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Estuarine Habitat Assessment Protocol

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ESTUARINE HABITAT ASSESSMENT PROTOCOL

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EXECUTIVE SUMMARY

The **Estuarine Habitat Assessment Protocol** (hereafter termed "the Protocol") is the product of a concept that emerged in the *ad hoc* Urbanized Estuary Mitigation Working Group (UEMWG*) and was adapted and completed by the Wetland Ecosystem Team (W.E.T.) of the Fisheries Research Institute, University of Washington, for the U.S. Environmental Protection Agency's Office of Puget Sound and Wetland Program.

The Protocol was developed in response to the need for procedures that quantitatively assess the function of estuarine wetlands and associated nearshore habitats for fish and wildlife. While all the various functions of estuarine wetlands and nearshore habitats need better identification and documentation, we felt it imperative to restrict the Protocol to one prominent function in terms of regulatory emphasis in Pacific Northwest estuaries: use as habitat by fish and wildlife species. We trust that protocols for assessing other functions and for freshwater wetlands will be developed in the future, perhaps following the same process.

The functional approach was taken in preference to simply assessing fish and wildlife use because the latter, usual approach to habitat assessment does not measure habitat quality directly, nor does it identify the characteristics of the habitat that *explicitly* promote fish and wildlife utilization. Thus far, a systematic approach for measuring the function of estuarine wetlands and nearshore habitats that considers these factors has not been available. Consequently, restoration and creation of estuarine habitats have proceeded haphazardly. Without such procedures, the scientific knowledge required to link the characteristics of estuarine habitats to their functions in support of fish and wildlife will not emerge. In the absence of this critical understanding, the present uncertainty inherent in the emerging technology of habitat restoration and creation will not be resolved, and we will not progress toward a goal of minimizing future habitat loss. The approach of the Protocol, and of the procedures and measurements it recommends, is intended to bridge this gap between estuarine habitat characteristics and the habitat's function to support fish and wildlife.

Fish and wildlife utilization of estuarine wetlands and nearshore habitats *per se* continues to be extremely difficult and expensive to document. Any reliable assessment methodology must, therefore, measure characteristics (termed attributes in the Protocol) of estuarine habitats that promote fish and wildlife utilization and fitness. This Protocol is not intended to supplant monitoring of the fish and wildlife themselves, which can often be a direct measure of habitat function. Instead, it should be considered a supportive measure of the *potential* to provide a specific function which, unlike utilization information, provides design criteria for habitat restoration.

The goal of the Protocol is to initiate systematic, on-site measurement of estuarine wetland and nearshore habitat function for fish and wildlife utilization *by assessing the attributes of the habitats identified as being functionally important to fish and wildlife.* The Protocol is intended as a supplement, not a replacement, to more comprehensive but basically qualitative procedures for assessing overall habitat quality (e.g., Wetland Evaluation Technique [WET], Habitat Evaluation Procedure [HEP]). As such, it could be looked upon as a higher resolution extension of the fish and wildlife function component of those procedures. In addition, it should not be used as a methodology for assessing other habitat functions (e.g., those that maintain water quality). Finally, as a *strictly scientific tool*, the Protocol does not promote a procedure for attaching "values" to wetlands and associated habitats, which we consider to be the purview of policy and

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*Terms that are defined in the Glossary are underlined at their first occurrence.

management disciplines. Probably the single aspect of the Protocol which differentiates it from these other approaches is that it is designed to accrue information for developing more successful approaches to habitat restoration.

The Protocol is organized so that it can be accessed to answer a particular question from three different perspectives: habitat type, fish and wildlife assemblage species, and attribute. Definitions of habitat types, the fish and wildlife species considered representative of each habitat type, and the attributes of the habitats that are considered important to these fish and wildlife are presented and cross-referenced so that the investigator can determine which parameters and methods are most appropriate to a particular situation. Basic concepts of sampling theory that are pertinent to estuarine wetland and nearshore habitats are also reviewed to assist the investigator in deploying sampling in a statistically valid design. Using these considerations, the endpoint of the Protocol's use should be an appropriate monitoring plan for assessing estuarine habitat *function* from any of these perspectives.

The separately bound supplements (Simenstad et al. 1990) that accompany this document include the following: (1) the result of the initial surveys (matrices) that guided the Protocol development; (2) an annotated bibliography assembled from material provided by the survey respondents and a computerized literature search; (3) individual, detailed descriptions of the attributes; (4) citations of literature that provide detailed information about the attributes; and (5) a list of the resource management agency personnel and other scientific experts who contributed to the development of the Protocol.

Under separate programs, the U.S. Environmental Protection Agency is supporting the preparation of other documents and products that will also contribute to the objective of the Protocol: (1) a computerized estuarine habitat database in R:Base format and (2) a computer software version of the Protocol that guides a habitat manager to the appropriate procedure for assessing their particular habitat.

In examining the results of this edition of the **Estuarine Habitat Assessment Protocol**, we reinforce the concern that further attention must be given to evaluations of habitat function and performance not included in the Protocol, which is restricted solely to the assessment of the fish and wildlife support function of estuarine wetland and nearshore habitats. Similar assessment protocols need to be developed for other wetland functions (e.g., shoreline stabilization, water quality) and for non-estuarine (e.g., riparian, palustrine, lacustrine) wetland habitats. We also strongly recommend the testing and verification of the Protocol in restoration and mitigation sites as well as in the natural estuarine wetland and nearshore habitats upon which it was based. For testing at natural sites, "reference" wetland sites need to be established for long-term monitoring of the more important, or potentially most inherently variable, estuarine habitat attributes.

Finally, the Protocol is designed as a "living" management tool, in the sense that it was developed using open-ended matrices and databases that can be expanded and attributes identified from new studies can be incorporated into future versions. Once the Protocol has been tested and applied in a variety of situations, the gaps and inadequacies that emerge should be addressed by ardent revision. This would preferably be done via a workshop similar to the Wetland Restoration Protocol Development Workshop, which was a critical step in the development of this edition.

Key words: *estuarine habitat, wetlands, monitoring protocols, fish and wildlife*

INTRODUCTION

> Ecosystem restoration is an activity at which everyone wins: when successful, we are rewarded by having returned a fragment of the earth's surface to its former state; when we fail, we learn an immense amount about how ecosystems work, *provided we are able to determine why the failure occurred.*
>
> Ewel (1987)

In environmental science, *protocols* are "formulas defined at the beginning of a preliminary document." Two words of this definition are most germane to sampling and analyzing environmental data from Puget Sound: *formulas* and *preliminary*. In this instance, "formulas" mean standardized, scientifically based, consistent procedures. As formulas, protocols are critical to any long-term evaluation of the state of a system, especially one as complex as Puget Sound. Only through the accumulation of common equivalent data can the results of studies be compared and a historic time sequence examined for trends that indicate declines or establish improvements in environmental quality. Protocols must also must be considered "preliminary" because the acquisition of information using protocols should result in revision and refinement of the original procedures. In sampling theory, a protocol is analogous to the sampling frame, which is a list of all sampling units (see Sampling Design section) that logically changes with sampling experience.

That the **Estuarine Habitat Assessment Protocol** (hereafter known as the "Protocol") is preliminary has two implications. First, it describes procedures that have been evaluated and generally accepted, typically through expert consensus, *prior* to a study. Second, the procedures are not mandated but advisory, and it is accepted that they will be revised as newer, more effective tools evolve. Ewel's quote at the beginning of this introduction, and especially the italicized segment, summarizes our rationale: *Neither the science nor the technology of estuarine habitat restoration can progress until we treat restoration as rigorous experimentation in which consistent data are gathered in a inductive format.*

The Protocol applies only to the *functional assessment of fish and wildlife support in estuarine wetlands and certain adjacent habitats of the Puget Sound trough.* Although the information used to develop the Protocol was derived from natural estuarine habitats, it emphasizes evaluation of restoration in disturbed estuaries, such as the many urbanized estuaries that surround Puget Sound. Therefore, the Protocol is designed (1) to assess the function to support fish and wildlife and (2) to monitor the comparative performance of the site after restoration or of a mitigation site designed to replace the development site. The information base of the Protocol was assembled by identifying the attributes of estuarine habitats that promote high use by fish and wildlife; these attributes form the focus of the Protocol.

The sections immediately following provide a detailed discussion of the impetus and development, the intended purpose and application, and the structure of the Protocol. This may not be of direct interest to the reader whose immediate need is to apply the Protocol to a particular situation; a quick perusal of the rest of the Introduction, however, will better acquaint the reader with the logic and philosophy behind the Protocol. We recommend that you read the Utility and Limitations section in order to understand the Protocol's limitations. Using the Protocol is a road map to the body of the Protocol. Finally, readers who are interested in the concept of this approach to assessing estuarine habitat function is directed to the Discussion section, which addresses several aspects of the implementation, testing, and enhancement of this approach.

In addition to this document, Simenstad et al. (1990) provide eight supplemental materials that constitute the basis of the Protocol. These include the following: (1) the result of the initial surveys (matrices) that guided the Protocol development; (2) an annotated bibliography assembled from material provided by the survey respondents and a computerized literature search; (3) individual, detailed descriptions of the biological attributes; (4) citations of literature with specific information on the attributes; and (5) a list of resource management agency personnel and other scientific experts who contributed to the development of the Protocol. These materials are reproduced in their original form and do not contain taxonomic and other corrections that have been incorporated subsequently into the Protocol.

STATEMENT OF PROBLEM

The demand for and pace of wetland creation/restoration appears to be accelerating beyond our abilities to either technically evaluate or effectively manage it (Zedler 1986). In a recent analysis of 35 projects in Washington receiving wetland development permits requiring mitigation (<5% of all Clean Water Act Section 404 permits), Kunz et al. (1988) documented that as a best possible case, on the basis of acreage, *only 67% of the natural wetlands that were developed were replaced by created/restored wetlands, and only 68% of the wetland habitat types lost were replaced.* The Kunz et al. (1988) study did not evaluate the functional status of the creation/restoration sites, but Kusler et al. (1988) has documented on the national level that, where the degree of wetland creation/restoration has been measured over the short term, about half have failed in some respect.

However, mitigation is only a policy tool designed to prevent continued loss of wetland resources. Mitigation does not inherently prescribe the optimum approaches to achieving this end, nor does it necessarily generate information useful for wetland habitat restoration. Kunz et al. (1988) illustrated that pervasive inadequacies in the negotiation, planning, and enforcement on the part of agency administration of Section 404 of the Clean Water Act have resulted in dramatic mismatches between the habitats impacted and those generated as their "replacements."

While the Kunz et al. (1988) and subsequent updates (Rylko and Storm 1991) identified primarily the administrative and policy faults inhibiting successful mitigation, two technical problems also lie at the root of the problem: (1) a lack of technical knowledge of the structure of Pacific Northwest wetlands and how they function; and (2) an inability of regulators and managers to uniformly assess mitigation projects and their outcome (Cooper 1987; Kunz et al. 1988). These inadequacies impede both habitat restoration and enhancement and are particularly germane to estuarine habitats, because most of the considerable development pressure is focused on the remaining wetland habitats in Puget Sound and other coastal estuaries of Washington and Oregon (Cooper 1987, Good 1987). Of primary concern is the dependence of many commercially and recreationally important fish and wildlife resources upon estuarine habitats. The Protocol is a technical approach to standardize techniques for quantitatively assessing estuarine habitat quality specific to functions enhancing fish and wildlife utilization.

At the core of the mismatch between the pressures to develop estuarine habitats and our meager track record in habitat restoration/creation is the incomplete understanding of natural estuarine ecosystems in this region and how they might be created in a manner so as to function naturally. However, in both cases, an important contributing cause is the lack of uniform procedures, or protocols, for objectively assessing habitat function. Protocols are critical for the evaluation of the functional status of estuarine wetland and nearshore habitats proposed for development, as well as for the subsequent monitoring of compensatory habitats to evaluate stated project objectives. This lack of standardized, objective protocols has resulted in (1) habitat assessments that inadequately, inaccurately or inappropriately quantify habitat functions; (2) the slow emergence of design criteria from the results of past mitigation projects; (3) inconclusive assessments of mitigation results due to inadequate monitoring; and (4) a lack of identifiable remedial actions to take if a mitigation site does not meet predetermined criteria.

Habitat restoration should extend beyond the mitigation associated with permit processes, which are limited in number and scope and constrained by regulatory mandates. This broader interest in habitat restoration has resulted in the inclusion of a habitat restoration component in the 1991 Puget Sound Water Quality Management Plan (PSWQAMMC 1991) and has instituted a variety of public, governmental, and private efforts toward estuarine habitat enhancement not directly related to compensation.

At the time of preparing this Protocol, there was no broadly accepted set of procedures for monitoring estuarine habitats to evaluate fish and wildlife utilization, nor did any advocate collecting quantitative data. The quantitative data that were available usually documented abundance and infrequently biomass. Time series of density and standing stock, and actual functional variables such as reproduction and feeding, were rare and not measured consistently. The Urbanized Estuary Mitigation Working Group (UEMWG, see Protocol Development section) identified five principal problems associated with present estuarine habitat monitoring: (1) monitoring criteria were not applied uniformly or consistently among projects; (2) data were not rigorously quantitative, if quantitative at all; (3) the focus on target species ignored the more complex ecological relationships among the other components of the community and the physical characteristics and processes of the habitat; (4) the interpretation of the results from these diverse approaches was highly subjective; and (5) criteria for design of habitat restoration projects were not being tested and refined. Therefore, the UEMWG determined that the principal need was to identify quantitative procedures to monitor the functional relationship between fish and wildlife and the other components, biological and physical, of estuarine wetland and nearshore habitats.

OBJECTIVE

The objective of the Protocol is to describe and recommend techniques for quantitatively measuring attributes of estuarine habitats that characterize the potential ecological function of that habitat for fish and wildlife.

We direct this objective to estuarine habitat managers, planners, consultants, and applied scientists who require a community-based procedure to assess estuarine habitat function and quality relative to restoration and mitigation of fish and wildlife habitat. In doing so, we have assumed that a common approach has definite benefits. Habitat managers would benefit from the uniform format of habitat assessments they review in their permit evaluations. Planners would have at hand a rapidly accumulating baseline of the design elements that did or did not create functioning fish and wildlife habitat. Developers should find the process of permit preparation, review, and approval more cost efficient and objective. Scientists will benefit considerably from the acquisition of data that have scientific value in interpreting habitat function and in comparing both applied and basic research.

The Protocol, however, is not the panacea for all problems faced in estuarine habitat management. It is *not* intended to culminate in a single, comprehensive determination of habitat "value" that would encourage comparison of different habitat types. It does not address flood desynchronization, groundwater recharge, or any other functions outside those directly applicable to fish and wildlife habitat and food chain support. It does not account for all attributes of Pacific Northwest estuarine habitats, nor does it constitute the final or only methodology that might be used for assessing estuarine habitat function in this region.

Estuarine management demands technically sound decisions regarding the relative quantities (i.e., habitat balance) that would be desirable in any given estuary. However, the Protocol is based upon the science of estuarine ecology as we understand it for this region, not on policy considerations. We have intentionally avoided the strategy of assigning habitat value because we find no ecological basis for ranking one habitat higher than another. Each habitat contributes to the estuarine ecosystem in a unique way, supports discrete fish and wildlife, and varies in its ability to generate, assimilate, or process nutrients, organic matter, and sediments. Furthermore, estuarine habitats are linked integrally through pathways of materials (the transport of nutrients, detritus) and organisms (the movements of fish, macroinvertebrates, mammals, birds); they should never be considered as disjunct communities. The natural continuum of habitats across an estuary must be preserved or restored if we are to maintain and eventually restore estuarine habitat function for fish and wildlife. For this reason, any decisions relative to habitat value, if they must be made, belong in the realm of policy and management and outside the scientific scope of the Protocol.

Physical and biochemical processes (and their associated wetland functions) often regulate ecological functions. It is probably a truism that most failures of estuarine mitigation projects can ultimately be attributed to disfunction of physical and biochemical processes upon which biotic processes are dependent. Unfortunately, we do not have the resources to address the physical and biochemical functions at the same level as we have addressed the biotic. (We recommend that the reader pursue more information in these respects by reading Chapters 3 and 4 of Strickland 1986). Instead, we have sought to distinguish the physical and biochemical conditions that are required by the biotic attributes of estuarine habitats, and trust that these are the more important indicators of abiotic functions.

PROTOCOL DEVELOPMENT

In 1988, Wetland Ecosystem Team (WET; Fisheries Research Institute, University of Washington) proposed that the UEMWG objectives be expanded to include the development of an estuarine habitat restoration protocol (Fig. 1). The expansion was funded by the EPA, Region 10 Wetland Protection Program, and EPA's Office of Puget Sound. The Port of Seattle also contributed resources.

The initial goals of the UEMWG were to improve predictability in the mitigation planning and review process, and to increase the success of mitigation projects in urbanized estuaries. From the beginning, the UEMWG confined its activities to technical aspects of implementing measures for compensation of fish and wildlife habitat lost due to development activity. As a result, its discussions did not consider mitigation policy. Any products of the UEMWG, therefore, were considered tools to be used in a broader management framework for estuarine habitat restoration.

In addition, the development of the fish and wildlife assemblages upon which the final protocol is based was restricted to regional information and data because the Protocol was designed to be applied to Puget Sound and its various "sub-estuaries." In identifying and describing what we know about the attributes, however, information from estuaries outside Puget Sound was considered when local data were otherwise unavailable. In this case, the geographic limitation was northern California to British Columbia, Canada. The data associating the assemblage species with wetland and nearshore habitat attributes originated from regional resource scientists and our combined knowledge of the applicable scientific literature. We acknowledge that this emphasis on empirical relationships has the advantage of being based on "hard" scientific evidence. However, a disadvantage is that it does not encompass subjective associations, which may represent an important but undocumented relationship.

The simple presence of fish and wildlife in a habitat does not necessarily constitute utilization *per se* and does not quantify the causal association between attributes of the habitat and the function of the habitat to support fish and wildlife. The strategy used to develop the Protocol was to *define important habitat attributes*. This attribute definition involved identifying biological and physical attributes of estuarine wetlands and nearshore habitats that determine the extent of fish and wildlife utilization. In addition, by building the structure of the Protocol around matrices associating fish and wildlife species (e.g., columns) with the functional attributes of the habitat (e.g., rows), an attribute could be identified as being important to the biotic community overall,

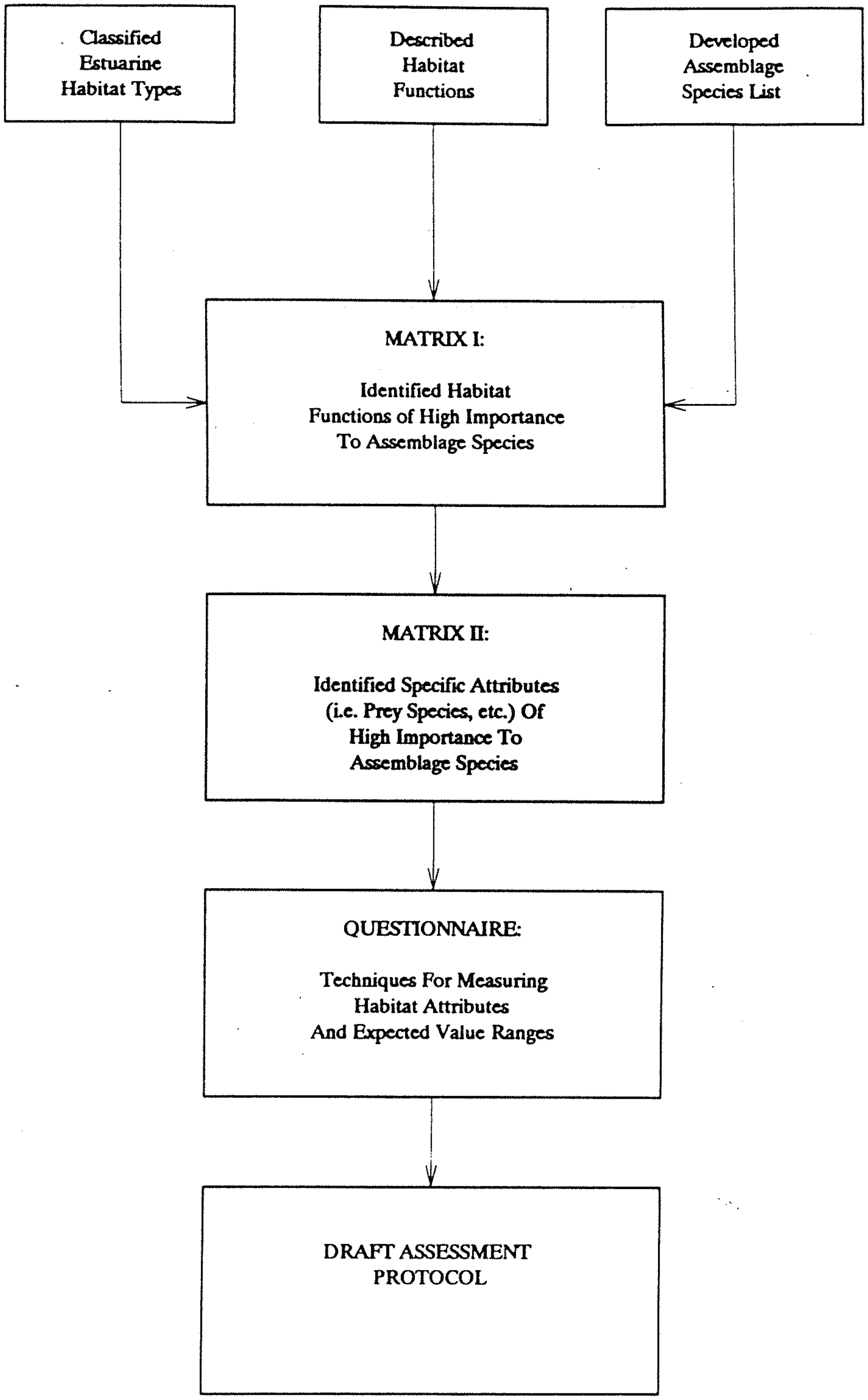

Figure 1. Flowchart of the sequence and interrelationships among the various processes in developing the structure of the **Estuarine Habitat Assessment Protocol**.

rather than just to a single target species. It then followed that incorporating such an attribute in a habitat restoration and mitigation design would increase the utility of the habitat for the maximum number of fish and wildlife species.

DESIGN

A detailed process was devised to categorize estuarine habitats, the fish and wildlife assemblages that typify them, and the attributes of those wetland that are functionally important to the fish and wildlife fauna (Fig. 1). Each of the following stages of the process is described in detail in the following section. The first stage of this process involved defining eight estuarine habitat categories. The next stage involved identifying fish and wildlife species that are considered representative of these habitats. This was not intended to result in a comprehensive list, but one which encompassed the fish and wildlife niches evident in each of the eight habitats, thus representing the overall scope of fish and wildlife use in the respective estuarine habitats. The next stage in the process of designing the Protocol employed a hierarchical sequence of increasingly specific matrices in which wetland and nearshore habitat attributes were ultimately ranked according to their functional importance. Fish and wildlife support functions were defined as (1) reproduction, (2) feeding and related activities (e.g., "graveling" of waterfowl), and (3) refuge and physiology (the use of habitats to adapt to physiological changes). These definitions are intended to include *both* the "fish and wildlife habitat" and "food web support" (exclusively for fish and wildlife) functions of estuarine habitats. Two matrices were developed. The first (Matrix I) ranked habitat functions that were important to specific fish and wildlife assemblages. The second (Matrix II) ranked the importance of specific attributes that accounted for these functions. The last task involved using the results of Matrix II to generate a questionnaire for quantitative data and associated information on the highest-ranked attributes.

CLASSIFICATION OF ESTUARINE HABITATS

Because of the dynamic nature of estuaries, estuarine habitats tend to be complex. Different habitats are often imbedded within a natural matrix that changes along subtle gradations in wave exposure, salinity regimes, sediment character, etc. However, habitat restoration and mitigation are currently practiced at a rather low level of resolution, and so we chose to structure the protocols around broad habitat categories. We designated eight estuarine habitat categories: Five of them—emergent marsh, mudflat, sandflat, gravel/cobble, and eelgrass—are acknowledged intertidal wetlands. The other three categories—water column, subtidal soft bottom, and subtidal hard substrate—are not wetland habitats, but they are integrally associated with intertidal wetlands in the estuarine landscape. Because of these broad definitions, the habitat types can be equated to a habitat class in any of the several hierarchical wetland and associated habitat classifications schemes currently being used (e.g., Cowardin et al. 1979 and as modified for this region [Dethier 1990]). See the Habitat Descriptions and Examples section for the specific translation among the Protocol habitat categories and those of Dethier (1990).

IDENTIFICATION OF REPRESENTATIVE FISH AND WILDLIFE ASSEMBLAGES

The second step was to develop a list of representative fish and wildlife species for each habitat type. These lists were designed to be *representative,* rather than inclusive, in order to avoid creation of unmanageable matrices. Thus, not every fish, bird and mammal which occurs in estuarine habitats of the Pacific Northwest is included in these lists, but their feeding mode, reproductive habits, behavior, etc., should be represented on the list by at least one sympatric species. As a consequence, the assemblages are considered to be composed of "functional species."

The specific assemblage species were also selected (rather than their functional analogies) because of the extent of information available on their use of estuaries. In effect, we optimized our ability to complete the matrices with relevant data. The list of assemblage species (see Appendix A) was generated during UEMWG discussions, then distributed to estuarine habitat and fish and wildlife experts in management agencies and other institutions (e.g., universities), and subsequently revised based upon the review comments.

CATEGORIZATION OF HABITAT FUNCTIONS

Estuarine habitat functions for fish and wildlife are categorized as (1) reproduction, (2) feeding, and (3) refuge and physiological adaptation (Simenstad 1987). While there are a number of additional or supplemental functions (e.g., migration corridors), we felt that these three functions explained the primary mechanisms.

Within each function, we developed a hierarchy of categories or factors which influence the outcome of the function (Table 1). For instance, fish or wildlife reproduction can occur exclusively on a particular substrate or over a restricted tidal range, and can be influenced heavily by ambient sound, light, temperature, etc. Each of these factors constituted a row in the first evaluation matrix (below).

For the sake of clarity, we have limited the functions that the habitat serves in supporting fish and wildlife to *direct* interactions (Table 1), by which we mean those habitat attributes that influence fish and wildlife survival rates. Of course, certain indirect functions also contribute to the production of fish and wildlife populations; for example, primary production by wetland plants is an ultimate energy source for the habitat as well as for the estuarine ecosystem as a whole (Simenstad and Wissmar 1985). However, we have excluded total production as a direct function because fish and wildlife do not depend directly upon total production *per se*. Functions such as primary production should be addressed, as fish and wildlife support is in this document, as discrete functions in their own right (see Discussion).

IDENTIFICATION OF IMPORTANT RELATIONSHIPS AMONG ASSEMBLAGE SPECIES AND HABITAT FUNCTIONS (MATRIX I)

For each habitat type, the association of the assemblage species (columns) and habitat function categories (rows) formed an "evaluation matrix." Again, we turned to technical experts in resource agencies, asking them this time to rank the importance of each habitat function category for an assemblage species within their expertise. Ranks were to be assigned as 0 (no relationship), 1 (low), 2 (medium) or 3 (high), or as 4 (unknown but believed important). The resulting compilation of ranks was referred to as Matrix I (Supplement 1, Simenstad et al. 1990). Bear in mind that at this point in the process the data input originated from only six sources (resource agencies, universities, and developer/consultants), although the number of experts contributing to each source's response to the matrix was considerably higher.

LISTING AND RANKING HABITAT ATTRIBUTES (MATRIX II)

The results of Matrix I were refined to identify specific habitat attributes that were indicated as being of high importance. All matrix cells (i.e., function category/assemblage species associations) receiving at least one rank 3 were selected to compose a high priority matrix, called Matrix II (Supplement 2, Simenstad et al. 1990).

Table 1. Hierarchies of functions of estuarine habitats for fish and wildlife used to structure the **Estuarine Habitat Assessment Protocol**.

- I. REPRODUCTION
  - A. General
    - 1. light
    - 2. salinity
    - 3. sound
    - 4. temperature
    - 5. turbidity
    - 6. water/sediment quality
  - B. Elevation
    - 1. intertidal
    - 2. subtidal
    - 3. riparian
  - C. Substrate
    - 1. sediment
    - 2. emergent vascular plants
    - 3. macroalgae
    - 4. riparian vegetation
- II. FEEDING
  - A. General
    - 1. carrion
    - 2. detritus
    - 3. graveling
    - 4. light
    - 5. salinity
    - 6. sound
    - 7. temperature
    - 8. turbidity
    - 9. water/sediment quality
  - B. Plants
    - 1. microalgae
    - 2. macroalgae
    - 3. emergent vascular
    - 4. submergent vascular
  - C. Invertebrates
    - 1. benthic
    - 2. epibenthic
    - 3. neustonic
    - 4. pelagic
  - D. Vertebrates
    - 1. demersal
    - 2. water column
    - 3. neustonic
    - 4. terrestrial
- III. REFUGE AND PHYSIOLOGY
  - A. General
    - 1. light
    - 2. salinity
    - 3. sound
    - 4. temperature
    - 5. turbidity
    - 6. water/sediment quality
  - B. Physical complexity
    - 1. bathymetric features
    - 2. horizontal edges
    - 3. vertical relief
    - 4. water movement
  - C. Biological complexity
    - 1. macron
    - 2. emergent vascular plants
    - 3. submergent vascular plants

For each matrix cell (habitat function and assemblage species) identified as high priority, the same UEMWG participants and their colleagues were again asked to list and then, using the same ranking system, rank the function category/assemblage species relationship, but this time listing specifically the attributes of the habitat that accounted for the acknowledged high function. Thus, compilation of the responses to Matrix II resulted in a new list: the attributes of estuarine habitats that were considered important determinants of fish and wildlife use.

LITERATURE REVIEW OF HABITAT REQUIREMENTS OF ESTUARINE HABITAT ASSEMBLAGE SPECIES

During and after the period of Matrix II evaluation, an independent search was conducted through the scientific literature for basic habitat requirements of the assemblage species. Particular attention was given to those that had received no input or had received ranks that indicated high importance but with no substantiation. This search was conducted primarily through a computerized query (using the Compact Cambridge retrieval system) of the Aquatic Sciences and Fisheries Abstracts (ASFA) and Life Sciences Collection (LSC) installed on compact disk and available in the University of Washington library system. Retrievals were made using the scientific binomial and common names of the assemblage species.

The literature citations were printed as a bibliography and the titles and abstracts were scanned to select those citations specific to estuarine habitats. Citations that were relevant were then examined *in toto* to extract any information that identified attributes of estuarine habitats as important determinants of the assemblage species' use of that habitat. Those selected citations (Supplement 3, Simenstad et al. 1990) were added to the Matrix II database.

ATTRIBUTE DATA QUESTIONNAIRE

All attributes receiving moderate or high ranks in the Matrix II survey were chosen as the basis of the Wetland Attribute Assessment Questionnaire. Together with the information gathered from the parallel literature search (above), these attributes formed the criteria upon which the Protocol was subsequently structured. The questionnaire was sent to more than 200 habitat specialists; the mailing list was compiled using predominantly the Resource Guide to Wetland Scientists of the Pacific Northwest (Washington Department of Ecology 1988), and was augmented by a review of the contemporary literature on estuarine ecology in the Pacific Northwest and by our own familiarity with the researchers working in the region. It requested *documented* information about these attributes, and it also solicited information on (1) procedures and sampling designs the investigators had used to measure the attributes, (2) descriptions and comments on idealized sampling designs, (3) expected values, (4) data formats, and (5) published sources of these data.

The survey was conducted from the perspective of natural estuarine habitats common to the Pacific Northwest, and especially to the sub-estuaries of Puget Sound that were the primary focus of the UEMWG discussions. Despite the fact that the estuarine habitat mitigation and restoration being considered by this group was occurring predominantly in disturbed, highly modified habitats, the UEMWG determined that the functional relationships identified in the protocol development process should represent the optimum function of estuarine habitats. While it was recognized that habitat function in an urbanized estuary might be highly contingent upon external factors such as water quality or fish and wildlife population levels, the UEMWG considered the maximum potential function of a created or restored habitat to be the primary objective. This was one of the principal reasons for basing the Protocol on characteristics of the habitat that would *enhance* fish and wildlife utilization, rather than on an assessment of fish and wildlife occurrence and abundance *per se*. The results of the questionnaire are included as Supplement 4 in Simenstad et al. (1990).

REVIEW WORKSHOP

With the information received and compiled from the completed Attribute Data Questionnaires, a rough draft Protocol was prepared for review by a larger body of estuarine scientists in the region. The review took place at the Estuarine Wetland Restoration Monitoring Protocol Workshop, which was held at Fort Worden, Port Townsend, Washington, on April 16-19, 1989. Thirty-eight estuarine scientists, resource managers, consultants, and developers attended. Their task was to critically review both the process that shaped the Protocol and the information that the draft Protocol contained. Most of the workshop sessions involved step-by-step review of the formulative products of the Protocol's development, i.e., the estuarine habitats, assemblage species, and the results of Matrix I and Matrix II. Information from the Attribute Data Questionnaire was available in the distributed copies of the draft Protocol, and the attendees were encouraged to correct or augment these data.

The revisions to the Protocol that resulted from the workshop revealed two substantial problems: the lack of input from experts with wetland and marine mammal expertise, and the lack of an assemblage approach to emergent (marsh) plants. Both of these topics were addressed in later meetings with several of the region's experts on wetland and marine mammals and on estuarine marsh vegetation, and those sections of the Protocol were revised. In the case of emergent vascular plants (attributes), their association within discrete assemblages and the distribution of these assemblages according to salinity, tidal elevation, exposure, soil type, and other plants were incorporated into the Protocol structure.

UTILITY AND LIMITATIONS

Although caveats surrounding the application of the Protocol have been mentioned throughout this introduction, we should reinforce the conditions for which the Protocol is intended and in what cases we consider it inapplicable.

1. *Restricted application.* The Protocol is explicitly restricted to only fish and wildlife functions and to only estuarine habitats of Puget Sound. It may be more broadly applicable, with certain reservations, to estuaries outside Puget Sound, but it is not applicable to tidal fresh water, palustrine, and other freshwater habitats.
2. *Based on function.* The habitat attributes recommended for measurement are, by the process used to develop the Protocol, indicators of potential fish and wildlife support. The advantage of this approach is that it can be applied across habitats (is not dependent upon particular fish and wildlife species *per se*) and is probably a measure of overall community function because of the interactions among fish and wildlife and other organisms in the community.
3. *Augments, rather than supplants, other assessment procedures.* The Protocol is intended to meet the need for additional quantitative information that can be standardized across restoration and mitigation efforts. It does not assess the abundance of target fish and wildlife populations or their activities in estuarine habitats, nor does it attach a relative "value" to the habitat. Therefore, the Protocol should be considered as only one tool available among the many needed to effectively assess the function of estuarine habitats.
4. *Is deficient in terms of physical attributes.* Although it was initially designed to include physical attributes (e.g., sediment structure, salinity range), the Protocol lacks a quantification of physical characteristics that are important to fish and wildlife reproduction, foraging, or refuge/physiology. We found that such information was basically unavailable, and we entreat natural resource scientists to address this gap.
5. *Is not landscape-oriented.* The Protocol is based upon attributes exclusively within a habitat. This falls well short of considering the linkages between habitats and across ecosystems that should be considered to maximize the success of estuarine habitat restoration and mitigation projects and improve the overall function of estuarine ecosystems.

Approaches to these deficits in the Protocol are described in more detail in the Discussion section.

AFTER THE PROTOCOL

The potential for the Protocol to enhance estuarine habitat management will not be realized without application. We argue in the Discussion that, as with all protocols, its success resides in the accumulation of consistent, standardized, repeatable, and valid data. This needs to be accomplished by two methods: (1) application by resource agencies and environmental consultants in the process of addressing permit assessment and monitoring requirements; and, (2) explicit testing of the Protocol assumptions and methods in a purely scientific activity.

In addition, the need for long-term estuarine habitat reference sites is paramount to continued monitoring of the natural, seasonal, and interannual variability and range in Protocol parameters. These studies will verify both the concepts and the technical information embodied by the Protocol. With verification, revisions and additions to the attribute lists will ensure that the Protocol is biologically comprehensive and, we hope, will expand the technical information on physicochemical attributes.

USING THE PROTOCOL

The following sections describe recommended procedures for monitoring the function of estuarine habitats in supporting fish and wildlife in the Puget Sound region. Definitions and lists of fish and wildlife species and their associated attributes precede the protocol for each habitat such that the user can "access" it through a variety of topics, e.g., from the perspective of habitat, assemblage species, or attributes.

The protocols themselves are organized by attribute group, i.e., by those attributes which, because of location, behavior, etc., can be measured by similar procedures. Thus, the recommended protocol for monitoring parameters for each attribute group is applied across a number of habitats, but the list of specific attributes is unique for each habitat. An example of accessing the Protocol is included at the end of this section to acquaint the user with the process.

SELECTION OF ATTRIBUTES AND PARAMETERS

The process of selecting specific monitoring protocols requires you to answer two questions: (1) what is *appropriate* to the habitat(s) of concern? and (2) what is *applicable* to the specific habitat restoration/mitigation issue? This Protocol provides monitoring procedures for estuarine habitats as an assessment of the habitats' utility to the unique *community* of fish and wildlife that would potentially use specific restoration sites. We feel that these attributes are *appropriate* for measuring fish and wildlife support at the community level because the habitat assemblage species were chosen to be broadly representative of fish and wildlife use in each habitat. In addition, most of the attributes are important to more than one particular fish or wildlife species. Thus, the optimum approach to using the Protocol is to assess all the attributes relevant to the habitat(s) of concern if the desire is to gain a complete assessment of the "quality" of the estuarine habitat as a community.

Many constraints impinge upon your ability to measure the full spectrum of recommended parameters, even in the best of circumstances. In many cases, funding limits the number of measurements you can make and samples you can process, and often some parameters are not particularly relevant to certain sites. Thus, monitoring programs *applicable* to particular restoration or mitigation sites normally consist of a subset of the applicable parameters. Selection of which attributes and parameters to monitor presumably is the decision of the negotiating parties, i.e., the developer and their consultants, resource agencies, tribes, environmental groups, etc.

This process, the policy of implementing the Protocol, cannot be determined with this document. We have, however, organized in hierarchical order the parameters of the chosen attributes that should guide the decision process: (1) *minimum*, those parameters characterizing an attribute group that should be monitored under all circumstances; (2) ***recommended***, parameters that provide an adequate measure of attribute status; and (3) *preferred*, those parameters that provide an optimum, irrefutable assessment of the attribute and which should be used whenever an attribute is to receive special attention in mitigation assessment.

In addition to this hierarchy of monitoring parameters, we recommend sampling units, periodicity and frequency, methods and statistical considerations (e.g., sample replication). These are *literally* recommendations, because there will always be a need to use alternative methods and alter sampling designs for untypical sites and situations. Therefore, we have described *valid* monitoring procedures for such circumstances (see Considerations Common to all Habitats, below).

In sum, the Protocol is to used first to generate a list of the appropriate attributes to assess and, second, to set priorities on specific parameters and methods that provide a measure of each attribute.

SAMPLING DESIGN

The first thing you should do before searching for specific protocols is read the Considerations Common to all Habitats section on Sampling Protocols that apply to all habitats (Common to All Habitats section). This section is consolidated because (sampling) designs for assessing estuarine wetland and nearshore habitats are basically the same for all habitats, regardless of specific attributes. We urge you to become familiar with the principles of applying the Protocol using a valid sampling design before you proceed to the selection of the specific attributes.

SELECTION OF APPLICABLE PROTOCOL

Figure 2 illustrates the variety of methods by which users can locate the protocols that pertain to their objectives or situations. If you already know the specific assemblage species or attribute with which you are concerned, the fastest way to find the appropriate protocol is to use the Index. If, however, the interest is unspecific, you can access the appropriate protocols through the following hierarchy.

ESTUARINE HABITAT

From the descriptions of the eight estuarine habitats (see following section), select those that are applicable to your case or intent. Examine each of these to determine the appropriate estuarine habitat(s). Next, consult Appendix A—Habitat-Specific List of Assemblage Species—to determine the fish and wildlife *species* that constitute the representative assemblage for the habitat. Appendix B—Habitat-Specific List of Attributes and Associated Habitat Functions—lists all the *attributes* associated with specific fish and wildlife assemblages. Using these two lists, you can determine which protocols are applicable to most situations.

ASSEMBLAGE SPECIES

If you are specifically interested in the attributes associated with a particular assemblage species, use Appendix B to directly determine the attributes that are associated with the specific fish and wildlife species, and proceed to the appropriate protocol. Note that fish and wildlife species may occur in more than one habitat. If you are unable to find the species of concern in either Appendix A or Appendix B, check the Index. If it is not listed, consider examining the Assemblage Species for similar species; they may have been listed as ecologically analogous to the unlisted species.

ATTRIBUTE OR ATTRIBUTE GROUP

You can also directly locate specific estuarine habitat attributes by using the Index to locate it within the Attribute Group-Specific Protocols. Unlike the case of the assemblage species, an attribute occurs only once among the Attribute Groups, e.g., each attribute has only one protocol description.

REFERENCE MATERIAL

All literature citations used for the body of this report are included in the Literature Cited. Literature citations which contain the information about the importance of the attributes to the

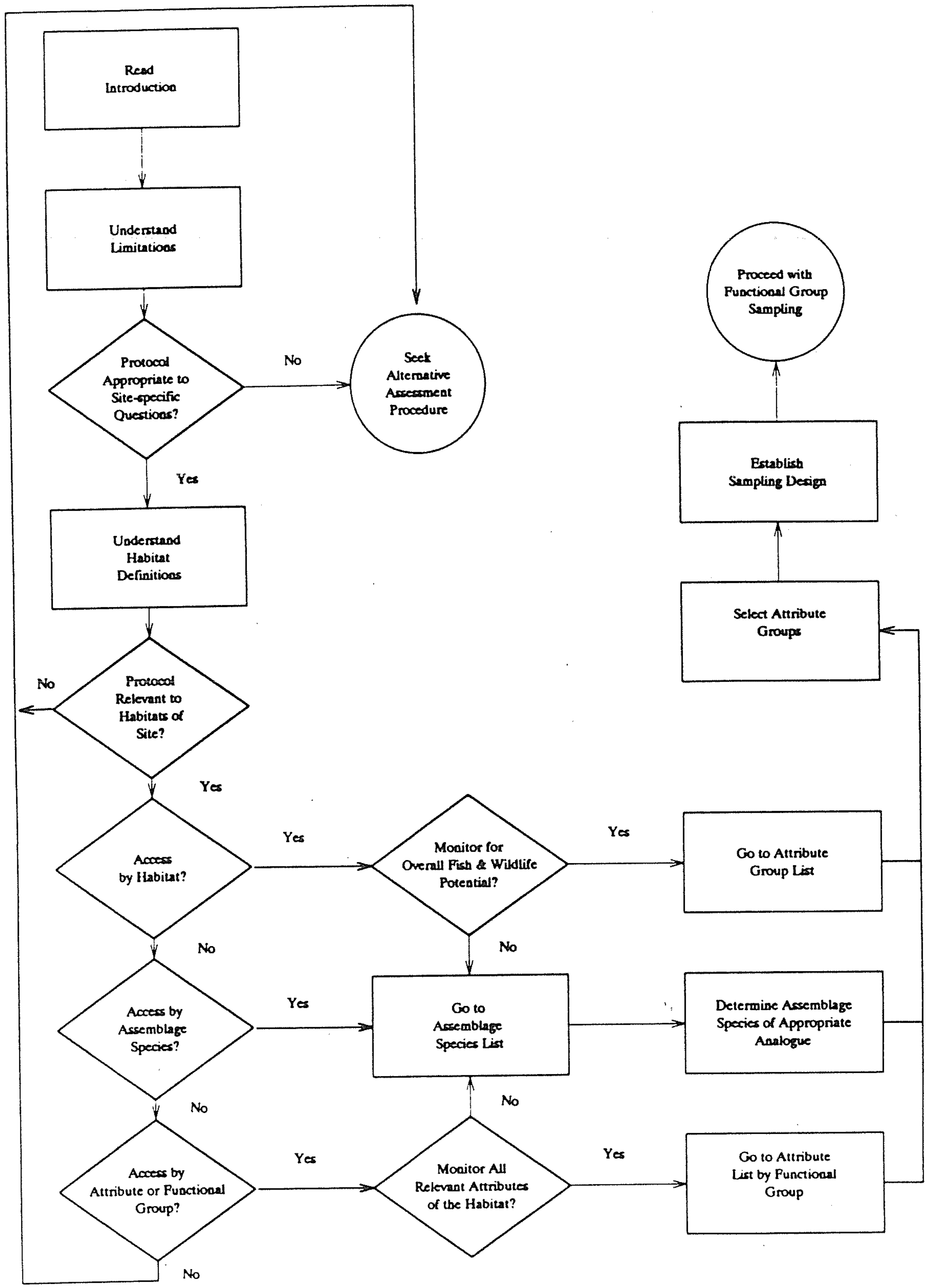

Figure 2. Decision tree associated with accessing the **Estuarine Habitat Assessment Protocol** on the basis of habitat, fish and wildlife assemblage species, or estuarine habitat attribute.

estuarine habitat assemblage species are listed in the Supplemental Bibliography (Supplement 3, Simenstad et al. 1990). Data and other information on each attribute, generated from the Attribute Data Questionnaire, are included as Supplement 4. Descriptions of the individual attributes are included as Supplement 5, and literature citations used for these descriptions, or recommended for further information, are cited in Supplement 6.

