of estimates in different quadrat sizes:
   a) compare average standard deviations and differences (from 1 above) at each quadrat size for each species.
4. Differences between variability of estimates of different species
   a) compare average standard deviations and differences (from 1 above) of different species for each quadrat size.

Horizontal Transect--RPQ Percent Cover and Determination of Scale (SS2B)

Line intercept sampling is sometimes used to measure vegetational percent cover of species with very discrete or clumped forms. A straight transect is laid out and distances along the transect covered by the species of interest are recorded. Percent cover of that species is the proportion of the total transect length that intercepted the species. Carlile *et al.* (1987) used this method to estimate percent cover of sagebrush and to investigate the scale at which sagebrush was distributed along the transect. Because many of the species in wetlands do not have such an easily measured, discrete form, we modified the technique and used systematic pq sampling along a transect. A 50-m meter tape was laid out along one edge of area SS2B. For the first 5 m, an rpq was placed every 5 cm (n = 100); for the next 15 meters, an rpq was placed every 10 cm (total n at 10 cm resolution = 200); and, from 20 to 50 m, an rpq was placed every 20 cm (total n at 20 cm resolution = 250). We will use the methods of Carlile *et al.* (1987), Markov chains, and time series

analysis to investigate pattern and scale along this transect, and to investigate how these methods compare in terms of ease of use and abilities to detect scale and pattern. The first two of these methods will also provide estimates of percent cover.

The estimates of scale from of the above methods will be compared to estimates of scale calculated from the two dimensional pq data from the plots. The probability of touching a species given a certain distance between points will be calculated for each method. At the distance this probability equals the probability of finding the species, samples will be considered independent. Distance to independence and estimates of percent cover will be compared from all the above techniques in terms of their point estimates and precision (confidence intervals and coefficients of variation), using bootstrapping techniques when necessary. Because the sampling techniques used are so different, and sampling units are not the same in each method, comparisons of the precision of each method will be made relative to time and effort needed to sample.

8.5.6 *Independent Tests of Below-ground Standing Stock*

Local variability (SS2B)

At site SS2B, below-ground standing stock samples were taken in the center of each of the 24 above-ground sample quadrats. These above- and below-ground samples will allow evaluation of local, within-patch variability of below-ground standing stock and direct comparison between above-ground standing stock, below ground standing stock, and percent cover.

8.5.7 *Independent Tests of Pore Water Salinity and Redox*

Local variability (SS2B)

Using the same methods described above for pore water, pore water readings were taken where each of the 24 below-ground standing stock cores were extracted in SS2B.

Comparison of methods (ELK1, ELK2, ELK3)

Because we were not certain about the methods used in collecting "pore water" samples, we conducted two independent tests of our methods. The first method was described above in the "Pore Water" Methods section. For the second method, we drilled holes through the entire column length of 5 catchment tubes that were then inserted into holes created next to those described in method one. It was hoped that these second tubes would allow greater water flux through the tube than the original method. In the third method, we took a small core of soil near the tube in a hypodermic syringe with a filter paper at the needle end. We then squeezed a drop of water (true pore water) from the soil

onto the refractometer by inserting and pressing the plunger into the syringe. A refractometer reading was recorded. Filter paper was replaced for each pore water sample. At some locations, this last method was the only reading possible, since no water had accumulated in the catchment tube. At ELK3, the last method was the only method used, since no tubes accumulated water.

The first test involved taking readings in three ways at all "pore water" stations at ELK1 and ELK2 (n=41). We first inserted a Yellow Spring Instrument™ [YSI] probe into the well and recorded temperature and salinity using the YSI™ meter. We then dripped water from the YSI™ probe onto a hand-held refractometer and took a reading. Finally, we used method three above (syringe) to measure the "true pore water" salinity with the refractometer. This series of tests will provide calibration of YSI™ readings with refractometer readings (relative bias), comparison of the salinity of water in the tubes with shallow sediment pore water, and comparison of water in tubes with inflow at the bottom and one sediment depth with water from tubes with inflow at the bottom and from all sediment depths.

Two people conducted the Elk River sampling and these people consulted on almost each reading. A different person took readings in South Slough with a different YSI™ meter. South Slough and Elk River readings will not be comparable, but all readings within an estuary will be.

In the future, we favor using the syringe method, extracting soil from the depth(s) of interest. Experimentation with the catchment tubes will continue using more sophisticated equipment and soil from different depths. We also advocate measuring redox potential using the method described by Faulkner *et al.* (1989). In this method, many redox probes can be made in the lab by welding copper wire to strips of platinum and encasing these in shrink wrap or plastic pipettes. The probes can then be inserted into the ground at multiple locations and depths and left to equilibrate for as long as necessary. The probes can be returned to later for rapid measurement of the extent of platinum electrolysis with a redox meter.

