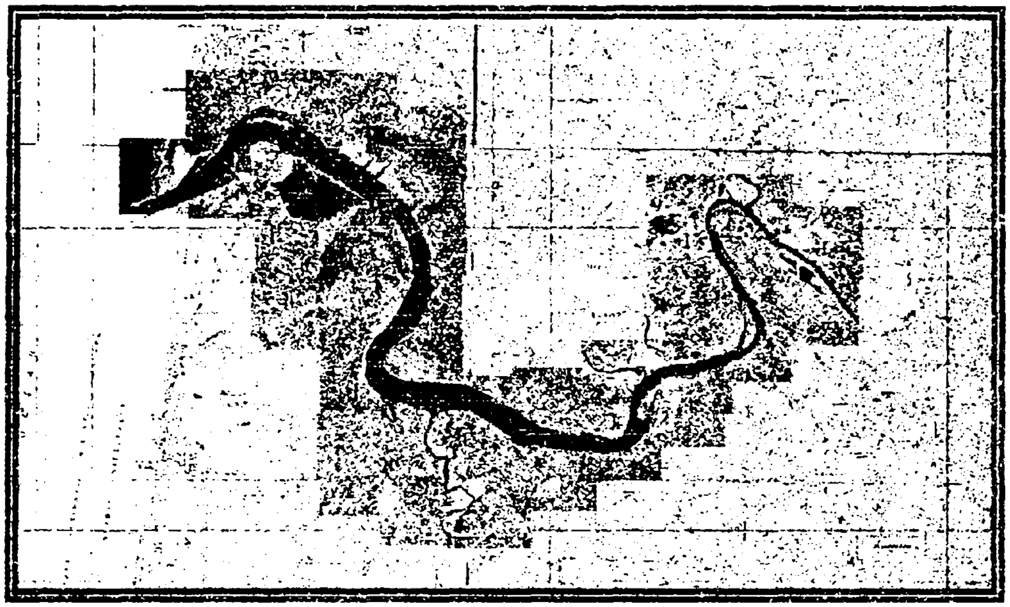
The Effects of Habitat Alteration by Estuarine Stressors on Ecological Resources of Pacific Northwest Estuaries

Research Plan

Coastal Ecology Branch
Western Ecology Division
National Health and Environmental Effects Research Laboratory
United States Environmental Protection Agency

February 1999



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ACROMYMS

AVS - Acid Volatile Sulfides
CEB - Coastal Ecology Branch
CIR - Color Infrared
CTDS - Conductivity-temperature-depth Sensors
CWAP - Clean Water Action Plan
DEM - Digital Elevation Model
DGPS - Differential-corrected Geographical Positioning System
DIN - Dissolved Inorganic Nitrogen
DIP - Dissolved Inorganic Phosphorous
EMAP - Environmental Monitoring and Assessment Program
FC - Full Color
GIS - Geographic Information System
GPS - Geographic Positioning System
LMER - Land Margin Ecosystem Research
MLLW - Mean Lower Low Water
NCR - National Research Council
NHEERL - National Health and Environmental Effects Research Laboratory
NOAA - National Oceanic and Atmospheric Administration
ORD - Office of Research and Development
ODFW - Oregon Department of Fisheries and Wildlife
OSU - Oregon State University
PNCERS - Pacific Northwest Coastal Ecosystem Study
PNW - Pacific Northwest
PAR - Photosynthetically Active Radiation
QA/QC - Quality Assurance/Quality Control
REB - Regional Ecology Branch
RPD - Redox Potential Discontinuity
SAV - Submerged Aquatic Vegetation
SAVEWS - Submerged Aquatic Vegetation Early Warning System
SEPM - Spatially Explicit Population Models
SOP - Standard Operating Procedures
STP - Sewage Treatment Plant
TOC - Total Organic Carbon
TSS - Total Suspended Sediment
TSS - Total Suspended Solids
US EPA - United States Environmental Protection Agency
USFWS - United States Fish and Wildlife Service
USGS - United States Geological Survey
WQV - Proposed Water Quality Values
WED - Western Ecology Division
YBE - Yaquina Bay Estuary

Abstract

The Coastal Ecology Branch (CEB), Western Ecology Division of the US Environmental Protection Agency will initiate a research program to evaluate the effects of alterations of estuarine habitats resulting from multiple stressor sources. The research will concentrate on stressor effects on the ecological resources of estuaries of the Pacific Northwest (PNW). The research program is designed to support the general mission of the US EPA which includes the safeguarding of the natural environment upon which the health and well being of the nation's population ultimately depends.

The goal of CEB research is to improve the ability to make key policy decisions on coastal environmental issues by defining key ecological processes and by developing models to predict stress-response relationships for ecological resources within Pacific Northwest estuaries at a range of spatial and temporal scales. CEB research objectives are to 1) evaluate how specific estuarine habitats respond to a range of potential stressors which may lead to habitat alteration, 2) understand the influences of these stress factors at spatial scales from local to regional, and 3) develop indicators of ecological condition which may be used to evaluate estuarine status across multiple spatial scales.

The research effort will concentrate on two habitats 1) submerged aquatic vegetation (SAV), and 2) burrowing shrimp, with lesser effort on other types of estuarine habitats. SAV and shrimp are selected as focal research habitats and important assessment endpoints because in each case the characteristic species which define the habitat do so because of their "physical ecosystem engineering" activities.

To accomplish the objectives of the CEB research plan, research will be organized in three thematic elements: A. Indicators of Ecological Condition for PNW Estuaries; B. Stressor-Response Modeling; C. Estuarine Physical-chemical Stressors.

The research projects under Research Theme A address the questions: 1) what are the biotic constituents of major estuarine benthic habitats of PNW estuaries, 2) what effects do various abiotic and biotic stressors have on the biotic composition of principal habitat types, 3) what role do biotic and abiotic stressors have in controlling the spatial extent and distribution patterns of major estuarine habitat types, and 4) what are appropriate indicators of ecological condition at the population, species, community and landscape levels for PNW estuarine systems. Stressors that will be examined include anthropogenic physical disturbances such as clam and burrowing shrimp harvesting, and sedimentation, salinity, and water column light field alterations potentially generated by elevated runoff resulting from changes in landscape-use patterns. Biotic stressors that will be examined include disturbances such as the smothering of seagrass habitat by mat-forming algae potentially promoted by elevated nutrients, and biotic stress induced by competition between native and exotic seagrass species and between burrowing shrimp and seagrasses.

Projects under Research Theme B will work at the population and community levels to develop modeling techniques to integrate the detailed studies of biological effects of estuarine stressors of Theme A with the spatial-temporal stressor distribution studies of Theme C. The principal current project is the development of spatially explicit modeling tools for estuarine benthic populations to allow predictions of population responses to the imposition of multiple stressors.

The research projects under Research Theme C will address the questions 1) what are the spatial and temporal distribution patterns of the primary physical and chemical factors determining estuarine habitat composition, 2) how are spatial variations in the physical-chemical stressors associated with variations in target estuarine habitats, 3) how do anthropogenic alterations of watershed characteristics influence the transport of dissolved and particulate materials into the estuary, and 4) what are the relative roles of oceanic versus riverine inputs of dissolved and particulate materials on water column physical-chemical processes.

1.0 Introduction

1.1 Context of the Research Program

The Coastal Ecology Branch (CEB), Western Ecology Division of the US Environmental Protection Agency proposes to initiate a research program to evaluate the effects of alterations of estuarine habitats resulting from multiple stressor sources. The research will concentrate on stressor effects on the ecological resources of estuaries of the Pacific Northwest. The research program is designed to support the general mission of the US EPA which includes the safeguarding of the natural environment upon which the health and well being of the nation's population ultimately depends.

CEB is a part of the National Health and Environmental Effects Laboratory (NHEERL), Office of Research and Development (ORD) of EPA. The CEB research program is a response to one of the six, high priority research areas identified in ORD's recent strategic evaluation of research needs (US EPA, 1997). The CEB research program will focus on the priority need for research to improve ecological risk assessment. Ecological risk assessment is defined as a determination of "the nature and likelihood of effects of our actions on animals, plants, and the environment" (SETAC, 1997). An increased focus on ecological risk assessment has emerged from the reorganization of the environmental research laboratories of ORD within the paradigm of risk assessment and risk management. The risk assessment approach provides a means to focus agency research to address increasingly complex environmental research issues.

The CEB research effort is also a response to the ORD strategic need to conduct research that allows EPA to improve its ability to identify and respond to emerging environmental issues. Agency research supporting development of environmental regulations has had considerable success in reducing the most extreme forms of environmental damage from toxic discharges into the nation's air, soil, fresh and marine waters, particularly from point source discharges. The critical need has now become to "better understand the vulnerability and sustainability of our ecological resources within the context of multiple stresses affecting multiple endpoints at multiple scales" (US EPA, 1997).

At the same time, the recent National Research Council review of EPA research (NRC, 1997) has pointed out that there is a need for an increased fundamental understanding of ecosystems and has recommended that EPA establish a core research agenda to advance such knowledge. To achieve this goal, the NRC review committee advocated development of more effective environmental research tools, including new instruments and measurement platforms, and development of more sophisticated environmental models (NRC, 1997).

The CEB research program will help support key action items of the Clean Water Action Plan which forms the core of the Presidential Clean Water Initiative (see section 3.0 below). Branch research will particularly help support actions for projects to Restore and Protect

America's Wetlands, Protect Coastal Waters, and Reduce Nutrient Over-enrichment.

Thus CEB research will operate within the broad strategic context of improved ecological risk assessment and in the support of key action requirements of the Clean Water Action Plan. The goal of CEB research is to improve the ability to make key policy decisions on coastal environmental issues by defining key ecological processes and by developing models to predict stress-response relationships for ecological resources within Pacific Northwest estuaries at a range of spatial and temporal scales. CEB research objectives are to 1) evaluate how specific estuarine habitats respond to a range of potential stressors which may lead to habitat alteration, 2) understand the influences of these stress factors at spatial scales from local to regional, and 3) develop indicators of ecological condition which may be used to evaluate estuarine status across multiple spatial scales.

1.2 Rationale for the Research Program

Nationwide, growth of the human population is disproportionally concentrated in the coastal zone (Culliton et al., 1990). Human population growth is a principal driver for many ecological stressors such as habitat loss, pollution, and nutrient enhancement which alter coastal ecosystems and affect the sustainability of coastal ecological resources. Increased globalization of the economy is a major driver influencing the introduction of exotic species into port and harbors. Major environmental policy decisions at local, state and federal levels related to land use planning, growth management, habitat restoration and resource utilization will determine the future trajectory for estuarine conditions of the western U.S. The results of policy decisions will have direct economic impacts on jobs, income, and population through effects on fish and shellfish harvest, property values, shipping and transportation, and quality of the coastal recreation experience. To optimize policy decisions, research is needed on the complex nature of the interactions among multiple stressors in estuarine systems to allow a greater ability to predict the outcome of policy choices.

Within the Pacific Northwest region, greatest population expansion to date has been in the major urban areas of Seattle and Portland. Although both cities have inland locations, both also border the largest coastal water bodies in their regions, Puget Sound and the Columbia River, respectively. While development around the outer coastal estuaries has been less intense, the coastal counties of Oregon have been growing from 1-2% per year over the last decade (R. L. Johnson, OSU, unpublished data), which if maintained, translates into nearly a 40% increase over the next 20 years. The coastal Oregon growth rate demonstrates the same inexorable development pressure found associated with estuarine systems in other areas of the country. With increased development comes associated environmental problems such as the loss of estuarine habitat, increased coastal resource utilization of all types, and increases in both point source and non-point source contamination (Copping and Bryant, 1993). Because of the current range of PNW estuaries in terms of state of development, CEB is ideally situated to conduct research on the impacts of development on estuarine systems of the region.

Within estuaries, benthic environments are areas where stressor impacts will tend to accumulate. Deposition of toxic materials, accumulation of sediment organics, and oxygen deficiency of bottom waters typically have a greater impact on benthic organisms than on planktonic and nectonic organisms because of their more sedentary nature. Long-term studies of the macrobenthos (Reish,, 1986, Holland and Shaughnessey, 1986) demonstrate that macrobenthos is a sensitive indicator of pollutant effects. Benthic assemblages are also closely linked to both lower and higher trophic levels, as well as to processes influencing water and sediment quality, and therefore appear to integrate responses of the entire estuarine system (Leppakoski, 1979; Holland and Shaughnessey, 1986). In the Pacific Northwest, the large tidal amplitude of the region means that a large proportion of total estuarine area is intertidal, and thus benthic resources are of primary importance. For example, up to 60% of the bottom of Yaquina Bay, OR and 90% of the bottom of Netarts Bay, OR may be exposed at low tide (Shirzad et al 1988). Benthic resources of PNW estuaries also provide important nursery habitat for juveniles of some salmonid species, provide direct economic and recreational benefits through oyster culture and clam harvest, and provide critical food resources for commercially and recreationally important fishes, waterfowl and shorebirds.

A secondary rationale for selecting estuarine benthic systems for study is the professional expertise of the research scientists of the Coastal Ecology Branch. The majority of branch scientists have spent much of their professional careers studying environmental toxicology related to contaminated marine sediments. The current redirection of research at CEB to focus on broad scale ecological issues means that the most efficient cross over for these individuals is to a focus on estuarine benthic resources, which is a study area where a considerable body of past professional knowledge of sediment properties and processes may be drawn upon. Recent hires have been specifically targeted to specialized expertise in areas such as fish population dynamics, marine benthic ecology, landscape ecology, estuarine ecological modeling, in order to provide a greater breadth and depth to the CEB research team.

While benthic habitats within Pacific Northwest estuaries may be classified in a variety of ways, general categories in the lower intertidal and subtidal regions include unvegetated intertidal sand and mud, two habitats dominated by species of burrowing shrimps (*Neotrypaea californiensis* and *Upogebia pugettensis*), intertidal and subtidal submerged aquatic vegetation (SAV; including the seagrasses *Zostera marina*, *Zostera japonica*, and the widgeon grass *Ruppia maritima*), and subtidal sand and mud. Additionally, there are at least eight categories of tidal marsh found from lower high water upward to the terrestrial fringe (Akins and Jefferson, 1973). In some estuaries, additional habitats include oyster beds and the introduced, emergent, smooth cordgrass, *Spartina alterniflora*. In order to achieve a goal of improving estuarine ecological risk assessment, it is necessary to first achieve some degree of mechanistic understanding of the processes which influence the development and maintenance of these estuarine ecological assemblages. Resource limitations preclude a simultaneous focus on all habitat types, and research expertise is greatest for lower intertidal systems. Therefore the research effort will concentrate on two habitats 1) submerged aquatic vegetation, and 2) burrowing shrimp, with lesser effort on other types of estuarine habitats.

SAV and shrimp are selected as focal research habitats and important assessment endpoints because in each case the characteristic species which define the habitat do so because of their "physical ecosystem engineering" activities (Jones et al., 1997). These activities alter the habitat in ways that have strong cascading ecological effects, either positive or negative, on other species in the habitat. In the broadest sense of the term, both SAV and burrowing shrimp can be considered keystone species (Menge et al., 1994) in that their removal from the habitat will result in a replacement by an alternate ecological system (Thayer et al., 1975). However, the multiple uses of the term "keystone species" has been criticized (Mills et al., 1993), and it is more useful to view the focal research organisms as ecosytem engineers. Both taxa exert important controls on estuarine biodiversity, with eelgrass and other SAV species having an enhancement effect and burrowing shrimp a negative effect. SAV is well known to affect nutrient cycling, sediment stability, and water turbidity (Dennison et al., 1993), and the extensive bioturbation activities of burrowing shrimp may also have strong effects on ecosystem properties (DeWitt et al., 1997). Mud shrimp create deep burrows and process massive amounts of water and sediment. As the major bioturbators in PNW estuaries, mud shrimp can affect nutrient regeneration, sediment deposition and transport, and water quality. By reducing sediment stability, they degrade habitat quality for oyster production and change the benthic community. In an attempt to control mud shrimp for the enhancement of the oyster industry, hundreds of acres in Willapa Bay are sprayed with carbaryl annually, which may have additional ramifications for estuarine communities.

The value of SAV habitats as nursery areas and sources of food to economically and recreationally important estuarine species is well established in many areas of the US (Dennison et al., 1993), and the more limited data from the Pacific Northwest also supports a similar importance in this region (Thom, 1987). Data from carbon isotope analysis has demonstrated that seagrass beds and their associated algae and animals are the food source for outmigrating juvenile chum salmon (*Oncorhynchus keta*) in an arm of Puget Sound (Simenstad and Wissmar, 1985, cited in Thom, 1987). Juvenile Dungeness crabs appear to utilize eelgrass meadows as refugia from predators (Armstrong et. al. 1982; cited in Thom, 1987), and are found in large numbers is these habitats. Eelgrass is an important substrate for the attachment of Pacific herring eggs (Thom, 1987). Eelgrass habitat can make up a large percentage of total estuarine habitat, for example representing 83% of the bottom habitat in Padilla Bay, WA (Thom, 1990). Coastline surveys in Washington state (Thom and Hallum, 1991) provide a probable underestimate of 25% of the shoreline as containing seagrass.

Although the assessment endpoints of our studies are primarily benthic, many of the stressors are transmitted to the benthic community through the water column (e.g. nutrients, suspended sediments). Thus we will also examine the spatial and temporal patterns of stressors within the estuarine water column.

At present, our ability to predict how estuarine ecosystems of the PNW will respond to single and multiple stressors is at a rudimentary level. Historically much of the marine research at major academic institutions of the region has focused either on blue water oceanography or open coast rocky intertidal systems, thus there are deficiencies in our basic knowledge about PNW estuaries. Our proposed research will contribute to addressing the basic informational deficiencies while evaluating the effects of multiple stressors on PNW estuaries.

Our predictive capabilities are also limited by a lack of appropriate ecosystem-level approaches. During the last two decades, there have been major advances in methods to evaluate the effects of point-source pollutant discharges to coastal systems. Many of these methods are based on toxic effects or bioaccumulation by a few laboratory bioassay species (e.g., Swartz 1987, Lee et al. 1993). Although the use of toxicity tests and bioaccumulation measurements of surrogate species has proven useful in regulating individual chemicals, toxic pollutants appear to play a fairly minor role in most PNW outer-coast estuaries. In addition, the classical approaches developed for single pollutants are generally inadequate to determine the effects of nonchemical stressors, the impact of multiple interacting stressors, the cumulative effects of habitat alteration, overall ecosystem response, or the linkages between the management of coastal watersheds and estuarine ecosystems. We have little quantitative information on the effects of watershed alteration and resultant loadings of estuaries by a variety of potential stressors, such as sediments, nutrients, and toxic substances. Knowledge of circulation, sedimentation, and runoff in Pacific Northwest coastal estuaries has not been well integrated, making it difficult to predict direct physical effects on biota or the physical transport of particulate-associated or dissolved stressors, such as nutrients. Similarly, quantitative relationships between the loss or alteration of specific habitats and changes in estuarine structure and functions generally are lacking.

If federal, state, tribal and local governments are to effectively manage this region, existing knowledge must be synthesized and analyzed from an ecosystem perspective, and critical data gaps must be filled. Management needs such as the requirements of the CWAP (section 3.0 below), together with the combination of societal and ecological relevance of estuarine benthic resources, make them an appropriate and important focus of CEB research.

1.2.1 Drivers and Stressors Affecting Pacific Northwest Estuaries

The great natural beauty and relatively low current population density of outer coast estuaries of the PNW gives an impression of systems that are less altered than those in other areas of the US. However, PNW estuaries are far from pristine. The list of potential disturbance agents that have affected PNW estuaries includes habitat loss or alteration, exotic species introduction, chemical contaminants, watershed alteration with changes in fluxes of freshwater, nutrients, and sediments, pathogens, over harvest of species, and various other forms of anthropogenic alteration of estuarine resources.

Over the last 200 years, at least six great waves of extractive industry have washed across the Pacific Northwest, constituting societal drivers producing a variety of stressors which have altered ecological systems. The nature of the ecological alterations are difficult in some cases to evaluate because of a lack of data from periods prior to exploitation (Durning, 1996).

In the first extractive wave, sea otters, a known ecological keystone species (Simenstad et al, 1978), were largely removed from coastal ecosystems in the PNW by 1810, and populations have never recovered. The consequence may have been a persistent transformation of near shore habitat from kelp forest to urchin barrens, although data are currently lacking to support this hypothesis.

In contrast to inland locations, the wave of western mining had little direct effect on the outer coast in terms of altering estuaries or causing chemical pollution. Open coast estuaries are believed to have generally low concentrations of toxic pollutants such as heavy metals, chlorinated hydrocarbons and polycyclic aromatic hydrocarbons because of relatively low population densities and low levels of heavy industry (Copping and Bryant, 1993). However, data for most estuaries are sparse, and there may be locations, e.g. Coos Bay, where chemical contamination such as the presence of tributyl tin associated with shipping does occur.

In the third wave, exploitative fishing devastated coastal fish and shellfish resources. Salmon catch peaked in Willapa Bay on the Washington coast by 1902, and on the Oregon Coast by 1911 (Durning, 1996). Native oyster populations were largely wiped out by the late 1800's, leading to attempts at replacement first by the unsuccessful introduction of the eastern oyster (*Crassostrea virginica*) followed by the successful introduction of the Pacific oyster (*Crassostrea gigas*)(Simenstad and French, 1995).

The following exploitative waves of agriculture, logging and damming each resulted in massive changes to land use practices throughout the region. In the Chesapeake Bay region, deforestation associated with human settlement and agricultural clearing led to a 100% increase in sediment accumulation rates (Cooper and Brush, 1991) during the 1800's. It is likely that similar changes in sediment accumulation rates may have occurred in PNW estuaries. Sedimentation problems associated with land use changes may be especially acute in the Pacific Northwest because of the combination of steep coastal watersheds, high rainfall, and timber harvesting. Dairy farming along streams entering PNW estuaries removed riparian vegetation and grazing damaged stream banks, generating additional anthropogenic modifications to estuarine sediment flux. The potential extent of changes due to sedimentation is illustrated in Tillamook Estuary, where the volume and average depth have been estimated to have declined by ~60% since the 1930s, as a result of high sediment loads caused by major forest fires in the watershed (referred to as the Tillamook Burn) and subsequent salvage logging and road building (James 1970).

To improve navagablity of coastal rivers, high numbers of large drift logs were removed and stream banks were clear cut to remove the source of logs. In the Coos River alone, the ASCE removed nearly 8,600 logs and blasted over 1,700 boulders prior to 1920 (Gonor et al., 1988). Removal of such structural elements must have inevitably altered the flux rates of sediments and organic particulates into the estuary, with concomitant alterations of estuarine water and habitat quality. While removal of large woody debris from freshwater streams is now known to have contributed to the decline in salmonid populations in the PNW, no information in available on what impacts debris removals may have had on estuarine ecological resources. SAV may have been particularly vulnerable, since it is especially susceptible to decreases is water column light penetration as a result of increased sediment loadings (Dennison et al., 1993). For a seagrass such as *Zostera marina* growing in a marginal habitat, even pulses of high turbidity of a month's duration may make the difference between local survival or extinction of a patch (Moore et al. 1996, 1997). Thus the development of a conceptual relationship between landscape and stream alterations, turbidity flux, and estuarine populations needs development.

To the six great waves of ecological resource exploitation in the PNW may be added two less immediately obvious processes, non-point source pollution and introduction of nonindigenous species, both of which may alter or degrade the estuarine ecological systems in the region. Non-point source pollution is primarily driven by the steady growth of the resident human population, which tends to concentrate near water bodies such as estuaries. Increases in the proportion of impervious surfaces (pavement, buildings, etc.) in the landscape, improperly designed septic tank drain fields, and storm water drains all may contribute to the problem. Runoff into the estuary will therefore tend to increase, particularly in high rainfall areas such as the PNW, bringing with it increased non-point source additions of nutrients (Osborne and Wiley, 1988), other chemical pollutants, and sediments (Howarth et al., 1991).

Nutrient and sediment loadings from population centers will augment the increased flux of these materials already resulting from the larger scale watershed alterations associated with logging of the coastal mountains (Howarth et al., 1991). The typical effects of nutrient elevation in coastal waters include an increase in hazardous algal blooms (Lancelot et al., 1987), and a shift in estuarine primary production from rooted SAV to benthic macroalgae and phytoplankton in the water column. Increased organic and inorganic particulate matter from runoff causes decreased light penetration (Stross and Sokal, 1989), and reduction in the depth range of the SAV. Increased macroalgae form large mats on the bottom where they may smother seagrass, cause localized oxygen depletion of sediments, and consequently result in major alterations of estuarine benthic habitats (Raffaelli et al., 1998). This alteration may have direct consequences to other trophic levels such as birds, marine invertebrates, and fishes dependent on the benthic resources that are degraded.

Increased flux of sediment can also drastically alter estuarine habitats. Most benthic species have a defined sediment grain size distribution which constitutes the preferred habitat (Rhoads, 1974; Gray, 1974). Additions of sediments may alter the sediment grain size, generating associated changes in benthic communities. Inorganic suspended material will also alter the light field, decreasing the ability of SAV to grow. A feedback then occurs, as vegetation, which binds sediments with roots and causes hydrodynamic damping to cause sediment deposition, is lost and physical resuspension of sediments becomes greater, further decreasing SAV. Synergistic effects of suspended sediments and organic particulates may greatly exacerbate the loss of SAV.

Increased sedimentation may also alter the bathymetric profile of an estuary, shifting the zones of tolerance for benthic species in the intertidal and potentially eliminating or significantly modifying the spatial distribution of some species. High sediment loadings may also clog the feeding structures of suspension feeders, causing their elimination (Young and Rhoads 1971). There is evidence that this has occurred in Yaquina Bay for oysters planted on the bottom (Fasten, 1931; cited in Baker et al. 1995).

The second of the more insidious stressors of PNW estuaries is the introduction of nonindigenous species. While the devastating effects of introduced species in island and other terrestrial ecosystems has been well described, the effects of nonindigenous species on estuarine habitats has only recently come under scrutiny (Carlton and Geller, 1993). The potential for ecological transformation is massive. Some 367 marine invertebrate taxa were recorded in the ballast water of ships arriving in Coos Bay, Oregon from Japan (Carlton and Geller, 1993). Introduction of a single species of comb jelly in the Black Sea resulted in the virtual destruction of pelagic fisheries valued at $250 million annually within only 10 years (Harbison and Volovik, 1994). Extensive natural and anthropogenic disturbance within estuarine systems may provide favorable conditions for invasion of exotic species (Cohen and Carlton, 1998), and thus there may be synergistic interactions among important estuarine stressors. In the PNW, one by-product of the attempted commercial introduction of the eastern oyster appears to have been the introduction of smooth cordgrass, *Spartina alterniflora*, into Willapa Bay, WA where the spread of this exotic species has resulted in the conversion of hundreds of hectares of mud flat to salt marsh habitat with consequences to the ecosystem that have not yet been fully defined (Simenstad and Thom, 1995).

In an independent assessment conducted by the Tillamook Bay National Estuary Program of EPA, priority management issues were habitat loss and alteration, sedimentation, introduced pest species, species loss, and pathogens. The CEB research program will contribute directly to addressing each of these management issues except pathogens, for which we have no current expertise.

1.2.2 Estuarine Scaling Issues

Many anthropogenic and natural stressors interact with estuarine habitats in ways that alter the normal spatial distribution pattern or mosaic of habitat patches. For example, channel dredging may dissect a shallow sand bottom habitat into sub patches separated by a deeper depositional environment characterized by finer sediments and altered biological communities. Various impacts to vegetated habitats will alter patch distribution and degree of fragmentation. Dredging, propeller scars, removal of vegetation for oyster planting, competitive displacement through bioturbation by burrowing shrimp, water column eutrophication and increased turbidity will alter patch size, contiguousness, and spatial distribution. Such alterations have important implications for estuarine ecosystem function. Recent work from terrestrial systems (Wardle et al., 1997) has shown that island area is related to relative carbon partitioning, humus accumulation, nitrogen acquisition and mineralization, standing biomass, and litter decomposition. There is reason to believe that there may be important relationships of habitat patch size to estuarine ecosystem functions as well (Robbins and Bell, 1994).

For example, the patch integrity of SAV may influence sedimentation rates through relative degree of hydrodynamic damping, which may in turn influence passive larval recruitment and sedimentary accumulation. Sediment accumulation rates may affect local accumulation rates of particulate associated toxic substances. SAV patch integrity may influence habitat resistance to some forms of disturbance. For example, large clonal patches may have greater nutrient reserves in rhizomes as compared to small patches, which can be shunted to damaged areas receiving either natural (burrowing shrimp, feeding pits from fishes or crabs) or anthropogenic (clam digging, prop damage) disturbance. Similarly, large patches may be more tolerant of lowered light levels due to seasonal pulse events causing elevated turbidity and lowered light levels. Other ecosystem properties that can be influenced by patch size for SAV are:

- relative proportion of exogenous organic carbon accumulated in sediments,
- percentage of in situ Net Primary Production retained within the patch as detritus versus exported from the patch,
- sediment chemistry properties such as depth of the Redox Potential Discontinuity (RPD) or Acid Volatile Sulfides (AVS),
- characteristics of associated animal populations (abundance, size structure),
- relative contribution of different function groups (detritivores, carnivores, suspension feeders),
- detrital breakdown rates,
- relative degrees of epiphytic algal cover,
- relative degree of drift algal trapping.

The influence of the relative scale of habitat patches on functional properties within the patches may also have a cascading effect to other trophic levels. For example, larval trapping and detrital accumulation may be greater in larger habitat patches, resulting in superior success for juvenile fish and shellfish.

These examples suggest that overall estuarine ecosystem response to stressors will be a function both of the tolerance of individual species and habitat elements to a given stressor, and the spatial distributional aspects of the habitat. Habitat fragmentation has become a crucial issue in terrestrial conservation ecology. As examples, forest fragmentation has been shown to negatively impact reproductive output of migratory birds (Robinson et al. 1995), above ground biomass of Amazonian trees (Lawrence et al., 1997), and old field fragmentation affects small mammal population dynamics and the population persistence of clonal plants (Robinson et al., 1992). For clonal SAV species, habitat fragmentation is similarly of potentially great importance in estuarine systems with linked effects to other faunal components associated with the habitat. The understanding of estuarine habitat scaling relative to stressor impacts is crucial to the development of a predictive evaluation capability for stressor response within estuarine systems. One approach to dealing with habitat scaling relative to stressor impacts is the development of spatially explicit models of estuarine populations which allow both the spatial extent of the target population and the stressor to be varied. One research element of the proposed research plan will be to use spatially explicit models to examine the two dimensional geometry of stressor effects on model populations of estuarine organisms.

In addition to the use of spatially explicit modeling to deal with issues of scaling and estuarine stressor responses, CEB has recently hired two principal investigator with research skills that may be applied to landscape scale issues. A landscape ecologist with research interests in estuarine habitat distribution patterns and organism responses has been hired. A biogeographer that is a shared position with the Regional Ecology Branch of WED located in Corvallis has considerable expertise in dealing with regional scale research questions. An element of the CEB research plan will develop linkages between estuarine habitat distributions and landscape alterations and land use patterns surrounding the estuary. As the research develops, we plan to link estuarine system responses into land use patterns in the estuarine watershed with the help of the biogeographer, and by means of developing research collaboration with the Regional Ecology Branch of WED located in Corvallis.

In attempting to extrapolate research results it is important to define the set of estuaries to which the results of the CEB research plan will apply. There are many physical types of estuaries in the PNW region, and differences in physical aspects of estuaries clearly must be considered. At the same time, the geographic region from panhandle Alaska to Northern California is classed as a single biogeographic province based on similarities of biotic composition. Estuaries within this province will thus share broad similarities of species composition. For example, the same seagrass species are distributed across much of the biogeographic province, and thus CEB research on stressor effects on SAV will generally be relevant across the region. None the less, placement of CEB research within the context of an estuarine classification scheme based on physical properties will be helpful.

There is an existing literature which may help to provide a better definition of estuarine typology in the PNW (Hansen and Rattray, 1966; jay and Smith, 1988; Dethier, 1990; Montgomery and Buffington, 1993, Jay et al, 1997). CEB will compile in data base format as much physical information on PNW estuaries as possible and will investigate potential approaches to categorization of estuaries in order to allow the CEB research effort to be placed into a proper framework for extrapolation. For example, work has shown that the ratio of drainage basin area to area of the estuary proper is highly correlated with estuarine fish species richness (Bottom and Jones, 1990). This ratio provides a metric for categorizing the relative amount of riverine versus marine influence. Other such metrics will be investigated in relation to the types of parameters which CEB is investigating wherever data sets are available. Some compilation of comparative data for PNW estuaries has already been done by other organizations (e.g NOAA, 1988, 1991).

1.3 Scientific Questions

The overall research goal is to improve the assessment of risk associated with alterations of ecological resources of PNW estuaries by multiple physical, chemical, and biological stressors at a range of temporal and spatial scales.

The principal scientific questions to be addressed are:

- How do stressors affect "physical ecosystem engineering" species, and thereby lead to alterations in estuarine habitats?
- What is the range of habitat alteration outcomes which result from individual and multiple stressor effects?
- How may results of small scale, process-oriented research on stressor effects leading to habitat alteration, be scaled to provide predictions for entire estuaries?
- What indicators of ecological status can be used to assess the effects of multiple stressors on estuarine habitats?
- What are the consequences of estuarine habitat change on economically and ecologically important estuarine species?

Each broad scientific question will be examined by means of a series of individual research elements which are integrated among principal investigators. Research elements are often mutually supporting, with data collected by one project providing important information to other projects. The initial research approach will include field observations of stressor magnitude, temporal variability, and spatial distribution concentrated at single estuary scale. Stressor measures will be combined with a determination of the distributional patterns and organismal composition of major habitats within the estuary. Use of remote sensing technologies to acquire estuarine- scale habitat distributions will be explored. Multivariate, correlational and geostatistical analytical tools will seek to associate large-scale, within-estuary distributions of target habitats to stressor distributions. In conjunction with field observations, both field and laboratory experimentation will be used to test specific hypotheses (defined in individual research elements, Section 3) related to the interactions between specific abiotic and biotic stressors and habitat types. As the research program matures, additional emphasis will be placed on comparative studies among estuaries to determine how specific stressor-response relationships vary among PNW estuaries. In order to provide the capability to develop predictive stressor response models at estuarine scales, CEB has hired an estuarine modeler to develop models that couple the spatial and temporal distribution of stressors to predicted habitat alteration outcomes. The expanded modeling effort will be supported by current research elements which are developing spatially explicit population models and which are testing existing estuarine models of water column light field responses to nutrient additions.

2.0 Regional Characteristics and Study Area

2.1 Pacific Northwest Estuaries

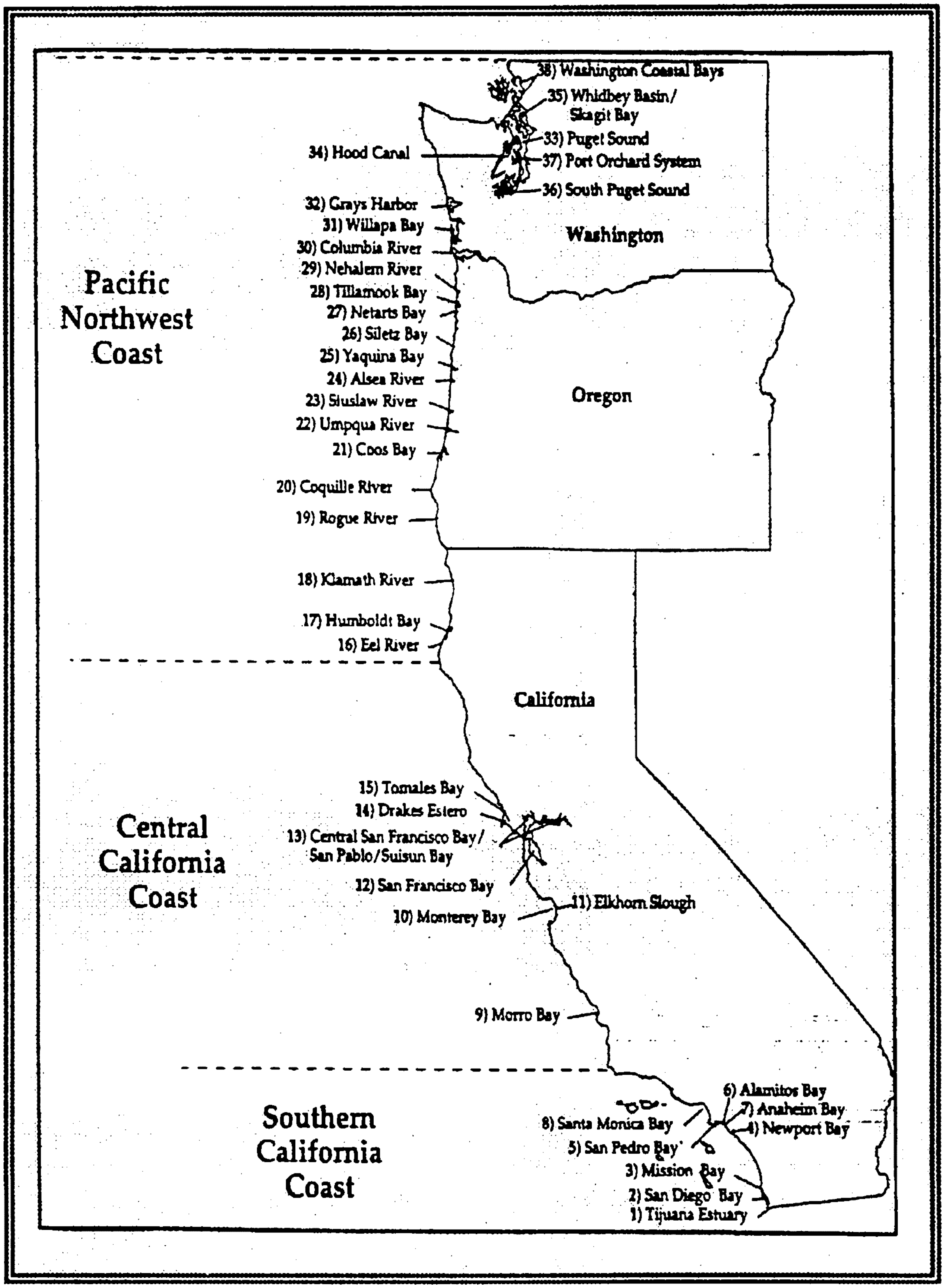
The Pacific Northwest coastal region and the associated Columbian biogeographic province extends from Puget Sound in the north to approximately Cape Mendocino in California (Fig. 1). The region contains at least 23 estuaries and coastal embayments with a total estuarine surface area of 4,504 km^2 (NOAA, 1998). The region contains a wide variety of types and sizes of estuaries, ranging in scale from the fjord like Puget Sound where numerous mid-sized streams form subestuaries as they drain into the main body of the sound, to small coastal streams emptying out into the ocean with estuarine basins less than a fraction of a square kilometer. The majority of estuaries along the outer coast of the Pacific Northwest are either of the shallow river mouth type or the drowned river valley type, including the large Columbia River estuary (Copping and Bryant, 1993). Because of their large size, geomorphology, hydrology, and proximity to large population centers, Puget Sound and the Columbia River estuary differ considerably from the smaller coastal estuaries of the Pacific Northwest (Copping and Bryant, 1993).

The estuaries of the Pacific Northwest are geologically young (Simenstad and Fresh, 1995). Estuarine geomorphology and ecology is periodically affected by tectonic events which may result in catastrophic changes. For example, subsidence events of 0.5 - 2.0 m along the coast appear to have taken place at least six times in the last 7,000 years (Atwater, 1987). Geological studies indicate that as little as three hundred years ago, a massive tsunami impacted coastal estuaries of the Pacific Northwest, causing transformation of coastal forests into salt marsh (Yamaguchi et al., 1997).

The climate of the region is strongly marine dominated, with seasonal weather patterns being heavily influenced by offshore current patterns. The dominance of northerly winds during the summer results in the southerly flow of the California current with associated Ekman transport of surface waters away from the coast generates coastal upwelling. The nutrient-rich upwelling water reaches the photic zone and stimulates the surface primary production near shore, helping support the rich commercial fisheries of the region. Summer fog as a result of the upwelling is also a common summer occurrence. In winter, wind direction is primarily from the south or southwest, generating a seasonal countercurrent, the Davidson current, which flows northward near the coast. The northward flow of warmer water ameliorates winter temperatures in the region. Strong winter storms from the southwest off the Pacific produce high rainfall amounts.

The proximity of the Coast Range of mountains to the outer coast along the Pacific Northwest means that, with the exception of the massive Columbia River, watershed drainage areas of coastal estuaries are relatively small. However, there are steep stream gradients and large volumes of freshwater runoff typically enter the estuary shortly after rainfall events (Copping

Figure 1. Map of estuaries of the west coast of the United States. Outer coast estuaries in the Columbian biogeographic province include those from Eel River CA to Grays Harbor, WA (numbers 16-32). Map from National Oceanic and Atmospheric Administration, 1998.



and Bryant, 1993). Freshwater inflow to the coastal zone in the Pacific Northwest is totally dominated by the Columbia River which accounts for 90% of flow in the summer, and 60% of total flow in the winter (Simenstad et al., 1990).

Much of the land comprising the watersheds of PNW estuaries is still forested, with silviculture being the dominant land use practice. For some estuaries, a high percentage of the forested watershed is in public ownership, which means that it may be possible to deal with some estuarine stressor problems through public policy changes.

With the exceptions of the Columbia River estuary and Puget Sound, the remaining coastal estuaries appear to differ primarily in estuarine area, rather than in the fundamental nature of the physical processes and stressors which influence the environmental conditions within the estuary (Shirzad et al., 1988; NOAA, 1997). Detailed comparative studies of benthic infauna among Pacific Northwest estuaries is presently lacking, but occurrence of benthic invertebrates and fish of commercial importance is broadly similar among estuaries of the region, although relative abundances vary considerably (Monaco et al., 1990). Variations among estuaries of the Pacific Northwest in the extent of tidal flushing, retention times of water masses, relative contributions of river runoff and marine waters to estuarine nutrient loadings, and land use patterns within the estuarine watershed offer the potential to achieve significant insights into estuarine risk assessments by comparative studies among estuaries.