HABITAT DESCRIPTIONS AND EXAMPLES

For the purposes of the Protocol, we have adopted a comprehensive definition of "estuarine wetlands and nearshore habitats" and have incorporated three aquatic habitats which are not wetlands technically but which are integrally associated with wetlands. Protocols were developed for these associated habitats because fish and wildlife commonly move among habitats, and therefore it is usually impossible, and undesirable from an ecological standpoint, to ignore interactions between estuarine wetlands and adjacent habitats. Other associated habitats (e.g., riparian, tidal freshwater) that we have not included certainly serve similar functions, but the we lack adequate information about ecological interactions between these terrestrial habitats and the estuarine habitats.

In general, we have based our habitat types on the classification of Cowardin et al. (1979), in part because it includes both vegetated and unvegetated habitats. This recognizes that some types of habitats lack vascular plant vegetation (e.g., mudflats, sandflats, rocky shores, gravel beaches, sand bars) (Federal Interagency Committee for Wetland Delineation 1989). Fish and wildlife typically use unvegetated habitats and the deepwater or unvegetated shallows adjacent to wetland habitats. In certain cases, such as definition of vegetation types, we have adopted some elements of the Dethier (1990) modification of the Cowardin classification system in terms of dominant functional groups.

All habitats addressed by the Protocol are *estuarine*; that is, they are usually semi-enclosed by land but have open, partially obstructed, or sporadic access to the open ocean, and occur where sea water is at least occasionally diluted by freshwater runoff from the land (Pritchard 1967). The upstream/landward limit to this system is normally defined as the point at which the concentration of ocean-derived salts measures less than 0.5°/oo during the period of average annual low flow, and the seaward limit by an imaginary line closing the mouth of a river, bay, or sound (Cowardin et al. 1979). In the Venice System for the classification of brackish waters, these habitats would usually occur in the oligohaline (5-0.5°/oo), mesohaline (18-5°/oo), and polyhaline (30-18°/oo) salinity zones, which together encompass the mixohaline (30-0.5°/oo) zone (McLusky 1981). Accordingly, these habitats are generally not defined as occurring in either limnetic (<0.5°/oo) or euhaline (40-30°/oo) salinity zones.

If the reader has some question as to whether a particular habitat meets the statutory definition of wetland, they should consult Cowardin et al. (1979) for more detailed definitions or the Federal Wetland Delineation Manual (Federal Interagency Committee for Wetland Delineation 1989) for the definitive procedure to make this determination.

EMERGENT MARSH

Emergent marshes occur as intertidal shores of unconsolidated substrate that are colonized by erect, rooted, herbaceous hydrophytes, excluding mosses and lichens (Cowardin et al. 1979); perennial plant vegetation dominates most of the growing season in most years. Sediments vary from compacted clays and fine, unconsolidated muds to a mixture of coarse sand and gravel. Where the topography permits and extensive diking and channelization have not occurred, emergent marshes tend to form conspicuously in the mixing region, where tidal energy generates flood tide periods with high settling of suspended sediments.

Except in the most industrialized estuaries, emergent marshes occur prominently in the deltas of the major rivers entering Puget Sound and are usually present at the margins ("fringing marshes") of other estuarine habitats in the region. Prominent estuarine emergent marshes can be found on the deltas of the Skagit, Stilliguamish, Snohomish, Nisqually, and Skokomish rivers.

MUDFLAT

Mudflats are intertidal shores not vegetated by macrophytes and with unconsolidated sediment particles smaller than stones, predominantly silt (4ø to 8ø;[1] 0.0625 to 0.00391 mm particle diameter) and clay (8ø to 12ø; 0.00391 to 0.00024 mm); the substrate usually has high organic content, and anaerobic conditions often exist below the surface (Cowardin et al. 1979). This habitat occurs over low gradient shorelines, often encompassing broad expanses (>1 km^2).

In Puget Sound, mudflats often occur between the vegetated, emergent marsh habitats and the MLLW line along channels, and at the delta foreshores of major rivers. Prime examples include the mudflats of Skagit Bay and Nisqually Reach.

SANDFLAT

Sandflats are intertidal shores that are not vegetated by macrophytes and have unconsolidated sediment in which particles smaller than stones are predominantly sand (-1ø to 4ø; 2.0 to 0.074 mm). They occur as low gradient flats and bars where the substrate material is exposed to sorting by wave and current action. In the Pacific Northwest, the sand particles originate primarily from fluvial, rather than marine, sources.

Sandflats are often found along the more exposed boundaries of mudflats in estuarine river deltas, particularly adjacent to the river channels, as typified by locations in the outer, foreshore areas of the Nisqually, Skokomish, and Skagit river estuaries. Moderately exposed embayments without measurable riverine input, such as Birch Bay in northern Puget Sound, also have extensive sandflat habitats. Elsewhere, sandflats often form as 50–100 m benches along the moderately exposed, high bank shores of the Sound, as in the Saratoga Passage area between Whidbey and Camano islands.

GRAVEL-COBBLE

These habitats are intertidal shores which have substrates composed of a mixture of cobble (-8ø to -7ø; 256 to 76 mm) and gravel (-7ø to -2ø; 76.2 to 4.76 mm). Owing to wave and current action, and seldom as flats, the habitat tends to be formed as beaches and bars.

Gravel-cobble beaches tend to be distributed in the more exposed regions of embayments and along the margins of Puget Sound. Thus, most of the central basin of the sound and the shores of Hood Canal would be classified as gravel-cobble habitat, and prominent monotypic locations include headland points such as Three Tree Point, Alki Point, West Point, and Point-No-Point. This habitat also persists in many of the more developed sub-estuaries of Puget Sound, such as Elliott and Commencement bays, because it was the least desirable habitat for dredging and filling activities.

EELGRASS

This habitat consists of intertidal and shallow subtidal, unconsolidated sand to mud shores which are colonized by rooted vascular angiosperms (seagrasses) of the genus *Zostera*. Two

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[1] Units of the phi grade scale; a logarithmic transformation of the Udden grade scale of sediment particle size categories based on the negative log to the base 2 of the particle diameter in millimeters. For example, between 4ø and 8ø is silt, and between 8ø to 12ø is clay.

species are prominent in the Pacific Northwest: *Zostera marina*, the endemic eelgrass, and *Z. japonica*, an introduced congeneric.

Undoubtedly the largest contiguous expanse (3500 ha) of eelgrass habitat in the Puget Sound trough occurs in Padilla Bay, within the boundaries of the Padilla Bay National Estuarine Research Reserve. Other prominent intertidal flat eelgrass habitats include many of the pocket estuaries along Hood Canal, e.g., Duckabush and Dosewallips river deltas. Throughout most of the rest of Puget Sound, eelgrass (*Z. marina*) tends to form a 2–10 m band along the lower edge of the intertidal. Further descriptions of the characteristics of eelgrass habitats can be found in the excellent synthesis by Phillips (1984).

NEARSHORE SUBTIDAL SOFT BOTTOM

The nearshore subtidal soft bottom habitat occurs in the shallow subtidal (<20 m) portion of estuaries, usually in the channels and the shallower portions of embayments. The bottom is unconsolidated gravel to silt, with no or minimal relief.

NEARSHORE SUBTIDAL HARD SUBSTRATE

Nearshore subtidal hard substrate habitats are those rare outcrops, erratics, and other rock formations that are scattered around the predominantly unconsolidated shoreline of Puget Sound. Such habitats are usually rare within discrete estuaries, where the heavy sedimentation has often resulted in complete burial since the last glaciation; they persist, however, where waves and currents maintain exposure of rocks and rock outcroppings. They are notable as being among the rare estuarine habitats in which subtidal macroalgae (seaweeds and kelps) can colonize and persist.

WATER COLUMN

The water column habitat is that portion of the estuarine subtidal not associated with the shore or bottom. This does not include the benthic boundary layer, which we include in bottom habitats, but does include the neustonic layer at the surface.

EXAMPLE USE OF PROTOCOL

An example of accessing the specific protocols illustrates these different approaches. Consider a situation where an emergent marsh restoration project is to be assessed. The first task would be to verify that the Protocol's definition of an emergent marsh matches the habitat desired by reviewing the Habitat Descriptions and Examples (pp. 15–17). If the Protocol's definition of an emergent marsh is appropriate to the situation, the next step might be to examine the list of species included in the emergent marsh fish and wildlife assemblage, listed in Appendix A. If the decision is to assess the restored habitat relative to the entire assemblage, you would select all of the applicable attribute groups from the sampling protocols section (pp. 40–112). For the emergent marsh habitat, this would include the following functional groups: rooted vascular plants, benthic macroalgae, benthic microbiota, surface epifauna, sedentary infauna, epibenthic plankters, pelagic zooplankton, neustonic drift invertebrates, evasive macroepifauna, sedentary fish, motile fish, avifauna, and herbivorous mammals.

A second, alternative approach would be to access the Protocol from the standpoint of selected species from the emergent marsh fish and wildlife assemblage. You would then examine Appendix B, which lists the attributes associated with each of emergent marsh assemblage species. For instance, choice of chum salmon and cutthroat trout as species of concern would involve twelve distinct attributes, three of which are common to both fish species (e.g., Chironomid [unid.], *Eogammarus confervicolus*, and *Neomysis mercedis*). Examination of these attributes in the Index would reveal that the sampling protocols for assessing all twelve attributes are found under five functional attribute groups: rooted vascular plants (pp. 40–45), benthic microbiota (pp. 49–51), epibenthic plankters (pp. 69–74), neustonic and drift insects (pp. 80–82), and motile fish (pp. 98–105).

Finally, a user might wish to access attribute-specific sampling protocols directly if they already know the attributes or functional attribute groups of interest. For instance, suppose that the gammarid amphipods *Corophium* spp. are of interest. Not only are *Corophium* spp. associated with the emergent marsh habitat, but also the mudflat, sandflat, and eelgrass habitats, and they are important attributes of at least 12 fish and wildlife species that utilize these four habitats. The Index will direct the user specifically to the sampling protocols for epibenthic plankters (pp. 69–74).

SAMPLING CONSIDERATIONS COMMON TO ALL HABITATS

A primary goal of the Protocol is to promote both scientific *sufficiency* and *adequacy* in the assessment of fish and wildlife support in restored or constructed estuarine habitats. By sufficiency, we mean that enough information about the habitat must be gathered to adequately characterize the various support functions. Adequacy addresses the requirement for data of high quality, which relates to the way it is collected and analyzed.

By identifying the attributes of estuarine habitats, the Protocol is intended to encompass measurement criteria indicative of ecological function. Scientific adequacy, however, depends upon the quality of the sampling design, i.e., of the procedures and methods used in making those measurements. An adequate sampling design always incorporates three principal components of scientific quality: (1) *repeatability* in terms of the potential to be exactly repeated, (2) *reliability* as the quality to sustain scientific confidence, and (3) *validity* because it is based on precedence and evidence (National Academy of Science 1989). In practice, we have adopted as protocols procedures that represent the current state of good practice as defined by their ability to pass peer review.

We have addressed sampling in estuarine habitats as methodologies which, although they often vary in exact design depending upon the habitat type, are determined by a common group of rules based on accepted concepts of sampling theory and the combined experiences from previous scientific studies. You will note that, although we generally recommend one or two specific sampling procedures, the Protocol contains information on alternative methods or procedures. The diversity of estuarine habitats and attributes will always present situations where prescribed procedures are insufficient, and the alternatives represent possible solutions to those situations. We stress that the most important consideration is that sampling conform to rules and precepts that provide *quantitative, precise* information which is *compatible* with the Protocol.

The following section is a *review* of sampling theory and design, and one that we consider particularly appropriate to monitoring projects for estuarine habitat restoration. Wetland investigators familiar with biostatistics may consider it obvious and prefer to move to the next section. We encourage the reader, however experienced, to review this section because it will explain in part the statistical basis behind many of the Protocol recommendations. If you are not familiar with statistical concepts or have never applied them to field situations, we strongly advise using the Protocol as a guide in consultation with a biostatistician before implementing a sampling design.

SAMPLING DESIGN IN ESTUARINE HABITATS

Sampling estuarine habitats is usually necessary, as most habitats are too complex to monitor in their entirety. Proper sampling design minimizes the effect of inherent variability, or "noise," in the monitoring parameters that conceals real differences in the systems under study. It is a rare habitat that is so small or uncomplicated that every characteristic, or sampling unit, can be measured *in toto*, i.e., by statistically measuring (censusing) the total populations. One of the dominant traits of most estuarine habitats is that the distribution of physical, chemical, and biological attributes are complex over space and time. Thus, although sampling estuarine habitats to provide an accurate picture of their status and quality is a difficult task, it is *required* if the objective of estuarine habitat restoration or compensation is to be achieved.

Natural variability is inherent in most attribute parameters in estuarine habitats. In addition, most sampling techniques used in research produce sampling variability, which is often unrelated to, and in addition to, the inherent variability. If extreme, both kinds of variability can obliterate any distinction of real differences among populations. A rigorous sampling design should ensure that errors associated with sampling can be reduced to the point that real differences in populations can be detected with acceptable confidence.

Adoption of a sampling strategy requires attention to what Stuart (1984) terms the "credentials" of a sample to appreciate how much our interpretations depend upon them. Essentially, our ability to detect differences in parameters, attributes. and estuarine habitats overall will depend *entirely* upon our approach to sampling the habitats. In addition, sampling to determine the success or failure of estuarine habitat mitigation or restoration includes the cost of misrepresenting the status or quality of a habitat, an ominous potential for error when your decision could result in the loss of acres of rapidly disappearing natural habitat or many thousands of dollars if the mitigation is considered a failure.

As the most important credential, *samples must be reliable, precise, free of bias*. Secondly, both the sampling design and the samples must be reproducible, i.e., systematic, a specific objective of the Protocol. In addition, adoption of particular sampling designs, requiring specific statistical tests, will require that the data meet certain assumptions, such as independent and normal error distribution, homogeneity of variance, additivity of effects. It is beyond the scope of the Protocol to discuss the concepts of sampling theory that impose these constraints. We strongly suggest that readers who are sampling estuarine habitats read a pertinent technical reference on sampling design, such as Cochran and Cox (1957), Poole (1974), Green (1979), Scheafer et al. (1979), or Clarke (1980). Stuart (1984) provides a particularly lucid explanation of the objectives of sampling.

Achieving acceptable sampling credentials, however, is not the only constraint on estuarine habitat sampling. Proper sampling requires considerable time and effort. Thus, the dilemma: how do you balance sampling rigor with the resources at hand? How do you distribute the sampling effort and intensity to minimize the damage that destructive methods will cause to the site? The following is intended to provide some guidance in the selection of sampling designs within these constraints.

SAMPLING STRATEGIES

The first question should always be: What is the question? For instance, there is a big difference in sampling strategy and statistical evaluation if the question is "Can we quantitatively typify emergent marshes in an estuary?" or it is "Is this *particular* restored or created emergent marsh (e.g., one at a development site) functionally equivalent to a natural emergent marsh?" The former question requires characterizing the spectrum of emergent marshes occurring in the estuary. Assuming that the "developed" and "mitigated" habitats are located in the same general environmental conditions (e.g., geography, energy level) of the estuary, the second, more specific question is a simpler situation of comparing the two specific habitats. However, placement of the mitigation habitat at a significant distance from the developed habitat (often called "off-site" mitigation) violates this assumption and implies the former, more complex and estuary-wide question.

Random sampling is the most statistically valid approach to characterizing most complex habitats. On most scales, estuarine habitats tend to be mosaics of different substrates, plants, animals (sometimes termed microhabitats) that are distributed in a nonrandom (e.g., aggregated, clustered) manner. Thus, we are typically applying a *hierarchy* of questions relative to the habitat complexity: (1) Is this an assessment of a discrete (isolated) habitat or a contiguous array (encompassing a variety of types and locations) of habitat types? (2) If it is a discrete habitat, is it a complex of habitat types? (3) If it is one habitat type, is it structurally or biologically diverse? In the case of a negative response to the last question, a simple random sampling design could be applied to the habitat overall. If, however, there is any pattern to the habitat's heterogeneity, you should consider a modification to random sampling that permits stratification or some other technique to reduce variability and the required level of replication.

Therefore, the first Protocol rule in developing a sampling design is: *Know the habitat you are proposing to sample*. This admonishes the investigators, whether they are the ones doing the field assessment or not, to conduct a site visit and familiarize themselves with the habitats to be monitored. If the site is confined to one habitat type with no obvious pattern in either structural (e.g., sediment, tidal elevation, topography) or biological character (e.g., vegetation type or distribution, polychaete tubes, bivalve or shrimp burrows), a purely random sampling design is justified. If these complexities are at all obvious, however, it will both advantageous and statistically proper to use stratified random, cluster, or systematic sampling.

Random sampling

Simple random sampling is defined specifically as the procedure in which "a sample of size n is drawn from a population of N in such a way that every possible sample of size n has the same chance of being selected" (Scheaffer et al. 1979). This dependence upon the mechanism of chance in selecting a sample, in theory, guarantees freedom from selection bias, the essential item in the credentials of a sample (Stuart 1984). Random samples should be taken ("drawn" in the statistics sense) from a population by an intentionally random manner by the investigator. Some sampling approaches, such as haphazard sampling or representative sampling, have been called random but are, in fact, still vulnerable to the biases of the investigator who makes these haphazard or representative selections.

Such selection bias can be both explicit and unconscious. Explicit bias results when an investigator *intentionally* avoids spots that are difficult to sample or are "unrepresentative" of the sampling universe, or suspects that the measurements will be unreasonably low or high, or rejects locations that are too close to previous samples. Unconscious bias results when the investigator is *unaware* of gradients or other factors affecting a nonrandom distribution of the element (attribute), such as sampling along a steep intertidal gradient, not knowing that the organisms being counted are highly clustered within narrow tidal elevations.

The avoidance of selection biases leads to the second Protocol rule in developing a sampling design: *Know the sampling response (attribute) you are monitoring*. In the case of biological attributes, this means having some knowledge of the basic ecology, life history, and expected distribution in the habitat being monitored. To give a simple example, knowledgeable investigators do not attempt a simple random sampling program for barnacles on a mixed gravel-boulder beach when they know that barnacles will be found only on the boulders and only in a restricted tidal elevation. If, however, the sampling universe (habitat) is defined in a restrictive case as the barnacle habitat, simple random sampling would be appropriate.

In the case of physical/chemical attributes, this means understanding something about their temporal and spatial dynamics in estuarine habitats. One can use measuring salinity as another obvious example. In most locations in an estuary, salinity as a single measurement in time is relatively meaningless because tides, and thus water masses with discrete salinities, flux in and out of the estuary over mixed diurnal cycles, and freshwater flow during winter and spring discharge periods mediates the tidal effects.

To avoid the selection bias inherent in haphazard and representative sampling, samples should be selected methodically using a verified random technique such as a random numbers table or generator. Thus, the third Protocol rule is: *Select samples using a consistent, standardized technique*. Random numbers can be applied over both space and time as long as the assumption of homogeneity of habitat (actually, the attribute's distribution within it) holds. Random numbers are sets of integers generated so that all ten integers (0-9) occur in approximately equal probability, with no trends in the pattern of their appearance. Most elementary statistics texts or reference books explain how to use random numbers tables, and most hand calculators and computers have random number generators.

The process of selecting sampling points within the habitat requires that the investigator establish some decimally graduated reference, either one-dimensional, such as a transect line, or orthogonal, such as a grid, *which encompasses the population to be sampled.* Transects would normally be utilized to sample elongated habitats, such as fringing marshes, while grids should be used to sample broadly uniform habitats, such as mudflats. Sampling points are selected by using the random numbers to locate points along the transect or coordinates on the grid. The number of sampling points is best determined by a preliminary pilot project, or by precedence (see Replication section). If selecting sampling elements from a transect, the random numbers should be used to proportion the sampling locations along the whole transect; e.g., use two-digit random numbers to select percentage points along its total length.

Stratified random sampling

Stratified sampling is adopted when, based upon supplementary information about the population, there is any indication that the population is separated into nonoverlapping groups or strata in which the subpopulations are more homogeneous than the population at large. The reason for stratifying a sampling design is to gain precision through compartmentalizing the variability; you can never lose precision by stratifying, and you may gain considerable precision (Stuart 1984). The resulting reduction in within-strata variability can also reduce the number of samples (replicates) required to maintain the level of precision.

This approach is common in estuaries because estuarine habitats tend to be stratified across a variety of gradients. The most obvious estuarine gradient is that of salinity, but tidal elevation, exposure to wave and current energy, and many other factors are responsible for habitat strata. Strata are usually obvious or have obvious manifestations in the plants and animals that have adapted to narrow physiological, morphological, or behavioral regimens. Thus, the factors associated with tidal exposure in part produce distinct bands of macroalgae and sessile animals colonizing hard substrates such as boulders on intertidal beaches or prominent emergent plants in low versus high elevation marshes.

The absence of visually distinct plant, animal, substrate, or other strata should not, however, be construed as the lack of stratification in an attribute. For instance, tidal elevation on an outwardly uniform gravel beach will nonetheless affect the occurrence and abundance of certain animals living within the substrate whose distribution is highly structured by tidal inundation/ exposure. Epibenthic meiofauna, for instance, whose populations are commonly assessed as prey resources of juvenile salmon, are improperly sampled as a homogeneous population across broad tidal zones, because different taxa are actually concentrated along narrow tidal elevation zones. Biotic interactions, such as grazing/predation and competition, also affect the distribution of plants and animals and should be considered in recognizing and defining strata. These contingencies reinforce our second rule of estuarine habitat sampling, in that you should be familiar enough with the attribute you intend to sample to know the space and time over which it should occur as a homogeneous population in the habitat.

Once you have legitimate reason and evidence to establish sampling strata, a fourth Protocol rule should be observed: *Clearly specify your strata.* Over some sharp gradients, this means that *precise* documentation is necessary, such as verifying tidal ranges of the strata through surveying or correlating with a local, verified NOAA tide gauge. In other instances, the more important descriptor may be the biological community, as in a mussel or barnacle band, which can vary in tidal elevation relative to wave energy.

Although increasing the number of strata incurs a direct multiplicative increase in the cost of your sampling program, remember that it is preferable to designate more strata than fewer. While it is possible to pool samples from strata that show no significant differences in critical parameters, it is virtually impossible to stratify your samples after the fact. A statistical *stratification principle* is "maximize precision by constructing strata so that their averages are as different as possible and their variances are as small as possible" (Stuart 1984).

Once strata have been designated, simple random sampling can be conducted within each stratum according to the principles described earlier. The statistically "safest" method is to allocate the samples uniformly across all strata where the strata themselves are uniform in size, i.e., as *uniform sampling fractions*. The alternative strategy, of sampling some strata more intensively than others, i.e., using *variable sampling fractions*, is not necessarily improper as long as you know the extent to which you are under- or oversampling the respective strata.

In many cases, there is no option but to establish unequal strata, such as in sampling sparse patches of eelgrass on an otherwise unvegetated littoral flat. A common sampling strategy then is that of the *proportional allocation method*, in which the number of sampling units in each stratum is proportional to the size of the stratum. A less common strategy, the *optimal allocation method*, can be applied when preliminary information exists on the sample variability in each stratum. This can be a distinct advantage because limited sampling resources can be focused on the most variable strata. But, because there is a critical disadvantage to allocating a smaller fraction to the more variable strata, it is imperative to have some indication of the sample variation within each stratum, another argument for a pilot study. With pilot study data in hand, you have a high probability of increasing your precision at the same or lower cost by adjusting the sampling fraction accordingly; the common rule is to make the sampling fraction proportional to the square root of the variance for each stratum.

If time and financial resources exist, the more rigorous test of differences between heterogeneous habitats would be accomplished with optimum allocation among strata based upon a pilot study. In lieu of such rare opportunities, however, use of equal strata and uniform sampling fractions is the most prudent approach.

Cluster sampling

In cluster sampling, groups, or clusters, are selected randomly and all elements are sampled within the selected clusters. Cluster sampling is an economical modification of stratified random sampling, useful in those cases where obtaining information from an entire sampling frame is too costly, or becomes so more costly as the distances separating the sampling elements increase (Scheaffer et al. 1979). Cluster sampling can also be particularly appropriate to estuarine habitat sampling because habitats are often composed of a mosaic of patches, such as in eelgrass habitats, which are natural, convenient sampling units. The primary difference from stratified random sampling is that not all clusters are sampled, but the whole subpopulation in each selected cluster is. A critical rule for maximizing precision through cluster identification is that "clusters should be formed so that individuals *(elements)* within a cluster are as different as possible" (Stuart 1984). With this in mind, cluster sampling should be applied with care in estuarine habitat sampling because obvious habitat clusters tend to be contiguous and, thus, many parameters of attributes within a cluster may be highly correlated, and contrary to the cluster-formation rule.

Cluster sampling can also be *staged*; i.e., a random selection of elements can be selected ("subsampled") from within a cluster. Theoretically, there is no limit to the number of clusters that can form a (usually) hierarchical sampling strategy. These strategies are typically hierarchical in the sense that the first population of clusters is the largest or the most diffuse, the second consist of smaller subunits, and so forth. There are also instances in which cluster sampling can be combined with stratified sampling to increase precision at one or more stages of cluster selection, such that clusters can be stratified at any stage in the design and the final elements to be sampled can also be stratified. Procedures for determining the overall unbiased estimators of the population average and associated variance of such complex designs are available from most advanced sampling design references.

Systematic sampling

Random sampling is essential if the objective is to determine the mean and variance of the population, which will be the case in most monitoring programs applying the Protocol. However, there are a few instances in which systematic sampling is often more appropriate; probably the most applicable situation would be when the objective is to determine attribute characteristics in relation to position in the habitat. In systematic sampling, a random number is determined for the first sampling unit and the units that follow are selected at fixed intervals. There are two distinct advantages—the ease of determining the sampling scheme when there is only one random number, and the fact that the units are distributed evenly across the population—and two disadvantages—the standard error of the sample mean cannot be reliably determined, and the sample may be very biased when the interval between units in the sample happens to coincide with a periodic variation in the population (Elliott 1977).

The first disadvantage can be negated by adopting a variant of systematic sampling, called repeated systematic sampling, in which more than one systematic sample is collected randomly (Scheaffer et al. 1979). Using this method, the variance of the mean $\hat{V}\hat{\mu}$ can be estimated by using the square of the deviations of the multiple sample means about their overall mean:

$$\hat{V}\hat{\mu} = (N-n/N)(\Sigma[\bar{y}_i - \hat{\mu}]^2/n_s[n_s - 1]),$$

where $\bar{y}_i$ represents the average of the *i*th systematic sample and n_s equals the number of systematic samples in the populations N.

REPLICATION

At the heart of any sampling design is replication, because sampling error and the "natural" variability of estuarine habitats ultimately determine our ability to detect and interpret differences between pre-development or control habitats and "treatment" (restored, created, or otherwise mitigated) sites. However, the results of a sampling design can have several interpretations depending upon whether or not both sample and treatment replications were carried out and to what extent.

Sample replication

Determination of the number of samples (sample size, *n*) is not a trivial problem in any monitoring program. The sensitivity of the sampling design to detect differences in the populations sampled depends upon adequate sample replication and, thus, should be the first consideration. On the other hand, economic and effort constraints are valid reasons for revising the complexity of the sampling design and rethinking the level of response that is acceptable for the question asked. Oversampling is a waste of time and money, and too many samples are as hard to interpret as too few. Therefore, a fifth Protocol rule would be: *Determine the optimum sample size statistically, given the purposes and resources of the study and considering the potential to damage the site with excessive destructive sampling.*

The sample size required to satisfactorily test for significant differences is a function of at least the following factors: (1) inherent variability (as measured by the standard deviation or the coefficient of variation); (2) the size of the effect that it is desired to detect, or the desired length of a confidence interval in case of estimation; (3) the level of significance of the statistical test or, conversely, the level of confidence attached to a particular estimator; and (4) the level of statistical power, or the probability of being able to detect a change if one has actually occurred. It is possible, however, to make some general statements about the required range of sample sizes under certain conditions. Assuming normal distributions and equal variances, the standardized minimum detectable effect (MDE) generally gets smaller as the sample size per group in a two-sample t-test increases (Fig. 3). For sample sizes over 30, the curve flattens out and there is not much drop in the MDE; similarly, the curve is nearly flat for sample sizes between 20 and 30. Of course, if the distributions are non-normal, or the variances unequal, the required sample size for a given MDE value will be larger.

One of the most important considerations in establishing sampling size is the precision (error on estimation, B) of the population mean which is acceptable for accepting or rejecting the hypothesis; the precision is defined as the ratio of the standard error to the mean. A relatively simple method for estimating sample size based upon this error bound is to set two standard deviations of the sample mean equal to this error level and to solve for *n* via the equation for estimating the error around the population mean (Scheaffer et al. 1979):

$$B = 2\sqrt{\sigma^2/n(N-n/N-1)},$$

thus,

$$n = N\sigma^2/(N-1)D+\sigma^2,$$

where

$$D = B^2/4.$$

The critical problem in this procedure is the requirement of the population variance (σ^2). Although this is usually unknown, a sample variance (s^2) can be used for a close approximation of *n*. This implies that *some* prior data are necessary for estimation of s^2, another solid case for conducting a pilot study! Another, somewhat less acceptable method is to use 1/4 of the range of the parameter as a gross approximation of $\hat{\sigma}$ if a normal distribution can be generally assumed.

Bros and Cowell (1987) have recently presented an alternative method for determining sample size when a minimum detectable difference cannot be specified *a priori*. Their method considers both resolving power as a primary factor and expended effort as a secondary factor, thus presenting a mechanism for evaluating the cost of additional samples versus the increased resolving power. The standard error (SE) of the mean is used as a measure of the resolving power, and the optimum sample size is determined graphically as the inflection in the rate of change of SE versus sample size where this relationship originates from a number of random selections over a range of sample sizes. These repeated selections can be generated using a "bootstrap" method. A graph superimposing the mean, minimum, and maximum standard error upon the density and detectable difference as a function of sample size will provide an opportune illustration of the trade-off between sampling effort and resolving power. For example, Figure 4 indicates that a sample size of n=6 to n=7 will permit detection of a 100% change in the densities of an organism (in this case, an epibenthic zooplankter) at a 95% confidence level. However, there will be no measurable increase in resolving power >n=25. Detailed explanations and examples of this procedure are available in the source paper.

However, even if the natural variability cannot be measured exactly, the *range* of the coefficient of variation (CV = standard deviation/mean) is often sufficient to compute a trial sample size. In such a case, for the sake of expediency, historic data might be substituted for a pilot study. For example, if you are considering change with respect to a background or control mean, then the change, δ, can be expressed as a relative change with respect to the baseline value, μ_c:

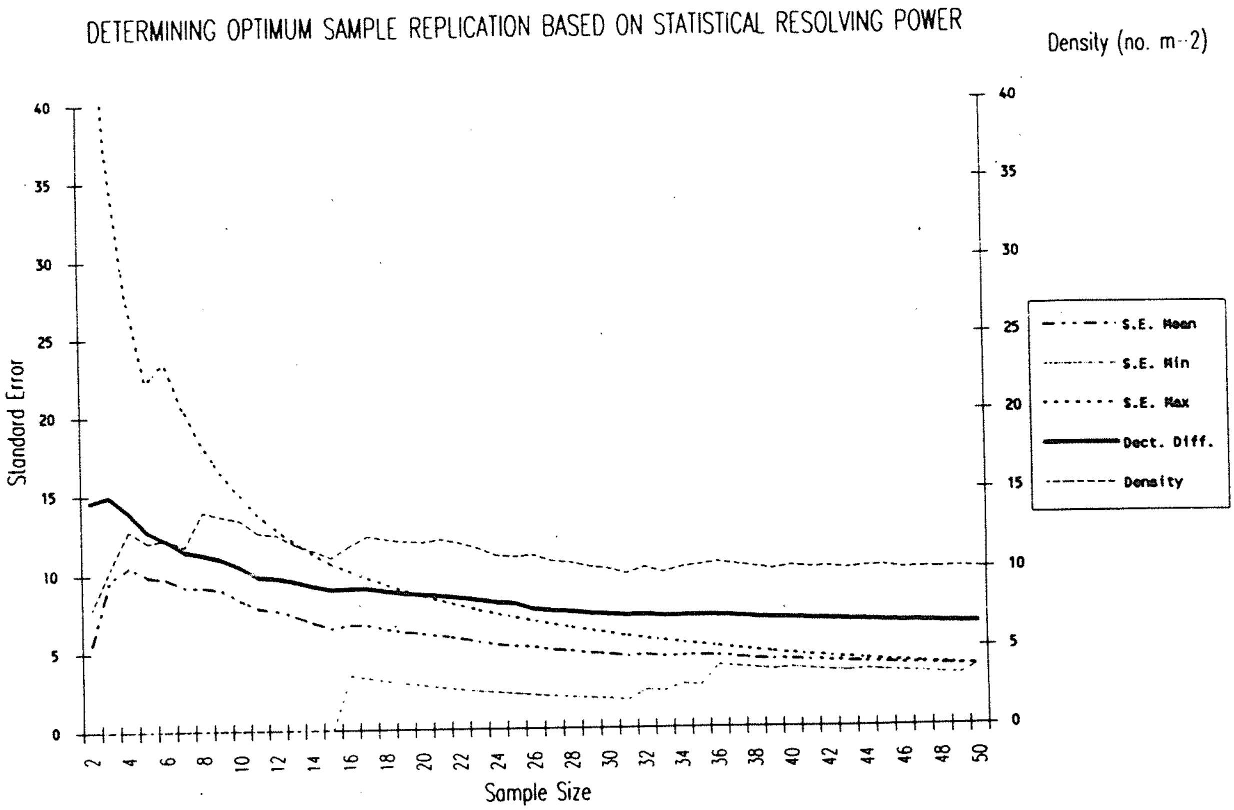

Figure 4. Example of Minimum Detectable Effect (MDE) as a function of sample size for three levels of power for a two-sample t-test at 5% level of significance (Source: L. Conquest, Center for Quantitative Science, Univ. Washington, Seattle, WA).

$$\frac{\delta}{\mu_c} = \frac{\sigma}{\mu_c} \times \frac{\delta}{\sigma}$$

where $\frac{\delta}{\mu_c}$ is the *relative* change from baseline, $\frac{\sigma}{\mu_c}$ is the coefficient of variation around the baseline, and $\frac{\delta}{\sigma}$ is the MDE in standard deviations. One may plot, for example, the lowest required CV required to detect a 50% difference between two population means as an increasing function of sample size. Other examples showing power calculations with only a range value as a guess for the CV may be found in Conquest (1983).

Treatment replication

The variation among plots, habitats, or sites that receive the same "treatments" is evaluated through treatment replication. Most habitat restoration or mitigation situations will not allow treatment replication because the projects are seldom large enough to allow separate restoration or creation of the same habitat within the same site. Restoration and creation of habitats at different sites could conceivably be considered replicates, but only if there were convincing evidence that all other factors were constant among the different sites—probably an impossible condition to meet.

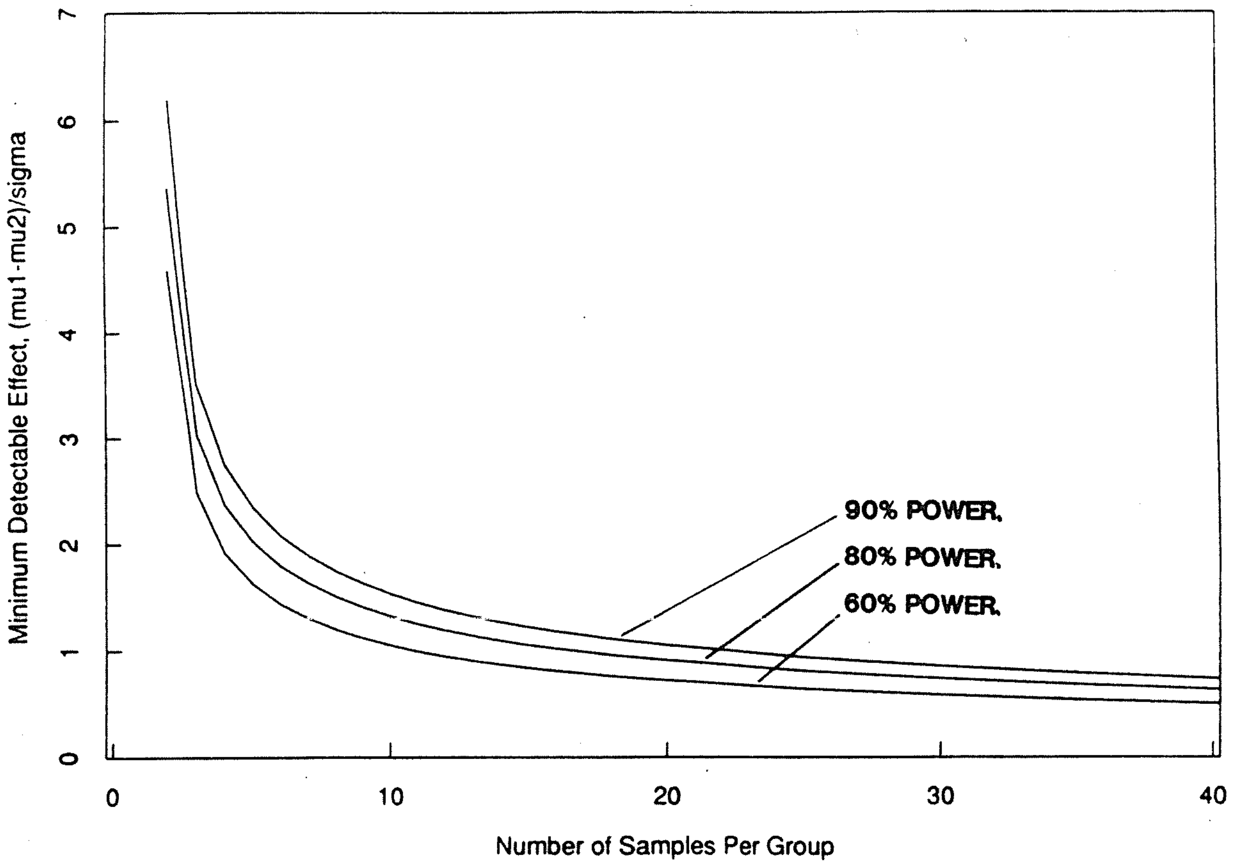

Figure 3. Example of mean, minimum, and maximum standard error, and detectable differences and density of organisms as a function of cumulative sample size (number of replicates).

Thus, in most situations, monitoring for the purpose of mitigation evaluation is limited to the comparison of two samples.

It should be remembered, however, that this implies statistically that the question "Does this mitigation design for restoring/creating this habitat provide values of these parameters equal to or greater than the development or control site?" cannot be answered because there is no treatment replication to provide a variance estimate; *the assessment of the design applies only to this particular case.* In a rigorous sense, this question can be answered only by *multiple application* of the same treatment (i.e., restoration/creation design) in more than two estuaries with all other conditions held equal. Thus, until such broad-scale experiments are conducted for a variety of habitat restoration/creation designs, *in a statistical sense, assessment of the outcome of every mitigation project must be conducted by monitoring.*

SAMPLE DIMENSION

The total number of sampling units in a population depends upon the relationship between the area of the population (the total sampling area) and the area of the sampling unit (the sample dimension) (Elliott 1977). The sample dimension basically affects the sampling efficiency, the relative sampling effort required to give estimates of equal precision over the total sampling area. In general, most sampling theorists conclude that a small unit is more efficient than a larger one when the dispersion of a population is contagious because: (1) more small units can be taken for the same amount of labor; (2) statistical error can be reduced by a sample of many small units, which has more degrees of freedom than a sample of a few large units; and (3) a sample of many small units is more representative of the habitat than a few large units. Noncontagious populations, however, impose the problem of scaling the sampling unit to the distribution, such as the dimension of recognizable patches.

In practice, the sample dimension is ultimately determined by the attribute being measured. Size, abundance, distribution, and motility of the attribute are the primary considerations. Obviously, a sample dimension must be scaled for the size of the attribute. For example, the dimension required to sample kelp plants (probably 1 m^2) is going to be larger than that required to sample diatoms (1 cm^2). Density, distribution, and motility can be considered in a similar sense. Attributes that are numerous and immobile, such as barnacles or small plankton, can be sampled reliably using a much smaller sample dimension (0.25 m^2) than those that are less numerous and more evasive, such as fish or active macroinvertebrates (e.g., 100 m^2). In addition, the smaller the sample dimension, the proportionally greater the sampling error at the edge of the unit. This effect can be additionally intensified when dealing with evasive animals, which are more likely to avoid a small sampler than a larger one.

Of course, field investigators always want to sample as many attributes as possible with the same sample in order to optimize sampling effort. In this case, the larger sample dimension will be required, but the effort required to measure the more numerous attributes will be intensive. A compromise design can often be developed by nesting sampling units. For example, large benthic samples can be obtained from large dimension samples, which are sampled in totality for large or rare attributes but can be subsampled by a smaller sampling unit for the more evasive, contagious attributes.

In the section on sampling protocols, we have attempted to recommend sample dimensions that are most appropriate to the attributes within each of the attribute groups. Where multiple attributes can be sampled in the same sampling unit, any subsampling strategy that might increase efficiency is also described. However, if there is any question or there is a lack of precedence in the scientific literature, we encourage you to test statistically the efficiency of the sample dimension to ensure independence of your statistical analysis from your sample size. One such method would be that proposed by Goodall (1961, 1973), which recommends that the minimum area should be "that of a square with the side equal to the distance at which the variance between samples ceases to be a function of their spatial separation"; alternatively, a similar rule might be applied to decreasing the sample dimension until it is much smaller than the scale of spatial pattern (Green 1979).

A final, sixth Protocol rule concerning all aspects of establishing and implementing your sampling design: *Document all procedures completely and maintain active notes of the modifications to any of the original procedures that had to be made in the field at the time of sampling.*

STATISTICAL STRUCTURE

Inherent in the application of the Protocol is the intent to compare the resulting parameters against a benchmark of some kind, presumably a "control," the habitat proposed for development, or an established reference site. To perform this "control-treatment" comparison in an exact manner, statistics are generally used to provide unambiguous interpretation of the results by testing their significance. The use of biostatistics to evaluate natural biological populations is an elaborate scientific discipline in itself. Determination of which specific statistical method is appropriate, and how to apply it, can be obtained by consulting standard biostatistics texts such as Zar (1974), Sokal and Rohlf (1969), and Snedecor and Cochran (1980). We herein only emphasize several important basic concepts which should guide the user of the Protocol in designing their monitoring program for statistical application.

Field biologists all too often wait until they have collected a suite of data to determine what statistics they need to use. As a result, their usually astute intuitive understanding about what is going on in the natural environment is often compromised by their inattention to how to illustrate it statistically. The application of statistics should depend upon the question, or hypothesis being asked. In almost every case, a hypothesis *must* be posed in order for statistical tests to be applied. The most acceptable form of hypothesis is the *a priori* null hypothesis (H_0), which can never be accepted but can be rejected with known risks of error in doing so. This is to say that, while you can legitimately reject the hypothesis that there is no significant difference between Habitat A and Habitat B, you cannot say with any known error that they are the same. A cardinal rule is *Null hypotheses must be falsifiable*; that is, the results must provide a definitive test of the hypothesis.

Hypotheses should be formulated using Occam's Razor, which states that the hypothesis should be the simplest one possible consistent with the evidence, and with the fewest possible unknowable explanatory factors. Rejection of H_0 implies an alternative hypothesis (H_A), the next most probable explanation in the hierarchy. *The relationship between H_0 and H_A lies at the heart of the Protocol's design.* H_0 establishes a format for testing the significance of an attribute parameter, e.g., that the density of shorebird benthic prey taxa in a restored marsh is not statistically different from a natural marsh; H_A provides the means by which the hypothesized *cause* of a significant difference may be tested, e.g., that the substrate structure (which is presumed to affect the density of these organisms) is significantly different. Thus, *as many interrelated attributes as possible should be monitored if the causal mechanism or mechanisms behind a significant difference in a key attribute are to be elucidated and this information incorporated into subsequent, alternative mitigation design criteria.*

Once a hypothesis has been formulated, the sampling design will determine the optimum statistical analysis. In other words, the statistical analysis will only be as good as the structure of the sampling design; you can always step back to a simpler test if a vital assumption is violated, but you can seldom step up to a more complex (and usually more precise) analysis if the sampling design is inappropriate. This argues for a seventh Protocol rule: *Develop the hypothesis (or hypotheses) and the expected methods of analysis before collecting any samples* (other than a pilot study).

The statistical methods available for testing significance are too diverse and complex to describe here. More important are the constraints upon their application, i.e., the assumptions about the data which should be met before specific significance tests can be used legitimately. Green (1979) argues that (1) these assumptions should be understood at the time the statistical design is chosen; (2) the likelihood and consequences of violation (of these assumptions) should be assessed (with the aid of data from preliminary sampling); and (3) application of the statistical test should proceed with awareness of the risks and the possible remedies.

Parametric tests have somewhat obdurate assumptions such as normality and homogeneity of within-group variation. We say *somewhat* because, in fact, a test may be quite robust under the assumptions used to derive it (Harris 1975). Among Harris's guidelines, at least three are quite applicable to analyses of habitat data: (1) two-tailed tests with F- and t-statistics will generally be valid, even on extremely non-normal populations; (2) the ratio of the largest to the smallest sample variance should not exceed 20, and the ratio of the largest to the smallest sample size should not exceed 4; and (3) the error degrees of freedom should be 10 or more.