9.0 SAMPLE CUSTODY

9.1 Sample Custody

Transport of samples in this project is limited to transport from the field to School of Fisheries storage areas and from storage areas to labs of appropriate staff for analysis. Detailed tracking of samples is not necessary; the following procedures assure that sample whereabouts are known.

1. All sampling and data generation in the field and subsequent analyses of field-collected samples are conducted by one team of investigators (WET) and one laboratory (WET facilities at the Fisheries Research Institute, University of Washington, Seattle, WA).
2. Sample collection and labeling is documented in field sampling logbooks and a daily inventory list of all samples collected is compiled and checked against the samples at the end of each day and site visit.
3. Sample labels containing site locations and code numbers, date of collection, name or initials of sample collector and the type of sample are added to samples in the field. A specific hierarchical code series was developed for each estuary, habitat, transect, grid and plot in order to guarantee that samples can be traced to each other and to data gathered on environmental conditions at the time of sampling.
4. All samples are returned to the WET laboratories at the University of Washington, where they are stored under secure (e.g., locked) conditions.
5. Archived specimens are maintained with either the WET laboratories (e.g., epibenthic zooplankton) or at the School of Fisheries (e.g., macroinvertebrates).

Table 3 describes containers, transport and storage of samples.

9.2 Sample Labeling

All sample labels are prepared before going into the field and contain the following information:

1. project acronym (e.g., WET RARE)
2. date
3. Site code
4. sample method and sample number (e.g. MB T1 #12 = Microbiota Transect 1, #12)

Sorted samples may also include additional labels (e.g., species, dead/live below-ground organic matter, etc.) Field books contain clear enumerations of site/sample codes.

10.0 CALIBRATION PROCEDURES AND FREQUENCY

All field instruments are calibrated in the laboratory prior to deployment in the field and recalibrated upon return to the laboratory in order to detect any changes from the pre-field calibration. The redox/pH meter is calibrated with solutions of known pH that are

provided by the manufacturing company. The YSI meter is borrowed from Ocean Tech Services at the UW School of Oceanography, that is responsible for servicing and maintaining equipment in good working condition. The Leitz Total System used for elevation data was borrowed from Geoline, (Bellevue, WA). The company provided training in the use of the equipment, guaranteeing precision of measurements, and is also responsible for maintaining equipment in good working condition. Extra batteries were carried in the field to assure that all equipment was operating with required power sources.

WET Lab and School of Fisheries scales are serviced regularly and scales are calibrated periodically with weights of known measure. The fluorometer is maintained by and calibrated before each use by passing a sample of known chlorophyll content (see "Objectives for Measurement, Precision, Microbiota").

Calibration for field estimation and sampling procedures is achieved through staff training prior to work in the field. Whenever possible, reference material is made available in the field and questions about plant identification cross-referenced to the University of Washington or Oregon State University Herbaria.

Table 4 describes the field and laboratory equipment associated with monitoring parameters.

11.0 ANALYTICAL PROCEDURES

Analytical procedures for parameters (i.e., benthic diatom standing stock, Table 1) determined through laboratory analyses are specifically described in the *Protocol.*

12.0 DATA REDUCTION, VALIDATION, AND REPORTING

12.1 Epibenthic Plankters and Benthic Infauna

Species identifications, counts, and standing stock for epibenthic and benthic meiofauna are entered in a hierarchical computer-coded form structure that provides direct data entry/retrieval into/out of a computer (relational) data base. The data structure is based upon, and whenever possible utilizes, the National Oceanographic Data Center (NODC) system for recording and archiving oceanographic records. The basic field data form was designed as a variant of NODC format #100, the Intertidal/Subtidal series of data records developed for research in Puget Sound. For instance, the WET laboratory uses a modification of the Species Identification Record (Record #4) for benthic organisms and has created a form for epibenthic and pelagic zooplankton that nests within

this data series. Data entry is performed by the Data Entry Services of the School of Fisheries, who have their own data checking procedures.

12.2 All Other Attributes

Units of measurement for all parameters are listed in Table 1. Field and laboratory data entry forms were designed uniquely for this project and are included in Appendix 1. Data is entered from field books into Microsoft Excel for Windows™, a spreadsheet format that has database capabilities.

One original and two backup copies of each file is always made. Two Xerox copies of field books and database print-outs are also made; one copy stored at the WET lab and one at the Center for Quantitative Science. Survey (elevation) data are stored in ASCII files with two backups for use in plotting software.