2.2 Yaquina Bay Study Area

In order to take advantage of the body of historical research carried out by a variety of state agencies, federal agencies, and university scientists housed at the Hatfield Marine Science Center, the Yaquina Bay (Fig. 2) estuarine system has been selected as the primary study area. This choice also allows optimum utilization of limited logistical support funds available to CEB. After the research methods and approaches have been tested in Yaquina Bay, supplementary observations to broaden the inference base will be obtained from Siletz Bay and Alsea Bay, estuaries located immediately to the north and south of Yaquina Bay (Fig. 1). Some chemical and biological observations in Willapa Bay, WA (Fig. 1) will also be continued in order to take advantage of a longer time series of data than is available from Yaquina Bay, and to provide the basis for an initial comparisons of ecological conditions among estuaries.

A further advantage of focusing CEB research on Yaquina Bay is that various research agencies are currently conducting or initiating research in other Pacific Northwest estuaries, and it will be possible to develop a broader spatial extrapolation of results from inter-estuary comparisons by coordination with these research efforts. South Slough at Coos Bay, a part of the National Estuarine Sanctuary Program, is the site of an estuarine research program conducted under sponsorship of NOAA. Research in Tillamook Bay is being conducted as part of the EPA National Estuary Program, and ancillary research in this estuary is being conducted by McManus et al. of Oregon State University under sponsorship of the EPA STAR grants program. The effects of removal of salt marsh dikes on the ecosystem function of the

Salmon River estuary in being conducted by Simenstad et al. of the University of Washington. The Pacific Northwest Coastal Ecosystem Study (PNCERS), funded by the NOAA Coastal Ocean Program, will focus on Willapa Bay and Coos Bay. CEB is a collaborator on the PNCERS project. Willapa Bay is also the site of previous research under the EPA Pacific Northwest Research Program.

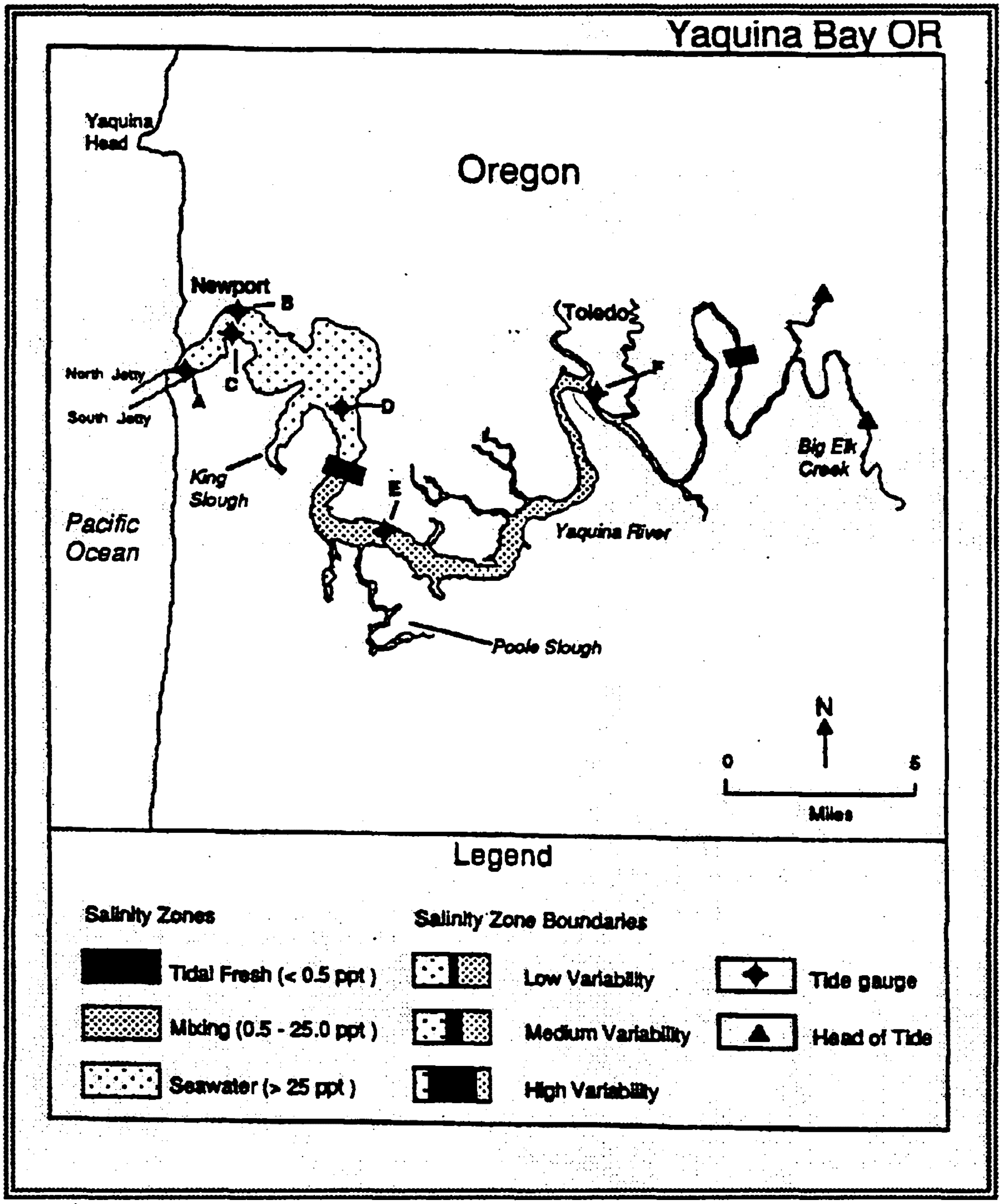
Yaquina Bay estuary lies at 44° 37' north latitude in the cool temperate Columbian biogeographic province. The estuary, formed by the submergence of a portion of the Yaquina River drainage basin, is approximately 17 km^2 with an intertidal area representing approximately 35% of estuarine area on average, but up to 60% on extreme tides. The river drainage basin is approximately 655 km^2, with a mean tidal range at the entrance of the estuary of 1.8 m and a range of 1.92 m at Toledo, 20 km up estuary. Mean flow of the Yaquina River is 30.5 $m^3 s^{-1}$, although flows may range from 1.3 to 87 $m^3 s^{-1}$ (Callaway and Specht, 1982). Rainfall in the Newport area averages 152 cm yr^{-1}, with most rainfall occurring in the period of November through March (Good, 1975). The estuary is classified as well mixed under low flow summer conditions, but is classed as partially mixed with some vertical stratification of waters of differing salinity under winter high-flow conditions (Shirzad et al., 1988). The estimated retention time of freshwater within the estuary is approximately five days (Shirzad et al., 1988).

Land use within the watershed of Yaquina Bay is currently approximately 95% forest, with the remainder as urban, agricultural, and rangeland uses. Estimates suggest that inputs of nitrogen are primarily nonpoint source runoff from forests, while phosphorus comes predominantly from wastewater treatment plants (NOAA/EPA, 1991). Yaquina Bay is estimated to be in the high range among west coast estuaries in terms of potential for concentrating dissolved substances, but is in the medium range for existing levels of nitrogen and phosphorus (NOAA/EPA, 1991). Based on limited data, the 1998 NOAA Estuarine Eutrophication Survey stated that turbidity and chlorophyll *a* concentrations are at medium levels in Yaquina Bay relative to other west coast estuaries, and that anoxia and hypoxia have not been observed within the bay. These data are almost anecdotal in nature, and it is probably premature to conclude that the ecological systems of Yaquina Bay are not being stressed at least seasonally by these factors.

While the primary focus of the Research Plan in the near term will be in and around the Yaquina Bay ecosystem, future comparisons to other Pacific Northwest estuaries will be important in the long term. Research results from Yaquina Bay should be relevant in a fairly broad geographic context, including many of the outer coast estuarine systems from the panhandle of Alaska to northern California. The suggested inference space should not be construed as an attempt by CEB to investigate all estuaries in this region. The logistical details of the research plan (Section 4) clearly focus research primarily on Yaquina Bay estuary in the initial phases of research. Some research on Willapa Bay is being maintained by two projects (Sections 4.1:A1 Ferraro/Cole, 4.3:C3, Sigleo). Minimal resources are being committed to the Sigleo sampling effort, primarily to allow continuation of a time series of data she has collected in Willapa. A greater level of effort for the Ferraro/Cole project in Willapa Bay has been provided because a comparison of habitat composition among estuaries is an important component of the research project. The overall commitment by CEB to estuarine locations other than Yaquina Bay for research is presently low.

There is the potential for a research effort in Tillamook Bay in FY 99 in support of a Region 10 interest in an EMAP intensive study of this estuary. Expansion of research efforts to other PNW estuaries is being approached cautiously by CEB, and will only be initiated in a major way after research approaches are thoroughly confirmed by work in Yaquina Bay.

Figure 2. Map of the Yaquina Bay estuarine system. Map from National Oceanic and Atmospheric Administration, 1998.



3.0 Research Approach

3.1 Research Theme Organization

To accomplish the objectives of the CEB research plan, research will be organized in three thematic elements:

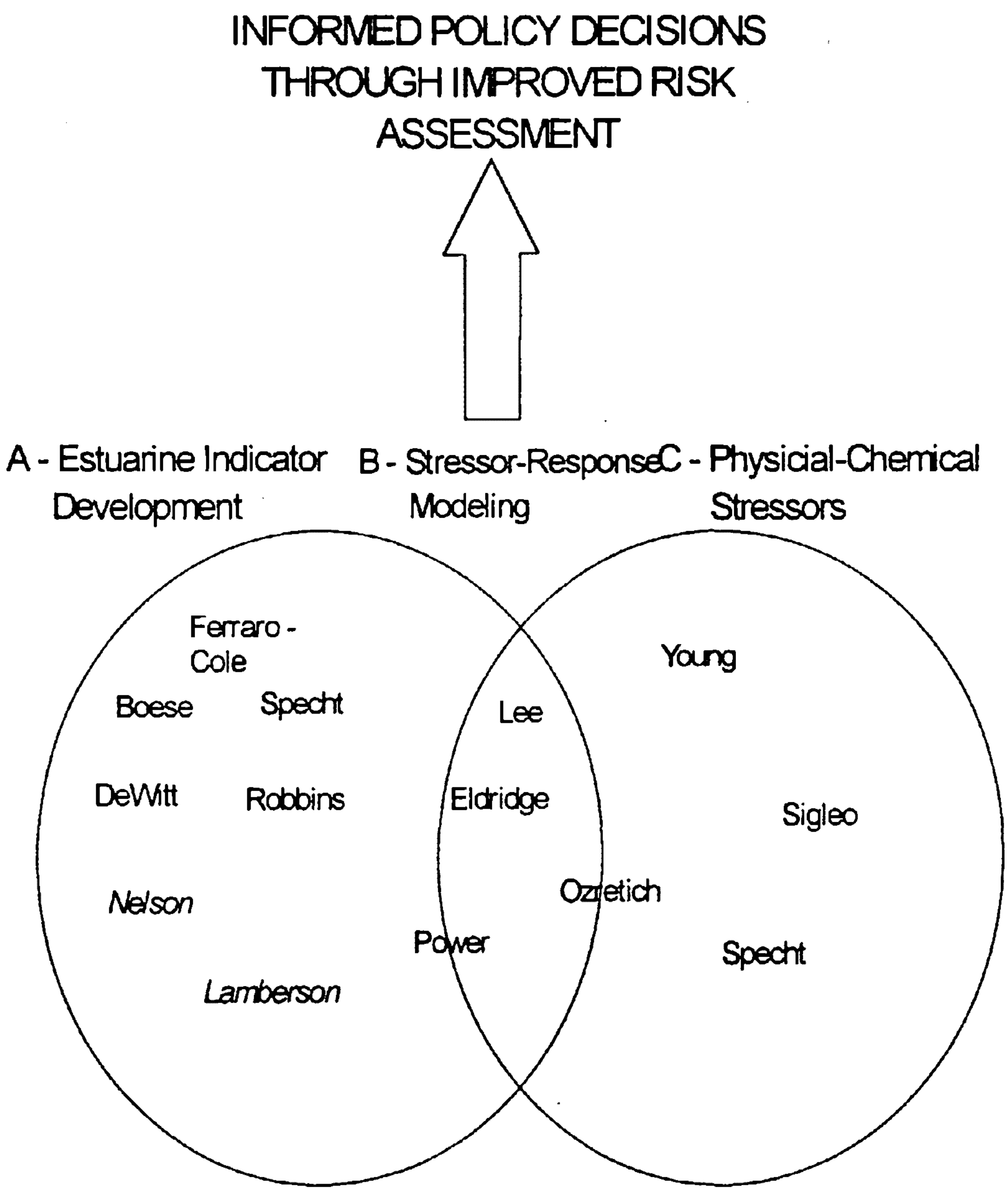
A. Indicators of Ecological Condition for PNW Estuaries
B. Stressor-Response Modeling
C. Estuarine Physical-chemical Stressors

Within each of the above research elements, a number of individual research projects are proposed. Individual research projects integrate both within and across research themes. A schematic indicating the organization of the research themes is provided in Figure 3. Research projects are described in detail in sections 4.1 - 4.3 below.

The research projects under Research Theme A seek to address the questions: 1) what are the biotic constituents of major estuarine benthic habitats of PNW estuaries, 2) what effects do various abiotic and biotic stressors have on the biotic composition of principal habitat types, 3) what role do biotic and abiotic stressors have in controlling the spatial extent and distribution patterns of major estuarine habitat types, and 4) what are appropriate indicators of ecological condition at the population, species, community and landscape levels for PNW estuarine systems. A list of research projects for this theme is given in Table 1. Stressors that will be examined include anthropogenic physical disturbances such as clam and burrowing shrimp harvesting, and sedimentation, salinity, and water column light field alterations potentially generated by elevated runoff resulting from changes in landscape-use patterns. Biotic stressors that will be examined include disturbances such as the smothering of seagrass habitat by mat-forming algae potentially promoted by elevated nutrients, and biotic stress induced by competition between native and exotic seagrass species and between burrowing shrimp and seagrasses.

Projects under Research Theme B will work at the population and community levels to develop modeling techniques to integrate the detailed studies of biological effects of estuarine stressors of Theme A with the spatial-temporal stressor distribution studies of Theme C. The principal current project is the development of spatially explicit modeling tools for estuarine benthic populations to allow predictions of population responses to the imposition of multiple stressors. While initial model development will focus on the specific example of a bivalve population, the modeling approach is a general one which can be applied to fish, crustacean, or even seagrass populations. The ecological modeler currently being hired by CEB will conduct modeling efforts to integrate research results at the community and landscape levels of organization. Until this individual has joined the research team, a precise research program can not be defined.

Figure 3. Schematic diagram of interactions among research plan elements. Names placed on the lines of the Integration section indicate projects with a secondary modeling component. Names in italics indicate CEB staff currently developing additional research elements.



Research Element Interactions - Coastal Ecology Branch

Table 1. Summary of individual research projects in Research Theme A, Indicators of Ecological Condition for PNW Estuaries, indicating the ecological level of the estuarine indicator being studied, the primary stressors affecting the indicator, and selected assessment endpoints.

Project No.	PI	Level of ecological indicator	Stressors	Assessment Endpoints
A1	Ferraro Cole	community landscape	altered habitat structure altered water chemistry (nutrients), exotic species, toxics, watershed alterations	community structure, mosaic of ecosystem types
A2	Robbins	community, landscape	altered sediment dynamics, altered hydrology, altered geomorphology altered habitat structure	relative coverage of ecosystem types, spatial-temporal patterns of habitat
A3	DeWitt	population, species, community, landscape	altered sediment dynamics, altered hydrology, altered geomorphology altered habitat structure	seagrass habitat quality and quantity, ecological condition of burrowing shrimp (ecological role)
A4	Boese	species, community	physical disturbance, altered water chemistry (nutrients)	seagrass habitat quality and quantity, community structure
A5	Specht	population, species, landscape	altered sediment dynamics, altered water chemistry (nutrients), exotic species	seagrass habitat quantity, relative coverage of ecosystem types
A6	Power	population, species	altered sediment dynamics, altered habitat structure, exotic species	ecological condition of population and species, trophic relationships

The research projects under Research Theme C seek to address the questions 1) what are the spatial and temporal distribution patterns of the primary physical and chemical factors determining estuarine habitat composition, 2) how are spatial variations in the physical-chemical stressors associated with variations in target estuarine habitats, 3) how do anthropogenic alterations of watershed characteristics influence the transport of dissolved and particulate materials into the estuary, and 4) what are the relative roles of oceanic versus riverine inputs of dissolved and particulate materials on water column physical-chemical processes. Collection of data is integrated among the various project elements to the greatest extent possible. Collection of data for physical and chemical parameters and their spatial and temporal variation within the estuary will consist of deployment of multiple instruments capable of logging data along the upstream axis of the estuary. Fixed station data will be augmented by periodic cruises to deploy equivalent instrument packages to obtain more extensive information on spatial variation of these parameters. A state-of-the-art in situ nitrate analyzer with data logger will be deployed at the mouth of the estuary to determine the temporal variation in nutrient inputs to the estuary from offshore sources. Data collected by CEB scientists will be supplemented from sources such as the State of Oregon Dept. of Water Resources which maintains a river flow gauge on a main stem of the Yaquina River. Arrangements to obtain these data from the state on a near real time basis have been made.

As one example of how interactions among projects are structured, the projects of Boese, DeWitt and Kentula, and Specht all will provide data on various aspects of the population dynamics of the seagrass *Zostera marina*. Boese will evaluate effects of physical disturbance and algal smothering, DeWitt and Kentula will evaluate effects of salinity, sedimentation, and burrowing shrimp, and Specht will examine sediment nutrients, temperature and geomorphology as controlling factors on *Zostera* populations. Young and Ozretich will provide data on water column light fields, sedimentation rates, and large scale distribution patterns of *Zostera* relative to physical stressors. Lee, with the spatially explicit population model will be able to input population characteristics, growth and mortality rates in response to stressors, and the spatial distribution of stressors in a spatially explicit population model which will allow specific predictions of population survival given different combinations and spatial distributions of stressors. The community structure assessments of Ferraro and Cole can be used to extrapolate the effects of stressors on the seagrass species to other co-occurring species in the habitat.

3.2 Key Infrastructure Support Elements

CEB has as a part of its research plan the establishment of a series of 5 permanent environmental monitoring stations for continuous acquisition of environmental data. The instruments for these stations have been acquired, and the details of deployment are currently being worked out (Section 4.3:C2, Ozretich). For a number of logistic reasons, the instruments will be deployed from pilings rather than buoys. Instruments will measure conductivity (salinity), temperature, depth, turbidity, and photosynthetically active radiation. One CTD has been deployed at the HMSC dock, and additional CTD's have already been deployed in temporary moorings up river. CEB is also investigating the possibility of obtaining instrumental data in real time using radio modems.

CEB has an in situ instrument for continuous measurement of nitrate deployed at the HMSC dock, and an additional nitrate monitor scheduled for deployment in the freshwater area near the head of tide. Deployment of additional continuous nitrate monitors within saline areas of the estuary will require the location of a source of more cost effective instrumentation. The instrument currently deployed cost nearly $25,000, and acquisition of 4 more instruments for nitrate analysis at all stations would be prohibitive given current CEB Expense dollar budgets. More cost effective instrumentation is currently in the developmental stage at several institutions, and we are hopeful that in the near future they will be commercially available so that CEB will be able to add a full array of nutrient monitors to the CTD instrumentation currently available.

CEB will also commit internal resources to the establishment of a mesocosm facility for controlled experiments which is viewed as an important part of the research program Section 4.1:A3, DeWitt). However, CEB financial resources are insufficient to allow construction of a mesocosm facility with optimal research capabilities in a single phase of construction. Resource limitation will mean that expansion of the mesocosm will require 2-3 years to complete, and the scientific capabilities of the facility will be concomitantly limited over this period.

3.3 Research Integration, Interactions and Continuous Improvement

The importance of information exchange and research interactions among all CEB research scientists in order to achieve the CEB research plan is recognized. CEB will conduct weekly team meetings, and biweekly research update discussions by all team members will be introduced. Additionally, ad hoc meetings to coordinate field work, to optimize sample collection, and to plan for allocation of technical support resources are frequent. CEB also plans to establish an annual research retreat which will be used to provide a forum for a more extensive internal presentation of research results by all investigators. Annual progress towards meeting individual project goals and objectives will be reviewed, and progress towards overall CEB objectives will be summarized at this retreat.

Establishment of a variety of partnerships is also essential to the success of the CEB research mission. Principal Investigators (PI's) are encouraged to interact with academic institutions by becoming adjunct faculty members in appropriate university departments. Individual PI's have established cooperative efforts with researchers at the USGS and US Army COE. CEB has made a concerted effort to identify state and local agencies which have research interests in PNW estuaries, and has some cooperative activities already in place. For example, CEB is a cooperator with the NOAA funded Pacific Northwest Coastal Ecosystems Regional Study (PNCERS), and has provided GIS support to habitat restoration efforts in Yaquina Bay sponsored by the Mid-Coast Watershed Council. CEB is interacting with Washington Sea

Grant, Oregon Sea Grant, and the Pacific Estuarine Research Society to sponsor a special symposium session on research in outer coastal estuaries of the Pacific Northwest. CEB has collaborated with Oregon Sea Grant to sponsor the establishment of an on line bibliography of research publications on Yaquina Bay. CEB will continue to expand collaborative research contacts.

An increase in contacts with outstanding scientists in a variety of relevant research areas would also be valuable to branch scientists. We recognize the need to increase our interactions with physical scientists in particular. CEB has already initiated discussions with estuarine researchers with international reputations for potential short term visits. The most likely mechanism to provide funding would be through the existing National Research Council fellowship program by providing support to selected Senior Fellows. CEB will continue to actively explore mechanisms for attracting visiting investigators to the laboratory.

CEB staff are strongly encouraged to present research results at scientific conferences and to publish results in a timely manner. CEB strives to provide travel support to each scientist to present at least one paper at a major scientific meeting annually. CEB will also cosponsor the Pacific Estuarine Research Society meeting in April, 1997, and CEB scientists are being encouraged to present their preliminary results at this conference. CEB scientists provide an annual Individual Publication Plan, and overall performance evaluation of scientists is strongly weighted to such items as meeting presentations and journal publications.

An important area for branch improvement is that CEB lacks in house scientific expertise in physical oceanography and numerical circulation modeling, areas which are critical to an integrated estuarine research program. One mechanism to achieve such in house expertise is via an NHEERL postdoctoral term position. In the event such a position can not be obtained, we believe that by establishing partnerships with other academic and agency groups, the need can be met. CEB has already let a contract for production of a two dimensional numerical model for Yaquina Bay circulation. Dr. Peter Eldridge, the new ecological modeler at CEB, has worked with numerical models of estuarine circulation. While he is not a physical oceanographer, he is able to apply modeling techniques to examine predictions of distribution of chemical parameters in estuaries. Other potential cooperators include oceanographers at the College of Oceanic and Atmospheric Sciences at Oregon State University, and numerical modelers with other laboratories of EPA (NERL), the US Army Corps of Engineers, and NOAA.

3.4 Programmatic Support of Research Plan

The CEB research plan will support important federal initiatives such as the Clean Water Action Plan. Examples of the relationship between CEB research elements and a number of specific action areas of the CWAP are given in Table 2. As one example, CEB research to determine the factors controlling distribution of eelgrass, which is a major wetland type in

Table 2. Coastal Ecology Branch research activities in support of Clean Water Action Plan priorities.

Priority 1) Restore and Protect American's Wetlands

Key Action: EPA and the Corps will emphasize restoration and mitigation of wetlands as remedies for section 404 violations.

Key Action: EPA {and other agencies} will provide technical.... assistance to states and tribes to integrate habitat considerations into geographic-based planning programs ...

CEB Research

Determination of parameters controlling distribution of submerged aquatic vegetation (SAV), a major wetland type, in Pacific Northwest estuaries.

Development of spatially explicit models for assessing population-level effects of stressors on estuarine species including (SAV).

Determine historical trends in landscape use, including riparian vegetation, around a west coast estuary and relate alterations to changes in SAV distribution patterns.

Priority 2) Protect Coastal Waters

Key Action: EPA {and other agencies} will develop a plan by the end of 1999 for coordinated monitoring of coastal waters and will, by the end of 2000, develop a comprehensive report to the public on the condition of the nation's coastal waters

Key Action: NOAA and EPA will further develop and support partnerships with state, tribal, and local governments and organizations to provide technical assistance and information to local decision makers in coastal areas...

CEB Research

Development and evaluation of new indicators of estuarine condition at the population, community and landscape levels of ecological organization.

Priority 3) Reduce Nutrient Over-enrichment

Key Action: {EPA and other agencies} will ... model and produce estimates of inputs, nutrient utilization (by major source category), transport, and net contributions of nitrogen and phosphorus in watersheds across the nation.

CEB Research

Modification of water quality models for SAV growth for west coast estuaries.

Determine magnitude, variation and sources of inputs of nutrients within selected west coast estuaries.

Priority 4) Unified Watershed Assessments

Key Action: Federal agencies will provide technical assistance or funding support for state efforts to develop unified assessments of watershed health.

CEB Research

Determine historical trends in landscape use, including riparian vegetation, around a west coast estuary and relate alterations to changes in submerged aquatic vegetation distribution pattems.

Development and evaluation of new indicators of estuarine condition at the population, community and landscape levels of ecological organization.

Pacific Northwest estuaries, will support the CWAP action of encouraging the restoration of wetlands. Without a detailed knowledge of limiting factors for eelgrass growth, it is difficult for resource managers to determine appropriate restoration targets. Such knowledge is also required to maximize the success of actual restoration projects which may be carried out.

The research program proposed is a five year program which will lead to the goal of developing a framework for prediction of ecological changes to PNW estuaries resulting from multiple stressors by the year 2003. The precise nature of the prediction framework can not be stated at this time. Two pathways are being considered. One is the use of integrative models to generate prediciton of resource alteration based on stressor levels. The seonnd possible approach is to develop CEB research results into an "alternative futures framework. In this approach, future scenarios are developed based on projections of current ecological conditions and economic and management policies and contrasted with alternative scenarios based on variations in important factors influencing ecological change. Development of an alternative futures approach would require building significant interactions with academic research programs outside of CEB.

Milestones for the production of scientific products from the research are outlined in Table 3. Research will be initiated in the summer of 1998.

Individual project descriptions follow in Section 4. Each project description is structured as a research proposal containing a statement of the research goal, the rationale for the individual research component, the specific research objectives, the scientific approach describing the methods to achieve the research objectives, and the expected benefits of the research.

Table 3. CEB Research Goal and Product Milestones.

Goal:
Development of a Framework for Prediction of Ecological Changes to Pacific Northwest Estuaries Resulting from Multiple Stressors - YR 03.

CEB Goal Milestones:

1) Selection of approach for development of prediction framework - YR 00
2) Development of prediction framework - YR 03

CEB Research Project Product Milestones:

Theme A. Indicators of Ecological Condition for PNW Estuaries

(1) Peer-reviewed scientific papers on estuarine habitats as ecological indicators - YR 00, 02, 03
(2) GIS maps of estuarine resources. Technical assistance to Mid-Coast Watershed Council - YR 98, 99, 00.
(3) Peer-reviewed scientific papers on landscape scale ecological indicators - YR 98, 99, 00, 01.
(4) Peer-reviewed scientific papers on exotic species as indicators of estuarine stress - YR 00, 01, 02.

Theme B.Stressor-Response Modeling

(1) Peer-reviewed scientific papers on spatially explicit models for assessing population -level effects of stressors on estuarine species including (SAV) - YR 01, 02, 03
(2) Peer-reviewed scientific papers on models to integrate multiple stressor effects on estuarine habitats - YR 01, 02, 03

Theme C. Estuarine Physical-Chemical Stressors

(1) Peer-reviewed scientific papers on water quality models for submerged aquatic vegetation for west coast estuaries - YR 00, 01, 02, 03
(2) Peer-reviewed scientific papers on the magnitude, variation and source of input for nutrients and other estuarine physical-chemical stressors - YR 00, 01, 02
(3) Peer-reviewed scientific papers on parameters controlling distribution of submerged aquatic vegetation - YR 99, 00, 01

4.0 Detailed Research Project Descriptions

4.1 Research Theme A. Indicators of Ecological Condition for PNW Estuaries

Project A1 - Estuarine Biota-habitat Relationships

Principal Investigator: Steven P. Ferraro
Co-Principal Investigator: Faith A. Cole

Goals: This research project contributes to the programmatic goal of developing cost-effective methods, measures and models for predicting the cumulative effects of natural and anthropogenic stressors on ecologically and economically important biotic resources and ecosystem services values (e.g., food production) (Costanza et al. 1997) of Pacific Northwest (PNW) estuaries. Specifically, the primary goal of this research project is to determine the functional value of major PNW estuarine habitats in terms of selected measurement and assessment endpoints. The primary anticipated products are empirical models of biota-habitat relationships determined at a sufficient level of resolution for use in alternative futures analysis (U.S. EPA 1995) and large-scale ecological risk ("ecorisk") assessments (e.g., Landis and Wiegers 1997; Wiegers et al. 1997). Within-estuary biota-habitat relationships will be determined by testing the null hypothesis (H_1): There are no significant differences on endpoints of interest between habitats in a given estuary. Temporal variability of within-estuary biota-habitat relationships will be determined by testing the null hypothesis (H_2): For a given habitat type and estuary, there are no significant differences on endpoints of interest between sampling events. Among-estuary biota-habitat relationships will be determined by testing the null hypothesis (H_3): For a given habitat type, there are no significant differences on endpoints of interest among estuaries. The measurement and assessment endpoints will include ecologically and economically important population and community metrics (Hunsaker and Carpenter 1990), the densities of societal-valued species and their prey (Simenstad et al. 1991), ecosystem-level distress syndrome indicators (Rapport et al. 1985), and indicators of biotic integrity (Nelson 1990; Weisberg et al. 1997; Deegan et al. 1997; Karr and Chu 1997).

The basic approach and sampling and statistical design we will use is described in detail in Ferraro and Cole (1996a,b). In summary, an EMAP-type (U.S. EPA 1992a) stratified (by habitat) random sampling design will be used to estimate summary statistics estuary-wide for each of the habitats investigated. Single classification ANOVA (Sokal and Rohlf 1995) will be used to test the null hypotheses of no differences in endpoints among habitats (H_1), time intervals (H_2), and estuaries (H_3). Within-estuary biota-habitat relationships will be determined by the magnitude of the mean difference, if any, in endpoints (A, B,...) among habitats (X, Y,...) in a given estuary. If H_1 is accepted, endpoint A is statistically indistinguishable in habitats X and Y in estuary Q, and the common mean value of endpoint A is the best estimate for habitats X and Y in estuary Q. If H_1 is rejected, endpoint A is statistically different in habitats X and Y in estuary Q, and there are different best estimates of endpoint A for habitats X and Y in estuary Q. The ability to extrapolate biota-habitat relationships over time is tested by H_2. If H_2 is accepted, the biota-habitat relationship holds over the time period tested; if H_2 is rejected, they do not. The ability to extrapolate biota-habitat relationships across estuaries is tested by H_3. Fidelity of an among-estuary biota-habitat relationship for a given habitat is indicated if H_3 is accepted, i.e., there is no significant difference in the mean values of a given endpoint in habitat X among estuaries (Q, R), and the common value of endpoint A is the best estimate for habitat X in estuaries Q and R. If H_3 is rejected, endpoint A is statistically distinguishable in habitat X in estuary Q and R, and best estimates of endpoint A in habitat X differ in estuaries Q and R.

Biota-habitat relationships will be determined based on the statistical outcomes of tests of H_1, H_2 and H_3. The strengths of the biota-habitat relationships will be assessed by significance testing at three alpha levels: $\alpha = 0.05, 0.20, 0.50$, with the three α-levels taken to be indicative of strong, intermediate, and weak relationships, respectively. Since each of the four parameters of statistical inference (α, β, n, δ) are a function of the other three, comparing statistical inferences at different α-levels, as we propose, is analogous to comparing the ability to detect "small," "medium," and "large" effect sizes (δ) (Cohen 1977), respectively.

Rationale: Alternative futures analyses and ecorisk assessments depend on estimating exposures on receptors or habitats and estimating the effect of the exposure on the receptors or habitats to predict future conditions (futures analysis) or to assess risk (risk assessment) (U.S. EPA 1992b, 1995; Freemark et al. 1996; Landis and Wiegers 1997; White et al. 1997). Estimates of exposure and effects may be obtained by measurements or models. The typical futures analysis and ecorisk assessment components are:

$$\text{Stressor} \xrightarrow{\text{exposure}} \text{Receptor or Habitat} \xrightarrow{\text{effect}} \text{Response.}$$

In futures analyses, and in ecorisk assessments where the receptors are habitats, habitat area may be an assessment endpoint, if the habitat itself is of direct environmental value, or habitat area may be a measurement endpoint for one or more assessment endpoints associated with the habitat. In the latter case, knowledge of biota-habitat associations provide one with the ability to translate observed or predicted changes in habitat areas to changes in habitat-associated assessment and measurement endpoints.

Three premises underlie the rationale for using a habitat-based approach for futures analyses and ecorisk assessments in PNW estuaries. Premise 1: Most estuarine species exhibit habitat preferences and are distributed non-randomly among habitats. There is much evidence which supports this premise (see, e.g., Briggs and O'Connor (1971), den Hartog (1977), Bayer (1979, 1981) Albright and Bouthillette (1982), Kneib (1984), Zimmerman and Minello (1984), Phillips (1984), Wenner and Beatty (1988), Dethier (1990), Heck et al. (1995), Zipperer (1996), and Bostrom and Bonsdorff (1997)). Premise 2: The major ecological stressors in PNW estuaries are sedimentation, nutrients, and the spread of nuisance exotic species, e.g., the Atlantic smooth cordgrass, *Spartina alterniflora*. This was the consensus of a group of experts (Williams and Zedler 1992); also see Baker et al. (1995). Premise 3: First-order effects of the major stressors on PNW estuaries will be to change the type (e.g., from unvegetated to *Spartina,* subtidal to intertidal, etc.) and area of habitats in PNW estuaries. If premises 1-3 are correct, biota-habitat relationships can be used in ecorisk and futures analyses to translate observed or predicted changes in habitat areas into estuary-wide biotic effects by simple mathematics. For example, if estuary area is lost by sedimentation or diking, the biota in the estuary is decreased in proportion to the area of the habitat lost. If habitat areas change in the estuary, there will be biotic winners and losers. An increase in *Spartina* habitat, for example, results in an increase in the biota associated with *Spartina* and a decrease in the biota associated with the habitat(s) displaced by *Spartina*. In habitat-based ecorisk assessments, risk is inferred where stressors intersect habitats, and if they intersect, the magnitude of the risk is inferred from the importance of the habitat (Landis and Wiegers 1997), which may be quantified by the magnitude of the endpoints of interest associated with the habitat. Other projects (e.g., NOAA 1997; Simenstad et al. 1997a,b; Young 1998) are being implemented to map PNW estuarine habitats. The Coastal Ecology Branch (CEB) and others (Grue 1995) also plan to map habitat distributions in PNW estuaries using data obtained from historical records.

Estuarine habitats are determined by bio-geophysical parameters, in particular, salinity, temperature, substrate type (sediment grain size, vegetated versus unvegetated), bathymetry, and the presence of keystone species (Kendall 1983; Kneib 1984; Phillips 1984; Dethier 1990; Zipperer 1996). We have tentatively identified eight major PNW estuarine habitats- (1) *Spartina alterniflora*, (2) *Zostera marina*, (3) muddy sand, (4) ghost shrimp (*Neotrypaea*)-dominant, (5) mud shrimp (*Upogebia*)-dominant, (6) oyster, (7) subtidal, undisturbed, and (8) subtidal, dredged- which together account for most of the area of most PNW estuaries. We chose this habitat classification because we believe these habitats support substantially different biota, their sizes and areal distributions are likely to change as a function of the major stressors, and because they can be relatively easily identified and mapped. The subtidal, dredged habitat will not be included in our studies as a considerable amount of information already exists on the effects of dredging (U.S. Army Corps of Engineers 1996). Other estuarine habitats, such as macroalgae-covered, mud, and algal mats, may also be included in our study if they are a significant component of the estuary. We will critically evaluate the results of our analyses on our tentatively defined habitats, and may redefine "habitats," by combining our habitats which do not support appreciably different biota, or by splitting our habitats into finer categories based on post stratification analyses with other potential habitat-defining variables (e.g., grain size, vegetation density, salinity, temperature).

Our measurement and assessment endpoints will include population and community metrics of ecologic and economic importance (e.g., valued fish and crustacean species and their primary prey (Simenstad et al. 1991)), keystone species (Hunsaker and Carpenter 1990), ecosystem-level distress syndrome indicators (Rapport et al. 1985), anthropogenic-effects indicators (e.g., abundance and % non-indigenous species), and components of Nelson's (1990) index of biotic integrity, the Estuarine Benthic Index of Biotic Integrity (Weisberg et al. 1997) and the Estuarine Biotic Integrity Index (Deegan et al. 1997). Specific measurement endpoints will include the total number of species, numerical abundance, total biomass, numerical abundance and biomass by major taxa (crustaceans, polychaetes, molluscs, fish), diversity (e.g., Brillioun's, McIntosh's, Simpson's, and Swartz's indices), and number and biomass of fish prey, bird prey, and habitat-engineering species (e.g., *Neotrypaea* and *Upogebia*). Metrics based on life history traits (e.g., density of short-lived opportunistic species and long-lived persistent species), trophic level, or feeding mode (e.g., density of deposit and suspension feeders) may also be used to assess habitat differences with respect to faunal types. These endpoints were chosen for their ecological and/or societal relevance and their susceptibility to the major stressors (U.S. EPA 1992b; Suter 1993). We focus on benthic fauna because they are generally more sedentary and have stronger links to habitats, they are easier to quantitatively sample, they are sensitive to stressors, and they often mediate effects at higher trophic levels (U.S. EPA 1991). Strongly habitat-related endpoints will be identified as those which can be reliably ($\alpha = 0.05$, $1-\beta \geq 0.80$) detected as statistically different among habitats using the optimum sampling protocol (see "Optimum Sampling Protocols" section, below). The relative sensitivity of the endpoints to habitat type will be determined by power analysis (Ferraro et al. 1989, 1994).

Objectives: The primary objective of this research project is to determine biota (macroinfauna, megainfauna, megaepifauna)-habitat relationships for major PNW estuarine habitats. Other complimentary objectives are to determine optimum sampling protocols and designs for determining biota-habitat relationships, and to identify cost-effective, habitat-associated indicators of estuarine productive capacity, ecosystem services values, and integrity.

Scientific Approach:

Optimum Sampling Protocols: The sampling objective of this study is to obtain sufficiently accurate estuary-wide ("universe") estimates of the macroinfauna, megainfauna, and megaepifauna in major PNW estuary habitats for ecorisk assessments and futures analysis. Many different types of sampling gear and

Figure 1. Chart of Willapa Bay showing intertidal habitats (based on best available information) and random station locations within habitats. Due to inaccuracies in habitat maps, some stations appear to be located outside habitats.