Transforming the data can reduce the violations of assumptions, such as that of nonhomogeneity of variance. It is common when dealing with data from estuarine organisms to find the variance depending upon the mean. As pointed out by Green (1979), the resulting *heterogeneity of variances associated with differences in means among groups can be removed by transformation, but differing within-group variances resulting from other, unknown causes cannot.* Transformation, therefore, should not be treated as the all-encompassing answer for resolving assumptions behind parametric tests. You should always *test* your data for violations of assumptions first, then decide whether a transformation of the data is appropriate and, if it is, choose the transformation. A detailed sequence for making these evaluations and decisions is described in detail in Green (1979).

When the assumptions of parametric significance tests cannot be met by the data, and the robustness of the tests is in question, non-parametric or so-called *distribution-free* tests are usually acceptable alternatives. These tests are particularly applicable to small or unequal samples from contagious distributions, and are usually based on ranking. It is important to remember that many non-parametric tests are almost as efficient as their parametric equivalents when all the "normal" assumptions for the parametric tests are fulfilled (Elliott 1977), which argues for the much simpler computations required by the non-parametric tests under certain circumstances.

The Mann-Whitney U-test is a common non-parametric tests for comparing two samples. Other common tests that compare more than two samples are the following: (1) Quenouille's test of the difference in mean level; (2) Kruskal-Wallis one-way analysis by ranks; (3) Friedman two-way analysis by ranks; (4) rank correlation coefficient; and (5) X^2 and contingency tables. The power-efficiency of these tests can, in fact, be quite high, e.g., between 90% and 96% for normal data in the case of the Mann-Whitney U-test and 96% for the Kruskal-Wallis. In addition to these "central value" oriented tests, the Kolmogorov-Smirnov two-sample test is applicable to testing the overall goodness-of-fit of two samples to see if they come from the same distribution. Deciding which test to use, relative to the data and the conditions required of it, should be done by consulting with a biostatistician and exploring one or more of the many texts and reference volumes. Elliott (1977), for instance, provides a good guide to the various methods and examples of their use, and Siegel (1956) and Wilcoxon and Wilcox (1964) are good general sources.

Several new tests to deal with non-random data have also been developed recently. Use of such "randomization" or "permutation" tests does not require that the test statistic under the null hypothesis (and the subsequent calculation of the P-value) depend upon normal distributions; they also do not require equal variances in many instances. One "simply computes" the values of the test statistic under all possible arrangements of the data (under the null hypothesis, each distinct rearrangement of the data values to the various treatment groups is equally likely). The resulting frequency distribution is known as the randomization distribution or the permutation distribution, and the P-value attached to the observed test statistic can be directly computed from this distribution. Box et al. (1978, pp. 94-97) provide a nice illustration of the robustness of the two-sample t-test under non-normality using a randomization approach.

Of course, it is no simple computing task to compute exact P-values. However, there are a variety of computer software packages available to perform the calculations. One such package, "StatXact"[2] developed by C. Mehta at Harvard University, has demonstrated that if sample sizes are equal (see summary of this section), using ordinary t-tests and F-tests even on non-normal data gives remarkably valid results. As for the case of unequal sample sizes, StatXact will compute either the exact P-values or an approximate P-value taken from an estimated randomization distribution. If there are simply too many random rearrangements of the data to compute them all, the computer will take a very large random sample (e.g., 25,000) from the randomization distribution and compute the P-values from the approximation to the actual randomization distribution. The P-values approximated in this manner have been found to be quite reliable.

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[2] Mention of this trademark product does not constitute an endorsement.

SAMPLE LOCATION

Sampling locations should be repeatable, either from the standpoint of returning to the same precise location for continued, nondestructive sampling or to avoid sampling a previous sampling point that had been sampled destructively.

Permanent markers (sometimes called datum markers) are the most reliable method to locate sampling points, transects of grids. They should be constructed of material that does not deteriorate, such as metal rebar or plastic PVC pipe, and implanted in the substrate firmly enough to resist displacement or loss by wave action. At sites with extreme wave or current exposure, heavier markers should be used, such as a concrete pier block with a metal pipe driven through it into the substrate. At the time of installation, all markers should be located relative to a known, pre-surveyed marker using standard survey techniques. Not only will this serve to locate sampling locations should markers be lost, but tidal elevations can be surveyed at the same time.

Care should always be taken to make the markers as unobtrusive as possible, both to minimize visual pollution of the site and to reduce the temptation to remove them without authorization.

Location of subtidal sampling points requires more elaborate procedures. Sampling for sedentary and motile fishes and evasive macroepifauna, for example, should employ accurate navigation, generating data on position, depth, and direction and length of towed samplers. In the simplest form, sampling points or the beginning and ending points of trawls and other tows can be established roughly by dropping buoys at the beginning and end of the sample; after sampling, the positions of the buoys should be determined by triangulation and marked on a fine-scale navigation chart. Optimally, sampling location should be established precisely using electronic means such as day-screen radar with built-in variable range markers (VRM), high-resolution lorans with multiple navigation capabilities, satellite navigation using a global positioning system (GPS), and video depth sounders and plotters. Puget Sound Estuary Program (1990a) describes in detail the use of these devices for precision trawl sampling.

SAMPLE PRESERVATION

Standardized procedures are required for preserving samples retained from field collections, and may vary if the samples are presorted in the field. In addition to preservatives, relaxants may also be needed to minimize distortion and autotomization of certain organisms (such as polychaete annelids) and to facilitate retention of organisms during sieving, and staining may be required for laboratory sorting. The basic rule, however, is to preserve material *immediately*! If circumstances prevent immediate preservation, short-term storage of material on ice in a cooler may be permissible, but identification of soft-bodied organisms (e.g., polychaete annelids) and organisms from fish stomach contents will not be reliable from iced samples.

Further discussion of standardized procedures for handling biological samples applicable to Protocol sampling are included in the Puget Sound Protocols for benthic infauna (Puget Sound Estuary Program 1990b) and soft-bottom demersal fishes (Puget Sound Estuary Program 1990a). More detailed information about fixation and preservation techniques for plankton are available in Steedman (1976) and Omori and Ikeda (1984) and for benthic infauna in Birkett and McIntyre (1971).

RELAXANTS

Relaxants are most important for soft-bodied organisms that tend to distort their body shape or autotomize, because their identification is often dependent upon certain taxonomic characteristics that are obvious only in live or relaxed specimens. Relaxants are most commonly used for benthic (infauna) macroinvertebrate samples and seldom used for crustaceans, molluscs, and fish. The most common general relaxant is probably isotonic magnesium chloride (e.g., 73 grams [g] $MgCl_2 \cdot 6 H_2O$ per liter [l^{-1}] of tap water), although propylene phenoxectol, epsom salts ($MgSO_4$), alcohol, menthol, and tobacco have also been recommended (Birkett and McIntyre 1971; Smith and Carlton 1975).

Relaxants have not been used in most benthic infauna studies in Puget Sound, and the Subtidal Benthic Macroinvertebrate Protocols (Puget Sound Estuary Program 1990b) do not recommend their use because of the potential conflict introduced in the level of taxonomic identification. Because the few soft-bodied infaunal organisms listed as attributes (e.g., *Manayunkia aestuarina*, *Neanthes limnicola*, *Abarenicola pacifica*) should not require relaxation prior to preservation for identification, we also recommend against using relaxants for basic sample collections.

However, the use of relaxants is encouraged when collecting fish for stomach contents analysis, because fish introduced directly into preservative often regurgitate. In fact, the ASIH, AFS, and AIFRB strongly recommend that fish be anesthetized in these instances (ASIH 1971). Two of the most common anesthetics used for fish relaxation are Tricaine (MS-222; 3-Aminobenzoic acid ethyl ester methanesulfonate) and Quinaldine (2-Methylquinoline). Other fish anesthetics are sodium pentobarbital, hydrous chlorobutanol, and urethane;[3] Cailliet et al. (1986) described the promise of Etomidate (1-[1-Phenylethyl]-1H-imidazole-5-carboxylic acid ethyl ester) but cautioned against its field use at that time. An appropriate dosage of MS-222 for fish relaxation before preservation would be 50-100 ppm.

From a humane standpoint, anesthetics are also required for handling small mammals (e.g., Townsend vole) and all other vertebrates collected in the field except those released immediately. Because the anesthetics and dosages used are very species- and size-specific, we suggest that you consult an experienced veterinarian or other expert before attempting anesthesis of these animals.

PRESERVATIVES

Formalin (37% by weight solution of formaldehyde gas in water, usually with 10%-15% methanol added to prevent polymerization) is the most common biological preservative, at least for immediate fixing. The normal concentration varies according to the mass of the organisms being preserved, but it generally ranges from 4% for zooplankton and benthic infauna to 10% for fish. In all cases, to prevent the preservative from becoming acidic over time, the formalin solution should be buffered by adding borax (sodium tetraborate), hexamine, or calcium carbonate (e.g., marble chips) to excess; in the case of borax, 20 g l^{-1} is recommended. In the case of large organisms such as fish >3-5 g in weight, incisions should be made in the abdominal cavity to allow rapid penetration of the preservative to internal organs.

Immediate freezing of samples may be necessary under a few circumstances but is generally not recommended because most soft tissues usually deteriorate when thawed. Fish and macroinvertebrates should never be frozen for later gut contents analyses for this reason.

All samples held for long-term storage should be transferred after fixation in the buffered formalin solution to an alcohol solution, usually 45% isopropanol or 70% ethanol. Fixation in formalin is usually sufficient after 1 day, but 7 to 10 days are recommended to reduce the risk of decalcifying molluscs and echinoderms (Puget Sound Estuary Program 1990b).

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[3] Caution: Urethane is reported to be carcinogenic; use and dispose with care.

STAINS

Vital stains may be added to samples at the time of preservation to enhance subsequent sorting because of the selective uptake of the stain by some organisms (most benthic infauna and meiofauna) and the resulting contrast with mineral material in the sample. Conversely, certain taxonomic features can be obscured in the staining process, and organic detritus also stains and can confuse the objective of contrasting the fauna. While staining is generally a help in sorting, we reiterate Puget Sound Estuary Program (1990b) in stating that it is not a substitute for proper sorting efficiency. A proper quality control program should ensure correct taxonomic identifications whether or not staining is used.

The most commonly recommended stain is rose bengal (4,5,6,7-Tetrachloro-3',6'-dihydroxy-2',4',5',7'-tetraiodospiro[isobenzofiuran-1(3H),9'-9[H]xanthen]-3-one dipotassium salt), added to samples either as a powder or in solution. A solution of between 1-4 g l^{-1} is recommended (Birkett and McIntyre 1971; Eleftheriou and Holme 1984; Puget Sound Estuary Program 1990b). Rhodamine B has also been used as a stain, with sorting performed under longwave ultraviolet light (Hamilton 1969).

COLOR PRESERVATIVES

For archival storage, it may be preferable to add an antioxidant or other chemical to retard color loss for some samples, especially those for which color may be an important taxonomic character (e.g., some fishes). A 40% emulsifiable concentrate of butylated hydroxytoluene (BHT, 2,6-Bis(1,1-dimethyl-ethyl)-4-methylphenol; sold by Shell under the name of Ional CP-40) or butylated hydroxyamisole (BHA, mixture of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol; sold by Universal Oil Products under the name of Sustane I-F) mixed as a 2 ppt solution in 10% formalin or 70% ethanol is recommended for both crustaceans and fish (Omori and Ikeda 1984). Two other color preservatives recommended for fish are erythorbic acid (isoascorbic acid; D-erythro-Hex-2-enonic acid y-lactone) and Ional CP-40 (butylated hydroxytoluene; both are normally diluted to 1% in 10% formalin (Cailliet et al. 1986).

SAMPLE PROCESSING

The same principles of repeatability, reliability, and validity that apply to the samples apply similarly to how those samples are processed (prepared and measured) in the field and in the laboratory. In addition, some control and assurance (so-called quality control, QC, and quality assurance, QA) of processing precision must be guaranteed, and some systematic documentation must be maintained on the discrete samples so that the data resulting from this processing can be fit unambiguously to the sampling design. We stress that individual elements of any sample should be *discretely* identifiable, and that the procedures for obtaining them should be *exactly* repeatable.

FIELD

Acquisition of data or sorting of samples on-site is generally encouraged, especially if data can be obtained undestructively. Even if destructive samples (e.g., benthic grab, bottom trawl) are collected, we recommend processing them on-site if possible, because most of the organisms can be returned to the general vicinity alive, and subsamples will usually suffice for more extensive laboratory analyses. This is especially the case for large organisms, e.g., benthic infauna and fish, samples of which occupy a large volume and mass and require immediate preservation.

In the case of most benthic samples, organisms must be sorted from mineral and detrital material, which is usually performed on the deck of a vessel by washing the contents of a sample (e.g., bottom grab) through a sieve (i.e., 1 mm; Puget Sound Estuary Program 1990b) over a sorting table. This procedure, as well as more elaborate sorting mechanisms, are described in more detail in Birkett and McIntyre (1971).

Similarly, except in cases of extremely low catches, we recommend subsampling fish catches immediately after collection and returning the residual catch alive. However, care should be taken to avoid returning fish to an area that will be immediately sampled again. The preferred procedure is to hold the earlier catches in containers (such as washtubs or plastic garbage cans) while subsequent samples are collected and release all residual catches after all sampling has occurred.

All field samples, subsamples, and any elements separated from the samples at the time of acquisition should be labeled with a unique sample number or code. *At a minimum*, this label should contain the following information: (1) date; (2) time; (3) sample location (include distinguishing reference number, such as the sample coordinates or transect location); (4) identifying stratum number, if necessary, e.g., transect number, elevation; (5) replicate number; and (6) initials of person recording sample data and making out label in the field. In addition, any comments relative to the potential efficiency of a sample (e.g., "beach seine snagged briefly on retrieval") should be noted at this time.

LABORATORY

Having been presorted in the field, most samples can be dealt with directly. In addition, compared with field-sorting, sorting of samples in the laboratory can obviously be maintained with greater precision and accuracy and will obviously be the only option when collecting zooplankton and meiofauna.

Any processing of samples in the laboratory that involves subsampling should follow the same precepts of sampling theory that have been described previously in the section on sampling design. In particular, subsampling should be random and the dimension proportional to the occurrence of the sampling unit in the population. As in field sampling procedures, these laboratory subsampling procedures should be well documented.

Sorting and concentrating

Zooplankton, meiofauna, and similar samples should preferably be extracted from the remainder of the sample while still alive. This is particularly advantageous because the organisms' behaviors (e.g., rheotaxic, phototaxis) can be used to concentrate them in the sample so that they can be more easily extracted. Samples may also be concentrated by decanting after repeated shaking and swirling, or by elutriation with a variety of liquids (e.g., sugar in solution, magnesium sulfate, sodium chloride, zinc chloride, carbon tetrachloride, detergent). Accepted methods for doing samples extractions (and source references) are described in more detail in Birkett and McIntyre (1971).

Subsampling

Samples with high abundances of organisms should be *systematically* subsampled. In practice, only epibenthic plankters, pelagic zooplankton, and neustonic and drift invertebrates might require subsampling in the laboratory.

The process of subsampling involves fractioning successive subsamples until the accepted abundance of the most prominent organisms is efficiently countable but well represented in the subsample. For most zooplankton studies, 400-500 organisms are considered an acceptable subsample; however, because the Protocol focuses on specific taxa, a good rule would be that *at least* 100 of the attribute organisms should be retained in the subsample to be counted.

A variety of splitting devices are available, each appropriate for certain types of collections. The Henson-Stempel pipette, Folsom splitter, and Motoda splitter are the most common devices used in zooplankton studies, and split (e.g., quartered) Petri dishes are often used for benthic insects and neuston. The important principle in selecting a subsampling device is to choose one that will not introduce bias into the subsampling process. For instance, the Henson-Stempel pipette is recommended for epibenthic zooplankters as long as they are the same relative size that will easily fit through the pipette aperture; large zooplankters such as gammarid amphipods and mysids should be picked from the sample before using this device. The Folsom and Motoda splitters, with their considerably larger apertures, are more appropriately designed for large samples of mixed sizes of organisms. In all devices, the samples should be agitated excessively prior to splitting in order to suspend all taxa equally.

INSTRUMENTATION

Any instruments used for any data acquisition, whether direct or indirectly associated with measuring attribute parameters, should be periodically calibrated and their precision documented. Some instruments, such as salinometers, have internal calibration (e.g., zero setting) or external calibration (e.g., resistance loops) available for repeated checking and adjustment in the field, but these should also be periodically checked against a known, documented standard (e.g., USBS Copenhagen water).

QUALITY CONTROL AND DATA HANDLING

QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance/quality control must play a prominent role in any monitoring program and should extend all the way from the field to the final analytical stage. As pointed out in PTI et al. (in press), quality assurance/quality control are an essential product of professional competence.

In the field, quality assurance/quality control must guarantee sample integrity. Foremost, established sampling designs must be adhered to, or, if there are deviations, they must be documented completely; such deviations are immediately reviewed for consideration before modifying the sampling design accordingly. Field quality assurance/quality control is actually implemented before the first sample is taken, by the fact that all sampling equipment should be tested for proper operation prior to deployment. In particular, all instruments must be checked under field conditions before actually field use. If feasible, back up sampling gear, instruments and spare parts should be carried into the field during any intensive sampling. When in the field, strict attention should be paid to established procedures to ensure that samples are precisely replicated. Any problematic sample which is suspected of harming the integrity of the sample should be discarded and that sample repeated. All environmental and sample parameter data must be recorded according to established format.

In the laboratory, the processing must follow similarly rigorous procedures to provide propagation of the sample integrity to the point of recording the data. Similarly, data reporting must adhere to a standardized format. All instruments should be calibrated periodically. Where sorting and identification of plants and organisms is involved, a complete reference collection should be maintained and additions to it verified by a relevant specialist for the taxa.

In data analysis, raw data files should be stored separately and all statistical and other analytical procedures well documented.

DATA FORMATTING AND ARCHIVING

It is *strongly* recommended that data collection be organized from the beginning around the eventual computer entry, manipulation and archiving using some form of relational database. The optimum method would be to use so-called data loggers, which you can program and use for *in situ* entry and storage of data in a magnetic medium (e.g., internal memory or disks) which can be downloaded to a computer upon return from the field; these are powerful and useful pieces of equipment, but attention should be made to download frequently, or maintain backup records, to avoid the impact of memory loss in the data logger. Data recording, analysis and archiving is most effective if it can be recorded in the same format that it is used for analysis and archiving, and that it conform with a basic data composition format used in similar investigations. It was with this in mind that the National Oceanographic Data Center (NODC) has generated a suite of data formats for acquiring data from nationally-funded programs. This format series has been adopted for many biological and physical studies of Puget Sound and, although it is not archived as a unified Puget Sound database, these data are available in NODC format from their national office in Washington, D. C.[4] The NODC format is one of the data formats which, in addition to several EPA formats, which has been incorporated into a recommended standard format and procedure for automated transfer of data among agencies involved in the Puget Sound Ambient Monitoring Program (PSAMP) (PSWQA 1991). Given the broad applicability of the PSAMP data format, including physical (e.g., sediment), chemical (e.g., contaminants) and biological (e.g., fish, shellfish) parameters, and its comparability to the NODC format system, *we urge the user of the Protocol to adopt this system for recording and archiving their estuarine habitat data.*

DATABASES

Even if in a common format, the data resulting from the description and monitoring of restoration/mitigation projects (and their natural "control" habitats) will not contribute to an accumulating, standardized base of knowledge about how we measure estuarine habitat function, and how habitat restoration/mitigation performs relative to fish and wildlife support, if it is not readily available. Both for your own analyses and for transfer of these data to other investigators and resource agencies, *we advocate that the raw data be entered into, and manipulated from, one of the common, commercially available computer databases.* Such databases can be adapted from simple "spreadsheet" programs or the more complex, dedicated database programs can be utilized. Most of these programs directly, or through utilities, have procedures for exporting data in unstructured (e.g., ASCII) formats, which enables any other program to access them. The only recommendation we make is that documentation be generated which describes the structure (e.g., field names, lengths, imbedded characters [e.g., decimals], missing data, etc.) and content of the original database. In addition, the database should be accompanied be a description of all sampling sites, including their formal place names, latitude and longitude, a detailed description of each sampling point or transect or grid, and any other locating characteristics (e.g., Loran coordinates, MESA station location number).

STATISTICAL ANALYSIS AND INTERPRETATION

As described in the Sampling Design in Estuarine Habitats section, statistical analyses should be conceptualized at the time of formulating the sampling design, not after all the data has been collected. If a proper hypothesis structure is employed, the statistical analyses will be direct

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[4] For further information on the NODC data formats used in the Pacific Northwest, and for accessing NODC data which has been collected in the region, contact the NOAA/NESDIS Northwest Liaison Office in Seattle, attn: Mr. Sid Stillwaugh.

and conclusive. The interpretation of the results should be just as straightforward, but seldom is. Whereas the rejection of distinctly falsifiable null hypothesis may be conclusive, the alternative hypotheses which may explain why there was a significant difference must be based on logical deductions from ancillary data, observations or other studies. Thus, we stress the importance of discussing your results in the context of any comparable prior data from a similar estuarine habitat, especially if the new information is contradictory.

GRAPHICAL AND TABULAR ILLUSTRATION

While we do not advocate any particular format and style, we do prescribe a number of standards for presenting any tabular or graphical illustration of data or analytical results with clarity, precision and efficiency:

1. *insert raw or basic statistical summaries of raw data*; except in extremely simple sampling designs with few attributes and parameters, the data will be presented in a reduced form, but the reader should in most cases have accessible the data from which the summary tables and graphs were derived; if raw data is extremely voluminous or detailed, include in the first paragraph of the Results how this data may be obtained from the investigators in hard copy or magnetic form;
2. *include tables of all statistical tests*, either in substantiation of the results or, if extremely numerous, in appendices; any significance tests should explicitly list the significance level and the test criteria for the test;
3. *identify the source of all data presented graphically*; state in the legend or labelling of the graph itself whether raw data is presented or, if not, what summary or subset of the data is graphed;
4. *explain and/or reference all complex analytical graphs and tables*; complex graphics which are often the product of computerized statistical analyses (e.g., cluster analysis, principal components analysis) are not always intuitive and explicit; include in the legend any clarifying comments and reference the particular segment of the text which describes the analytical procedure, and clearly label potentially confusing elements of the graph; all table legends should identify measurement units (in a common dimension such as m^{-2}, cm^{-2}, $100\ km^{-2}$, etc.) if they are not indicated directly in table;
5. *ensure that all graph and labels and patterns are readable*; reduction or better-than-perfect copying of small font size labels often destroys graph readability and, similarly, the differentiation of fill patterns; use large, high resolution/contrast font sizes and fill patterns and avoid extensive reduction of graphs;
6. *present many numbers in a small space*; as long as it is readable and not overtly complex, the more detail which can be presented, the better the overall picture of the relationship among the data;
7. *encourage direct comparison of different datasets*; overlay, stack or otherwise arrange data with common axes so that comparisons are easily visualized;
8. *show the whole dataset at several levels of detail*; use a hierarchical structure, first showing the data at its broadest level and subsequently in finer detail; and
9. *don't distort the data to show a result or interpretation that they don't show*; use the figures to show obvious results.

We highly recommend consulting Tufte (1983), from whom we adopted many of these concepts, for his exquisite synopsis of what constitutes effective visual display of quantitative information.

SUMMARY

In summary, it is important to repeat that care must be exercised in establishing the sampling design upon which the protocols will be applied. In doing so, we encourage attention to Green's (1979) 10 basic principles of sampling design and statistical analysis, most of which are eminently applicable to the design, analysis, and interpretation of estuarine habitat monitoring parameters.

1. Be able to state concisely to someone else what question you are asking. Your results will be as coherent and as comprehensible as your initial conception of the problem.
2. Take replicate samples within each combination of time, location, and any other controlled variable. Differences among can only be demonstrated by comparison to differences within.
3. Take an equal number of randomly allocated replicate samples for each combination of controlled variables. Putting samples in "representative" or "typical" places is **not** random sampling.
4. To test whether a condition has an effect, collect samples both where the condition is present and where the condition is absent but all else the same. An effect can only be demonstrated by comparison with a control.
5. Carry out some preliminary sampling to provide a basis for evaluation of sampling design and statistical analysis options. Those who skip this step because they do not have enough time usually end up losing time.
6. Verify that your sampling device or method is sampling the population you think you are sampling, and with equal and adequate efficiency over the entire range of sampling conditions to be encountered. Variation in efficiency of sampling from area to area biases among-area comparisons.
7. If the area to be sampled has a large-scale environmental pattern, break the area up into relatively homogeneous subareas and allocate samples to each in proportion to the size of the subarea. If it is an estimate of total abundance over the entire area that is desired, make the allocation proportional to the number of organisms in the subarea.
8. Verify that your sample unit is appropriate to the sizes, densities and spatial distributions of the organisms you are sampling. Then estimate the number of replicate samples required to obtain the precision you want.
9. Test your data to determine whether the error variation is homogeneous, normally distributed and independent of the mean. If it is not, as will be the case for most field data, then (a) appropriately transform the data, (b) use a distribution-free (non-parametric) procedure, (c) use an appropriate sequential sampling design, or (d) test against simulated H_0 data.
10. Having chosen the best statistical methods to test your hypothesis, stick with the result. An unexpected or undesired result is not a valid reason for rejecting the method and hunting for a "better" one.

Finally, when considering allocation of our usually limited sampling effort, we would add a final caveat. When developing a sampling design and the statistical tests of significance to test differences in habitat attributes, applicability and realism are of primary importance, while precision can, within limits, be sacrificed. However, given the critical nature of estuarine habitat loss, we suggest that in mitigation and other situations involving "replacement" of natural habitat function, both realism and precision are mandatory.

SAMPLING PROTOCOLS

ROOTED VASCULAR PLANTS

While the protocol development process resulted in a list of individual vascular plant taxa, we recognized that these plants generally occur in complexes or assemblages. Thus, in developing the Protocol, we chose to describe parameters and sampling methodology for these attributes within the context of their assemblages. Therefore, the assemblages defined below are an expansion of the individual attributes listed earlier in this document and in Supplements 2-6 (Simenstad et al. 1990).

HABITAT OCCURRENCE

All rooted vascular plants but the seagrasses (*Zostera* spp., *Zostera japonica*, and *Z. marina*, which occur predominantly in the Eelgrass Habitat) are included by us in the Emergent Marsh Habitat. They can occur as the basic "matrix" in the habitat or as patches in a matrix of different habitat type. Eelgrass, in particular, occurs as patches in mudflat or sandflat habitats. The distributions of habitat patches within habitat matrices must be considered in developing your sampling design, especially when considered sampling strata.

CHARACTERISTICS

This group of macroscopic plants have below-ground structures (e.g., roots, rhizomes, etc.) and above-ground structures consisting of stems or shoots. The species vary widely in morphology from simple upright single shoots to a complex system of branching stems. Trees and shrubs are considered part of this group. All members of this group grow rooted in sediments. They are distributed from the upper fringes of tidal influence down to shallow subtidal depths. Estuarine marsh plants and eelgrass are often epiphytized by smaller plants (usually algae). Epiphytes can be important members, in terms of biomass and productivity, of some vascular plant assemblages.

Estuarine habitat assemblages are distributed along elevational, salinity, substrata and exposure gradients in Puget Sound. Estuarine habitat assemblages at the lowest elevations, especially those in polyhaline salinity conditions, are typically dominated by one species (i.e., eelgrass and certain kelp habitats). In contrast, many estuarine marshlands contain several species in variable quantities which can make these assemblages difficult to characterize. A number of studies of estuarine marshlands, including Disraeli (1977), Burg et al. (1980), Boule' (1981), Boule' and Dybdahl (1981), Bradfield and Porter (1982), Ewing (1982), Hutchinson (1982), Granger and Burg (1986) and Hutchinson (1989), characterize the assemblages. Although each of these studies identified one or more marsh assemblages based upon quantitative data on species cover or abundance, the criteria for delineating an assemblage type were never established. There is a need for a unified approach to classifying estuarine marsh species into assemblages. To meet this need, a marine and estuarine habitat classification system has been developed by the Department of Natural Resources (Dethier 1990). Although quantitative criteria are not given, the system covers all estuarine habitats in Washington State. The system, therefore represents the broadest scheme presently available for estuarine plant assemblages of the Puget Sound trough, and will be used as part of a region-wide nearshore habitat monitoring program. The Dethier (1990) system was adapted for the Protocol because of its broad coverage and predicted long-term use. At this time, it is the broadest systematically-derived spectrum of assemblage types that one would encounter in the study region.

Dethier (1990) identified assemblages that consist of single species (i.e., monotypic stands) or species groups that are diagnostic for each habitat type. In general, a species is diagnostic of a habitat if it is the most abundant or obvious species at the end of the growing season. Other factors such as functional importance or fidelity for a habitat may have also been used when identifying a diagnostic assemblage.

The assemblages (monotypic or multiple species complexes) that are diagnostic of estuarine habitats in the State are listed in Table 2; these are based upon Dethier (1990) and a survey of acknowledged estuarine plant experts. Also listed are plant taxa that were identified as important to fish and wildlife during the protocol development process. Taxa that were identified as being important to fish and wildlife through the protocol development procedure and are included in Dethier's (1990) diagnostic assemblages are identified by an asterisk (*). Taxa designated by a double asterisk (**) are not among Dethier's diagnostic species but were listed as important to fish and wildlife during the protocol development process. Other plant taxa and assemblages are obviously important for fish and wildlife; however, they were not identified by direct connection (e.g., food, reproduction or refuge) to fish and wildlife based upon data from previous investigations. Although not diagnostic by definition, virtually all of these latter taxa were listed as common in at least one estuarine habitat by Dethier (1990).

Several invasive, introduced species (e.g., *Phragmites communis*, *Zostera japonica*) that have become well-established in some Pacific Northwest estuaries are included in this list. We have incorporated these into the region's wetland plant assemblages not because they necessarily important to fish and wildlife, but because a better understanding of their role in wetland communities would be gained by assessing them equivalently with the endemic plant taxa.

MONITORING PARAMETERS

Critical parameters for assessing the structure and function of rooted vascular plant assemblages are:

Minimum;
(1) presence/absence of species;
(2) percent cover;
(3) shoot or stem density (not applicable to species with extensive branching);

Recommended;
(3) above-ground standing stock (live and dead);
(4) shoot or stem length;
(5) density of flowering shoots;

Preferred;
(6) net aerial primary productivity; and
(7) below-ground standing stock.

Percent cover

Percent cover is the percentage of substrate area covered by a taxon or group of taxa. If vegetation layers are present (i.e., canopy, understory, surface), separate measures of each layer are necessary. The total percent cover in quadrat could exceed 100% in these latter situations. Percent cover within a quadrat can be measured using a photogrammetric technique. This involves taking a photograph of the area enclosed by the quadrat from vertically above the center of the

Table 2. Plant taxa and assemblages of estuarine habitats and that were identified as being important to fish and wildlife during the protocol development process; all but assemblage numbers 1, 14, 23, 25, 26, 50 (all macroalgae), 51 and 52 (seagrasses), and 3, 29, 46 and 48 (trees) are vascular marsh plant taxa. * = taxa considered diagnostic of habitats in Dethier (1990); ** = taxa not considered diagnostic of habitats in Dethier (1990). a = non-native invasive; b = estuarine fringe, freshwater species.

1. _Agarum_ spp.
2. _Agrostis alba_*
3. _Alnus rubra_**[b]
4. _Carex lyngbyei_*
5. _Carex lyngbyei_*/_Distichlis spicata_*
6. _Carex lyngbyei_*/_Potentilla pacifica_
7. _Deschampsia caespitosa_*/_Distichlis spicata_*
8. _Deschampsia caespitosa_*/_Distichlis spicata_*/_Salicornia virginica_*
9. _Deschampsia caespitosa_*/_Juncus balticus_/_Potentilla pacifica_
10. _Distichlis spicata_*
11. _Distichlis spicata_*/_Salicornia virginica_*
12. _Distichlis spicata_*/_Salicornia virginica_*/_Triglochin maritimum_*
13. _Eleocharis palustris_**
14. _Enteromorpha_ spp.**
15. _Festuca rubra_
16. _Glaux maritima_
17. _Grindelia integrifolia_*
18. _Jaumea carnosa_/_Salicornia virginica_*
19. _Jaumea carnosa_/_Salicornia virginica_*/_Triglochin maritimum_*
20. _Juncus balticus_*/_Potentilla pacifica_
21. _Juncus balticus_*/_Potentilla pacifica_ /forb
22. _Juncus gerardii_
23. _Laminaria_ spp.**
24. _Lilaeopsis occidentalis_
25. _Macrocystis integrifolia_**
26. _Nereocystis luetkeana_
27. _Phalaris arundinacea_**[a]
28. _Phragmites communis_**[a]
29. _Picea sitchensis_*
30. _Polygonum hydropiperoides_**[a]
31. _Polygonum_ spp.**
32. _Populus trichocarpa_**[b]
33. _Potentilla pacifica_
34. _Rumex_ spp.**
35. _Ruppia maritima_**
36. _Salicornia virginica_*
37. _Salicornia virginica_*/_Triglochin maritimum_*
38. _Salix_ spp.**
39. _Scirpus acutus_*
40. _Scirpus americanus_*
41. _Scirpus maritimus_*
42. _Scirpus validus_**
43. _Sium suave_**
44. _Spartina_ spp.**[a]
45. _Spergularia canadium_**
46. _Thuja plicata_*
47. _Triglochin maritimum_*
48. _Tsuga heteraphylla_*
49. _Typha latifolia_*
50. _Ulva_ spp.*
51. _Zostera japonica_**[a]
52. _Zostera marina_*

quadrat. The color (or infrared) slide can then be projected onto a screen containing random dots (50-100 dots per quadrat are typically used). The plant taxa and number of dots it overlays is recorded and converted to a percent. Another method involves visually estimating a cover class for each taxon in the quadrat. For example, a taxon may cover <1%, 1-5%, 6-15%, 16-25%, 26-50%, 51-75%, 76-95% or 96-100% of the space within the quadrat. The median percent cover is used when calculating the mean percent cover for a taxon within a habitat. For example, _Carex_ is estimated to cover 16-25% of the space in the quadrat. The median cover assigned _Carex_ for this quadrat would be 20.5%. Trees and shrub percent cover is generally estimated using this latter method. For trees it is useful to record the cover of the crown and the base of the trunk.

Shoot density

Shoot density is the number of shoots per unit area of ground. This parameter is sampled by counting the number of shoots of each taxon within the quadrat. Standing live shoots (i.e., those with green leaves) should be distinguished from standing dead shoots in the counts.

Above-ground species standing stock

Above-ground species standing stock is a measure of the biomass (weight) of plant material per unit area above the substrate surface (i.e., quadrat area). Above-ground standing stock is sampled by removing all plant material by cutting the plants at the level of the sediment surface. Care must be taken to remove only the shoot or stem portion of the plant and not the root or rhizome. The plants are then separated by species, and by live and dead shoots, and the wet weight recorded. In most cases, it is advisable to dry the plants at approximately 80°C until a constant weight it attained, and report the dry weight. Because wet weight varies widely depending upon the degree of drying that takes place between collection and measurement, wet weight should only be used in situations where time is extremely limited. The methods (i.e., time and handling) between harvest and weighing must be consistent for all samples in order to reduce variability in the net weight measurements. Weights are typically recorded to the nearest 0.1 g. However, very small plants may require weight to be recorded to the nearest mg.

Shoot length

Shoot length can be recorded by measuring the length of all or a randomly selected group of shoots within the quadrat using a ruler or tape measure. Shoot lengths can also be measured for plants removed for the assessment of above-ground standing stock. Shoot length measurements of highly branched species (e.g., *Salicornia virginica*) is not recommended.

Flowering

Flowering can be quantified as the number of shoots with flowers, the total number of flowers per species or simply the presence of flowers in the quadrat.

Net aerial primary productivity

Net aerial primary productivity (NAPP) is defined as the amount of organic matter produced per unit area and per unit time through photosynthesis after plant respiratory losses are subtracted. NAPP is a measure of the rate and amount of food produced by plants in a system. This parameter can be assessed by taking samples of above-ground standing stock periodically during the growing season or once during the period of peak biomass. When sampling occurs several times over the growing season, the incremental changes in mean standing stock per species and per assemblage are added to compute a total net annual productivity estimate. In addition, productivity per week or month (depending upon the sampling interval) can also be calculated.

Species which can lose significant amount of biomass during the growing season (by sloughing or grazing) will require a modification of the above method. Among the group of taxa of rooted macrophytes listed in Table 2, *Zostera* spp. (eelgrass) is the most prone to tissue loss due to natural sloughing of leaves and its relatively high frequency of submergence. Eelgrass productivity is best measured using a shoot marking technique. In this technique, individual shoots are tagged and a small hole is poked through the leaves in the region immediately above the youngest sheath. Upon return to the site in 2-4 weeks, the shoot is removed at its base, and the biomass in the portion of the leaves relative to the basal hole in the oldest leaf (generally slow or no growth) is recorded and used as an estimate of net productivity (Kentula and McIntire 1986).

Values of productivity are given in grams of tissue (dry weight) produced per m^2 per unit time. A quick method for determining a rough estimate of net annual productivity using standing stock samples and standard curves of production for selected species, is given in Kibby et al. (1980).

Below-ground species standing stock

Below-ground standing stock is the weight of plant matter comprising the roots and rhizome system of a plant per unit area. This is estimated for most species by removing a core (usually smaller than the above-ground quadrat) of sediment containing plant material from the center of the quadrat or at a randomly determined point within the quadrat. The plant material is separated from the sediment and the wet and/or dry weight of the plant material is determined.

Species presence/absence and percent cover of each taxon within the study area or strata provide the basic measurement of assemblage structure, and shoot or stem density is a more precise quantitative measure which can be derived out of the same sampling unit; both involve non-destructive sampling. Indicators of actual productivity, however, are recommended if emergent plants are an extensive proportion of the habitat area; the disadvantage, however, is that measurement of productivity can involve effort-intensive and destructive sampling. These are most easily measured as above-ground standing stock (also termed "biomass"), shoot or stem length and density of flowering shoots. Below-ground standing stock is less often measured, but can be a much more sensitive measure of the success of emergent marsh plant transplants than analogous above-ground parameters. Monitoring of the Recommended or Preferred parameters should be conducted under any circumstances where stipulated criteria for a restoration or mitigation activity involves comparable productivity to a natural habitat.

RECOMMENDED SAMPLING METHODOLOGY

Method:	**benthic quadrat**
Unit:	emergent vegetation, **0.25 m^2**; submergent vegetation, **0.1 m^2**

Measurement of these parameters is most often accomplished through the use of quadrat samples appropriately stratified, positioned and replicated (see Sampling Design in Estuarine Habitats section). Other methods do exist (e.g., line-intercept), which are generally quicker to carry out or better suited for a particular situation. The quadrat method is recommended here because of its wide-spread application.

Quadrat size is an important consideration. Square quadrats that enclose 1.0 m^2 (i.e., 1.0 x 1.0 m), 0.25 m^2 (0.50 x 0.50 m) and 0.1 m^2 (0.32 x 0.32 m) are used to sample rooted vascular plants. The chosen quadrat size must allow the measurement of all the target parameters. In general, high replication using smaller quadrats yields a lower variance in most parameters as compared to larger quadrats using fewer replicates (see Sampling Design in Estuarine Habitats). The size of the plants will help determine the appropriate quadrat size. For example, stands of large (i.e., 3-m shoot length) *Typha* may be best sampled with a 0.25, 0.5, or 1.0 m^2 quadrat. Whereas, *Zostera marina* may be best sampled using a smaller quadrat (e.g., 0.1 m^2) except where plants are sparse, in which case a 0.25 or 0.5 m^2 quadrat dimension is more appropriate. Sampling larger macrophytes such as large submergent macroalgae (e.g., kelps) or wetland trees and large shrubs may require a much larger "quadrat" size, e.g., 4 m^2 to 100 m^2).

Periodicity and Frequency:	emergent vegetation, **once yr^{-1} late July-early September**; submergent vegetation, **once yr^{-1} July-August**
Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; emergent vegetation, **n=5**; submergent vegetation, **n=5; n=10** may be found necessary for both vegetation types where the communities are diverse or areal cover/standing stock of plants is relatively low

While replication depends upon the area of the sampling unit relative to the area of the habitat, typical replication for quadrat sampling has been five to fifteen. To optimize the potential and power of statistical analyses, we suggest adopting completely overlapping sampling of vegetation, e.g., sampling macroalgae within the same quadrats as submergent vegetation and extracting sediment cores for microalgae from within the same quadrat. Thus, sample replication (as well as dimension) should adopt the maximum required for any one component.

CASE STUDIES

Studies which assessed all or some of these parameters in Pacific Northwest estuarine rooted vascular plant assemblages include Burg et al. (1980), Thom (1981), Eilers (1975) and Ewing (1982). For assessing the performance of a restored marsh, consult the studies of Mitchell (1981), Shreffler (1990), Thom et al. (1988, 1990, 1991) and Frenkel and Morlan (1990). Quantitative studies on eelgrass include Kentula and McIntire (1986) and Thom (1988, 1989, 1990a). A review of eelgrass meadow mitigation and construction projects can be found in Thom (1990b).

BENTHIC MACROALGAE

HABITAT OCCURRENCE

Eelgrass;	*Ulva* spp. epiphytic algae/animals
Emergent Marsh;	*Enteromorpha* spp. *Ulva* spp.
Gravel-Cobble;	*Laminaria* spp. *Macrocystis pyrifera* *Ulva* spp.

CHARACTERISTICS

Macroalgae, commonly referred to as both seaweeds and kelps, (although there is some vague distinction between these two categories) are generally found attached to rocky substrata. However, seaweeds do not need to be attached to live and many species in estuaries can be found free floating in the intertidal zone. Macroalgae are also commonly found as epiphytes on other algae or rooted vascular plants. Macroalgae do not have below-ground parts (i.e., roots and rhizomes). Almost all, however, have attachment structures (e.g., holdfasts). Among the estuarine plant assemblages delineated in Table 2, assemblages 1, 14, 23, 25, 26 and 50 are macroalgal taxa.

PARAMETERS

Critical parameters for characterizing the macroalgal assemblage are:

Minimum;
(1) percent cover;

Recommended;
(2) species standing stock; and

Preferred;
(3) net and gross primary productivity.

Percent cover and species standing stock are the most often used parameters for characterizing the structure of a benthic macroalgal assemblage, are sampled similar to the methods described for rooted vascular plants, and should be used in most cases for assessing the performance of created systems.

Percent cover

Percent cover is the percentage of ground area covered by a taxon or group of taxa. Total percent cover for a quadrat would be 100%, which may include vegetation, sessile animals and bare ground. If vegetation layers are present (i.e., canopy, understory), separate measures of each layer are necessary. The total percent cover in the quadrat could exceed 100% in these latter situations. Percent cover within a quadrat can be measured using a photogrammetric technique. This involves taking a photograph of the area enclosed by the quadrat from vertically above the center of the quadrat. The color (or infrared) slide can then be projected onto a screen containing random dots (50-100 dots per quadrat are typically used). The plant taxa and number of dots it overlays are recorded and converted to a percent. Another method involves visually estimating a cover class for each taxon in the quadrat. For example, a taxon may cover <1%, 1-5%, 6-15%, 16-25%, 26-50%, 51-75%, 76-95% or 96-100% of the space within the quadrat. As with rooted vascular plants, median percent cover is used when calculating the mean percent cover for a taxon within a habitat.

Species standing stock

Species standing stock is a measure of the biomass (weight) of plant material that occurs per unit area of substrate. Standing stock is sampled by removing all plant material by total removal or, for those that have holdfast attachments, cutting the plants' holdfasts at the level of the substrata surface. Care must be taken to remove as much of the plant as possible, or to cut the holdfasts systematically. The plants are then separated by species and the wet weight recorded. In most cases, it is advisable to dry the plants at approximately 80°C until a constant weight it attained, and report the dry weight. Because wet weight varies widely depending upon the degree of drying that takes place between collection and measurement, wet weight should only be used in situations where time is extremely limited. Weights are typically recorded to the nearest 0.1 g. However, very small plants may require weight to be recorded to the nearest mg.

Net and gross primary productivity

Because seaweeds can loose much of their tissues in a short period of time due to sloughing, and because significant amounts of organic matter can leak from the tissues of the plants and not become incorporated into plant material, net and gross primary productivity assessment is often done using oxygen flux (photosynthesis and respiration) measurements.

Net primary productivity (NPP) and gross primary productivity (GPP) is measured using standard light-dark bottle techniques (Littler and Arnold 1985). First, a fresh piece of a species is placed in a glass bottle (usually a 300-ml BOD bottle). The bottle is filled with filtered seawater, and the initial dissolved oxygen (DO) in the water is measured. The bottle is sealed and incubated under ambient day-light and sea temperature conditions for a period of time (i.e., this varies from 0.5-3.0 hrs depending upon how quickly photosynthesis is taking place). The final dissolved oxygen is then recorded. The bottle can then be darkened to exclude all light, and incubated under ambient sea temperature for a period of time [generally much longer than the light treatment because respiration (R) rates are generally slow in seaweeds]. The final dissolved oxygen concentration is then recorded. The surface area of the piece of plant use is recorded and the dry weight is determined. NPP and GPP are related as follows:

$$GPP = NPP + R$$

Oxygen produced in the light treatment represents net productivity. This change in oxygen should be divided by either the dry weight of the plant used or by the surface area to give oxygen produced per mg or cm^2 of tissue. The rate is calculated by dividing the result by the incubation time. Respiration is the loss of oxygen in the dark bottle, which must be normalized per unit time (usually 1 hour) and per unit of tissue (mg, cm^2). Biomass data from the quadrat sampling can then be used to calculate the NPP, R or GPP per m^2 (or other suitable unit) in the habitat under study.