Validation of data entry is ensured and assessed by two methods: (1) the investigators who collect the data perform data entry to minimize illogical entries; and (2) computer printouts of data are cross -checked with the field/laboratory data sheets and scanned for out-of-range values by the Project Leader and the person responsible for data entry. If out of range values are encountered, laboratory samples can be reprocessed to insure that the value was not caused by measurement errors. If the "outlier" is valid or the result of a field estimation that can not be repeated, it is of interest to the project to investigate possible reasons for the outlying value. Outliers can be the most interesting data points, providing information about scales, factors, or types of measurement errors not assessed. In all cases, the reasons for the outlier would be investigated, and if no reason could be found, the value would be used as is, and qualifying statements would accompany data summaries or analyses. Interpolations of missing or outlying data points will rarely be used.

Statistical analysis and graphical illustration of data will be carried out with the aid of commercially-available computer programs. Simple graphs can be generated with Microsoft Excel for Windows™ directly from data retrieved from that database. For more sophisticated analyses and graphs data can be exported from Excel as ASCII files and imported into one of the more dedicated programs, such as Statgraphics™, SigmaPlot™, Graftool™, and Axum™. Most data files are relatively small and sample sizes for each parameter known, so that the effectiveness of file transfer programs will be readily apparent. Statistical tests to be performed have been described under the appropriate Methods sections.

13.0 INTERNAL QUALITY CONTROL CHECKS

Internal quality control checks are described in "Objectives for Measurement, Precision".

14.0 PERFORMANCE AND SYSTEM AUDITS

The QA staff of the Environmental Research Laboratory-Corvallis performed a technical systems audit (TSA) of this project in September 1992. TSAs are conducted prior to or concurrent with initial data collection activities to: (1) familiarize the project's staff with EPA QA requirements and procedures; (2) evaluate the implementation of the QA activities as specified in this document; and (3) provide assistance in attaining the objective to collect data of known and documented quality.

15.0 PREVENTIVE MAINTENANCE

Equipment maintenance and calibration is described under "Calibration Procedures and Frequency". Duplicate equipment is always carried, for ALL methods, to be used in the event of equipment breakage or loss. Only rented equipment is not carried in duplicate. For those parameters requiring meters or pumps that could break, equipment was serviced before going in the field, sampling was conducted on only one sampling trip, and equipment was serviced again on return from the sampling trip.

16.0 SPECIFIC ROUTINE PROCEDURES USED TO ASSESS DATA PRECISION ACCURACY, AND COMPLETENESS

Precision, accuracy, and completeness, will be assessed in the field and the lab as described as in earlier sections (see, especially "Methods").

16.1 Precision

Precision is defined, here, as standard error (SE) about the sampling population mean. When possible, we will try to distinguish the two components of this variability, natural variability in the distribution of the attribute population and sampling error (e.g., variability due to estimation techniques).

Boot-strapping methods can be used to generate distributions of data and investigate variability as related to sample size. In some cases bootstraps can be performed using existing data. In other cases, it may be more meaningful to generate a

distribution that fits the data at hand and sample from that distribution. The appropriateness of each method will be assessed as we explore the data gathered. In these cases, desired sample size desired will be that required to detect differences between means equal to the mean or one half the mean (at $\alpha(2) = .05$, $\beta(1) = .1$) for the attribute and species of interest. The procedures for such power analyses will use the methods in Zar (1894) for two sample t tests and ANOVA.

$Var_{total}(x) = Var_{nat}(x) + Var_{meas}(x)$

$Var_{nat}(x)$ = Natural variability of x
$Var_{meas}(x)$ = Measurement error of x

$Var(x) = \Sigma(Xi - \bar{X})2/(n-1)$
calculated as: $\Sigma X_i^2 - (\Sigma X_i)^2/n)$

Xi - = value of ith measurement
$\bar{X}$ = mean of all measurements
n = sample size

Normal SE = sqrt(Var(x)/n)
Binomial SE = $\hat{p}\hat{q}/(n-1)$

$\hat{p}$ = estimated proportion
$\hat{q} = 1 - \hat{p}$

16.2 Accuracy

In our project, accuracy criteria can be applied to the calibration of instruments the taxonomic identification of species, and blank processing using the fluorometer (see previous section on accuracy under "Data Quality Objectives"). No accuracy evaluations are possible for parameters such as percent cover of rooted vascular plants. Accuracy will be assessed, respectively, as product specifications for instruments, percentages of specimens properly identified, divergence (bias) of reading from known values.

16.3 Bias

Bias will be calculated as both the absolute and the percentage deviation of the measurement/processing estimate from a known reference sample (both B=X-T and B=100 (X-T)/T). Relative bias of different sampling methods will be calculated as percentage of positive and negative estimates relative to another method (B= number high/total number of samples), magnitude of maximum difference between two methods (max (X_1-X_2)).