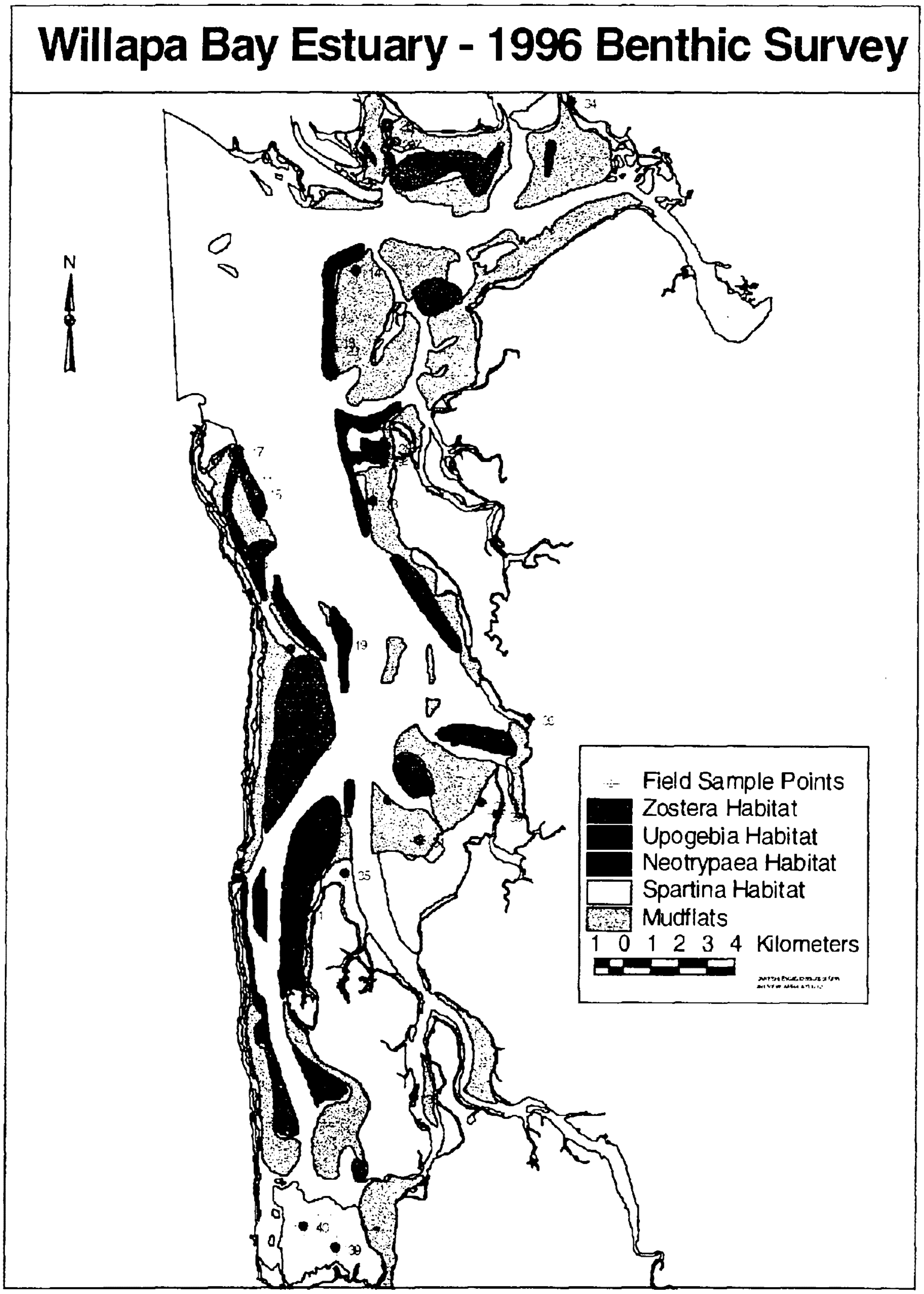
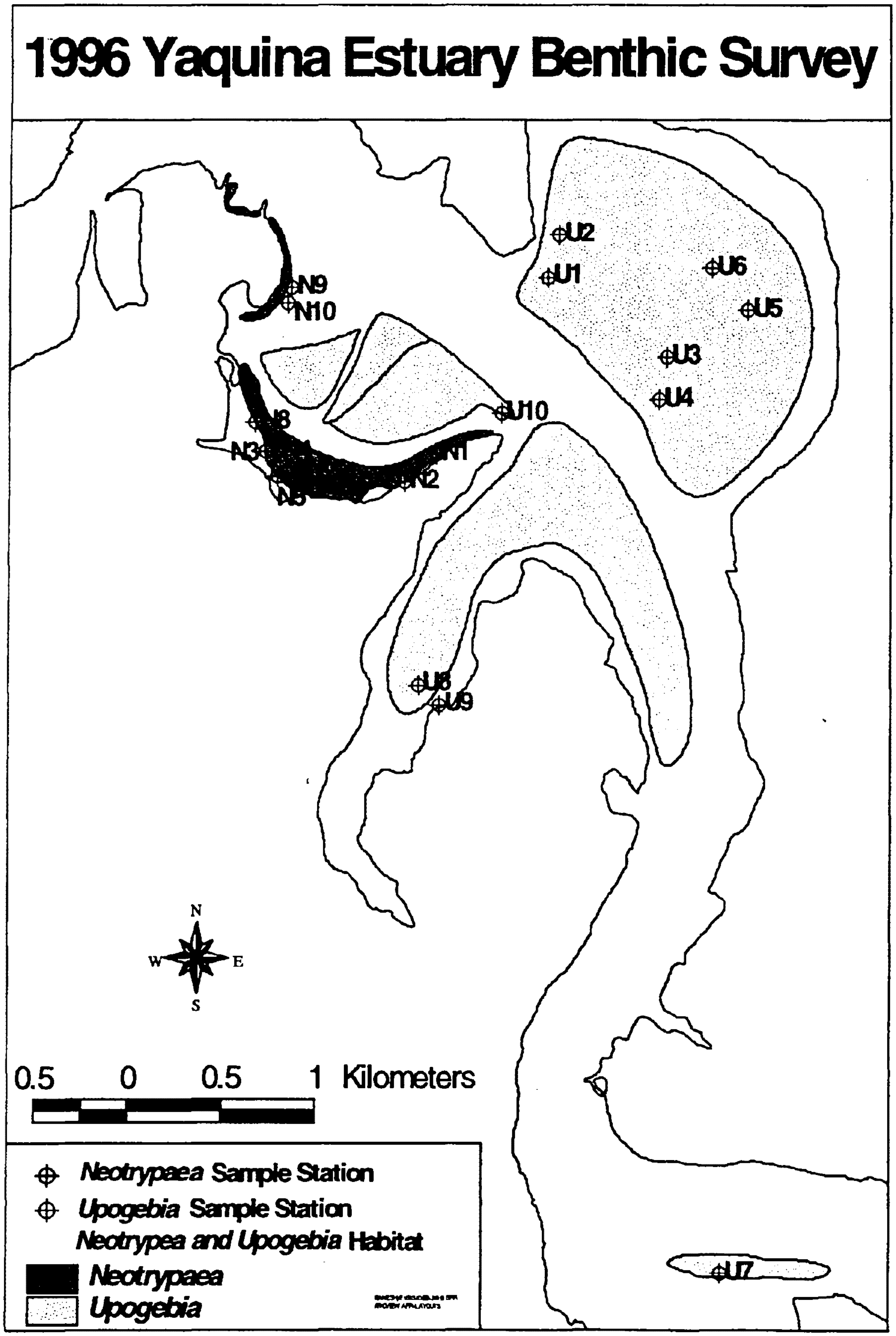


Figure 2. Chart of Yaquina Bay showing Neotrypaea and Upogebia habitats (based on best available information) and random station locations within habitats. Due to inaccuracies in habitat maps, some stations appear to be located outside habitats.



protocols have been used to sample different target species in different estuarine habitats (e.g., Puget Sound Estuary Program 1987, 1990; Simenstad et al. 1989, 1991; Rozas and Minello 1997). Since large-scale field studies of the type necessary to determine estuary-wide biota-habitat associations are likely to be costly, and the optimum (i.e., most cost-effective) sampling protocol is unknown, we will, as part of this study, test and compare a variety of alternative sampling protocols using Ferraro et al.'s (1989) power-cost efficiency approach. The optimal sampling protocol will be determined as the least costly sampling protocol capable of reliably detecting significant differences ($\alpha = 0.05$, $1\text{-}\beta \geq 0.80$) among habitats on most of the measurement and assessment endpoints of interest (Ferraro et al. 1989, 1994). Standard statistical methods (e.g., Krebs 1989; p. 213-223) will be used to determine the optimal allocation of samples within habitats. By identifying and then implementing the optimum sampling protocol and design early in the study, we will maximize the probability of meeting our research objectives and minimize cost (Green 1979; Ferraro et al. 1989, 1994).

Details of our sampling and statistical design for determining macroinfauna-habitat relationships and the optimum macroinfauna sampling protocol for detecting differences among PNW estuary habitats are in Ferraro and Cole (1996a,b) and Boese et al. (1996). In brief, benthic habitat maps will be drawn using best available information (published charts, written reports, scientific papers, personal interviews with experts familiar with the estuary, aerial photographs, etc.). As the benthic habitat maps may be inaccurate, prior to sampling a sufficiently large number (~40) of random station locations will be identified within each of the habitat map areas using the EMAP protocol. Macroinfaunal samples will be collected at the first ~10 (in 1996) or ~15 (in 1998 and thereafter) random stations correctly identified with respect to habitat. The difference in number of samples per habitat in 1996 and 1998 and thereafter is a result of a preliminary determination of the optimum sampling protocol capable of reliably detecting differences in the number of species and numerical abundance among habitats in Willapa Bay in 1996 (Ferraro and Cole 1998; QAPP98.05 Estuary Biota-Habitat Relationships, revised 8/11/98).

In August-September 1996 nine or ten stratified random samples were taken in each of four habitats in Willapa Bay (*Spartina*, *Zostera*, *Neotrypaea*, *Upogebia*) (Figure 1) and two habitats in Yaquina Bay (*Neotrypaea*, *Upogebia*) (Figure 2). The samples collected at each station were divided into 28 subsamples [six 8-cm internal diameter (0.005 m^2) cores x 2 depth strata (0-5 cm and 5-10 cm) x 2 sieve mesh sizes (1.0 mm and 0.5 mm) = 24 + 4 (2 cores x 2 depth strata x 1 mesh size (0.25 mm) = 28]. The original plan was to sequentially process (processing includes sorting, taxonomic identification, enumeration, and quality assurance) the subsamples (core 1 from each station, then core 2 from each station, etc.) and to continue processing the subsamples until compositing subsamples into progressively larger sample units no longer appreciably increased the statistical power to distinguish differences among habitats on the majority of the endpoints of interest. In light of preliminary data showing deeper (5-10 cm) strata had far fewer individuals and few new species (Table 1), and in response to a management decision to place new discrete time and resource limits on ongoing research, sample processing was limited to four (of the six) 0-5 cm deep cores, 1.0 and 0.5 mm mesh samples and one (of the two) 0-5 cm deep core, 0.25 mm mesh samples per station. Our future macroinfaunal sampling protocol and target endpoints will be determined based on the results of this currently ongoing investigation (see preliminary results in the "Expected Results and Benefits" section, below). To the extent possible, the timing and location of the field sampling of macroinfauna, megainfauna and megaepifauna components of this study will be same.

DeWitt (1998) is conducting a pilot study to determine the effectiveness and relative cost efficiency of three megainfauna (>3 mm) sampling protocols, including two core sizes and 2 extraction and processing methods for 40-cm diameter x 80-cm deep cores, in burrowing shrimp habitat in Yaquina Bay. CEB staff will also test a suction "flushing-coring" sampler (van Arkel and Mulder 1975; Grussendorf 1981). The sampling gear and protocol determined as best in CEB pilot studies will be used in our project to determine megainfauna-habitat relationships.

For our investigations of megaepifauna-habitat relationships, we will use a drop sampler similar to that used by Zimmerman et al. (1984) if, after testing, it proves to be an effective quantitative sampler of the primary target species in the intertidal habitats of our investigation. A drop sampler is being considered first because it was the only sampling gear recommended by Rozas and Minello (1997) for quantitative sampling in all major shallow-water estuarine habitats and because Simenstad (pers. comm.) recommended it for our study. Other possible samplers are a beach seine and a 3-m staffbeam trawl (Gunderson and Ellis 1986). The primary target megaepifaunal species will be juvenile Dungeness crabs (*Cancer magister*) and English sole (*Parophrys vetulus*), as they are valued species which rely heavily on estuaries as nursery areas and they appear to have specific estuarine habitat preferences (Bayer 1979, 1981; Hedgepeth and Obreski 1981; Gunderson et al. 1990).

Macroinfauna-Biota Habitat Relationships: The presence and strength of macroinfauna-habitat relationships will be determined by tests of differences on endpoints of interest among estuarine habitats (see "Goals" section). In August-September 1996, macrofaunal samples were collected at ten random stations from four habitats (*Spartina*, *Zostera*, *Neotrypaea*, *Upogebia*) in Willapa Bay and two habitats (*Neotrypaea* and *Upogebia*) in Yaquina Bay (Figures 1 and 2) (Ferraro and Cole 1996a,b). In a similar manner, we will periodically (Table 2) collect macroinfaunal samples using the optimum sampling protocol at approximately fifteen random stations from targeted habitats in Willapa Bay and Yaquina Bay and at least one other PNW estuary in order to test the ability to extrapolate the relationships over time and across estuaries. All macroinfauna sampling will occur in the index period July-September to avoid seasonal variability, for consistency with other estuarine research programs (e.g., the EMAP-Estuaries program's sampling index period is June-September (U.S. EPA 1992a)), and because most fish and invertebrates, including the primary target megaepifauna species (Dungeness crab and English sole), are usually most abundant during that time of year in PNW estuaries (Swartz et al. 1974; Bayer 1979, 1981; DeBen et al. 1990; Gunderson et al. 1990; Hinton et al. 1992; McCabe et al. 1993). Sampling more than one index period is impractical due to resource constraints, and field sampling during the winter/early spring is logistically much more difficult due to frequent inclement weather and infrequent daylight low tides. Inferences from our research, therefore, will necessarily be limited to our sampling index period. The amount and frequency of sampling will ultimately be dictated by available resources. We anticipate that macroinfaunal sampling will be conducted biannually, at a minimum, according to the priorities and the tentative schedule in Table 2. Resources permitting, we will increase the frequency of macroinfaunal sampling from biannually to annually. Macroinfaunal sampling will be performed in cooperation with another CEB research project (Lamberson 1998).

Megainfauna-Biota Habitat Relationships: The presence and strength of megainfauna-habitat relationships will be determined by tests of differences on endpoints of interest among estuarine habitats (see "Goals" section). The sampling gear and protocol found to be the most cost-effective in CEB pilot studies will be used. Megainfaunal sampling will occur during the same times (July-September) and locations as the macroinfauna sampling according to the priorities and the tentative schedule in Table 3. Resources permitting, we will increase the frequency of megainfauna sampling from biannually to annually. Megainfaunal sampling will be performed in cooperation with two other CEB research projects (Boese 1998; DeWitt 1998).

Megaepifauna-Biota Habitat Relationships: The presence and strength of megaepifauna-habitat relationships will be determined by tests of differences on endpoints of interest among estuarine habitats (see "Goals" section). Since the temporal within-season variability of the target megaepifauna species can be high (De Ben et al. 1990; Gunderson et al. 1990), megaepifaunal sampling will occur monthly (July, August, and September) at approximately the same locations as the infauna sampling and according to the priorities and tentative schedule in Table 4. Resources permitting, we will increase the frequency of megaepifauna sampling from biannually to annually. Megaepifaunal sampling will be performed in cooperation with another CEB research project (Power 1998).

Scientific Merit: Estuaries have high ecosystem services value, providing services (food production, habitat refugia (e.g., nursery grounds), recreation, etc.) with a estimated value of at least $22,832/ha/yr (Costanza et al. 1997). As estuaries are stressed, habitat areas may change resulting in increases or decreases in their ecosystem services value. Since ecosystem services values are not distributed equally

Table 1. Willapa Bay macroinfauna samples, core 1, stations 1-5.

0.5 mm sieve			
Individuals			
	Top 5 cm	Bottom 5 cm	
Neotrypaea	91	58	
Spartina	2127	115	
Upogebia	352	134	
Zostera	1253	64	
TOTAL	3823	371	
Species			
	Top	Bottom	Species not in core top
Neotrypaea	20	13	6
Spartina	36	16	0
Upogebia	44	25	8
Zostera	48	23	4

1.0 mm sieve			
Individuals			
	Top 5 cm	Bottom 5 cm	
Neotrypaea	29	25	
Spartina	278	88	
Upogebia	397	60	
Zostera	524	338	
TOTAL	1228	511	
Species			
	Top	Bottom	Species not in core top
Neotrypaea	15	6	4
Spartina	31	13	2
Upogebia	41	23	7
Zostera	50	22	3

Table 2. Macroinfauna field sampling completed (+) and planned in priority order by habitat type. "-" indicates absent or minor habitat.

Macroinfauna						
Habitats	Willapa Bay		Yaquina Bay			Other PNW Bay
Year:	1996	1998	1996	1998	2000	2000
Spartina	+	5	-	-	-	-
Zostera	+	2		1	1	1
Neotrypaea	+	7	+	4	4	3
Upogebia	+	6	+	3	3	2
oyster		1	-	-	-	-
mud		3		2	2	4
subtidal		4		5	5	5

Table 3. Megainfauna field sampling locations in priority order by habitat type. "-" indicates absent or minor habitat.

Megainfauna			
Intertidal Habitats	Yaquina Bay		Other PNW Bay
Year:	1998	2000	2000
Spartina	-	-	-
Zostera	1	1	1
Neotrypaea	2	2	2
Upogebia	3	3	3
oyster	-	-	-
mud	4	4	4

Table 4. Megaepifauna field sampling locations in priority order by habitat type. "-" indicates absent or minor habitat.

Megaepifauna			
Intertidal Habitats	Yaquina Bay		Other PNW Bay
Year:	1998	2000	2000
Spartina	-	-	-
Zostera	1	1	1
Neotrypaea	3	3	3
Upogebia	2	2	2
oyster	-	-	-
mud	4	4	4

Table 5. Mean (SE) numerical abundance and mean (SE) number of macroinfauna per 0.005 m^2 in four habitats in Willapa Bay. Analyses below are based on collections using a 0.005 m^2 x 5 cm deep core sample unit, ≥0.5 mm animal size fraction, and $n = 5$ samples per habitat.

	Mean (SE) Abundance	Mean (SE) Number Species
Spartina (S)	480.4 (82.8)	17.8 (2.32)
Zostera (Z)	342.4 (207.6)	26.8 (3.09)
Upogebia (U)	149.4 (116.4)	19.8 (2.60)
Neotrypaea (N)	21.4 (22.8)	7.6 (2.31)
Mean Square Error	22868	33.7
Tukey's test:	$S > N$	$Z > N$
($p < 0.05$)	$S > U$	$U > N$
	$Z > N$	

among habitats, valuations on habitats within estuaries are needed to determine stressor effects on estuaries.

The primary purpose of this research is to quantify the functional value of habitats within estuaries so that important effects of stressors on estuaries can be predicted using the tools of ecorisk assessment and futures analysis (U.S. EPA 1992b; Landis and Wiegers 1997; Wiegers et al. 1997). This information can then be conveyed to the public and used by resource managers to make better informed decisions regarding actions to minimize or eliminate the risks. This research is consistent with EPA/ORD's high priority and long-term mission goals to conduct "research to improve ecosystem risk assessment," "to develop scientifically sound approaches to assessing and characterizing risks to human health and the environment," and "to provide common sense and cost effective approaches for preventing and managing risks" (U.S. EPA 1996, 1997). This research will also identify optimal sampling protocols and designs for estuary-wide habitat sampling, determine the sensitivity of measurement and assessment endpoints in relation to habitat type, and increase basic knowledge in estuarine ecology.

Expected Results and Benefits: The principal expected results of this research are empirical models of macroinfauna-, megainfauna-, and megaepifauna-habitat relationships for major PNW estuary habitats. Since neither sufficient data nor models currently exist to conduct habitat-based ecorisk analyses or futures analyses, these models will significantly improve our ability to perform ecorisk assessments and futures analyses in PNW estuaries. The expected primary beneficiary is the public, on whose behalf better informed decisions can be made to protect the environment.

Optimum sampling protocols and designs will be identified for determining biota-habitat relationships in PNW estuaries, thus maximizing the probability of the success of determining biota-habitat relationships and minimizing the cost. Preliminary results suggest that useful macroinfauna-habitat relationships may be established among the four habitats investigated in Willapa Bay with a smaller sampling effort than is typically used in macroinfaunal studies (Table 5). Additional analyses will be performed with sample units of up to four composited cores (total surface area = 0.02 m^2), $\geq$1.0 mm versus $\geq$0.5 mm animal size fraction, and $n = 10$ to determine the overall optimal sampling protocol and design for determining macroinfauna-habitat relationships for a suite of measurement and assessment endpoints of interest.

This research will also increase our basic scientific knowledge and understanding of estuarine ecology, especially the spatial and temporal distribution of estuarine organisms and their habitat preferences.

Project A2 - Dynamics of an Estuarine Landscape: Spatial and Temporal Patterns with Regard to Coastal Shoreland Development

Principal Investigator: Bradley D. Robbins

Goals: The goal of the proposed research is to quantify the spatial organization (*i.e.* heterogeneity) and the temporal dynamics of the habitats (elements) that define the Yaquina Bay landscape and to evaluate historic and current impacts on this landscape by anthropogenic stressors. Because the landscape mosaic is formed and maintained by processes acting across a suite of spatial and temporal scales, information on landscape heterogeneity and the spatial and/or temporal distribution of biological patterns provide a new perspective on the dynamics of habitat change and the impact this change may have on associated landscape elements (Forman and Godron 1984). Investigations aimed at the detection and quantification of patterns at the landscape level of analysis (*i.e.* coastal change detection analysis; C-CAP; Dobson et al. 1995) may provide insight into processes that influence these patterns which themselves appear only at larger levels of spatial aggregations. Principles drawn from terrestrial landscape ecology (see Robbins and Bell 1994) will be used to analyze and interpret my data because of the expected interdependence of landscape elements. Specifically, this proposed research will explore how the heterogeneous combination of landscape elements is structured (space), how it functions (interactions), and how it changes (time).

Rationale: The research proposed here is intended to address ORD's primary goal to develop scientifically sound approaches to assess and characterize risks to the environment (EPA 1996). Specifically, this research will use a large-scale or a landscape ecology-based perspective to assess the risks of anthropogenic (non-chemical and/or chemical) stressors to ecosystems within the Yaquina Bay landscape introduced from adjacent shoreland development. Because natural systems are hierarchically structured and thus are inherently complex (Allen and Starr 1982), the recognition of the interdependence of ecosystems and the evaluation of environmental risk across multiple scales is important. Specifically, biological patterns seen at one scale may control or be controlled by factors operating at another larger or smaller scale. However, identification of the appropriate scale of inquiry for environmental studies is often difficult and is necessarily related to the target organism and/or habitat (Weins 1989; Robbins and Bell 1994). Alternatively, the ability to extrapolate results across spatial scales may not be possible. For example, McNeill and Fairweather (1993) found that the smaller patches supported significantly more species of fish and macroinvertebrates than larger patches because the increased perimeter:area ratio of smaller patches allowed greater recruitment to occur. Others examining the influence of seagrass patch size on the accumulation of drift algae (Bell *et al.* 1995), the growth and survivorship of infaunal bivalves (Irlandi 1996, 1997) and patch use by hermit crabs and gastropods (Robbins 1998) also found that directly scaling from a small area

($1m^2$) to a large area ($4m^2$) was not possible. These data suggest that extrapolation from the small to the large scale may lead to an erroneous interpretation. These difficulties may be alleviated by taking a multi-scale approach to ecosystem risk assessment and management. For example, seagrass-dominated landscapes can be viewed as an hierarchical arrangement of floral patches (*i.e.* seagrass and attached macroalgae) embedded in a matrix of unvegetated sediment (*i.e.* sand and/or mud) (Robbins and Bell 1994; Robbins 1998). The floral patches, in turn, can be considered hierarchically from individual short shoots to kilometer-wide areas of contiguous cover (Robbins and Bell 1994). Knowledge of the shoot to km patterns of seagrass distribution allows questions to be asked such as what is the impact of a process which occurs at one scale on a pattern seen at another? Specifically, does the propagation of disturbance effects (stressors) depend upon the characteristics (*i.e.* patch distribution, size, shape, or type) of the landscape?

In contrast to efforts directed toward quantifying spatial patterns in terrestrial habitats, analogous information on intertidal/subtidal soft bottom marine habitats is limited (Robbins and Bell 1994). However, the available results from estuarine studies have demonstrated the feasibility of large scale (km) studies of seagrass patches and their associated fauna (e.g. Ferguson and Korfmacher 1997; Lehmann *et al.* 1997; Malthus and George 1997; Norris *et al.* 1997; Robbins 1997,1998; Schmieder 1997; Ward *et al.* 1997; Williams and Lyon 1997). These studies show how trophic organization, as evidenced by fish feeding guilds or the linkage between macroinvertebrates and their resources, varied with landscape features. Using the underlying principles of landscape ecology, questions concerning processes which influence ecological spatial heterogeneity can be addressed in spatially explicit terms (Weins *et al.* 1993) which will contribute to our overall understanding of the functional aspects of the landscape. Presently, it is not well known how factors responsible for creating estuarine landscape patterns may have changed over time, especially in areas where anthropogenic effects have not been documented. Therefore, utilizing an approach to measure spatial and temporal patterns of the landscape, as well as the processes that produce these patterns, may help identify sites that are similar in ecosystem characteristics beyond the mere presence or absence of the target habitat and/or organism or interest.

The purpose of this study is to develop an understanding of the structure and function of a PNW estuarine landscape as well as its temporal dynamics (change). Anthropogenic activities along the estuary's adjacent shorelands have altered the structure of the estuary and subsequently, its function. Thus, an understanding of the coastal shoreland development over time will result in a better conceptual picture of the estuary. My initial efforts must necessarily be directed to the development of a spatial representation of a PNW estuary (*i.e.* Yaquina Bay). It is my intent to document the number, size, and location of the Yaquina Bay landscape's structural elements across the subtidal, intertidal, and coastal shorelands (defined as boundary lands within 1000m of MLLW). Because the tempo of habitat change, and the speed of associated

processes whether subtle or complex (Franklin 1989) are critical to the understanding of landscape dynamics and function I am proposing the use of historical aerial photographs to extend the temporal component of this study. Temporal studies not only allow for comparisons to be made across time but also provide insight into the placement of appropriate field experiments and/or sampling sites for measuring and evaluating ecosystem function.
Yaquina Bay, the fifth largest estuary in Oregon, encompasses an area of approximately 1582ha with 35% intertidal and 65% subtidal (Oregon Estuaries 1973). Yaquina Bay has been classified as a deep developed estuary (Estuary Plan Book) with the city of Newport (population ~9500) situated at its mouth and the city of Toledo (population ~3500) located nine miles upriver. Shorelands immediately adjacent to the estuary can be classified as either urban-developed, rural-developed (agriculture), or rural-undeveloped (forested) following protocols developed by NOAA's C-CAP (Dobson et al. 1995). Within the Yaquina Bay estuary, seagrass patches are arranged either as fringing patches along the lower (*Zostera marina*) or upper (*Z. japonica*) intertidal margins or as large contiguous or small highly convoluted patches (*Z. marina*) found both intertidally and subtidally across the mud/sandflats. A second dominant landscape element within Yaquina Bay are extensive aggregations of burrowing shrimp (*Neotypaea californiensis* and *Upogebia pugettensis*). These two element types offer an interesting dichotomy in that they may be spatial competitors while both have economic and ecological importance. Seagrasses act as sediment traps, stabilizing sediments and filtering suspended particulates from the water column (Dawes 1981) and are nursery areas for many commercially important invertebrates and vertebrates. Alternatively, the shrimp destabilize the substrate by excavating large quantities of sediment while digging their extensive burrows and thus change the physical and chemical properties of the sediment (Posey *et al.* 1991). The interaction between eelgrass (*Zostera marina*) and burrowing shrimp (*Neotypaea californiensis* and *Upogebia pugettensis*) within Yaquina Bay may provide an unique system in which to explore questions of landscape dynamics both spatially and temporally across a suite of spatial scales. For example, a stress such as increased sediment deposition as the result of clear cutting along the boundary of the bay may result in an areal decline in seagrass and subsequent increase in the areal extent of burrowing shrimp. Alternatively, burrowing shrimp may increase both oxygen and nutrient availability within sediment porewaters thus enhancing the growth of seagrass. Macroalgae compose a third element found within the bay. However, because of the ephemeral nature of macroalgae in Yaquina Bay, it is unclear what role this vegetation may play in influencing the distribution and spatial organization of seagrass and/or burrowing shrimp.

The time series maps developed in this study will be used to address how the spatial arrangement of landscape elements within Yaquina Bay have been impacted by coastal shoreland development. For example, the dredge and fill activities which have historically taken place within Yaquina Bay have resulted in the loss of several hectares (~7%) of the intertidal region. The loss of intertidal flats to fill and the subsequent blockage of natural drainage channels can modify both current patterns and velocities which may alter sediment erosion and deposition patterns resulting in the loss of valuable habitat. Alternatively, the construction of mooring areas, docks, and wing dams within the bay may also have a negative impact on seagrass habitats either because of shading after a dock has been built or the direct removal of seagrass during construction (see Simenstad *et al.* 1997). Thus the obvious question is how has the areal extent of seagrass changed over time and can this change be correlated with anthropogenic behaviors (development) along the coastal shorelands?

Objectives:
1: To develop of a detailed depiction of the spatial organization of the Yaquina Bay marine landscape;
2: To evaluate the impact of both historic and modern coastal shoreland development on the landscape's spatial heterogeneity;
3: To examine the power of mapped variables for predicting the distribution of a dominant landscape element within the Yaquina Bay landscape.

Methods: The project will have two phases with the acquisition of historic aerial photographs and collateral materials (e.g. reports) of the Yaquina Bay estuary (Objective 1) occurring first. This phase of my proposal will be coordinated with the research of Dr. David Young (CEB/EPA, Newport, OR). Aerial photographs have been successfully used to produce historic land-use maps to monitor terrestrial development (Adeniyi 1980) and should also be suitable for reconstructing the historical distribution of aquatic habitats and for subsequently monitoring coastal shoreland development (see Benner 1991; Coulton *et al.* 1996; Benner and Sedell 1997). Historical photographs are especially useful in identifying potentially hazardous sites that have become inactive and/or subsequently developed for other purposes (Evans and Mata 1984) yet still represent an environmental threat (stressor). A description of the spatial heterogeneity of the Yaquina Bay intertidal/subtidal landscape will be accomplished using information derived from the interpretation of historic and modern aerial photography. The length of the time period studied will be dependent upon the availability and quality of historic aerial photographs. The source media for the historic maps will most likely be black and white monoscopic aerial photographs taken at a scale of 1:24000 or greater which may limit the recognition of some landscape element types (see Robbins 1997). Other potential problems associated with historic aerial photographs include the ambient environmental conditions (e.g. sun angle, tidal height, wind speed, etc.) at the time the photographs were taken and the lack of groundtruthing; *in situ* sampling is used to check the accuracy of interpretations made on the basis of sensor data. Archived photographs are available from the City of Newport, Oregon State University, the Oregon Department of Fish and Wildlife. Other potential sources of photographs are the USGS, NOAA, Lincoln County DMV, Lincoln County Planning Office, the University of Oregon, the Oregon Department of

Transportation, and the US Army Corp of Engineers.

The source media for the modern aerial photographs will be first generation positive transparencies (diapositives) from color infra-red (CIR) negatives at a scale of 1:7200. Modern aerial photographs will be taken based on the following specifications: 1) a standard calibrated aerial photographic camera with an antivignetting on a 6in lens with the final product a series of 1:7200 stereoscopic color-infrared diapositives with a 60% forward lap on 9x9in film; 2) low tide; 3) minimal haze and cloud cover with a water surface glint of <5%/frame; 4) wind speeds of <5mph, no visible white caps, offshore winds preferable to onshore winds; 5) water clarity should be high such that seagrass patch boundaries can be clearly delineated. Although the NOAA Coastal Change Analysis Program (Dobson et al. 1995) recommends the use of natural color film for the detection of SAVs this is based on the need to detect subtidal seagrasses and macro algae. In Yaquina Bay as in many PNW estuaries, the majority of seagrass and macroalgae is intertidal and thus exposed during summer daylight hours. Thus CIR, extensively utilized in the detection of terrestrial vegetation, is an appropriate choice for the detection of exposed vegetation.

All modern aerial photographs will be scanned at 20 to 25micron resolution using a high precision/ high accuracy laser scanner resulting in digital images with a nominal image resolution ranging from 0.29m^2 to 0.36m^2. All scanned images will be orthorectified using the appropriate digital elevation model (DEM) and entered into the Branch GIS for digital data interpretation and classification. Landscape attributes including patch type (*i.e.* SAV, burrowing shrimp), size, shape, and position within the landscape will be measured. *In situ* (groundtruth) samples will be used to assess the accuracy of each element classification. The number of necessary groundtruth samples is dependent upon several factors including the number and size of each category. A general rule of thumb is that enough groundtruth samples need be taken to cover at least 5% of the overall area and 10% of each category. The estimated number of groundtruth points will be further modified to remain within logistic sampling constraints.

The second phase of this project will be further subdivided into two parts. First, I will evaluate the impact of coastal shoreland development on the Yaquina Bay landscape (Objective 2) examining two hypotheses:

H$_1$: The spatial organization of landscape elements is not correlated with historic large- scale anthropogenic disturbances (e.g. dredge/fill operation); and

H2: The development of coastal shorelands along the boundaries of the estuary is not correlated to a change in the areal extent of SAVs and/or the addition of upper marshland.

Thematic data layers will be created based on a land-use classification scheme modified from those proposed by C-CAP (Dobson et al . 1995). These data will be analyzed using a composite mapping analysis technique which entails combining the separate thematic data layers using their spatial coincidence (Lowery *et al.* 1995). Changes in land-use classification with time within a thematic data layer will be correlated to the spatial adjacency of classifications within other data layers. Several additional approaches can be utilized to evaluate spatially representative data. I will employ three analytical techniques: 1) simple pattern comparison between maps generated at different times; 2) spatial autocorrelation and temporal cross-correlation analyses; and 3) trend and residual analysis. The first step in my analytical approach will be a comparison of maps by calculating the difference (isopachs) between maps representing different time periods. For example, the seagrass map generated from 1997 aerial photographs will be compared to the seagrass map generated from the 1998 aerial photographs by "subtracting" 1997 seagrass areas from the 1998 seagrass areas which will reveal the annual addition of seagrass. The second step is to measure neighborly influence, defined as the spatial autocorrelation or the self-similarity of adjacent data. Analogously, the relationship between spatial points through time can be examined using cross-correlation. Both of these procedures assume that data points lying in close proximity (whether in time or space) to one another are more similar than widely separated data points. Specifically, one can ask whether an area has changed through time in terms of the elements that define the area and those areas which are adjacent. Third, if trends are found to be present in the spatial and/or temporal configuration of the data, these can be removed and relationships can be examined using residual trend analysis. Differences in the areal extent and configuration of the seagrass will be compared using parametric (regression and/or correlation analyses), non-parametric (correspondence analysis), and/or geostatistical (semivariography) techniques.

The second part of Phase 2 will be to examine the power of mapped variables for predicting the distribution of a dominant landscape element within Yaquina Bay (Objective 3).

> **H3:** The distribution of landscape elements can not be predicted by measurable environmental variables.

To achieve this objective, I will develop a map of predicted seagrass coverage based on differences in seagrass areal extent between 1997 and 1998 as derived from the interpretation of aerial photographs projected one year into the future. This predicted seagrass map will then be compared to observed seagrass aerial extent derived from 1999 aerial photographs. A comparison of predicted and observed maps will result in a residual map which will be interpreted using thematic layers of environmental variables which should have some influence on seagrass distribution. These thematic maps will be derived from data collected by other EPA scientists at the Newport facility:

burrowing shrimp (Dr. Ted DeWitt), water quality (Dr. Bob Ozretich), suspended and bottom sediments, bathymetry (Dr. David Young), and nutrient availability (Dr. Ann Sigleo). In essence I will answer the following questions:

1) What is the total area and patch size distribution of observed seagrass habitat in 1997 and 1998?
2) What is the total area and patch size distribution of seagrass habitat predicted on observed differences in seagrass habitat from 1997 to 1998?
3) How are areas where predicted and observed maps disagree distributed with respect to other landscape elements (e.g. burrowing shrimp and/or macroalgae), water quality, differences in sediments attributes, and nutrient availability?

The correspondence between maps will be measured by testing for the non-random distribution of residuals using spatial measures of contiguity or spatial autocorrelation (Cliff and Ord 1981) or by developing a contingency or correlation matrix without regard for spatial position (Phipps 1981).

An alternative to the use of an aerial platform to remotely sense intertidal and subtidal landscape elements will be the use of videography. While this technology is dependent upon water clarity and thus may not be suitable for use in Yaquina Bay, the technique has been used in other estuaries and may prove feasible here. The method is as follows. Video data will be collected using an 8mm camcorder housed in an underwater video housing mounted on a modified roller trawl or a flying sled from six 100x100m areas located haphazardly in Sally's Bend, Idaho Flats, and Raccoon Flats. The system will be towed behind a small skiff at a fixed rate of speed along predetermined transects. Also attached to the system will be a depth transducer and a DGPS. Depth data will be collected using a commercial data logger and a laptop computer connected via a RS-232 cable, at a rate of once every 0.3sec. DGPS readings will be recorded on the video every 0.5m. This research will be conducted in cooperation with CEB scientist Dr. DeWitt and compared to similar data collected using a 420-KHz hydroacoustic echolocation system operated by Dr. Bruce Sabol (USACE Waterways Experiment Station) a collaborator on DeWitt's proposed research. Comparisons between the methods will be made by directly comparing the hydroacoustic signature to the video recording by transect and scoring the percentage of agreements. Ground truthing will be conducted at all six sites following the protocol outlined in Appendix A.

Expected Results/benefits: The proposed research will quantify the spatial heterogeneity of an estuarine landscape and document its temporal dynamics. Change detection analysis is a useful tool in detecting and evaluating areas particularly vulnerable to environmental risk. Once these areas have been identified, they can then be targeted for experimental manipulations or as areas requiring restoration and/or mitigation. This research is unique in that few if any studies exist which document the spatial and temporal dynamics of a marine estuary at this scale of resolution.

An assessment of ecological risks to the landscape via anthropogenic stressors will also be made and addressed. Specifically, the impact of historical and modern coastal shoreland development to the abundance and distribution the major landscape elements within Yaquina Bay will be evaluated. These data will be useful for the development of future risk management plans. The model developed by this research will be a useful tool in the recognition and management of future environmental risks and will allow predictions concerning the distribution of the landscape's dominant elements based on several environmental variables such as water quality, nutrient availability, and sediment chemistry, organization, and dynamics to be made.

APPENDIX A: IN SITU MAPPING PROTOCOL

Six sites (100x100m) will be mapped by a 4 member team of researchers working in pairs. Each pair will consist of a "caller" and a "recorder." The caller's job is to identify the dominant element(s) along each transect. The recorder maintains a written record on provided data sheets by crossing out the appropriate code signifying the presence of an element. Recorders are also responsible for recording Quadrat (site) and Transect number, Date and Time, and the names of both the caller and recorder. The team works in concert with each pair mapping half of the site (50x100m area).

Specifically, 3150 data points will be collected by each team from each site. Collected data will consist of identifying the presence of five elements: seagrass, *Zostera* sp.(G), green macroalgae (Ag), shrimp (S), bare substrate (B), and brown/red algae (Ab) for all sites. In addition, at Site 6 the shrimp classification will be further subdivided into two categories by shrimp genera: *Upogebia* (Su) and *Neotrypaea* (Sn).

Data will be collected across each site by walking 21 transects positioned at five meter intervals. Each transect will be 3m wide and delineated by a 100m polypropylene rope marked at 1m intervals. The rope will be initially positioned at the first transect (A-D). One caller will begin at their end of the transect (corner A) and will walk 50m to the middle of the transect (toward corner D) while the second caller will begin at the 50m mark and walk to their 0m mark (corner D). A recorder will be at each end of the transect line. Data will be collected every 2m in the direction of the transect and every 1m across the width of the transect. When each caller completes their 50m walk, the transect rope will be moved 5m. The presence of a caller in the middle of the transect will aid in the movement of the transect line. After positioning the rope along the next transect the callers will again walk 50m stopping every 2m for data collection. At the end of this transect the callers will be in their original positions on the transect line. This procedure will be repeated until each site is mapped.

Project A3 - Changes in The Abundance And Distribution of Estuarine Keystone Species in Response to Multiple Abiotic Stressors

Principal Investigator: Ted DeWitt
Coprincipal Investigators: Mary Kentula, Bob Ozretich, Brad Robbins, David Young

Goals:The goal of the proposed research project is to assess the ecological risks posed by multiple abiotic stressors to eelgrass and burrowing shrimp, which are ecologically- and economically-important, habitat-creating, keystone species living in Pacific coast estuaries. The proposed research will use field and mesocosm experiments to measure the effects of abiotic stressors on the population dynamics of eelgrass and burrowing shrimp and to measure the effects of stressors on the outcome of competition among these keystone species.

Rationale: Eelgrass (*Zostera marina* and *Z. japonica*) and burrowing shrimp (particularly *Neotrypaea californiensis* and *Upogebia pugettensis*) create or extensively modify large areas of intertidal and subtidal benthic habitat within Pacific estuaries. These species occur in estuaries from Alaska to Baja California where they are often the ecologically dominant benthic species. They are keystone species because they determine the structure of associated benthic communities (Thom 1987, DeWitt et al. 1997). Eelgrass beds are habitats for resident species of fish and invertebrates, provide temporary refuge and foraging habitat for migratory fish (e.g., salmonids), and provide feeding habitats for waterfowl and migratory shorebirds (Griffin 1997). The economic value of eelgrass habitats in Pacific Northwest estuaries includes providing habitat for salmonids, herring, and Dungeness crabs (Thom 1987, (Doty et al. 1990, Berkeley 1998, Simenstad and Fresh 1995).

Estuarine sandflats and mudflats often harbor high densities of burrowing shrimp. These crustaceans dig extensive galleries below the sediment surface, excavating large quantities of sediment, thereby changing the physical and chemical properties of the sediment and the stability of the substrate. Sessile species are often excluded from burrowing shrimp beds, whereas abundances of mobile species can be enhanced (Posey 1986a, Posey et al. 1991, Brooks 1995). Densities of epibenthic megafauna, such as crabs, are reduced over burrowing shrimp beds because bioturbation by the shrimp buries or kills objects that provide physical structure for cover or foraging habitat (i.e., eelgrass, oysters) (Armstrong et al 1989, Doty et al. 1990). As compared to eelgrass or oyster-bed habitats, burrowing shrimp habitat harbors much lower diversity of benthic invertebrates (Posey et al. 1991, Brooks 1995). Modification of benthic habitats by burrowing shrimp has direct negative economic impact to commercial oyster mariculture in the PNW (DeWitt et al. 1997, Griffin 1997). The economic damage to oyster culture is so great that since the 1950's growers apply the pesticide, carbaryl, to over 320 ha of intertidal, estuarine oyster beds in Willapa Bay and Grays Harbor (WA) (DeWitt et al. 1997).

Eelgrass beds and burrowing shrimp beds affect the flux and storage of nutrients and carbon within estuaries (Phillips 1984). *Zostera* and associated epiphytes take up nutrients from the water column, and release it seasonally in the form of dead leaves and blades. As zones of hydrodynamic damping (i.e., depositional areas), eelgrass beds may also trap particulate organic matter from the water column which then accumulates within the bed (Fonseca et al. 1982). This can have important consequences for the deposition and spatial distribution of persistent hydrophobic or particulate-bound contaminants (e.g., metals, PAHs, PCBs, etc.) bound to sediments within the estuary. Conversely, bioturbation and burrow irrigation by burrowing shrimp may result in the flux of nutrients and other chemicals (including persistent contaminants) from the sediment into the water column. Resuspension of sediments by the actively burrowing *Neotrypea* leads to the winnowing of fine-grained sediments from tide flats, thereby changing the textural properties of the sediments as well as the concentration of organic carbon (DeWitt et al. 1997). Mud shrimp (*Upogebia*) pump large volumes of water through their burrows and feed on suspended particulate matter; large populations of mud shrimp may be significant consumers of planktonic primary production and thus possibly affect the productivity of other suspension feeders within the estuary.

Relatively large annual fluctuations in the distribution and abundance of eelgrass and burrowing shrimp have been observed in outer-coast estuaries of Oregon and Washington over the last 20-50 years (Thom and Hallum 1990; Ellis 1997; Griffin 1997). These variations are unusual as compared to other areas such as Puget Sound (Thom and Hallum 1990). In some cases, long-term reduction (e.g. 10+ yr) in the distribution and abundance of eelgrass beds has been recorded (Ellis 1997; Griffin 1997), whereas the distribution and abundance of burrowing shrimp (particularly *Neotrypea*) is reported to have increased within several estuaries (DeWitt et al. 1997). In Yaquina Bay eelgrass beds, mapped in the mesohaline portions of Yaquina estuary in 1968 and 1979 (FWS 1968, Bayer 1979), have disappeared. Proposed causes include regional environmental changes (i.e., El Niño, drought cycles, Columbia River water management) and local anthropogenic disturbance such as dredging, channelization, shoreline modification, construction of docks, commercial and recreational shellfish farming and harvesting, sedimentation associated with upland erosion, flood control and water diversion (Thom and Hallum 1990, Simenstad and Fresh 1995, Rumrill and Christy 1996). An important scientific need is a greater understanding of how abiotic stressors affect the population dynamics of these keystone species. Research is also needed to identify and rank the abiotic stressors that affect eelgrass and burrowing shrimp populations, in order to manage water quality for enhanced resource value or to classify sites by their suitability for habitat restoration (Thom 1990a).