Daily NPP is calculated by multiplying the hourly rate by the number of hours of non-light limited photosynthesis. This varies from approximately 10 hours around the summer solstice to 4 hours around the winter solstice. Daily R is simply the hourly rate multiplied by 24 hours, since respiration is assumed to occur all the time. Hence, for a daily GPP, daily NPP and R must be calculated. Annual rates of productivity are estimated by integration between productivity experiments. It is strongly recommended that the time between experiments not exceed one month, and preferably be shorter periods in late-winter through early summer. Seaweeds are most abundant in Puget Sound estuaries during May through September, and any comprehensive sampling program should span this period.

RECOMMENDED SAMPLING METHODOLOGY

Method:	**benthic quadrat**
Unit:	**0.1 m^2**
Periodicity and Frequency:	**twice yr^{-1} June-July**
Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**, distributed randomly within each intertidal elevation strata (transect); at least **three tidal elevations** should be sampled, e.g., (1) between +3 ft and +4 ft MLLW; (2) between 0 ft and +1 ft MLLW; and, (3) between -4 ft and -3 ft MLLW.

While replication depends upon the area of the sampling unit relative to the area of the habitat, typical replication for quadrat sampling has been five to ten. The more effort-intensive production measurements, however, usually involve only three to five replicates. To optimize the potential and power of statistical analyses, we suggest adopting completely overlapping sampling of vegetation, e.g., sampling macroalgae within the same quadrats as submergent vegetation and extracting sediment cores for microalgae from within the same quadrat. Thus, sample replication (as well as dimension) should adopt the maximum required for any one component.

CASE STUDIES

Studies which have documented benthic macroalgal assemblages in Puget Sound include Thom et al. (1976), Thom (1978, 1980) and Thom and Albright (in press). Studies of benthic macroalgae in restored estuarine habitats are not available.

BENTHIC MICROBIOTA

HABITAT OCCURRENCE

Eelgrass;	epiphytic algae/animals
Emergent marsh;	epiphytic algae/animals filamentous algae

CHARACTERISTICS

This group of plants consists of microscopic algae (primarily diatoms and blue-greens) that occur on the surface of sediment grains or attached to larger rocky substrata. It is a diverse group, consisting potentially of hundreds of species. Although not conspicuous, this group can account for significant amount of primary productivity in some habitats due to their high productivity rates. Measurement of microalgal biomass and productivity are advised in situations where benthic microalgae may be important system components and particularly where benthic infauna and epibenthic meiofauna are being monitored.

PARAMETERS

Typical parameters for measuring estuarine microalgal assemblages are:

Minimum;
(1) total standing stock; and

Recommended;
(2) net and gross primary production.

Preferred;
—

Total standing stock

Total standing stock or biomass is estimated as chlorophyll *a* concentration per unit area. In general, chlorophyll *a* is sampled using 1-cm diameter benthic cores that are 1 cm deep. The chlorophyll *a* biomass is commonly used as a direct indicator of total standing stock. Conversion factors can be used to convert chlorophyll *a* to carbon equivalents, but this factor can range from 25 to 250 depending upon environmental conditions, nutrient limitation, and growth phase of the algae (Parsons et al. 1977) and are based predominantly upon phytoplankton taxa; Strickland (1960) recommends a C/chlorophyll *a* ratio of 30.

Net and gross primary productivity

Productivity (NPP, GPP) measurements follow those of oxygen flux (photosynthesis and respiration) for macroalgae using standard light-dark bottle techniques (Littler and Arnold 1985). Sediment cores are extracted from the habitat and these are incubated intact in chambers designed to hold the cores. For productivity measurements, cores of 5-10 cm in diameter are commonly used. First, the core is placed in the chamber. The chamber is filled with filtered seawater, and the initial dissolved oxygen (DO) in the water is measured. The chamber is sealed and incubated under ambient day-light and sea temperature conditions for a period of time (i.e., this varies from 0.5-3.0 hrs depending upon how quickly photosynthesis is taking place). The final dissolved oxygen is then recorded. The chamber can then be darkened to exclude all light, and incubated under ambient sea temperature for a period of time (generally much longer than the light treatment because respiration (R) rates are generally slow in seaweeds). The final dissolved oxygen concentration is then recorded. The surface area of the core and the total standing stock (chlorophyll *a*) are determined. NPP and GPP are related as follows:

$$NPP = GPP - R$$

Oxygen produced in the light treatment represents net productivity. This change in oxygen should be divided by either the chlorophyll *a* concentration or by the surface area of the core to give oxygen produced per unit of chlorophyll *a* or cm^2 of sediment surface. The rate is calculated by dividing the result by the incubation time. Respiration is the loss of oxygen in the dark bottle, which must be normalized per unit time (usually 1 hour) and per unit of plant matter (chlorophyll *a*). Standing stock is estimated by the chlorophyll *a* determinations is used to calculate the NPP, R or GPP per cm^2 (or other suitable unit) in the habitat under study. Using GPP = NPP + R, daily NPP is calculated by multiplying the hourly rate by the number of hours of non-light limited photosynthesis. This varies from approximately ten hours around the summer solstice to four hours around the winter solstice. Daily R is simply the hourly rate multiplied by 24 hours, since respiration is assumed to occur all the time. Hence, for a daily GPP, daily NPP and R must be calculated. Annual rates of productivity are estimated by integration between productivity experiments. It is strongly recommended that the time between experiments not exceed one month, and preferably be shorter periods in late-winter through early summer. Because oxygen may be lost due to heterotrophic respiration associated with sediments, gross productivity is referred to as gross *community* productivity.

RECOMMENDED SAMPLING METHODOLOGY

Method:	**benthic core**
Unit:	**0.79 cm^2 (1.0-cm dia.)**
Periodicity and Frequency:	**monthly, March-May**
Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**

When sampled in conjunction with quadrat sampling for other attributes (e.g., benthic macroalgae, sedentary infauna), one core is usually removed for benthic microalgae, thus replicating at the same level as the other attributes. Historical studies of sampling just for benthic microbiota have involved five to fifteen replicates. Similar to the productivity measurements for benthic macroalgae, normally only three to five replicates are run. To optimize the potential and power of statistical analyses, we suggest adopting completely overlapping sampling of vegetation, e.g., sampling macroalgae within the same quadrats as submergent vegetation and extracting sediment cores for microalgae from within the same quadrat. Thus, sample replication (as well as dimension) should adopt the maximum required for any one component.

CASE STUDIES

Studies where benthic microalgae have been assessed in Puget Sound include Thom et al. (1984, 1985), Thom (1988, 1989) and Thom and Albright (in press). The biomass of microalgae in estuarine habitat reconstruction monitoring has been measured by Thom et al. (1986, 1987, 1988). Pertinent studies in the Northwest estuaries include Amspoker and McIntire (1978) and McIntire and Amspoker (1986).

DEMERSAL ADHESIVE FISH EGGS

HABITAT OCCURRENCE

Gravel-Cobble;	*Clupea harengus pallasi* (eggs)
Eelgrass;	*Clupea harengus pallasi* (eggs)

CHARACTERISTICS

The cream-colored eggs of Pacific herring are typically laid in layered masses which adhere to macroalgae (e.g., kelps) or eelgrass in shallow subtidal and low intertidal habitats. For still indiscernible reasons, herring spawning appears to be extremely site-specific, and we could not find any information which might provide criteria for optimizing herring spawning on kelp and eelgrass. Spawning occurs between March and June, although local herring stocks seem to have relatively specific spawning times[5]. When intensive, the spawning is visually obvious as an extensive milky cloud along the shoreline, coincident with considerable fish and seabird activity. Spawning can occur, however, without such obvious manifestations and measurement of actual spawn deposition is the more accepted approach.

PARAMETERS

Minimum;
(1) number of egg layers;

Recommended;
(2) egg density;

Preferred;
(3) egg survival and viability.

Egg layer (spawning intensity)

Counts of the distinct layers of eggs are made from eggs masses on intact vegetation, discarding any which have been disrupted in sampling, using a grappling rake. Sampling with the rake is conducted at longshore extremes and distances from shore until no or few egg masses are found, providing some quantitative delineation of the area in which spawning occurred.

Egg density

This measurement is the number of eggs per unit of eelgrass or kelp substrate. Samples of vegetation with eggs intact are removed from within (a minimum of) 0.10- to 0.25-m^2 quadrats distributed over a random sampling grid or stratified (where working with a beach gradient) 250-m to 500-m long transects. The eggs are stripped off each eelgrass shoot or kelp frond and counted. Under extremely high densities, the eggs can be weighed and the total egg counts extrapolated

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[5] If monitoring of Pacific herring spawning is considered, the investigator should contact the Marine Fish Division of the Washington Department of Fisheries for more specific information on the timing and location of spawning at the location under consideration.

from the weight of a known subsample of eggs. The vegetation is measured for surface area and the egg density estimated as a total or as a mean of the individual shoot or frond densities.

Egg survival and viability

Two methods can be used to evaluate egg survival and viability, one *in situ* and one laboratory method. On the spawning ground, discrete egg masses can be enclosed in a mesh cage which permits maximum water movement but limits macroinvertebrate and fish predation. These cages should be monitored coincident with each spawning ground survey and the number and state of the eggs recorded relative to the progress of the natural egg masses on the spawning ground. Eggs removed to a laboratory with flowing seawater of the same temperature and salinity regime as the spawning ground can be held in conjunction with egg masses from a comparable natural spawning ground; these eggs should be monitored frequently to assess the number of successful eggs hatching relative to the control.

The egg layer method is a standard methodology for assessing spawning intensity and has been used by the Washington Department of Fisheries from the early 1970s (Trumble et al. 1977) to estimate spawning escapement; this extensive baseline should be considered an important precedence for sampling Pacific herring eggs in any estuarine habitat in the Puget Sound region. Although limited to intertidal and shallow subtidal habitats where sampling units can be deployed, egg density per unit of spawning substrate is a more precise method which allows variance estimates and statistical comparison among treatment and control sites. Egg survival and viability is a more intensive monitoring which should be considered for situations in which the spawning substrate is not considered to be the sole limiting factor, e.g., water quality or higher bird predation are potential limiting factors.

RECOMMENDED SAMPLING METHODOLOGY

Method:	**benthic quadrat**
Unit:	**0.25-m^2 quadrat**
Periodicity and Frequency:	**semi-weekly, during documented spawning period or, if unknown, February-July**

Consult the Washington Department of Fisheries for information on spawning periodicity of local herring stocks.

Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=10** within an identifiable spawn concentration

ALTERNATIVE PROCEDURES

Using a small skiff, samples of vegetation are collected at 350-500 m intervals along the spawning grounds using a grappling rake. The herring spawn (egg masses) are sampled directly from the vegetation. The number of replicate transects will depend upon the shoreline area being monitored, as the 250-m to 500-m sampling segments would constitute replicate samples as long as tidal elevation and habitat type (strata) does not change.

CASE STUDIES

Descriptions of the accustomed spawning ground surveys occur in Trumble et al. (1982).

SURFACE EPIFAUNA

HABITAT OCCURRENCE

Emergent Marsh;	*Balanus* spp. Chironomidae (larvae) *Corbicula manilensis* Diptera (unid.) Hemiptera (unid.) Insecta (unid.) *Littorina* spp. *Mytilus* spp. *Saunderia* spp.
Gravel-Cobble;	*Balanus* spp. *Corbicula manilensis* *Dendraster excentricus* *Mytilus* spp. *Nucella* spp.
Mudflat;	*Corbicula manilensis* *Corophium salmonis* *Corophium* spp. Diptera (unid.) *Ostrea lurida*
Nearshore Subtidal Soft Bottom;	*Ampelisca agassizi* *Clinocardium nutalli*
Nearshore Subtidal Hard Substrate;	*Balanus crenatus* *Balanus glandula*
Sandflat;	*Clinocardium nutalli* *Corophium* spp. Oligochaeta (unid.)

CHARACTERISTICS

Surface epifauna are organisms which are attached to (sessile) or relatively closely associated with the surface of the bottom. As defined, this functional group does not include those organisms that penetrate benthic sediments (infauna) or move off the surface of the substrate into the benthic boundary layer (epibenthic plankters), although some of the same taxa may appear in these other groups.

PARAMETERS

<u>Minimum</u>;

(1) species occurrence;
(2) percent cover;

Recommended:

(3) density and standing stock (wet weight biomass);

Preferred;

(4) population structure (i.e., length frequency distribution, life history stage, reproductive status);
(5) age and growth;
(6) standing stock (dry weight or ash free dry weight).

Species occurrence

When sampling is distributed intensively over time and space, or when dealing with non-motile fauna, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine habitats and associated aquatic habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because surface epifauna are often distributed in patches.

Percent cover

For sessile animals that cover sediment surfaces, a minimal measure of their abundance is the percentage of a standard (quadrat) area which they cover. Total percent cover for a quadrat would be 100%, which may also include vegetation and bare ground. The total percent cover in quadrat could exceed 100% in these latter situations. Percent cover within a quadrat can be measured using a photogrammetric technique. This involves taking a photograph of the area enclosed by the quadrat from vertically above the center of the quadrat. The color (or infrared) slide can then be projected onto a screen containing random dots (50-100 dots per quadrat are typically used). The sessile epifauna taxa and number of dots it overlays is recorded and converted to a percent. Another method involves visually estimating a cover class for each taxon in the quadrat. For example, a taxon may cover <1%, 1-5%, 6-15%, 16-25%, 26-50%, 51-75%, 76-95% or 96-100% of the space within the quadrat. As with plants, the median percent cover is used when calculating the mean percent cover for a taxon within a habitat.

Density and standing stock

Density and standing stock are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the surface epifauna and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. If feasible, surface epifauna are best weighed fresh (damp) after excess water is toweled off. If preserved in formalin or alcohol, specimens can also be weighed in this manner but the standing stock values should *expressly* state that they are preserved wet weights and the time out of the preservative should be standardized because drying can be quite rapid under these circumstances.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are extremely labor- and time-intensive measures for epifaunal biomass estimates and are generally not warranted unless production or some other sensitive bioenergetic indices are intended.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Population structure for may epifaunal taxa may an important indication of the function of an estuarine habitat because critical assessment criteria such as reproduction and recruitment can only be assessed by data on reproductive state and life history stage, respectively. This is particularly the case for organisms with intermittent and episodic recruitment. At a minimum, life history stages should be classified for a subsample of the collections. In the case of most epifauna, this will take form of three broad life history classes: (1) juvenile, recently settled through all instar stages; (2) subadult or non-reproducing; and (3) reproductive individuals.

Size, as measured by dimension and less commonly by weight, frequency distributions can often be used to differentiate different size classes within these life history stanzas, and can be important indications of different recruitment events, i.e., short-term age differences. Organism size can be measured by a variety of standard techniques and several "standards" are used for different epifaunal taxa: (1) maximum valve length for bivalves such as *Mytilus*, *Ostrea* and *Corbicula*; (2) total shell length or aperture width for gastropods such as *Littorina*; (3) basal surface area for barnacles (*Balanus*); (4) maximum disk diameter for circular or oval organisms such as *Dendraster*; and (5) anterior tip of the carapace (including rostrum if present) to tip of telson with the animal stretched to full length in the case of amphipods. A sample for length measurements must be completely random and of sufficient size to represent the distribution; typically, a *minimum* of 25 to 50, and often 100, are required depending upon the variability.

Age and growth

Although similar to size distributions, age and growth data are some of the most optimal parameters to assess habitat quality. Not only will this indicate the age at which the population likely recruited to the habitat, but these data can also indicate the relative productivity of these organisms. Both bivalves and gastropods can be aged by analysis of thin sections of their shells; some bivalves can actually be aged by external annual striations.

RECOMMENDED SAMPLING METHODOLOGY

Method:	emergent marsh, mudflat, sandflat habitats, **benthic core**; gravel-cobble, **excavation within benthic quadrat**; nearshore subtidal soft- and hard-bottom, **benthic quadrat in combination with airlift (Venturi) suction sampler**; all samples washed through **1.0 mm** mesh sieve

Sediment cores have provided satisfactory samples of estuarine insect larvae and pupae. Quadrat sampling may be either non-destructive or destructive, depending upon the size of the attributes being sampled and the taxonomic verification required. Airlift or Venturi samplers commonly used for sampling in unconsolidated sediments (e.g., Brett 1964; Barnet and Hardy 1967; Christie and Allen 1972; Tunberg 1983; Benson 1989) can be used effectively in conjunction with destructive quadrat sampling. In this context, they are used to suction up all fauna (unless limited by large size) which is scraped from within the quadrat and retained a 500-µm mesh or smaller sampling bag.

Unit: **benthic core, 176.7 cm^2 (15-cm dia.) excavated to 10 cm depth; benthic quadrat, 0.25 m^2 surface evacuation**

Given their size, abundance and distribution, cores with diameters of 10-cm to 20-cm would be expected to provide adequate samples; LaSalle and Bishop (1987) described samples from 16.5-cm diameter cores used principally for sampling molluscs. Quadrat size will also vary, depending upon the size, density and distribution of the attributes being sampled. For instance, assessment of *Balanus* spp., which can reach high densities, has been assessed using (a minimum of) 0.10- to 0.25-m^2 quadrats, while *Ostrea lurida* and other less dense attributes will involve larger quadrat sizes, e.g. 0.5- or 1.0-m^2. Obviously, the size of the quadrat to be used for monitoring many attributes will be set by the larger or rarer attribute, but staged sampling may be considered in the case of extremely divergent attributes (see Sampling Design in Estuarine Habitats).

Periodicity and Frequency: **quarterly, e.g., February, May, August, November, to capture recruitment events**

The Puget Sound Protocols (Puget Sound Estuary Program 1990b) suggest that subtidal benthic macroinvertebrates be sampled in April and May. However, there is other evidence that this period would encapsulate the primary spawning and recruitment events for many Puget Sound macroinvertebrate taxa, and thus introduce considerable variance in the estimation of organism density and standing stock. In a compilation of spawning and reproduction of benthic macroinvertebrates from Puget Sound compiled by Shimek (1990), it was illustrated that spawning and reproductive behavior tends to be more concentrated between April to August, and peak settlement occurred from June through August. The results of this compilation was compared to selected benthic infauna data for January, June and August. This comparison indicated that, although differences were not significant, statistical variation tended to be lowest in January for most taxa. This pattern is presumably due to the early mortality of organisms recruiting to the benthos in the prior spring or summer, and accompanying stabilization of organism densities. Only in the case of arthropods was the coefficient of variation greater in January than in one of the other two months, and the disproportional representation of pagurid crabs (which reproduce in the winter) may have biased this result.

Given these results, we recommend seasonal (quarterly) sampling in order to capture the potentially different recruitment events for diverse taxa. This could be abbreviated to twice yr^{-1} for specific attribute taxa if sufficient data is available on their life history patterns to identify representative pre-recruitment and post-recruitment sampling seasons.

Statistical Considerations (Replication): in lieu of statistical predetermination using pilot study or historical data; **n=10**, distributed randomly within each tidal elevation strata (transect); at least **three tidal elevations** should be sampled in intertidal habitats, e.g., (1) between +3 ft and +4 ft MLLW;

(2) between 0 ft and +1 ft MLLW; and, (3) between
-4 ft and -3 ft MLLW

As in the cases of other core and quadrat studies, replication will depend in part upon the relationship between the quadrat dimension and the areal extent of habitat being monitored. Typical replication in historic studies has ranged from five to 25 samples.

CASE STUDIES

Albright and Armstrong (1982) describe a sampling program for *Corophium* spp. in the mudflat habitats of Grays Harbor based on coring. Hueckel (1980), Hueckel and Buckley (1987) and Benson (1989) have described the use of an air-lift sampler in assessing fish prey organisms scraped from quadrat samples on a subtidal artificial reef.

SEDENTARY INFAUNA

HABITAT OCCURRENCE

Eelgrass;	Capitellidae (unid.) *Cryptomya californica* *Macoma* spp. *Mya arenaria* *Tellina* spp.
Emergent Marsh;	*Manayunkia aestuarina* *Neanthes limnicola* Nematoda (unid.) Nereidae (unid.) *Tanais* spp. *Transennella tantilla*
Gravel-Cobble;	*Mya arenaria* *Tellina nuculoides*
Mudflat;	*Abarenicola pacifica* *Macoma* spp. Nematoda (unid.) *Tapes (Ruditapes) philipinarum*
Nearshore Subtidal Soft Bottom;	*Transennella tantilla*
Sandflat;	*Mya arenaria*

CHARACTERISTICS

Sedentary infauna are those which are relatively immobile within the benthic substrate. These can include not only animals which form burrows (e.g., clams such as *Mya arenaria*, *Macoma* spp., etc.) but free-living nematodes (Nematoda) and polychaetes (Nereidae). Their general lack of rapid motility means that they can be easily sampled with core and grab devices, which are generally inappropriate for the more evasive taxa. In cases where populations densities and standing stocks are being used as primary assessment criteria, grabs or box corers are preferred to most coring methods because of their higher reliability in assessing meiofauna (Elmgren 1973).

PARAMETERS

Minimum;

(1) species occurrence;

Recommended:

(2) density and standing stock (wet weight biomass);

Preferred;

(3) population structure (i.e., length frequency distribution, life history stage, reproductive status);
(4) age and growth;
(5) standing stock (dry weight or ash free dry weight).

Species occurrence

When sampling is distributed intensively over time and space, or when dealing with non-motile fauna, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is indicative of a greater supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because infauna are often distributed in patches.

Density and standing stock

Density and standing stock are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the infauna and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. If feasible, infauna are best weighed fresh (damp) after excess water is toweled off. If preserved in formalin or alcohol, specimens can also be weighed in this manner but the standing stock values should *expressly* state that they are preserved wet weights and the time out of the preservative should be standardized because drying can be quite rapid under these circumstances.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are extremely labor- and time-intensive measures for infaunal biomass estimates and are generally not warranted unless production or some other sensitive bioenergetic indices are intended.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Population structure for may infaunal taxa may an important indication of the function of an estuarine habitat because critical assessment criteria such as reproduction and recruitment can only be assessed by data on reproductive state and life history stage, respectively. This is particularly the case for organisms with intermittent and episodic recruitment. At a minimum, life history stages should be classified for a subsample of the collections. In the case of most infauna, this will take form of three broad life history classes: (1) juvenile, recently settled through all instar stages; (2) subadult or non-reproducing; and (3) reproductive individuals.

Size, as measured by dimension and less commonly by weight, frequency distributions can often be used to differentiate different size classes within these life history stanzas, and can be important indications of different recruitment events, i.e., short-term age differences. Organism size can be measured by a variety of standard techniques and several "standards" are used for different infaunal taxa: (1) maximum valve length for bivalves; (2) total overall, stretched length for free-living polychaetes; and (3) anterior tip of the carapace (including rostrum if present) to tip of telson with the animal stretched to full length in the case of most microcrustacean like tanaids and amphipods. A sample for length measurements must be completely random and of sufficient size to represent the distribution; typically, a **minimum** of 25 to 50, and often 100, are required depending upon the variability.

Age and growth

Although similar to size distributions, age and growth data are some of the most optimal parameters to assess habitat quality. Not only will this indicate the age at which the population likely recruited to the habitat, but these data can also indicate the relative productivity of these organisms. Both bivalves and gastropods can be aged by analysis of thin sections of their shells; some bivalves can actually be aged by external annual striations.

RECOMMENDED SAMPLING METHODOLOGY

Method: emergent marsh, mudflat, sandflat and gravel-cobble habitats, **excavation within benthic quadrat**; nearshore subtidal soft- and hard-bottom, **benthic quadrat in combination with airlift (Venturi) suction sampler**; all samples washed through **1.0-mm mesh sieve**

Diver-controlled airlift or Venturi samplers commonly used for sampling in unconsolidated sediments (e.g., Brett 1964; Barnet and Hardy 1967; Christie and Allen 1972; Tunberg 1983; Benson 1989) can be used effectively in conjunction with destructive quadrat sampling. In this situation, the air-lift sampler is used to excavate the area inside a quadrat (size may vary between 0.25 m^2 and 1.0 m^2, depending upon the size, density and depth of the infaunal organism being monitored) to a standard depth (e.g. 12 cm to 25 cm) and the infauna are retained in the collection bag, which is usually constructed of 0.5-mm to 7.0-mm mesh netting. With extremely large fauna such as *Mya arenaria*, they may be removed by hand by the diver during or after the excavation of the quadrat area.

Unit: **0.25 m^2 excavated to 25 cm depth**

As for surface epifauna, the sizes of sampling quadrats used to assess sedentary infauna vary depending upon the size, density and distribution of the attributes being sampled. The large sizes of many of the attribute organisms, particularly the bivalves, suggests that the 0.25-m^2 quadrat size and deep penetration would be necessary to effectively sample their populations. Obviously, the size of the quadrat to be used for monitoring many attributes will be set by the larger or rarer attribute, but staged sampling may be considered in the case of extremely divergent attributes (see Sampling Design in Estuarine Habitats).

Periodicity and Frequency:	**quarterly, e.g., February, May, August, November, to capture recruitment events**

The Puget Sound Protocols (Puget Sound Estuary Program 1990b) suggest that subtidal benthic macroinvertebrates be sampled in April and May. However, there is other evidence that this period would encapsulate the primary spawning and recruitment events for many Puget Sound macroinvertebrate taxa, and thus introduce considerable variance in the estimation of organism density and standing stock. In a compilation of spawning and reproduction of benthic macroinvertebrates from Puget Sound compiled by Shimek (1990), it was illustrated that spawning and reproductive behavior tends to be more concentrated between April to August, and peak settlement occurred from June through August. The results of this compilation was compared to selected benthic infauna data for January, June and August. This comparison indicated that, although differences were not significant, statistical variation tended to be lowest in January for most taxa. This pattern is presumably due to the early mortality of organisms recruiting to the benthos in the prior spring or summer, and accompanying stabilization of organism densities. Only in the case of arthropods was the coefficient of variation greater in January than in one of the other two months, and the disproportional representation of pagurid crabs (which reproduce in the winter) may have biased this result.

Given these results, we recommend seasonal (quarterly) sampling in order to capture the potentially different recruitment events for diverse taxa. This could be abbreviated to twice yr^{-1} for specific attribute taxa if sufficient data is available on their life history patterns to identify representative pre-recruitment and post-recruitment sampling seasons.

Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**, in intertidal habitats distributed randomly within each tidal elevation strata (transect); **at least three tidal elevations** should be sampled in intertidal habitats, e.g., (1) between +3 ft and +4 ft MLLW; (2) between 0 ft and +1 ft MLLW; and, (3) between -4 ft and -3 ft MLLW

As in the cases of other quadrat studies, replication will depend in part upon the relationship between the quadrat dimension and the areal extent of habitat being monitored. Typical quadrat replication ranges from five to 25 samples. Benthic grab samples are often not replicated (n = 1) but many of the historical studies in Puget Sound have involved four to five samples and this is the recommended sample size established by the Puget Sound Estuary Program (1990b), and we recommend five for reasonable statistical analyses.

All other sampling considerations should be adopted in accordance with the Puget Sound Protocols (Puget Sound Estuary Program 1990b).

ALTERNATIVE PROCEDURES

(1) Benthic grabs:

The benthic equivalent to ring nets in sampling zooplankton is the benthic grab for sampling sedentary infauna in moderate (e.g., below diving depths of ~30 m) to deep depths; grabs are commonly employed from boats and usually require powered spools. Over the decades of benthic grab sampling, a plethora of different types and modifications have evolved (see Eleftheriou and Holme 1984). Word (1977) provided a synopsis and comparison of just seven of the more common devices used for assessing benthic infauna which are important as prey of fishes; his evaluation was based on consistency of sampling area, consistency of sampling below the depth where the majority of the species and specimens occur, minimum surface disturbance caused by pressure waves and minimum disturbance due to leakage. Using these criteria for evaluation, Word concluded that the chain-rigged 0.1-m^2 Van Veen and the 0.1-m^2 Smith-McIntyre grabs were preferable, and that the Van Veen was preferred overall because it was slightly more effective in capturing surface-dwelling microcrustacea and was simpler to use. Most Van Veen grabs are now modified by a trap door or screen to minimize the pressure wave effect. Modified Van Veen grabs are available which sample a smaller surface area (e.g., 0.025 m^2) but these should not be used unless the larger, deeper burrowing infauna are not attributes to be monitored.

(2) Benthic cores:

In intertidal habitats, or sometime also subtidal habitats, benthic cores are easier to utilize for sampling sedentary infauna. These are simply cylinders which are open-ended and are pushed or driven to a standard depth into the substrate. When the sediment is extremely unconsolidated, it may be necessary to excavate around the core and place a shovel blade beneath it before removal; in muds and more consolidated sediments, the suction is usually sufficient to contain the core sample within the corer. It is extremely important to avoid disturbance of the sediment surface when placing the core, especially when deploying under water. The diameter of the core will depend upon the taxa being monitored. Small cores are almost mandatory when assessing extremely small, dense taxa such as nematodes but larger cores are required for larger taxa which typically occur at lower densities, such as most of the bivalves. Word (1977) used 5.4-cm diameter cores to evaluate the performance of the various grab samplers. In the Seahurst Baseline Studies, Thom et al. (1984) used two sizes and penetration depths, a 2.74-mm core inserted 10 cm deep and a 14.1 cm core inserted 30 cm deep, to sample small and larger infauna, respectively. In the intertidal and shallow subtidal benthos studies of Nyblade (1979) and Webber (1980), 500-cm^2 core 15 cm deep was utilized.

CASE STUDIES

Quadrat excavations, surface scrapes and cores were used in intertidal and shallow subtidal investigations in Puget Sound by Houghton (1973) and along the Strait of Juan de Fuca by Nyblade (1979) and Webber (1980). Simenstad et al. (1988b) describes the use of an air-lift suction pump to quantitatively sample deep-burrowing bivalves within a 0.25-m^2 quadrat in shallow subtidal sand and eelgrass habitats in Neah Bay. The modified 0.1-m^2 Van Veen grab was used to sample subtidal infauna in the extensive Seahurst Baseline Study in central Puget Sound (Word et al. 1984); the smaller 0.025-m^2 modified Van Veen was used to sample small infauna in the Simenstad et al. (1988) studies of Neah Bay. Benthic cores of various sizes have been used to sample intertidal infauna and the most extensive database is probably that of Thom et al. (1984) from the Seahurst Bight area of central Puget Sound. Thom et al. (1988) have also utilized a 5.1-cm core inserted 10 cm deep to sample benthic infauna in intertidal habitats of the restored Lincoln Avenue Wetland in the Puyallup River estuary.

ACTIVE INFAUNA

HABITAT OCCURRENCE

Eelgrass;	*Callianassa californiensis*
Mudflat;	*Callianassa californiensis*
Nearshore Subtidal Soft Bottom;	*Callianassa californiensis*
Sandflat;	Callianassidae (unid.)

CHARACTERISTICS

Active infauna are exclusively the deep-burrowing, active ghost shrimp. Their deep burrows and rapid digging capability makes them extremely difficult to sample properly.

PARAMETERS

Minimum;
(1) species occurrence;

Recommended;
(2) density and standing stock (wet weight biomass);

Preferred;
(3) population structure (e.g., size, sex and reproductive state).

Species occurrence

When sampling is distributed intensively over time and space, or when dealing with non-motile fauna, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is indicative of a greater supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because infauna are often distributed in patches.

Density and standing stock

Density and standing stock are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the infauna and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. If feasible, infauna are best weighed fresh (damp) after excess water is toweled off. If preserved in formalin or alcohol, specimens can also be weighed in this manner but the standing stock values should *expressly* state that they are preserved wet weights and the time out of the preservative should be standardized because drying can be quite rapid under these circumstances.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are extremely labor- and time-intensive measures for infaunal biomass estimates and are generally not warranted unless production or some other sensitive bioenergetic indices are intended. As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Some investigators have found the density of borrow openings visually evident at the sediment surface to be a reliable indicator of subsurface shrimp density. Although this may be feasible in other situations, it has been found to be unreliable in recent, intensive studies of *Callianassa californiensis* in Puget Sound (Weitkamp 1991) and is not recommended as an optional protocol.

Population structure

Population structure for may infaunal taxa may an important indication of the function of an estuarine habitat because critical assessment criteria such as reproduction and recruitment can only be assessed by data on reproductive state and life history stage, respectively. This is particularly the case for organisms with intermittent and episodic recruitment. At a minimum, life history stages should be classified for a subsample of the collections. In the case of most infauna, this will take form of three broad life history classes: (1) juvenile, recently settled through all instar stages; (2) subadult or non-reproducing; and (3) reproductive individuals.

Size, as measured by dimension and less commonly by weight, frequency distributions can often be used to differentiate different size classes within these life history stanzas, and can be important indications of different recruitment events, i.e., short-term age differences. Organism size can be measured by a variety of standard techniques and several "standards" are used for different infaunal taxa: (1) maximum valve length for bivalves; (2) total overall, stretched length for free-living polychaetes; and (3) anterior tip of the carapace (including rostrum if present) to tip of telson with the animal stretched to full length in the case of most microcrustacean like tanaids and amphipods. A sample for length measurements must be completely random and of sufficient size to represent the distribution; typically, a **minimum** of 25 to 50, and often 100, are required depending upon the variability.

RECOMMENDED SAMPLING METHODOLOGY

Method:	intertidal habitats, **benthic cores**; shallow subtidal habitats, **benthic cores evacuated with airlift (Venturi) samplers**; samples washed through **5.0-mm mesh sieve**

Studies of burrowing shrimp in other regions have used coring devices on the order of 10-cm diameter x 50-cm deep in size. Dumbauld et al. (1988), however, consider the small dimension of the core relative to the usually complex shrimp burrow morphology and the lack of consistent sediment core retention to be a disadvantage of this method; they recommend either hand pumps or excavation of a larger core sample. In the latter case, those investigators have used a 40-

cm diameter x 60-cm corer in combination with a Venturi pump system and hand excavating the sediment core with a small shovel. In shallow subtidal habitat, the diver-controlled airlift or Venturi samplers commonly used for sampling in unconsolidated sediments (e.g., Brett 1964; Barnet and Hardy 1967; Christie and Allen 1972; Tudhope and Scoffin 1984; Tunberg 1983; Benson 1989) could feasibly be used to sample deep-burrowing shrimp in conjunction with quadrat sampling or if a box is inserted into the sediment to form the sampling volume (as in Brett 1964). In this situation, the air-lift sampler would be used to excavate the area inside a quadrat to a standard depth (e.g., 1 m) and the shrimp retained in a collection bag constructed of 0.5-mm mesh netting. The active digging, swimming shrimp may, however, be too evasive to be effectively sampled with this method.

Unit:	**0.25 m^2, excavated to 1.0 m**
Sampling Periodicity and Frequency:	**twice yr^{-1}, in April and September** to sample pre- and post-recruitment population structure and reproduction
Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**, in intertidal habitats distributed randomly within each tidal elevation strata (transect); at least **two tidal elevations** should be sampled in intertidal habitats, e.g., (1) between +3 ft and +4 ft MLLW; and (2) between 0 ft and +1 ft MLLW

There are few studies upon which to base any recommendation for replication. We suggest in this instance that a pilot study be conducted to determine the necessary replication level for statistical precision.

ALTERNATIVE PROCEDURES

(1) Hand suction pump:

Many surveyors of ghost/mud shrimp have adopted a hand suction pump which is derived from sport (bait) shrimp harvesters. Modified from the design of hand bilge-pumps, this device is usually made of PVC plastic pipe 5-cm to 8-cm in diameter, approximately 1-m long, and operates by pulling a plunger attached to a rubber gasket (ball) up through the pipe with the suction end enclosing a shrimp burrow; pushing the plunger back down expels the shrimp from the sampler if one has been captured. To our knowledge, no one has conducted a field verification of the sampling efficiency of the hand suction pump, and it is dubious that it would be effective for more than documentation of *Callianassa* presence or absence.

(2) Hydraulic infusion pumps:

The most common method used specifically for sampling *Callianassa* is the infusion of water deep (at least 1 m) into the bottom to liquify the sediments and force the shrimp to the surface in the "boil" of surfacing water. This is duplicated from the common commercial method of harvesting ghost and mud shrimp and is conducted during low tide exposure of sand and mudflats where shrimp densities are on the order of >100 m^{-2}. A gasoline-powered pumps injects approximately 50-100 gal min^{-1} of water drawn from a nearby channel. The shrimp are typically dipped up with dip nets. No standardization or quantification of surface area being liquified has been attempted, and estimation of density and standing stock may be a problem. This method, however, does provide a large number of shrimp from which population structure data could be obtained.

(3) Deep-penetrating box corers:

Although they have not been utilized for these taxa, large (0.5-m^2 to 1.0-m^2) box corers may have the ability to sample certain active infauna, but it is questionable that they would be able to sample much deeper than 0.5 m. Such penetration would be insufficient, for instance, for sampling *Callianassa californiensis* because this species may burrow 0.8 to 1.0 m deep in sediments. Box corers are also notoriously poor samplers in loose, unconsolidated sediments (e.g., sand) that tend to drain out of the sampler when hauled to the surface. Box corers could, however, be used in combination with SCUBA diver controlled airlift (Venturi) samplers to evacuate the organisms contained within a box corer at the bottom.

CASE STUDIES

To our knowledge, the only published account of data for quantitative sampling of ghost or mud shrimp in Puget Sound or coastal estuaries are those of Dumbauld et al. (1988) and Weitkamp (1991). Unpublished data may also be available from the Washington Department of Fisheries, Point Whitney Shellfish Laboratory (J. Odell, WDF, pers. comm.). Posey (1986) provides some quantitative data on burrowing shrimp from Oregon estuaries.

EPIBENTHIC PLANKTERS

HABITAT OCCURRENCE

Eelgrass;	*Caprella laeviuscula* *Corophium* spp. (males) *Corophium* spp. *Cumella vulgaris* *Eogammarus confervicolus* *Eogammarus* spp. *Harpacticus spinulosus* *Harpacticus* spp. *Harpacticus uniremis* *Ischyrocerus* spp. *Pontogeneia rostrata* *Tisbe* spp. *Zaus* spp.
Emergent Marsh;	*Corophium salmonis* *Corophium spinicorne* *Corophium* spp. *Cumella vulgaris* *Eogammarus confervicolus* *Eogammarus* spp. *Gnorimosphaeroma oregonese*
Gravel-Cobble;	*Ampithoe* spp. *Anisogammarus pugettensis* *Corophium* spp. *Cumella vulgaris* *Eogammarus* spp. *Eusiroides* spp. *Gnorimosphaeroma oregonese*
Mudflat;	*Atylus* spp. *Corophium salmonis* *Corophium* spp. *Cumella vulgaris* *Eogammarus confervicolus* *Eogammarus* spp. *Eohaustorius washingtonianus* *Harpacticus uniremis* *Megalorchestia pugettensis* *Pancolus californiensis* *Tisbe* spp.
Nearshore Subtidal Soft Bottom;	*Orchomene minuta* *Photis lacia*

Sandflat;	*Cumella vulgaris* *Eogammarus confervicolus* *Megalorchestia pugettensis* *Phoxocephalidae* spp.

CHARACTERISTICS

Epibenthic plankters are very small macrofaunal or meiofaunal (e.g., 0.065-mm to 2-mm long) organisms, predominantly crustaceans, which live at the interface between the bottom substrate and the water column, either in the very surface layer or in the benthic boundary layer. These include predominantly harpacticoid copepods, gammarid amphipods, cumaceans, and isopods; one tanaid (*Pancolus californiensis*) occurs. In many cases, this is an operational definition and assemblage which is formed simply by turbulent processes in the boundary layer and entrainment from organisms sinking out of the water column. However, most of the organisms identified as attributes in this group are associated with the surface layer of unconsolidated sediments and benthic epiphytes but which are described to actively (behaviorally) emerge a short distance into the adjoining water column. Sampling methodologies recommended are those which cause minimal disruption of and do not penetrate above and below the benthic boundary layer (thus, air-lift suction samplers are not recommended), sample a known area of the benthic surface reliably (thus, samplers which contact the bottom intermittently are not recommended), and which include a long precedence of comparable data in the region.

PARAMETERS

Minimum;
(1) species occurrence;
(2) settled volume;

Recommended;
(3) density and standing stock;

Preferred;
(4) population structure (e.g., naupliar, copepodid stages; ovigerous females); and
(5) diel and tidal stage sampling.

Species occurrence

When produced from an intensive sampling effort over time and space, or in the case of relatively non-motile organisms, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat or different microhabitats (e.g., depth strata) by reproductive individuals and different larval stages may a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because epibenthic plankters occurrence can be exceedingly patchy over space and time.

Settled volume

One straightforward, quantitative assessment which can be relatively analogous to total standing stock is settled volume. This is measured as the volume which the epibenthic plankters constitute when allowed to settle in a graduated flask or cylinder and is normally reported in milliliters (ml).

Density and standing stock

Density and standing stock are preferred monitoring parameters because they are more definitive indicators of subtle differences in habitat quality. Habitat and microhabitat characteristics can effect density-dependent interactions between zooplankton and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. Wet weights of epibenthic plankters are usually obtained as preserved after excess preservative is absorbed; the time out of the preservative should be standardized because drying can be quite rapid under these circumstances, especially if an alcohol is used as the preservative.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours for fish). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are somewhat more labor- and time-intensive methods for epibenthic plankters but are a more precise measure and should be encouraged in production estimates are intended.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Epibenthic plankters population structure can an important indication of estuarine habitat function because verification of resident populations can only be determined through verification of reproduction and contiguous life history stages. At a minimum, life history stages should be classified for a subsample of the collections. In the case of most epibenthic plankters, this will take form of five broad life history classes: (1) nauplii; (2) copepodids; (3) nonreproductive females; (4) males; and (5) ovigerous females. If it is desirable to document these stages in more detail, the various naupliar and copepodid stages should also be distinguished.

Diel and tidal stage sampling

The occurrence of epibenthic plankters can vary appreciably over diel and tidal cycles due to actual behavioral migration through the water column. For this reason, monitoring focused on the identification of viable epibenthic plankter populations should document density, standing stock and population structure during different diel periods and tidal stages. Minimally, this should include sampling during (1) diurnal, nocturnal and crepuscular periods and (2) ebb and flood and slack tide stages.

RECOMMENDED SAMPLING METHODOLOGY

Methods:	**epibenthic suction pump**; all samples washed through **0.253-µm mesh sieve**

Efficient, quantitative sampling of epibenthic plankters has been a distinct challenge because of their small size, their discrete occurrence in the benthic boundary layer and their avoidance capabilities. Standard benthic samplers (e.g., cores, air-lift suction pumps, benthic grabs), even when modified to address these organisms, are either ineffective because they disturb the sediment surface extensively (often prior to actually enclosing a sample), produce a shock wave which the animals avoid, or make sample processing ambiguous because of the high level of contamination by infaunal organism of the same general taxa (e.g., interstitial harpacticoid copepods and gammarid amphipods). The following sampling techniques have been developed in this region specifically for sampling epibenthic plankters which are the preferred prey of juvenile salmon foraging in estuarine habitats and, as such, appear to sample these general assemblages efficiently.

Epibenthic suction pumps have been developed for sampling epibenthic organism in a variety of habitats, microhabitats, and conditions in Pacific Northwest estuaries. They all have the same basic design, in which a sampling cylinder with fine-mesh (usually 0.130-mm or 0.252-mm) screened ports is very slowly lowered to enclose an area of the bottom and segment of the adjoining benthic boundary layer. The water volume in this sampling cylinder is then evacuated with a water pump; all water entering the cylinder passes through the screened ports and is thus not contaminated. Usually three to five times the volume of the cylinder is pumped. The pumped water and epibenthos is captured and screened to one of several sieve sizes, or nested nets; the usual geometric mesh series is 0.130-mm, 0.253-mm and 0.500-mm and selection depends upon the specific taxa and life history stages which are to be monitored.

For large evasive epibenthic plankters, e.g., mysids and large amphipods, a large, gasoline-powered (13 L min.$^{-1}$) pump system has been used; that used by Simenstad and colleagues in the Columbia River estuary and other sites encompasses 0.1 m^2 of the bottom and (since it is 25 cm high) the sampling cylinder encloses 0.025 m^3; this system pumps water into a sampling apparatus in which the water gravity flows through three nested nets (see above). The system can be deployed from a small boat to depths of at least 10 m and does not require SCUBA diver assistance.

Another version of the same design is designed for the smaller, less evasive meiofauna. It incorporates a battery-powered bilge pump into the sampling cylinder and is thus portable and small enough to be handled by one person if necessary. This epibenthic pump has been used effectively in water depths approaching 2.5 m. The sampling cylinder encloses an area of 179 cm^2 of the sediment surface and is equipped with 0.130-mm ports; it is often mounted on a staff for ease of use and for control of placement of the sampling cylinder in waters >1 m deep.

Most of these sampling methods are employed along constant-elevation intertidal and shallow subtidal transects during brief (~1 hr.), discrete periods of the tidal cycle.

Unit:	meiofauna (e.g., harpacticoids and juvenile amphipods), **0.018 m^2**; small macrofauna (e.g., amphipods, cumaceans, isopods, **0.10 m^2**
Periodicity and Frequency:	**biweekly, March-June**

Most epibenthic zooplankters have very rapid population turnover rates, especially the harpacticoid copepods, which may turn over in two weeks. Thus, biweekly sampling is necessary to adequate represent densities and standing stock of taxa that are univoltine, e.g., have only one generation, and appear in only one ephemeral pulse.

Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=15**, in intertidal habitats distributed randomly within uniform microhabitats along tidal elevation strata (transect); **optimum tidal elevation = 0.0 ft (MLLW), with additional transects recommended at +2.0 ft, -2.0 ft, +4.0 ft and -4.0 ft MLLW tidal elevations** in that relative order of priority

Historical sampling of epibenthic plankters Pacific Northwest estuaries has varied from essentially no replication to 10 replicates. However, in a recent study of epibenthic crustaceans in mudflat and sandflat habitats, including many of the attribute taxa, the level of replication found necessary for statistical testing of treatment differences was found to be fifteen to twenty-five, depending upon the natural variability of the taxa (Simenstad et al. 1991). We suggest that fifteen replicates as a *minimum* number of replicates for monitoring these organisms. We also recommend that a mesh size for sample retention or sieving be 0.253-mm at the largest, and smaller mesh if early life history stages are to be monitored.

ALTERNATIVE PROCEDURES

(1) Amphipod traps:

Traps to sample epiphytal amphipods such as *Anisogammarus pugettensis* have been used in several estuarine studies. These are usually 0.500-mm mesh bags in which algae such as *Fucus* are enclosed and the bags are set at intertidal and shallow subtidal stations over a tidal cycle. Although they provide species occurrence data, there is no estimate of the area of habitat they effectively sample.

(2) Epibenthic sleds:

Epibenthic sleds or, in the European vernacular "sledges," have been used in the Atlantic for several decades to sample epibenthic crustaceans and, if large enough, can effectively sample the more evasive taxa such as the mysids *Alienacanthomysis macropsis* and *Neomysis mercedis*. The more quantitative sleds have opening and closing mechanisms and flow meters (Bossanyi 1951; Wickstead 1953; Beyer 1958; Frolander and Pratt 1962; Macer 1967; Higley et al. 1977) when deployed from boats and others are designed to be pushed in the intertidal (Colman and Segrove 1955) or by SCUBA-equipped divers (Sibert et al. 1977). Of these, the Sibert et al. (1977) sled is the only one which has been documented for quantitative use in this region. The larger, modified Higley et al. (1977) variable-height sled used in the Columbia River estuary (Simenstad 1984; Simenstad and Cordell 1985) to sample evasive macroinvertebrates (see Evasive Macroinvertebrates section, below) could be used in situations where water depths allow and where very large samples are desired, but this gear is basically too large and cumbersome for most shallow estuarine use. Yocum and Tesar (1980) also describe a similar sled which is small enough to be utilized effectively in shallow water habitats.

(3) Epiphyte sampling:

Recent investigations suggest that many of these organisms may be associated uniquely with epiphyte communities on eelgrass, kelps and rock (Simenstad et al. 1988a). To sample these communities, the epiphytes must be removed intact and with as little disturbance as possible (e.g., slowly and under water). In the case of eelgrass, whole blades or measured segments (if there is a consideration of difference in epibenthos in different epiphytes) of eelgrass can be slowly enclosed in a sampling bag or cylinder and preserved whole. In the laboratory, these can be repeatedly washed over fine-meshed sieves to remove the epibenthic organism. Parameters are usually related to the area of the substrate, including both sides of the eelgrass blades, and/or the standing stock of the epiphyte (normally dry weight).

CASE STUDIES

Epibenthic pump techniques as recommended have been utilized in a large variety of Puget Sound locations and estuarine habitats such as eelgrass meadows and mud/sandflats in northern Puget Sound (Simenstad et al. 1988a; Thom et al. 1989); eelgrass, sandflat, and gravel/cobble in central Puget Sound (Thom et al. 1984; Hiss et al. 1988); and in emergent marsh, mudflat, channel and gravel/cobble habitats (including restored/created habitats) in the Puyallup River estuary (Thom et al. 1986, 1989; Shreffler et al. 1990, 1991). These systems have also been used in coastal estuaries such as Grays Harbor (Simenstad and Eggers 1981; Cordell and Simenstad 1981), Willapa Bay (Simenstad and Cordell 1988) and the Columbia River estuary (Simenstad 1984; Simenstad and Cordell 1985), as well as coastal embayments such as Neah Bay (Simenstad et al. 1988b).

PELAGIC ZOOPLANKTON

HABITAT OCCURRENCE

Water Column;	Decapoda (larvae) *Cancer* spp. (zoea)
Emergent Marsh;	*Daphnia* spp.
Gravel-Cobble;	Decapoda (larvae)
Mudflat;	*Callianassa californiensis* (larvae) *Upogebia pugettensis* (larvae)
Water Column;	*Acartia californiensis* *Acartia clausi* *Callianassa californiensis* (larvae) *Cancer* spp. (larvae) *Cancer* spp. (megalops) *Corycaeus anglicus* *Corycaeus* spp. *Daphnia* spp. Decapoda (larvae) *Epilabidocera amphitrites* *Epilabidocera* spp. *Euphausia pacifica* *Eurytemora affinis* *Oikopleura* spp. *Oithona similis* *Parathemisto pacifica* *Pseudocalanus minutus* Teleostei (larvae)

CHARACTERISTICS

Pelagic zooplankton are those organisms which occupy the water column and are passive or only weakly-swimming. Due to low motility, they are prone to the advection effects of currents and are often found in aggregations along tidal fronts. However, they can also have the capability in water columns with no vertical mixing and turbulence for vertical migrations over surprising depth ranges. They can be both permanent members of the plankton (holoplankton) or temporary members (meroplankton) before they metamorphose and recruit to benthic habitats. Except for larvaceans (*Oikopleura* spp.) and fish (Teleostei) larvae, these are exclusively crustaceans, including predominantly calanoid and cyclopoid copepods, as well as several decapods (larvae), euphausiids, hyperiid amphipods, and cladocerans.

PARAMETERS

Minimum;

(1) species occurrence;

(2) settled volume;

Recommended:
(3) density and standing stock;

Preferred;
(4) population structure (e.g., naupliar, copepodid stages; ovigerous females); and
(5) depth-stratified diel sampling.

Species occurrence

When produced from an intensive sampling effort over time and space, or in the case of comparatively non-motile taxa, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat or different microhabitats (e.g., depth strata) by reproductive individuals and different larval stages may a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because zooplankton occurrence can be exceedingly patchy over space and time.

Settled volume

One straightforward, quantitative assessment which can be relatively analogous to total standing stock is settled volume. This is measured as the volume which the zooplankton constitute when allowed to settle in a graduated flask or cylinder and is normally reported in milliliters (ml).

Density and standing stock

Density and standing stock are preferred monitoring parameters because they are more definitive indicators of subtle differences in habitat quality. Habitat and microhabitat characteristics can effect density-dependent interactions between zooplankton and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. Wet weights of zooplankton are usually obtained as preserved after excess preservative is absorbed; the time out of the preservative should be standardized because drying can be quite rapid under these circumstances, especially if an alcohol is used as the preservative.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours for fish). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are somewhat more labor- and time-intensive methods for zooplankton but are a more precise measure and should be encouraged in production estimates are intended.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Zooplankton population structure can be an important indication of habitat function because verification of resident populations can only be determined through verification of reproduction and contiguous life history stages. At a minimum, life history stages should be classified for a subsample of the collections. In the case of most zooplankton, this will take form of five broad life history classes: (1) nauplii; (2) copepodids; (3) nonreproductive females; (4) males; and (5) ovigerous females. If it is desirable to document these stages in more detail, the various naupliar and copepodid stages should also be distinguished.

Depth-stratified diel sampling

Despite a planktonic lifestyle, vertical distribution of zooplankton can vary considerably over diel cycles due to actual behavioral migration through the water column. For this reason, monitoring of viable zooplankton populations should document density, standing stock and population structure in different depth strata of the water column over diel periods. At a minimal, this would entail sampling during representative nocturnal and diurnal times, but would optimally cover crepuscular periods as well.

RECOMMENDED SAMPLING METHODS

Method: **paired (bongo) nets, towed at fixed depths equipped with 333-µm mesh**

Many zooplankton and ichthyoplankton collections have been made in Puget Sound using paired nets in a "bongo" configuration. The most common bongo net configuration uses a 60-cm diameter mouth opening equipped with 0.333-mm or 0.500-mm mesh nets, and often with one of each due to the efficiency of each mesh type for zooplankton versus ichthyoplankton. In estuaries, which may have strongly-stratified water masses, tows should be conducted over a fixed time (5- to 10-min.) at a constant depth (determined by wire angle and length or by time-depth recorder). Although fixed sampling time will somewhat standardize the volume of water passing through the nets (as long as vessel speed is constant), one or (preferably) two flow meters should be installed inside the net openings to provide precise sampling volumes. In some situations, such as strongly mixed water masses, an oblique haul from depth to the surface, or a combination of the two as a stepped tow which involves continuous towing at several depths may be appropriate. Sampling should be conducted in surface layer, as many attribute taxa are found concentrated in this microhabitat. Sampling "offboard" using a davit or boom is also preferred in order to avoid effects of the vessel's propwash.

Unit: **60-cm dia. net openings**

Collection mesh and sieve mesh sizes can vary by attribute, but 0.333-mm is used often for zooplankton in the same general size ranges as the identified attributes; 0.500-mm may be used if only larger taxa are being monitored.

Periodicity and Frequency: **monthly, March-August**

Statistical Considerations (Replication): in lieu of statistical predetermination using pilot study or historical data; **n=5**, standardized or, if determining vertical migration, stratified by time of day

Even when using multiple-net sampling methods, it is worthwhile to obtain replicated pelagic zooplankton samples because of the usual heterogeneous spatial distribution of these organisms. Most studies take three to five samples. Optimum sampling times would be associated with dawn and dusk periods, when vertical migrators are nearest the surface layer. Estuarine sampling, however, should be stratified more by tidal stage, and encompass at least flood and ebb slack tides during both neap and spring tidal series.

ALTERNATIVE PROCEDURES

(1) Ring and other single towed nets:

Ring plankton nets have been the archetypical zooplankton sampling gear throughout the contemporary era of marine science. Their inherent problem, however, is that they are difficult to use in a quantitative manner and are extremely vulnerable to contamination when used to sample subsurface depth strata. For this reason, we recommend using the "Puget Sound" net (Miller et al. 1984), which was developed specifically for efficiently sampling large, relatively evasive zooplankton in this region. This net is a vertically-hauled closing net which has a 1-m diameter mouth opening and is messenger operated. While the net's mesh size can vary according to the size of the attribute(s) specifically desired, the net equipped with 0.333-mm mesh has been used to sample most taxa and life history stages of concern as attributes which are preferred prey taxa of fishes.

Rey et al. (1988) describe a towed net which is designed to sample zooplankton just beneath the water surface in very shallow estuarine habitats, such as over tidal flats and in emergent marshes. The net was 91.4 cm deep X 20.3 cm wide X 167.6 cm long and was constructed of either 0.202-mm or 0.065-mm mesh. In their comparison with a shallow water pumping apparatus (see below), the authors recommended combining the two gears to obtain optimum sampling efficiency.

(2) Multiple, opening-closing net systems:

An alternative to using consecutive hauls to sample different depth strata with a single opening-closing net is a multiple net sampling devise. One such sampler is that described by Bé (1962), as modified by Weikert and John (1981) to carry five nets programmed to open and close at predetermined depths.

(3) Vertical profiling pumps:

Pumps which suction water from discrete depth strata, often simultaneously, can be effective in sampling zooplankton, although there is some question of zooplankton detection and evasion of the velocity field in small aperture (e.g., 4-mm) suction intakes (Singarajah 1969, 1975). Miller and Judkins (1981) compile a number of advantages of vertical pump systems, including: (1) reliable measurement of the volume filtered under all conditions of clogging, ship velocity, and depth of sampling; (2) reliable control of the filtration process with the potential for sequential use of several mesh sizes; (3) absolute reliability of the "opening-closing" mechanism when vertically stratified samplers are desired; (4) simplicity in control and monitoring of the depth of sampling; (5) capability for sampling all of the lower trophic levels, all of the physical parameters, and all of the nutrient, pollutant, and trace element concentrations in exactly the same water at exactly the same time; and (6) capability to take long sets of sequential samples of analysis of small-scale distribution. Due to their low filtration rates, relative to towed nets, pumps may only be useful were plankton densities can be assessed in at most 5 to 10 m^3 of water. Miller and Judkins (1981) describe several pump systems which have been proven effective in sampling coastal and estuarine zooplankton; this reference would be most useful in examining the different options for a pump system to sample zooplankton in particular situations. Although to our knowledge they have not been used in this region, Coughlan and Fleming (1978) and Rey et al. (1987) also describe pump sampling apparatii specifically designed for sampling in very shallow estuarine habitats such as emergent marsh channels; this apparatus should be investigated for these special situations in which the larger pump systems would not be operable. In their comparison with a shallow water pumping apparatus (see below), the authors recommended combining the two gears to obtain optimum sampling efficiency.

(4) Plankton purse seine:

A plankton purse seine which was found under some circumstances to be more effective in sampling fish larvae was described by Murphy and Clutter (1972). Similar to the purse seines described for sampling motile fishes (see below), this net is 30-m long and relatively shallow (6.4 m) and is set and pursed in a circle which was found during tests to enclose 356 m^2; the net, however, is constructed of 0.333-mm mesh plankton netting. This could be an effective sampling technique for the more evasive components of the zooplankton community, particularly larval fishes, but its large aspect relative to water flow through it may cause tremendous problems under even minor current and wind conditions.

CASE STUDIES

Both the "Puget Sound" and Bé multiple net zooplankton sampler have been used in Puget Sound, and particularly in Hood Canal, by Frost (1985, 1988) and his students and coworkers (Ohman 1985, 1986; Runge 1985). Miller et al. (1977) and Fresh et al. (1979) provide quantitative data using a bongo net in southern and northern Puget Sound, respectively, and Chester et al. (1979) provide similar data for the Strait of Juan de Fuca. One of the few documentations of zooplankton sampling with a vertical profile pumping system is that of Beers et al. (1967).

NEUSTONIC AND DRIFT INVERTEBRATES

HABITAT OCCURRENCE

Emergent Marsh;	Araneae (unid.)
	Chironomidae (unid.)
	Diptera (unid.)
	Ephydridae (unid.)
	Heleidae (unid.)
Water Column;	Insecta (unid.)
	Insecta (larvae)
	Scoloidea (unid.)

CHARACTERISTICS

A distinct assemblage of organism is comprised of adult and larvae insects which are deposited onto or emerge into the surface layer, and certain other aquatic insects and crustaceans which spend most of their time in the surface layer of the water column.

PARAMETERS

Minimum;
(1) species occurrence;

Recommended;
(2) density and standing stock; and

Preferred;
(3) diel emergence.

Species occurrence

When produced from an intensive sampling effort over time and space, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat or different microhabitats (e.g., depth strata) by reproductive individuals and different larval stages may a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because neustonic invertebrates occurrence can be exceedingly patchy over space and time.

Density and standing stock

Density and standing stock are preferred monitoring parameters because they are more definitive indicators of subtle differences in habitat quality. Habitat and microhabitat characteristics can effect density-dependent interactions between zooplankton and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. Wet weights of neustonic invertebrates are usually obtained as preserved after excess preservative is absorbed; the time out of the preservative should be standardized because drying can be quite rapid under these circumstances, especially if an alcohol is used as the preservative.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours for fish). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are somewhat more labor- and time-intensive methods for neustonic invertebrates but are a more precise measure and should be encouraged in production estimates are intended.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Diel sampling

The occurrence and distribution of neustonic invertebrates can vary appreciably over diel cycles due to actual behavioral migration through the water column, which often occurs specifically during crepuscular or nocturnal periods. For this reason, monitoring focused on the identification of neuston should document density, standing stock and population structure over diel periods. At a minimum, this would entail sampling during representative nocturnal and diurnal times, but would optimally cover crepuscular periods as well.

RECOMMENDED SAMPLING METHODOLOGY

Method: **neuston net equipped with 253-µm mesh net**

Neuston nets are essentially modified plankton nets which are designed to float on the surface of the water with their mouth opening sampling just the water surface layer. Usually these are hand towed, held in an active current, or towed outboard of a small boat. Maintenance of a constant depth (position at which the net sits in the water) and *in situ* (within the net) or simultaneous measurements of current velocities are required in order to determine the sampling volume. Hodson et al. (1981) describes one example of a surface microlayer/neuston net which is designed for estuarine habitats. A more elaborate system of two 0.36-m^2 nets operated in front of a small boat is described by Miller (1973), and would be highly effective for long-shore and open water transect sampling.

Unit: **0.025 m^2 mouth opening**

Periodicity and Frequency: **monthly, during discrete freshwater and tidal flow phases**

Sampling should be conducted during representative periods of tidal inundation (flood tide) and exposure (ebb) in order to evaluate neuston import and export, respectively, into/out of the habitat.

Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**. In the few studies of estuarine neuston which have taken place in the Pacific Northwest, the sample size has varied from three to five.

ALTERNATIVE PROCEDURES

(1) Emergence traps:

Unlike neuston nets, which are designed to sample what is available in the neuston, emergence traps sample insects and crustaceans which enter the neuston. They can either entrain only the surface, in sampling the insects which emerge as adults, or they can encompass the whole water column in order to sample those organisms which migrate to the water column but don't necessarily leave it. Youngbluth (1982) describes three traps to sample migrating demersal zooplankton which could be modified to encompass the entire water column and surface layer. However, to our knowledge, only the emergence trap described by LeSage and Harrison (1979) has been employed in the Pacific Northwest. These traps cover 0.5 m^2 and are constructed of 0.202-mm mesh netting.

CASE STUDIES

While neustonic organisms are recognized to comprise a unique, important component of estuarine habitats, the only discrete documentation of this assemblage in this region of which we are aware is that of the neuston entering the restored Lincoln Avenue Wetland in the Puyallup River estuary (Schreffler et al. Submitted a & b). Whitehouse et al. (1989) provide data from emergence trap deployment in both natural and developed estuarine habitats in British Columbia. Recent studies (Thom et al., in prep.) have utilized both neuston nets and emergence traps in the same restored wetland system to document fish prey availability and production over a diel period.

EVASIVE MACROEPIFAUNA

HABITAT OCCURRENCE

Eelgrass;	*Alienacanthomysis macropsis*
	Cancer magister
	Crangon spp.
	Hemigrapsus nudis
	Hippolytidae (unid.)
Emergent Marsh;	*Cancer productus*
	Crangon spp.
	Hemigrapsus spp.
	Neomysis mercedis
	Orchestia traskiana
	Pacificastacus leniusculus
Gravel-Cobble;	*Cancer magister*
	Cancer spp.
	Crangon spp.
	Hemigrapsus nudis
	Hemigrapsus spp.
	Heptacarpus spp.
	Hippolytidae (unid.)
	Neomysis mercedis
	Pacificastacus leniusculus
	Pugettia gracilis
Mudflat;	*Archaeomysis grebnitzkii*
	Cancer spp.
	Crangon spp.
	Hemigrapsus spp.
Nearshore Subtidal Soft Bottom;	*Crangon* spp.
	Hemigrapsus spp.
	Neomysis mercedis
Nearshore Subtidal Hard Substrate;	*Eualus* spp.
	Heptacarpus spp.
	Lebbeus spp.
	Octopus spp.
	Pandalus danae
	Spirontocaris spp.
Sandflat;	*Cancer magister*
	Crangon spp.

Water Column;	Gonatidae (unid.) *Loligo opalescens*

CHARACTERISTICS

Evasive macroepifauna include those macroinvertebrates which are associated with the bottom, either on it or epipelagic in moving up into the water column immediately above it, and which are highly motile. They therefore have the ability to avoid static and slow-moving sampling gears.

PARAMETERS

Minimum;
(1) species occurrence;

Recommended:
(2) density and standing stock (wet weight biomass);

Preferred;
(3) population structure (e.g., length frequency distribution, life history stage, reproductive status).

Species occurrence

When produced from an intensive sampling effort over time and space, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because macroinvertebrate occurrence is typically patchy over space and time.

Density and standing stock

Density and standing stock are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the these macroinvertebrates and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. Like fish, macroinvertebrates are usually weighed fresh (damp) after excess water is toweled off. Macroinvertebrates preserved in formalin or alcohol can also be weighed in this manner but the standing stock values should *expressly* state that they are preserved wet weights and the time out of the preservative should be standardized because drying can be quite rapid under these circumstances.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours for fish). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are extremely labor- and time-intensive measures for macroinvertebrate biomass estimates and are generally not warranted unless production or some other sensitive bioenergetic indices are intended.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Macroinvertebrate population structure may an important indication of the function of an estuarine habitat because critical assessment criteria such as reproduction and recruitment can only be assessed by data on reproductive state and life history stage, respectively. At a minimum, life history stages should be classified for a subsample of the collections. In the case of most macroinvertebrates, this will take form of three broad life history classes: (1) juvenile, recently settled through all instar stages; (2) subadult or non-reproducing; and (3) ovigerous females; in addition, mating pairs (for crabs) and recently molted individuals may also be recorded.

Length, and less commonly weight, frequency distributions can often be used to differentiate different size classes within these life history stanzas, and can be important indications of different recruitment events, i.e., short-term age differences. Macroinvertebrate length can be measured by a variety of standard techniques and several "standards" are used for different macroinvertebrates in this region: (1) maximum carapace width (excluding lateral teeth) is commonly used for all crabs; (2) tip of rostrum to the tip of the telson for shrimps and mysids; and (3) anterior tip of the carapace (including rostrum if present) to tip of telson with the animal stretched to full length in the case of amphipods. A sample for length measurements must be completely random and of sufficient size to represent the distribution; typically, a *minimum* of 25 to 50, and often 100, are required depending upon the variability.

RECOMMENDED SAMPLING METHODOLOGY

Method: emergent marsh and gravel-cobble habitat, **SCUBA transect observations**; mudflat and sandflat habitats, **sinking beach seine**; eelgrass, and nearshore subtidal soft-bottom habitats, **bottom (staffbeam) trawl**; nearshore subtidal hard substrate habitat, **SCUBA quadrat sampling with airlift (Venturi) sampler**; water column, **mid-water trawl**

(1) SCUBA transect observations and sampling:

While there are variants on the general methodology, all SCUBA transect observations basically involve SCUBA-equipped divers recording observations or taking quadrat samples along measured underwater "strip" transects on the species, size, activity, etc. of fish and macroinvertebrates. Transects can be either permanently fixed (e.g., with markers or line), so they can be consistently revisited, or impermanent and measured before or after the SCUBA observations. The length of permanent transects are usually established by the length of the line or by the distances between markers as measured *in situ*; impermanent transects are usually measured as between buoys which are typically released at the beginning and the end of the dive and the distance measured directly or with navigation aids. While the permanent transect, or some similar modification (e.g., a temporary transect line) establishes the exact transect length, the impermanent transect should be considered a minimum distance, since dives seldom involve linear paths.

In the case of strip transects (i.e., continuous observations), the area swept is estimated as the transect distance multiplied by a transect path width, which can either be a (minimal) set distance or estimated as the visibility (at that time) for effective observation of the fishes; given the variable visibility in Puget Sound estuaries, the latter method is recommended for optimum standardization of data collection. A common method of establishing effective transect width is to measure the distance at which a standard target (Secchi disk) disappears and reducing this by a factor (usually 1/2) to account for the visibility required to make reliable observations (see Moulton 1979). Examples of permanent, set area transects are those of 23-m X 6.1-m and 53-m X 6.1-m used by Hueckel et al. (1983) in their studies of artificial reefs in Puget Sound and Simenstad et al.'s (1988) continuous transects 1-m wide to evaluate the demersal fishes in the subtidal portions of Neah Bay. Strip transects observations can either be made by two divers independently, covering the entire transect width, or by two divers each making simultaneous observations along each side of the transect line. The data treatment is, however, is different in these two cases; in the former case, a mean or consensus must be generated from the two divers' observations, and in the latter case the two divers' observations are additive. In both cases, however, true replicate sampling of the transect should involve repeated sampling of the transect.

In the case of quadrat sampling, the quadrats are usually placed randomly, sometimes systematically, along the transect using measured distances along the transect. Counts and samples are then taken of the fauna within the quadrat. Quadrat size varies according to the size of the larger, more evasive epifauna, but usually 0.25-m^2 and 0.50-m^2 quadrats are used in sampling shrimps. In order to sample the more evasive shrimps (Hippolytidae, *Eualus* spp., *Heptacarpus* spp., *Lebbeus* spp., *Pandalus danae*, *Spironticaris* spp.) and amphipods (*Orchestia traskiana*), an air-lift or Venturi suction sampler is utilized to vacuum up these animals from the turf algae and other substrate material, sometimes in conjunction with it being scraped (see Surface Epifauna). These are essentially the same air-lift samplers used for sampling in unconsolidated sediments (e.g., Brett 1964; Barnet and Hardy 1967; Christie and Allen 1972; Tunberg 1983; Benson 1989).

(2) Sinking beach seine:

The 37- X 2-m beach seine used to sample evasive macroinvertebrates is the same as that advocated for sampling sedentary fish (see below) and is designed to sink to the bottom in depths >2 m. The 18-m wings composed of 3-cm mesh are joined to a 2-m H X 2.4-m W X 2.3-m D bag of 6-mm mesh. A detailed description of the net and the protocols associated with its deployment occurs in Puget Sound Estuary Program (1990a). It is usually deployed 30 m from shore and parallel to the shoreline from an outboard or row boat. Hauling lines are attached to poles at each end of the wings and the net is pulled simultaneously and evenly by two shore parties spaced 40-m apart. After 20 m of the lines have been hauled in, and the wings are perpendicular to shore, the shore parties move to within 10 m of each other and the net is hauled directly to the beach for the final 10-m to shore. During all times that the net is sweeping shallow water, effort should be made to keep the leadline in direct contact with the bottom. Deployed in this manner, the net samples 520 m^2 and 790 m^3. Some differences in sampling efficiency of beach seines (for fish) have been documented when the net is deployed over fine (higher efficiency) versus coarse (lower) substrates (Parsley et al. 1989), although the nets efficiency for macroinvertebrates has not been examined. Nonetheless, sampling efficiency should be a major consideration when comparing habitats which differs significantly in substrate size.

(3) Bottom and midwater trawls:

Due to its performance and the ground-rope or "tickler chain" effect in increasing the capture efficiency of burrowing macroinvertebrates (Kruipers 1975; Creutzberg et al. 1987), a beam trawl is recommended as the methodology of choice for the demersal taxa among these attributes, and specifically for *Cancer magister*, and *Crangon* spp. In this region, the 3-m plumb staffbeam trawl has been used almost exclusively in estuarine sampling. This trawl is composed of similar materials (the codend liner is of 0.4-cm material) but differs in that a solid pipe or beam keeps the net opening constant, as compared to the trynet in which the net opening can be distorted in different conditions, even during the same tow. When deployed, the net has an effective width of 2.3 m (Gunderson and Ellis 1986). The method of using this specific gear, however, is otherwise similar to the trynet. Gunderson et al. (1985) develop a concise case for bottom trawl sampling and describe a comprehensive, systematic sampling design appropriate for assessing *Cancer magister* density and population dynamics.

Slightly larger (e.g., 5.5-m and 6-m) beam trawls have been adopted by investigators and research institutions in other regions such as the North Sea (Kuipers 1975; Cruetzberg et al. 1987). Although some trawls vary in the number of ground-ropes or "tickler chains," this does not appear to affect gear efficiency for different taxa in muddy habitats. However, different tickler chains have definitely been found to affect the gears' selectivity for some taxa which burrow in or cling to sandy substrates. Thus, if maximally efficiency is required in estimating density and standing crop of these taxa, consideration should be given to increasing the ground-rope effect; strict comparison of habitats, however, would not *per se* require such modification.

While most of the taxa in this attribute group are demersal forms, the squids (Gonatidae, *Loligo opalescens*) and shrimp (*Pandalus danae*) tend to be epipelagic or at least extensively epibenthic and thus not effectively sampled by bottom trawl gear. When sampling these particular taxa, a midwater trawl will be required. A 9.2-m midwater trawl which has been used extensively for assessment of spawning herring populations in Puget Sound is appropriate for sampling squid and shrimp if it can be deployed in near-bottom depths. This net is a four panel trawl with a 9.2-m headrope and footrope and 9.2-m sides designed to open 6.1 m vertically and horizontally (Trumble et al. 1982). Mesh sizes taper from 7.6-cm stretch mesh in the wings to 1.3-cm stretch mesh in the cod end liner. Floats were used on the headrope and chain on the footrope to optimize the vertical opening. However, we know of no exact measurement of the net opening and the variability of this areal dimension under fishing conditions. In the absence of this measurement, 37.2 m^2 will have to be assumed. The net was fished with 31 kg, metal, V-type trawl doors, which were connected to the side panels by 55-m dandylines. Standard tow times (e.g., 30-60 min) must be maintained as a common unit of effort; in addition, it is highly recommended that the net be equipped with flow meters to provide more precise measurement of the distance sampled by the trawl; alternatively, exact positions at which the net was deployed and retrieved should be determined by loran or other high-resolution geographic location system. Trawl depth should be maintained using a depth sensor mounted on the trawl door, with telemetry sent to the vessel.

Unit:	**variable according to method; SCUBA transect, seine and trawl should sample at least 250-1,000 m^2; quadrat samples should be 1 m^2**
Periodicity and Frequency:	**monthly, March-September**
Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**

A minimum of three replicate samples is recommended for trawls, beach seines and epibenthic sleds, and five to ten samples should be considered for subtidal quadrat sampling with the airlift sampler.

ALTERNATIVE PROCEDURES

(1) Epibenthic sleds:

Epibenthic sleds or, in the European vernacular "sledges," have been used in the Atlantic for several decades to sample epibenthic crustaceans and, if large enough, can effectively sample the more evasive taxa such as the mysids *Alienacanthomysis macropsis* and *Neomysis mercedis*. The more quantitative sleds have opening and closing mechanisms and flow meters (Bossanyi 1951; Wickstead 1953; Beyer 1958; Frolander and Pratt 1962; Macer 1967; Higley et al. 1977) when deployed from boats and others are designed to be pushed in the intertidal (Colman and Segrove 1955) or by SCUBA-equipped divers (Sibert et al. 1977). To our knowledge, the only sled used specifically to sample evasive macroinvertebrates in this region is the modification of the Higley et al. (1977) variable-height sled used in the Columbia River estuary (Simenstad 1984; Simenstad and Cordell 1985). The Columbia River version has a mouth opening of 1-m X 0.25-m and could be used with one net or two nets sampling half the mouth area. Net mesh sizes were variable, depending upon the epibenthic organisms being targeted in the sampling, but were generally 0.253-mm or 0.500-mm when sampling mysids (specifically *Neomysis mercedis*). The sled can be opened and closed by a cable-mounted triggering device, which should especially be used when deploying the net in deep channels, when contamination from water column organisms is particularly desirable. Propeller flowmeters installed at the mouth of the net(s) recorded the sampling volume.

All subtidal sampling locations should be established precisely using electronic means such as day-screen radar with built-in variable range markers (VRM), high-resolution lorans with multiple navigation capabilities, satellite navigation using a global positioning system (GPS), and video depth sounders and plotters. PTI Environmental Services and the School of Fisheries, University of Washington (1991) describe in detail the use of these devices for precision trawl sampling.

CASE STUDIES

Bottom trawls, particularly the 3-m staffbeam trawl, have been used recently and in a diverse array of locations and habitats in Puget Sound to sample quantitatively juvenile Dungeness crab (Dinnel et al. 1986, 1987; Armstrong et al. 1987). A more extensive database is available for the same gear and sampling strategy from coastal estuaries of Washington, particularly Grays Harbor (Stevens and Armstrong 1984, 1985; Gunderson et al. 1985, 1990; Dumbauld et al. 1986; Rogers et al. 1989); the Grays Harbor collections are particularly illustrative of sampling for population dynamics parameters. Both bottom trawls and epibenthic sleds were utilized by Simenstad (1984) and Simenstad and Cordell (1985) to gather quantitative data on the distribution and abundance of divergent groups of evasive macroinvertebrates in the Columbia River estuary. Hueckel and Buckley (1987) and Benson (1989) have conducted quadrat sampling of shrimps and amphipods from the turf algae habitat which has developed on many of the artificial reefs built in Puget Sound. Simenstad et al. (1988b) included both bottom trawl and SCUBA transect sampling of evasive macrofauna in Neah Bay.

SEDENTARY FISH

HABITAT OCCURRENCE

Eelgrass;	*Leptocottus armatus* *Lumpenus sagitta* *Platichthys stellatus* *Porichthys notatus* *Psettichthys melanostictus*
Emergent Marsh;	*Cottus* spp. *Leptocottus armatus* *Platichthys stellatus*
Gravel-Cobble;	*Apodichthys flavidus* *Pholis laeta* *Platichthys stellatus*
Mudflat;	*Lepidogobius lepidus* *Leptocottus armatus* *Platichthys stellatus* *Pleuronectes vetulus*
Nearshore Subtidal Soft Bottom;	*Leptocottus armatus*
Sandflat;	*Citharichthys* spp. Cottidae (unid.) *Leptocottus armatus* *Platichthys stellatus*

CHARACTERISTICS

Sedentary fish, which includes those termed "demersal" fish, are those which are directly associated with the substrate and are typically slow or relatively passive in their movements along the bottom. As a result, they can be collected with sampling gears which are would be otherwise biased by fish with avoidance capabilities (see #13 Motile fish). Thus, in most cases, sampling efficiencies of the different methods will be more alike for the different taxa than for motile fish. Sedentary fish can be both transient, such as the flatfishes (e.g., *Citharichthys, Platichthys* and *Pleuronectes*) and relatively intransient or even territorial (e.g., *Lepidogobius, Pholis* and *Apodichthys*).

PARAMETERS

Minimum;

(1) species occurrence;

Recommended;

(2) density and standing stock (wet weight biomass);

Preferred;

(3) population structure (i.e., length frequency distribution, life history stage, reproductive status);
(4) spawning activity;
(5) diet composition and daily ration;
(6) growth rate; and
(7) standing stock (dry weight or ash free dry weight).

Species occurrence

When produced from an intensive sampling effort over time and space, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of specie/life history stage by all gears over space or time because different gears have selection biases and because fish occurrence is typically patchy over space and time.

Density and standing stock

Density and standing stock are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the fish and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. Usually fish are weighed fresh (damp) after excess water is toweled off. Fish preserved in formalin or alcohol can also be weighed in this manner but the standing stock values should *expressly* state that they are preserved wet weights and the time out of the preservative should be standardized because drying can be quite rapid under these circumstances.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours for fish). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are extremely labor- and time-intensive measures and are not warranted unless fish production or some other sensitive bioenergetic index are demanded.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Population structure is an important indication of the function of an estuarine habitat for fish because critical measures such as reproduction and recruitment can only be assessed by data on reproductive state and life history stage, respectively. While a few populations may be assumed relatively homogeneous in some estuarine habitats, e.g. young-of-year recruits of *Pleuronectes vetulus*, because the other life history stages utilize predominantly other habitats, basic information on length, weight, sex, sexual maturation, and age may be required to describe the population structure. At a minimum, life history stages should be classified for a subsample of the collections. By classifying by life history stage, "relative" classes are ascribed on the basis of some knowledge of the taxa. Usually, this takes form of classifying life history stage by: (1) embryo; (2) larva; (3) juvenile; (4) subadult or non-spawning adult; (5) spawning or sexually mature adult; and (6) senescent adult; see Balon (1975) for more details about distinguishing these intervals of fish development. Sexual maturity or spawning state can be made by normal internal examination of the gonads, although in some instances can be made externally. In addition to individual fish examination, non-destructive observations can also form important measures of reproductive activity. For instance, *Porichthys notatus* spawns in shallow sublittoral and intertidal habitats under rocks, and can be observed either by SCUBA-equipped diving or by gently checking under rocks during low tide periods.

Length, and less commonly weight, frequency distributions can often be used to distinguish size classes within these life history stanzas, and can be important indications of different recruitment events, i.e., short-term age differences. Fish length can be measured by a variety of standard techniques and several "standards" have evolved for different taxa among the Pacific Northwest fishes: (1) total length (tip of snout to tip of tail) is commonly used for all taxa with robust snouts and caudal fins, such as sculpins and rockfish; (2) fork length (fork of tail to tip of snout) is more commonly used for fishes which have deeply incised caudal fins which can often erode, e.g., herring, smelts and salmon; and (3) standard length (tip of snout to end of hypural plate) is not common for any of the sedentary fish included as attributes in the Protocol (see Fig. 4 of Wydoski and Whitney [1979] for an illustration of the differences in these measurement techniques). A sample for length measurements must be completely random and of sufficient size to represent the distribution; typically, a *minimum* of 25 to 50, and often 100, are required depending upon the variability.

Aging of fish is an effort- and time-intensive process which is seldom required for monitoring habitat function, especially in transient fish populations. It does, however, have some value in assessing the potential recruitment chronology of certain fish taxa which are known to be highly territorial and intransient (e.g., certain species of *Sebastes*). Aging is conducted normally by counting distinguishable yearly growth rings (annuli) on the scales or in the otoliths of the fish.

Anderson and Gutreuter (1983) and Cailliet et al. (1986) are valuable references for further information and detailed descriptions of procedures for length, weight, age, sex, diet and other standard determinations for fishes.

Spawning activity

In addition to the basic enumeration of fish species, life history stages and estimates of sizes, quantitative observations can often be gathered on spawning activity. In particular, spawning activity by sedentary fishes in shallow subtidal rocky substrate habitats can be assessed during SCUBA surveys either directly as the occurrence of spawning or of nest guarding or indirectly as territorial defense by fishes which indicates an egg mass nearby. In gravel-cobble habitats, spawning of fishes in the low intertidal zone can also be assessed during transect surveys by examining beneath rocks for nests of pholids, gunnels and *Porichthys*.

Diet composition and daily ration

In contrast to motile fishes, which we cannot assume have necessarily fed within the habitat being sampled, most sedentary fishes remain within their habitats long enough to make their diets useful indicators of habitat quality. Taxonomic composition of the diet is useful for fish which are known to be selective feeders but, conversely, is of no real utility for generalistic feeders. Thus, extensive baseline data is required on the universality of specific prey of these fishes in different estuarine habitats; in general, this is only available for a restricted number of fishes in a few habitats. Therefore, at this time, the significance of diet differences cannot be interpreted to necessarily imply habitat differences.

In general, the description of diet must be made at the population level and document both numerical and gravimetric (biomass) or volumetric measures of food item "importance." The volume of food items should provide a comparable measure as weight, but is not used as often because it is more difficult to measure in small amounts. Therefore, stomach contents analyses to gather this information need to be conducted on individual fish rather than for pooled samples in order to maintain a measure of the population utilization of the item, i.e. as frequency of occurrence, and of the extent of any feeding selectivity. In addition to counts and weights (wet damped, dry, or ash-free dry) of the food items in each stomach, some indices of stomach fullness and digestion rate should also be assessed; these often involve a scale between 1 to 6 and 1 to 10. Recording of the total stomach contents weight before separation into identifiable food items will also provide, by subtraction, a measure of the weight and proportion of unidentifiable (digested) material. One protocol for fish stomach contents analysis at this level of detail is described by Terry (1977), and Cailliet et al. (1986) provide a helpful discussion of the important considerations in fish diet studies.

Fish diet composition using this combination of measures is commonly represented and illustrated according to an Index of Relative Importance (IRI) which is created for each food item i;

$$\text{I.R.I}_i = \{\%\text{F.O}_i\ (\%\text{N.C.}_i + \%\text{G.C.}_i)\}$$

where %F.O. = the percent frequency of occurrence, %N.C. = percent numerical composition, and %G.C. = percent gravimetric/volumetric composition. Because the resulting numerical values for the I.R.I depend greatly upon sample size, the relative importance is often converted to the percent of the total I.R.I (%I.R.I.). Graphically, this is illustrated by a two dimensional figure in which the abscissa is %F.O. and the ordinate is %N.C. above the abscissa and %.G.C. below the abscissa. The original I.R.I. description occurs in Pinkas et al. (1971), and the approach is modified and described further in Cailliet (1977) and Cailliet et al. (1986).

Consumption rate, as commonly measured as daily ration, is less ambiguous a measure of the foraging success than diet composition, and would be an extremely valuable index if coupled with growth estimates. Daily ration estimates generated from *in situ* sampling involves periodic estimates of the mean stomach contents biomass over a diel period.

Non-destructive assessment of diet can also be made by enumerating the number of feeding attacks on specific groups of prey taxa per unit of observation time during SCUBA transect surveys. For instance, Hueckel (1980) observed feeding behavior of three species of fishes on artificial reefs over 5-min. periods, noting the fish species, its length interval and the type of substrate in which it fed (all three fish species were of the "turf picker-winnower" feeding type); this information was then related to quantitative data on the invertebrate organisms found in the particular substrate type.

Growth rate

A mean growth rate can also be derived for a population of fish from mark and recapture experiments, but are constrained in open systems due to the lack of information on emigration and immigration. An alternative is the use of the widths of daily increments laid down on the otoliths, which has been used to estimate relative growth rate (Volk et al. 1984; Neilson et al. 1985). The distinct advantage of the otolith-derived growth estimation is that distributions of individual growth and variance estimates are obtained, rather than a population mean. The lack of verification from *in situ* experiments, however, implies that verification of the otolith increment width-ration relationship should be tested under natural conditions in the field if absolute rates are desired. In theory, comparison of the relative growth of fishes in a mitigation/restoration habitat as compared to a natural habitat can be validly derived from otolith microstructure; however, this remains to be attempted at this time.

RECOMMENDED SAMPLING METHODOLOGY

Method:	eelgrass, mudflat and sandflat habitats, **37-m x 2-m sinking beach seine with 6-mm mesh codend**; emergent marsh habitat, **channel trap nets with 6-mm mesh**; gravel-cobble habitat, **SCUBA transect observations**; nearshore subtidal soft bottom, **3-m bottom** (staffbeam) **trawl with 4-mm codend**

(1) Sinking beach seine:

The 37- X 2-m beach seine used to sample sedentary fish is the same as that advocated for sampling motile fish (see below) but does not have flotation along the toprope, such that it sinks to the bottom when set over depths >2 m. The 18-m wings composed of 3-cm mesh are joined to a 2-m H X 2.4-m W X 2.3-m D bag of 6-mm mesh. A detailed description of the net and the protocols associated with its deployment occurs in the Puget Sound Estuary Program (1990a). It is usually deployed 30 m and parallel to the shoreline from an outboard or row boat. Hauling lines are attached to poles at each end of the wings and the net is pulled simultaneously and evenly by two shore parties spaced 40-m apart. After 20 m of the lines have been hauled in, and the wings are perpendicular to shore, the shore parties move to within 10 m of each other and the net is hauled directly to the beach for the final 10-m to shore. Puget Sound Estuary Program (1990a) recommend a hauling speed of 10 m min^{-1}. During all times that the net is sweeping shallow water, effort should be made to keep the leadline in direct contact with the bottom. Fished in this manner, the net samples 520 m^2 and 790 m^3. Some differences in sampling efficiency of beach seines has been documented when the net is deployed over fine (higher efficiency) versus coarse (lower) substrates (Parsley et al. 1989), which should be a major consideration when comparing habitats which differ significantly in substrate size.

(2) Channel trap net:

Channel trap nets are designed to capture fishes as they emigrate tidal habitats during an ebbing tide. Most sedentary fishes will be captured by trap nets except for those that occupy burrows and depressions during tidal exposure. Although they have been adopted extensively on the east coast, channel trap nets (e.g., Cain and Dean 1976) and the similar flume nets (McIvor and Odum 1986) have not been utilized extensively in this region. The intertidal trap net utilized by Levy and Northcote (1982) to sample emergent marshes is one of the few and better examples of this sampling method. Their net was 2.4-m deep and constructed of 0.6-cm mesh. It is deployed at high tide, stretched across a tidal channel, immediately preceding a steep tidal drop, and fastened to stakes set into the bank along the channel. As the tide ebbs, most of the fish in the marsh drainage area of the channel above the net, and in the channel proper, are forced into the trap or into the pools of the channel remaining at low tide. When the channel is as dewatered as will occur at low tide, fish are collected from within the trap and seined from the remaining pools. A most effective modification, also described by Levy and Northcote, is a removable trap box which acts as a live collection trap and permits periodic checking of the temporal distribution and abundance of fish exiting the channel over the ebb tide.

(3) SCUBA transect observations:

While there are variants on the general methodology, all SCUBA transect observations basically involve SCUBA-equipped divers recording observations or taking quadrat samples along measured underwater "strip" transects on the species, size, activity, etc. of fish and macroinvertebrates. Transects can be either permanently fixed (e.g., with markers or line), so they can be consistently revisited, or impermanent and measured before or after the SCUBA observations. The length of permanent transects are usually established by the length of the line or by the distances between markers as measured *in situ*; impermanent transects are usually measured as between buoys which are typically released at the beginning and the end of the dive and the distance measured directly or with navigation aids. While the permanent transect, or some similar modification (e.g., a temporary transect line) establishes the exact transect length, the impermanent transect should be considered a minimum distance, since dives seldom involve linear paths.

The area swept is estimated as the transect distance multiplied by a transect path width, which can either be a (minimal) set distance or estimated as the visibility (at that time) for effective observation of the fishes; given the variable visibility in Puget Sound estuaries, the latter method is recommended for optimum standardization of data collection. A common method of establishing effective transect width is to measure the distance at which a standard target (Secchi disk) disappears and reducing this by a factor (usually 1/2) to account for the visibility required to make reliable observations (see Moulton 1979). Examples of permanent, set area transects are those of 23-m X 6.1-m and 53-m X 6.1-m used by Hueckel et al. (1983) in their studies of artificial reefs in Puget Sound and Simenstad et al.'s (1988) continuous transects 1-m wide to evaluate the demersal fishes in the subtidal portions of Neah Bay.