16.4 Completeness

Completeness of sampling efforts, laboratory analysis, and data analysis will be assessed as the ratio of the number of data intended versus the number actually completed. Because much of our data gathering is exploratory, if the full sampling effort does not achieve desired precision levels, additional required sampling will be recommended.

16.5 Representativeness

See above under "Data Quality Objectives - Representativeness".

16.6 Comparability

See above under "Data Quality Objectives -Comparability". The *Protocol* specifically recommends that all monitoring procedures be deployed such that the data generated are maximally comparable, and are standardized. For each attribute group (e.g., monitoring parameter), data were gathered from the different estuarine locations and habitats using exactly the same procedures. In addition, all data were standardized to common scientific dimensional (e.g., area, volume) references (e.g., grams wet m^{-2}). When measurement error could not be tested, it was controlled by training and methods described in "Sampling Procedures". Because the methods for gathering data for each parameter are standardized, the validity of comparisons will be based on biological interest in the comparison.

16.7 System Error

System error is not an appropriate criterion for the purposes of this QA/QC project. In this case, locating and correcting errors in specific procedures and estimates will be the focus of this effort.

17.0 CORRECTIVE ACTIONS

All deviations in accuracy or other quality indices discovered in quality control checks will be investigated by the Project Leader (epibenthos, benthic infauna) or the Statistical Consultant (all other attributes). The error source and the extent of affected samples will be determined. If a directly translatable error can be identified (e.g., calling species X species Z), all prior data will be corrected and spot checks of these samples will be conducted to verify that the correction is proper. If a non-specific error is found, 25% of the prior samples will be examined to determine the extent of the error. If more than 10% of these samples illustrate the error, all samples will be reprocessed.

18.0 QUALITY ASSURANCE REPORTS (TO MANAGEMENT)

A final report is being submitted to EPA, including the results of all internal quality control checks, documentation of accuracy and precision determinations, corrective actions implemented if required, and other problems encountered that potentially affected data quality.

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Table 1 Parameters proposed for selective testing of Estuarine Habitat Assessment Protocol; see QA Plan Appendix A for full description of methodology; NA = no "true values" available for these parameters. Accuracy = Accuracy of Instrumentation Precision = precision required on repeated measurement

Functional Attribute	Attributes	Parameter	Units of Measurement	Accuracy/ Precision	Protocol page
Site Establishment & characterization		elevation relative to Mean Water	ft relative to benchmark	accuracy, 0.01 ft.; precision, 0.03 ft. on "closing"	
		porewater salinity	ppt	YSI precision, 0.1; refractometer, 1.0	
		redox	millivolts	precision, 0.1	
Rooted Vascular Plants (Emergent Marsh Vegetation)	(approximately 40 plant asseemblages; see final Protocol, p. 42)	percent cover	% / sampling unit	accuracy, NA; precision, to nearest whole number	41-44
		above-ground biomass/standing stock	g dry wt/m^2	accuracy, 1 mg; precision, 5%	43
		below-ground biomass/standing stock			44
Benthic microbiota	benthic/epiphytic algae (diatoms)	standing stock	mg/m^2	accuracy, 0.1 mg; precision, 1%	49-50
Sedentary Infauna	*Manayunkia aestuarina* *Macoma* spp *Mya arenaria* *Neanthes limnicola* *Tanais* spp. *Transenella tantilla*	density	no./m2	accuracy, NA; precision, 1%	61-62
		standing stock	g dry wt/m^2	accuracy, NA; precision, 2%	61-62
Epibenthic Plankters	*Corophium* spp *Eogammarus confervicolus* *Cumella vulgaris*	density	no./m^2	accuracy, NA; prec. -subsampling, 5%, -sorting, 2%, -counting, 1%	71-72
		standing stock	g wet (preserved/ m^2	accuracy, 1 mg; prec.-subsampling, 5%, -sorting, 2%, -weighing, 5%	71-72

% ovigerous females	% total density	accuracy, NA; prec. -subsampling, 5%, -sorting, 1%, -counting, 1%	71-72

Table 2 Sample design and sample sizes, by site and stratum for selected attributes (see text for explanation of methods).