In addition to abiotic stressors, interspecific interactions undoubtably affects the distribution and abundance of eelgrass and burrowing shrimp, which are limited in spatial distribution to the intertidal and shallow subtidal by light availability (eelgrass; Phillips 1984) and predation (burrowing shrimp; Posey 1986b). Competition for space potentially occurs between eelgrass and burrowing shrimp, and large-scale changes in the distribution and abundances of these species may have been caused by competition (Dumbauld 1998, Brooks 1997, Thom 1997, Bennett 1997, Wilson 1996). Burrowing by these shrimps may be responsible for substantial reduction in the abundance of eelgrass in PNW estuaries (Swinbanks and Luternauer 1987, Dumbauld et al. 1997), and such effects occur in other marine ecosystems (Suchanek 1983, Woods and Shiel 1997). Eelgrass populations in Willapa Bay, WA, often expand into intertidal oyster beds following the use of pesticide to control burrowing shrimp (Dumbauld 1998, Dumbauld et al. 1997, Bennet 1997, Wilson 1996, DeWitt et al. 1997). Potential interaction mechanisms are sediment resuspension which reduces light levels and thereby eelgrass growth, and depletion of sediment nutrients by burrow irrigation. Conversely, Harrison (1987) showed that eelgrass may out-compete burrowing shrimp following an increase of water clarity, possibly because the increased water clarity allowed eelgrass to overgrow *Neotrypea* burrows before the shrimp became active. It is also known that *Z. marina* rhizome mats can impede burrowing by *Neotrypea* and *Upogebia* (Brenchley (1982).

Competition is most evident between *Zostera* and *Neotrypea*, and although their spatial distributions are largely disjunct, populations can abut one another (Dumbauld et al. 1997). *Zostera* and *Upogebia* populations often overlap, but the nature of interactions is unknown. *Neotrypea* and *Upogebia* populations are largely disjunct, but do overlap along their common borders. Aggressive interactions occur between individuals that inadvertently come to share the same burrow (personal observation). There may be ecologically important three-way interactions may change under different environmental conditions. If *Upogebia* has no effect on *Zostera*, but competes for space with *Neotrypea*, then *Upogebia* may be beneficial to *Zostera* by suppressing *Neotrypea*. One of the scientific contributions of the proposed research will be to provide a better understanding of the mechanisms and dynamics of competition between *Zostera* and burrowing shrimp in Pacific estuaries.

Laboratory and field experiments will be conducted to determine whether these species do in fact compete for space, and to identify the mechanisms by which competitive displacement occurs. Subsequent experiments will focus on whether natural and anthropogenic stressors, particularly those associated with water quality, affect the outcome of competition. Given that changes in water quality or climate may have contributed to changes in the distribution and abundance of eelgrass and burrowing shrimp (discussed above), it is important to know whether natural or human-caused changes in the abiotic environment can influence the outcome of competition among these species. Changes in water quality, reduction in the abundance of predators or herbivores, increase in physical disturbance, or combinations of these stressors can provide one species with a competitive advantage over the other. These interactions are not well understood, and can not yet be incorporated into estuarine management practices.

Light and salinity appear to be the dominant abiotic factors affecting eelgrass and burrowing shrimp populations in PNW estuaries (Phillips 1984, Thom 1990, Bird 1982, Posey 1987). Light probably controls the spatial distribution of *Z. marina* populations (Olson et al. 1997), and the light penetration regime in PNW estuaries may have changed historically due to increased input of fine suspended particulates (associated with erosion) or phytoplankton abundance (associated with increased input of nutrients, especially nitrogen) (Thom and Hallum 1990). Light stressor studies will be coordinated with Dr. Robert Ozretich (CEB) who is testing the efficacy of water quality (including light) based models to predict the performance of *Z. marina* populations. Low salinity is strongly suspected to limit the up-estuary distribution of eelgrass and burrowing shrimp (Phillips 1984, Posey 1987) and episodic freshets may affect population distribution in the lower estuary too. The salinity dynamics of Pacific Northwest estuaries are likely being altered because of long-term changes in rainfall, increased runoff due to land use alterations, and water diversion. Other abiotic stressors will be studied in future experiments as their relative importance is determined.

Objectives: The goal of the proposed research is to assess the ecological risks posed by multiple abiotic stressors to eelgrass and burrowing shrimp. This will be accomplished through:

1. Field experiments to evaluate coring and remote sensing methods to detect, identify, quantify, and map eelgrass and burrowing shrimp;
2. Laboratory and field experiments to identify, measure, and map the population-level effects of single and multiple abiotic stressors on eelgrass and burrowing shrimp;
3. Laboratory and field experiments to measure competition among eelgrass and burrowing shrimp species, and to measure how abiotic stressors affect the outcome of that competition;

Methods: The research questions that will be addressed are:

1) What are efficient and accurate methods to sample and map the abundances and distributions of eelgrass and burrowing shrimp?;
2) Which population-level characteristics of eelgrass and burrowing shrimp show the strongest and least variable responses to abiotic stressors?;
3) Does competition between eelgrass and burrowing shrimp influence the abundance or spatial distribution of these species?; and
4) What are the most important stressors affecting the abundance and distribution of eelgrass and burrowing shrimp in Pacific Northwest estuaries?

1. Population Sampling Methods Evaluation: Experiment 1.A. Comparison of Coring Methods to Sample Burrowing Shrimp: A limited-effort pilot study will be conducted to compare at least four methods for collecting quantitative core samples for burrowing shrimp. These methods are 1) clam gun cores (15-cm diameter x 60-cm length, which are extracted by manually lifting the corer and sample from the sediment), 2) manually excavated megacorer (40-cm diameter x 80-cm length, in which sediments are manually dug out of the core barrel), 3) hydraulically flushed megacorer (40-cm diameter x 80-cm length, in which sediments flushed from the corer using high pressure water jet, with the water, sediment, and animals directed through a spout and through a screen to collect the sample), and 4) a suction sampler (15-cm to 20-cm diameter x 100-cm length, excavated by venturi-action caused by high pressure water jet; Grussendorf 1981). Each corer will be used to collect replicate samples of *Neotrypea* and *Upogebia* within intertidal habitats. Corers will be compared on the basis of the among-sample variability of shrimp abundance and their ease of use. The mega-infaunal sampling method selected in this study will be used in many of the field experiments described below, and by other researchers at CEB (i.e., Boese, Cole, Ferraro, and Robbins).

Additionally, we will evaluate the accuracy and precision of burrow-hole density as a means of measuring the abundance of *Neotrypaea* and *Upogebia*. Burrow-hole density is used widely as a simple measure of shrimp abundance, but is subject to inaccuracy due to incorrect species identification, seasonal variation, and habitat variation (i.e, grain size, current strength) (DeWitt et al. 1997). Burrow-hole density is determined by counts of burrow openings per unit area (typically a 0.25m^2, 0.5m^2, or 1.0m^2 quadrat) (Dumbauld 1994). The relationship between burrow-hole density and shrimp density has only been determined for Willapa Bay estuary (Dumbauld 1994). We will characterize this relationship for Yaquina estuary by measuring burrow-hole density (using a 0.25m^2 quadrat) at all sites where mega-infaunal cores samples are collected; burrow-hole counts will be made prior to coring. Additionally, temporal and environmental data will be collected (i.e., date, sediment grain size, overlying water salinity and temperature, sediment temperature). These data will be stored in a database which subsequently will be used to statistically evaluate (i.e., using regression analysis) how species, season, and environmental factors affect the relationship between burrow-hole density and burrowing shrimp density.

Experiment 1.B. Comparison of Acoustic, Photographic, and Videographic Remote-Sensing Methods to Identify and Map Estuarine Submerged Aquatic Vegetation: An experiment will be conducted to compare the accuracy of three remote sensing techniques (acoustic, photographic, and videographic) in terms of their ability to represent the spatial distribution of SAV and burrowing shrimp within Yaquina estuary. The abundance patterns of each target organism, *Zostera marina*, macroalgae (including *Ulva, Enteromorpha, Chaetomorpha*, and *Fucus*), and burrowing shrimp burrow openings will be mapped using each of the remote sensing methods and then comparing these maps to ground-truth data. Six study sites 10,000m^2 in area (*i.e.* 100m x 100m) will be selected within the central bay of Yaquina estuary (Figure 1) based on 1997 aerial photographs. Site selection will be stratified to include areas with continuous and patchy flora and fauna.

The accuracy of each map produced by the three remote sensing methods will be compared to the corresponding map generated from ground-truth data by three methods, across four spatial scales: 1-m^2, 10-m^2, 100-m^2, and 1000-m^2. First, the 1-m^2 maps will be smoothed by resampling. At each spatial scale, pixels will be reclassified by the floral or faunal type of greatest proportion. A correlation coefficient will then be calculated between all common points among maps to estimate the overall correspondence between map pairs. At each spatial scale, we will test whether there is agreement between the remotely sensed data and the ground-truth data in terms of element type within each pixel. Second, difference maps (isopachs) will be constructed by subtracting one map from another, and will be used to look for small areas of large deviation between map pairs which may have unduly influenced the correlation coefficient (Davis 1984). Third, map pairs will be examined using a cross-tabulation matrix which will be analyzed for similarities in pixel type using the kappa coefficient, κ (Carstensen 1987). This direct comparison of mapping accuracy will give us the ability to recommend a method of remote sensing best suited for the study of estuarine SAV. Additional technical details can be found in Appendix A.

Experiment 1.C. Evaluation of Remote Sensing Methods to Detect, Identify, and Quantify Burrowing Shrimp: This project will compare several acoustic remote sensing methods and videographic remote sensing for their abilities to detect, identify, and quantify *Neotrypea* and *Upogebia* in intertidal sediments. Preliminary trials (summer-fall 1998) will be conducted with each method to determine whether any signal corresponding to the presence or absence of burrowing shrimp can be detected. If any of the methods show capability to detect the shrimp, then methods-comparison experiment will be conducted (summer-fall 1999).

Test sites (patches approximately 2-m x 2-m) will be designated within Yaquina estuary (Figure 1), pre-classified by ground survey into six patch types: 1) uninhabited sand (*Neotrypaea* habitat), 2) low density *Neotrypaea* (<100 holes m^{-2}), 3) high density *Neotrypaea* (>300 holes m^{-2}), 4) uninhabited muddy-sand (*Upogebia* habitat), 5) low density *Upogebia* (<100 holes m^{-2}), and 6) high density *Upogebia* (>300 holes m^{-2}). Minimally, eight sites of each type will be identified; three sites will be used for calibration of the classification method, and the remaining sites will be used for testing the accuracy of the classification. The sites will also be selected to represent a wide variety of sediment characteristics (i.e., sediment grain size, organic matter content, water content, and compaction). Field staff will pre-classify sites at low tide by identifying the shrimp (i.e., by the configuration of the burrow openings; qualitative core samples can also be collected to verify the species identity of shrimp) and by measuring the density of burrow openings (i.e., number of burrow holes per 0.25-m^2). Crew operating the remote sensing methods will be blind to identity of each site. The boat pilot will maneuver and anchor the boat such that the acoustic transponder or video camera is positioned directly over a site, and the investigator will be allowed to sample the site for a fixed of period time (nominally, two minutes).

After all sites have been sampled, the classification of the "calibration" sites will be revealed to each investigator. Each investigator will then analyze data they collected, classify the remaining "unidentified" sites, and report the results (along with raw data and the rationale for making the classification). After the investigators report their findings, the correct classification of the unidentified sites will be revealed. The accuracy of classifying burrowing shrimp patches by each remote sensing method will be assessed by chi-square analysis. Each investigator also will be asked to evaluate how their system could be modified to improve its accuracy, to discuss any logistical or analytical difficulties that were encountered, and to assess the feasibility and cost of adapting their remote sensing method to a geographically-accurate system for mapping burrowing shrimp. Additional technical details can be found in Appendix A.

Experiment 1.D. Evaluation of Remote-Sensing Methods to Map Burrowing Shrimp Populations: If experiment 1.C. is successful, a study will be conducted (summer-fall 2000) to determine the accuracy of maps of burrowing shrimp populations generated by one or more remote sensing methods. Selection of the remote sensing method(s) used in this study will be based on the results of experiment 1.C. The specific experimental design and methods will be similar to those used in experiment 1.B. First, we will compare the accuracy of the remote sensing methods. Test sites will be designated throughout estuary, encompassing a variety of shrimp population densities, and maps of each site will be prepared using each remote sensing method and by ground-truth surveys. Mapping accuracy of each method will be evaluated using the methods of experiment 1.B. Second, we will evaluate whether seasonal changes in shrimp burrowing activity affects the accuracy of the maps. Shrimp beds will be sampled and mapped by remote sensing and ground survey in summer (when burrowing activity is maximum) and in winter (when burrowing is minimum, and *Neotrypea* burrow openings can be filled with sediment), and map accuracy will be determined as above. And third, shrimp populations inside and adjacent to eelgrass beds will be included to evaluate whether SAV affects the accuracy of the maps produced by remote sensing. The results of this study will be used to select a remote sensing method to use in mapping the large-scale spatial distribution of burrowing shrimp.

II. Population Dynamics of Eelgrass and Burrowing Shrimp Along Stressor Gradients: Experiment 2.A. Population Characteristics of Eelgrass and Burrowing Shrimp Along the Dominant Salinity-Temperature Gradient in Yaquina Estuary, Oregon: This study will provide a baseline of eelgrass and burrowing shrimp population dynamics, and will evaluate population-level response variables that may be used for subsequent work.

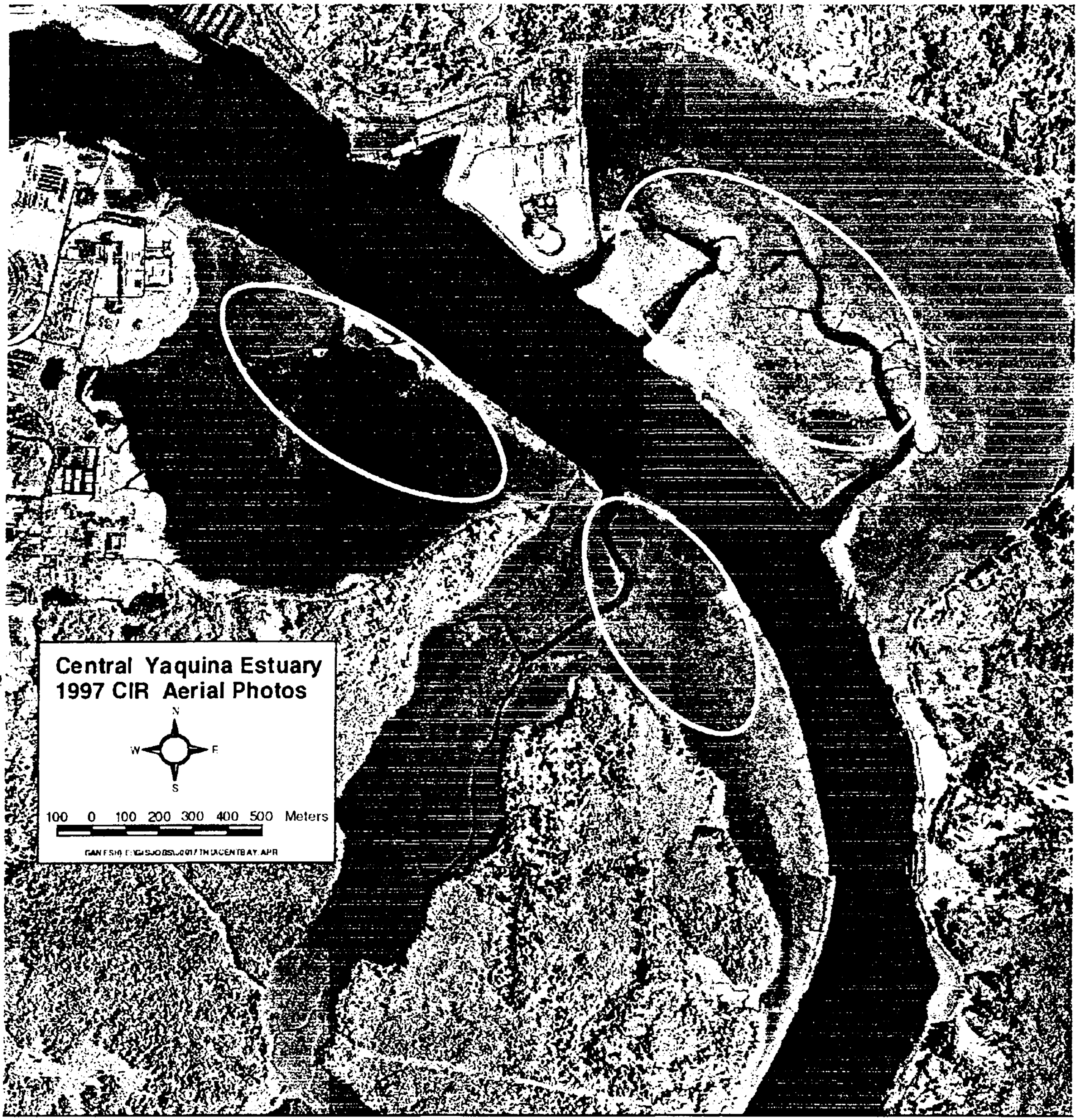


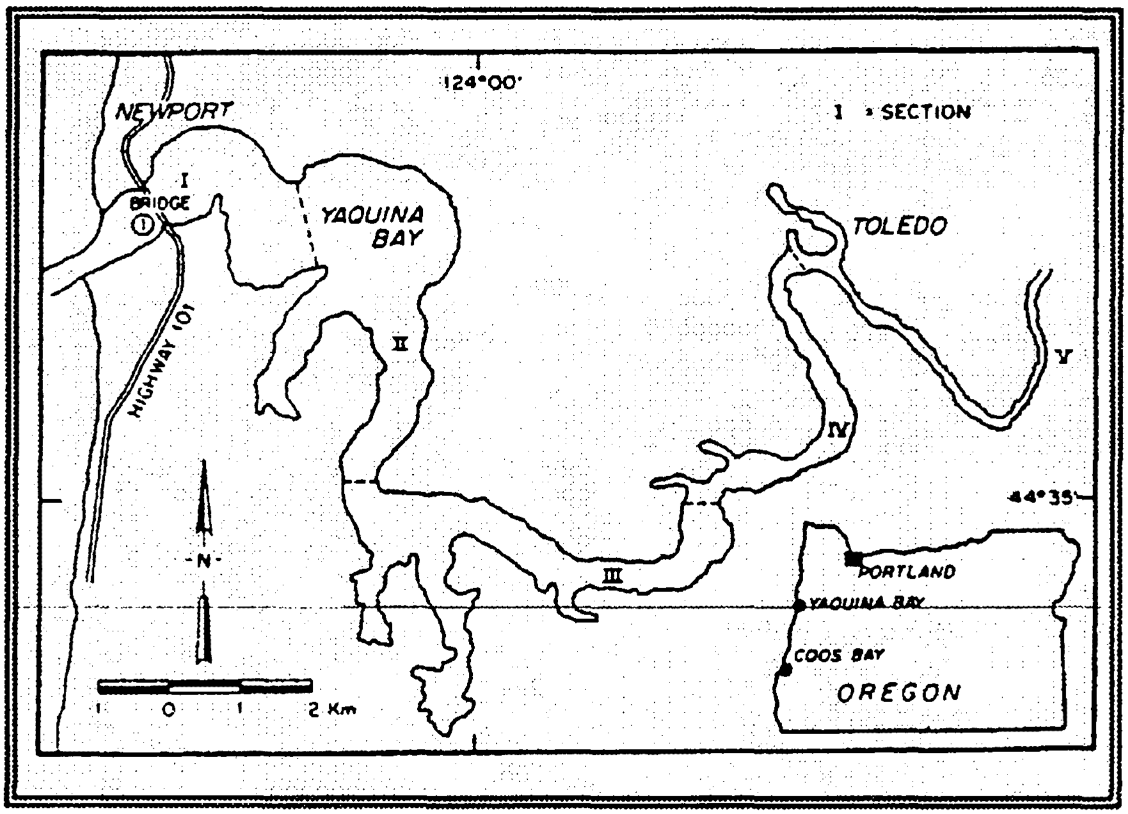
Figure 1. Aerial color infrared photograph-mosaic of central Yaquina estuary showing three areas where remote-sensing methods-comparisons studies may be conducted.

The ability to assess the ecological risks posed by multiple stressors to eelgrass and burrowing shrimp depends on defining the current condition of the resource to serve as a benchmark from which to judge their responses to natural or anthropogenic stressors. Defining these benchmarks is important for field experiments proposed later in this proposal, as well as for research proposed by other CEB scientists (i.e., Boese, Ozretich, Young, and Robbins). Because salinity and temperature are important natural "stressors" affecting the distribution of organisms within estuaries, we will characterize the population dynamics of *Zostera, Nerotrypaea,* and *Upogebia* along the of temperature-salinity gradient of Yaquina Bay (as defined by DeBen et al. 1990; Figure 2). Specifically, the population studies on these three species will: 1) document the demographic characteristics for each species along a natural stressor-gradient; 2) investigate the utility of various measures of population characteristics as indicators of population-level response to natural stressors; 3) evaluate the frequency at which various demographic characteristics need to be sampled; and 4) evaluate methods that could be used for rapid assessments of condition for *Zostera*.

These objectives will be met by using "intensive" and "extensive" sampling approaches. The "intensive" sampling effort will be done at large patches of *Zostera, Neotrypea*, and *Upogebia* co-occurring in Zones I, II, and III as classified by DeBen et al. (1990) (Figure 2). No intensive sampling site will be located in section IV as these species are uncommon in this part of the estuary. The rationale for sampling large patches is to characterize the population dynamics of each species where it grows best as indicated by extent of the patch and to sample an area large enough that sampling and the associated trampling are unlikely to destroy the patch. The "extensive" sampling effort will employ collecting random samples of *Zostera*, *Neotrypea*, and *Upogebia* throughout the entire estuary taken in conjunction with the sampling being done by Ferraro and Cole (CEB). The intensive samples will be used to develop the detailed characterization of the population dynamics of each species, while the extensive sample will provide insight into whether population information from the intensive sample sites can be extrapolated throughout the estuary. Both types of samples will provide information on the relative utility of various demographic variables and methods used to collect population data. Technical details for this study can be found in Appendix A.

The DeBen et al. (1990; Fig. 2) salinity-temperature stratification represents annual average environmental conditions, which undoubtably vary seasonally. Ideally, the stressor gradient will be sufficiently steep that eelgrass and shrimp at each study site will experience distinctly different salinity and temperature regimes. However, preliminary data is not available to make this determination, and physical/chemical environmental measurements made during each sampling will be used to characterize the seasonal variation of environmental conditions at each site. If the environmental conditions are not distinctly different among sites, the study may be changed after the first year to include other eelgrass or shrimp beds.

Fig 2. Stratification of the Yaquina estuary by salinity and temperature regime, using the [redacted] studies will be conducted in Sections (zones) I, II, and III.



Experiment 2.B. Development and Testing of Biophysical Population Models: This study will develop and test "biophysical population models" that predict whether *Zostera marina*, *Neotrypea*, or *Upogebia* should be present or absent at a site based on physical and chemical properties of the site. These models only classify sites as to their potential as suitable habitat for the target organism, and cannot unequivocally identify the factors that cause an organism to be absent. These models are also useful for ranking the importance abiotic stressors that affect the distributions of eelgrass and burrowing shrimp populations within estuaries, and are useful for guiding habitat restoration efforts (Thom 1990). The biophysical population models will consist of the range of conditions in which populations of each species have been found in Pacific Northwest estuaries, as reported in previous studies. A preliminary biophysical model for *Z. marina* is presented in Table 1, which was derived by Thom (1990) as a list of the habitat characteristics for eelgrass in Pacific Northwest estuaries. The model for *Z. marina* will be updated by review of the literature since Thom (1990), and similar models will be prepared similarly for *Neotrypea* and *Upogebia* based on review of the literature, much of which has been compiled already (see DeWitt et al. 1997; Feldman et al. in prep.).

Table 1. Preliminary list of components for a biophysical model for *Zostera marina* populations in the Pacific Northwest. Table from Thom (1990).

Parameter	Condition	Notes
Depth	0.0 to -6.6 m MLLW	optimal
Light	20-30% of surface irradiance	max. biomass recorded
	70-175 FE m^{-2} s^{-1}	saturates photosynthesis; temp. affects saturation point
	0.95 X mean annual Secchi depth (m)	max. depth limit
Nutrients	ample inorganic nitrogen and phosphate	growth can be nutrient limited; sources are sediment and water column; excess nutrients can reduce growth due to high epiphyte biomass
Salinity	10-30‰	optimal
Sediment	mixed sand and mud	optimal
Slope	flat to very slight incline	optimal

Temperature	10-20^{0}C	optimal

To test the biophysical population models, we will sample for the presence of each species (i.e., *Z. marina*, *Neotrypea*, and *Upogebia*) and benthic and water-column environmental parameters (see Table A.4, Appendix A) at approximately 100 sites within Yaquina estuary. Geographic positioning system (GPS) coordinates for sampling sites will be designated using a tesselated stratified-random design (Stevens 1997), with the estuary stratified by depth and salinity-temperature zones (Figure 2). Water-column parameters will be measured three or four times per year (spring, summer, fall, and possibly winter), whereas the ground-based habitat abiotic and biotic variables will be measured once during the active growing and burrowing seasons for eelgrass and shrimp (i.e., between April and October). Water column parameters may be collected over the span of two years to obtain a better measure of their temporal variation. Site-selection, water-column sampling, and benthic sampling will be coordinated with Bob Ozretich and David Young (CEB) whose research call for similar site dispersion and data.

One unresolved issue is how best to represent the temporal variability in water column parameters at each site. Clearly, water column parameters will be more variable temporally than sediment-associated variables. Single samples at high tide collected once per season will not be sufficient to characterize this variability. One approach is to report the maximum and minimum extremes for each parameter. We will also investigate linking measurements made at the sites to 1) water quality data collected continuously by moored CTD/PAR units (see proposal by David Specht, CEB) using regression techniques, and 2) to a circulation model for the Yaquina estuary, such as the 2-dimensional model used by the NOAA/PMEL Tsumami Project (Kamphaus 1998). In any case, we will consult with other estuarine scientists to identify other ways to integrate these temporally-variable data. These approaches could may eventually allow CEB to have the capability to accurately model water column parameters at sites within the estuary.

The goodness of fit of the predicted and measured presence and absence data for each species will be tested using the G-test (Sokal and Rohlf 1981). The null hypothesis to be tested is that the measured data are not different from the predicted data (i.e., that the model correctly predicts the presence and absence of each species). Additional technical details of this experiment may be found in Appendix A.

Experiment 2.C. Effects of Multiple Abiotic Stressors on the Population Biology of Keystone Species: Laboratory (mesocosm) and field experiments will be conducted to measure the effects of single and multiple abiotic stressors on population characteristics (i.e., survival, growth, and fecundity) of eelgrass and burrowing shrimp under controlled conditions. The purpose of these experiments will be to 1) determine whether the interacting effects of the stressors is greater than the sum of the effects of each independent stressor, and 2) to determine which stressor(s) has the greatest impact on eelgrass or shrimp survival or growth. Mesocosms are simplistic mimics of the natural environment that offer an opportunity to conduct stressor-response experiments under controlled conditions. Field experiments face the risk that unanticipated, uncontrolled, local events may confound the experimental treatments, such as vandalism, accidental human disturbance, erosion, sediment deposition, or rapid changes in water quality. Mesocosm experiments will be conducted first, followed by field experiments to verify the results of the laboratory studies.

Mesocosm Experiments: CEB plans to construct mesocosms that are appropriate for conducting these experiments during FY1998 (Appendix A). Experiments to measure the presence and outcome of competition (expt. 3.A. and 3.B.) will be conducted in the mesocosms before the multiple stressor-response experiments are conducted. The schedule for starting the multiple-stressor experiments is dependent on the availability of the mesocosms and the results of the competition experiments, and could thus start either in spring/summer 1999 (if competition is not measurable) or sometime in 2000. Three experiments that are anticipated are:

1) *Zostera* (1 density) x salinity (3 conc.) x light (2 intensities) x temperature (3 levels) x 4 replicates = 72 compartments;
2) *Neotrypea* (1 density) x salinity (3 conc.) x temperature (3 levels) x 4 replicates = 72 compartments; and
3) *Upogebia* (1 density) x salinity (3 conc.) x temperature (3 levels) x 4 replicates = 72 compartments.

Aside from specifying the species and stressors, the methods used for all stressor-response mesocosm experiment will be identical. Population response variables will be similar to those used in experiment 2.A. Technical details of the experiments are described in Appendix A.

<u>Field Experiments</u>: Field studies to measure the responses of eelgrass and burrowing shrimp to multiple stressors will verify results of the mesocosm experiments. To the greatest extent possible, fully-factorial designs will be used in the field experiments, although that might be difficult to achieve for stressors that co-vary spatially or temporally (i.e., depth and mean temperature, salinity and temperature, light and temperature). Natural gradients in abiotic stressors will be used when possible, particularly for water-column variables (i.e., salinity, nutrients, turbidity, temperature, dissolved oxygen). Some variables are amenable to experimental manipulation, such as light (by shading), substrate-type, sedimentation rate, sediment nutrient concentration, and depth. Selection of stressor treatments will be guided by the mesocosm multiple-stressor experiments, the biophysical models (expt. 2.B.), and the

logistics of finding or creating spatially and temporally reliable gradients of the stressor. The population-level response variables will be similar or identical to those used in previous mesocosm and field experiments. Technical details of the experiments are described in Appendix A.

<u>Container or Cage Artifacts</u>: The cylinders in the mesocosm experiments (and below-ground cages in field experiments) may affect the growth of eelgrass or burrowing behavior of the shrimp. It is suspected that the latter will be more of an issue than the former because eelgrass has been successfully grown in very small containers (i.e., 1-gallon pots) in laboratory and field experiments (pers. obs. of work by Ron Thom). Controlling for container effects with burrowing shrimp may be very difficult, especially in the mesocosm studies. However, as the same chambers (cylinders or cages) will be used for all treatments within the mesocosm and field experiments, any artifacts will be equally distributed among replicates and treatments. Additionally, I will compare the behavior and physiological/ecological properties of shrimp in experimental chambers (mesocosm or field experiments) with those of shrimp in field populations to determine the magnitude of laboratory artefacts. Variables measured could include the surface area: volume ratio and the number of angular turns of burrows (determined from resin casts at the end of experiments), sediment deposition rate, oxygen consumption rate, burrow irrigation rate, or elemental flux rates. If experimental artefacts are minimal, I would expect the characteristics of shrimp measured in the experiments to fall within the range of characteristics measured in the field populations, as determined by non-parametric statistical comparisons of median values. (This also assumes that important environmental variables, such as temperature, salinity, and sediment grain size are comparable in the experimental and field populations). It would be impractical to change the size or physical characteristics of the cylinders used in the mesocosm and field experiments, as suggested by the reviewers, without sacrificing other important experimental design considerations, particularly the number of replicates. Thus, I will first measure the magnitude of artefacts, and then adjust the design of the chambers or experiments only if artefact effects are substantial.

Experiment 2.D. Mapping Changes in Eelgrass and Burrowing Shrimp Populations Along Stressor Gradients: This study will measure whether the spatial distributions of eelgrass or burrowing shrimp change along abiotic stressor gradients in the manner predicted from laboratory and field experiments, and to determine whether changes in the spatial distribution of a species are characteristic of particular classes of stressor. One possible product from this work could be a landscape-scale indicator of stress to estuarine benthic habitats (i.e., eelgrass or burrowing shrimp beds). The design of this project is incomplete and will be developed with other CEB collaborators (Drs. Brad Robbins (landscape ecology), Denis White (biogeographer), and David Young (aerial photography)). Very broadly, our goals are 1) to develop population change maps SAVs and burrowing shrimp for portions of Yaquina estuary, 2) to develop similar maps for other estuaries in order to measure differences in rates of population change across

the region, and 3) to develop population change maps along stressor gradients to test hypotheses concerning detection and measurement of changes in species' spatial distribution in relation to abiotic stress. Population change maps (= difference maps, isopachs) will be constructed using remote sensing methods and the techniques for comparing maps described in experiments 1.B. and 1.D.

Several issues will need to be addressed prior to testing hypotheses about population spatial changes in relation to stress. First, we need to know what spatial scale to map for each species. The mapping accuracy study (expt. 1.B.) will examine how accuracy changes with spatial scale for each of three remote-sensing methods. Results from that study will be balanced against the resolution of available data (to be acquired from other researchers or public agencies) and the cost of obtaining high-resolution spatial data. Second, we need to know the temporal scale at which to map population change. We anticipate that annual or biennial maps may be adequate for these purposes, but also recognize that eelgrass and burrowing shrimp distributions can change dramatically seasonally. One approach to resolving this question would be to generate population change maps for select sites on a seasonal basis for 2+ yr, measure the rate of population change over different temporal scales, and select the scale at which the rate of change reaches an asymptote. Third, we need to know whether natural rates of population change vary geographically across the PNW. It seems likely that latitudinal gradients in day length, temperature, rainfall, or storm intensity could affect natural rates of population expansion and contraction. Underlying natural environmental gradients will have to be identified and their influence measured before we can confidently use population change maps to detect and measure the effects of anthropogenic stress.

A plan for this project will be developed over the next year. In the meantime, we can start to measure rates of annual change in spatial distribution of eelgrass using aerial photographic data for Yaquina estuary obtained by David Young (CEB) in 1997 and 1998. We will also seek data from previous aerial photographs of Yaquina estuary (an inventory of which is being prepared by David Specht, CEB), and time-sequenced remote-sensing data from other estuaries. We anticipate forming collaborations with researchers within the region who have similar interests in mapping the large-scale distributions of these species in other estuaries. These include, potentially, Roxanna Hinzman (Tillamook Bay National Estuary Project), Steve Rumrill (South Slough National Estuarine Research Reserve), Si Simenstad (Univ. Washington), Doug Bulthuis (Padilla Bay National Estuarine Research Reserve), Tom Mumford (Washington Dept. of Natural Resources), and Ron Thom (Battelle Marine Sciences Laboratory). Additional technical issues are discussed in Appendix A.

III. Effects of Multiple Abiotic Stressors on Competition Among Eelgrass and Burrowing Shrimp - Laboratory and field experiments will be conducted to measure the presence of interspecific competition for space among *Zostera*, *Neotrpea*, and *Upogebia* (expt.

3.A.). The goals and design of subsequent laboratory and field experiments will depend on the presence of competition among these species. If competition is important, the next laboratory and field experiments will be directed to measuring whether water quality affects the outcome of the competitive interaction (expt. 3.B.). If competition is not important, laboratory and field experiments will focus on measuring the effects of multiple stressors on each species individually (i.e., experiment 2.C.).

Experiment 3.A. Measuring Interspecific Competition Among Eelgrass and Burrowing Shrimp: Laboratory and field experiments will be conducted to test the null hypothesis that interspecific competition does not occur between *Zostera* and *Neotrypea*, between *Neotrypea* and *Upogebia*, and between *Zostera* and *Upogebia*. Mesocosm experiment to test this hypothesis will be conducted prior to conducting field experiments. Definitive laboratory experiments will be conducted during fall 1998 - summer 1999; while the mesocosms are being constructed, preliminary laboratory and field experiments may be conducted during summer 1998.

Mesocosm Experiment: The methods used for these experiments will be identical to those described for the mesocosm stress-response experiments (expt. 2.C.), with the experimental treatments consisting of pairs of species at different population densities. The first experiment will measure competition between *Zostera* and *Neotrypaea* because this interaction is expected to have the greatest impact on the distribution and abundance of benthic habitats (and associated species) in PNW estuaries. The experimental design will be: *Zostera* (4 densities: 0, 100, 200, 400 shoots m^{-2}) x *Neotrypaea* (4 densities: 0, 100, 200, 400 shrimp m^{-2}) x 4 replicates. The second and third experiments will measure competition between *Zostera* and *Upogebia*, and between *Neotrypaea* and *Upogebia*. The densities will be comparable to those used in the *Zostera-Neotrypaea* competition experiment.

The competition experiments will be very similar to those described for the multiple stressor experiment, employing the same methods as described for experiment 2.C. to harvest, acclimate, and plant test organisms, and to sample population variables (Appendix A, Table A.4). The duration of the competition experiments will be 8 to 16 weeks, depending on whether effects are measurable by non-destructive sampling. Results will be analyzed using analysis of variance to test the null hypothesis that changes in the density of one species did not affect the demographic variables of the second species. If competition between *Zostera* and either species of shrimp is significant, we will probably move ahead to conducting experiments on the effects of water quality on the outcome of competition, rather than immediately conduct the experiments to measure competition between the shrimp species. We will return to conduct that experiment after completing experiments on the effects of abiotic stressors on the competition between eelgrass and shrimp. Additional experiments may be conducted to examine three-way interactions among these species after the two-way interspecific interactions have been explored.

Field Experiment: Reciprocal-transplant field experiments will be conducted to verify that interspecific competition can be measured in the field. *Zostera* will be transplanted at different densities into *Neotrypaea* or *Upogebia* beds, *Neotrypaea* within *Zostera* or *Upogebia* patches, and *Upogebia* within *Zostera* or *Neotrypaea* patches. All possible pairings of these three species will be tested simultaneously. For each transplanting, pairs of competitors will be identified as "invader" (the species that is placed into the patch) and "resident" (the species occupying the patch prior to the experiment). Eelgrass or shrimp "invaders" will be harvested from nearby populations, sorted to uniform size and condition, and "planted" inside caged plots (40-cm diameter) within large patches of the "resident" species. The plots will be created by excavating a 40-cm diameter x 60-cm depth core of sediment, lining the hole with 3-mm mesh plastic screen "underground fence" to a depth of 60 cm, and refilling the hole with the excavated sediment which will be sieved to <3-mm to remove burrowing shrimp, eelgrass, and other megafauna. Initially, the plots will covered with 3-mm mesh cages to prevent immigration or emigration of conspecifics, predators, or competitors. After an acclimation period of a few days, all cages will be removed, and underground fences will be removed from some plots but retained in others.

Three treatment-types will be deployed for each species pair: control, fenced invader, and unfenced invader (Figure 4). For controls, shrimp or eelgrass will be back-transplanted into beds of their own species, which will measure the effects of transplant-shock on the invader. Fenced out-plant plots will measure both transplant-shock effects and the suitability of the "resident" habitat to support the survival and growth of the invader. The un-fenced invader treatments will measure the combined effects of the presence of the "resident" species (i.e., the competition), habitat suitability, and transplant shock. The replication rate will be based on results of a power analysis of key response variables (i.e., %cover and net primary productivity for *Zostera,* abundance for the shrimp) using variance estimates from field populations (i.e., Kentula and McIntire 1986, Dumbauld et al. 1996). The duration of the experiments could be extended for as long as 1-yr if there is no apparent effect of competition; in this case, a bi-monthly sampling frequency will be used. The long duration may be required for competition to manifest itself, although we expect the effects to be revealed within weeks, particularly between *Neotrypaea* and *Zostera* because of the high rate of sediment turnover caused by ghost shrimp.

Population-level responses of resident species measured within experimental plots will be similar or identical to those used in previous mesocosm and field experiments (Appendix A, Table A.4). Non-destructive samples will be measured approximately every 4 weeks (depending on site accessibility due to tides and weather). The field experiment will be conducted for 6 to 20 weeks, depending on the rate at which responses were observed in mesocosm experiments and on whether responses are observed and are measurable in the field. Analysis of variance will be used to test the null hypothesis that the effect of competition on population characteristics is the same as the effects of transplant shock and habitat suitability (i.e., that population characteristics of the invader in the un-fenced treatment are not significantly different from those of the invader in the fenced or control treatments).

Experiment 3.B. Effects of Abiotic Stressors on the Outcome of Competition Between Keystone Species: Laboratory experiments, and subsequent field experiments, will measure the effects of water quality or benthic habitat variables on the outcome of competition between eelgrass and burrowing shrimp. The laboratory experiments will be very similar to those described above (expt. 3.A.), except that fewer population-density treatments will be used, and one or more abiotic stressor levels will be used. The first abiotic stressor to be studied is light, based on the suggestion by Harrison (1987) that improvement of water clarity led to a increase in eelgrass, to the detriment of burrowing shrimp.

Mesocosm Experiments: The experimental design will be something along the lines of: *Zostera* (3 densities) x shrimp (3 densities) x light (2 intensities) x 4 replicates. The species to be used will be determined from experiments in 3.A. The methods to conduct this experiment and to measure responses of the eelgrass and shrimp will be the same those described for experiment 3.A. Subsequent mesocosm experiments may be conducted to examine the effect of salinity, temperature, nutrients, or substrate-type on competition between eelgrass and the shrimp (or between the two species of burrowing shrimp). Results of the biophysical population-model study (expt. 2.B.) will be used to select the stressors. Later experiments may examine the effects of two interacting stressors on competition.

Field Experiments: If the above mesocosm experiment demonstrates that abiotic stressors can affect the outcome of competition, field experiments will be conducted to verify this finding in the presence of "strong" and "weak" abiotic stressors. It is impractical to plan these experiments in detail at this time, but they are expected to employ either the reciprocal-transplant methods described above (expt. 3.A.) using "resident" populations distributed along natural stressor gradients, or to outplant pairs of species at different population densities into plots distributed along natural or artificial stressor gradients. Varying abiotic stressor levels in the field can be achieved by either conducting the experiment along existing stressor gradients (i.e., salinity-temperature, depth, nutrient, etc.) or by manipulating habitat characteristics (i.e., using shade cloth to reduce light levels; Bulthuis 1997). The duration of these experiments, the measurement variables, and statistical analytical methods will likely be the same as those used in field experiments described previously (expt. 2.C. and 3.A.).

Expected Results and Benefits: The research proposed here will contribute to the understanding of the population ecology of keystone species in Pacific temperate estuaries, and their responses to abiotic stressors, which is required to assess risks of multiple stressors to estuarine ecosystems and to manage the living resources therein.

One strength of the proposed work is that it integrates ecological processes occurring at several spatial and temporal scales, from responses measured after several weeks in laboratory and field experiments, to annual (or longer) changes in landscape-scale patterns of populations among coastal estuaries of Pacific Northwest. The proposed research project uses several different approaches to study these problems (including laboratory and field experiments, estuarine-scale population surveys, and remote sensing) and integrates the resulting data into GIS layers that will be incorporated into the Yaquina estuary GIS database maintained by the US EPA WED/Coast Ecology Branch. The proposed experiments are highly integrated, sharing methods and information, conserving resources by coordinating efforts, and organized to be conducted in a hierarchical fashion (i.e., baseline studies conducted first, which build into more complex studies). Results from these studies will be complied into manuscripts prepared for submission to peer-reviewed scientific and environmental management journals.