Transect observations can either be made by two divers independently, covering the entire transect width, or by two divers each making simultaneous observations along each side of the transect line. The data treatment, however, is different in these two cases; in the former case, a mean or consensus must be generated from the two divers' observations, and in the latter case the two divers' observations are additive. In both cases, however, true replicate sampling of the transect should involve repeated sampling of the transect.

In soft sediment habitats, a modification of the strip transect method developed by Walton and Bartoo (1976) is recommended in which a 2-m rod is pushed along the surface of the substrate to disrupt flatfish (e.g., *Platichthys* and *Pleuronectes*) hiding in the surface layer.

(4) Bottom trawl:

Bottom trawls are sock-like nets which are usually towed with one cable behind one boat with the mouth of the net spread apart by either flat "doors" attached to the bridles of each wing or by a pole or "beam." Their use as sampling gear derives from their long history as a commercial fishing technique, although their application can be limited in situations where there are obstructions which will snag the towed net or where the habitat is limited in space (e.g., there is no room to maneuver the boat and deploy the net effectively). It is, however, one of the few methods for capturing fishes, especially moderately evasive taxa, in subtidal habitats.

The trawl net that has been used extensively in sampling estuarine fishes in this region is the 3-m plumb staffbeam trawl. This trawl is composed of similar materials (the codend liner is of 0.4-cm material) to the trynet (see Alternative Procedures) but differs in that a solid pipe or beam keeps the net opening constant, as compared to the trynet in which the net opening can be distorted in different conditions, even during the same tow. When deployed, the net has an effective width of 2.3 m (Gunderson and Ellis 1986). The method of using this specific gear, however, is otherwise similar to the trynet.

It should be acknowledged that the plumb staffbeam trawl and trynet do have some differences in efficiency due to their selection biases for/against certain taxa or fish intervals.

Donnelly (In prep.) has conducted a detailed comparison among the 4.9-m and 7.6-m bottom trawls and 3-m staffbeam trawls commonly used for research collections in Puget Sound and coastal estuaries. Although his analyses at this time have not directly compared the 4.9-m trynet with the 3-m staffbeam trawl, preliminary examination of the data illustrate some notable differences. For instance, while the 4.9-m trynet effectively captures *Pleuronectes vetulus* between 120 mm and approximately 250 mm total length, the 3-m staffbeam trawl is biased against fish >170 mm in length; similarly, the 3-m staffbeam trawl is less effective in sampling *Citharichthys sordidus* >150 mm in length. Efficiency for sampling other taxa, such as *Porichthys notatus*, were not significantly different between the two gears. These differences must be taken into consideration if emphasis is being placed on attribute taxa which are particularly biased against by one gear or if the population structure characteristics are included in the monitoring parameters.

No matter what the trawl gear chosen, the effort should be standardized. A minimal, and not overly acceptable standard is the time during which the net is towed, e.g., 5- or 10-min. The difficulty using this method is that the areal extent of the sampling is prone to extreme variability due to current and other effects. A better alternative is either by measuring the distance towed (through a benthic metering wheel mounted on the net, navigational positioning or measuring the distance between buoys released at the beginning and end of the trawl) or by the volume filtered (measured as the water volume passing though the net by a flowmeter).

Location should be established precisely using electronic means such as day-screen radar with built-in variable range markers (VRM), high-resolution lorans with multiple navigation capabilities, satellite navigation using a global positioning system (GPS), and video depth sounders and plotters. PTI Environmental Services and the School of Fisheries, University of Washington (1991) describe in detail the use of these devices and a standard protocol for precision trawl sampling.

Unit: beach seine, **~500 m^2**; channel trapnets, **dimensions dependent upon channel**; SCUBA transects, **50-m x 6-m**; staffbeam trawl, **~1000 m^2**

Periodicity and Frequency: **monthly, March-September**

Statistical Considerations (Replication): in lieu of statistical predetermination using pilot study or historical data; **n=5**

Kellogg and Shreffler (1985) illustrated that if species richness or diversity are important monitoring parameters, the necessary sample size for beach seine collections of nearshore fish ranged between three and six, depending upon habitat. They also compared trynet samples and found that five to ten samples were necessary to establish the representative number of species in a shallow subtidal soft sediment habitat. These studies would suggest that, in general, sample size should be set at no less than five replicates. If monitoring is focused on the density or standing stock of one or two attributes is the criteria, somewhat fewer replicates may be justified if a pilot project illustrates lower variability. Given the sedentary nature of these fish, all replicates should be non-overlapping but in identical habitat conditions.

ALTERNATIVE PROCEDURES

(1) Other beach seines:

The Puget Sound Estuary Program (1990a) recommends as an alternative to the 37-m beach seine a 9-m seine. Because the catchability of this net is likely to be more biased against larger or more evasive fishes, we do not recommend this net except in unique circumstances. For instance, if data on small and comparatively slow-moving fish such as young-of-the-year English sole (*Pleuronectes vetulus*) are the primary monitoring objective, this method would be appropriate. This net is 8.8 m in total length, with 3.6-m long wings. The wings are constructed of 3.9-mm square, knotless mesh and the bag of 2.4-mm square, knotless mesh. A 6.5 m check line is installed between the poles to ensure a constant mouth opening. Deployment is different from the 37-m beach seine; the 9-m seine is set perpendicular to the beach gradient (waterline) and pulled manually along (parallel to) the beach at a speed of approximately 15 m min^{-1}, usually for a previously-measured distance of 30 m, before being turned into the beach to concentrate and collect the catch. If the mouth opening is maintained at a constant length (6.5 m), the sampling area effort will be approximately 195-200 m^2.

(2) Other trawls;

A net used often in shallow habitats (e.g., intertidal mud- and sandflats), where larger vessels are prohibited from operating, is a 4.9-m trawl, often referred to as a "trynet." The body and codend are normally constructed of 1.9-cm mesh and the codend is, in addition, lined with a bag of 0.5-cm mesh. The net can be deployed from an outboard powered boat as long as two requirements are met: (1) the power is enough to move the net across the bottom at 1.0 to 2.0 m s^{-1}; and (2) forward motion of the boat can be maintained when the net is retrieved from the bottom. In the case of the latter requirement, this often means that the towing cable is stored on a powered spool or drum which can be driven independently of the boat's power. If the boat loses momentum or stops during retrieval, many fish have the capability to swim out of the net. In the absence of current effects and deployed over an even bottom type and depth range, 5-min. tows of this net have been estimated to sample an area and volume of 750 m^2 and 375 m^3, respectively.

Slightly larger (e.g., 5.5-m and 6-m) beam trawls have been adopted by investigators and research institutions in other regions such as the North Sea (Kuipers 1975; Cruetzberg et al. 1987). Although some trawls vary in the number of ground-ropes or "tickler chains," this does not appear to affect gear efficiency for different taxa in muddy habitats. However, different tickler chains have definitely been found to affect the gears' selectivity for some taxa which burrow in or cling to sandy substrates. Puget Sound Estuary Program (1990a) advocate a towing speed of 2.6 km hr^{-1} for the 3-m staffbeam trawl.

(3) Drop and pop nets:

In vegetated habitats such as eelgrass and emergent marshes, beach seine and trawl sampling can be both destructive and ineffective samplers of the fish which are associated specifically with these habitats because they tend to integrate both vegetated and unvegetated habitats.

Borton (1982) used a drop net to sample shallow subtidal eelgrass and sand habitats in central Puget Sound (Alki Point) discretely.

Buoyant pop nets have been shown to effectively sample shallow unvegetated habitats and to be useful in heavily-vegetated freshwater habitats by Dewey et al. (1989), although their size may limit the assessment of species richness. While there is no precedence of their use in estuarine habitats in this region, both drop and pop nets should be considered for habitats such as eelgrass. The pop nets used by Dewey et al. (1989) were 1.8-m wide X 3.1-m long X 1.8-m high when released and enclosed an area of 5.6 m^2, the top of the net was attached to a rectangular frame made buoyant with foam floatation. Different net arrangements had to be used for vegetated and unvegetated habitats; the net used in unvegetated habitats had a bottom net panel, while the net used in vegetated habitats had net panels which could be drawn (pursed) over the vegetation once the net had been deployed.

(3) Intertidal transects:

Several of these sedentary fishes occur commonly in the intertidal zone, most notably *Apodichthys flavidus, Pholis laeta*, several cottids, and *Porichthys notatus* during its spawning period. Many of these can be enumerated effectively during low tide transect studies. Fishes occurring within quadrats positioned along the transect should be thoroughly evaluated by examining under all large rocks and cobble. If tidepools fall within the sampling unit, they should be drained by bucketing or siphoning the water out; the fish can also be narcotized by the application of an ichthyocide such as Quinaldine in low enough concentrations to cause the fish to leave hiding places but not enough to kill them. Whenever possible, basic data should be gathered on the live fish and they should then be returned to the pool after it has been restored with fresh seawater.

(4) Push net:

Another gear designed in many respect to sample the same small, less-evasive fishes as the 9-m beach seine is a push net, which is especially effective for juvenile flatfishes (Kendall 1966). One version that has been utilized in this region is the 1.5-m push net design of Riley and Holford (1965). This is a rigid-frame net with an opening 1.5-m wide and 0.3-m high, equipped with a 2-mm mesh net. The frame and net are mounted on skis and have a handle. Sampling involves pushing the net parallel to the depth gradient (e.g., along the 0.0 m MLLW contour) for a measured distance (e.g., 100 m).

CASE STUDIES

Pertinent references for techniques and comparable data for sedentary fishes in Puget Sound are Miller et al. (1977, 1980), Fresh et al. (1979), Donnelly et al. (1984) and Thom et al. (1989); in addition to the estuarine habitats of Puget Sound, these techniques have been used to sample motile fishes in coastal estuaries (Simenstad and Eggers 1981; Rogers et al. 1988) and other coastal habitats (Simenstad et al. 1988b). Miller et al. (1977), Moulton (1979), Hueckel et al. (1983), Hueckel and Buckley (1987) all provide relatively comparable data on density and feeding behavior of sedentary fishes observed along SCUBA strip transects in shallow subtidal hard substrate communities; Walton and Bartoo (1976) and Walton (1982) provide similar data for soft sediment communities. Weitkamp (1991) also describes the use of, and data on, juvenile flatfishes using a 1.5-m push net.

MOTILE FISH

HABITAT OCCURRENCE

Eelgrass;	*Clupea harengus pallasi* *Cymatogaster aggregata* Embiotocidae (unid.) *Engraulis mordax* *Oncorhynchus* spp. (fry) *Thaleichthys pacificus*
Emergent Marsh;	*Cymatogaster aggregata* *Gasterosteus aculeatus* *Oncorhynchus gorbuscha* (fry) *Oncorhynchus keta* (fry) *Oncorhynchus* spp. (fry) *Thaleichthys pacificus*
Gravel-Cobble;	*Ammodytes hexapterus* *Clupea harengus pallasi* *Cymatogaster aggregata* *Gasterosteus aculeatus* *Oncorhynchus* spp. (fry) *Sebastes caurinus* *Thaleichthys pacificus*
Mudflat;	*Engraulis mordax* *Gasterosteus aculeatus* *Oncorhynchus* spp. (fry)
Nearshore Subtidal Soft Bottom;	*Ammodytes hexapterus* *Clupea harengus pallasi* *Microgadus proximus* *Thaleichthys pacificus*
Nearshore Subtidal Hard Substrate;	*Ammodytes hexapterus* *Clupea harengus pallasi* *Phanerodon furcatus*
Sandflat;	*Engraulis mordax*
Water Column;	*Ammodytes hexapterus* *Clupea harengus pallasi* *Cymatogaster aggregata* *Engraulis mordax* *Gadus macrocephalus* *Gasterosteus aculeatus* *Hypomesus pretiosus* *Lampetra tridentatus* *Mallotus villosus* *Merluccius productus* *Microgadus proximus*

Oncorhynchus gorbuscha (fry)
Oncorhynchus spp.
Oncorhynchus spp. (fry)
Scorpaenidae (unid.)
Sebastes spp.
Spirinchus thaleichthys
Thaleichthys pacificus
Theragra chalcogramma

CHARACTERISTICS

As distinguished from sedentary fish, motile fish are those species which are active swimmers, tend to be epipelagic or water column (also termed "neritic") and quite often are schooling species. This offers some particular problems to sampling them quantitatively. Sampling methods have stressed "active" gears such as seines and trawls instead of "passive" gears such as gillnets because sampling effort is difficult to impossible to quantify for passive gears. We also recommend specific methods which either have extensive precedence in sampling motile fishes (particularly juvenile salmonids) in Puget Sound estuarine habitats, which have the least potential for biased catches, and in which the sampling effort can be effectively quantified.

PARAMETERS

Minimum;
(1) species occurrence;

Recommended;
(2) density and standing stock;

Preferred;
(3) population structure (e.g., length frequency distribution, life history stage, reproductive status; and
(4) residence time and growth rate.

Species occurrence

When produced from an intensive sampling effort over time and space, the relative occurrence of species (providing species richness and timing) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of water and/or sediment quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages per sample by gear and as (2) the cumulative number of species/life history stage by all gears over space or time because different gears have selection biases and because fish occurrence is typically patchy over space and time.

Density and standing stock

Density and standing stock are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the fish and their habitat which would otherwise not be reflected by species richness. Density and standing stock are expressed, respectively, as the number and biomass per unit area (e.g. no. m^{-2}, g wet m^{-2}) or volume (e.g., no. m^{-3}, g wet m^{-3}) of the habitat.

Standing stock is normally expressed as wet weight (biomass), which is acceptable as long as the weighing method is standardized to minimize variability due to different amounts of moisture content. Usually fish are weighed fresh (damp) after excess water is toweled off. Fish preserved in formalin or alcohol can also be weighed in this manner but the standing stock values should *expressly* state that they are preserved wet weights and the time out of the preservative should be standardized because drying can be quite rapid under these circumstances.

Due to these sources of variability, which can often introduce considerable bias, the optimum method to express standing stock is as dry weight or as ashfree dry weight. Dry weight is defined as the biomass after drying at 100°C until the weight has stabilized (typically >48 hours for fish). Ashfree dry weight is the biomass of organic matter combusted at 500-600°C for >24 hr. Both of these, especially the latter, are extremely labor- and time-intensive measures and are not warranted unless fish production or some other sensitive bioenergetic index are demanded.

As in species occurrence, density and standing stock should be expressed relative to species and life history stage.

Population structure

Population structure is an important indication of the function of a habitat for fish because critical measures such as reproduction and recruitment can only be assessed by data on reproductive state and life history stage, respectively. While a few populations may be assumed relatively homogeneous in some estuarine habitats, e.g. young-of-year recruits of *Pleuronectes vetulus*, because the other life history stages utilize predominantly other habitats, basic information on length, weight, sex, sexual maturation, and age may be required to describe the population structure. At a minimum, life history stages should be classified for a subsample of the collections. By classifying by life history stage, "relative" classes are ascribed on the basis of some knowledge of the taxa. Usually, this takes form of classifying life history stage by: (1) embryo; (2) larva; (3) juvenile; (4) subadult or non-spawning adult; (5) spawning or sexually mature adult; and (6) senescent adult; see Balon (1975) for more details about distinguishing these intervals of fish development. Sexual maturity or spawning state can be made by normal internal examination of the gonads, although in some instances can be made externally. In addition to individual fish examination, non-destructive observations can also form important measures of reproductive activity. For instance, *Porichthys notatus* spawns in shallow sublittoral and intertidal habitats under rocks, and can be observed either by SCUBA-equipped diving or by gently checking under rocks during low tide periods.

Length, and less commonly weight, frequency distributions can often be used to differentiate different size classes within these life history stanzas, and can be important indications of different recruitment events, i.e., short-term age differences. Fish length can be measured by a variety of standard techniques and several "standards" have evolved for different taxa among the Pacific Northwest fishes: (1) total length (tip of snout to tip of tail) is commonly used for all taxa with robust snouts and caudal fins, such as sculpins and rockfish; (2) fork length (fork of tail to tip of snout) is more commonly used for fishes which have deeply incised caudal fins which can often erode, e.g., herring, smelts and salmon; and (3) standard length (tip of snout to end of hypural plate) is not common for any of the sedentary fish included as attributes in the Protocol (see Fig. 4 of Wydoski and Whitney [1979] for an illustration of the differences in these measurement techniques). A sample for length measurements must be completely random and of sufficient size to represent the distribution; typically, a *minimum* of 25 to 50, and often 100, are required depending upon the variability.

Aging of fish is an effort- and time-intensive process which is seldom required for monitoring habitat function, especially in transient fish populations. It does, however, have some value in assessing the potential recruitment chronology of certain fish taxa which are known to be highly territorial and intransient (e.g., certain species of *Sebastes*). Aging is conducted normally by counting distinguishable yearly growth rings (annuli) on the scales or in the otoliths of the fish.

Anderson and Gutreuter (1983) and Cailliet et al. (1986) are valuable references for further information and detailed descriptions of procedures for length, weight, age, sex, diet and other standard determinations for fishes.

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Residence time and growth rate

In the case of motile fishes, the period of occupation of a habitat may be an important index of the habitat's functional support of the fish. It has been argued (Simenstad and Salo 1982; Simenstad et al. 1982; Simenstad and Wissmar 1984; Simenstad 1987; Wissmar and Simenstad 1988; Shreffler et al. 1990, 1991) that for some species of juvenile salmon (e.g., fry of pink, chum and chinook) estuarine residence time is an important survival factor. Thus, the time spent in specific estuarine habitats and the rate of growth of fish during that occupancy can be a valid indication of the habitats' importance relative to natural habitats.

Residence time is most often determined by routine mark-recapture studies, which can be complicated in open systems sampled sporadically or relatively straightforward in closed systems (see the design for sampling the Lincoln Avenue Wetland, Thom et al. 1988, Shreffler et al. 1990, 1991). Burnham et al. (1987) effectively present the requirements for release-recapture experiments.

A mean growth rate can also be derived from mark and recapture experiments, but have the same constraints in open systems due to the lack of information on emigration and immigration. An alternative is the use of the widths of daily increments laid down on the otoliths, which has been used to estimate relative growth rate (Volk et al. 1984; Neilson et al. 1985). The distinct advantage of the otolith-derived growth estimation is that distributions of individual growth and variance estimates are obtained, rather than a population mean. The lack of verification from *in situ* experiments, however, implies that verification of the otolith increment width-ration relationship should be tested under natural conditions in the field if absolute rates are desired. In theory, comparison of the relative growth of fishes in a mitigation/restoration habitat as compared to a natural habitat can be validly derived from otolith microstructure; however, this remains to be attempted at this time.

RECOMMENDED SAMPLING METHODOLOGY

Method: eelgrass, emergent marsh, gravel-cobble, mudflat, sandflat and water column habitats, **53-m purse seine with 6-mm mesh bag**; nearshore subtidal soft bottom habitat, **7.6-m bottom trawl with**

6-mm mesh codend; nearshore subtidal hard substrate habitat, **SCUBA transect observations**

Due to the avoidance capabilities of motile fishes, sampling methods employed to catch them must either encompass a large area to minimize disturbance from the sampling gear or be rapid to minimize the avoidance, and preferably both. Purse seines and surface and midwater trawls are the most common gears used and have selection biases for different groups of fishes and limitations on the habitat in which they can be deployed; a floating beach seine has been used to sample these fishes near shore but may be limited in its efficiency.

(1) Purse seine:

A modification of the commercial purse seine technique has been adapted for biological sampling from smaller boats in the confined embayments and channels of estuaries. The basic requirements for this sampling is that the net be long enough to effectively enclose schooling fish, composed of fine mesh in order to effectively capture small and juvenile estuarine fishes, that it be deep enough to inhibit avoidance by fish sounding, and that it be deployed and pursed in a rapid manner. One seine meeting these criteria has been utilized by the Washington Department of Fisheries and Fisheries Research Institute (see case studies). This net is 58-m long and is composed of 13-mm mesh in the wings and 6-mm mesh in the bunt (the end section of the net where the fish are concentrated). It is deployed rapidly from a 5-m outboard-powered boat in "round haul" fashion, which if fished efficiently samples and area and volume of 268 m^2 and 835 m^3, respectively. Setting, pursing and retrieving the net by hand can, with experience, take only 15 to 30 min.; modification of the boat to deploy, retrieve and store the net on a hydraulic-powered reel can reduce the sampling time considerably. The round haul technique is an important requirement, as opposed to the strategy of holding the net open into the prevailing current or towing it, principally because the sampling area/volume will be known and consistent; in addition, the "holding open" method requires two boats to work effectively. Although not deployed extensively, the net employed in Pearce et al.'s (1982) investigations in the Nisqually River estuary, while slightly larger (61-m), has the same mesh size and should have performed similar to the WDF net. Parametrix, Inc. (1980, 1982) has utilized a somewhat smaller (38.1-m x 4.6-m) "research seine" equipped with 9.5-mm mesh in sampling juvenile salmonids in the Duwamish River estuary; this net, which samples approximately 230 m^2, should perform similar to the WDF net but no tests have been conducted to compare gear efficiency. A larger net, 63-m long by 7-m deep, with 25-mm to 6-mm mesh, that samples 300 m^2 and 2000 m^2 has also been used in Puget Sound and coastal estuaries (see case studies); its size, however, requires a larger (e.g., 7 to 10-m) vessel, preferably one equipped with a reel, to be fished effectively and safely.

(2) Bottom trawl:

Motile, demersal fishes are generally less vulnerable to small bottom trawls such as the 4.9-m trawl and the 3-m staffbeam trawl. For quantitative monitoring of fishes such as *Microgadus proximus, Thaleichthys pacificus,* and *Theragra chalcogramma*, the 7.6-m "otter" trawl described in detail by Puget Sound Estuary Program (1990a) is recommended. This trawl has a headrope and footrope 7.6-m and 8.1-m long, respectively. Its body is constructed of 38-mm stretch mesh and the body is of 32-mm stretch mesh lined with a 6-mm mesh bag. The 7.6-m otter trawl is commonly equipped with 23-kg doors and it towed at approximately 4.6 km hr^{-1}. The scope of wire cable out to sampling depth varies from about 5:1 in shallower depths to 3:1 at deeper depths (PTI in press). Pair-wise comparisons of the 7.6-m trawl with the 4.9-m trawl by Donnelly (In prep.) indicate that the larger net is a more effective sampler of *Microgadus proximus*, *Theragra chalcogramma*, total flatfish and total fish; in addition, the larger trawl was able to sample much larger *Microgadus* than the smaller trawl.

Unit:	purse seine, **~268 m^2**; bottom trawl; SCUBA transects, **50-m x 6-m**
Periodicity and Frequency:	**monthly, March-September**
Statistical Considerations (Replication):	in lieu of statistical predetermination using pilot study or historical data; **n=5**

Because catches of motile fish tend to be highly variable, we recommend a *minimum* of five replicate samples for all methods and as many as ten if the monitoring criteria requires detecting significant differences in the density or standing stock of attributes which are particularly patchy (e.g., schooling taxa such as herring, sand lance and small juvenile salmon).

ALTERNATIVE PROCEDURES

(1) Surface trawl:

The Kvichak surface trawl or "townet" has been used extensively for sampling near-surface (i.e., 3-m) neritic fishes in Puget Sound since early research on juvenile salmon in Bellingham Bay and provided the sampling basis of the Washington Department of Natural Resources (DNR) Nearshore Fish Survey of neritic fishes in northern Puget Sound and NOAA's Marine Ecosystem Analysis (MESA) studies in the Strait of Juan de Fuca in the mid- to late 1970s (see case studies). In general, this is the "accepted" technique for sampling juvenile salmonids once they have moved away from shallow shorelines. This net has a mouth opening of 18.3 M (3.0 x 6.1 M) and mesh size grading from 8.9-cm at the mouth to 0.6-cm at the cod end. Two pipes with large floats at the top and weights at the bottom were attached to the sides of the net to keep the mouth open vertically. The net is towed between two boats at an average tow speed of 80 cm s^{-1} (range 50-100 cm s^{-1}), which Bollens and Frost (1989) determined to result in sampling volumes of between 7,300 and 31,700 m^3.

(2) Midwater trawl:

A midwater trawl used extensively for assessment of spawning herring populations in Puget Sound is a four panel trawl with a 9.2-m headrope and footrope and 9.2-m sides designed to open 6.1 m vertically and horizontally (Trumble et al. 1982). Mesh sizes taper from 7.6-cm stretch mesh in the wings to 1.3-cm stretch mesh in the cod end liner. Floats were used on the headrope and chain on the footrope to optimize the vertical opening. However, we know of no exact measurement of the net opening, and the variability of this areal dimension, under fishing conditions; in the absence of this measurement, 37.2 m^2 will have to be assumed. The net was fished with 31 kg, metal, V-type trawl doors, which were connected to the side panels by 55-m dandylines. Standard tow times (e.g., 30-60 min) must be maintained as a common unit of effort; in addition, it is highly recommended that the net be equipped with flow meters to provide more precise measurement of the distance sampled by the trawl; alternatively, exact positions at which the net was deployed and retrieved should be determined by loran or other high-resolution geographic location system. Trawl depth should be maintained using a depth sensor mounted on the trawl door, with telemetry sent to the vessel.

A similarly-sized trawl has been used for the capture of midwater fishes within 50 m of the surface of Hood Canal by the University of Washington School of Oceanography (Bollens and Frost 1989) and should provide comparable catches if deployed similarly. This net has a 81.0 m^2 (9.0 X 9.0 m) mouth area and mesh size ranging from 8.9-cm stretch measure at the mouth of the net to 1.3-cm mesh in the cod end. The mouth of the net was kept open by 230-kg doors. Tow speed averaged 150 cm s^{-1} (range 120-180 cm s^{-1}), thus the volume filtered by the net ranged between 115,000 and 449,000 m^3.

(3) Floating beach seine:

The 37- X 2-m beach seine used to sample sedentary fish (see above) has been modified by adding enough large floats (usually the "bullet" floats) to the toprope to keep the net at the surface. As with the sinking net, the 18-m wings composed of 3-cm mesh are joined to a 2-m H X 2.4-m W X 2.3-m D bag of 6-mm mesh. A detailed description of the net and the protocols associated with its deployment occurs in PTI et al. (in press). It is deployed and retrieved in a similar manner, i.e. 30-m and parallel to shore, with the two wings pulled simultaneously toward shore for the first 20-m at a distance of 40-m, and from a distance of 10-m apart for the final 10-m to shore; in this configuration it samples 520 m^2 and 790 m^3. As utilized by Parametrix (1980, 1982) and Lipovsky (1985), a smaller (30-m) beach seine which encompasses an area of 230 m^2 may sample comparably in shallow water but several variances in the gear and deployment (i.e., 9.5-mm mesh; setting the net with one end attached to shore) suggest that these two nets may have significantly different efficiencies, and the associated data should not be directly compared without conducting paired efficiency tests.

(4) Channel trap net:

Tidal channels in emergent marshes and mud- and sandflats provide particular problems in sampling fishes. In many cases, the traditional beach seine techniques are inferior to methods which sample through an ebb tide cycle because fish which are utilizing the flats and emergent vegetation are comparatively invulnerable.

Channel trap nets are designed to capture fishes as they emigrate tidal habitats during an ebbing tide. Most motile fishes will be captured by trap nets because few will occupy burrows and depressions during tidal exposure as will some sedentary fishes. Although they have been adopted extensively on the east coast, channel trap nets (e.g., Cain and Dean 1976) and the similar flume nets (McIvor and Odum 1986) have not been utilized extensively in this region. The intertidal trap net utilized initially by Levy and Levings (1978) and later by Levy and Northcote (1982) to sample emergent marshes is one of the few and better examples of this sampling method. Their net was 2.4-m deep and constructed of 0.6-cm mesh. It is deployed at high tide, stretched across a tidal channel, immediately preceding a steep tidal drop, and fastened to stakes set into the bank along the channel. As the tide ebbs, most of the fish in the marsh drainage area of the channel above the net, and in the channel proper, are forced into the trap or into the pools of the channel remaining at low tide. When the channel is as dewatered as will occur at low tide, fish are collected from within the trap and seined from the remaining pools. A most effective modification, also described by Levy and Northcote, is a removable trap box which acts as a live collection trap and permits periodic checking of the temporal distribution and abundance of fish exiting the channel over the ebb tide.

Shreffler et al. (1988, 1990, 1991) and Thom et al. (1988) describe a modification to a channel trap net which should be considered when residence time, growth or consumption rate estimates are required. In this case, the trap net is equipped with two side-by-side "fyke" traps, one facing up-channel and one down-channel, which are designed to sample fish actively moving into and out of the channel. The net is set in the lower intertidal portion of the channel such that it can operate (because it requires some water remaining in the trap area) at low tide, unlike the Levy and Northcote (1982) net.

(5) Oneida trap:

Another "fyke-net" type of fixed net is the Oneida trap which can be used where currents or fish movements are predominantly unidirectional. As use by Parametrix, Inc. (1982), the Oneida trap is constructed of 4.8-mm stretched mesh webbing and is formed of two 12.8-m wings and a

40.3-m center lead which funnel into a 2-m x 2.1-m x 2.0-m "crib" which traps and holds the fish alive. This is one of the few gears which can be deployed in a habitat with extensive obstructions, such as under a pier apron. However, unlike the trapnet which samples a comparatively known area of habitat (i.e., the channel "watershed"), it is difficult if not impossible to quantify the sampling effort of the Oneida net without several tenuous assumptions, such as uniform distribution of fish across the total water surface which the net is supposed to be subsampling, equal fishing efficiency during different tidal stages, etc. If the Oneida trap offers the only option for sampling an estuarine habitat, the effects of these assumptions should be minimized by test fishing in several locations, sampling during short periods within tidal stages, etc.

For all purse seining and trawling, location should be established precisely using electronic means such as day-screen radar with built-in variable range markers (VRM), high-resolution lorans with multiple navigation capabilities, satellite navigation using a global positioning system (GPS), and video depth sounders and plotters. PTI Environmental Services and the School of Fisheries, University of Washington (1991) describe in detail the use of these devices and a standard protocol for precision trawl sampling.

CASE STUDIES

The recommended beach seine, purse seine, and surface trawl fish sampling techniques have been applied extensively to sampling juvenile salmon and baitfish in estuarine and nearshore habitats of Puget Sound and the Strait of Georgia in the studies of Tyler (1964), Miller et al. (1977, 1980), Schreiner et al. (1977), Levy and Levings (1978), Bax et al. (1978), Fresh et al. (1979), Meyer et al. (1981), Simenstad et al. (1981), Parametrix, Inc. (1982) and Donnelly et al. (1984); in addition to the estuarine habitats of Puget Sound, these techniques were utilized to sample juvenile salmonids and other motile fishes in Grays Harbor (Simenstad and Eggers 1981) and Neah Bay (Simenstad et al. 1988b). Trumble et al. (1982) used midwater trawling to sample spawning herring in northern Puget Sound during 1976-1979, although they did not report species other than Pacific herring. Bollens and Frost's (1989) studies of motile neritic fishes in the surface waters of Hood Canal provide one of the few highly quantitative datasets of its kind. Levy and Northcote (1982) provide data from channel trap net collections in an emergent (*Carex*) marsh in the Fraser River delta and Shreffler et al. (1988, 1990, 1991) and Thom et al. (1988) provide analogous data for a restored wetland system in the Puyallup River estuary using the fyke-modified trap net. The use of an Oneida trap is relatively exclusively reported by Parametrix, Inc. (1982).

GENERAL AVIFAUNA

HABITAT OCCURRENCE

Eelgrass;	Aves (unid.)
Emergent marsh;	Aves (unid.) Charadiidae (unid.)
Mudflat;	Aves (unid.)

CHARACTERISTICS

Birds in general, but especially those associated with estuarine habitats, including most of the shorebirds and waterfowl occurring in the habitat assemblage groups.

PARAMETERS

Minimum;
(1) species occurrence;

Recommended;
(2) density;

Preferred;
(3) activity patterns; and
(4) diet.

Species occurrence

When produced from an intensive sampling effort over time and space, the relative occurrence of species (providing species richness over space and time) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as an assessment of gross levels of habitat quality. Species richness should be defined as relative to sex and life history stage, especially because the use of the habitat by reproductive individuals and juveniles is a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages and sexes by microhabitat and activity category and as (2) the cumulative number of species/life history stages and sexes, and times because different methodologies have selection biases and because avifauna movement and migration patterns can be highly variable.

Density

Density measurements are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the birds and their habitat which would otherwise not be reflected by species richness. Density is expressed as the number per unit area (no. km^{-2}) of the habitat or, if expressed for the total transect or area surveyed, the transect/plot area should be established and any variances associated with areal coverage documented. As in species occurrence, density and standing stock should be expressed relative to species, life history stage and sex, and relative to microhabitat, tidal cycle and activity pattern. These effects can also influence estimation precision; for instance, Herman and Bulger (1981) reported that the best counts were obtained two to three hours on either side of high tide, i.e., when the tideline was closest to the observers. While individuals can be counted for most species, counts of shorebirds and waterfowl must be scaled to tens and hundreds when they occur in high densities. Particularly when counting dense groups of birds, counts should be made repeatedly or by different observers and a consensus count agreed upon.

Activity pattern

In conducting either individual or group observations, it is recommended to categorize the counts as to activity pattern. These can include, but not be limited to: (1) feeding; (2) roosting/resting; (3) flight, including direction; (4) mating; (5) territoriality/aggression; and (6) nesting. It may be particularly informative if one behavior can be linked to another (e.g., aggression to nesting in mated pairs), or an area/habitat used for one behavior can be linked by flight patterns to an area/habitat used for another behavior, such as from a feeding area to a roosting area. In addition to recording the incidence of different behaviors, long-term observation of individuals or groups should gather data which measures time (inferring energy) budgets, preferably over the diurnal period of activity.

Diet

Food habits of estuarine avifauna in this region are usually extrapolated from the same or related species in other regions and we generally know little about their feeding dependencies upon specific plant or animal taxa unique to Pacific Northwest estuarine habitats. Therefore, any quantitative observations or sampling of avifauna to determine food habits would contribute measurably to our understanding of the habitats' function. At a minimum, any detailed observations of feeding birds should note their basic feeding strategy. If prey are visible, they should be categorized if not identified. In the case of nesting birds, food remnants around the chicks may provide information on the diet during that period. Non-destructive sampling of stomach contents might involve obtaining regurgitated food items when the adults return to feed nestlings. Although biased by digestion, analysis of prey hard parts (e.g., bones, shell, otoliths) in fecal remains may also provide ancillary information. If feeding is a function upon which the habitat assessment is dependent, and none of these methods provide conclusive evidence, destructive sampling of birds utilizing the habitat may have to be considered in order to verify feeding by detailed stomach contents analyses.

In the case of birds, both the crop (expansion of the esophagus in front of the stomach) and the proper stomach should be examined for food items. In general, the description of diet must be made at the population level and document both numerical and gravimetric (biomass) or volumetric measures of food item "importance." The volume of food items should provide a comparable measure as weight, but is not used as often because it is more difficult to measure at low levels. Thus, stomach contents analyses to gather this information need to be conducted on individual birds rather than for pooled samples in order to maintain a measure of the population utilization of the item, i.e. as frequency of occurrence, and of the extent of any feeding selectivity. In addition to counts and weights (wet damped, dry, or ash-free dry) of the food items in each stomach, some indices of stomach fullness and digestion rate should also be assessed; these often involve a scale between 1 to 6 and 1 to 10. Recording of the total stomach contents weight before separation into identifiable food items will also provide, by subtraction, a measure of the weight and proportion of unidentifiable (digested) material.

Diet composition can be described using this combination of measures as an Index of Relative Importance (IRI) which is created for each food item i;

$$I.R.I_i = \{\%F.O._i\ (\%N.C._i + \%G.C._i)\}$$

where %F.O. = the percent frequency of occurrence, %N.C. = percent numerical composition, and %G.C. = percent gravimetric/volumetric composition. Because the resulting numerical values for the I.R.I depend greatly upon sample size, the relative importance is often converted to the percent of the total I.R.I (%I.R.I.). Graphically, this is illustrated by a two dimensional figure in which the abscissa is %F.O. and the ordinate is %N.C. above the abscissa and %.G.C. below the abscissa. The original I.R.I. description occurs in Pinkas et al. (1971), and the approach is modified and described further in Cailliet (1977) and Cailliet et al. (1986).

RECOMMENDED SAMPLING METHODOLOGY

Method: **transect/plot observations**

(1) Quantitative observations:

Most estuarine avifauna surveys are conducted by systematic observations, either along transects (Hazel 1984) or from established sites or plots (Herman and Bulger 1981). When undertaken for broad regions such as large bays, sounds, and inlets, transects are often conducted from vessels or from aircraft (Maunal et al. 1979; Wahl et al. 1981). Observations are made with the assistance of binoculars and spotting scopes for standard times and areas and species, relative sizes, sexes, and activity categories are recorded. It is important that all observations be stratified or otherwise categorized by tidal stage because many of these birds are feeding, resting, etc. according to these cycles.

Unit: **dependent upon areal extent of habitat, 100-m^2 transects/plots in large habitats**

Periodicity and Frequency: **weekly during peak migration periods (Spring, Fall), monthly during other periods**

Statistical Considerations (Replication): in lieu of statistical predetermination using pilot study or historical data; **n=3**

Replicate sampling is not always a consideration in avifauna studies, particularly because the observations are continuous or are designed to assess the whole population(s) in the study area. It is recommended, however, that some element of replication be incorporated into the sampling design in order to evaluate sampling error. In particular, repeated sampling of the same population by different observers should be examined for observer bias and estimation of that source of error incorporated into the sampling design.

CASE STUDIES

The principal quantitative studies of estuarine avifauna in this region are those conducted during the MESA program in northern Puget Sound by Manual et al. (1979) and Wahl et al. (1981) and in Grays Harbor by Herman and Bulger (1981) and the Columbia River estuary by Hazel (1984). Couch (1972) provides one of the few quantitative accounts of avifauna feeding ecology in this region.

HERBIVOROUS MAMMALS

HABITAT OCCURRENCE

Emergent Marsh;	*Microtus* spp. *Microtus townsendii* *Ondatra zibethicus* *Peromyscus maniculatus* *Sorex bendirii*

CHARACTERISTICS

These are small (voles) to moderate-sized (muskrat) mammals which either live entirely in or venture into emergent marsh habitats; they may also range from adjoining upland habitats to forage along the intertidal zone. While detailed examination of the habitat can often provide some information on small mammal use, sampling with traps will usually be required to generate quantitative information on species, density and standing stock, and population structure.

PARAMETERS

Minimum;
(1) species occurrence;

Recommended;
(2) density and standing stock;
(3) birth and rearing sites;

Preferred;
(4) population structure (e.g., reproductive state); and
(5) diet;
(6) activity patterns.

Species occurrence

When produced from an intensive sampling effort over time and space, the relative occurrence of species (providing species richness over space and time) can provide a minimal indication of the viability of estuarine wetland and nearshore habitats, principally as a gross assessment of habitat quality. Species richness should be defined as relative to life history stage, especially because the use of the habitat by reproductive individuals and juveniles is a more important supportive function than if only adults occur. Species occurrence should be represented as both (1) mean number of species/life history stages and sexes by microhabitat and activity category and as (2) the cumulative number of specie/life history stages and sexes, and times because different gears have selection biases and because small mammal movement and migration patterns can be highly variable.

Density and standing stock

Density measurements are more definitive indicators of subtle differences in habitat quality because habitat characteristics can effect density-dependent interactions between the birds and their habitat which would otherwise not be reflected by species richness. Density is expressed as the number per unit area (no. km^{-2}) of the habitat or, if expressed for the total transect or area surveyed, the transect/plot area should be established and any variances associated with areal coverage documented. As in species occurrence, density and standing stock should be expressed relative to species, life history stage and sex, and relative to microhabitat, tidal cycle and activity pattern. These effects can also influence estimation precision; for instance, Herman and Bulger (1981) reported that the best counts were obtained two to three hours on either side of high tide, i.e., when the tideline was closest to the observers. While individuals can be counted for most species, counts of shorebirds and waterfowl must be scaled to tens and hundreds when they occur in high densities. Particularly when counting dense groups of birds, counts should be made repeatedly or by different observers and a consensus count agreed upon.

Birth and rearing sites

Birth and rearing sites are usually inferred from the capture of pregnant or lactating females, as reproductive activities generally occur within the limited home ranges of small mammals (Howerton 1984).

Population structure

Population structure information is derived principally from trapped individuals. Each animal is examined for size, sex, age, reproductive status and general condition. Age can be determined by either sectioning teeth, using the sequence of tooth eruption, using the method of Hugget and Widdas (1951) which is based on fetal weight at birth (estimated by capture of recently-born young or from literature) and the gestation period (estimated from the literature), or inferring from growth curve data in the literature (e.g., Dorney and Rusch 1953 for muskrats). Reproductive status can be determined from examination of females for visible signs of pregnancy or recent birth (e.g., fetuses, recent placental scars, lactation).

Diet

Food composition of the small mammals can be inferred from the remains of food items left at documented feeding areas (along transect lines), from analysis of the plant fragments in the stomach contents and in fecal remains, and from direct observation; trapped individuals can only be used for stomach analyses if they were trapped without bait or with traps which did not allow the animal to the bait.

In the case of identification at the feeding site, the presence of tracks, scats and or species-specific evidence (e.g., cuttings, diggings, etc.) are used to identify the species of mammals feeding at the site. These data are usually represented as the percent occurrence of mammal species feeding on a specific plant taxa. Direct observation generates similar data except that the time energy allocation to feeding on specific plant taxa can provide a more quantitative, comprehensive measure of feeding specificity and food item dependency.

Stomach and fecal remains analyses can provide more detailed information because the weight (biomass) contribution of plant taxa to the diet can be determined and an index of the variance over the population (percent frequency) for each food item can be estimated. Plant fragments are usually measured microscopically, using methods similar to those of Sparks and Malcheck (1968), Davitt and Nelson (1980) and Korfhage et al. (1980). Data generated are expressed as frequency of occurrence of identifiable food taxa in the sample and food item weight over the individuals. In the absence of meaningful measures of food "abundance," an I.R.I. approach such as used to assess food item importance to fishes (see Sedentary and Motile Fishes, above) cannot be used.

Activity patterns

Whether generated by real-time observations or radio telemetry of small mammals, the activity "budget" over diel periods, usually represented as the time or percentage of the daily record which was spent in a particular activity in different habitats. In the case of the telemetry data, some observation or supplemental information (e.g., recent feeding evidence) will be necessary to validate assumptions of unobserved activities.

RECOMMENDED SAMPLING METHODOLOGY

Method: **trapping transects/plots**

(1) Trapping transects/plots:

Seasonal and permanent transects are typically established along which traps and baits are used to capture individuals. In the Columbia River estuary (Howerton 1984) these transects consisted of four trapping stations spaced 25 m apart; each trapping station was assumed to have a diameter of approximately 8 m or a surface area of ~50 m^2. The transects were placed along the mean high tide elevation, which allowed they traps to function at high tides and still sample mammals foraging into the intertidal zone. At each trapping station, traps are placed within microhabitats where the animals are most likely to occur, such as runways, burrows and cavities. Howerton (1984) operated the trapping transects seasonally for three consecutive nights at each transect.

Unit: **50 m^2**

Periodicity and Frequency: **nightly for three nights, bimonthly March-September**

Statistical Considerations (Replication): in lieu of statistical predetermination using pilot study or historical data; **n=3**

Like quadrat sampling, trapping and other sampling of mammals along transects or in plots should be designed in proportion to the overall habitat area. In the case of standard transect distances/areas, these are usually conducted in adjoining segments through the center of the habitat. Sampling plots, such as trapping stations, are often distributed along these transects, and average four to five in number, within which as many as ten traps are deployed. Howerton (1984), for instance, used this general design to analyze estuarine mammal population indices based on trap catch per 100 trap nights. Several historical studies have monitored study sites for five days per season sampling interval.

ALTERNATIVE PROCEDURES

(1) Radio telemetry:

Activity patterns and distribution in the habitat and among microhabitats can be obtained for large mammals (*Ondatra*) by the mounting of radio transmitters on individuals and locating or monitoring their locations over periodic periods. Howerton (1984) used surgically-implanted transmitters for monitoring muskrat and nutria activities and habitat use in the Columbia River estuary.

CASE STUDIES

To our knowledge, the only documented quantitative studies of these small herbivorous mammals in estuarine habitats are those of Schoen (1972) and Howerton (1984).

PHYSICOCHEMICAL ATTRIBUTES

Although physical and chemical attributes were often identified as important attributes of estuarine habitats promoting fish and wildlife use, no values *per se* were identified in the protocol development process. Such associations between a habitat's structure and physiochemical environment, although based only on qualitative observations, should not be discounted. Physiochemical attributes are particularly important to habitat restoration because many of these parameters can be directly incorporated into the initial project design, e.g., sites can be selected within specific salinity ranges and exact bathymetric/topographic features can be created at the project's inception. The results of this portion of the Protocol development highlights the limitations of our understanding of fish and wildlife habitat requirements. Therefore, an important aspect of the Protocol is to accumulate physiochemical information that will enhance our *quantitative* understanding of these factors to fish and wildlife support.

Appendix C and Supplement 7 (Simenstad et al. 1990) lists those attributes which were identified as potentially being important, even if no specific value, range, etc. could be listed. We recommend monitoring of as many of these as possible because of their suspected importance to fish and wildlife species. Based upon these responses and our expert opinions, we have placed the following priorities on the measurement of physiochemical attributes and our recommendations for their measurement:

RECOMMENDED

(1) Topography/bathymetry:

If not available or recent, a complete survey should be conducted of the site. The survey should be linked to established benchmarks and referenced to the closest NOAA tide gauge. The land portion of the survey should extend as far into the intertidal as feasible, and preferably conducted during a strong (e.g., minus) spring tide series. If subtidal habitats are involved as extension of intertidal habitats, a comparable bathymetric survey should be conducted, overlapping with and tied to the intertidal survey. An elevation datum should be installed permanently in a protected area of the site, where it would not likely be lost to erosion/accretion processes. All station positions should be exactly located on this survey. Protocols for station positioning are found in Puget Sound Estuary Program (1990c). In addition to these surveys, horizontally-corrected aerial photographs should be acquired of the site annually. These should be flown at the lowest elevation possible that encompasses the site, preferably 500 ft.