Site	Vertical transect 1 (all strata)	2	3	25x10m plot (hm)	3x5 m plot (hm)	Visual Estimate (hm)	Horizontal transect 40m (mud)	50m (hm)	160m (mud)
ELK1	EL 8 AG 8 BG 8 PW	EL 15 AG 15 BG 15 PW	EL	104 RPQ 24 AG			20 EPI 20 MEI 10 MB 20 pH 20 RED 20 T		
ELK2	EL 5 AG 5 BG 5 PW	EL 7 AG 7 BG 7 PW	EL 7 AG 7 BG 7 PW	104 RPQ 24 AG					80 EPI 80 MEI 20 MB 20 pH 20 RED 20 T
ELK3	EL 7 AG 7 BG 7 PW	EL 3 AG 3 BG 3 PW	EL 7 AG 7 BG 7 PW	104 RPQ 12 AG					
SS1	EL 10 AG 10 BG 10 PW	EL 15 AG 15 BG 15 PW	EL	104 RPQ 24 AG			20 EPI 20 MEI 10 MB 20 pH 20 RED 20 T		
SS2	EL	EL 8 AG 8 BG 8 PW	EL 8 AG 8 BG 8 PW	104 RPQ 24 AG 24 BG 24 PW	67 RPQ(a) 220 RPQ(r)	24 quad ; (teams) 5 quad; (repeat meas)		RPQ (syst)	80 EPI 80 MEI 20 MB
SS3	EL	EL 8 AG 8 BG 8 PW	EL	104 RPQ 24 AG					

AG = Above-ground standing stock
BG = Below-ground standing stock
RPQ = random point quadrat (% cover veg)
hm = high marsh
EL = Elevation
EPI = Epibenthos
(a) = all species
mud = mudflat
MEI = Meiofauna
MB = Microbiota
(r) = rare species
repeat meas = 3 sizes, 4 people, 3-4 reps (% cover veg)
PW = Porewater
RED = Redox potential
T = Temperature

Table 3 Transport and storage of samples

Functional Attribute	Parameter	Container	Transport	Storage
Site Establishment & characterization	elevation porewater salinity redox potential	NA	NA	NA
Rooted Vascular Plants	percent cover	NA	NA	NA
	above ground biomass	12"x24" plastic bags	ice chests	immediately sorted and dried
	below ground biomass	12"x224" plastic bags	ice chests	immediately sorted and dried
Benthic microbiota	standing stock	darkened glass or plastic jars	ice chests	frozen until analysis
Sedentary Infauna	density standing stock	16 oz. pvc jars, buffered formalin	buckets boxes	shelved at Fisheries until analysis
Epibenthic Plankters	density standing stock % ovigerous females	8 oz. pvc jars buffered formalin buffered formalin	buckets boxes	shelved at Fisheries until analysis

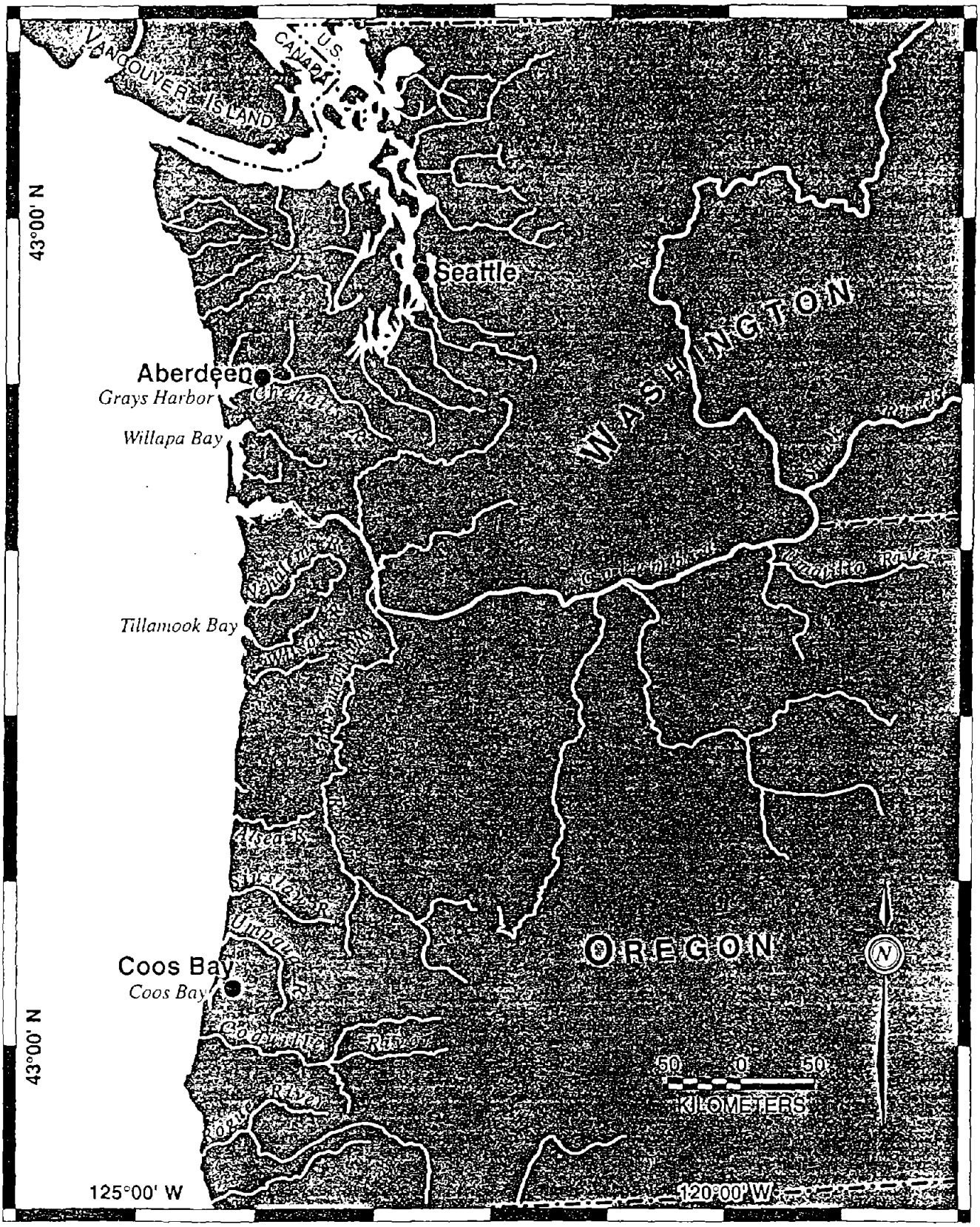
Table 4 Lab and field equipment for measuring parameters