From a basic-science perspective, the proposed research will provide important and unique information about the population and community ecology of estuarine keystone species. In particular, this research will evaluate the importance of competition among keystone species which is believed to be a fundamental, but overlooked, mechanism that determines the distribution and abundance of valuable eelgrass habitat. From an environmental-management perspective, the proposed research will provide critical information on the effects of abiotic stressors on the dynamics of habitat dominated by eelgrass or burrowing shrimp. These are arguably the most ecologically and economically important benthic species within Pacific estuaries because of their habitat-generating and habitat-modifying activities. Data from this research may be useful in the establishment of water quality guidelines or other management tools, for siting ecological restoration projects, or for habitat enhancement programs. New information on the ecology of burrowing shrimp may lead to improved methods to control their population growth within commercial oyster beds, and thereby lead to reduced need for applying Sevin pesticide in Willapa Bay and Grays Harbor, WA. Additionally, the contemporary and historical data collected for Yaquina and other Pacific estuaries, stored in GIS-database format, will be useful to community-based environmental management of Pacific coast watersheds and estuaries.

Project A4 - The Impact of Disturbances on an Eelgrass Habitat

Principal Investigator: Bruce L. Boese

Goals: Project goals will be to determine the benthic effects of disturbance (i.e., recreational clam harvesting and algal smothering) on eelgrass (*Zostera marina*) habitat. The hypothesis is that disturbance of an eelgrass patch reduces shoot density resulting in a reduction in eelgrass primary productivity and the diversity and abundance of associated mega and macrobenthos. Results of the clam harvesting experiments will be placed in a larger spatial context by comparison to the results of a field survey conducted in an eelgrass meadow which is subjected to heavy recreational clamming activity.

Rationale: Eelgrass is an important habitat in Pacific Northwest (PNW) estuaries. *Zostera marina* meadows serve as a nursery ground for juveniles of commercially important species such as herring and as a refuge for juvenile salmonids (Griffin, 1997; Simenstad and Wissmar, 1985; Levings, 1990; den Hertog , 1977). Eelgrass meadows are significant sites of primary production and eelgrass shoots can be utilized directly for food by some water fowl such as the western black brant (Griffin, 1997; Kentula, 1982) and indirectly by many species via consumption of detritus (Thayer *et al.*, 1975). Eelgrass roots stabilize the sediment (Thayer *et al.* 1975) and the presence of eelgrass dampens wave energy which may serve to reduce erosion and to enhance larval settlement (Orth, 1992). Because of these characteristics species abundances in eelgrass patches are usually greater than in other estuarine habitats (Everett *et al.*, 1995).

Mechanical disturbances associated with commercial shell fish and bait harvesting operations have been shown to reduce mudflat biodiversity (Brown and Wilson, 1997) and to adversely affect SAV growth (Fonseca *et al.*, 1984; Peterson *et al.*, 1987; Everett *et al.*, 1995). Although the obvious factor effecting the upper limit of SAV growth is tidal exposure (Thayer, *et al.*, 1975), bioturbation by a burrowing sand shrimp (*Neotrypaea*) may have an effect on the upper growth limit of *Z. marina* and may prevent a high intertidal eelgrass species (*Z. japonica*) from inhabiting the full extent of its intertidal range (Dumbauld *et al.*, 1997). The establishment of shrimp beds has been shown to be capable of excluding seagrasses (Suchanek, 1983), conversely, dense eelgrass roots have also been shown to inhibit the burrowing ability of shrimp (Benchley, 1982). In addition, natural eelgrass colonization of sandflats and eelgrass transplant experiments have been shown to reduce *Neotrypaea* abundance (Harrison, 1987).

A relatively unexplored eelgrass disturbance factor is the potential for macroalga (*Ulva* and *Enteromorpha*) to overgrow and smother relatively large eelgrass patches. Macroalgal blooms which are often associated with increased nutrients may be a contributing factor to the apparent world wide decline in seagrasses (see review by Raffaelli *et al.*, in press). For example, den Hartog (1994) documented a sudden and complete disappearance of *Zostera* species from a British estuary and concluded that increased nutrient loads from a new sewage treatment plant and a warm/dry fall resulted in a massive increase in *Enteromorpha* which appeared to smother and kill eelgrass patches. While observations such as this suggest a direct linkage between macroalgal increases and eelgrass declines, increased nutrient loads may have a direct adverse effect on eelgrass by stimulating epiphyte growth on eelgrass leaves which serves to reduce available light for photosynthesis and by reducing water clarity via increased phytoplankton densities (Dennison *et al.*, 1993). Thus it may be difficult to separate the direct effects macroalgal growth on eelgrass from nutrient effects (Raffaelli *et al.*, in press). In Oregon estuaries, floating *Ulva* mats have been observed to similarly cover and kill *Z. marina* patches (Kentula, 1982), especially during late summer low tides (Kentula, pers. comm.), however, the mechanism by which this occurs, its spatial impact and recovery time are unknown.

As eelgrass has been shown to affect current velocity and turbulence (Gambi *et al.*, 1990), reductions in eelgrass densities by mechanical disturbances or by smothering with *Ulva* mats or storm deposited sediments (Onuf, 1987) may serve to alter the wave or current energy at the affected site (Peterson, *et al.*, 1987). Colonization of these sites by *Neotrypaea* and the potential for increased wave energy at these denuded sites may perpetuate the disturbance, making recolonization by eelgrass difficult. This sort of interaction has led to speculation that sandflats and seagrass habitats may be alternative stable states (Peterson *et al.*, 1987).

The habitat relationships between sandflats dominated by *Neotrypaea* and eelgrass is of particular interest in the PNW. The oyster culturing methods used in Yaquina Bay (stake and rack culture) have been shown to be destructive to eelgrass (Everett *et al.*, 1995) , however, a definitive study of oyster ground culture (method used in Willapa bay) on eelgrass patches, has not been done (John Johnson, ODFW, pers. comm.). One possibility is that persistent disturbance from activities associated with oyster culture creates a disturbed environment in which burrowing shrimp can thrive at the expense of eelgrass (Simenstad and Fresh, 1995) indirectly affecting the utility of the habitat for continued oyster culture. Regardless of the cause, *Neotrypaea* and to a lesser extent burrowing mud shrimp (*Upogebia*) are considered pest species by oyster growers and are periodically sprayed in Washington State with carbaryl as a control measure. In Oregon, carbaryl spraying for the same purposes is a recurring proposal but is currently illegal.

Recreational clamming is a significant activity in PNW estuaries which may have an impact on eelgrass. Locally, gaper (*Tresus capax*) and butter clams (*Saxidomus giganteus)* are harvested by digging in lower intertidal *Z. marina* meadows while cockles (*Clinocardium nuttalli*) are often raked from the sediment surface of higher intertidal areas. Clamming activity is not limited to intertidal areas of eelgrass meadows as some clam diggers wade at low tide into two or three feet of water to dig subtidal clams. Thus, this mechanical disturbance could affect eelgrass to -6 ft MLLW.

An additional potentially disruptive activity involves the taking of bait shrimp. This is done both recreationally using a hand operated slurp gun and commercially in which a high pressure stream of water is directed into the sediment. Commercial shrimp operations are highly destructive as the sediment is liquefied, collapsing burrows with the surviving shrimp harvested as they are force to the surface by the injected water. Although most of the local commercial activity has been directed at *Neotrypaea* populations in Alsea Bay, some *Upogebia* are harvested from eelgrass meadows in Yaquina and Netarts Bays.

It is apparent from the above discussion that physical disturbance of eelgrass meadows has the potential to cause deleterious effects on an estuarine scale. As human coastal populations and associated tourism increases, the potential for physical disturbance from recreational and commercial activities are also likely to increase. The proposed research describes field experiments which will address the potential for some of these physical disturbance to affect eelgrass patches and their associated biota.

Objectives: 1. To examine the short and long-term effects of mechanical disturbance (recreational clamming) and algal smothering on eelgrass patches and associated biota.

2. To compare recreational clamming experimental results to the results of a field survey conducted at a site which has been subjected to prolonged and intense recreational clamming.

Experimental Methods:

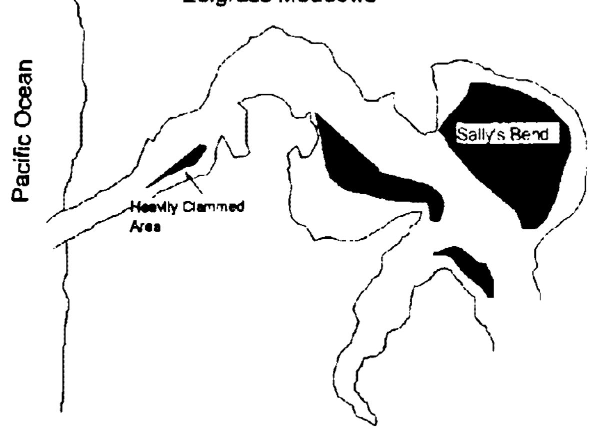
Recreational Clamming Simulation Experiments: The effects of two recreational clamming methods (raking and digging) on *Z. marina* and associated biota will be examined beginning in May 1998. Clamming treatments will be applied to experimental plots multiple times to simulate a high level of recreational clamming activity. The digging treatment will be applied to *Z. marina* experimental plots where *T. capax* and *S. giganteus* are likely to be present and the raking treatment will be applied to experimental plots at which *C. nuttalli* are likely to be present. As *T. capax* and *S. giganteus* are found in low intertidal eelgrass meadows, the two treatment types will be applied in vertically separate areas which are also likely to have differing eelgrass densities.

The locations of the two study sites will be selected using the existing 1997 aerial survey of Yaquina Bay and its associated ground truth survey. Preliminary surveys will also be conducted in March and April 1998 to locate clam beds within these eelgrass habitats. The most ideal location for both of these experiments is the Sally's Bend mud flat (Figure 1) which contains the most extensive eelgrass meadows in Yaquina Bay. Sally's Bend is relatively flat, thus it should be possible to locate all plots within a narrow tidal range. Public access to this eelgrass meadow is limited as the central areas of the meadow are accessible at low tide only via hovercraft.

Once the locations of the two study areas are known, transects or plot grids will be established in each. Along each transect or within each of the plot grids a series of 1.5 m X 1.5 m contiguous potential sampling plots will be numbered and randomly designated as control or treatment plots. Each of these plots will visited in order and visually accessed using the following acceptance criteria: 1) eelgrass is present, 2) the plot is undisturbed (e.g., no obvious signs of clam harvesting), and 3) the plot is at least 1.5 m away from previously selected control or treatment plots. If the plot meets these criteria it will be marked with a numbered wooden stake located at a specified distance and direction from the center of the plot and the GPS coordinates noted. Locating marking stakes away from plots will help ensure that drift alga entrapped by the stake will not effect the plots.

Clam Raking Experiment: The clam raking experiment will begin in May 1998 and consist of 50 treatment and 50 undisturbed control plots which are randomly assigned along a transect or within a plot grid. All 50 treatment plots will be raked using a four-tined hoe (each tine ≈ 20 cm in length) to remove cockles. This treatment will be repeated on these same 50 plots two additional times (June and July 1998).

Figure 1: Major Yaquina Bay Eelgrass Meadows

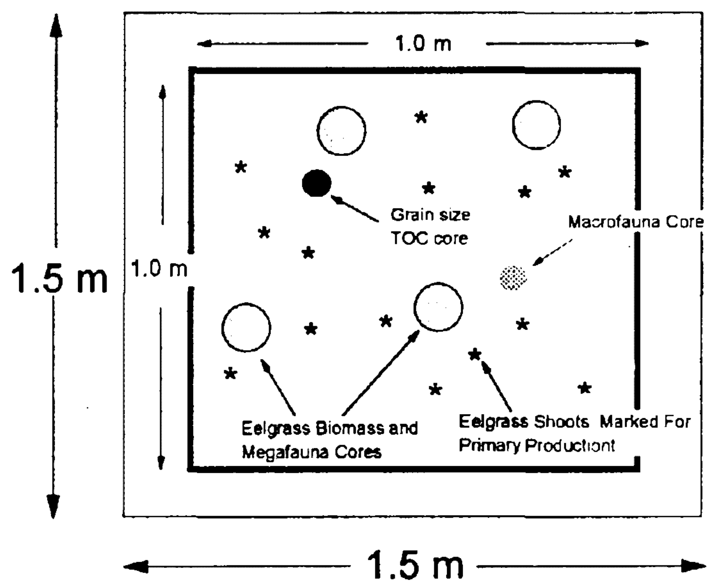


Concurrent with the July raking treatment, ten control and ten treatment plots will be selected for destructive sampling in August 1998. Fifteen eelgrass shoots will be marked in each of these plots for net primary production measurements. Shoot marking techniques are described in Kentula (1982), Kentula and McIntire (1986), and Zieman (1974). The first five of these shoots which appear undamaged will be collected when these plots are destructively sampled at the next sampling interval (≈1 month) and analyzed (leaf width, length, growth) using the procedures described in Zieman(1974) and Kentula and McIntire (1986).

Destructive sampling of randomly selected plots will begin in August 1998 (Table 1).

This sampling will accomplished by placing a 1.0 m^2 quadrat in the center of each 2.25 m^2 plot (Figure 2). Single cores for macrofauna (8 cm dia., 5 cm deep) and grain size/TOC (3.4 cm dia., 5 cm deep) will be taken at random sites within the quadrat. Also at random locations within each of these quadrats *Z. marina* will be harvested from four 15 cm diameter areas (Figure 2) and transported to the laboratory for biomass measurements. Megafauna will then be sampled from these harvested areas using four cores (15 cm dia., 60 cm deep)[1]. These cores will be combined and sieved (3.0 mm) in the field. All samples will be numbered as to plot and transported on ice to the laboratory. Macrofaunal cores will be sieved (0.5 mm) in the laboratory. Material retained on sieves from mega and marcofaunal cores will be preserved (10% formalin) then transferred into 70% ethanol for later sorting, taxonomic identification (species level), and biomass determinations. This sampling pattern will continue until recovery (no significant difference between treatments/controls) or for up to two years post-treatment (Table 1).

Figure 2: Plot Design
Destructive Sampling



[1]An alternative method is currently under development at our laboratory. This method uses a flushing-coring device similar to one used to collect bivalves to a depth of 70 cm (Grussendorf, 1981). This should allow us to sample a larger area to depth with less effort than that required when using large diameter manual corers.

Table 1: Sampling Strategy for the Clam Raking Experiment

Date	Plots destruc-tively sampled	Macrofauna Cores taken	Megafauna Cores taken	# Plots harveste d	Grain Size TOC cores	1° Production Measurements	# Plots Raked
May 1998							50
June 1998							50
July 1998						10 plots marked 10 plots marked	50
Aug. 1998	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10	10 measured 10 measured	
May 1999	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10		
July 1999						10 plots marked 10 plots marked	
Aug. 1999	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10	10 measured 10 measured	
May 2000	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10		
July 200 0						10 plots marked 10 plots marked	
Aug. 2000	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10	10 measured 10 measured	
Total	**50 Control 50 Treatment**	**50 50**	**50 composites 50 composites**	**50 50**	**50 50**	**30 30**	**50 plots 3 X each**

Clam Digging Experiment: During spring and summer 1998 low tides, lower intertidal eelgrass patches will be visited to locate relatively undisturbed areas were *T. capax* and *S. giganteus* populations are present. As with the clam raking experiment, 50 treatment and 50 control plots will be randomly assigned along a transect or within a plot grid. All 50 treatment plots will be dug using a shovel to remove all large, recreationally important clam species. This treatment will be repeated on these same 50 plots two additional times (June and July 1998). Although the tentative sampling design for this experiment (Table 2) is essentially identical to that used in the clam raking experiments, it will be more difficult to accomplish as these low intertidal areas are exposed for a shorter duration and treating plots by digging will involve more time than raking. Hopefully the number of plots to be sampled can be reduced based upon the preliminary results of the clam raking experiment and those of an ongoing study being conducted at our laboratory to determine the optimal sampling strategy for defining habitat-biota relationships (Ferraro and Cole, 1996). As with the clam raking experiments, sampling will continue until recovery (no significant difference between treatments and controls) or for up to two years post-treatment.

Table 2: Tentative Sampling Strategy for the Clam Digging Experiment

Date	Plots destruc-tively sampled	Macrofauna Cores taken	Megafauna Cores taken	# Plots harvested	Grain Size TOC cores	1° Production Measurements	# Plots Dug
May 1999							50
June 1999							50
July 1999						10 plots marked 10 plots marked	50
Aug. 1999	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10	10 measured 10 measured	
May 2000	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10		
July 2000						10 plots marked 10 plots marked	

Aug. 2000	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10	10 measured 10 measured	
May 2001	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10		
July 2001						10 plots marked 10 plots marked	
Aug. 2001	10 Control 10 Treatment	10 10	10 composites 10 composites	10 10	10 10	10 measured 10 measured	
Total	**50 Control 50 Treatment**	**50 50**	**50 composites 50 composites**	**50 50**	**50 50**	**30 30**	**50 plots 3 X each**

Field Survey: Concurrent with the clam digging experiment, a similar sampling strategy (Figure 2) will be employed in an eelgrass meadow which is harvested by the public for *T. capax* and *S. giganteus* during summer low tides. Comparison between experimental and field survey plot data will at least qualitatively indicate how well the experimental manipulations mimic actual long-term clamming activities. The sampling design for this study (Table 3) differs from the previous designs as an adjacent undisturbed site is not available for a control and primary productivity measurements will not be attempted due to the likelihood of disturbance by clam harvesters. If recovery occurs in the clam digging experiment, the field survey will be discontinued.

Table 3: Sampling Strategy for the Field Survey

Date	Plots Destruc-tively sampled	Macrofauna Cores taken	Megafauna Cores	# Plots harvested	Grain Size /TOC cores
Aug. 1999	10	10	10 composites	10	10
May 2000	10	10	10 composites	10	10
Aug. 2000	10	10	10 composites	10	10

May 2001	10	10	10 composites	10	10
Aug. 2001	10	10	10 composites	10	10
Total	**50**	**50**	**50 composites**	**50**	**50**

Locating a suitable site for the field survey in Yaquina Bay may prove difficult. The most extensively harvested area in the bay is located on the south shore from the public fishing pier west to the nearest wing dam on the south jetty (Figure 1). However, this site is approximately 2 km from the probable experimental sites in Sally's Bend and may not be readily comparable. Another possible site is located on the far east side of Sally's Bend. Although considerably closer to the primary study area, the site is only occasionally clammed and is considerably smaller in size than the south jetty area. Raking for cockles is not a major activity in Yaquina Bay, although this technique is used at other sites on the Oregon Coast (e.g., Alsea Bay). A comparative site for the clam raking experiment may be added to this project if a suitable site is located.

Algal Smothering Experiment: Preliminary work will be done in 1998 and 1999 to determine: 1) when algal mats are present, 2) where algal smothering occurred, 3) the duration of smothering, and 4) a practical method to experimentally smother eelgrass with algae. Algal smothering experiments will begin in mid to late summer, 1999 or 2000 depending the continuing need to sample clam raking and digging plots.

In this experiment the effects of single, acute smothering events will be assessed, and site recovery monitored over 1 to 3 years. As before, 2.25 m^2 plots will be selected along a transect or within a plot grid, and control (no disturbance) or treatments (algal smothering) randomly assigned to the plots. The smothering treatment will be accomplished by covering plots with algal mats which will be collected from adjacent areas and restrained over the treatment plots in mesh bags or under nets which will be staked down at the periphery of each plot. This method should allow the alga to settle on the eelgrass at low tide yet provide for some circulation of oxygenated water at high tide. These smothering treatments will be maintained for a duration that is similar to that observed in pre-experimental surveys.

After the smothering treatment is removed, control and experimental plots will be sampled using the same sampling methods and plot design used in the previous experiments (Figure 2). Measurement procedure will be repeated within each plot until recovery (no significant difference between treatment and control) or for up to two years post-treatment. A proposed sampling strategy is presented in Table 4.

Table 4: Tentative Sampling Strategy for Algal Smothering Experiment

Date	Plots distructively sampled	Macrofauna Cores taken	Megafauna Cores taken	# Plots harvested	Grain Size TOC cores	1° Production Measurements	# Plots smothered
July 2000							50
Aug. 2000	10 Control* 10 Treatment	10 10	10 Composites 10 Composites	10 10	10 10		
May 2001	10 Control 10 Treatment	10 10	10 Composites 10 Composites	10 10	10 10		
July 2001						10 plots marked 10 plots marked	
Aug. 2001	10 Control 10 Treatment	10 10	10 Composites 10 Composites	10 10	10 10	10 measured 10 measured	
May 2002	10 Control 10 Treatment	10 10	10 Composites 10 Composites	10 10	10 10		
July 2002						10 plots marked 10 plots marked	
Aug. 2002	10 Control 10 Treatment	10 10	10 Composites 10 Composites	10 10	10 10	10 measured 10 measured	
Total	**50 Control 50 Treatment**	**50 50**	**50 Composites 50 Composites**	**50 50**	**50 50**	**20 20**	**50 plots 1 time each**

*Depending on the method used to smother plots, treatment control plot (i.e., algal entrapment method but without algae) maybe needed in addition to undisturbed control.

Data Analysis: Comparisons between controls and treatments within each of these experiments is basically the same. Each experiment is a completely randomized design which will be analyzed by two-way ANOVA. Sources of variation to be analyzed are as follows:

Source of Variation	Number	Degrees of Freedom
Time (Fixed)	a=5	a-1
Treatment (Fixed)	b=2	b-1
Raking and Control		
Algal Smothering and Control		
Interaction		(a-1)(b-1)
Error	n=10	ab(n-1)

The exception to this will be the clam digging experiment in which samples taken from the field survey will be included as an additional treatment. Although comparing controls and digging plots located in Sally's Bend to a heavily clammed area located two kilometers away is far from ideal, it does allow for some interpretation of the field survey results. Sources of variation for this two-way ANOVA will be as follows:

Source of Variation	Number	Degrees of Freedom
Time (Fixed)	a=5	a-1
Treatment (Fixed)	b=3	b-1
Digging, Field Survey, Control		
Interaction		(a-1)(b-1)
Error	n=10	ab(n-1)

The plot design (Figure 2) gives the appearance that multiple sampling strategies are being employed within a plot. This is not the case as samples will either be composited into one sample (e.g., megafaunal cores and harvested *Zostera*) or mean values determined (e.g., primary production values) and compared among plots.

Expected Results and Benefits: Experimental treatments will initially reduce eelgrass biomass and possibly primary production. This is likely to have deleterious effects on macrofaunal abundance and diversity. Effects are likely to be enhanced and of greater duration at the higher intertidal sites which generally have lower initial eelgrass densities. Reductions in the eelgrass shoot density may result in the colonization of the disturbed plots by opportunistic species such as exotic amphipods (e.g., *Grandidierella japonica*) and *Neotrypaea*. The extent and time course of colonization by *Neotrypaea* would increase our understanding of the possible amensalistic relationship between eelgrass and burrowing shrimp. In addition measurements made on eelgrass productivity under stressed and unstressed conditions may be useful in developing a method for assessing the relative health of eelgrass patches.

The present experiments are being conducted to determine if a significant treatment effect could be observed in an area in which physical/chemical variation is minimal. This design maximizes the ability to detected a statistically significant effect. If ecologically important treatment effects are detected within the Sally's Bend study site, experiments would need to be conducted to expand the inference space, either across Yaquina Bay and/or among several estuaries. If treatment effects are not observed in the present study (as suggest by preliminary results), the likelihood of finding a significant effect across a more heterogeneous environment would be minimal and the next experiment (clam digging, algal smothering) would be started ahead of schedule.

Results of the clamming experiments and the associated field survey would be useful in predicting the effects of increased utilization of eelgrass meadows for recreational clamming and may help in estimating the effects of other mechanical disturbances (e.g. those associated with oyster culture and commercial shrimping). These data could be combined with Oregon Department of Fisheries and Wildlife (ODFW) data on the extent of recreational clamming activity in Yaquina Bay and other Oregon estuaries in order to make projections on how this activity could impact eelgrass meadows as human population and tourism increases. However, the proposed experiments are only designed to determine if a significant treatment effect can be observed in an area in which physical/chemical variation is minimal. The design maximizes the ability to detected a statistically significant effect. If ecologically important treatment effects are detected within the Sally's Bend study site caution should be used in extrapolating these results across the entire Yaquina Bay estuary or among several estuaries. Additional experiments may need to be conducted to expand the inference space in order to make statistically valid large scale impact predictions. However, if treatment effects are not observed in the present study, the likelihood of finding a significant effect across a more heterogeneous environment would be minimal and an expanded experimental protocol would not be necessary.

Results of algal smothering experiments could be scaled up to estimate the effect on the eelgrass in the entire Yaquina Bay estuary using existing (1997) aerial photographs which provide a snap-shot of the extent of algal rafts in Yaquina Bay. This sort of information would be useful in estuarine risk assessments. The link between increased nutrients and enhanced drift algae suggests that as the nutrient loads of PNW estuaries increase with increasing human population, the smothering of eelgrass by drift algae is likely to increase. The present study would provide a first look at the associated benthic effects.

The results of this research will be communicated to the scientific community through three or more peer reviewed journal publications and several regional and national meeting presentations. This research should significantly advance our understanding of how physical disturbance effects eelgrass and associated biota.

Project A5 - Comparison of Factors Affecting the Distribution of the Non-indigenous Seagrass *Zostera japonica* with Those Controlling the Native *Zostera marina* in Yaquina Bay, Oregon.

Principal Investigator: David T. Specht

Goals: The project goal will be to determine the predominant factors affecting the distribution of the non-indigenous seagrass *Zostera japonica* as compared to the native seagrass *Zostera marina* in Yaquina Bay, Oregon.

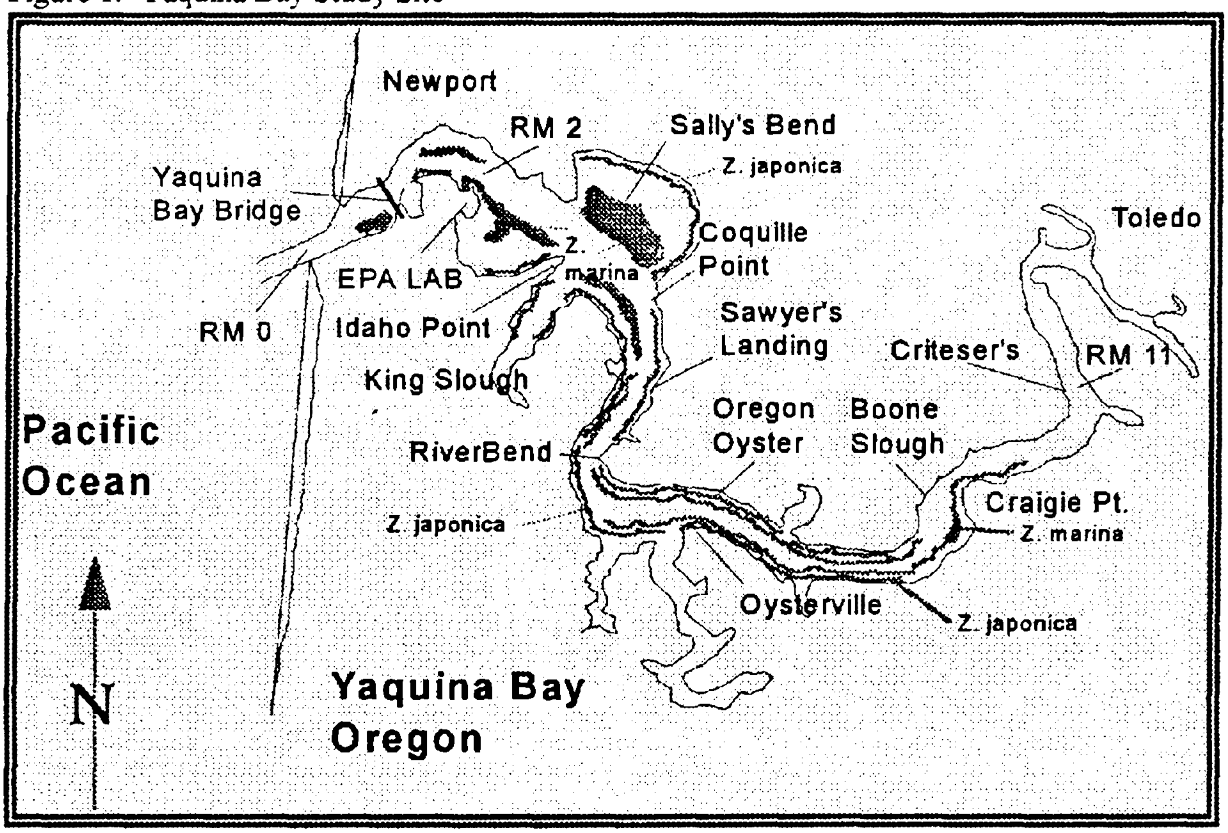
Rationale: The native seagrass *Zostera marina* L. and the non-indigenous *Z. japonica* Aschers. & Graebn. co-occur in many Pacific Northwest (PNW) estuaries. Phillips and Meñez (1988) establish the introduction of the non-indigenous *Z. japonica* on the Pacific Coast of North America concurrent with the importation of Japanese oysters and spat to Willapa Bay, Washington, in 1925, and it has spread south to at least Coos Bay, Oregon (Harrison and Bigley, 1982). Bayer (1996) concludes that *Z. japonica* was introduced to Yaquina Bay in the early to mid-1970s; he first collected specimens in 1976, but had not encountered any in his prior eelgrass survey of 1974-75 (Bayer, 1979). There appear to be no other local records of recognition of or collection of *Z. japonica* (*cf. Z. nolti*) prior to that time.

In Yaquina Bay *Zostera* species are present in the intertidal zone along a salinity gradient upriver from the area of the Yaquina Bay Bridge, approximately 1 river mile (RM) inland from the mouth of the estuary, to a point upstream near Boone Slough, approximately RM 10, ~3 statute miles south and west of Toledo (Figure 1). Lateral distribution differences among the two species are evident. The vertical distribution of *Z. japonica* in Yaquina Bay appears to be concentrated in the upper intertidal, immediately adjacent to emergent aquatic rooted species of grasses, reeds and sedges (seaward edge of the emergent salt marsh), occurring in bands paralleling the shore from ~1 m to as much as 30-100 m wide, at elevations ~+0.4 to +1.8 m above mean lower low water (MLLW), the bulk occurring at ~+1.3 m (Bayer, 1996). Harrison (1979, 1982) describes *Z. japonica* as an opportunist with an ability to grow in the subtidal, and to "escape" direct competition with a taller, light-shading *Z. marina*, by enduring dessication at low tide at the upper intertidal level. Nomme and Harrison (1991), studying *Zostera* spp. distributions just south of Vancouver, B.C., using transplant experiments, assert that abiotic conditions could not be responsible for the absence of *Z. japonica* from deeper zones typically occupied by *Z. marina*, because transplanted monospecific clones grew well at all cross-transplanted inter- and subtidal elevations during the growth season. Unlike Yaquina Bay habitats, their study site typically endures considerably harsher winter exposures, and the *Z. japonica* population is largely annual, growing from seed each season. Thom (1990), studying *Z. japonica* populations in Padilla Bay, WA (northern Puget Sound), states that it is virtually absent from the bay in winter. Thom (1990) also observed considerable overlap of vertical distribution of *Z. marina* and *Z. japonica*, the maximum biomass of the combined populations occurring at ~+0.3m above MLLW; peak standing stocks of both species were centered at about +0.5 m (*Z. japonica*) and MLLW (*Z. marina*).

Yaquina Bay distribution patterns differ from those previously described, in that the populations do not overlap. *Z. marina* habitats grade from immediately adjacent to *Z. japonica* populations (typically up-bay), to widely separated intervals (meters to hundreds of meters laterally and ~0.0 to ~1.5 meters vertically - typically lower-bay). This distribution gap may be due to several factors, among them are wind-generated wave action in the intertidal area (Phillips and Meñez, 1988), species-specific dessication and insolation effects (McRoy and McMillan, 1977), predation pressure by herbivorous waterfowl (Keller, 1963; McRoy, 1966, 1970) and non-herbivorous waterfowl, which uproot and ingest eelgrass covered with herring eggs (Bayer, 1979), and differential temperature tolerance of intertidal vs. subtidal populations (Biebl and McRoy, 1971). The influence of shading by the more physically-imposing *Z. marina* is not likely to be the operant mechanism in Yaquina Bay populations where, in most cases, the two are physically separated by unvegetated expanses.

Bayer (1979, 1996) concluded that in Yaquina Bay, *Z. marina* reproduced by seed in the upper intertidal, while lower intertidal and subtidal beds reproduced vegetatively. The subtidal limit of distribution of *Z. marina* in Yaquina Bay may be dictated by

Figure 1. Yaquina Bay Study Site



insufficient light penetration and/or physical disturbance (episodic hydraulic erosion of substrate along dredged or natural tidal channels due to storm or wind events). Bayer (1996) further inferred that because of its higher intertidal elevation, the ability of *Z. japonica* to persist throughout the winter season could be due to lack of the uprooting suffered by *Z. marina* from wind-generated wave erosion in the intertidal and less intensive waterfowl grazing. Whereas *Z. japonica* is reported to exist as an annual, reproducing by seed in other Pacific Northwest estuaries (Thom, 1990; Nomme & Harrison, 1991), it persists over winter as a perennial in most habitats in Yaquina Bay (Bayer, 1996, Specht, pers. obs.) in apparent good health, although declining in biomass during the winter. Phillips and Meñez (1988) conclude that *Z. marina* "is known to remain active all winter along a broad latitudinal gradient in North America", and that, "Above 22°C the plants either produce flowers and seed, becoming annual, or become moribund. According to Phillips et al. (1983), flowering is a response to warming water temperatures which interact with local genotypes". Bayer's (1996) observations might be interpreted to conclude that the intertidal exposure during summer months in Yaquina Bay provides enough warming to provide such stimulus for flowering, whereas subtidal populations, which are subjected to tidal prism flooding influenced by cooler waters provided by summer upwelling, are inhibited from flowering.

Kentula (pers. obs., May 1, 1998) observed *Z. marina* flowering at several subtidal locations in the upper bay, at Craigie Point (~RM 9), Oysterville (~RM 7) and the west shore south of King Slough opposite Sawyer's Landing (~RM 4) (where a specimen was collected), but no flowering was observed seaward of RM 4. The surface water temperature at Sawyer's Landing was 14°C. The maximum surface water temperature (1 m depth) on May 1 was ≤14.5°C, averaging ~12.5 °C. The average temperature in the prior month was ~12.5°C, with a range of 10-14°C (recorded at the HMSC small boat dock, RM 2). Water column temperature varies seasonally, with colder water during the winter rainy season draining off the watershed (typically ~8 °C upstream, ~9.5 °C downbay); summer wind-induced upwelling reduces temperatures in the lower bay subject to the tidal prism (typically ~22 °C upstream, ~13 °C down-bay). Periodic anomalies, such as "el nino" weather, cancel this pattern during the summer (no upwelling), with lower bay temperatures on the order of ~20°C; severe events are relatively infrequent. Historical records (Specht, unpublished data, 1976-78) for that zone show surface water temperatures not exceeding ~13°C during March and April periods; surface temperatures in late June did not exceed 20°C at Criteser's Dock (RM 11) or seaward. Surface temperatures approached 20-22°C from RM 11 or landward only during the period of July-August; lower bay temperatures average 12-14°C during the same period. The observations suggest that the guidelines of Phillips and Meñez (1988) on flowering of *Z. marina* may require further examination.

Thum (1972), investigating the ecology of an estuarine flatworm in Yaquina Bay adjacent to the Hatfield Marine Science Center (intertidal flats ~ RM 2), characterized the production of macrophytes (to include *Z. marina*, *Enteromorpha tubulosa*, *E. intestinalis* and *Ulva "angusta"*) as approaching "100 gm/30 cm^{2}" (dry weight basis) (≈ 1.1 kg dw/m^2, assuming Thum meant a 30 cm x 30 cm plot). He observed that while stations in the upper intertidal were "devoid of macrophytic material by January, ... young *Zostera marina*, 1 to 2 cm in length were present a month later. The *Zostera* at the lower stations (-2.0 and 0.0 feet) was cropped severely by the black Brant (*Branta nigricans*) during the winter, allowing sediment erosion to proceed." Notwithstanding this observation, his data indicate that the subtidal populations maintained a minimum biomass level of ~"20 gm/30 cm^{2}" (≈ 0.22 kg dw/m^2, using the previous assumption) over winter. Thum also stated that permanent, mature *Zostera* beds occurred below 0.0 feet, while seasonally transitory beds occurred between 3.0 and 0.0 feet. Kentula (1982) measured *Zostera marina* maximum standing crop in Netarts Bay, a similar environment, as ranging from a low of 143 g dw m^{-2} (high intertidal) to a high of 463 g dw m^{-2} (low intertidal). She reported net annual production, including above- and below ground parts, as 3.1 kg dw m^{-2} yr^{-1}.

A variety of factors may interact to explain spatial distribution patterns of seagrasses. That *Zostera japonica* flourishes in the high intertidal while *Z. marina* does not may indicate that *Z. japonica* is more tolerant of high levels of insolation and desiccation during daylight low-tide exposures. Alternately it may be more tolerant of exposure to freshwater flows off the land than *Z. marina*, although *Z. marina* is known to be remarkably tolerant of low (~6 PSU - practical salinity units) salinity (Phillips and Meñez, 1988).

Controlling factors may differ at each end of the estuarine depth gradient. Since much of the lateral growth of beds of *Zostera marina* is by vegetative extension of roots and rhizomes, growth of shoots at any given depth descending a steep gradient may not be light-dependent, as nutrition would initially be supplied by the parent plant through the rhizomes until the leaf blade growth is sufficient to reach into the euphotic zone. The physical environment at the lower edge of distribution, being on the edge of a channel, is likely quite severe compared to life on the intertidal flat. Although the average channel current speed is approximately 1.5 knots (Goodwin *et al.* 1970), episodic runoff events approach 15 knots (Callaway and Specht, 1982), which could induce substantial bedded sediment erosion, especially in low-stability sediment; high-flow induced turbidity is known to suppress eelgrass photosynthesis (Stephan and Bigford, 1997). Nomme and Harrison (1991) report that the ability of *Z. marina* to resume growth after transplanting to subtidal depths was reduced with increasing depth, and assumed this was due to reduced light availability. While light reduction may be the ultimate cause of growth reduction, isolation of the transplant plug may be significant. Isolation from an extensive network of rhizomes of a parent plant will prevent transfer of nutrients, resulting in growth inhibition of plugs at depths where connected plants might grow successfully.

Knowledge of the potential role of nutrient distribution patterns in directly influencing seagrass distribution patterns in west coast estuaries is limited. Estuarine circulation patterns may be important. Kentula (1982) describes circulation patterns in Netarts Bay that result in two distinct water masses: "bay water" and "ocean water". Little mixing occurs between the two water masses within single tidal cycles. Factors affecting the degree of mixing include substantial differences in temperature and salinity. The clear implication is that these circulation pattern phenomena (typical of PNW estuaries subject to upwelling influence) can reflect the sources of and affect the distribution of nutrients for the estuarine primary producers.

It is likely that nutrient distribution patterns along the estuary have changed considerably in the last 100 years. A significant proportion of the nutrient supply in Yaquina Bay in earlier times (~late 1800's through ~1980) may have come from disposal of untreated and digested process waste from the Georgia Pacific Kraft Paper Mill and predecessors, sawmill wood processing waste (e.g., sawdust, bark), leachate from failing shore side septic tank drain fields (e.g., from Coquille Point upriver to the city of Toledo), and normal and storm overflow from the chronically under capacity municipal sewage treatment plant (STP) at Toledo. Due to repairs and upgrading of septic tank drain fields bordering the estuary, improvements of the domestic sewage treatment plant at Toledo and industrial effluent treatment systems at the Georgia-Pacific Mill since the 1980's, this seasonally variable fraction of the supply of nutrients to the estuary may have significantly declined (although significant episodes of rainfall-related STP combined overflows still occur at Toledo with some regularity).

Winter and spring growth of both *Zostera* sp. in Yaquina Bay is likely sustained by sediment-bound and microbially-generated N, in addition to runoff-derived nutrients, but growth of most primary producers is limited by light availability (Davis, 1982). Late summer growth of *Zostera marina*, epiphytes, phytoplankton and macroalgae may be largely fueled by additional N and P contained in the coastal upwelling water in the flooding tidal prism, and ammonia generated from infauna feeding on plankton and detritus (Reusch and Williams, 1998). *Zostera japonica*, with its location higher in the intertidal, may have less opportunity for extraction of nutrients from the flooding tidal prism than *Z. marina*. It may thus be more efficient in intercepting ground-water borne nutrients flowing across the exposed upper intertidal or arising from land-based seepage, or in obtaining nutrients directly from recycled excreta from waterfowl feeding in the area. In the maritime climate of Yaquina Bay, *Z. japonica* is exposed to rainfall inundation for more extended periods of time during winter-spring months than *Z. marina* because of its elevated position in the upper intertidal. The relatively high levels of excrement-derived nutrients from migratory wildfowl present in the estuary during the fall months (Bayer, 1979, and Thum, 1972) will tend to cycle as slowly converted refractory material in the sediment, in addition to nutrients supplied by populations of resident wading birds, fish, invertebrates and marine mammals (e.g., harbor seals). Much of this material, because it occurs in relatively shallow water, is probably retained in the intertidal during tidal ebb, and may be tidally pumped upstream as either dissolved or particulate matter as it is released along the tide interface, and thus may also influence upstream distributions.

Zostera marina, however, may be able to compete in relatively N-poor sediment areas because of a difference in microbial associations that provide N-fixation, and/or scavenge critical nutrients from the salt wedge during upwelling. Additionally, episodic river floods supply nitrogen off the watershed. The results of such floods are seen most clearly in the "spike" in primary production following the first fall rains and in the growth response in populations of phytoplankton-consuming bivalves (e.g., *Macoma nasuta*) (Specht and Lee, in prep.). Laboratory phytoplankton biostimulation bioassays (Specht, 1974, 1976, and unpublished data, 1975-76 samples) also suggest such rapid responses to nutrient pulses. These nutrient sources should be relatively independent.