If only subtidal habitats are being considered, a bathymetric survey should be conducted if none is available, current or of reasonable resolution. This should preferably be conducted with a digitally recording echosounder linked to a positioning system (e.g., microwave triangulation, Loran or Global Positioning System).

(2) Water character/quality:

At a minimum, basic water characteristics should always be monitored during sampling to describe temporary conditions during sampling. These measurements should include *at least*: (1) temperature; (2) salinity; (3) turbidity, and (4) dissolved oxygen. These samples or measurements should be collected at the immediate location and time of sampling, including at the sampling depth if submerged.

Given the dynamic nature of estuarine habitats, however, such extremely episodic measurements will not *characterize* the habitat; much more extensive, long-term data acquisition is required. Temperature, salinity, tidal elevation and *any* constituent of estuarine water should be gathered over *representative* tidal cycles and freshwater flow periods. Ideally, a permanent sampling station, such as a tide gauge or a CTD (Conductivity-Temperature-Depth) or STD (Salinity-Temperature-Depth) instrument package, should be installed over a years period. If only short-term sampling can be conducted, it should occur over the entire tidal cycle (e.g., at least 26-30 hr) at least once during each stage of the tidal month (e.g., weak and strong neap and spring tidal series) and seasonally during the maximal and minimal freshwater flow periods. Optimally, short-term sampling should occur during all stanzas of the local hydroperiod, including during storm events. All measurement procedures should follow the Puget Sound Estuary Program's recommended protocols for measuring water-column variables (Puget Sound Estuary Program 1990c) or a method of superior precision and validity.

(3) Sediment structure (for all but water column habitat):

Sediment structure should be sampled at least yearly and potentially more frequently in habitats subject to sporadic erosion/accretion activity, including bioturbation. Although dedicated samples can be used, sediment may also be retained from samples for benthic infauna, below-ground vegetation biomass and other soil/sediment attributes. Measurements and analyses should include *at least*: (1) mean grain size distribution; (2) statistical characterization of sorting, skewness and kurtosis; and (3) percentage of organic matter. The recommended procedures for determining these parameters is contained in Dyer (1979).

SUGGESTED

(1) Water flow levels:

The relationship between the flow (quantity of fluid moved) of fresh water and tidal water through estuarine habitats and the structure of biotic communities in these habitats is poorly understood. These data are important from the standpoint of both the transport of constituents such as suspended sediments, nutrients and organic matter and of the erosion and deposition of sediments within the habitat. In habitats where flow can be feasibly estimated, e.g., tidal channels, it is recommended that recording instrumentation such as current meters be deployed over representative tidal month periods (e.g., one each during low and high freshwater flow). Due to the complex nature of water velocities in shallow water, these measurements should optimally be taken at several points in the water column, and particularly close to the sediment surface.

(2) Sound/light levels:

Due to the lack of information on responses of estuarine organisms to disturbance, you are encouraged to conduct some sampling of sound and light levels in the vicinity of the sampling site. These would preferably be monitored continuously for one year or at least during representative seasonal periods. We know of no published sound/light standards or protocols. The accepted measurement unit for human health effects is "A-weight" sound levels, and 55dBa is a common threshold for A-weighted sound averaged over 24 hours. This level may provide an appropriate benchmark from which to make comparisons with fish and wildlife behavior.

Light levels are even more nebulous, even in human health considerations. Work standards often use 3-7 foot candles averaged over the work area as a standard, with 0.5-1.5 foot candles acceptable in perimeter work areas. However, there are many categories of artificial light, e.g., diffuse, indirect/reflected, and direct glare, and these have yet to be standardized. We suggest considering all these aspects when monitoring light impacts.

(3) Surface sediment contaminants:

Both prior and recurrent sampling of sediment contamination in development, restoration, and reference sites is important to understanding the limitations upon biotic communities existing or anticipated to develop within estuarine habitats. Estuarine habitats have not only been direct depositories of contaminants, but also sinks of contaminants transported to the site from other regions of the estuary or outside the estuary entirely. Recurrent sampling, rather than sampling on one occasion, is also meaningful because the rate of contaminant accumulation may be a pivotal parameter in determining the biological effects or uptake by biota. Particularly in the case of habitats in urbanized estuaries, we recommend that sediment contaminant sampling be incorporated into the initial assessment and later monitoring designs.

At the minimum, a basic screening should conducted for the State of Washington's *Pollutants of Concern*, i.e., organics, heavy metals, PCBs (Tetra Tech, Inc. 1986). If any of these are found in threshold concentrations (low Apparent Effects Threshold [AET]), further analysis will be required using the State of Washington's sediment management standards. For these analyses, the reader should consult the State of Washington's sediment management standards (Chapter 173-204 WAC; State of Washington Department of Ecology 1991) for: (1) contaminant standards for the quality of surface sediments; (2) biological effects criteria; (3) clean-up standards for contaminated sediments; and (4) sampling and testing standards.

DISCUSSION

APPLICATION AND TESTING

The potential of the Protocol to foster more effective estuarine habitat restoration and mitigation can be realized only if an attempt is made to apply it. The most likely avenue for application will be the habitat or aquatic resources permit process (e.g., Shorelines, Clean Water Act Section 404). In addition, the information required for revisions and modifications can only be gained through application on a broad scale across a variety of habitat types and estuarine situations. It has been designed essentially as a "toolbox" to be used by all parties involved in habitat restoration/ mitigation activities, from the developer and consultants to the agency representative reviewing the permit applications and environmental impact assessments. Therefore, it is designed, not to specify what must be measured and what procedures must be used, but to provide the means to develop a "template" for procedures to assess both the estuarine resources to be lost to development and the mitigation project designed to replace those resources.

Using this strategy, the Protocol should be applied in an adaptive manner. Because every estuarine site, every development activity, every restoration or mitigation project is unique, we realize that each situation will require a different subset of tools from those available in our toolbox. In most cases, the agency representatives and biological consultants are better equipped to isolate the fish and wildlife issues involved with particular sites, and the site characteristics which constrain various assessment procedures. These parties should collectively negotiate the habitat type(s), attributes, parameters and procedures relevant to the particular situation. For instance, in the case of Clean Water Act-Section 404 Permit and Washington State Hydraulic Permit Application (HPA) processes, the Protocol would provide the basis for a common template to be negotiated in the initial pre-scoping meetings intended to guide subsequent impact assessments and mitigation designs.

In addition to routine application in the permit process, the concept and approach of the Protocol also need to be verified formally in an explicit test outside the constraints imposed by mitigation requirements. In such a test, the Protocol would be applied in a research project to compare natural and restored/created estuarine habitats in Puget Sound with the coastal estuaries of the region in order to validate the importance of the ecological relationships upon which the Protocol is based and to generate at least one comparable dataset that encompasses as many attributes and parameters as feasible.

REQUIREMENT FOR REFERENCE SITES

Testing and implementation of the Protocol and its inherent strategy for monitoring may reduce or eliminate many of our current problems with estuarine habitat restoration and mitigation. However, the *need still remains to relate Protocol-generated data to "benchmark" or reference habitats that function in a comparatively natural manner*. Any study that looks at the effects of a real or predicted impact requires some sort of "control" or baseline with which to compare affected sites. This is a *major restriction* on the long-term utility of the Protocol because there are no such programs for consecutive monitoring of any of these attributes in estuarine habitats of Puget Sound. Even with site- and project-specific control habitats, which will *always* be required without such reference site monitoring, any interpretation of the function of restoration or mitigation sites will be inhibited by the absence of any long-term data that indicate the magnitude and trends in variation of attribute parameters.

Reference site monitoring would enable local coastal resource managers to evaluate more precisely the result of site-specific monitoring in terms of long-term trends. In addition, such data would be the only means by which the optimum sampling frequency and duration for restoration/mitigation monitoring can be derived because permit-required monitoring will unlikely be of the intensity and resolution to define natural cycles and variation.

The reader is referred to Hughes (1985), Hughes et al. (1986), and Hughes et al. (1990) for further, more detailed arguments for development and application of reference sites.

REVISION, MODIFICATION, AND EXPANSION OF THE PROTOCOL

As stated in the Introduction, the Protocol is a somewhat basic, untested approach to a complex scientific and policy situation. It is, however, an approach that shows promise, and one that can be tested through both regulatory (permit) and scientific (grant and contract) activities over the next few years. As such, *this version should be considered preliminary and certainly subject to revision or rejection after a reasonable test period.* Just as the Protocol approach was validated initially at the Estuarine Wetland Restoration Monitoring Protocol Workshop in Port Townsend, Washington, on 16-19 April 1989 (see section on Review Workshop), the results of the first two to three years of application and testing of the Protocol would be best critiqued in a workshop with the original workshop participants and any additional estuarine specialists who have used or dealt with the Protocol on any level.

At such a "reject or revise" workshop, the participants should examine critically the successes and failures of the Protocol in achieving its objectives. If the results have been less than successful, the participants should evaluate whether or not this arises from the concept of the Protocol, from its application to actual situations, or because of inappropriate parameters, attributes, and other driving variables. It would then follow that the workshop would work toward a consensus to (1) reject the Protocol as being ineffective, (2) recommend changes in the way it is implemented in the regulatory process, or (3) identify additions and modifications that would enhance its applicability. The product of the workshop would be either recommendations for different approaches to monitoring estuarine habitat functions or changes to the existing Protocol.

In particular, further revision of the Protocol *must* address the lack of identifiable physical/chemical attributes which promote fish and wildlife support. In the sense that our lack of understanding of these factors is manifested in the paucity of abiotic parameters in the existing Protocol, a major objective of the "revision" objective of the workshop will be to compile any new information and propose studies that might gain information about these attributes.

It must also be remembered that the Protocol is but one approach to monitoring only one function (fish and wildlife support) in one subset of wetland and aquatic habitat types (estuaries). If the Protocol is effective, this approach would have to be repeated for other acknowledged aquatic habitat functions and for other types of aquatic habitats, specifically those in fresh water (e.g., lacustrine, palustrine). This may or may not be appropriate, and will depend upon the opinion of hydrologists, sedimentologists, geomorphologists, ecologists and other wetland scientists as to attributes identifiable with other functions.

A useful example of a different approach to wetland habitat function assessment is that of Horner and Raedeke (1989) in their **Guide for Wetland Mitigation Project Monitoring**, in which they have attempted to address the more important functions of freshwater wetlands and monitoring. The approach of Horner and Raedeke is more intensively focused on a few select parameters over a wide range of functions, while this Protocol is comprehensive for only the primary function of fish and wildlife habitat, and secondarily for food web support. These different approaches should be compared and evaluated as their use grows, and the best of each should be integrated into subsequent approaches.

BEYOND THE PROTOCOL

The Protocol should be considered only the first step in a sequence of activities designed to provide more scientific rigor to wetland and other aquatic resource policy decisions and restoration/ mitigation projects. If the objectives for which the Protocol was developed are to be fulfilled, several other endeavors should follow or, even better, occur simultaneously.

EVOLUTION OF DESIGN CRITERIA

While one of the goals driving the development of the Protocol was to develop a system that results in successful design criteria for habitat restoration and mitigation, these criteria will not evolve without gathering information about the relationships between the attributes (and the specific parameters) and the habitat characteristics that influence them. Some of this information can be obtained by examining the distribution of attribute parameter values relative to the structure of the habitat. Thus, it is important when interpreting attribute parameters to consider them in the context of their location in the habitat, their relationships to microhabitats, morphology, bathymetry, surface runoff and seepage, etc. For example, we have identified larval insects (e.g., Chironomidae) as important attributes (as prey) of many emergent marsh and mudflat fish and wildlife. Yet, we have little or no information on the basic habitat requirements of these infaunal/ epibenthic forms of flies. We do not know if they require specific sediment structure and organic content, whether they are attached to specific emergent plants, and where and when they are available to foraging fish and wildlife.

In and of itself, attribute correlation does not necessarily imply functional relationships. However, correlation among physical and biological attributes, and with other characteristics of the habitats, can suggest testable hypotheses about the relationships between the habitat structure and the distribution and abundance of attributes. Such hypotheses could be examined specifically in dedicated field or laboratory experiments. This coupling between the regulatory process of restoration and mitigation and applied ecological research is one of the few ways in which we will be able to provide feedback into, and ultimately advance, the "ecotechnology" of habitat restoration/ creation. This feedback cycle is a specifically scientific application of the policy of adaptive management, which has been advocated as a strategy for optimizing Pacific salmon enhancement (Larkin 1974; Holling 1978; Lee and Lawrence 1986).

SCALING FACTORS AND OTHER CONSIDERATIONS BEYOND HABITAT TYPE

From its initiation, the protocol development process was based on the concept of a single uniform habitat, regardless of its size, shape, and relationship to other habitats. This was necessary, in part, because interactions between fish and wildlife and the habitat were considered so complex as to limit our initial scope. In fact, the extent of habitat support for many fish and wildlife species may be limited not so much by the attribute parameters in the habitat as by the diversity and arrangement of the attributes within the habitat, the habitat's morphology, the scale and configuration of the habitat, and its connections to other wetland or upland habitats. Yet, the Protocol fails to provide criteria for any of these "second order" influences simply because we had no information upon which to make such judgments. This is to say that we may now have the building blocks (the attributes) to assess habitat function but we need to know how they fit together before we are able to provide a working structure (functioning habitat).

One obvious way to address this deficiency in the Protocol is to test how adding and diversifying attributes affect the number of fish and wildlife species using the habitat; we presume intuitively that this is a positive relationship but have no empirical knowledge of its form, e.g., whether it is linear or exponential, etc. Obviously, selecting attributes that are functionally important to many assemblage species in that habitat would be the most direct way to maximize utilization in terms of the number of fish and wildlife potentially attracted to the habitat. But, if we wish to incorporate criteria promoting these attributes into designs for restoration or mitigation projects, we need to understand the tradeoffs incorporating many attributes versus focusing effort on a few, more "universal" attributes.

Habitat scale, a concept of the emerging science of landscape ecology, logically should be an important determinant of fish and wildlife support although its influence remains relatively untested. Obviously, the size of a habitat will determine the level of any space-limited attribute, such as attached (sessile) epifauna that are limited to hard substrate within a narrow tidal range. But, the functional thresholds for even these seemingly straightforward relationships are unknown. For instance, we do not know the minimum dimensions of an eelgrass or marsh vegetation patch that ensure benefit to fish and wildlife. Similarly, the configuration of the habitat, and its component microhabitats, may well produce variable results in terms of fish and wildlife utilization. Following the same example; is there a difference in the way fish and wildlife respond to many scattered, dense patches of eelgrass or marsh vegetation as compared with one large but contiguous sparser patch? Finally, there is the question of how important connectivity (such as corridors) and transition habitats (buffers zones) are in determining the extent of fish and wildlife use: All the highest parameter values for a plethora of attributes will not benefit a fish or wildlife species if the boundaries of the habitat do not have the characteristics that the species requires for access.

Until questions of scale, configuration, complexity, and connectivity are examined directly, this approach to enhancing the success of estuarine habitat restoration and mitigation through better design criteria will not achieve its full potential.

COMPREHENSIVE, ESTUARY-WIDE PLANNING OF RESTORATION AND MITIGATION

Finally, we suggest that in both policy and practice, *restored and created estuarine habitats will never achieve full function as long as the location and composition of estuarine habitats are not considered in the context of the estuarine ecosystem.* Until we consider the ecosystem of which we are manipulating but a segment, we face the constant possibility of failure because of factors outside our sphere of consideration. In this context, it is worth noting Diamond's (1987) observation that,

> We're not restoring the natural landscape but tiny pieces of it. The integrity of these tiny pieces will inevitably depend on the influences from outside their borders. Hence restored communities tend to be small, and to support small populations prone to extinction partly because they depend upon resources beyond the boundaries of the community. We are the ones who will have to supply those resources to small, isolated communities to prevent extinctions and so to preserve the community itself. And doing this is a form of restoration—or at least active maintenance.

The roots of the Protocol are based in estuarine scientists' and managers' studies and observations over a broad number and spectrum of natural estuarine habitats, but over only a few restoration/mitigation projects. Thus, the attributes they have identified as being most important in determining fish and wildlife utilization originate from the way the habitats function in the estuarine system, not as isolated habitats. In contrast, both (1) evaluating the function of the habitat to be developed and (2) determining an appropriate restoration/mitigation plan presently treat the habitat in isolation because it is almost exclusively the applicant's option to choose the habitat and location. In most cases, but not necessarily all, the driving constraints are cost: cost of the land, cost of the restoration/mitigation design, cost of monitoring.

Yet, the success of any particular restoration/mitigation project may depend just as much on where it is located in the estuary as on how it is designed. Circulation and sedimentation patterns, salinity distribution, freshwater discharge cycles, water quality, location of critical estuarine features such as the turbidity maximum (null zone), migration corridors of fish and wildlife: These factors are important considerations in determining where and in what form restored/created habitats should occur.

If restoration and mitigation projects are to succeed on a large scale, they must be planned and implemented on a large scale. At a minimum, this scale must encompass all factors that determine the occurrence, distribution, and ecological role of habitats in our remaining estuarine ecosystems.

A primary consideration, for instance, should be the historic habitat composition and distribution in the estuary before the advent of dredging, filling, and diking. The historic structure of the estuary's natural estuarine habitats thus could provide a template both for planning how restoration/mitigation can best reestablish viable habitats and for determining where chosen projects have a high probability of succeeding.

It is not our position to advocate a particular restoration/mitigation policy but to argue the scientific basic for planning on an estuary-wide scale. The policy alternatives in such a planning effort are being treated elsewhere (Tanner 1990) and are outside the scope and mandate of the Protocol.

SUMMARY

The **Estuarine Habitat Assessment Protocol** described in this document is an *ad hoc* approach to assessing estuarine habitat function for fish and wildlife using quantitative parameters. In developing the Protocol, we drew on the accumulated knowledge of estuarine scientists and managers in the region using a hierarchical series of questionnaires and a workshop to synthesize their expertise. Although the Protocol may be found inadequate, it will likely be because the scientific information about fish and wildlife use of habitat in the Pacific Northwest, and estuarine habitat attributes that account for it, is quite restricted. Of course, the impetus to the Protocol and its novel structure is, in part, intended to systematically expand this meager base of knowledge.

Our focus from the beginning has also been exceedingly narrow, limited to estuarine habitats and only their function in support of fish and wildlife. This in no way discounts the importance of other habitats to fish and wildlife, nor the other functions of all habitats; there is just as much a need for standardized, quantitative monitoring protocols for these as there is for the habitats and functions we have addressed. We invite similar attempts in these other arenas.

Finally, we repeat our common goal to be effective in reconstructing damaged estuarine ecosystems and to ensure that no net loss is incurred in the remaining undeveloped estuarine habitats. The Protocol is one attempt to facilitate this goal, by establishing tools to (1) learn not only from our successes but also from our mistakes; (2) emphasize restoration/mitigation based on ecological processes (i.e., function) rather than just structure; (3) provide insights into the dynamics of estuarine communities; and (4) provide "basic" information about the way estuarine communities develop. A perspective on restoration pointed out by Leopold (1934) long before habitat restoration/mitigation was a viable concept, much less a practice, is that ecologists can take the same approach as chemists in establishing the elemental composition of a substance by analysis; but, as in chemistry, the proof of structure lies in synthesizing the compound. Similarly, restoration of complex habitats can only benefit from its treatment as experimentation into the processes which determine their "function" to man. We must make this opportunity work to our advantage.

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GLOSSARY

algae (pl. of alga)	simple plant form having no true roots, stems or leaves; ranging in size from microscopic, single-celled plants (microalgae) to seaweeds (macroalgae)
amphipod	crustaceans in the Order Amphipoda, of Class Malacostraca
attribute	physical and biological characteristic of wetland habitats which foster fish and wildlife utilization by facilitating reproduction, foraging, refugia (from predation, disturbance) and physiological adaptation
attribute definition	the process by which the estuarine wetland attributes were determined; involving (1) the selection of process representative wetland habitats, (2) fish and wildlife assemblages associated with them, (3) their primary functions determining fish and wildlife utilization, and the (4) ranking of these functions and (5) the attributes of the wetland which are responsible for or indicative of the wetlands' functional support
benthic	pertaining to the bottom of a body of water
brackish	pertaining to water with a salt content ranging between that of sea water and fresh water; commonly used, however, to refer to oligohaline waters
calanoid copepods	crustaceans in the Order Calanoida, of the Class Copepoda
chlorophyll	green pigments essential to the process of photosynthesis, found primarily in plants; chlorophyll *a* is a specific type of chlorophyll pigment often used as an indicator of plant biomass
cladocerans	crustaceans in the Order Cladocera, of the Subclass Branchiopoda
copepod	crustacean in the Class Copepoda; includes both pelagic (Calanoida, Cyclopoida) and benthic/epibenthic (Harpacticoida) orders
cyclopoid copepods	crustaceans in the Order Cyclopoida, of the Class Copepoda (also generally includes those in the Order Poecilostomatoida)
creation	modification of estuarine shoreline to create a wetland which was not historically present
cumaceans	crustaceans in the Order Cumacean, of the Class Malacostraca
demersal	pertaining to an organism, such as a fish, living close to or on the bottom of a body of water; describing the habitat close to or on the bottom
density	the number of organisms per unit of area or volume eelgrass (habitat) intertidal and shallow subtidal, unconsolidated sand to mud shores which are colonized by rooted vascular angiosperms (seagrasses) of the genus *Zostera*; two species are prominent in the

	Pacific Northwest: *Zostera marina*, the endemic eelgrass, and Z. *japonica*, an introduced congeneric
eelgrass (habitat)	intertidal and shallow subtidal, unconsolidated sand to mud shores that are colonized by rooted vascular angiosperms (seagrasses) of the genus *Zostera*. Two species predominate in the Pacific Northwest: *Zostera marina*, the endemic eelgrass, and Z. *japonica*, an introduced congeneric
elutriation	method of removing organisms from a sediment sample by continuous flushing with water or another aqueous chemical
emergent marsh	intertidal shores of unconsolidated substrate which are colonized by erect, rooted, herbaceous hydrophytes, excluding mosses and lichens
enhancement	an effort to improve habitat quantity and quality specifically for fish and wildlife utilization, and unrelated to mitigation or other development activity
epibenthic	pertaining to the benthic boundary layer habitat at the interface between the bottom surface and the overlying water column, or to the organisms living in this habitat
epipelagic	pertaining to organisms which, though associated with the bottom, actively migrate off it into the water column, sometimes to the surface in shallow depths
estuarine	occurring within the boundaries of the estuary, as defined seaward by the measurable dilution of seawater, usually between headlands enclosing an embayment, upstream by the limit of tidal influence, and landward by mean higher high water, but including transition riparian and upland habitat margins
euhaline	waters in the salinity range of 40-30 ppt
euphausiids	crustaceans in the Order Euphausiacea, of the Class Malacostraca
fish and wildlife assemblages	groups of species that are representative of all fish and wildlife species that commonly utilize specific estuarine habitats; not inclusive of all species, but each feeding, reproduction, etc., use is represented; not guilds
gammarid amphipods	crustaceans in the Suborder Gammaridea, of the Order Amphipoda (see amphipod)
gravel-cobble (habitat)	intertidal shores which have substrates composed of a mixture of cobble (-8ø to -7ø; 256 to 76 mm) and gravel (-7ø to -2ø; 76.2 to 4.76 mm); the habitat tends to be formed as beaches and bars, due to wave and current action, and seldom flats
habitat	sum total of environmental conditions of a specific place that is occupied by an organism, a population, or a community
haphazard sampling	selecting samples using a subjective judgment of randomness

hard substrate (habitat)	outcrops, erratics and other rock formations scattered around the predominantly unconsolidated shoreline of Puget Sound; this habitat is usually rare within discrete estuaries, where the heavy sedimentation has often resulted in complete burial since the last glaciation; they persist, however, where wave and current exposure maintain exposure of rocks and rock outcroppings
harpacticoid copepods	crustaceans in the Order Harpacticoida, of the Class Copepoda
hydric (soils)	a soil that is saturated, flooded, or ponded long enough during the growing season to develop anaerobic conditions in the upper part
hydrophytes	any macrophyte that grows in water or in a substrate that is at least periodically deficient in oxygen as a result of excessive water content; plants typically found in wetlands and other aquatic habitats
hyperiid amphipods	crustaceans in the Suborder Hyperiidea, of the Order Amphipoda (see amphipod)
hypothesis	an idea, usually in the form of a statement of question or theory, which, without itself having been proved, is taken for granted as a premise from which to test or discover an assured conclusion; typically framed in the form of a null hypothesis
infauna	those organisms living within the sediments underlying a body of water
intertidal	pertaining to the area exposed at low tides and inundated at high tides; defined as the area between Extreme Low Tide and Extreme High Tide
isopods	crustaceans in the Order Isopoda, of the Class Malacostraca
larvae (pl. of larva)	the immature form of a animals which is unlike the adult form and which requires fundamental changes before reaching the basic adult form
limnetic	waters in the salinity range of 0-0.5 ppt
macrofauna	animals with lengths equal to or larger than 0.5 mm (sometimes 1.0 mm) and (usually) less than 5 cm
meiofauna	animals (usually benthic/epibenthic) between 0.063 and 1 mm long
mesohaline	waters in the salinity range of 18-5 ppt
microbiota	animals less than 0.063 mm long
mitigation	compensation for unavoidable losses of fish and wildlife habitat in the form of wetland creation or restoration
mixohaline	water in the salinity range of 30-0.5 ppt
mudflat	intertidal shores not vegetated by macrophytes and with unconsolidated sediment particles smaller than stones, predominantly silt (4ø to 8ø; 0.0625 to 0.00391 mm particle diameter) and clay (8ø to 12ø; 0.00391 to 0.00024 mm); the substrate usually has high

	organic content and anaerobic conditions often exist below the surface
natural variability	error associated with estimates of populations which is attributed to their natural fluctuations, heterogeneous distribution, or dispersal in the environment
nearshore subtidal	subtidal (depths >ELLW and <20 m) zone adjacent to shoreline or within estuary
oligohaline	waters in the salinity range of 5-0.5 ppt
parameter	a measurement or index of an attribute characteristic; e.g., organism density, vegetation height
pelagic	pertaining to the water column or to an organism living within the water column
photosynthesis	the process by which plants utilize radiant energy from the sun to synthesize carbohydrates from carbon dioxide and water
planktonic	pertaining to organisms which drift passively or swim weakly in the water column
polychaete	segmented worms of the phylum Annelida
polyhaline	waters with salinity in the range of 30-18 ppt
population	all individual of one or more species within a prescribed area or, statistically, all individuals under investigation
production	the amount of organic matter generated by a plant or an animal
productivity	the rate at which plants or animals generate organic matter
replication	repetition of another independent sample or experiment to obtain more information for estimating experimental error
representative sampling	selecting samples that are considered typical or representative of the population
restoration	modification of developed estuarine shoreline to return it to historic wetland state
sample	a collection of sampling units drawn from a (sampling) frame
sample size	the number of sampling units within/upon which measurements are acquired
sampling	the process of collecting a subset of measurements that are representative of a larger population
sampling element	an object or character on which a measurement (parameter) is taken
sampling frame	a list of sampling units
sampling units	nonoverlapping collections of (parameter) measurements from the population
sandflat	intertidal shores which are not vegetated by macrophytes and have unconsolidated sediment in which particles smaller than stones are

	predominantly sand (-1ø to 4ø; 2.0 to 0.074 mm); they occur as low gradient flats and bars where the substrate material is exposed to sorting by wave and current action
sediments	the organic and inorganic particulate materials, including gravel, sand, silt and clay, that cover the bottom of the estuary, including intertidal areas
soft bottom (habitat)	shallow subtidal (i.e., <20 m depth) portion of estuaries, usually in the channels and shallower portion of embayments in which the bottom is unconsolidated gravel to silt, with no or minimal relief.
standing stock	the weight (biomass) of a group of organisms per unit area or volume at a given time
subtidal	the area below Extreme Lower Low Water (ELLW)
tanaid	crustaceans in the Suborder Tanaidacea of the Class Malacostraca
tidal channel	a channel through which water drains and fills intertidal areas
UEMWG	Urbanized Estuary Mitigation Working Group; an ad hoc technical group of government agency, tribal, university, and industry representatives which has met since 1986 to discuss mitigation in urbanized estuaries (a listing of the entities and their representatives of the UEMWG can be found in Supplement 5 [Simenstad et al. 1990])
variability	error associated with estimates of populations which is attributed to sampling
water column	that portion of the estuarine subtidal not associated with the shore of bottom; this does not include the benthic boundary layer, which we include in bottom habitats, but does include the neustonic layer at the surface.
zooplankton	the group of small (primarily microscopic) passively suspended or weakly swimming animals in the water column

INDEX

APPENDICES

APPENDIX A: Habitat-Specific List of Assemblage Species

Emergent Marsh Habitat

Birds

American coot	*Fulica americana*
American goldfinch	*Spinus tristis*
American wigeon	*Anas americana*
black brant	*Branta bernicla*
bufflehead	*Bucephala albeola*
Canada goose	*Branta canadensis*
common goldeneye	*Bucephala clangula*
common snipe	*Capella gallinago*
dark-eyed junco	*Junco hyemalis*
gadwall	*Anas strepera*
great blue heron	*Ardea herodias*
greater yellowlegs	*Tringa melanoleuca*
green-winged teal	*Anas crecca*
killdeer	*Charadrius vociferus*
least sandpiper	*Calidris minutilla*
mallard	*Anas platyrhynchos*
merlin	*Falco columbarius*
northern oriole	*Icerus galbula*
osprey	*Pandion haliaetus*
red-tail hawk	*Buteo jamaicensis*
redwing blackbird	*Agelaius phoeniceus*
savannah sparrow	*Passerculus sandwichensis*
short-billed dowitcher	*Limnodromus griseus*
short-eared owl	*Asio flammeus*
song sparrow	*Melospiza melodia*
spotted sandpiper	*Actitis macularia*
Virginia rail	*Rallus limicola*
western sandpiper	*Calidris mauri*

Fishes

chinook salmon	*Oncorhynchus tshawytscha*
chum salmon	*Oncorhynchus keta*
cutthroat trout	*Oncorhynchus (Salmo) clarki*
Pacific staghorn sculpin	*Leptocottus armatus*
prickly sculpin	*Cottus asper*
threespine stickleback	*Gasterosteus aculeatus*

Emergent Marsh Habitat - cont.

Mammals

muskrat	*Ondatra zibethica*
Pacific harbor seal	*Phoca vitulina*
raccoon	*Procyon lotor*
river otter	*Lutra canadensis*
Townsend vole	*Microtus townsendii*

Mudflat Habitat

Birds	
bufflehead	*Bucephala albeola*
Canada goose	*Branta canadensis*
common goldeneye	*Bucephala clangula*
common snipe	*Capella gallinago*
dunlin	*Calidris alpina*
great blue heron	*Ardea herodias*
greater yellowlegs	*Tringa melanoleuca*
least sandpiper	*Calidris minutilla*
short-billed dowitcher	*Limnodromus griseus*
spotted sandpiper	*Actitis macularia*
western sandpiper	*Calidris mauri*

Fish	
bay goby	*Lepidogobius lepidus*
chum salmon	*Oncorhynchus keta*
English sole	*Pleureonectes (Parophrys) vetulus*
green sturgeon	*Acipenser medirostris*
Pacific staghorn sculpin	*Leptocottus armatus*
starry flounder	*Platichthys stellatus*

Invertebrates	
Dungeness crab	*Cancer magister*

Mammals	
Pacific harbor seal	*Phoca vitulina*
raccoon	*Procyon lotor*

Sandflat Habitat

Birds	
common goldeneye	*Bucephala clangula*
common snipe	*Capella gallinago*
dunlin	*Calidris alpina*
great blue heron	*Ardea herodias*
greater yellowlegs	*Tringa melanoleuca*
horned grebe	*Podiceps auritus*
least sandpiper	*Calidris minutilla*
spotted sandpiper	*Actitis macularia*
Fish	
bay goby	*Lepidogobius lepidus*
chinook salmon	*Oncorhynchus tshawytscha*
Pacific sand lance	*Ammodytes hexapterus*
Pacific sanddab	*Citharichthys sordidus*
Pacific staghorn sculpin	*Leptocottus armatus*
sand sole	*Psettichthys melanostictus*
speckled sanddab	*Citharichthys stigmaeus*
Invertebrates	
Dungeness crab	*Cancer magister*
Mammals	
Pacific harbor seal	*Phoca vitulina*
Gray whale	*Eschrichtius robustus*

Gravel-Cobble Habitat

Birds	
black brant	*Branta bernicla*
black turnstone	*Arenaria melanocephala*
bufflehead	*Bucephala albeola*
common goldeneye	*Bucephala clangula*
double-crested cormorant	*Phalacrocorax auritus*
horned grebe	*Podiceps auritus*
least sandpiper	*Calidris minutilla*
mew gull	*Larus canus*
spotted sandpiper	*Actitis macularia*
western grebe	*Aechmophorus occidentalis*
Fish	
buffalo sculpin	*Enophrys bison*
coho salmon	*Oncorhynchus kisutch*
copper rockfish	*Sebastes caurinus*
cutthroat trout	*Oncorhynchus (Salmo) clarki*
dolly varden	*Salvelinus malma*
great sculpin	*Myoxocephalus polyacanthocephalus*
Pacific herring	*Clupea harengus pallasi*
Pacific tomcod	*Microgadus proximus*
padded sculpin	*Artedius fenestralis*
pile perch	*Rhacochilus vacca*
river lamprey	*Lampetra ayresi*
rock sole	*Pleuronectes bilineata*
starry flounder	*Platichthys stellatus*
surf smelt	*Hypomesus pretiosus*
whitespotted greenling	*Hexagrammos stelleri*
Invertebrates	
red rock crab	*Cancer productus*
Mammals	
raccoon	*Procyon lotor*
river otter	*Lutra canadensis*

Eelgrass Habitat

Birds	
black brant	*Branta bernicla*
bufflehead	*Bucephala albeola*
Canada goose	*Branta canadensis*
common snipe	*Capella gallinago*
glaucous-winged gull	*Larus glaucescens*
great blue heron	*Ardea herodias*
greater yellowlegs	*Tringa melanoleuca*
least sandpiper	*Calidris minutilla*
osprey	*Pandion haliaetus*
spotted sandpiper	*Actitis macularia*
Fish	
bay pipefish	*Syngnathus leoptorhynchus*
chum salmon	*Oncorhynchus keta*
crescent gunnel	*Pholis laeta*
kelp perch	*Brachyistius frenatus*
lingcod	*Ophiodon elongatus*
Pacific herring	*Clupea harengus pallasi*
penpoint gunnel	*Apodichthys flavidus*
shiner perch	*Cymatogaster aggregata*
snake prickleback	*Lumpenus sagitta*
striped seaperch	*Embiotoca lateralis*
tube-snout	*Aulorhynchus flavidus*
Invertebrates	
Dungeness crab	*Cancer magister*
Mammals	
Pacific harbor seal	*Phoca vitulina*
river otter	*Lutra canadensis*

Nearshore Subtidal Soft Bottom Habitat

Birds	
common murre	*Uria aalge*
Fish	
C-O sole	*Pleuronichthys coenosus*
Dover sole	*Microstomus pacificus*
hybrid sole	*Inopsetta ischyra*
largescale sucker	*Catostomus macrocheilus*
mountain whitefish	*Prosopium williamsoni*
northern squawfish	*Ptychocheilus oregonensis*
Pacific cod	*Gadus macrocephalus*
Pacific tomcod	*Microgadus proximus*
ratfish	*Hydrolagus colliei*
rough sculpin	*Chitonotus pugetensis*
sturgeon poacher	*Agonus acipenserinus*
walleye pollock	*Theragra chalcogramma*
Invertebrates	
Dungeness crab	*Cancer magister*
Mammals	
gray whale	*Eschrichtius robustus*

Nearshore Subtidal Hard Substrate Habitat

Fish

black rockfish	*Sebastes melanops*
brown rockfish	*Sebastes auriculatus*
cabezon	*Scorpaenichthys marmoratus*
copper rockfish	*Sebastes caurinus*
kelp greenling	*Hexagrammos decagrammus*
lingcod	*Ophiodon elongatus*
pile perch	*Rhacochilus vacca*
quillback rockfish	*Sebastes maliger*
striped seaperch	*Embiotoca lateralis*

Water Column Habitat

Birds

bufflehead	*Bucephala albeola*
cassin's auklet	*Ptychoramphus aleuticus*
common goldeneye	*Bucephala clangula*
common merganser	*Mergus merganser*
double-crested cormorant	*Phalacrocorax auritus*
gadwall	*Anas strepera*
mew gull	*Larus canus*
osprey	*Pandion haliaetus*
red-breasted merganser	*Mergus serrator*
western grebe	*Aechmophorus occidentalis*

Fish

chinook salmon	*Oncorhynchus tshawytscha*
chum salmon	*Oncorhynchus keta*
coho salmon	*Oncorhynchus kisutch*
longfin smelt	*Spirinchus thaleichthys*
northern anchovy	*Engraulis mordax*
Pacific hake	*Merluccius productus*
Pacific herring	*Clupea harengus pallasi*
Pacific sand lance	*Ammodytes hexapterus*
pink salmon	*Oncorhynchus gorbuscha*
river lamprey	*Lampetra ayresi*
soft sculpin	*Gilbertidia sigalutes*
steelhead (rainbow) trout	*Oncorhynchus mykiss (Salmo gairdneri)*
surf smelt	*Hypomesus pretiosus*
threespine stickleback	*Gasterosteus aculeatus*
western brook lamprey	*Lampetra richardsoni*

Mammals

northern sea lion	*Eumetopias jubatus*
Pacific harbor seal	*Phoca vitulina*

APPENDIX B: Habitat-Specific List of Attributes and Associated Habitat Functions

Emergent Marsh Habitat

Assemblage Attribute	Function
Birds	
American coot	
Scirpus spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
Scirpus acutus	Feeding: Plants, Emergent Vascular
Typha latifolia	Feeding: Plants, Emergent Vascular
American goldfinch	
Alnus rubra	Feeding: Plants, Emergent Vascular
Phalaris arundinacea	Feeding: Plants, Emergent Vascular
American wigeon	
Balanus spp.	Feeding: Invertebrates, Epibenthic
Enteromorpha spp.	Feeding: Plants, Macro Algae
Mytilus edulis	Feeding: Invertebrates, Epibenthic
Ruppia maritima	Feeding: Plants, Submergent Vascular
Scirpus spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
Typha latifolia	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
Ulva spp.	Feeding: Plants, Macro Algae
bufflehead	
Balanus spp.	Feeding: Invertebrates, Epibenthic
Insecta (larvae)	Feeding: Invertebrates, Epibenthic
Mytilus edulis	Feeding: Invertebrates, Epibenthic

Emergent Marsh Habitat - cont.

Assemblage	Attribute	Function
black brant		
	Carex spp.	Feeding: Plants, Emergent Vascular
	Enteromorpha spp.	Feeding: Plants, Macro Algae
	Scirpus spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Typha latifolia	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Ulva spp.	Feeding: Plants, Macro Algae
Canada goose		
	Carex lyngbyei	Feeding: Plants, Emergent Vascular
	Distichlis spicata	Feeding: Plants, Emergent Vascular
	Scirpus americanus	Feeding: Plants, Emergent Vascular
	Scirpus spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Triglochin maritimum	Feeding: Plants, Emergent Vascular
	Triglochin palustris	Feeding: Plants, Emergent Vascular
	Typha latifolia	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
common goldeneye		
	Hemigrapsus spp.	Feeding: Invertebrates, Epibenthic
	Littorina spp.	Feeding: Invertebrates, Epibenthic
	Scirpus spp.	Feeding: Plants, Emergent Vascular
common snipe		
	Balanus spp.	Feeding: Invertebrates, Epibenthic
	Orchestia traskiana	Feeding: Invertebrates, Epibenthic
	Scirpus spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Transennella tantilla	Feeding: Invertebrates, Benthic
	Typha latifolia	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants

Emergent Marsh Habitat - cont.

Assemblage	Attribute	Function
dunlin		
	Balanus spp.	Feeding: Invertebrates, Epibenthic
	Transennella tantilla	Feeding: Invertebrates, Benthic
great blue heron		
	Cymatogaster aggregata	Feeding: Vertebrates, Water Column
	Gasterosteus aculeatus	Feeding: Vertebrates, Water Column
	Leptocottus armatus	Feeding: Vertebrates, Demersal
	Microtus spp.	Feeding: Vertebrates, Terrestrial
	Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
	Picea sitchensis	Reproduction: Substrate, Riparian Vegetation
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Populus trichocarpa	Reproduction: Substrate, Riparian Vegetation
gadwall		
	Carex spp.	Feeding: Plants, Emergent Vascular
	Eleocharis palustris	Feeding: Plants, Emergent Vascular
	Enteromorpha spp.	Feeding: Plants, Macro Algae
	filamentous algae	Feeding: Plants, Micro Algae
	Insecta (larvae)	Feeding: Invertebrates, Epibenthic
	Ruppia maritima	Feeding: Plants, Submergent Vascular
	Scirpus acutis	Feeding: Plants, Emergent Vascular
	Scirpus spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Typha latifolia	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Ulva spp.	Feeding: Plants, Macro Algae
greater yellowlegs		
	Balanus spp.	Feeding: Invertebrates, Epibenthic
	Transennella tantilla	Feeding: Invertebrates, Benthic

Emergent Marsh Habitat - cont.

Assemblage Attribute		Function
green-winged teal		
	Carex spp.	Feeding: Plants, Emergent Vascular
	Distichlis spicata	Feeding: Plants, Emergent Vascular
	Eleocharis palustris	Feeding: Plants, Emergent Vascular
	Eleocharis spp.	Feeding: Plants, Emergent Vascular
	Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
	Manayunkia aestuarina	Feeding: Invertebrates, Benthic
	Polygonum spp.	Feeding: Plants, Emergent Vascular
	Polygonum hydropiperoides	Feeding: Plants, Emergent Vascular
	Potomogeton spp.	Feeding: Plants, Emergent Vascular
	Rumex spp.	Feeding: Plants, Emergent Vascular
	Salicornia virginica	Feeding: Plants, Emergent Vascular
	Saunderia spp.	Feeding: Invertebrates, Epibenthic
	Scirpus americanus	Feeding: Plants, Emergent Vascular
	Scirpus spp.	Feeding: Plants, Emergent Vascular
	Scirpus acutus	Feeding: Plants, Emergent Vascular
	Triglochin maritimum	Feeding: Plants, Emergent Vascular
	Typha latifolia	Reproduction: Substrate, Emergent Vasc. Plants
killdeer		
	Insecta (larvae)	Feeding: Invertebrates, Epibenthic
	Littorina spp.	Feeding: Invertebrates, Epibenthic
mallard		
	Carex lyngbyei	Feeding: Plants, Emergent Vascular
	Carex spp.	Feeding: Plants, Emergent Vascular
	Distichlis spicata	Feeding: Plants, Emergent Vascular
	Eleocharis palustris	Feeding: Plants, Emergent Vascular
	Eleocharis spp.	Feeding: Plants, Emergent Vascular
	Insecta (larvae)	Feeding: Invertebrates, Epibenthic
	Picea sitchensis	Reproduction: Substrate, Riparian Vegetation
	Polygonom spp.	Feeding: Plants, Emergent Vascular

Emergent Marsh Habitat - cont.

Assemblage Attribute	Function
mallard (cont.)	
Polygonum hydropiperoides	Feeding: Plants, Emergent Vascular
Populus trichocarpa	Reproduction: Substrate, Riparian Vegetation
Potomogeton spp.	Feeding: Plants, Emergent Vascular
Rumex spp.	Feeding: Plants, Emergent Vascular
Scirpus americanus	Feeding: Plants, Emergent Vascular
Scirpus spp.	Feeding: Plants, Emergent Vascular
Scirpus acutus	Feeding: Plants, Emergent Vascular
Triglochin maritimum	Feeding: Plants, Emergent Vascular
Typha latifolia	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
merlin	
Aves (unid.)	Feeding: Vertebrates, Terrestrial
Charadriiformes (unid.)	Feeding: Vertebrates, Terrestrial
red-tail hawk	
Charadriiformes (unid.)	Feeding: Vertebrates, Terrestrial
Microtus townsendii	Feeding: Vertebrates, Terrestrial
Ondatra zibethicus	Feeding: Vertebrates, Terrestrial
Peromyscus maniculatus	Feeding: Vertebrates, Terrestrial
Sorex bendirii	Feeding: Vertebrates, Terrestrial
redwing blackbird	
Typha latifolia	Reproduction: Substrate, Emergent Vasc. Plants
Typha latifolia	Feeding: Plants, Emergent Vascular
short-billed dowitcher	
Orchestia traskiana	Feeding: Invertebrates, Epibenthic

Emergent Marsh Habitat - cont.