Functional Attribute	Parameter	Field Equipment	Lab Equipment
Site Establishment & characterization	elevation	Leitz Total Station	Sokkia software
	porewater salinity	YSI meter, refractometer	NA
	redox potential	Beckkmann pH redox meter (PHI 11) with Fisher no. 13-620-82 electrode	NA
Rooted Vascular Plants (Emergent Marsh Vegetation)	percent cover	visual estimates in benthic quadrats; 50 m tape measures for transects and delineating areas, wooden stakes, thin rod	NA
	above-ground biomass	.125 m^2 quadrat	Metler top-loading analytic balance
	below ground biomass	1.25" pvc core, 20 inches long	Metler top-loading analytic balance
Benthic microbiota	standing stock	.75" plastic syringe	Turner 111 flourometer
Sedentary Infauna	density	1.25" pvc core, 20 inches long	dissecting microscope
	standing stock	1.25" pvc core, 20 inches long	Metler top-loading analytic balance
Epibenthic Plankters	density		dissecting microscope
	standing stock	epibenthic suction pump	Metler top-loading analytic balance
	% ovigerous females		dissecting microscope

Table 5 Methods of assuring precision, accuracy, and completeness of Protocol assessment parameters; RPQ = random point quadrat, wrt = with respect to.

Parameters	Precision	Accuracy	Completeness
Taxonomic id Epibenthic plankters, benthic meiofauna, % ovigerous females	mean overlap in two independent assessment	Id's checked by Project Leader	% samples completed
Standing stock Epibenthic plankters benthic meiofauna above-ground biomass below-ground biomass	mean difference in 2 independent sample counts applied to 5% of samples	NA	% samples completed
Percent Cover Rooted vascular plants	**RPQ:** binomial CI's, vary sampling density **Visual**: test for team effects (ANOVA); mean, std dev of individual measurement error, wrt quadrat size, species, individual; # hi, low estimations/individual **Scale**: compare CI's wrt to constant n, constant sampling effort (time, cost); Time series, Markov chain, Carlile et.al.	NA	% samples completed

Figure 1. Locator map of estuaries sampled: Grays Harbor, WA and South Slough, Coos Bay, OR