In order to assess the degree in which physical factors may be controlling plant distribution, it is necessary to have indicators of plant stress. In addition to measurements of overall plant growth reflected in shoot counts or biomass measurements, Phillips & Meñez (1988) cite a number of studies in which leaf width appears to be an indicator of stress (Phillips and Lewis, 1983; McMillan, 1979; McMillan and Phillips, 1979). Setchell (1920, 1929) noted that leaves of *Z. marina* were narrower in the intertidal than subtidal, and that leaves are narrower in winter than in summer. Setchell suggested that these narrower leaves were due to stress from greater annual or even tidal exposure or ranges of temperature. Ostenfeld (1908) recorded narrower subtidal leaves when the plants grew in sand than in mud. In this substrate example, the stress could also be a nutrient one. Field transplants across gradients have confirmed some of these observations. In certain cases populations show phenotypic plasticity and adapt readily to new sites with an accompanying increase or decrease in leaf width. In other cases populations show little change in leaf width and are said to be genotypically differentiated. It is thought that the latter populations are native to stressed locations (Phillips & Meñez, 1988).

If we are able to show what mechanisms are involved in restricting the distribution of the exotic *Zostera japonica* to the upper intertidal in this habitat, we may be able to isolate those mechanisms eligible for control strategies that would be effective in restricting the establishment of other non-indigenous invasive plant species, such as the smooth cordgrass *Spartina alterniflora*, a significant threat to the ecological health of PNW estuaries.

Objectives: The overall goal of the research effort is to determine factors responsible for the differential distribution patterns of the exotic seagrass *Zostera japonica* versus the native seagrass *Zostera marina*. Because other research at Coastal Ecology Branch (CEB) will focus on the role of light availability in the water column on seagrass distributions patterns, the focus of my research will be on nutrients as a potential factor affecting seagrass distribution. Specific objectives and hypotheses to be examined are described below.

Objective 1. To compare the sediment nutrient (N species, P) concentrations for *Zostera japonica* and *Zostera marina* habitats within the root/rhizome horizon in selected sample sites within Yaquina Bay.

Hypotheses 1.1 - Sediment nutrient (nitrate-N, ammonia-N, soluble reactive phosphate) concentrations and their bioavailability to resident SAV are not significantly different between populations of *Zostera marina* and *Z. japonica* at equivalent locations along the salinity gradient from Yaquina Bay Bridge upstream to the vicinity of Boone Slough. Factors which may affect this bioavailability include a) sediment temperature regimes, b) infauna distribution and composition and c) nutrients from land-margin/high marsh groundwater drainage. These factors are addressed below.

Objective 2. To compare the a) water column nutrient (N species, P) concentrations, b) PAR penetration through the overlying water column, and c) sediment temperature, sediment grain size distribution, and salinity of interstitial water within the root/rhizome mat among selected Yaquina Bay *Z. japonica* and *Z. marina* beds. Sampling will be on a monthly basis over at least one calendar year, with higher frequency sampling (weekly) at selected sites during the period of maximum growth (May through June). Site-specific values of water column parameters will be compared to longer term records of water quality parameters across the estuarine gradient using moored conductivity-temperature-depth sensors (CTDs), at surface or bottom locations axially distributed upstream from the OSU dock (~RM 2). Parameters to be measured will include salinity, temperature, turbidity, tide level, and, in selected installations, dissolved oxygen, PAR light intensity, and in-situ fluorometric determination of chlorophyll "a" concentration. To benchmark current conditions, comparisons will be made to water column nutrient concentrations from a 1976 survey of the Yaquina estuary (Callaway and Specht, 1982; Callaway, Specht and Ditsworth, 1988). Specific hypotheses are:

Hypotheses 2.1 - Surficial sediment temperature ranges and averages are not significantly different between equivalent locations for populations of *Z. marina* and *Z. japonica* along the estuarine salinity gradient.

Hypotheses 2.2 - Salinity, temperature, and light availability to the seagrass canopy are not significantly different in the water-column at tidal extremes between populations of *Z. marina* and *Z. japonica* at similar locations along the estuarine salinity gradient.

Objective 3. To compare the growth, survival and reproductive success of reciprocally transplanted specimens of *Z. japonica* and *Z. marina* over a minimum two-year period, to demonstrate potential physiological differences in local habitat-adapted monospecific clones of the two species. Stress measures may include reduction in blade width compared to control, time of onset of flowering and seed set, and shoot density and mean shoot length.

Hypotheses 3.1 - Reciprocal transplants of monospecific clones of *Z. japonica* and clones from MLLW elevation of *Z. marina* will show no difference in growth or "stress" measures at equivalent locations along the estuarine salinity gradient.

Methods:

Objective 1 - A - Using Yaquina Bay aerial photography from 1997 (Young *et al.* in prep.) And field survey, we will locate appropriate sites of monospecific populations of *Zostera marina* and *Z. japonica* to sample for sediment nutrient concentrations, sediment characterization, and sources of transplantation clones. I propose to establish lateral transects at three locations at which both *Zostera* species co-occur (at MHHW and MLLW): the north shore at Sally's Bend, the north shore at RiverBend, and the south shore south of Craigie Point (Fig. 1).

A survey of the sites will be conducted by the PI or an outside contractor to establish the vertical elevations of the sampling sites to an appropriate tolerance level. Vertical zonation is perceived as critical to establishment and maintenance of these populations (see D. Young, CEB, proposal). This survey will be conducted in cooperation with on-site contractor and EPA staff concerned with geographic information system (GIS), geodetic control, and photogrammetric aspects of the FY97 Yaquina Bay mapping exercise (Young *et al.*, in prep.).

Objective 1 - B - Sediments will be sampled within the selected habitats and analyzed for N and P species (nitrate-nitrogen, ammonia-nitrogen, ortho- or soluble reactive phosphate) in both interstitial water and that fraction sorbed to sediment particulates.

Objective 1 - C - Infaunal distribution and abundance will be addressed in the DeWitt *et al.* proposal surveys, and data compared to Boese *et al.* (1997, 1998) flood survey site results, where coincident with *Zostera japonica* occurrence.

Objective 1 - D - Interstitial water will be sampled periodically at the upper intertidal margin in sediment a depth of ~5 cm. at the upper terminus of transplant transects for N-P analyses.

Objective 2 - A -We will deploy CTDs at strategic locations in the estuary to characterize the salinity and temperature structure over time. These instruments will undergo quality assurance/quality control (QA/QC) checks and will be deployed, operated and maintained by the on-site contractor, at the technical direction of the PI (see also Ozretich and Young research proposals). Stations are proposed at the following locations:

1) OSU pump dock (~RM 2), at which a surface unit (i.e., attached to float dock to read 1.0 m below the surface) and a bottom unit (located at approximate bottom of the euphotic zone, i.e., the mean lower level of *Zostera marina* beds), this unit will also have a PAR light sensor attached, a fluorometer and turbidity sensor; the surface unit will also measure turbidity.
2) Between Idaho Point and Sawyer's Landing (~ RM 3.5), at which a surface unit with a turbidity sensor will be deployed to float 1 m below the surface.
3) RiverBend (~RM 5.5), at which a surface unit with a turbidity sensor will be deployed to float 1 m below the surface.
4) Oregon Oyster, or Oysterville (~RM 7), at which a surface unit with a turbidity sensor will be deployed to float 1 m below the surface.
5) Criteser's Moorage (~RM 11), at which a surface unit with a turbidity sensor will be deployed to float 1 m below the surface.
6) One unit equipped with a PAR light sensor and a fluorometer will be reserved for vertical casts, to profile the water column at optimal times preceding and immediately following episodic climatic events (heavy rain and flooding, for instance).

Objective 2 - B - Sediment temperature over time will be recorded by in-situ placement of temperature loggers at ~5 cm depth along each transect, with an additional transect at the west bank south of King Slough ("Racoon Flats"), where *Zostera marina* grows all the way up through the intertidal to the MHHW line, and a transect on the north shore of Idaho Point. Initially, five loggers will be placed on the Sally's Bend transect, three at RiverBend, three at Craigie Point, two at Racoon Flats, and two on the north side of Idaho Point. The distribution is designed to measure sediment temperature variance during tidal submersion and intertidal emersion in a number of different exposures. We will also read instantaneous sediment temperature at the root/rhizome level (~5 cm depth) during sampling operations

Objective 3 - At three locations along a salinity gradient, we will transplant monospecific clones of *Zostera japonica* and *Z. marina* (from MLLW habitat) reciprocally on a lateral transect to determine survival, growth and reproductive success in non-native habitats, monitoring the transplanted clones over at least a two-year period. Transplanting should be accomplished prior to the maximum growth season (May). Transect lines will be located by Geographic Positioning System (GPS) and marked. Elevations will be determined subsequent to transplanting activity by GPS and laser range-finder in conjunction with standard surveying techniques. Reaction to stress will be measured by determining significant differences in leaf blade width in new growth immediately above the intercalary meristem, shoot density and mean shoot length on a periodic basis (Nomme and Harrison, 1991).

Expected Results and Benefits: Determination of the physical parameters characterizing the present habitats and sources of nutrient supply for the different populations of *Zostera* species could allow the potential for control strategies of the non-indigenous *Z. japonica*, if that is determined to be a desirable end. Such knowledge may be of significant import in controlling or preventing the establishment of other potential invading species, such as *Spartina alterniflora* (smooth cordgrass, a significant problem in a growing number of west coast estuaries), which could utilize the same nutrient source or tolerate the physical environment. Success in determining levels of stress in local populations of *Zostera* spp. could prove a useful tool in evaluation of declining SAV populations.

Project A6 - Spatial Variation of Growth and Condition in Juvenile English Sole Relative to Substrate Characteristics

Principal Investigator: James H. Power

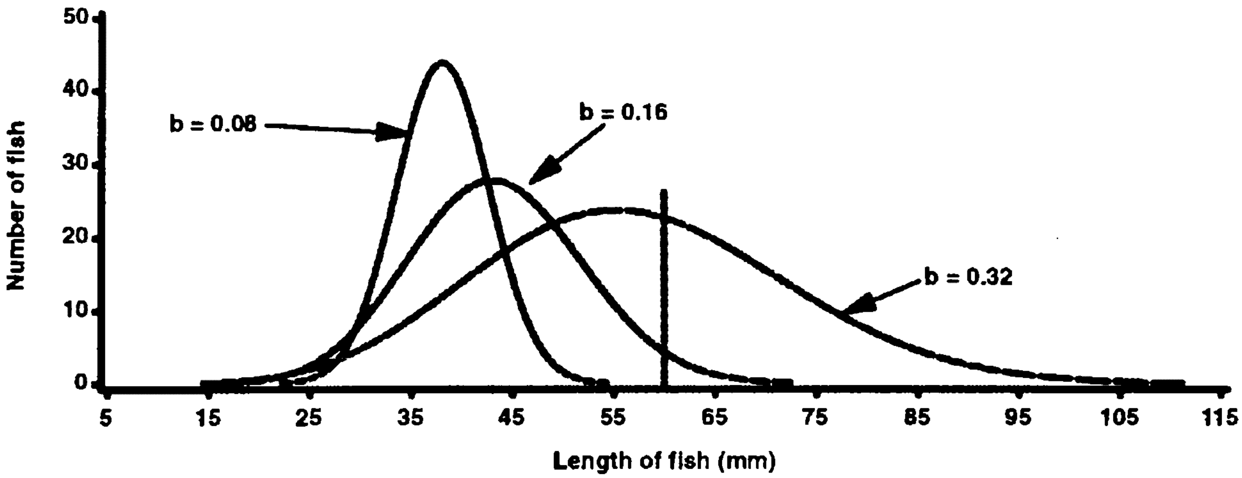
Goal: The goal of the proposed research is to determine how the growth rates of an important estuarine flatfish, the juvenile English sole (*Pleuronectes vetulus*, formerly *Parophrys vetulus*), varies in time and space, and particularly to relate these patterns to spatial variations in the sediment substrate of Yaquina Bay. I will further interpret this information with respect to the consequences of natural or anthropogenic alterations in the Yaquina Bay substrate.

Estuaries, as the region of transition between rivers and the coastal ocean, are influenced by the combined action of freshwater inflow, tidal processes, watershed features, and anthropogenic effects. The result is an environment where important physical, chemical, and biological parameters can vary greatly in both space and time (Virnstein 1990; Moore et al. 1996; Jassby et al. 1997). Any natural or anthropogenic process that alters those spatial or temporal patterns, or imposes greater variability, will in turn affect the ecology of the residents and their role in the estuarine ecosystem. Recent studies have begun to highlight the importance of spatial patterns in the benthic flora and fauna of estuaries, and the causative agents that might give rise to those patterns (Kneib 1994; Brandt and Mason 1994; Deegan and Garritt 1997; see also the Journal of Experimental Marine Biology and Ecology Volume 216, Nos. 1-2 which is devoted to a series of papers organized under the theme "The ecology of soft-bottomed habitats: Matching spatial patterns with dynamic processes"). With regard to estuarine fishes, Sogard (1994) discusses how a number of factors, such as competition, predator avoidance, or habitat degradation, might act to constrain estuarine fishes to suboptimal habitats. This proposal concerns the association between estuarine-watershed processes, as reflected by the sediment substrate composition, and the proximal growth and condition of an important estuarine flatfish that lives in association with those sediments during part of its life cycle.

This research is being proposed both for its intrinsic scientific merit and because the EPA Office of Research and Development (EPA-ORD) has identified "Research to Improve Ecosystem Risk Assessment" as a high priority research topic (EPA 1997). This EPA-ORD Strategic Planning document lists the four components to ecosystem risk assessment as: 1) Hazard identification; 2) Dose-response assessment; 3) Exposure assessment; and 4) Risk characterization. The research proposed here is intended to identify a heretofore little recognized hazard to an important estuarine species: the relationship between variations in bottom type and the important physiological process of growth. It will do this by undertaking a field-based "dose-response" assessment of the effects of the hazard. Both the identification and assessment can be then used to characterize the risks associated with alterations to the estuary and its surrounding watershed. It is then expected that the results of this work will contribute to a regional scale "exposure assessment", whereby the consequences of natural or anthropogenic alterations to the distribution and composition of estuarine sediments might be anticipated.

Rationale: The growth of juvenile fish has been extensively studied because of its importance in fish population biology. A fish's rate of growth is perhaps one of the most important indicators of its condition (physiological health), and the growth rate in turn reflects the relative suitability of the fish's local environment. Fish that are growing at comparatively higher rates can do so because they are obtaining a sufficient amount and quality of food, and they do not have to divert much energy towards coping with external stressors. Conversely, fish that are not growing well are either not locating sufficient food resources, are being subjected to some energetically taxing stress, or both. The rate of growth is believed to be especially important for the larval and juvenile (pre-reproductive) stages in fish. Rapid growth to larger size in larvae and juveniles is beneficial, because with growth the larger fish then become unavailable to predators that cannot successfully handle them. Growth may also confer greater foraging ability or the ability to avoid stressors.

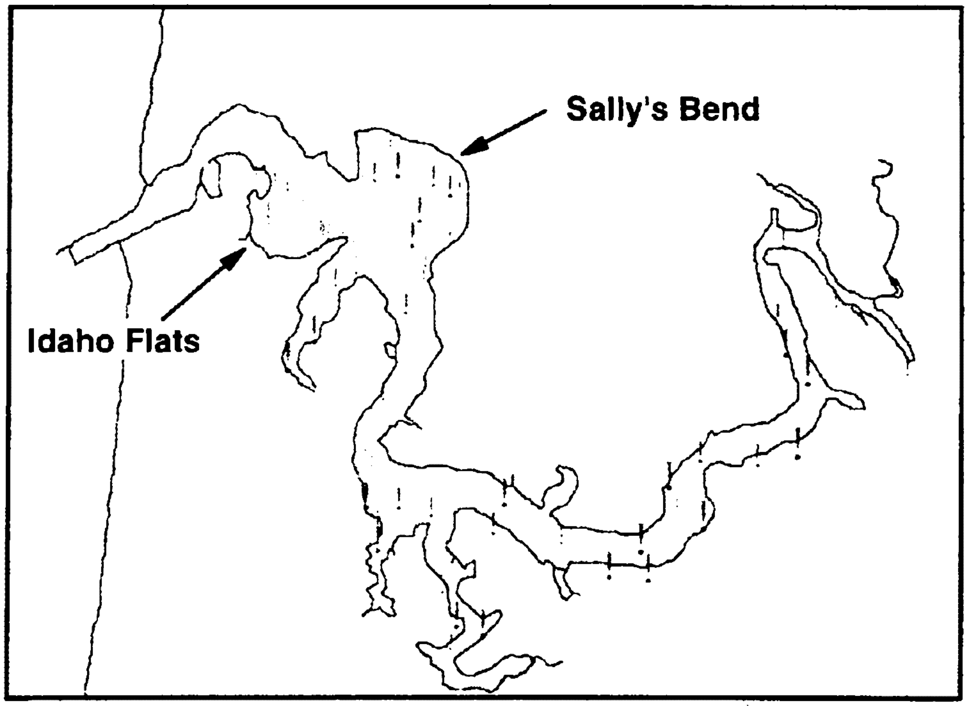
The results provided by DeAngelis et al. (1993) can be used to illustrate how spatial variations in growth can have important implications for a fish population. They provide an analytical solution to a partial differential equation that expresses how the size-frequency distribution of a fish cohort develops over time under specified growth and mortality conditions. In this model (their "Case 1") all fish begin growth at the same time, and at the same size. An important feature of the model is that fish mortality rate is inversely proportional to fish size. Each of the individual fish grows at a constant growth rate, but it's assumed that growth rates vary among the fish, being normally distributed with mean g_{0m} and standard deviation b. Below are the population frequency distributions that are predicted by this model after 60 days of growth. These curves were created keeping all parameter values provided by DeAngelis et al. (1993) constant, <u>except</u> for the standard deviation of the growth rate (b).



Note that when variability in growth rate is increased (to *b*=0.32) there is a greater proportion of larger fish in that population, and a larger mean fish size. Further, there are more fish surviving: the curve for *b*=0.32 represents 911 fish, while there are 506 and 596 survivors for *b*=0.08 and *b*=0.16 respectively. This result occurs in spite of the fact that the mean growth rate was identical and constant for all three of these "populations". What occurs is that the few fish fortunate enough to have higher growth rates (those from the right hand tail of the growth rate distributions) can capitalize on this advantage by the combination of rapid growth and by escaping the higher mortality at smaller sizes. The vertical line at a size of 60 mm in the graph is intended to illustrate the further consequences of variability in growth rate. Hypothetically, this could represent the size at which an important size-dependent life history event might occur, such as spawning or emigration from an estuarine nursery area. Variability in growth rate again has important implications for the numbers of fish that achieve this critical size in a timely manner.

These theoretical calculations underlie the premise of the research proposed here: that the magnitude of growth rate variability within a real-world estuary, possibly occurring because of spatial patterns in the substrate and food resources available, has a significant impact on the real fish population. These ideas are in accordance with those expressed by Underwood (1991), who points out that a natural or anthropogenic stress on an ecosystem may not necessarily alter the mean value of an ecological parameter. Instead, such stress may affect the spatial or temporal variability of that parameter (in this case fish growth rate), with important consequences for the population and ecosystem.

One such ecological parameter of importance to benthic fish is the substrate they live upon and from which they obtain their food. It is well known that a wide variety of natural and anthropogenic processes act to alter the bottom sediment characteristics in estuaries, and this is true of Pacific Northwest estuaries. Changes in the watershed that affect sediment distribution include the clear cutting of timber and other agricultural practices, alterations of the stream flows, or changes in land use such as residential development and urbanization that affects runoff and erosion of the adjoining land. The proposed research will be done in Yaquina Bay, Oregon, and the Bay's channel is periodically dredged, which also redistributes the sediments and affects the circulation patterns. However, detailed, historical data on the spatial distribution of infaunal species and the associated substrate characteristics of Yaquina Bay appears to be lacking. Data on grain size distribution at 88 sites in Yaquina Bay were provided by Bruce Boese (U.S. EPA) in the form of the percentages of sand, silt, and clay. These data were summarized in a principal component analysis, and the first principal component axis accounted for 96.6% of the variability in the data. As a result, the first principal component score of each location in Yaquina Bay is a convenient scalar to summarize sediment types in Yaquina Bay, and these are plotted below with the size of the circle being proportional to the component score.



Smaller circles in this plot indicate locations where the sediments have a high sand content, while larger circles indicate sediments that are mixtures of silt and clay. Note that the intertidal sediments in the Idaho Flats region are dominated by sand, while the substrate directly across the channel in Sally's Bend has substantial percentages of silt and clay. These variations in substrate composition can in turn be expected to influence the abundance and persistence of the infaunal organisms that are available to English sole as food. J. Chapman (Oregon State Univ.) and B. Boese (pers. commun.) have indeed noted considerable small scale variations in the abundances of infaunal amphipods (*Corophium* sp.) sampled within Yaquina Bay. Similar small-scale patchiness is also apparent in the distribution of submerged aquatic vegetation, such as *Zostera*. English sole, however, are widely distributed in the Bay and can be captured at essentially all locations in lower Yaquina Bay (De Ben et al. 1990).

In spite of the spatial structuring evident in estuaries, there have been comparatively few studies that have actually examined differences in fish feeding and growth rates wholly within an estuary, especially at spatial scales of several kilometers or less. Shaw and Jenkins (1992) found that feeding conditions for the flounder *Rhombosolea tapirina* were much better in a small embayment than they were in more open waters that were several kilometers away. The flounders in the better environment were feeding at a rate 3-4 times greater than that of the poorer location. Using caging experiments, Sogard (1992) found differences in growth, measured as change in fish length over 15 days, of three species at four New Jersey locations separated by roughly 15 km. Her study sites varied in bottom type and in the amount and kind of vegetation, but vegetation did not appear to be related to the growth of two of the species. Working at a larger scale, Sogard and Able (1992) found that winter flounder at one of four locations on the New Jersey coast were growing at a lower rate compared to the other three sites. They calculated flounder growth rates from otolith increment counts and widths. Berghahn et al. (1995) also used otolith microstructural analysis to find that plaice (*Pleuronectes platessa*) juveniles grew faster in a food-rich area when compared with a food-poor area about 5 km distant. In a controlled laboratory experiment Gibson and Batty (1990) did not find a direct effect of substrate type on plaice growth rates. However, they do emphasize that under field conditions the substrate will affect the food resources available to this flatfish, and so will have an indirect effect on its growth. The premise of the proposed research, suggested by these studies, is that variations in the substrate and immediate environment occupied by benthic fish can be regarded as a stressor, or indirect (proxy) indicator of associated stressors, particularly reduced food resources, that affect the fish's recent growth rate.

The adult English sole occupies coastal waters, but the larvae and juveniles utilize Pacific coast estuaries as nursery grounds (Boehlert and Mundy 1987; Gunderson et al. 1990; Chamberlain and Barnhart 1993). English sole enter Yaquina Bay as larvae beginning in late February, and benthic juveniles later emigrate from the estuary beginning in late May after overwintering one season (Boehlert and Mundy 1987). Previous studies have shown that English sole are widely distributed throughout Yaquina Bay, and can be captured at locations ranging from the dredged central channel to mudflats that are exposed at low tides (Westrheim 1955; Toole 1980; Krygier and Pearcy 1986; De Ben et al. 1990). Although direct study of juvenile English sole substrate preferences have not been reported, Becker (1988) did find that adults in Puget Sound appear to segregate by sex depending on bottom grain size characteristics. As a flatfish, the juvenile English sole lives in close association with the bottom sediments, and food habit studies have shown that it relies on infaunal organisms for a majority of its food (Hogue and Carey 1982; Becker and Chew 1987; Barry et al. 1996). Growth rates of juvenile English sole have been reported in a number of studies (Misitano 1976; Williams and Caldwell 1978; Rosenberg 1982; Rosenberg and Laroche 1982; Kreuz et al. 1982; Laroche et al. 1982; Shi et al. 1997), but none of these have reported growth rates relative to bottom type, or have made explicit comparisons among specific localities. The English sole's comparatively sedentary habit, numerical abundance, and widespread occurrence at a variety of within-estuary locations, all combine to make it an ideal subject for this study.

There are several approaches to evaluating fish growth. Mark-recapture methods are often used with adults, but are impractical with juveniles that are not the target of a fishery. Observation of the progression of modal values in length-frequency distributions has also been used to evaluate English sole growth rates, but that approach has coarse resolution and does not permit spatial comparisons. The principal approach used in this study will be a biochemical analysis of fish muscle tissue, specifically an evaluation of the animal's relative concentrations of RNA and DNA. The DNA content of an animal's tissues remains constant. However, the RNA content of that same tissue will vary according to the amount of protein synthesis that was being done at the time the animal was sacrificed. Hence, a measure of RNA content, scaled by the DNA content of the same tissue, provides a snapshot of recent animal protein synthesis rate and nutritional status (see Ferron and Leggett 1994 for a recent review). The RNA:DNA ratio was used by Malloy and Targett (1994a, b) to compare growth of juvenile summer flounder from Delaware and North Carolina. Although data are limited, the time course of changes in fish RNA:DNA ratios in response to changes in food is on the order of several days to a week, depending on the species and its developmental stage. For example, Clemmesen and Doan (1996) observed a significant change in larval cod RNA:DNA ratios after 5 days of starvation immediately after yolk absorption. Grant (1996) noted a significant decline in adult brown trout RNA:DNA ratios after one week in fish given rations of 1% body weight day^{-1} when compared to fish receiving 5% of their body weight day^{-1}.

The RNA:DNA ratio provides a measure of the fish's recent growth performance. It is of considerable interest to determine whether the fish otoliths might provide a more permanent record of fish growth rates. Daily increment formation has been reported in English sole otoliths (Rosenberg 1982), and these provide a measure of the time that has elapsed during their deposition. Measures that relate otolith to somatic growth have used either overall otolith size, or the comparative widths of the otolith increments, as indicators of fish somatic growth. However, there is continuing debate as to whether otolith and somatic growth in fish are coupled or decoupled, and whether the relationship is linear or nonlinear (Campana 1990), and in fact slow growth in some fish results in larger otoliths. Investigators such as Folkvord et al. (1997) assert that there is an otolith-somatic growth relationship in herring (*Clupea harengus*). However, others such as Mosegaard (1988), Milicich and Choat (1992), and Fitzhugh et al. (1997) have noted that temperature variations will affect, and possibly obscure, any otolith-somatic growth relationship. Wright et al. (1990) also did not find any relation between somatic and otolith growth in salmon parr, and further argued (Wright 1991) that salmon otolith growth is more closely related to fish metabolic rates, as measured by oxygen consumption, rather than somatic growth. Molony and Choat (1990) noted there may be a considerable (ca. 15 days) lag between reductions in somatic growth and otolith growth, because a fish placed under reduced rations may first mobilize stored food reserves for an extended period, allowing it to sustain otolith growth for some time before the effect of reduced food resources is reflected in the otolith growth pattern. A key assumption in using otoliths to indicate fish growth is that increments are formed daily, but another confounding difficulty in this usage is that slow growing fish may not deposit daily increments, or the increments may be too narrowly spaced to be resolved (Bailey and Stehr 1988). It spite of this controversy, it appears worthwhile, at least on a limited basis, to explore whether English sole otoliths provide an indication of somatic growth that can be used as an alternative to the RNA:DNA ratio.

Objectives: The objectives of the proposed research are:

1. To examine the spatial and temporal patterns of two indicators of recent growth rates (the RNA:DNA ratio and otolith increment deposition) in an important estuarine fish, the English sole.
2. To evaluate those patterns with respect to the fish's immediate environment, especially the characteristics of the sediment where the animals are collected.
3. To interpret the combined dataset of fish growth and sediment parameters at larger spatial scales, particularly with respect to anthropogenic effects.

Scientific Approach: Two kinds of field observations will be conducted in this study, and the first is the periodic collection of free-living fish. Collection of fish will be coordinated with the research of Ferraro and Cole of the EPA Newport laboratory, who will undertake sampling to evaluate estuary biota-habitat relationships in Yaquina Bay. A sampling method that Ferraro and Cole plan to use is the "drop sampler". The drop sampler is a fiberglass cylinder, five feet in diameter and five feet in height. It is suspended from the bow of a boat, and the boat is allowed to drift to the sample site so as to not startle the resident organisms. The sampler is released and allowed to quickly fall to the bottom, enclosing a sample of the water column and the substrate. It is pushed further into the sediment after deployment, and a pump is used to evacuate the water from the interior. The organisms are then collected from the interior, so that it captures a quantitative sample of the resident epifauna and infauna. The drop sampler was compared with other sampling methods by Rozas and Minnelo (1997), and they advocate its use as the most effective gear for sampling shallow estuarine habitats. Ferraro and Cole plan on deploying the sampler in a variety of contrasting habitats in Yaquina Bay, and these contrasts will contribute to the objective of this study: to make spatial comparisons using English sole captured at a variety of locations in Yaquina Bay. Up to ten individual English sole will be collected at each deployment of the drop sampler, and transported alive back to the laboratory for analysis. Temperature and salinity will be recorded, and sediment samples will also be collected for later analysis of grain size distribution and total organic content using methodology reviewed by Larson et al. (1997). Further, Ferraro and Cole plan to characterize the benthic infauna as part of their work, and this will contribute information to this project concerning the food resources available to the co-occurring English sole.

We fully anticipate that the drop sampler will be as effective a sampler in Yaquina Bay as it has been in other regions, but we are presently unaware of others that have used it in Pacific Northwest estuaries. It is therefore appropriate to discuss alternative sampling strategies to be used in case the drop sampler is ineffective for some unforeseen reason. De Ben et al. (1990) have reported on year-round trawling for bottom fish in Yaquina Bay at a sequence of stations from near the Bay mouth to nearly freshwater locations. Their data indicates that English sole were readily captured at their first six stations, and as an alternative to the drop sampler we may choose to reoccupy three of those (De Ben et al. stations 1, 3, and 5) on a monthly basis between May and October. These stations are near, or in, the main channel. In this event we would also sample three additional stations lateral to the channel, but near to shore, using a beach seine or trawl depending on tide height and bathymetry. Again, water temperature and salinity will be recorded for each trawl, and a core of bottom sediments will be collected for later grain-size and total organic carbon analysis. Up to ten individual sole will be selected from each trawl, and this selection will be done so as to collect a range of the fish sizes captured in each sample.

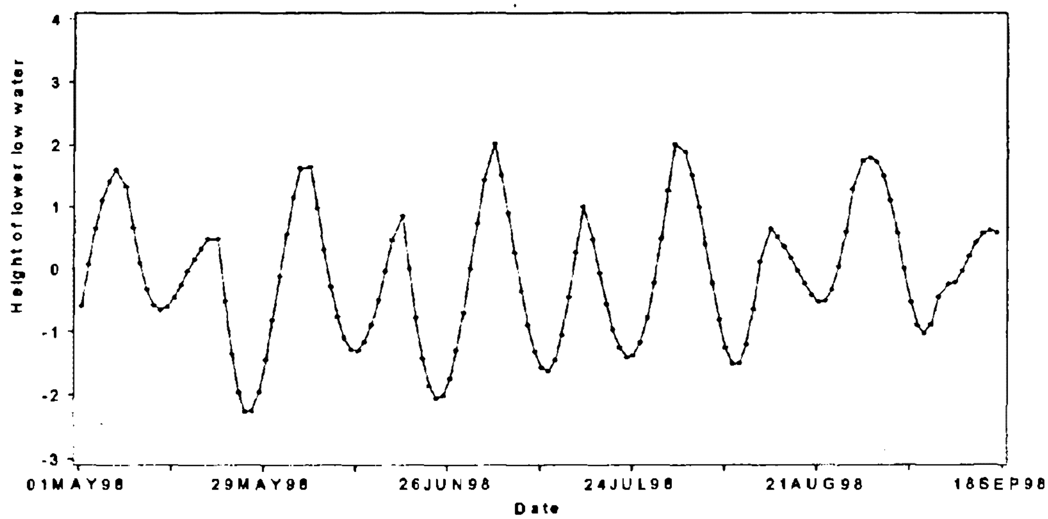
Finally, National Marine Fisheries Service scientists have indicated they will be conducting beach seine sampling for juvenile salmon in twelve west coast estuaries (including Yaquina Bay) ranging from San Francisco Bay in California to the Nisqually River in the state of Washington. They have generously offered to provide English sole they might incidentally capture to this project, and so we will opportunistically examine fish collected from these other locations as they might become available. If sufficient numbers of fish are obtained this way then larger scale, regional inferences about variation in sole growth rates may be possible.

Regardless of how they are captured, the sole will be held in separate buckets associated with the individual sampling site and returned to the laboratory. They will be anaesthetized in a 100 ppm solution of tricaine methanesulfonate (MS222), after which they will be weighed, measured, and sacrificed. Samples of muscle tissue will be collected and frozen at -80° for later biochemical analysis, and sagittal otoliths will be removed from the fish for later examination. Some additional fish will also be collected for experiments described below.

It is not known whether juvenile English sole exhibit any sort of site fidelity, although anecdotal information indicates that estuarine flatfish do not move much between adjoining habitats (S. Sogard pers. commun.). Continual movements of English sole between differing substrate types would inhibit detecting any relationship between bottom type and fish growth. Unfortunately, undertaking a conclusive field assessment of juvenile English sole site fidelity is problematic. This would ordinarily be done by conducting a mark-recapture experiment, and the recapture of a significant number of previously marked fish at or near the same site where they were marked would suggest the sole had maintained residence in that area over the intervening time. However, the large areal extent of Yaquina Bay habitat that is available to the sole is in contrast to the limited areal extent that can be effectively resampled using a trawl or beach seine. This makes it doubtful that sufficient numbers of sole could be marked, and then recaptured at a later date, to allow a conclusion about the fish's site fidelity to be made, even if fish dispersal from the site of marking is minimal.

With these considerations in mind it is also proposed to undertake caging experiments as the second set of field observations, so that juvenile English sole can be restricted to selected substrates for known periods of time. This portion of the research will follow the methodology of Sogard (1992). Cages will be constructed with open bottoms to allow the fish access to the substrate, and then anchored at two locations of contrasting substrate composition: one in the Sally's Bend area and the other in the Idaho Flats region (see previous figure on sediment types in Yaquina Bay). A "skirt" will be sunk around each cage's perimeter to prevent escape of the fish that might locate an opening under the bottom edge. Some of the sole collected during the trawling described above will be held in the laboratory and fed *ad libitum* for periods no less than one week, to allow them to equilibrate from their previous growth and feeding history while in the wild. These fish will be marked by subcutaneous injection of colored acrylic paint, so that individual fish identities can be known during the experiments. The otoliths of these fish will also be marked by immersing them in a solution of oxytetracycline (Secor et al. 1991a). Uptake of oxytetracycline into the otoliths leaves a fluorescent mark in the otolith that can later be viewed under a microscope and used as a reference point.

After being held in the laboratory, the fish will be weighed and measured, and one to five individuals will be placed in the cages and held there for varying periods depending on anticipated tide heights. Tidal height patterns for Yaquina Bay have been examined, and a series of windows during the first field season have been identified where a very low tide is followed by extended periods of higher low tide levels (corresponding to the times between spring tides). The plot below shows the heights of lower low tide versus date in the 1998 field season. Fish will be placed in a cage during a period of especially low spring tides (e.g. late May), and recovered during the next occurrence of equivalent low water levels, roughly 14 days later.



Choosing these dates for caging experiments will help ensure that the cages, and fish, are not exposed during intervening low tides (of course anomalous weather or river discharge conditions may alter predicted tide height, but these cannot be anticipated and countered).

Sample sizes (number of fish/cage) will initially be low (one or two per cage), and then revised upward as we gain an understanding of fish growth rate patterns and can be assured that fish growth is not depressed due to overcrowding of the cages. At the conclusion of a caging experiment the fish will be recovered, returned to the laboratory, and processed the same way as the fish captured during the trawl sampling: weighed, measured, and otoliths and tissue collected. An expectation of this research is that spatial differences in growth rate among cage locations will be detected. If this is found to be the case, then the magnitude of the observed growth rate differences will permit at least a qualitative assessment of how much between-location movement must occur to equilibrate the fish growth rates between sites, and this information can then further be compared with the growth rate variations observed among the free-living fish captured with the drop sampler or trawl.

The quantitation of fish tissue RNA and DNA content from both trawl samples and fish from caging experiments will follow the methodology developed by Burger (1998; his protocol is presently being revised to form a SOP). Briefly, the fish tissue is sonicated, then frozen, both of which disrupt the cells and free the nucleic acids. The sample is then treated with proteinase to further remove any nucleoproteins bound to the nucleic acids that might interfere with the assay. At this step the sample is divided into two equal aliquots. The fluorochrome dye thiazole orange is added to one of these aliquots. This dye binds to both RNA and DNA, and the amount bound to the nucleic acids is determined using a spectrofluorometer. The enzyme RNase is added to the second aliquot to digest the RNA in the sample, leaving the DNA behind. The DNA remaining in this sample is also determined using thiazole orange. The amount of RNA that was in the tissue is then calculated as the difference between the two determinations, and the ratio of RNA to DNA content is interpreted as an index of recent protein synthesis and growth.

Otoliths will be prepared and examined using the standard methodology described by Secor et al. (1991b). They will be cleaned, embedded in Spurr media, mounted on microscope slides, and polished until thin enough so that the daily increments that were deposited in them can be viewed using a video microscopy setup. The number of recently deposited rings in each otolith will be counted and interpreted as the number of days, while the widths of a span of one or more increments will be interpreted as proportional to the fish's growth during that time period. English sole, like other flatfish, form secondary otolith primordia when they metamorphose to the benthic form, and the increments formed around the secondary primordia are especially easy to count (Sogard pers commun.). Xiao (1996), Schirripa and Goodyear (1997), and Gallego and Heath (1997) provide mathematical analyses of the process under which otolith and somatic growth might be linked and recognizable, and their results will be used as guidance in this study.

The data from these experiments will be examined as follows:

1. The RNA:DNA ratio determined for a fish will be compared with the recent otolith increment growth of the same fish, to establish the degree of correlation between them. The two approaches provide different representations of recent fish growth, and each has its own advantages and limitations. There presently appear to be only a few studies that have made explicit comparisons between biochemical and otolith growth measures (Clemmesen and Doan 1996; Suthers 1996).

2. The RNA:DNA ratio and otolith growth information will be evaluated in both wild-caught and caged fish with respect to both bottom type (location) and date to characterize the spatial and seasonal patterns of juvenile English sole growth in Yaquina Bay. It is anticipated the same principal component analysis approach of sediment samples described previously can be usefully applied to sediment samples collected as part of this study, i.e. that grain size distribution can be summarized as a principal component axis score. The sediment PCA score can then be used as a metric describing the substrate that can be used in a correlative analysis of that score and sole growth data.

3. The expectation of this research is that, after controlling for season, the growth rates will differ significantly among locations in wild fish, and it will also differ significantly among locations in caged fish. If this is found to be the case, then comparisons between growth rates of caged fish and wild fish caught at the same location and time will be made with regard to the sign and magnitude of

their correlation. If wild fish are remaining near a site, and not moving among substrate types, then the expectation is that growth between these will be positively correlated: wild fish caught near rapidly growing caged fish will also have elevated growth rates, while fish caught near slow growing caged fish will have a similarly depressed growth rate.

4. The combination of growth rate information and substrate characteristics will be used to make inferences about how changes in sediment input, dredging, or other changes to the surrounding watershed that will potentially affect the substrate, might in turn enhance or diminish the growth of juvenile English sole while resident in the estuary.

5. Finally it is appropriate to also include an evaluation of the statistical properties of the observed growth data as an early outcome of the work proposed here. A detailed statistical design for the above measurements hasn't been provided because this work represents an effort to measure variability in growth rates, and since that variability is presently unknown it is not possible to define the sample sizes necessary for statistical comparisons. The continuing controversy over the utility of otoliths as a direct measure of growth has been mentioned previously. Although others have measured the RNA:DNA ratio in fish, the majority of the published work has been done on larvae and/or as tightly controlled lab experiments. Because of these considerations the proposed research must proceed adaptively. The anticipated approach is to continually reevaluate the data as it is acquired, so that the validity of statistical comparisons (or requisite sample sizes) can be ascertained as early as possible.

Expected Results and Benefits: The proposed research will examine a vital rate process in an important fish of Yaquina Bay, and will relate variations in that process to an attribute of the Bay (substrate composition). Sediment composition is subject to change from both natural and anthropogenic processes; the changes in substrate characteristics will affect changes in the English sole food supply that substrate supports. There is ample evidence in the existing literature that spatial differences in fish growth rate occurs, but these differences have not been studied at smaller spatial scales and have not been linked to environmental variations at those same scales. The methodology proposed for this study is well established, and the combined application of otolith and biochemical approaches at smaller spatial scales represents a significant strength of this research.

It is anticipated that the proposed research will document the relative sensitivity of juvenile English sole to habitat alterations. The substrate composition is viewed as an indicator of the juvenile English sole's habitat quality. This proposal does not include studies of infaunal community composition, or English sole feeding habits, in order to keep the scope of work within manageable limits. However, it is easily recognized that a suite of environmental factors associated with substrate composition, especially associated flora and fauna, will have bearing upon English sole growth. As a result, this study will integrate with companion EPA studies concerning infaunal and submerged aquatic vegetation community dynamics. Finally, the results of this research can be utilized by management personnel responsible for decisions concerning sediment input and alterations in Pacific Northwest estuaries.

4.2 Research Theme B. - Stressor-Response Modeling

Project B1. An Evaluation of the Geometry of Stress Using Spatially Explicit Population Models

Principal Investigator: Henry Lee II
Co-Investigators: Chuck Bodeen, Ted DeWitt

Goals: The long range research goal is to generate insights into how marine/estuarine populations respond to single and multiple anthropogenic stressors, including the interactions among the spatial and temporal patterns of the populations and the stressors. Multiple stressors denotes both multiple sources of the same stressor and the combined effects of multiple chemical and/or non-chemical agents. The primary objective is to evaluate the effects of the spatial distribution of single and multiple sources of a stress on the qualitative patterns of population dynamics of a representative benthic resource species.