Assemblage Attribute	Function
song sparrow	
Grindelia integrifolia	Feeding: Plants, Emergent Vascular
Nereidae (unid.)	Feeding: Invertebrates, Epibenthic
Scirpus maritimus	Feeding: Plants, Emergent Vascular
spotted sandpiper	
Balanus spp.	Feeding: Invertebrates, Epibenthic
Transennella tantilla	Feeding: Invertebrates, Benthic
western sandpiper	
Deschampsia caespitosa	Feeding: Plants, Emergent Vascular
Nematoda (unid.)	Feeding: Invertebrates, Benthic
Spergularia marina	Feeding: Plants, Emergent Vascula
<u>Fishes</u>	
chinook salmon	
Araneae (unid.)	Feeding: Invertebrates, Neustonic
Carex spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
Chironomidae (unid.)	Feeding: Invertebrates, Neustonic
Chironomidae, larvae/pupae	Feeding: Invertebrates, Epibenthic
Corophium salmonis	Feeding: Invertebrates, Epibenthic
Corophium spinicorne	Feeding: Invertebrates, Epibenthic
Corophium spp.	Feeding: Invertebrates, Epibenthic
Daphnia spp.	Feeding: Invertebrates, Pelagic
Diptera (unid.)	Feeding: Invertebrates, Epibenthic
Ephydridae (unid.)	Feeding: Invertebrates, Neustonic
Heleidae (unid.)	Feeding: Invertebrates, Neustonic
Hemiptera (unid.)	Feeding: Invertebrates, Epibenthic
Insecta (larvae)	Feeding: Invertebrates, Epibenthic
Neomysis mercedis	Feeding: Invertebrates, Epibenthic

Emergent Marsh Habitat - cont.

Assemblage	Attribute	Function
chum salmon		
	Carex spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
	Chironomidae (unid.)	Feeding: Invertebrates, Neustonic
	Chironomidae, larvae/pupae	Feeding: Invertebrates, Epibenthic
	Corophium spinicorne	Feeding: Invertebrates, Epibenthic
	Corophium spp.	Feeding: Invertebrates, Epibenthic
	Diptera (unid.)	Feeding: Invertebrates, Epibenthic
	Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
	epiphytic algae/animals	Feeding: Plants, Micro Algae
	Hemiptera (unid.)	Feeding: Invertebrates, Epibenthic
	Neomysis mercedis	Feeding: Invertebrates, Epibenthic
cutthroat trout		
	Chironomidae (unid.)	Feeding: Invertebrates, Neustonic
	Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
	Gasterosteus aculeatus	Feeding: Vertebrates, Demersal
	Neomysis mercedis	Feeding: Invertebrates, Epibenthic
	Oncorhynchus gorbuscha (fry)	Feeding: Vertebrates, Water Column
	Oncorhynchus keta (fry)	Feeding: Vertebrates, Water Column
prickly sculpin		
	Chironomidae, larvae/pupae	Feeding: Invertebrates, Epibenthic
	Corophium salmonis	Feeding: Invertebrates, Epibenthic
	Gnorimosphaeroma oregonese	Feeding: Invertebrates, Epibenthic
	Neomysis mercedis	Feeding: Invertebrates, Epibenthic

Emergent Marsh Habitat - cont.

Assemblage Attribute	Function
Pacific staghorn sculpin	
Chironomidae, larvae/pupae	Feeding: Invertebrates, Epibenthic
Corophium salmonis	Feeding: Invertebrates, Epibenthic
Corophium spp.	Feeding: Invertebrates, Epibenthic
Cumella vulgaris	Feeding: Invertebrates, Epibenthic
Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
Gnorimosphaeroma oregonese	Feeding: Invertebrates, Epibenthic
Neanthes limnicola	Feeding: Invertebrates, Benthic
Neomysis mercedis	Feeding: Invertebrates, Epibenthic
Tanais spp.	Feeding: Invertebrates, Benthic
threespine stickleback	
Carex spp.	Refuge/Physiology: Biological Complexity, Emergent Vasc. Plants
Chironomidae, larvae/pupae	Feeding: Invertebrates, Epibenthic
Corophium salmonis	Feeding: Invertebrates, Epibenthic
Corophium spp.	Feeding: Invertebrates, Epibenthic
Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
Eogammarus spp.	Feeding: Invertebrates, Epibenthic
Insecta (larvae)	Feeding: Invertebrates, Epibenthic
Oncorhynchus gorbuscha (fry)	Feeding: Vertebrates, Water Column
Oncorhynchus keta (fry)	Feeding: Vertebrates, Water Column

Emergent Marsh Habitat - cont.

Assemblage	Attribute	Function
Mammals		
muskrat		
	Carex lyngbei	Feeding: Plants, Emergent Vascular
	Carex lyngbei (seeds)	Feeding: Plants, Emergent Vascular
	Phalaris arundinacea	Feeding: Plants, Emergent Vascular
	Scirpus americanus	Reproduction: Substrate, Emergent Vasc. Plants
	Scirpus maritimus	Feeding: Plants, Emergent Vascular
	Scirpus validus (seeds)	Feeding: Plants, Emergent Vascular
	Sium suave	Feeding: Plants, Emergent Vascular
	Typha latifolia	Feeding: Plants, Emergent Vascular
Pacific harbor seal		
	Cottus spp.	Feeding: Vertebrates, Demersal
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Platichthys stellatus	Feeding: Vertebrates, Demersal
raccoon		
	Aves (unid.)	Feeding: Vertebrates, Terrestrial
	Cancer productus	Feeding: Invertebrates, Epibenthic
	Corbicula manilensis	Feeding: Invertebrates, Benthic
	Cottus spp.	Feeding: Vertebrates, Demersal
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Engraulis mordax	Feeding: Vertebrates, Water Column
	Pacificastacus leniusculus	Feeding: Invertebrates, Epibenthic
	Picea sitchensis	Refuge/Physiology: Physical Complexity, Vertical Relief
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Salix spp.	Refuge/Physiology: Physical Complexity, Vertical Relief
	Thaleichthys pacificus	Feeding: Vertebrates, Water Column

Emergent Marsh Habitat - cont.

Assemblage	Attribute	Function
Townsend vole		
	bentgrass/aster	Feeding: Plants, Emergent Vascular
	Carex lyngbei	Reproduction: Substrate, Emergent Vasc. Plants
	Carex lyngbei (seeds)	Feeding: Plants, Emergent Vascular
	Salicornia spp.	Feeding: Plants, Emergent Vascular
	Scirpus americanus	Reproduction: Substrate, Emergent Vasc. Plants

Mudflat Habitat

Assemblage	Attribute	Function
Birds		
bufflehead		
	Diptera (larvae)	Feeding: Invertebrates, Epibenthic
	Lepidogobius lepidus	Feeding: Vertebrates, Demersal
	Leptocottus armatus	Feeding: Vertebrates, Demersal
	Macoma spp.	Feeding: Invertebrates, Benthic
	Ostrea lurida	Feeding: Invertebrates, Epibenthic
	Tapes (Ruditapes) philipinarum	Feeding: Invertebrates, Epibenthic
common goldeneye		
	Hemigrapsus spp.	Feeding: Invertebrates, Epibenthic
	Leptocottus armatus	Feeding: Vertebrates, Demersal
	Macoma spp.	Feeding: Invertebrates, Benthic
	Ostrea lurida	Feeding: Invertebrates, Epibenthic
	Tapes (Ruditapes) philipinarum	Feeding: Invertebrates, Epibenthic
common snipe		
	Diptera (larvae)	Feeding: Invertebrates, Epibenthic
	Megalorchestia pugettensis	Feeding: Invertebrates, Epibenthic
dunlin		
	Corophium salmonis	Feeding: Invertebrates, Benthic
	Corophium spp.	Feeding: Invertebrates, Benthic
	Diptera (larvae)	Feeding: Invertebrates, Epibenthic
	Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
	Eogammarus spp.	Feeding: Invertebrates, Epibenthic
	Megalorchestia pugettensis	Feeding: Invertebrates, Epibenthic

Mudflat Habitat (cont.)

Assemblage	Attribute	Function
great blue heron		
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Engraulis mordax	Feeding: Vertebrates, Water Column
	Lepidogobius lepidus	Feeding: Vertebrates, Demersal
	Leptocottus armatus	Feeding: Vertebrates, Demersal
	Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Pleuronichthys vetulus	Feeding: Vertebrates, Demersal
least sandpiper		
	Corophium spp.	Feeding: Invertebrates, Benthic
western sandpiper		
	Diptera (larvae)	Feeding: Invertebrates, Epibenthic
	Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
	Eohaustorius washingtonius	Feeding: Invertebrates, Epibenthic
	Nematoda (unid.)	Feeding: Invertebrates, Benthic
	Pancolus californiensis	Feeding: Invertebrates, Epibenthic
Fishes		
bay goby		
	Harpacticus uniremis	Feeding: Invertebrates, Epibenthic
	Tisbe spp.	Feeding: Invertebrates, Epibenthic
chum salmon		
	Callianassa californiensis (larvae)	Feeding: Invertebrates, Pelagic
	Upogebia pugettensis (larvae)	Feeding: Invertebrates, Pelagic

Mudflat Habitat (cont.)

Assemblage Attribute	Function
English sole	
Abarenicola pacifica	Feeding: Invertebrates, Benthic
Archaeomysis grebnitzkii	Feeding: Invertebrates, Epibenthic
Corophium salmonis	Feeding: Invertebrates, Epibenthic
Corophium spp.	Feeding: Invertebrates, Epibenthic
Cumella vulgaris	Feeding: Invertebrates, Epibenthic
Eogammarus spp.	Feeding: Invertebrates, Epibenthic
Macoma spp.	Feeding: Invertebrates, Benthic
Pacific staghorn sculpin	
Callianassa californiensis	Feeding: Invertebrates, Benthic
Crangon spp.	Feeding: Invertebrates, Epibenthic
Gasterosteus aculeatus	Feeding: Vertebrates, Water Column
starry flounder	
Atylus spp.	Feeding: Invertebrates, Epibenthic
Corophium spp.	Feeding: Invertebrates, Epibenthic
Cumella vulgaris	Feeding: Invertebrates, Epibenthic
<u>Invertebrates</u>	
Dungeness crab	
Crangon spp.	Feeding: Invertebrates, Epibenthic
Macoma spp.	Feeding: Invertebrates, Benthic
<u>Mammals</u>	
Pacific harbor seal	
Cancer spp.	Feeding: Invertebrates, Benthic
Crangon spp.	Feeding: Invertebrates, Epibenthic
Platichthys stellatus	Feeding: Vertebrates, Demersal

Mudflat Habitat (cont.)

Assemblage	Attribute	Function
raccoon		
	Aves (unid.)	Feeding: Vertebrates, Terrestrial
	Corbicula manilensis	Feeding: Invertebrates, Benthic
	Hemigrapsus spp.	Feeding: Invertebrates, Epibenthic
	Platichthys stellatus	Feeding: Vertebrates, Demersal

Sandflat Habitat

Assemblage Attribute	Function
Birds	
common goldeneye	
Leptocottus armatus	Feeding: Vertebrates, Demersal
dunlin	
Corophium spp.	Feeding: Invertebrates, Benthic
Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
Oligochaeta (unid.)	Feeding: Invertebrates, Benthic
Megalorchestia pugettensis	Feeding: Invertebrates, Epibenthic
Phoxocephalidae spp.	Feeding: Invertebrates, Epibenthic
great blue heron	
Citharichthys spp.	Feeding: Vertebrates, Demersal
Cottidae (unid.)	Feeding: Vertebrates, Water Column
Crangon spp.	Feeding: Invertebrates, Epibenthic
Engraulis mordax	Feeding: Vertebrates, Water Column
Leptocottus armatus	Feeding: Vertebrates, Demersal
Platichthys stellatus	Feeding: Vertebrates, Demersal
horned grebe	
Leptocottus armatus	Feeding: Vertebrates, Demersal
Fishes	
Pacific staghorn sculpin	
Callianassidae (unid.)	Feeding: Invertebrates, Benthic
Cancer magister	Feeding: Invertebrates, Epibenthic
Crangon spp.	Feeding: Invertebrates, Epibenthic
Cumella vulgaris	Feeding: Invertebrates, Epibenthic
Mya arenaria	Feeding: Invertebrates, Benthic

Sandflat Habitat (cont.)

Assemblage	Attribute	Function
sand sole		
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Engraulis mordax	Feeding: Vertebrates, Water Column
speckled sanddab		
	Callianassidae (unid.)	Feeding: Invertebrates, Benthic
Invertebrates		
Dungeness crab		
	Clinocardium nutalli	Feeding: Invertebrates, Benthic
	Crangon spp.	Feeding: Invertebrates, Epibenthic
Marine Mammals		
Gray whale		
	Callianassa californiensis	Feeding: Invertebrates, Benthic

Gravel-Cobble Habitat

Assemblage Attribute	Function
Birds	
American wigeon	
Clupea harengus pallasi (eggs)	Feeding: Vertebrates, Demersal
bufflehead	
Hemigrapsus spp.	Feeding: Invertebrates, Epibenthic
black brant	
Clupea harengus pallasi (eggs)	Feeding: Vertebrates, Demersal
black turnstone	
Balanus spp.	Feeding: Invertebrates, Epibenthic
Mytilus edulis	Feeding: Invertebrates, Epibenthic
double-crested cormorant	
Ammodytes hexapterus	Feeding: Vertebrates, Demersal
Apodichthys flavidus	Feeding: Vertebrates, Demersal
Cymatogaster aggregata	Feeding: Vertebrates, Water Column
Gasterosteus aculeatus	Feeding: Vertebrates, Demersal
Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
Pholis laeta	Feeding: Vertebrates, Demersal
Platichthys stellatus	Feeding: Vertebrates, Demersal
Sebastes caurinus	Feeding: Vertebrates, Water Column
Thaleichthys pacificus	Feeding: Vertebrates, Water Column

Gravel-Cobble Habitat (cont.)

Assemblage	Attribute	Function
horned grebe		
	Apodichthys flavidus	Feeding: Vertebrates, Demersal
	Gasterosteus aculeatus	Feeding: Vertebrates, Water Column
	Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
	Pholis laeta	Feeding: Vertebrates, Demersal
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Sebastes caurinus	Feeding: Vertebrates, Water Column
mew gull		
	Clupea harengus pallasi (eggs)	Feeding: Vertebrates, Demersal
osprey		
	Gasterosteus aculeatus	Feeding: Vertebrates, Water Column
	Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
	Sebastes caurinus	Feeding: Vertebrates, Water Column
western grebe		
	Clupea harengus pallasi	Feeding: Vertebrates, Water Column
	Cymatogaster aggregata	Feeding: Vertebrates, Water Column
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Thaleichthys pacificus	Feeding: Vertebrates, Water Column
<u>Fishes</u>		
buffalo sculpin		
	Ulva spp.	Feeding: Plants, Macro Algae

Gravel-Cobble Habitat (cont.)

Assemblage Attribute	Function
coho salmon	
Ammodytes hexapterus	Feeding: Vertebrates, Demersal
Anisogammarus pugettensis	Feeding: Invertebrates, Epibenthic
Corophium spp.	Feeding: Invertebrates, Epibenthic
Decapoda (larvae)	Feeding: Invertebrates, Pelagic
Gnorimosphaeroma oregonese	Feeding: Invertebrates, Epibenthic
Neomysis mercedis	Feeding: Invertebrates, Epibenthic
copper rockfish	
Macrocystis pyrifera	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant
cutthroat trout	
Ammodytes hexapterus	Feeding: Vertebrates, Water Column
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Corophium spp.	Feeding: Invertebrates, Epibenthic
Crangon spp.	Feeding: Invertebrates, Epibenthic
Cumella vulgaris	Feeding: Invertebrates, Epibenthic
Decapoda (larvae)	Feeding: Invertebrates, Pelagic
Gasterosteus aculeatus	Feeding: Vertebrates, Demersal
Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
dolly varden	
Ammodytes hexapterus	Feeding: Vertebrates, Water Column
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Crangon spp.	Feeding: Invertebrates, Epibenthic
Gasterosteus aculeatus	Feeding: Vertebrates, Demersal
great sculpin	
Crangon spp.	Feeding: Invertebrates, Epibenthic

Gravel-Cobble Habitat (cont.)

Assemblage	Attribute	Function
pile perch		
	Laminaria spp.	Refuge/Physiology: Biological Complexity, Macro Algae
	Macrocystis pyrifera	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant
	Ulva spp.	Refuge/Physiology: Biological Complexity, Macro Algae
padded sculpin		
	Hippolytidae (unid.)	Feeding: Invertebrates, Epibenthic
Pacific tomcod		
	Corophium spp.	Feeding: Invertebrates, Epibenthic
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Eogammarus spp.	Feeding: Invertebrates, Epibenthic
striped seaperch		
	Hemigrapsus nudis	Feeding: Invertebrates, Epibenthic
starry flounder		
	Corophium spp.	Feeding: Invertebrates, Epibenthic
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Cumella vulgaris	Feeding: Invertebrates, Epibenthic
	Dendraster excentricus	Feeding: Invertebrates, Benthic
	Eogammarus spp.	Feeding: Invertebrates, Epibenthic
	Mya arenaria	Feeding: Invertebrates, Benthic
	Tellina nuculoides	Feeding: Invertebrates, Benthic

Gravel-Cobble Habitat (cont.)

Assemblage	Attribute	Function
whitespotted greenling		
	Ampithoe spp.	Feeding: Invertebrates, Epibenthic
	Cancer spp.	Feeding: Invertebrates, Epibenthic
	Eusiroides spp.	Feeding: Invertebrates, Epibenthic
	Heptacarpus spp.	Feeding: Invertebrates, Epibenthic
	Hippolytidae (unid.)	Feeding: Invertebrates, Epibenthic
	Laminaria spp.	Refuge/Physiology: Biological Complexity, Macro Algae
	Pugettia gracilis	Feeding: Invertebrates, Epibenthic
	Ulva spp.	Refuge/Physiology: Biological Complexity, Macro Algae
Invertebrates		
Dungeness crab		
	Hemigrapsus spp.	Feeding: Invertebrates, Epibenthic
red rock crab		
	Balanus spp.	Feeding: Invertebrates, Epibenthic
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Ulva spp.	Refuge/Physiology: Biological Complexity, Macro Algae
Mammals		
raccoon		
	Cancer magister	Feeding: Invertebrates, Epibenthic
	Corbicula manilensis	Feeding: Invertebrates, Benthic
	Nucella spp.	Feeding: Invertebrates, Benthic
	Pacificastacus leviusculus	Feeding: Invertebrates, Epibenthic

Eelgrass Habitat

Assemblage Attribute	Function
<u>Birds</u>	
bufflehead	
Zostera japonica	Feeding: Plants, Submergent Vascular
Zostera marina	Feeding: Plants, Submergent Vascular
black brant	
Clupea harengus pallasi (eggs)	Feeding: Vertebrates, Demersal
Spartina spp.	Feeding: Plants, Emergent Vascular
Ulva spp.	Feeding: Plants, Macro Algae
Zostera japonica	Feeding: Plants, Submergent Vascular
Zostera marina	Feeding: Plants, Submergent Vascular
Canada goose	
Zostera japonica	Feeding: Plants, Submergent Vascular
Zostera marina	Feeding: Plants, Submergent Vascular
Zostera spp.	Feeding: Plants, Submergent Vascular
great blue heron	
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Cymatogaster aggregata	Feeding: Vertebrates, Water Column
Embiotocidae (unid.)	Feeding: Vertebrates, Water Column
Leptocottus armatus	Feeding: Vertebrates, Demersal
Platichthys stellatus	Feeding: Vertebrates, Demersal
glaucous-winged gull	
Aves (unid.)	Feeding: Vertebrates, Terrestrial
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Thaleichthys pacificus	Feeding: Vertebrates, Water Column

Eelgrass Habitat (cont.)

Assemblage	Attribute	Function
osprey		
	Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
	Thaleichthys pacificus	Feeding: Vertebrates, Water Column
western sandpiper		
	Eogammarus confervicolus	Feeding: Invertebrates, Epibenthic
<u>Fishes</u>		
bay pipefish		
	epiphytic algae/animals	Refuge/Physiology: Biological Complexity, Macro Algae
	Zostera spp.	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant
chum salmon		
	Corophium spp. (males)	Feeding: Invertebrates, Epibenthic
	Cumella vulgaris	Feeding: Invertebrates, Epibenthic
	Eogammarus spp.	Feeding: Invertebrates, Epibenthic
	Harpacticus spp.	Feeding: Invertebrates, Epibenthic
	Harpacticus uniremis	Feeding: Invertebrates, Epibenthic
	Tisbe spp.	Feeding: Invertebrates, Epibenthic
	Zaus spp.	Feeding: Invertebrates, Epibenthic
	Zostera spp.	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant
crescent gunnel		
	Caprella laeviuscula	Feeding: Invertebrates, Epibenthic
	epiphytic algae/animals	Refuge/Physiology: Biological Complexity, Macro Algae
	Ischyrocerus spp.	Feeding: Invertebrates, Epibenthic

Eelgrass Habitat (cont.)

Assemblage Attribute		Function
kelp perch		
	Cumella vulgaris	Feeding: Invertebrates, Epibenthic
	Ulva spp.	Refuge/Physiology: Biological Complexity, Macro Algae
	Zostera spp.	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant
penpoint gunnel		
	epiphytic algae/animals	Refuge/Physiology: Biological Complexity, Macro Algae
	Harpacticus spinulosus	Feeding: Invertebrates, Epibenthic
	Ischyrocerus spp.	Feeding: Invertebrates, Epibenthic
shiner perch		
	Capitellidae (unid.)	Feeding: Invertebrates, Benthic
	Corophium spp.	Feeding: Invertebrates, Benthic
	Corophium spp. (males)	Feeding: Invertebrates, Epibenthic
	Cumella vulgaris	Feeding: Invertebrates, Epibenthic
	Eogammarus spp.	Feeding: Invertebrates, Epibenthic
	Harpacticus spp.	Feeding: Invertebrates, Epibenthic
	Hippolytidae (unid.)	Feeding: Invertebrates, Epibenthic
	Ischyrocerus spp.	Feeding: Invertebrates, Epibenthic
	Pontogeneia rostrata	Feeding: Invertebrates, Epibenthic
	Tisbe spp.	Feeding: Invertebrates, Epibenthic
	Ulva spp.	Refuge/Physiology: Biological Complexity, Macro Algae
	Zostera spp.	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant

Eelgrass Habitat (cont.)

Assemblage Attribute	Function
striped seaperch	
Crangon spp.	Feeding: Invertebrates, Epibenthic
Cumella vulgaris	Feeding: Invertebrates, Epibenthic
Decapoda (larvae)	Feeding: Invertebrates, Pelagic
Harpacticus spp.	Feeding: Invertebrates, Epibenthic
Hemigrapsus nudis	Feeding: Invertebrates, Epibenthic
Hippolytidae (unid.)	Feeding: Invertebrates, Epibenthic
Ischyrocerus spp.	Feeding: Invertebrates, Epibenthic
Ulva spp.	Refuge/Physiology: Biological Complexity, Macro Algae
Zostera spp.	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant
tube-snout	
Alienacanthomysis macropsis	Feeding: Invertebrates, Epibenthic
Corycaeus anglicus	Feeding: Invertebrates, Pelagic
Harpacticus spinulosus	Feeding: Invertebrates, Epibenthic
Paracalanus spp.	Feeding: Invertebrates, Pelagic
Tisbe spp.	Feeding: Invertebrates, Epibenthic
Zostera spp.	Refuge/Physiology: Biological Complexity, Submergent Vasc. Plant

Eelgrass Habitat (cont.)

Assemblage Attribute	Function
Invertebrates	
Dungeness crab	
Callianassa californiensis	Feeding: Invertebrates, Benthic
Cancer magister	Feeding: Invertebrates, Epibenthic
Capitellidae (unid.)	Feeding: Invertebrates, Benthic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Crangon spp.	Feeding: Invertebrates, Epibenthic
Cryptomya californica	Feeding: Invertebrates, Benthic
Embiotocidae (unid.)	Feeding: Vertebrates, Water Column
Harpacticus spp.	Feeding: Invertebrates, Epibenthic
Leptocottus armatus	Feeding: Vertebrates, Demersal
Macoma spp.	Feeding: Invertebrates, Benthic
Mya arenaria	Feeding: Invertebrates, Benthic
Psettichthys melanostictus	Feeding: Vertebrates, Demersal
Tellina spp.	Feeding: Invertebrates, Benthic
Thaleichthys pacificus	Feeding: Vertebrates, Water Column
Zostera spp.	Feeding: Plants, Submergent Vascular
Mammals	
Pacific harbor seal	
Cancer magister	Feeding: Invertebrates, Epibenthic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Crangon spp.	Feeding: Invertebrates, Epibenthic
Lumpenus sagitta	Feeding: Vertebrates, Demersal
Porichthys notatus	Feeding: Vertebrates, Demersal
Thaleichthys pacificus	Feeding: Vertebrates, Water Column

Nearshore Subtidal Soft Bottom Habitat

Assemblage	Attribute	Function
Fishes		
Pacific cod		
	Clupea harengus pallasi	Feeding: Vertebrates, Water Column
	Decapoda (larvae)	Feeding: Invertebrates, Pelagic
	Neomysis mercedis	Feeding: Invertebrates, Epibenthic
Pacific tomcod		
	Crangon spp.	Feeding: Invertebrates, Epibenthic
	Neomysis mercedis	Feeding: Invertebrates, Epibenthic
ratfish		
	Hemigrapsus spp.	Feeding: Invertebrates, Epibenthic
	Yoldia spp.	Feeding: Invertebrates, Benthic
sturgeon poacher		
	Crangon spp.	Feeding: Invertebrates, Epibenthic
walleye pollock		
	Clupea harengus pallasi	Feeding: Vertebrates, Water Column
	Decapoda (larvae)	Feeding: Invertebrates, Pelagic

Nearshore Subtidal Soft Bottom Habitat (cont.)

Assemblage Attribute	Function
Invertebrates	
Dungeness crab	
Ammodytes hexapterus	Feeding: Vertebrates, Demersal
Callianassa californiensis	Feeding: Invertebrates, Benthic
Clinocardium nutalli	Feeding: Invertebrates, Benthic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Crangon spp.	Feeding: Invertebrates, Epibenthic
Leptocottus armatus	Feeding: Vertebrates, Demersal
Macoma spp.	Feeding: Invertebrates, Benthic
Microgadus proximus	Feeding: Vertebrates, Demersal
Mya arenaria	Feeding: Invertebrates, Benthic
Tellina spp.	Feeding: Invertebrates, Benthic
Thaleichthys pacificus	Feeding: Vertebrates, Water Column
Veneropsis japonica	Feeding: Invertebrates, Benthic
Mammals	
gray whale	
Ampelisca agassizi	Feeding: Invertebrates, Benthic
Ampelisca careyi	Feeding: Invertebrates, Benthic
Orchomene minuta	Feeding: Invertebrates, Benthic
Photis lacia	Feeding: Invertebrates, Benthic
Protomedeia penates	Feeding: Invertebrates, Benthic

Nearshore Subtidal Hard Substrate

Assemblage Attribute	Function
Fishes	
black rockfish	
Eualus spp.	Feeding: Invertebrates, Epibenthic
Heptacarpus spp.	Feeding: Invertebrates, Epibenthic
Lebbeus spp.	Feeding: Invertebrates, Epibenthic
Spirontocaris spp.	Feeding: Invertebrates, Epibenthic
brown rockfish	
Eualus spp.	Feeding: Invertebrates, Epibenthic
Heptacarpus spp.	Feeding: Invertebrates, Epibenthic
Lebbeus spp.	Feeding: Invertebrates, Epibenthic
Spirontocaris spp.	Feeding: Invertebrates, Epibenthic
copper rockfish	
Cancer spp. (zoea)	Feeding: Invertebrates, Pelagic
Eualus spp.	Feeding: Invertebrates, Epibenthic
Heptacarpus spp.	Feeding: Invertebrates, Epibenthic
Lebbeus spp.	Feeding: Invertebrates, Epibenthic
Pandalus danae	Feeding: Invertebrates, Epibenthic
Phanerodon furcatus	Feeding: Vertebrates, Water Column
Spirontocaris spp.	Feeding: Invertebrates, Epibenthic
kelp greenling	
Eualus spp.	Feeding: Invertebrates, Epibenthic
Heptacarpus spp.	Feeding: Invertebrates, Epibenthic
Lebbeus spp.	Feeding: Invertebrates, Epibenthic
Spirontocaris spp.	Feeding: Invertebrates, Epibenthic
lingcod	
Ammodytes hexapterus	Feeding: Vertebrates, Water Column
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Phanerodon furcatus	Feeding: Vertebrates, Water Column

Nearshore Subtidal Hard Substrate (cont.)

Assemblage	Attribute	Function
pile perch		
	Balanus crenatus	Feeding: Invertebrates, Epibenthic
	Balanus glandula	Feeding: Invertebrates, Epibenthic
	Transennella tantilla	Feeding: Invertebrates, Benthic
quillback rockfish		
	Cancer spp. (zoea)	Feeding: Invertebrates, Pelagic
	Eualus spp.	Feeding: Invertebrates, Epibenthic
	Heptacarpus spp.	Feeding: Invertebrates, Epibenthic
	Lebbeus spp.	Feeding: Invertebrates, Epibenthic
	Pandalus danae	Feeding: Invertebrates, Epibenthic
	Spirontocaris spp.	Feeding: Invertebrates, Epibenthic
striped seaperch		
	Cancer spp. (zoea)	Feeding: Invertebrates, Pelagic

Water Column

Assemblage Attribute	Function
Birds	
common merganser	
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Cymatogaster aggregata	Feeding: Vertebrates, Water Column
Gasterosteus aculeatus	Feeding: Vertebrates, Water Column
Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
Thaleichthys pacificus	Feeding: Vertebrates, Water Column
double-crested cormorant	
Cymatogaster aggregata	Feeding: Vertebrates, Water Column
Thaleichthys pacificus	Feeding: Vertebrates, Water Column
mew gull	
Cancer spp. (megalops)	Feeding: Invertebrates, Pelagic
osprey	
Thaleichthys pacificus	Feeding: Vertebrates, Water Column
red-breasted merganser	
Gasterosteus aculeatus	Feeding: Vertebrates, Water Column
Oncorhynchus spp. (fry)	Feeding: Vertebrates, Water Column
western grebe	
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Cymatogaster aggregata	Feeding: Vertebrates, Water Column

Water Column (cont.)

Assemblage Attribute	Function
Fishes	
coho salmon	
Ammodytes hexapterus	Feeding: Vertebrates, Water Column
Callianassa californiensis (larvae)	Feeding: Invertebrates, Pelagic
Cancer spp. (larvae)	Feeding: Invertebrates, Neustonic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Corophium spp.	Feeding: Invertebrates, Epibenthic
Engraulis mordax	Feeding: Vertebrates, Water Column
Epilabidocera amphitrites	Feeding: Invertebrates, Neustonic
Euphausia pacifica	Feeding: Invertebrates, Pelagic
Eurytemora affinis	Feeding: Invertebrates, Pelagic
Hypomesus pretiosus	Feeding: Vertebrates, Water Column
Insecta (drift)	Feeding: Invertebrates, Neustonic
Oncorhynchus gorbuscha (fry)	Feeding: Vertebrates, Water Column
Oncorhynchus keta (fry)	Feeding: Vertebrates, Water Column
Teleostei (larvae)	Feeding: Vertebrates, Water Column
chinook salmon	
Ammodytes hexapterus	Feeding: Vertebrates, Water Column
Callianassa californiensis (larvae)	Feeding: Invertebrates, Pelagic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Corophium spp.	Feeding: Invertebrates, Epibenthic
Daphnia spp.	Feeding: Invertebrates, Pelagic
Decapoda (larvae)	Feeding: Invertebrates, Pelagic
Euphausia pacifica	Feeding: Invertebrates, Pelagic
Oncorhynchus gorbuscha (fry)	Feeding: Vertebrates, Water Column
Oncorhynchus keta (fry)	Feeding: Vertebrates, Water Column
Teleostei (larvae)	Feeding: Vertebrates, Water Column

Water Column (cont.)

Assemblage	Attribute	Function
chum salmon		
	Acartia clausi	Feeding: Invertebrates, Pelagic
	Callianassa californiensis (larvae)	Feeding: Invertebrates, Pelagic
	Cancer spp. (larvae)	Feeding: Invertebrates, Neustonic
	Corophium spp.	Feeding: Invertebrates, Epibenthic
	Corycaeus anglicus	Feeding: Invertebrates, Pelagic
	Corycaeus spp.	Feeding: Invertebrates, Pelagic
	Daphnia spp.	Feeding: Invertebrates, Pelagic
	Decapoda (larvae)	Feeding: Invertebrates, Pelagic
	Epilabidocera amphitrites	Feeding: Invertebrates, Neustonic
	Epilabidocera spp.	Feeding: Invertebrates, Neustonic
	Euphausia pacifica	Feeding: Invertebrates, Pelagic
	Insecta (larvae)	Feeding: Invertebrates, Pelagic
	Oikopleura spp.	Feeding: Invertebrates, Pelagic
	Parathemisto pacifica	Feeding: Invertebrates, Pelagic
longfin smelt		
	Acartia clausi	Feeding: Invertebrates, Pelagic
	Corophium spp.	Feeding: Invertebrates, Epibenthic
	Eogammarus spp.	Feeding: Invertebrates, Epibenthic
northern anchovy		
	Acartia californiensis	Feeding: Invertebrates, Pelagic
	Acartia clausi	Feeding: Invertebrates, Pelagic
	Corycaeus anglicus	Feeding: Invertebrates, Pelagic
	Eurytemora affinis	Feeding: Invertebrates, Pelagic
Pacific herring		
	Acartia clausi	Feeding: Invertebrates, Pelagic
	Corycaeus anglicus	Feeding: Invertebrates, Pelagic
	Daphnia spp.	Feeding: Invertebrates, Pelagic
	Decapoda (larvae)	Feeding: Invertebrates, Pelagic
	Insecta (larvae)	Feeding: Invertebrates, Pelagic

Water Column (cont.)

Assemblage Attribute	Function
pink salmon	
Acartia clausi	Feeding: Invertebrates, Pelagic
Callianassa californiensis (larvae)	Feeding: Invertebrates, Pelagic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Euphausia pacifica	Feeding: Invertebrates, Pelagic
Insecta (larvae)	Feeding: Invertebrates, Pelagic
Oikopleura spp.	Feeding: Invertebrates, Pelagic
Oithona similis	Feeding: Invertebrates, Pelagic
Parathemisto pacifica	Feeding: Invertebrates, Pelagic
Pseudocalanus minutus	Feeding: Invertebrates, Pelagic
river lamprey	
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
surf smelt	
Acartia californiensis	Feeding: Invertebrates, Pelagic
Acartia clausi	Feeding: Invertebrates, Pelagic
Daphnia spp.	Feeding: Invertebrates, Pelagic
Decapoda (larvae)	Feeding: Invertebrates, Pelagic
Eurytemora affinis	Feeding: Invertebrates, Pelagic
Insecta (larvae)	Feeding: Invertebrates, Pelagic
Oikopleura spp.	Feeding: Invertebrates, Pelagic
steelhead (rainbow) trout	
Ammodytes hexapterus	Feeding: Vertebrates, Water Column
Callianassa californiensis (larvae)	Feeding: Invertebrates, Pelagic
Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Corophium spp.	Feeding: Invertebrates, Epibenthic
Decapoda (larvae)	Feeding: Invertebrates, Pelagic
Scoloidea (unid.)	Feeding: Invertebrates, Neustonic

Water Column (cont.)

Assemblage	Attribute	Function
western brook lamprey		
	Clupea harengus pallasi	Feeding: Vertebrates, Water Column
Mammals		
Pacific harbor seal		
	Ammodytes hexapterus	Feeding: Vertebrates, Water Column
	Cancer magister	Feeding: Invertebrates, Epibenthic
	Clupea harengus pallasi	Feeding: Vertebrates, Water Column
	Crangon franciscorum	Feeding: Invertebrates, Epibenthic
	Cymatogaster aggregata	Feeding: Vertebrates, Water Column
	Engraulis mordax	Feeding: Vertebrates, Water Column
	Gadus macrocephalus	Feeding: Vertebrates, Demersal
	Lampetra tridentatus	Feeding: Vertebrates, Water Column
	Leptocottus armatus	Feeding: Vertebrates, Demersal
	Loligo opalescens	Feeding: Invertebrates, Pelagic
	Lumpenus sagitta	Feeding: Vertebrates, Demersal
	Lycodopsis pacifica	Feeding: Vertebrates, Water Column
	Merluccius productus	Feeding: Vertebrates, Water Column
	Microgadus proximus	Feeding: Vertebrates, Demersal
	Pleuronectes (Parophrys) vetulus	Feeding: Vertebrates, Water Column
	Platichthys stellatus	Feeding: Vertebrates, Demersal
	Pleuronectidea (unid.)	Feeding: Vertebrates, Demersal
	Porichthys notatus	Feeding: Vertebrates, Demersal
	Spirinchus thaleichthys	Feeding: Vertebrates, Water Column
	Thaleichthys pacificus	Feeding: Vertebrates, Water Column
	Theragra chalcogramma	Feeding: Vertebrates, Demersal

Water Column (cont.)

Assemblage	Attribute	Function
northern sea lion		
	Clupea harengus pallasi	Feeding: Vertebrates, Water Column
	Cottidae (unid.)	Feeding: Vertebrates, Water Column
	Gadus macrocephalus	Feeding: Vertebrates, Demersal
	Gonatidae (unid.)	Feeding: Vertebrates, Water Column
	Mallotus villosus	Feeding: Vertebrates, Water Column
	Merluccius productus	Feeding: Vertebrates, Water Column
	Octopus spp.	Feeding: Invertebrates, Pelagic
	Oncorhynchus spp.	Feeding: Vertebrates, Water Column
	Pleuronectidea (unid.)	Feeding: Vertebrates, Demersal
	Scorpaenidae (unid.)	Feeding: Vertebrates, Water Column
	Sebastes spp.	Feeding: Vertebrates, Water Column
	Theragra chalcogramma	Feeding: Vertebrates, Demersal

APPENDIX C: Physicochemical attributes identified in Protocol as important to fish and wildlife utilization of estuarine habitats.

Parameter	Attributes	Function	Assemblage Species
(1) Bathymetry/ topography	(a) physical complexity, bathymetric features	(i) refuge/ physiology	buffalo sculpin chinook salmon chum salmon coho salmon copper rockfish cutthroat trout Dolly Varden great sculpin padded sculpin rock sole starry flounder surf smelt
			American coot bufflehead black brant Canada goose common goldeneye double-crested cormorant great blue heron greater yellowlegs
			Pacific harbor seal
	(b) physical complexity- horizontal edges	(i) refuge/ physiology	chinook salmon chum salmon coho salmon English sole starry flounder threespine stickleback
			American coot common snipe great blue heron green-winged teal mallard
			Pacific harbor seal
	(c) physical complexity- verical relief	(i) refuge/physiology	cutthroat trout chum salmon Dolly varden English sole

		shiner perch
		starry flounder
		threespine stickleback
		black brant
		bufflehead
		Canada goose
		common goldeneye
		common snipe
		double-crested cormorant
		dunlin
		gadwal
		glaucous-winged gull
		great blue heron
		greater yellowlegs
		green-winged teal
		least sandpiper
		mallard
		mew gull
		osprey
		Pacific staghorn sculpin
		red-winged blackbird
		rock sole
		short-billed dowitcher
		song sparrow
		spotted sandpiper
		western sandpiper
		northern sea lion
		Pacific harbor seal
		Townsend vole
(d) elevation-riparian	(i) reproduction	American coot
		American goldfinch
		American wigeon
		Canada goose
		common snipe
		dark-eyed junco
		gadwall
		great blue heron
		green-winged teal
		mallard
		merlin
		northern oriole
		osprey
		red-tailed hawk
		savannah sparrow
		song sparrow

			muskrat raccoon
	(e) elevation-intertidal	(i) reproduction	Pacific herring surf smelt
			American wigeon
	(f) elevation-subtidal	(i) reproduction	Dungeness crab
			copper rockfish English sole Pacific cod Pacific herring Pacific staghorn sculpin Pacific tomcod pile perch starry flounder threespine stickleback whitespotted greenling
(2) Water and sediment character and quality	(a) general	(i) feeding	red rock crab
			chinook salmon chum salmon coho salmon C-O sole cutthroat trout Dolly Varden Dover sole hybrid sole Pacific cod Pacific sanddab Pacific tomcod pink salmon ratfish rock sole rough sculpin sand sole speckled sanddab starry flounder steelhead (rainbow) trout sturgeon poacher

	surf smelt
	threespine stickleback
	walleye pollock
	American wigeon
	black brant
	bufflehead
	Canada goose
	common goldeneye
	common merganser
	common snipe
	double-crested cormorant
	dunlin
	gadwall
	glacous-winged gull
	great blue heron
	greater yellowlegs
	green-winged teal
	horned grebe
	least sandpiper
	mallard
	mew gull
	osprey
	short-billed dowitcher
	spotted sandpiper
	red-breasted merganser
	western grebe
	western sandpiper
	northern sea lion
	Pacific harbor seal
(ii) refuge/physiology	chinook salmon
	chum salmon
	coho salmon
	C-O sole
	cutthroat trout
	Dolly Varden
	Dover sole
	hybrid sole
	Pacific cod
	Pacific sanddab
	Pacific tomcod
	pink salmon
	ratfish
	rough sculpin
	sand sole
	speckled sanddab

		steelhead (rainbow) trout
		sturgeon poacher
		surf smelt
		threespine stickleback
		walleye pollock
		American wigeon
		black brant
		bufflehead
		Canada goose
		common goldeneye
		common merganser
		common snipe
		dunlin
		gadwall
		glaucous-winged gull
		greater yellowlegs
		green-winged teal
		horned grebe
		least sandpiper
		mallard
		mew gull
		osprey
		red-breasted merganser
		short-billed dowitcher
		spotted sandpiper
		western grebe
		western sandpiper
		northern sea lion
		Pacific harbor seal
	(iii) reproduction	Dungeness crab
		copper rockfish
		Pacific herring
		prickly sculpin
		surf smelt
		dunlin
		greater lellowlegs
		least sandpiper
		spotted sandpiper
		western sandpiper
(b) sediment size structure (gravel)	(i) feeding	black brant

(c) sediment organics (detritus)	(i) feeding	Dungeness crab
		chum salmon
		mew gull
(d) salinity	(i) feeding	Dungeness crab
		chinook salmon
		chum salmon
		C-O sole
		coho salmon
		copper rockfish
		cutthroat trout
		Dover sole
		English sole
		hybrid sole
		mountain whitefish
		northern squawfish
		Pacific cod
		ratfish
		sand sole
		surf smelt
		threespine stickleback
		walleye pollock
	(ii) refuge/physiology	chinook salmon
		chum salmon
		coho salmon
		cutthroat trout
		kelp perch
		northern squawfish
		Pacific cod
		Pacific herring
		Pacific sand lance
		Pacific staghorn sculpin
		pink salmon
		shiner perch
		striped seaperch
		surf smelt
	(iii) reproduction	Dungeness crab
		Pacific herring
		Pacific tomcod
		ratfish
		starry flounder
		surf smelt
		walleye pollock

(e) temperature	(i) feeding	chinook salmon
		chum salmon
		coho salmon
		cutthroat trout
		Dolly Varden
		English sole
		pink salmon
		steelhead (rainbow) trout
		surf smelt
		threespine stickleback
	(ii) refuge/physiology	chinook salmon
		chum salmon
		coho salmon
		cutthroat trout
		pink salmon
		steelhead (rainbow) trout
		surf smelt
	(iii) reproduction	starry flounder
(f) turbidity	(i) feeding	chinook salmon
		chum salmon
		coho salmon
		cutthroat trout
		Dolly Varden
		Pacific cod
		Pacific sanddab
		Pacific tomcod
		pink salmon
		ratfish
		sand sole
		speckled sanddab
		steelhead (rainbow) trout
		sturgeon poacher
		surf smelt
		walleye pollock
		black brant
		bufflehead
		common goldeneye
		common merganser
		double-crested cormorant
		gadwall
		great blue heron

			greater yellowlegs
			horned grebe
			mew gull
			osprey
			red-breasted merganser
			western grebe
			Pacific harbor seal
			raccoon
		(ii) refuge/physiology	chinook salmon
			chum salmon
			coho salmon
			cutthroat trout
			Dolly Varden
			Pacific cod
			Pacific tomcod
			pink salmon
			steelhead (rainbow) trout
			surf smelt
			threespine stickleback
			walleye pollock
			great blue heron
			greater yellowlegs
(3) Disturbance	(a) sound/pressure	(i) feeding	chinook salmon
			chum salmon
			cutthroat trout
			American goldfinch
			American wigeon
			black brant
			bufflehead
			Canada goose
			common snipe
			dark-eyed junco
			dunlin
			gadwall
			great blue heron
			greater yellowlegs
			green-winged teal
			least sandpiper
			merlin
			osprey
			red-tailed hawk
			savannah sparrow

		short-billed dowitcher
		song sparrow
		spotted sandpiper
		western sandpiper
		muskrat
		Pacific harbor seal
		raccoon
		Townsend vole
	(ii) refuge/physiology	chinook salmon
		chum salmon
		coho salmon
		cutthroat trout
		American wigeon
		black brant
		Canada goose
		common goldeneye
		common snipe
		dunlin
		gadwall
		great blue heron
		greater yellowlegs
		least sandpiper
		mallard
		red-tailed hawk
		short-billed dowitcher
		spotted sandpiper
		western sandpiper
		Pacific harbor seal
	(iii) reproduction	American wigeon
		Canada goose
		common snipe
		gadwall
		great blue heron
		green-winged teal
		mallard
		merlin
		red-tailed hawk
		short-billed dowitcher
		Pacific harbor seal
(b) light	(i) feeding	chinook salmon
		chum salmon
		cutthroat trout

			black brant
(4) Water movement	(a) general	(i) refuge/physiology	river lamprey Western brook lamprey