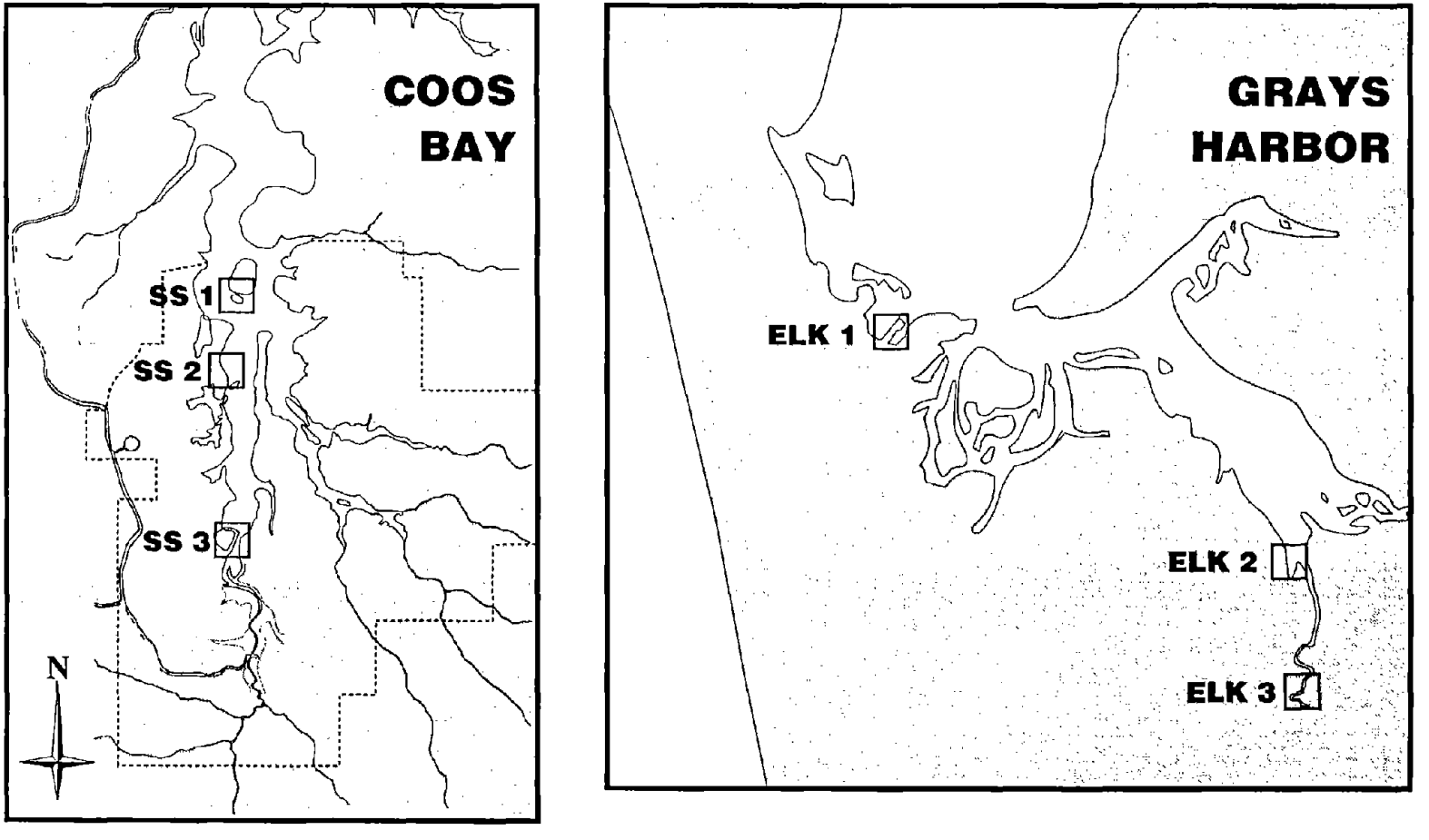
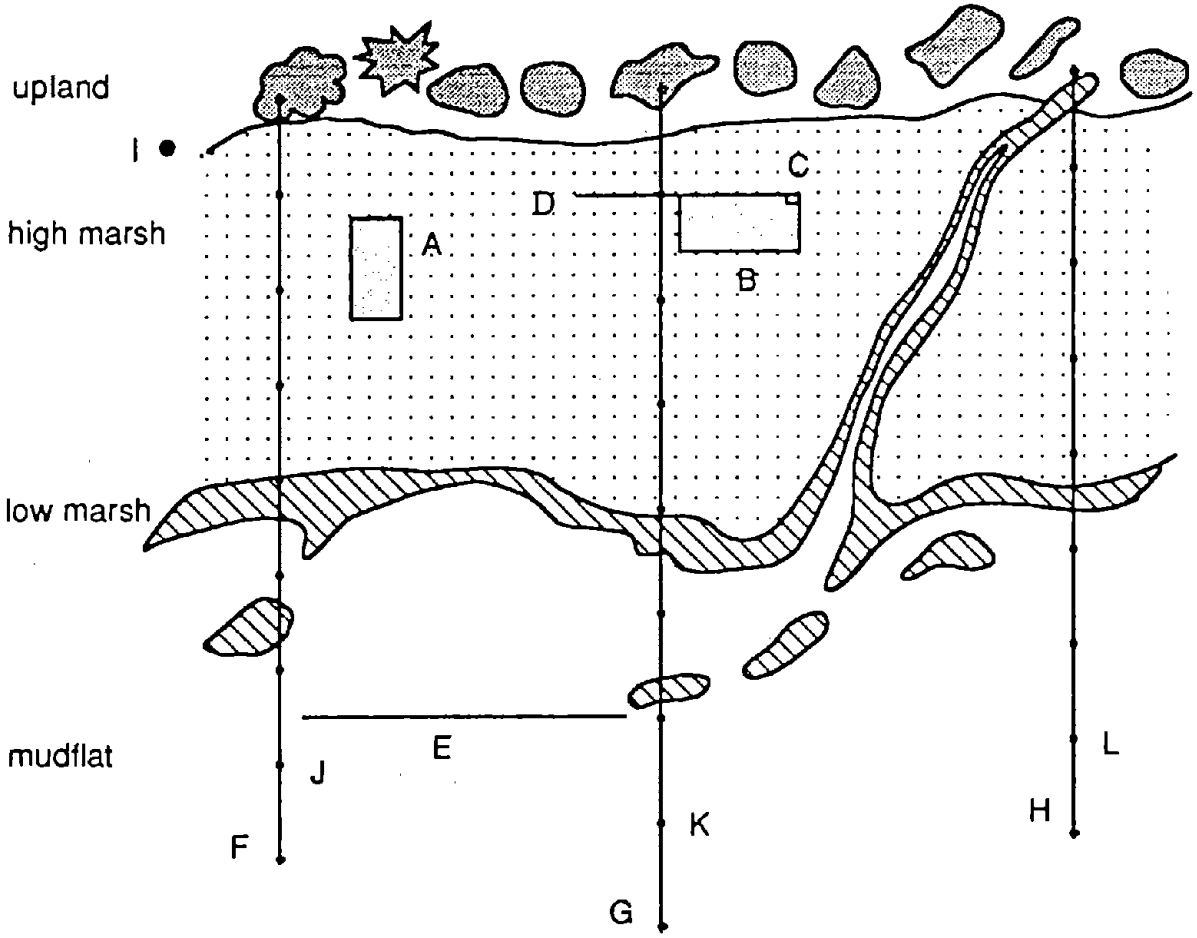