Rationale: A frequently stated maxim is "dilution is the solution to pollution." Based on this strategy, tens if not hundreds of millions of dollars have been spent designing and constructing high performance diffusers for municipal and industrial discharges. Maximizing dilution is inherent in several sections of the Clean Water Act (e.g., Section 301H) that compare ambient water concentrations with Water Quality Criteria at the edge of a zone of dilution. However, dilution is not considered the solution to all pollution. Dispersal of plutonium is rarely, if ever, suggested as the solution to radioactive waste disposal. The management strategy for dredge materials is to minimize loss during dredging and disposal (see U.S. EPA/ACE, 1991). While a few dispersive sites exist (e.g., the Alcatraz dredge disposal site), the potential for containment is a prime consideration in choosing most dredge disposal sites, especially for materials containing sediment contaminants. Similarly, a containment strategy is inherent in most of Superfund's remedial actions.

The point is that two diametrically opposed disposal strategies have been used in the management of anthropogenic stressors in marine and estuarine environments. These two strategies potentially differ dramatically in cost and in level of protection afforded to ecological resources. For example, the costs and near-field versus far-field ecological effects of a sewage outfall designed to minimize diffusion would be considerably different from one with a high performance diffuser. Nonetheless, there does not appear to have been a systematic evaluation of "dispersal" versus "containment" strategies. One key component of such an evaluation would be to predict the effects of different strategies on the population dynamics and sustainability of high value species, the assessment endpoints (U.S. EPA, 1991). This research addresses a subset of this problem by posing the question, "What is the relative risk to populations of benthic resource species exposed to a dispersed versus a contained stressor?"

The proposed use of population assessment endpoints differs from most marine/estuarine risk assessments that are usually conducted using indicators, or measurement endpoints (U.S. EPA, 1991), at the individual level (e.g., toxicity benchmarks) or the community/ecosystem level (e.g., benthic community structure). In part, this is due to the difficulty in quantitatively sampling populations. Population models are one potential method of addressing this problem both by integrating available knowledge and as an exploratory tool. Besides the obvious example of fisheries models (e.g., Ricker, 1975; Hilborn and Walters, 1992; Higgins et al., 1997), population endpoints have been used to address a variety of anthropogenic stressors. Population models have been used to predict the effects of contaminants or thermal pollution on fish populations (e.g., Van Winkle, 1977; Power and Power, 1995; Black et al., unpublished), population demographics have been used in laboratory toxicology studies (Gentile et al., 1982; Levin et al., 1996; DeWitt, unpublished), and simulation studies have been used to predict the effects of individual and multiple stressors on fish populations (Barnthouse et al., 1987, 1989, 1990; Marschall and Crowder, 1996).

A standard assumption of most population models, such as the ones mentioned above, is of a single, well-mixed population. This approach is inappropriate for the question posed in this research, which inherently implies a non-homogeneous habitat. Point discharges, such as sewage outfalls, create strong stress gradients while dredge disposal forms "localized" patches of disturbance. Besides the limitations due to stressor heterogeneity, there has been a growing recognition of the role habitat heterogeneity and interconnectiveness among subpopulations have on population dynamics (e.g., Roughgarden and Iwasa, 1986; Hastings and Harrison, 1994; Karelva and Wennegren, 1995). Spatially explicit population models (SEPMs) are a recent advancement in this area, where SEPMs "combine a population simulator with a landscape map that describes the spatial distribution of the landscape features" (Dunning et al., 1995). SEPMs are emerging as important management tools for terrestrial populations in fragmented habitats (e.g., spotted owls). However, this approach has been applied to only a few marine/estuarine resource species (Quinn, et al., 1993; Botsford et al., 1994; Botsford, 1995).

To address the relative risk associated with these dispersive versus containment disposal strategies, we propose to develop a SEPM. This proposal describes a two-year research project using this SEPM to generate insights into the relative ecological risks associated with exposure scenarios with different spatial configurations of single and multiple sources and to identify the relative importance of various environmental and demographic parameters in determining these risks. By focusing on multiple sources, this research is responding directly to the Agency's need for methods for assessing cumulative risk (U.S. EPA, 1997a).

Objectives : The goal of the research is to elucidate general patterns and relationships and not to predict population trends with sufficient accuracy for management of the target species. The research is framed in the context of a generalized stressor, though it can be conceptualized as a fixed mass of a contaminant discharged into a body of water. Given this mass of contaminant, what geometry of the pollutant source(s) (e.g., discharges, sediment hot spots) minimizes effects on the target population? The specific questions addressed are:

> Under what exposure scenarios should stressors be concentrated versus dispersed to minimize effects on benthic populations?
>
> What demographic characteristics determine whether benthic populations are more sensitive to a dispersed versus a contained stressor?
>
> What characteristics of the stressor determine whether benthic populations are more sensitive to a dispersed versus a contained stressor?
>
> How does the optimum mitigation strategy for maintenance of benthic populations vary with the total intensity of the stressor (e.g., total mass of contaminant)?

METHODS:
I. Model Overview: A theoretical model is used to address the above questions. A theoretical model allows us to focus on discovering the general qualitative patterns rather than on the details of a particular pollutant, species, or site. A theoretical approach also facilitates the integration of information and relationships from a diversity of pollutants and species. Addressing the effects of the geometry of stress requires sub-models for the habitat, stressor dispersal, stressor-response function, and population dynamics. To the extent practical, these sub-models are dimensionless, so many parameters are unitless and functions related to density are normalized to the equilibrium density. Detailed mathematical formulations are given in the Appendix while the model parameters are summarized in Appendix Table 1 and key assumptions are summarized in Appendix Table 2.

The habitat is modeled as independent, identical cells. In Phase I the habitat is models as a one-dimensional space while in the other Phases it is modeled as a two-dimensional space with circular symmetry. In Phase I the cells are of unit area spaced one unit apart along a line (i.e., a linear habitat). In other Phases cells are of unit area and placed in annular rings. This means that at radius **r** there are $\mathbf{2\pi r}$ cells. Fractional cells are accounted for by the numerical integration process (see Appendix).

The circular model was chosen for simplicity of analytical solutions and ease of computer programming. Every cell at radius ρ from a source is exposed to the same level of stress from that source. In spaces based on squares or hexagons, the number of cells at a particular distance from the source is a complicated function. If there are compelling reasons, the results calculated with our annular model can be translated into hexagonal cells within a nearly circular domain after the fact, but with additional programming.

The habitat is initially assumed to be homogeneous except for spatial differences in anthropogenic stress. Using life history characteristics for an equilibrium population, an equal number of the target species, the clam *Mya arenaria*, are introduced into each cell. Recruitment is simulated by summing the larval output from each cell into a single larval pool, which is evenly distributed among all cells. An age-structured population model (Leslie matrix) is used to predict natural population dynamics and the effects of anthropogenic stress.

A generalized stressor is then imposed in a "source" cell (point source). Depending on the exposure scenario, a single or multiple source cells will be distributed throughout the habitat. Stress level decays exponentially with distance from each source cell, with the steepness of the decline determined by a lapse rate. The stress level at the origin is adjusted so that the total stress from each stressed cell in the habitat (summed across all cells) is constant among the different exposure scenarios, which can be conceptualized as a fixed mass of a contaminant discharged into an estuary. This constraint makes the stress level at the origin a function of the lapse rate. For example, under high lapse rate (high dispersion) the stress (e.g., the fixed mass of contaminant) will be widely dispersed over the habitat resulting in a lower stress level in the source cell. Once the stressor level in a cell has been determined, its effect on post-metamorphic survival and/or fecundity is predicted using a logistic stressor-response function.

Both spatial and temporal variability and density-dependent interactions will be phased in as the research progresses, thereby relaxing many of the initial simplifying assumptions. Simulations will be used to predict the relative effects of different geometries of the stress (source cells) on population dynamics and sustainability. In the simplest case, all the stress is introduced into a single source cell. Other exposure scenarios simulate the stress being "discharged" into multiple source cells with different decay rates. The importance of exposure, stressor, and life-history parameters on population dynamics will be assessed by sensitivity analyses. The ultimate goal of the research is to approximate the stress pattern in the Southern California Bight, San Francisco Bay, and/or Yaquina Bay.

II. Research Phases: Our strategy is to divide the two-year research project into discrete conceptual and programming phases that will be addressed sequentially (Appendix Table 3). Phase I includes problem formulation and development of a one-dimensional model. These tasks have been or are nearly completed. Phase II develops the techniques for the two-dimensional model and derives preliminary functions for density dependence. These are ongoing tasks that will be completed by the end of April - early May 1998. Phase III begins the simulations with adult-adult density-dependent interactions but no stochastic variation. Stochastic variation on benthic habitat quality and life history parameters will be incorporated in Phase IV. We anticipate completing most of these tasks by late August 1998. Phase V incorporates adult-juvenile and larval-larval density-dependent interactions with a monthly time step. Because of the potentially large number of simulations, we do not anticipate completing this phase before the end of November or December 1998. In particular, comparing predicted and observed temporal variability may take longer. The incorporation of actual field data in Phase VI is the final suite of tasks, and is the most problematical both because it builds upon all the other phases and because of the need to gather and analyze field data from a variety of sources. We anticipate finishing the simulations in this phase by the end of June, 1999. As mentioned in the Budget/Resources section, full completion of Phase VI may require additional resources. These phases are a "road map" to help guide the research and will be modified as needed.

III. Habitat and Stressor Distribution: The model domain is broken into cells that represent uniform, independent sections of habitat. The size of the cell is sufficiently large that migration of post-metamorphic *Mya* among cells is assumed to be negligible. In the two-dimensional configuration, the model domain is a circle with a radius of 100 units (see Figure A-2 in Appendix). A circular domain was chosen to simplify the analytical calculations though the techniques can be adapted to rectangular, hexagonal, or even spatial approximations of actual estuaries. The assumption that the natural (unstressed) habitat quality is homogeneous across all cells will be relaxed as spatial and temporal variability are incorporated into the model (Appendix Table 3).

A generalized anthropogenic stressor is applied to one or more source cells. The source is assumed to be constant so the level of stress does not vary over time. Each source cell can be conceptualized as a point source, such as sewage discharge or contaminated sediment hot spot, emanating pollutants in all directions. The stress level in surrounding cells decays exponentionally from each source cell. Rapidity of the decay over space is determined by the lapse rate, bs. In the one-dimensional case, the normalized stress level in cells surrounding a source cell (cell 0) is calculated as:

$$S© = S_o e^{-c\ bs}$$

where:

S© = stress level in cell c
S_o = stress at the source cell
c = cell number (0-100 indicating distance from source cell)
bs = lapse rate

The Appendix gives the methods for calculating stress levels in the two-dimensional case and for multiple source cells with overlapping stressor distributions.

So as not to confound the effects of total stress and the geometry of stress, the total amount of stress in the model domain is constant regardless of the number of source cells. This can be conceptualized as discharging a fixed mass of effluent into an estuary. As more outfalls are added, the discharge per outfall declines proportionally but the total mass of contaminants remains constant. Total stress is a more appropriate measure to predict wide-spread effects than a localized measure of stress level, such as the pollutant concentration at the edge of the zone of dilution. For example, Mearns and O'Connor (1984) showed that the spatial scale of impacts on marine/estuarine environments was related to the mass discharged for a variety of pollutants.

One complexity with multiple source cells is how to calculate the dispersion of stress when some source cell is not in the center of the habitat (circle). The stress from such a source is dispersed exponentially over the circular domain, but is truncated at the domain edge. The strength of this source is determined by the fact that it contributes the same fixed mass of effluent as if it were at the center of the circle. This can be conceptualized as having two sewage discharges, one in the center of the estuary and other at the edge. Since the effluent can not leave the estuary (model domain), the effluent concentrations in cells surrounding the discharge at the edge are higher than those surrounding the discharge in the center. Mathematical details on addressing this problem are given in the Appendix.

Our initial approach to varying the spatial pattern of the stress is to expand the number of source cells at the center of the habitat in a geometric series, resulting in an expanding "circle" of source cells. At the limit, all of the cells in the domain are stress sources, with each receiving stress from all of the others. A major objective of these single-source simulations will be to determine how the effects of dispersing a stressor spatial geometry of the stressor interacts with stressor characteristics (e.g., slope of stressor-response function - see below) and population characteristics.

As multiple sources are added, the stress from each will be normalized so that the total stress to the domain will be constant in each scenario (e.g., fixed mass of effluent discharged through multiple outfalls). We will evaluate various exposure scenarios using different spatial configurations of source cells coupled with different decay rates. For example, we could evaluate the effects of locating discharges along the boundary of the estuary versus in the center with high and low decay rates. Another example is evaluating the effects of clustering multiple dredge disposal sites in a sector versus distributing them equi-distant throughout the estuary. Our ultimate goal is to approximate the distribution of stress patterns in San Francisco Bay, Yaquina Bay, and/or Southern California Bight, as discussed below.

IV. Population Biology of Target Species: While it is not our purpose to forecast population changes in any particular species, the model requires life history characteristics of a "target" species. We focus on commercial/ recreational species because they are important to the public and are heavily weighted in risk management decisions. The difficulty with mobile species, such as fishes and crabs, is their duration of exposure becomes a confounding factor that we felt was best not addressed in this initial effort. Of the potential clam species, *Mya arenaria* was chosen because of the availability of age-specific vital rates (Brousseau, 1978; Brousseau et al., 1982), including an estimate of equilibrium population structure and "larval" mortality (Appendix Table A-1). *Mya* is an important commercial species on the Atlantic coast and a recreational species on the Pacific coast, though historically there has been commercial fishing in Grays Harbor (David Armstrong, pers. comm.). On the Pacific coast, *Mya* occurs from Vancouver Island to San Diego and is locally abundant in several estuaries, including Yaquina Bay and San Francisco Bay.

Since we are not attempting to simulate *Mya* per se, we may modify the life history characteristics reported by Brousseau to simplify the modeling as needed. For example, when we go from the yearly time step to a month time step (Appendix), we may assume that the larval period lasts only one month. We recognize that life-history parameters can vary with habitat (e.g., Appeldoorn, 1995). By conducting a sensitivity analysis on the life-history parameters, it should be possible to determine to what extent spatial/temporal variations in *Mya*'s vital rates qualitatively affect the conclusions. These results will also offer insights into the confidence in extrapolating the conclusions to other clam species with generally similar life history characteristics. Because of fundamental differences in life histories, extrapolation to taxa that brood their young or are highly mobile is problematical.

V. Population Model: Leslie Matrix Model: An age-structured Leslie matrix, is used to model the population dynamics of *Mya*. The Leslie matrix predicts the population size in the next generation from age-specific vital rates for survival and fecundity (see Emlen, 1973; Caswell, 1989; Akçakaya, 1997). (An alternate model is a stage matrix which groups individuals by size or biological stage (egg, pupa, adult) instead of age). Anthropogenic effects are expressed through alterations in these vital rates. Leslie or stage matrix models have been applied to a variety of terrestrial and aquatic populations (e.g., Van Winkle, 1977; Caswell, 1989; Emlen 1989), including estuarine bivalves (Brousseau et. al., 1982; Weinberg, 1985, 1989). Recently, the approach was used to assess stressor effects on a marine polychaete (Levin et al., 1996).

Emlen (1989) concluded that matrix models "work well over broad circumstances." Though Power and Power (1995) concluded that an individual-based model (IBM) for brook trout was better than a Leslie matrix model, the Leslie matrix actually predicted total population size slightly more accurately. In any case, most of the differences were only on the order of a few percent. The U.S. EPA Risk Assessment Forum (U.S. EPA, 1991) concluded that Leslie or stage matrix models coupled with sensitivity analyses can be used to: 1) understand the effects of changes in the vital rates on population dynamics; 2) estimate the direct effects of stressors on population dynamics; 3) serve as a tool for evaluating alternative management strategies; 4) serve as a framework for interpreting the consequences of acute and sublethal stressors; and 5) provide a method of incorporating demographic properties into ecological risk characterization.

Application of Leslie Matrix Model to Mya: Details of applying the Leslie matrix to *Mya* and of incorporating a generalized stressor are given in the Appendix, while key assumptions are summarized in Appendix Table 2. Two specific modifications are summarized here. First, the production of female eggs from each cell is summed into a common "larval pool." It is initially assumed this larval pool is completely mixed and that the surviving larvae are distributed equally among all the cells. In other words, there is equal recruitment into each cell, though not necessarily equal survival of recruits as discussed below.

The second modification is a result that Brousseau et al. (1982) used a mesh too large to capture the 0-2 month post-settlement class (i.e., early recruits) when sampling the benthic population. Therefore, their calculated estimate of "larval" survival at equilibrium (their r_{seq} our p_{peq}; Appendix Table 1) includes both larval and early juvenile (post-settlement) mortality. Modeling adult-juvenile or larval-larval density-dependent interactions requires partitioning this total mortality, though as pointed out by Rumrill (1990), no one has accurately partitioned larval and early juvenile mortality. To address this uncertainty, we define a ratio (R_{jp}) of survival in the 0-2 month class to survival in the larval phase. R_{jp} changes the partitioning of the mortality among life history stages, not the total amount. Values for R_{jp} will be based on literature values for larval survival (Rumrill, 1990), though these reported values are likely to be lower estimates either because the studies did not cover the full larval stage or did not include losses due to unfertilized eggs.

Inhouse Model and RAMAS-GIS: We initiated this research with one of us (C.B.) writing a Fortran program to calculate Leslie matrices. Since then, we have obtained RAMAS-GIS (Akçakaya, 1997), which links age-structured population models with spatial data on habitat quality. Strengths of RAMAS-GIS include the number of built-in functions and links to GIS data. While a powerful program, the present version does not meet all our requirements. One limitation is that RAMAS-GIS is limited to 160 populations (= subpopulations in cells), which is insufficient for our two-dimensional simulations. Another limitation is that RAMAS has an upper limit of 20 x 20 age- or stage-matrices, which is insufficient if we use a month time step for *Mya*, which requires a 144 x 144 matrix (12 year life span of *Mya* X 12 months/year). An alternative to the month time step would be to convert the data to a stage matrix, using larvae and early juveniles as stages. However, converting the existing Brousseau Leslie matrix to a stage matrix would be difficult, or impossible, even for an unstressed population. For these reasons, we will continue the development of the inhouse program as the primary tool for this research. As appropriate, we will use RAMAS-GIS to address specific questions. To date, we have used RAMAS-GIS as a QA check on our inhouse program, and the comparisons have been excellent.

VI. Stressor-Response Function: A unitless generalized anthropogenic stressor is used to simulate the effects of chemical pollutants (e.g., neutral ogranics, heavy metals) as well as many non-pollutant stressors (e.g., temperature, hypoxia, organic enrichment). Natural mortality and the additional anthropogenic mortality are assumed to be independent. Therefore, their combined effect is calculated as the product of the survival rates and the order in which the stresses are applied does not affect the total mortality. The assumption of independence could be violated if one stressor increased susceptibility to new stress (e.g., pollutant reducing predator avoidance) or if one of the stressors operates in a highly density-dependent fashion (e.g., invasions by exotic). These cases will not be explored at this time.

The logistic function (Barnthouse et al. 1989, 1990) will be used as the stressor-response function for the generalized stressor. The specific equation used (equation #8 in Barnthouse et al. 1990) is:

$$\boldsymbol{P} = e^{(\alpha + \beta x)} / [1 + e^{(\alpha + \beta x)}]$$

where

$\boldsymbol{P}$ = fractional response in population
α = fitted parameter
β = fitted parameter
$\boldsymbol{X}$ = log10 concentration of contaminant

As explained in the Appendix, $\boldsymbol{x}$ is transformed from concentration to a unitless level of the generalized stress normalized to the stress level the source cell. The parameter α determines the extent of the effect (e.g., mortality) for a given stress level (e.g., toxicity of a discharge for a given concentration). β determines the slope of the stressor-response curve, with larger βs resulting in steeper stressor-response curves. Stressors with a high β have a narrow range between no or minimal effect and high effect. By altering α and/or β, it is possible to generate a family of sigmoid curves describing the dose-response relationship for individual pollutants, complex mixtures, and selected non-chemical stressors (e.g., temperature).

We will initially assume that all adult stages ($\geq$ 1 year) have the same sensitivity to the stressor. However, the effect of increased sensitivity of juveniles (<1 year) will be examined by increasing the value of α relative to the value used for adults while maintaining β constant. The importance of acute versus sublethal effects will be examined by varying the values of α for mortality (α_q) and fecundity (α_m). We will review the literature to obtain acute/chronic or acute/sublethal ratios as guides for the relative difference in sensitivity in these two vital rates.

The steepness of the stressor-response function, as determined by β, is likely to be a major driver in determining the cumulative effect of low to moderate levels of stress. Barnthouse et al. (1989) determined the range in β for mortality based on 77 chronic exposures of fish to organic pollutants or metals. They found no significant difference in the β values for eggs, larvae, and post-larval life stages, so the same range will be applied to all life history stages of *Mya*. Barnthouse et al. (1989) did not evaluate sublethal effects but as an initial assumption we will apply the same range in β to stressor effects on fecundity.

At this stage, it is assumed that the generalized stressor does not cause larval mortality. In general, water column effects should be minimized because of the high dilution with overlying water in marine/estuarine systems. One of us (H.L.) reviewed numerous 301H applications (variance for secondary sewage treatment in marine/estuarine waters), and effects on phytoplankton or larvae were rarely, if ever, documented. Even if there are effects on the water column from a discharge or contaminated sediment, they are likely to be localized around the source and thus have a negligible effect on overall larval survivorship.

It is important to recognize limitations of the stressor-response function as used in this model. First, the model does not consider indirect effects. Indirect effects may be physiological in nature (e.g., increased sensitivity to low salinity due to pollutant stress) or ecological (e.g., competitive release). To the extent that indirect effects are important, the model predicts the potential effects on the population rather than an actual population trajectory. Because the objective of the research is to rank order exposure scenarios, the occurrence of indirect effects alters the conclusions only if they change the relative risk associated with various exposure scenarios. For example, the ability of a pollutant-sensitive keystone predator to regulate the population of the target species may be a function of the scale of the contaminated area, and hence the lapse rate of the stressor. No attempt is made to simulate these complex ecological-toxicological interactions at this time. Second, the logistic function is likely to be inappropriate for many biotic stressors (e.g., exotic species) because of non-linear effects and feedbacks. The third case is habitat alteration, which is used here to denote multi-generational changes in the "natural" physical/chemical properties of the environment. Short-term changes (e.g., low salinity after a flood) would be modeled using the logistic stressor-response model. Long-term changes probably would be better modeled by redefining habitat quality by altering the natural rates of survival and fecundity.

VII. Density-Dependent Interactions: Density-dependent interactions can occur during three life-history stages in benthic organisms: larval, early juvenile, and adult ($\geq$1 year). Since arbitrary densities are used, density dependence will be scaled to the equilibrium densities of larvae or adults, as appropriate. Our strategy is to address adult-adult interactions first as these can be modeled using a yearly time step. Later in the research, we will incorporate adult-juvenile and larval-larval interactions, both of which require a monthly time step to be able to separate larval and early juvenile densities. In general, we will attempt to bracket the potential importance of density-dependent interactions by modeling three levels: none (base case), moderate, and high. If inclusion of density dependence qualitatively changes the conclusions, more detailed simulations will be conducted. The specifics for each life history stage are given below.

Adult-Adult Density-Dependent Interactions: Field experiments have documented cases where growth rates, fecundity, and survival of benthic organisms have declined at densities approximating natural levels (e.g., Peterson, 1982; Peterson and Black, 1988; Peterson and Black, 1993; Olafsson et al., 1994). It appears that fecundity is more sensitive to "crowding" than survival, so we will primarily focus density-dependent effects on fecundity and secondarily on adult survival. Because of the impracticality of converting the existing age-based Leslie matrix for *Mya* to a size-based stage matrix, we do not consider effects on growth. The direct changes in fecundity should capture indirect effects growth rate, and size, would have on fecundity. Based on Peterson's (1982, his Fig. 5) results with the clam *Protothaca staminea*, a ramp function is used to approximate the density-dependent effects on fecundity (Appendix). We will use Peterson's (1982) work and other lab/field experiments reported in the literature to bracket the input values for the ramp function.

Adult-Juvenile Density-Dependent Interactions: Adults clams may reduce recruitment success by directly preying on settling larvae, altering habitat suitability for early recruits, or through competition. While the evidence for intraspecific adult-juvenile interactions at natural densities is mixed (though very early recruits often are not sampled quantitatively), Olafsson et al. (1994, page 95) concluded that "there is some evidence that juvenile invertebrates also experience density-dependent mortality as a consequence of food limitation." Therefore, we will model survival of the youngest benthic age class (= recruitment success) as a function of the localized adult ($\geq$1 year) density. Unless a better function is found in the literature, we will assume a ramp function to describe this interaction. At this stage we will not consider facilitation, where adults enhance recruitment (e.g., Peterson and Black, 1993).

Larval-Larval Density-Dependent Interactions: Density-dependent interactions affecting larval survival are a function only of the size of the larval pool. Fisheries scientists have spent considerable effort evaluating larval density-dependent interactions and models (e.g., Ricker, 1973, 1975; Hall, 1988; Hilborn and Walters, 1992; Higgins et al., 1997). Given the current state-of-the-science, our approach is to use functions generally accepted among fisheries scientists, in particular the Ricker and the Beverton-Holt equations (Ricker, 1973, 1975). Akçakaya (1997) suggests that the Ricker equation better describes contest competition while the Beverton-Holt equation better describes scramble competition. Both functions will be used in selected cases to determine whether the nature of the relationship qualitatively affects the conclusions.

VIII. Spatial and Temporal Variability: Spatial and temporal variation is inherent in estuarine and marine populations and environments (e.g., Ulanowicz et al., 1982; Lipcus and Van Engel, 1990; Nelson and Virnstein, 1995). This variation can have "deterministic" components, such as the spatial patterns of habitat types, and stochastic components. While recognizing these sources of uncertainty, we will initially focus on deterministic, homogeneous models, which allows us to identify key variables and qualitative trends before adding additional layers of programming and conceptual complexity. After completion of the first suite of deterministic simulations, temporal and spatial variability in the "natural" vital rates will be phased into the simulations (Appendix Table 3). This natural variation is in addition to any variation due to the effects of the anthropogenic stressors.

Considerable effort is required to incorporate stochastic variation with precision. Akçakaya (1997) calculated that approximately 1000 simulations are needed to generate results with a 95% confidence interval of about ±3% in models incorporating environmental stochasticity. To keep the number of simulations to a reasonable number, the importance of parameters related to stressors (e.g., lapse rate, β) will be evaluated primarily through deterministic sensitivity analyses (Appendix Table 1). Parameters defining natural habitat quality and the associated vital rates will be varied both deterministically and stochastically. Because many of these vital rates are likely to have small mean values and high variation, a lognormal distribution for the rates is likely to be used (see Akçakaya, 1997). As appropriate, we will follow the EPA guidance on Monte Carlo analysis (U.S. EPA, 1997b).

The indicators used for habitat quality and the types of variation in are discussed below. The sources are broken into classes for clarity but several sources of variation will be combined in simulations (Appendix Table 3).

Indicators of Habitat Quality: Survival of early recruits and fecundity should be sensitive indicators of benthic habitat quality in terms of food availability, sediment suitability, physical-chemical environment, and interspecific interactions. Therefore, we will model the effects of spatial and temporal variation in benthic habitat quality primarily through changes in these rates, and secondarily on adult survival. Recruits are defined either as the 0-year class or the 0-2 month class, depending on whether yearly or monthly time steps are used, respectively. Survival rate is the only measure of habitat (water column) quality used for larvae. A more realistic approach that may be explored is to use instantaneous rates of mortality (see Rumrill, 1990) and make duration of the larval phase a function of habitat quality (e.g., temperature).

Spatial Variation in Benthic Habitat Quality: The first step will be to impose stochastic variation on the homogeneous benthic habitat, simulating within-habitat variation. Survival of recruits and fecundity within each cell will be varied randomly. The equilibrium values will be used for the means and variance estimates will be derived from studies of within-habitat variation, though we recognize that such estimates can be scale dependent. We assume that fecundity and juvenile survival are correlated (i.e., what is good for adults is good for juveniles), though different scenarios will be explored as time permits. Both spatial stochasticity and adult-adult density dependence will be imposed concurrently in these simulations. A refinement is to impose a spatial pattern of differing habitat types (e.g., high intertidal, deep water) on the model domain. Each habitat type would have different qualities as expressed through the vital rates. The habitat types would be discrete, though it is possible, and more complicated, to model gradations in habitat quality. Stochastic variation could then be imposed upon these habitat types.

We will explore the possibility of modeling habitat quality in San Francisco Bay, Yaquina Bay, or the Southern California Bight. Habitat types would be ranked for quality for bivalves based on expert judgement using factors such as water depth, salinity, and substrate type. Although this approach is admittedly crude, it is an initial step in incorporating field data. The specific geographic area would be chosen based on data availability, the extent to which the site conforms to the model assumptions, and Agency needs. Habitat data for Yaquina Bay are available from previous studies and from ongoing research projects at the Coastal Ecology Branch (CEB). Data for San Francisco Bay are available from the various Region 9 monitoring programs, previous research programs (e.g., Lee et al., 1994; NOAA, 1997), the San Francisco Estuarine Institute, and the CalFed program. Data from the Southern California Bight are available from the 1994 and 1998 Southern California Bight Monitoring Study (Janet Hashimoto, pers. comm.) as well as the extensive studies conducted around the Southern California outfalls (e.g., Swartz et al., 1986; Ferraro and Cole, 1990; Stull, 1995; ongoing Region 9 Superfund assessment of Palos Verdes shelf). Field data would be incorporated into the model through ArcInfo, which may require using RAMAS-GIS. Though it is a goal to simulate one or more of these sites, we will be cautious not to "get lost in the detail" for any particular site.

Spatial Pattern of Larval Recruitment: In the methods described above, benthic conditions drive the spatial variation in recruitment. Another approach to relaxing the assumption of homogeneous recruitment is to randomly distribute the larval pool among cells. This approach varies the supply of larvae to each cell, simulating stochasticity due to localized circulation patterns. The two approaches can be combined to generate a total spatial variation in recruitment.

<u>Temporal Variation in Benthic Habitat Quality:</u> Benthic habitats can vary from year to year due to climatic conditions affecting factors such as microalgal production or sediment type. This temporal variation in habitat quality will be modeled by randomly varying fecundity and juvenile survival among years. As with spatial variation, year-to-year variations in fecundity and recruit survival will be co-varied. Temporal variation will be applied uniformly across all cells and habitat types.

<u>Temporal Variation in Larval Survival:</u> Year-to-year variability in the recruitment of a variety of fishes and epibenthic invertebrates (e.g., Ulanowicz et al., 1982; Lipcus and Van Engel, 1990) suggests that temporal variation in larval survival has a greater impact on population dynamics than temporal changes in benthic habitat quality. Therefore, we will place a greater effort on capturing this source of uncertainty. This effect will be simulated by stochastically varying survival of the larval pool among years. Landing records for *Mya* or similar bivalves (e.g., 1890-1992 Maine soft-shell clam landings available on http://www. maine.com/mer/bpproj.htm; Ulanowicz et al., 1982) will be used as a guide to the variability in these vital rates. Landing records include variation due to both larval survival and survival to marketable size classes, so it will be necessary to estimate the relative contributions of these two components.

Stochastic variation can operate either in a density-independent or density-dependent fashion. In the former, the natural larval survival rate is varied randomly from year to year. By varying the probability of survival from the larval pool to the 3-12 month class (P_{peq}), it is possible to impose this type of variation while using the one-year time step. With density-dependent variation, the larval carrying capacity (input into the Ricker or Beverton-Holt equations) is varied randomly from year to year. This approach requires a monthly time step. It is not obvious which of these is more realistic or whether they would generate qualitatively different conclusions.

IX. Model Outputs and Analysis: The primary objective of this research is to generate rankings for the risk associated with various spatial distributions of stressors. To achieve this objective, we will examine a suite of population metrics that emphasize different life history characteristics or aspects of population dynamics. Qualitative agreement among outputs would increase our confidence in the conclusions. Disagreement would suggest that the results are sensitive to model assumptions or that different components of population dynamics respond differently to the spatial distribution of stress. In either case, further analysis would be needed.

Population Size and Age Structure: Total population size and age structure will be examined at specific times (e.g., 5, 10, 15 years) under various exposure scenarios. Additionally, the temporal variance in population size and vital rates will be examined since strongly fluctuating populations may have a greater probability of extinction. The effects of anthropogenic stress on the spatial pattern of the population will be examined by plotting population density on a cell-by-cell basis at various time steps. We will explore the possibility of importing the model outputs into geostatistic software for spatial analysis, including calculating varigrams and plotting out changes using kriging techniques (see Rossi et al., 1992). Changes in age structure of the total population and subpopulations (individual cells or spatial aggregates of cells) will also be examined, especially in relationship to the equilibrium age structure and densities of "marketable" clams.

Population Finite Rate of Increase: A population's finite rate of increase is a predictor of its trajectory. Compared to population size, the finite rate of increase integrates the anthropogenic effects on age structure and age-specific fecundity. Although changes in the finite rate of increase must be interpreted with caution especially in terms of projecting long-term population trends (Akçakaya, 1997), it is useful in analyzing near-term trends. It can be calculated from the Leslie matrix for the total population or subpopulations. These values are amenable to the same types of temporal and spatial analyses as population size. In addition, the relative importance of changes in specific vital rates on the finite rate of increase will be determined by sensitivity analysis (Weinberg, 1985; Caswell, 1989).

Quasi-Extinction: The susceptibility of a population to extinction is of direct management relevance. Because the model uses arbitrary values for population size, it cannot predict absolute extinction. Calculating quasi-extinction is possible, however, which is the probability that the population will fall below some threshold size. The thresholds will be set at decimal fractions of the equilibrium population size (e.g., 0.001). The deterministic simulations generate predictions, not probabilities, of whether the population will fall below the threshold, while the stochastic simulations generate probabilities of quasi-extinction within a specified time.

Size of Larval Pool: The size of the larval pool represents the total reproductive output or "fitness" of the population. As such, it is one indicator of the ability of the population to recover from stress and to colonize denuded or new habitats. How well this indicator predicts population trends is likely to depend on the nature and strength of density-dependent interactions.

Contiguous Areas with Population Above Management Thresholds: As time permits, we will examine two management thresholds. The economics of exploiting a resource such as *Mya* depend, in part, on the spatial distribution of "dense" beds. If dense beds are aggregated, costs associated with locating and fishing will be minimized. In contrast, if the dense beds have a "checker board" distribution, these costs will increase. This economic endpoint will be qualitatively evaluated by determining the fragmentation of dense beds as a function of stressor geometry. Various values will be used for the definition of "dense" and of the minimum fishing area (management unit). These analyses will be conducted by importing the model outputs into ArcView.

Due to public concern, it may not be possible to allow a localized population to fall below some minimum value. The public may not accept a "dead zone" regardless of population viability. To determine if the conclusions are sensitive to this political reality, we will evaluate the effect of maintaining minimum population densities on the conclusions. These analyses resemble the economic-based analyses except that they would use different thresholds and different scale for defining management units. Again, these analyses will be conducted by importing the model outputs into ArcView.

X. Model Validation: Approaches to Model Validation: The proposed model can be "validated" in several ways. The most straightforward is algorithm validation in which the specific mathematical functions are shown to produce the correct outputs. This ongoing QA/QC is being conducted by comparing the inhouse model predictions with those of RAMAS-GIS and by reproducing outputs for functions to the published results (e.g., duplicating the life history of *Mya* to that published in Brousseau et. al., 1982)

One approach to validating ecological models is given by Caswell (1976) who distinguishes between theoretical models, whose goal is to generate insights, and predictive models, whose goal is to forecast. Caswell concluded that statistical comparison of outputs to time series of data is not applicable with theoretical models. Rather, he suggests testing hypotheses generated by the model. Because of the complexity, it is often more practical to evaluate predictions from sub-models rather than the overall model. Caswell also suggests explicitly comparing alternate models. Though Caswell does not believe that sensitivity analysis can validate the "truth" of a theoretical model, it can generate estimates of uncertainty (see Miller, 1974; U.S. EPA, 1997b). We will combine these approaches to derive a qualitative estimate of confidence in the model predictions (Appendix Table 3).

Sensitivity Analysis and Stochastic Variation: As previously stated, deterministic sensitivity analyses will be conducted on a suite of parameters and stochastic variation will be added to another suite. These analyses will determine how robust the conclusions are to various assumptions or model inputs.

Alternate Models: Several sub-models are amenable to comparisons of alternate configurations. Generating the same, or very similar, predictions with different sub-models increases confidence in the robustness of the conclusions. The alternate sub-models will be evaluated on selected scenarios that capture the range in the biological and exposure parameters. The following alternate sub-models will be considered for evaluation:

> Stressor-Response Function: probit, quadratic (simulates hormetic or protective dose range), empirical dose-response function for sediment contaminants derived from Southern California sewage discharges (see below).

Stressor Decay from Source: Inverse relationship (stress proportional to 1/(distance to source))

Larval Density-Dependent Interactions: Ricker equation, Beverton-Holt equation.

Adult-Adult and Adult-Juvenile Density-Dependent Interactions: Alternate model(s) to the ramp function will be determined from a literature search.

Hypothesis Testing: The most rigorous, and most difficult, type of validation is testing model hypotheses. While conducting field or mesocosm experiments to test predictions from the overall model is theoretically possible, such tests would be prohibitively expensive. Therefore, any hypothesis testing must be conducted using available data sets and, most likely, on sub-models. We will test the following hypotheses, which are ordered in approximate order of their ability to reject the model or sub-model.

Population Variation: There is no significant difference in the coefficient of variation (or other measure of temporal variation) between that predicted for the stochastically varying unstressed population and that observed in natural populations of *Mya*. Predictions from the Leslie matrix would be compared with long-term catch records for *Mya* (e.g., Ulanowicz et al., 1982; http://www.maine.com/mer/bpproj.htm). The limitation of this test is that it is likely that the same data used to bracket input values would be used to evaluate the hypothesis, so this approach blends into curve fitting. An independent test is possible only if two independent data sets from geographically similar areas can be found.

Stressor Dispersion from Source Cell: There is no significant difference between the shape of the one-dimensional stressor distribution predicted by the exponential lapse rate and the observed pattern for sediment pollutants along a gradient away from Southern California sewage discharges. The Southern California outfalls will be used both because of the extensive data and because of the relatively uniform habitat along the 60 meter contour (e.g., Swartz et al., 1986; Ferraro and Cole, 1990; Stull, 1995).

Relationship of Population Distribution to Stressor Distribution: There is no significant difference in the spatial pattern of relative population density predicted by the one-dimensional model and the pattern in benthic populations observed in a gradient away from Southern California sewage discharges. The specifics of how to conduct this test need to be developed further, but our preliminary idea is to use the empirical relationship between population density of target benthic species and sediment contamination along an outfall gradient to develop an empirical "dose-response" function. The dose may be expressed as the summation of toxic units to account for mixtures of pollutants. Substituting this empirical dose-response for the logistic stressor-response function, the model would then be used to predict the shape of the population distribution along the gradient from a second, independent outfall. Comparison of the predicted and observed population patterns would evaluate the robustness of the model to predict responses at different sites with similar stressor types.

Population Extinction: The most rigorous test would be to evaluate model predictions concerning population extinction (or quasi-extinction) and stressor geometry. We will review the literature to determine if such data sets exist.

Expected Results and Benefits: One immediate benefit of the proposed research would be the development of a SEPM that captures the key characteristics of benthic populations and the direct effects of many of the dominant stressors in marine and estuarine ecosystems. This tool would be available for use by the CEB and Region 9. The model is a research tool and additional development would be required to make it "user friendly." Another near-term output is a draft of peer-reviewed publication on the effects of the geometry of stress which will be completed in FY98 or early FY99. Additional publications will follow in FY99 and FY00.

The proposed research directly addresses the Agency's need for the development of cumulative risk assessment methods. Regions 9 identified multiple stressors and cumulative effects as one of their priority scientific needs. The Agency's guidance on cumulative risk assessment (U.S. EPA, 1997a) stated that "... EPA's risk assessment emphasis has shifted increasingly to a more broad based approach characterized by greater consideration of multiple endpoints, sources, pathways and routes of exposure ....." In future research, the model could be modified to address other components of cumulative risk, such as evaluating the adequacy of standard ecological indicators (e.g., sediment bioassay) to detecting cumulative effects at the population level. Eventually, it should be possible to use SEPM's to make first-order predictions on the combined effects of a mosaic of stressor sources with multiple types of stressors.

Besides generating general insights into cumulative risk, the proposed research directly relates to the management of sewage discharges and dredge material. The research could suggest, for example, that one or the other of the present management strategies does not minimize risk to benthic populations. Any such conclusion would be considered preliminary, and additional lab-field and theoretical research would be required before changing management practices. Nonetheless, the proposed research could indicate whether this line of investigation should be pursued.

4.3 Research Theme C - Estuarine Physcial-chemical Stressors

Project C1 - Relationships Between Suspended and Bottom Sediment Conditions and Macrophyte Distributions in Yaquina Bay Estuary

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Goals: The goals of this research project are to determine the relationship of suspended and bottom sediment conditions to the distribution patterns of principal habitat-defining macrophytes such as eelgrass and green algae, in a small non-urbanized estuary typical of those found in the Pacific Northwest (PNW). Emphasis will be placed on identifying sediment-related physical/geochemical stressors that may affect such distributions. As part of a Coastal Ecology Branch (CEB) synthesis effort, results of related past and present studies on these and other candidate stressors (e.g., low salinity, excess nutrients, benthic competitors, grazing by waterfowl, natural and anthropogenic physical disturbances) will be compared and evaluted. Sources of those stressors determined to significantly impact macrophyte habitats in this test PNW estuary then can be sought to support improved ecosystem risk assessments required by Environmental Protection Agency (EPA) and other environmental managers.