Figure 2. Map of three gradient sites in South Slough and Elk River

Figure 3. : Example of a wetland site with strata, sampling transects, and sampling plots



A, B	25 x 10 m RPQ sampling plots	F,G, H	vertical elevationtransect
C	3 x 4 m RPQ sampling plot	J, K, L	topo point
D, E	horizontal sampling transect	I	control point

20.0 APPENDICES

20.1 Appendix A: Sample field and laboratory data sheets

A. Rpq sampling at 5 cm interval along horizontal transect

B. Visual estimates of % cover emergent vegetation by teams

C. RPQ sampling at random x,y coordinates in 25 x 10 m area

D. Repeat visual estimates of % cover emergent vegetation by individuals

E. pH, redox, temperature readings along horizontal transect

F. Pore water readings along elevation transect, different methods

G. Above-ground standing stock by species; laboratory weights of sample with aluminum foil

H. Epibenthos, Meiofauna

I. Microbiota Flourescence

TECHNICAL REPORT DATA
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16. ABSTRACT

Many estuarine wetland scientists and managers are of the opinion that present wetland habitat assessment procedures are inadequate for application to specific geographic regions and may be too subjective to provide consistent results. This is particularly the case in Pacific Northwest estuaries, where wetlands are structurally and functionally different from southeast and Gulf coast estuaries, for which most assessment approaches were originally developed. To increase the effectiveness of our management and conservation of estaurine habitats, we need assessment and monitoring procedures that: (1) are based explicitly on habitat function; (2) are specific to the region of applications; (3) use methods that are standardized, consistent and comparable; (4) generate quantitative data rather than qualitative indices; (5) are designed to be thoroughly objective among different users and sites; (6) will be adaptive in terms of building on prior results; and (7) are structured in a flexible form wherein both the biotic community and target resources can be addresses. This report represents such an approach to assessing the function of estuarine habitats for fish and wildlife, and specifically for the Pacific Northwest region. Habitat functions, such as maintenance of water quality or flood desynchronization, should be assessed with similar rigor. The report is intended to address the need for a systematic procedure that can be applied uniformly across a variety of wetland and associated nearshore habitats using objective, scientific methods. The approach is directly applicable for the study of natural wetland systems and in evaluating compensatory mitigation projects in estuarine habitats. It also has the potential to facilitate the development of design criteria for estuarine habitat restoration.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Wetlands, Quality assurance, Wetland assessment, Pacific Northwest, Estuarine wetlands.		

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