Rationale: Macrophytes constitute major habitats in estuaries. The reduction of Submerged Aquatic Vegetation (SAV) distributions in Atlantic and Gulf Coast estuaries over the last few decades has been a major source of concern (Orth et al., 1995). Extensive research into causative or associated factors has been reported (Ogata and Matsui, 1965; den Hartog and Polderman, 1975; Jupp and Spence, 1977; Orth, 1977; Rasmussen, 1977; Phillips et al., 1978; Thayer et al., 1984; Dawes and Lawrence, 1979; Whitmann and Ott, 1982; Bulthuis, 1983; Phillips, 1984; Dennison and Alberte, 1985; Libes, 1986; Wetzel and Neckles, 1986; Muehlstein et al., 1988; Orth and Moore, 1988; Giesen et al., 1990; Zimmerman et al., 1991; Batiuk et al., 1992; Burkholder et al., 1992; Dennison et al., 1993; Neckles et al., 1993; Stevenson et al., 1993; Thom et al. 1995; Bayer, 1996a, 1996b; Moore et al., 1996; Wetzel, 1996). A major finding is that reduced subsurface light (Photosynthetically Active Radiation or PAR) caused by increased turbidity has a major impact on SAV distributions. Moore et al. (1996) utilized growth and survival of transplanted eelgrass (*Zostera marina*) to investigate potential causative factors for the absence of this species in previously-vegetated sectors of the lower Chesapeake Bay. They suggested that water quality conditions causing seasonally-high turbidity and resultant water column light attenuation (K_d) was a major stressor. Accumulation of epiphytes also may have contributed to this stress. Suspended sediment (principally inorganic particulates) and Chlorophyll *a* best correlated with K_d. However, deposited sediments also can be a major stressor on eelgrass. Anthropogenic alterations to a small coastal lagoon in southern California apparently resulted in abnormal accumulation of storm-related sediments, permanently removing this eelgrass habitat (Onuf, 1987). Such findings suggest that natural events (e.g., storms; floods; coastal upwelling) and anthropogenic activities (e.g., enhanced watershed release of soil and nutrients; dredging; breakwaters; wastewater discharge) may interact in a complex fashion to alter water column and substrate conditions, potentially resulting in significant alterations of macrophyte-habitat distributions in PNW estuaries. One of the EPA Office of Research and Development (ORD) High Priority Research Topics is "Research to Improve Ecosystem Risk Assessment" (USEPA, 1997). The target areas of concern include causes of the destruction of critical habitat. Cost-effective actions to prevent or mitigate such habitat destruction require an understanding of the vulnerability and sustainability of these ecological resources regarding the effects of multiple stressors on multiple endpoints at multiple scales. This research project will focus on the relative importance of sediment-related stressors on one such critical habitat (SAV), as an assessment endpoint, at the scale of the coastal non-urbanized PNW estuary.

Objectives and Hypotheses: One of the persistent questions regarding alterations of PNW watersheds involves possible impacts on living estuarine resources. SAV is known to be an important habitat and/or food source for economically-important fisheries and waterfowl species in PNW estuaries. The following objectives (in conjunction with those of other Branch researchers) address possible relationships between living estuarine resources, SAV distributions, turbidity (regarding effects on PAR and SAV distributions), suspended solids (regarding effects on turbidity), deposited solids (regarding effects on substrate composition, elevation, and SAV distributions), and watershed alterations (regarding effects on sediment input to the estuary):

Objective 1. Document annually (1997-2000) the summer season distributions of the major SAV taxa throughout (or in selected sectors of)Yaquina Bay estuary (YBE). The primary focus will be on eelgrass (principally *Zostera marina)*, and the secondary focus on mixed green algae (principally *Enteromorpha* spp. and *Ulva* spp.). Test the hypotheses:

$H_o(1)$: There are no detectable annual changes in the summer distributions of eelgrass or mixed green algae in any of the target YBE sectors over the four year study period.

$H_o(2)$: There is no association between total suspended sediment (TSS) concentration or turbidity and the summer distribution of eelgrass in YBE.

$H_o(3)$: There is no association between bottom sediment composition (grain size distribution; total organic carbon [TOC]; total nitrogen [N]) and the summer distribution of eelgrass in YBE.

Objective 2. During summer/fall 1998, document the bathymetry of YBE. Determine the substrate elevation ranges within which (1) eelgrass and (2) mixed green algae occurred during summer 1998.

H_o(4): There is no association between substrate elevation and the summer distribution of eelgrass and mixed green algae in YBE.

Objective 3. Document bottom sediment accumulation rates and composition during the last century within key intertidal habitats in YBE. Test the hypothesis:

H_o(5): There is no association between historic alterations to the YBE watershed and any changes measured in bottom sediment accumulation rates or composition inYBE.

Scientific Approach:
Objective 1: The Remote Sensing (Aerial Photography - Ground Data) Pilot Project survey of Yaquina Bay estuary conducted in the summer of 1997 will be completed. Approximately 170 Color Infrared (CIR) photographs (scale of 1:7200) were taken of the entire estuary (Figure 1) at an extreme low tide (-2 to +1 feet around Mean Lower Low Water) on July 23, 1997. Another ~50 Full Color (FC) photographs were taken of the center part of the Bay within about one hour of the CIR photography. As part of the planned digital photogrammetry project, diapositives from about 90 of the CIR photographs providing stereo coverage of the intertidal and subtidal portions of the estuary between the Ocean Breakwater and the City of Toledo will be scanned at a resolution of about 25 microns. Based on ground control points (located by Differential-corrected Geographical Positioning System [DGPS] to within +/- 0.5 m), the images will be geocoded and then orthorectified by a professional digital photogrammetry contractor.

As part of the ground data ("truth") component of the project, we selected 10 categories of substrate cover which we termed "visually dominant taxon-density classes". By "visually dominant" we mean that classification of substrate cover that is most visable from above. The term "taxon-density" is included because we pre-selected 4 classes of estimated "percent cover" values (0-25, 26-50, 51-75, 76-100 percent) for quantifying the abundance of the endemic and dominant eelgrass *Zostera marina.* For each of 10 visually-dominant taxon-density classes, 6 stations (3 each on opposite sides of a sector) were preselected in each of 5 sectors (A-E) of YBE between Hatfield Marine Science Center (HMSC) and Boone Slough (Figure 2). As described above, 4 target density classes were assigned to *Zostera marina.* For the other 6 taxa (the exotic eelgrass *Zostera japonica,* mixed green algae, benthic diatoms, bare substrate control, mud shrimp, ghost shrimp), stations characterized as "heavy" density were selected. The ground data survey was initiated the day preceeding that of the aerial photography (July 23, 1997). Virtually all of the 240 SAV-related stations, constituting 80 percent of the 300 total ground data stations, were surveyed within 7 days of the aerial photograph survey. (The surveys of the two shrimp habitats, believed to have the least temporal variability, were completed later in the summer). A 0.25-m^2 quadrat strung with a 5 x 5 wiregrid was placed at one corner of a numbered stake (with a colored side north-facing, adjacent to a card indicating which compass sector [SE, SW, NW, NE] was being sampled). After taking a near-vertical color photo, an estimate of the Percent Cover of each of the 7 target taxa was made . Then a Percent Occurance measure of each taxon was made by assessing which taxon was "visually-dominant" at each of the 25 point-intercepts of the wire grid. Voucher specimens, and samples of surficial sediment (0-2 cm) once per station, also were collected. The frame then was moved to the other three compass positions and the sequence repeated, so that a total of 4 Percent Cover estimates and 100 Point-Intercept counts were made for the 7 target taxa (including bare substrate control) at each station (see Field Data Sheet, Figure 3A, and Ground Data Survey Instructions, Figure 3B) .

Quite satisfactory agreement was obtained between the Percent Occurance values from the point-intercept measures and the Percent Cover estimates for eelgrass *(Z. marina*, Figure 4*)* and mixed green algae (principally *Enteromorpha* spp. and *Ulva* spp., Figure 5). Given that remote sensing strategies usually include a significant ground data component, and the relatively narrow exposure time windows usually available for lower-intertidal habitats, a rapid and reliable method of quantifying the visually-dominant taxa recorded in an aerial photography is critical.

For each sector, half of the stations (15 total for YBE) sampled in the ground data survey for a given taxon-density class will be selected randomly for the digital image interpretation\analysis (supervised classification) step. The remaining 15 stations will be used to provide some general guidance regarding the reliability of the classification. To provide initial calibration data for the summer 1997 aerial photography test project, station positions were selected on the basis of a pre-flight visual assessment of taxon presence and density, as opposed to being probability-based. To improve the basis for comparison, the two lower and the two higher density classes of *Z. marina* may be combined to yield 30 classification and 30 general assessment stations each for the 0-50 percent and 51-100 percent cover classes. Based on a preliminary evaluation of the CIR photographs, it is anticipated that reliable identifications of at least "dense" beds of the endemic eelgrass (*Z. marina*) and mixed green algae in the 5 target sectors of YBE will be produced (Figure 6). (Beds of the exotic eelgrass *Z. japonica* also appear to be distinguished in the CIR photos). Thus, the results of the intensive ground data survey of the 120 *Z. marina* eelgrass stations and the 30 mixed green algae stations (all surveyed within 7 days of the aerial photography) are expected to provide a reasonably reliable classification, and resultant digital maps (projected image resolution: 0.5 m) of these two dominant SAV habitats in Yaquina Bay estuary during July 1997, to the limit of the edge of tide at the time of a given CIR photograph.

During summer 1998, aerial photography of YBE again will be conducted as part of a comparative study of three remote sensing methods for delineating SAV (and possible burrowing shrimp) populations in the intertidal zone. Based on a preliminary evaluation of the summer 1997 CIR aerial photographs, it appears that a large majority of the *Z. marina* growing in the estuary occurs within a 4.5 km x 4.5 km zone (Zone I) located near the mouth of the estuary (Figure 7). This zone encompasses the study areas for the underwater video survey proposed by Dr. Brad Robbins, and the study areas for the accoustical survey proposed by Dr. Ted DeWitt. Both studies will include ground data surveys. (Related studies of SAV and burrowing shrimp habitats proposed by Dr. Bruce Boese, and by Dr. Steven Ferraro and Faith Cole, also are expected to provide relevant ground data results). The total area of intertidal zone targeted for the five related studies in Zone I does not exceed about 10 km^2. To aid the planning process, a general matrix relating key variables of aerial photography (Figure 8) has been prepared from published algorithms (Avery and Berlin, 1992; ASPRS, 1996). The assumptions made in preparing this matrix are (1) use of a calibrated high-precision aerial camera (9 in. x 9 in. film) with a 6 inch focal length (available on short notice of a break in cloud/fog cover); (2) an average ground elevation of 3 m; (3) a standard square study area 5 km x 5 km; and (4) a digital scanner resolution (for diapositives) of 20 microns. The independent variable is photo scale (ranging from 1:1000 to 1:7500). The dependent variables related to product usefulness are minimum ground line separation, pixel size, and nominal image resolution in ground units (m). The limiting variables related to cost are the total number of photos (regarding acquisition and diapositive production/scanning costs) and digital file size (regarding minimum computer specifications).

For reference, the summer 1997 aerial photography survey of the entire YBE (1:7200) yielded a total of about 220 photos (~170 CIR + ~50 Full Color), at an acquistion cost of about $11,000. The minimum flight altidude (above ground level) over populated areas in Oregon is 1000 feet. Thus, the highest resolution available to us is tabululated under the 1:2000 photo scale column (for a flight altitude of 1010 feet). According to the results obtained from the theoretical relationships of aerial photography, this scale should yield (in ground units) a pixel size of about 4 cm x 4 cm, and a nominal image resolution (for blocks of 4 pixels) of about 8 cm x 8 cm (3 in. x 3 in). However, the projected file size per color (3 band) photo of almost 400 megabytes (MB) - near our present operational limit - may require that we have the diapositives scanned at a digital scanner resolution of 25 rather than 20 microns; this would yield a photo file size of about 250 MB, a pixel size of about 5 cm x 5 cm, and a nominal image resolution of about 10 cm x 10 cm. This range of 8 - 10 cm nominal image resolution appears to encompass our present operational limit for CIR or FC aerial photography. Although a "realistic" image resolution limit probably is several-fold higher, with enough ground data collection and digital photo interpretation effort, it may be possible to detect sediment ripple/mound patterns above dense beds of burrowing shrimp. (This possibility might be enhanced even futher by using an intermediate-grade metric camera to take low-altitude, rear sun position - low sun-angle oblique photographs, discussed below). Given that habitats related to populations of SAV and burrowing shrimp appear to account for a substantial percentage of the total area of intertidal mudflats in at least some PNW estuaries, acquiring an operational capability to detect and map these habitats via aerial photography, and other, remote sensing methods is a high-priority objective.

To obtain an estimated cost of acquisition of digital images for the ~10 km^2 intertidal mudflat study area of Zone I, we have scaled the projected 546 photos for a 25 km^2 area (Figure 7) by a factor of 0.4, yielding about 220 photos, similar to the total number obtained in the summer 1997 aerial survey. Thus, we accept the cost of about $11,000 (~$50 each) for that photo acquisition as our estimate for Zone I for 1998. Estimated costs of producing and scanning (to 20 or 25 micron resolution) a diapositive also is ~ $50; the corresponding estimated combined cost for 220 CIR photos is about $11,000.. Thus the estimated total cost of obtaining ~220 digital images, ready to enter into the Branch Geographical Information System (GIS), is about $22,000, or about $100 each (prorating the fixed aircraft mobilization cost).

Based on costs estimates made to date, converting the images from these 220 digital files into a geocoded, orthorectified state (to a ground positional accuracy of ~ 50 cm) via a commercial photogrammetry contractor could cost approximately $100,000. This would appear to be beyond the Branch Fiscal Year (FY98 or FY99) budgets. Costs could be reduced by decreasing the photo scale (moving to the right on the matrix of Figure 8), thereby increasing the pixel size and minimum ground separation/image resolution values while reducing the number of photos required to provide standard stereophotography coverage (60 percent frontlap; 30 percent sidelap). For example, decreasing the photo scale by a factor of 2 (from 1:2000 to 1:4000) reduces the minimum ground separation also by a factor of 2 (from 1.3 cm to 2.7 cm), but the number of photos (and proportional costs) by almost a factor of 4 (to 172 photos/25 km^2 or ~70 photos/10 km^2). This is similar to the number (~90) of summer 1997 CIR photos (1:7200) of the YBE (up to the City of Toledo) now being scanned, geocoded, and orthorectified for entry into the Branch GIS for digital image interpretation and classification. The commercial cost of producing digital orthophotos from such a summer 1998 set could be substantially lowered (perhaps by as much as 50 percent) by utilizing the Digital Elevation Model (DEM) to be generated as part of the present (1997) digital orthophotography project. Similar savings with substantially higher ground postitional accuracy may be obtainable by utilizing the X,Y,Z data (DEM) to be generated via the YBE 1998 bathymetry project, described below. Based on this logic, it is proposed that, during summer 1998, approximately 70 CIR photos of the ~ 10 km^2 intertidal mudflat area of Zone I will be collected at a photo scale of 1:4000. Utilizing the DEM obtained either from the 1997 CIR digital orthophoto production, or from the 1998 bathymetry project, the estimated cost of obtaining GIS-ready geocoded, orthorectified digital photographs of the intertidal mudflats of Zone I is ~ $15,000. Adding this to an estimated cost of $7000 to obtain and scan diapositives of the estimated 70 CIR aerial photos (~ $100 each) yields a combined photo acquistion - digital orthophotography cost for the ~10 km^2 study area of about $22,000.

As mentioned above, the square (4.5 km x 4.5 km = 20.25 km^2) Zone I contains most of the present distribution of *Z. marina*. (It includes the summer 1997 Sectors A-C, and the northern half of Sector D, Figure 2). However, a second rectangular (7.5 km x 2.5 km = 18.75 km^2) Zone II (Figure 7), beginning approximately halfway between Sawyer's Landing and River Bend Marina and extending to Babcock Creek (Figure 10), contains the remaining beds of this native eelgrass (southern half of Sector D, and Sector E). In addition, from the summer 1997 ground data survey and the corresponding CIR aerial photos, David Young concluded that a previously-vegetated area in Sector E between Grassy Point and Craigie Point (USFWS, 1968; Bayer, 1979) did not contain any substantial beds of *Z. marina* in summer 1997 (Figure 10). This fact makes this Zone II an especially important section of the YBE, as a natural laboratory for investigating the question: What changes in this part of the estuary have occurred that may have contributed to the loss (or substantial decrease) of the *Z. marina* beds reported there in the 1960's and 1970's? In addition, David Specht and David Young observed extensive distributions of the exotic eelgrass *Z. japonica* in the upper intertidal of Sector E (in Zone II) during their summer 1997 ground data survey. Further, David Specht has concluded from a preliminary evaluation of the corresponding CIR aerial photos that he is able to detect beds of this exotic eelgrass in these photos.

These facts support the argument that, at the least, the summer 1998 CIR photography planned for Zone I also should be conducted in Zone II. The estimated area of the river channel and intertidal mudflat area of this zone does not exceed about 10 km^2. Thus, based on the derivation above that about 70 aerial photos (1:4000) would be needed to provide stereo coverage of the ~10 km^2 intertidal mudflat area of Zone I, approximately another 70 CIR aerial photo (at an added acquisition cost of about $3000) would be needed for the river portion (and portions of the two major southern sloughs) in Zone II. The developed film from this set could be archived until a decision whether or not to order corresponding digital orthophotos, based on results of past or proposed surveys, is made. The estimated annual combined cost (through outside contractors) of conducting this two-tier aerial photography acquisition and partial digital orthophotography is about $25,000. A decision regarding whether on not to repeat (or modify) the summer 1998 surveys during summer 1999 and/or summer 2000, in order to provide sufficient data for a SAV Change Detection analysis (Lyon et al., 1998), would be made on the basis of results obtained from the 1997 and 1998 mapping (and associated ground data) projects, as well as those from the other SAV/benthic shrimp remote sensing projects proposed by Dr. Ted DeWitt and by Dr. Brad Robbins. The results of these three collaborative research projects will be used to test $\mathbf{H_0(1)}$.

As mentioned above, for the initial test project on aerial photography - digital photogrammetry of YBE conducted during summer 1997, the ground data sampling component was based on stations that were selected before the photography on the basis of desired taxa and estimated densities. This initial strategy was used to insure that enough relevant information on ground distributions of the 7 target taxa (including bare substrate control) would be obtained to adequately train the image analyst in recognizing the digital images of differing taxa (or habitats) obtained by this remote sensing project, which was new to our Branch scientists. However, because these ground data sites were not obtained using a probability-based sampling strategy, the validity of using them to conduct a traditional accuracy assessment of the image classifications is questionable.

Therefore, beginning in summer 1998, as part of the collaborative effort with Dr. Robert Ozretich and Dr. Ted DeWitt, a probability-based sampling program targeting the water column, bottom sediment, and distribution patterns of SAV and salt marsh will be conducted. To minimize the overall cost of sampling, the following common approach between the Principal Investigators has been developed. To provide satisfactory geographical coverage, the division of the estuary into the four "salinity" zones proposed by De Ben et al. (1990) will be adopted. (Their fourth, lowest salinity zone ending near Toledo City has been extended upriver somewhat to the Toledo Bridge near the mouth of Olalla Slough, Figure 10). Within each of these four zones, polygons within the classes listed below have been constructed using the Branch GIS:

1. Salt Marsh (generally higher than + 10 ft relative to MLLW)
2. +5 ft to +10 ft
3. +2 ft to + 5 ft
4. - 6 ft to + 2 ft
5. Deeper than - 6 ft

All of these polygon classes except that for "salt marsh" are based on the bathymetry summarized in 1953 (Figure 11). The salt marsh polygons are based on maps provided in The Oregon Estuary Plan Book (ODLCD, 1987). The elevation zone +5 ft to +10 ft is believed to contain the major distributions of the exotic eelgrass *Z. japonica*. The major distributions of mixed green algae are believed to occur betwee +2 ft and +5 ft, while most of the endemic eelgrass *Z. marina* is believed to occur between - 6 ft and + 2 ft. The lowest elevation zone (deeper than - 6 ft) will be sampled only for water and sediment.

The GIS-based polygons have been sent to Dr. Anthony Olson, the EPA/ORD Western Ecology Division (WED) statistician. Using established prochedures (Stevens, 1994;1997) developed for EPA's Environmental Monitoring and Assessment Program (EMAP), Dr. Olson will supervise production of the number of probability-based stations specified by each researcher within each polygon class for each of the four salinity zone strata. Dr. Ozretich has developed a station allocation strategy that is expected to yield more than 30 water column - surficial sediment stations each within and outside of beds of *Z. marina*, throughout the estuary. Drs. DeWitt and Young have specified a common suite of 8 stations each for elevation zone numbers 2, 3, and 4 above, for each of the four salinity zones, and an additional 8 stations per salinity zone have been specified for the salt marsh polygons. (The largest practical overlap between the probability-based stations of Ozretich and DeWitt & Young will be sought). Thus, at least 32 probability-based stations will be obtained for each elevation level in the estuary. (To insure that this minimum number of stations is obtained for each target polygon class, the production of three times as many probability-based station positions have been requested for each class). If, for a given sampling activity, the randomly selected station is judged to be inadequate for that sampling purpose, a standard procedure for obtaining an adequate station as close as possible to the specified postition will be developed in advance of the field sampling.

For the ground data ("truth") survey, a modification of the benthic sampling strategy successfully used during the summer 1997 ground data survey (Figures 4 and 5) will be employed to obtain Percent Cover and Percent Occurance data for SAV (two eelgrass species and two mixed green algae taxa) in a sufficiently rapid manner to permit the sampling of the 96 benthic stations (32 each for elevation zone numbers 2, 3, and 4 above) within two weeks of the planned date of aerial photography (weather permitting).

Based on sufficiently low tides (~ -1.7 ft. to -1.0 ft. below Mean Lower Low Water - MLLW) occuring during periods of sufficent daylight (8 a.m. to 4 p.m.), the present target period for the photography is the second week of either July or August . There are approximately 16 days of sufficiently low tides between late June and late July, and between late July and late August. Thus, using the CEB hovercraft for rapid transport over the mudflats, two benthic surveyors could be expected to complete 6 stations per low tide interval (~ 1.5 hr./day) if the time to sample and move on to the next station did not exceed about 15 minutes.

To meet this criterion, a 1.0 m x 1.0 m quadrat strung with a 5 x 5 wire grid (25 point intercepts) will be placed at the pre-marked probability-based positions, and a Percent Cover estimate and Percent Occurance measure of the target SAV taxa (at least *Z. marina, Z. japonica, Enteromorpha* spp. and *Ulva* spp.) will be obtained. Voucher specimens, and a sample of surficial sediment, also will be collected at each of the probability-based benthic stations. This strategy should enable the surveying of the 96 probability-based intertidal ground data stations as part of the summer 1998 aerial photography project. This is expected to support a valid accuracy assessment of the image classification resulting from this project (Congalton, 1991; Stehman, 1995; 1996). Based on an evaluation of this assessment, modifications will be made, as necessary, to the ground data surveys supporting any summer 1999 and summer 2000 aerial photography surveys.

CIR photography has the distinct advantage of distinquishing between different types of vegetation, such as eel grass and green algae. However, this remote sensing method has the disadvantage that it cannot reliably detect underwater images (owing to the very limited transmission of infrared radiation through water). Thus, once digital orthophotos have been produced from the summer 1997 CIR aerial photographs, efforts will be made to compare selected digitized Full Color aerial photos (taken the same morning) with their CIR "mates" (photocenters of the CIR and FC pictures often are separated by only about 30 m). The objective of such a comparison is to determine if the offshore boundaries of the eelgrass bed images from the CIR and FC photos systematically differ (i.e., does the CIR deep boundary generally occur inshore of the FC deep boundary). If so, by what percentage does the aerial distribution of eelgrass determined from the FC photography exceed that determined from the CIR photography. This exercise should provide some insight into the degree of bias associated with low-tide CIR aerial mapping of eelgrass beds, relative to the aerial distributions obtained from FC aerial photography.

A corresponding effort to evaluate the degree to which low tide CIR aerial maps of eelgrass beds underestimate the actual distribution will be made via a ground survey. During calm, full daylight periods of summer 1998 at the lowest navigatable tide, the lower edge of *Z. marina* beds in different sectors of Zones I will be mapped by driving a vessel-mounted DGPS unit slowly over the clearly visible deep boundary of the beds. The resultant deep boundaries will be compared to the apparant deep boundaries obtained from analysis of the CIR digital othrophotos from the summer 1998 aerial photography survey. (If substantial bias is measured, attempts to extend this comparison to Zone II also will be made).

Another important part of remote sensing via aerial photography is the collection and evaluation of historical photographs. This is more difficult for intertidal vegetative habitats, where aerial photographs taken for other purposes may be of limited usefulness owing to (1) black and white, monoscopic photographs; (2) photos taken at other than a low tide; (3) lack of any ground truth data. However, there still is the possibility of locating some historical aerial photos that could provide, at the least, an opportunity for estimating SAV distributions which are approximately consistent with present distributions. To date, David Specht and David Young have located potentially useful photographs archived by the City of Newort, the Oregon State University (OSU) Library, and the OR Dept. of Fish and Wildlife. Contacts also have been located for probable sources from the OR Dept. of Transportation in Salem, and the U.S. Corp of Engineers in Portland. During the three year study period, these and other candidate sources of historical photographs will be systematically contacted and surveyed. Any photos of the intertidal mudflat zones of Yaquina Bay Estuary, or other PNW estuaries that may be studied by CEB in the future, will be obtained and evaluated. If sufficiently promising photo images are located, a contract to delineate such images and then geocode and orthorectify the resulting polygons (assuming post-positioning of a sufficient number of photo-visable ground control points) may be sought. The methodology for this approach, using either mono or stereo aerial photographs in conjuction with computer-assisted analytical plotters, has been developed over the last decade by OSU's aerial photogrammetry expert, Professor Ward Carson (Carson, 1985; Warner and Carson, 1991, 1992; Warner et al. 1993). This technique also may be employed for geocoding and orthorectifying polygons (delineated SAV or burrowing shrimp distributionsons) on CIR or FC 35-mm oblique photos taken by CEB scientists from a small plane at low altitude over YBE. This technique already is being used by WED scientists to monitor flooding of riparian zones in the Willamette Valley (S. Klein, personal communication). Alternatively, a Windows-NT computer program to produce orthorectified digital photos (from geocoded photo-visable points and the Digital Elevation Model of the estuary) will be obtained for use by the Branch GIS specialists.

In collaboration with the research proposed by Dr. Robert Ozretich and by David Specht, beginning in summer 1998 near-surface and near-bottom water column measurements (Callaway and Specht, 1982; Callaway et al., 1988) of Turbidity will be initiated at 5 selected continuous sampling sites where the *in situ* sensors can be securely deployed (Figure 10). Collections of water for gravimetric determination of Total Suspend Solids (TSS) also will be made randomly at these stations in an effort to establish a significant relationship between Turbidity and TSS. Replicate samples of surficial bottom sediment will be collected quarterly at these stations for measurement of Total Organic Carbon and Nitrogen (Percent TOC/N) and Grain Size Distribution (Percent Sand, Silt, and Clay). Near-surface and near-bottom Turbidity measurements (again with random samples for TSS) also will be made twice during each quarter at more than 60 probability-based stations throughout the estuary. As discussed above, these stations are expected to include approximately equal numbers of sites within and outside areas containing beds of *Z. marina*. A surficial bottom sediment sample for determination of TOC/N concentrations and Grain Size Distribution also will be collected at the time of the water column *in situ* measurements. An empirical model relating the data from the continuous sampling sites and the probability-based sites will be developed under the guidance of WED statisticians. Based upon the recommendations of these statisticians, the results of these water column and surficial bottom sediment measurements made during the three year study period (1998 - 2000), in conjunction with those obtained from the corresponding three annual (summer) SAV distribution surveys described above, will be used to test hypotheses $\mathbf{H}_0(2)$ and $\mathbf{H}_0(3)$.

Objective 2: The most recent comprehensive bathymetry of YBE was compiled by the National Oceanic and Atmospheric Administration's (NOAA) National Ocean Survey (NOS) in 1953. This information has been used by the Branch GIS group to produce a bathymetric chart of the estuary (Figure 11). Because the data for this chart are approximately 50 years old, during a week of extreme high (daylight) tides during Fall 1998, a bathymetric survey of YBE will be conducted. Using DGPS for continuous positioning (accurate to within +/- 0.5 m), depth soundings via a fathometer mounted on a shallow-draft boat will be conducted along predetermined transects. A filter to remove most of the variation in depth measurements due to waves will be used, resulting in an estimated accuracy of 0.04- 0.08 m in the fathometer readings. Absolute depths will be determined from tidal height measurements obtained from the continuous tide gauge situated at the HMSC dock. Using laser-survey techniques, the tide height at various sectors of YBE during the survey will be related to a local Vertical Datum, thus permitting corrections for spatial (up-estuary) vertical displacement owing to substrate grade and atmospheric pressure differential. The proposed extent of this bathymetric survey is from the mouth of YBE at the Ocean Breakwater to the mouth of Olalla Slough southeast of the City of Toledo (Figure 10). Thus, the area off Boone Slough also will be obtained. This area includes the sites of the "eelgrass" bed reported in 1968 by the U.S. Fish & Wildlife Service (USFWS, 1968), and the *Z. marina* bed studied in 1974 by Bayer (1979); as described above, both beds were determined (from the ground data survey and CIR photographs) to be virtually absent in 1997. Thus, bathymetry for areas of both extant and previously-existing beds of *Z. marina* will be determined. The absolute depths will be compiled in a digital map and entered into the CEB Geographical Information System for comparison with corresponding summer 1998 SAV distributions, to determine their substrate ranges and test hypothesis $H_o(4)$.

Objective 3: Beginning in summer 1998, cores of bottom sediment will be collected from different sectors of YBE for geochronology (sediment core layer dating). A traditional approach in such a project is to seek out likely depositional sites in the vicinity of the various habitats of interest. An example of such a conceptual sampling plan is illustrated in Figure 10. In this plan an effort initially was made to select areas "to the side" of the main flow of water within various sectors of the estuary, in the hope of finding areas sufficiently exposed to sediment-laden waters to capture a long-term record of net deposition rates, but sufficiently removed from areas of periodic or episodic high-velocity currents that such sedimentary records could persist. For example, one likely area to find an undisturbed depositional record is the uppermost part of the intertidal zone, in the salt marshes at the estuary's edge. The approach was used in a preliminary study conducted in Willapa Bay, WA. In collaboration with Dr. Brian Atwater (USGS - University of WA), Professor James Phipps (Grays Harbor College, WA), and Professor Brent McKee (Tulane University, LA), large-volume cores were collected from shallow (~1.5 m) pits dug into natural marshes at river sites known to have experienced a subsidence of about 1 meter approximately 300 years ago (Yamaguchi et al., 1997). Preliminary results from Lead-210 (Pb-210) geochronology (Figure 12) show that, below a mixed surface layer a few cm deep, there is a regular decrease in the concentration of Pb-210 indicating a sediment accumulation rate of approximately 0.25 $\pm$ 0.05 (wet) cm/yr. This is consistent with the clearly visible "buried marsh soil" horizon at a depth of about 120 cm at this site (and the probability that the sediment accumulation rate was higher during the first century following the January 1700 subsidence - when the drowned estuarine marsh was inundated frequently, than was the rate during the last century - when the accumulated sediment had raised the substrate elevation and tidal inundation was much less frequent). Unfortunately, the practical limit of Pb-210 geochronology for estuarine cores (with relatively high deposition rates) is about 100 yr BP (Before the Present). This precludes a direct measurement of hypothesized alterations in sediment accumulation rate between the time of initial European colonization (~1850 A.D. - 150BP) and about 1900, during which interval the Old-Growth forests of the Pacific Northwest first were logged. However, the introduction of much more efficient logging techniques since the turn of the century, and the increased demand for timber since then, could have led to a significant increase in soil erosion, and mass "sediment wasting" via slides. This in turn could have caused an increase in estuarine sediment accumulation in YBE since the early 1900's that may be measurable.

Thus, the sediment core sampling plan proposed here will include salt marshes of YBE, seeking evidence of a change in sediment accumulation rate, and a correlation between the time such a change is initiated and the time of a major alteration in the YBE watershed. However, to permit conclusions to be made regarding changes in the very recent sediment history of the estuary as a whole, the probability-based sampling strategy described above for the other elevation zones also will be utilized. Owing to the limited number of cores that can be analyzed during the study period, the probability-based stations will be adjusted according to pre-established rules to obtain nearby sites that include, where feasible, the following classes:

1. Salt marshes (supra intertidal mudflats)
2. Beds of exotic eelgrass *Z. japonica* (upper intertidal mudflats)
3. Beds of mixed green algae (intermediate intertidal mudflats)
4. Beds of endemic eelgrass *Z. marina* (lower intertidal mudflats)
5. Sites of known or suspected previous distributions of *Z. marina*
6. Subtidal non-dredged "slopes"

The Channel proper is not proposed for sampling, in view of the low probability of long-term (decadal) sediment accumulation owing to flood runoff scouring or dredging.

If consistent with the recommendations of the WED statisiticians assisting us in the development of the sampling design, cores will be collected throughout the estuary at (or near) probability-based stations which fall within the above-described classes.

At subtidal stations, in collaboration with Dr. Dixon Landers (WED), a custom coring device deployed from the WED sediment coring vessel will be used to obtain cores up to 1 m in length. At mudflat stations, a 6-inch diameter core barrel will be used to obtain the 1-m sediment cores by hand. At salt marsh stations, pits will be dug and stainless steel three-sided box corers will be inserted horizontally to obtain the 1-m cores. The subtidal and mud flat samples will be maintained in a vertical position until returned to the CEB laboratory clean room and completely extruded; all cores will be sampled at 1-cm intervals and frozen for analysis of Pb-210 by Dr. McKee via chemical separation and alpha-ray spectrometry (Clifton and Hamilton, 1979; Nittrouer et al., 1979, 1983/84; Carpinter et al., 1982; Huh et al., 1990), or at CEB via gamma-ray spectrometry of whole samples in sealed containers at secular equilibrium (Cutshall et al, 1983; Appleby et al., 1986). Cesium-137 (Cs-137) profiles also will be obtained at the CEB laboratory on selected core samples, using the nuclear fallout peak of 1963 as an independent horizen to check the dating via the Pb-210 method.

These data will be used to document approximate dates of deposition of individual sediment core layer samples during about the last 100 years. The principal objective of this phase of the research plan is to determine if there have been any significant changes in sediment accumulation rate or composition over the last century, and the degree to which any increases in accumulate rate may have elevated the substrate in parts of YBE. In particular, is there evidence that areas previously vegetated by the endemic eelgrass *Z. marina,* or present beds of mixed green algae, have shoaled enough to exceed the present optimal range of *Z. marina* here. A comparison of the present bathymetry in such areas with that of the 1953 NOS survey, and with early bathmetric data, also will be made in an effort to answer, or shed light on, such questions.

As a part of this reseach project, the collaboration of a specialist in the "historical reconstruction" of PNW watersheds will be sought to document the history of such alterations in the YBE watershed. The goal will be to detect sufficiently precise data, from both the geochronology and the historical reconstruction, to obtain statistically-significant changes in rates, so that correlations between the times of such changes may be sought. For example, is there a correlation between the time of a major watershed alteration (e.g., a distinct change in logging rate or method), and the time when a significant increase in sediment accumulation rate occured. The guidance of WED statisticians will be sought in an effort to obtain data adequate to permit the testing of hypothesis $\mathbf{H_o}$(5).

Expected results and benefits: This research project is expected to answer/address the above-stated Question and Hypotheses which are of direct relevance to the Agency's goal of reducing uncertainty in the risk assessment process. In particular, the results should benefit EPA and other environmental scientists and managers concerned with minimizing the impact of anthropogenic activities on the relatively-small, non- or lightly-urbanized estuaries of the Pacific Northwest.

Project C2 - Evaluation of the Susceptibility of Eelgrass Beds in Oregon Estuaries to Changes in Watershed Uses

Principal Investigator: Robert Ozretich

GOALS: The overall goal of the proposed research is to assess the susceptibility of eelgrass beds to negative changes in water quality parameters caused by watershed alterations. This requires:

1. Determination of the vulnerability of *Zostera marina* (Zm) habitat in coastal Oregon estuaries to changes in water column conditions.

2. Determination of a sufficient set of parameters for the calculation of Zm production in Yaquina Bay.

3. Determination of the sources of suspended matter and nutrients entering the water column of these estuaries.

4. Determine the water column response to changes in the anthropogenic sources and masses of suspended solids and nutrients entering Pacific coastal estuaries.

RATIONALE: Submerged aquatic vegetation (SAV) provides food for waterfowl and critical habitat for fish and shellfish. It also affects nutrient cycling, sediment stability and water clarity. The extensive experience of east coast researchers with the impacts of urbanization on SAV has resulted in a variety of models explaining the relationships between eutrophication, suspended solids and the health of SAV. Dennison et al. (1993) proposed water quality values (WQVs) that were estimated using correspondence analysis on five water quality parameters that were associated with sites of varying SAV cover in Chesapeake Bay. The proposed WQVs for Chesapeake Bay are salinity-dependent, and include light attenuation, total suspended solids (TSS), chlorophyll a, dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorous (DIP) (Table 1). As an initial assessment of the current status of Oregon estuaries as suitable habitat for SAV, these water quality parameters could be determined and individually compared to the proposed WQVs. Interpretation of the results would need to consider that the dominant estuarine SAV in this region is Zm.

A recent areal and ground survey of the intertidal area of Yaquina Bay in 1997 has discovered that a complete loss of a significant Zm bed in Yaquina Bay has occurred since this bed was last reported in 1974 (Bayer, 1979), and apparent reductions in the extent of other beds have occurred since they were last described in 1971 (as reported in Good, 1975). Near the missing Zm bed (river distance ~15 km) nutrient and TSS WQVs were exceeded during the early spring of 1977 (Callaway et al., 1988 and unpublished nutrient data). The spring growth period has been shown to be critical for continued survival of Zm in Chesapeake Bay (Moore et al., 1996 and 1997). Average river flow in April in Yaquina Bay is 50% of the winter maxima (NOAA, 1988) which is the month that the growth season of Zm begins (Kentula and McIntire, 1986). A critical issue is whether watershed use practices have changed in the Yaquina watershed between the 1970s and the present in ways that contribute more solids to the still-significant river flows at this critical time of year. A second critical issue

Table 1
from Dennison, et al. (1993)

Water quality characteristics that are necessary to sustain SAV to a depth of -1 m MLW in Chesapeake System.

Salinity regime	Light attenuation coefficient (K_d; m^{-1})	Total suspended solids (mg/L)	Chlorophyll a (µg/L)	Dissolved inorganic nitrogen (µM)	Dissolved inorganic phosphorou (µM)
Tidal freshwater (0-0.5‰)	<2.0	<15	<15	--	<0.7
Oligohaline (0.5-5‰)	<2.0	<15	<15	--	<0.7
Mesohaline (5-18‰)	<1.5	<15	<15	<10	<0.3
Polyhaline (>18‰)	<1.5	<15	<15	<10	<0.7

is whether changes have occurred in the point and non-point discharges of algae-stimulating nutrients to the upper reaches of the estuary.

In a recent compilation of existing published and anecdotal information regarding the eutrophication status of Pacific coast estuaries, (NOAA, 1997 draft) no SAV information was available for 14 of the 38 estuaries (11% of the region's estuarine surface area) and no information was available on an additional 8 estuaries (26% of area) related to changes in SAV coverage between 1970 and 1995. Most of the estuaries for which a minimal amount of information was available were the smaller, outer coastal estuaries of the region, including Yaquina, Siletz, Alsea, Netarts and Tillamook Bays. Among these estuaries there is a five-fold difference in freshwater residence times and a range in extent of watershed urbanization.

With the baby-boom population of the U.S. approaching retirement age and the attractiveness of coastal areas for retirement, the pressures on coastal Oregon to accommodate a growing population will only increase over the next 25-40 years. As these people arrive their needs for housing, sewage disposal, drinking water, shopping centers, roads, etc. will impact the water quality of the estuaries around which they will live. The extent of water quality changes in response to added nutrients and/or solids would need to be estimated by accounting for source-dependent nutrient processes and the unique hydrodynamics of each estuary. These considerations would be part of a comprehensive watershed plan and would include estimates of the total maximum daily loads (TMDLs). A component of such an analysis would be an estimation of the response of the estuary to these inputs that could come from a comprehensive estuarine ecosystem model such as that of Hopkinson and Vallino (1995). This model was designed to account for residence times, sources and rates of nitrogen inputs and losses, and includes both settling algorithms for particulate matter and a 1-dimensional advection-dispersion model representing the hydrodynamics of the estuary. Future responses in the Dennison et al. water quality parameters (chlorophyll a and nitrogen concentrations) could be predicted with this model by varying those anthropogenically-controlled parameters to which these parameters respond.

OBJECTIVES: There are four major objectives of the proposed research. These are:
1. Determine in Yaquina estuary the temporal and spatial distribution of five water quality parameters (Table 1) that have been associated with growth of SAV. Differences between these parameters and the WQVs proposed by Dennison et al. will be compared with the distribution of Zm. Regional WQVs for Zm will be proposed if there are significant differences between Zm distributions and those predicted from measured water column parameters using Chesapeake Bay-derived WQVs. In addition, the role of light attenuation and desiccation in the distribution of Zm will be evaluated.

2. Determine the consequences of using different sources and kinds of data in calculating seasonal production of Zm. The hypotheses to be tested include equality of discrete and continuously determined light attenuation coefficients, equality of calculated and measured *in situ* irradiances. In addition, the comparability of full spectrum light sensor readings (under defined conditions) and PAR measurements. Determination of the consequences of using averaged attenuation coefficients in calculating seasonal production of Zm.

3. Determine the proportions of different sources of suspended matter and nutrients entering the water column using a variety of means including isotope ratios, carbon/chloropyll a composition, multiple component mixing models.

4. Provide a model that accurately reproduces the distribution of water column components that are linked to the habitat requirements of *Zostera marina*. Such a

model should account for the most important physical and biological processes that control the water column parameters affecting Zm growth. The accuracy of the Hopkinson and Vallino (1995) model in reproducing measured distributions of phytoplankton, dissolved inorganic nitrogen, and salinity in a single estuary (Yaquina) will be determined. If sufficient agreement is obtained, algorithms for tides and the coupling of suspended solids and phytoplankton to water column light attenuation will be added.

SCIENTIFIC MERIT OF OBJECTIVE **1**: Given a suitable substrate, the distribution of sea grasses appears to be controlled primarily by the availability of light that in turn is affected by water quality parameters. Light attenuation, nutrient, chlorophyll a and suspended solids concentrations have been found to correlate with the presence or absence of sea grasses in Chesapeake Bay (Dennison et al. 1993; Batiuk et al., 1992) and the success or failure of Zm transplants in that system (Moore et al., 1996; Batiuk et al., 1992). Water quality values (Table 1) that are associated with permanent SAV beds to a depth of –1 m (MLW) in the Chesapeake estuary have been proposed (Dennison et al. 1993) and strategies for restoration of beds to greater depths have been articulated (Gallegos, 1994; Gallegos and Kenworthy, 1996; Batiuk et al., 1992). Although nutrient limited systems would, generally, not respond with measurable increases in concentrations of the limiting nutrient (Ryther and Dunstan, 1971), the interactions of temporally competing limiting constituents apparently results in the associations of elevated nutrient concentration and diminished SAV abundance in the Chesapeake system.

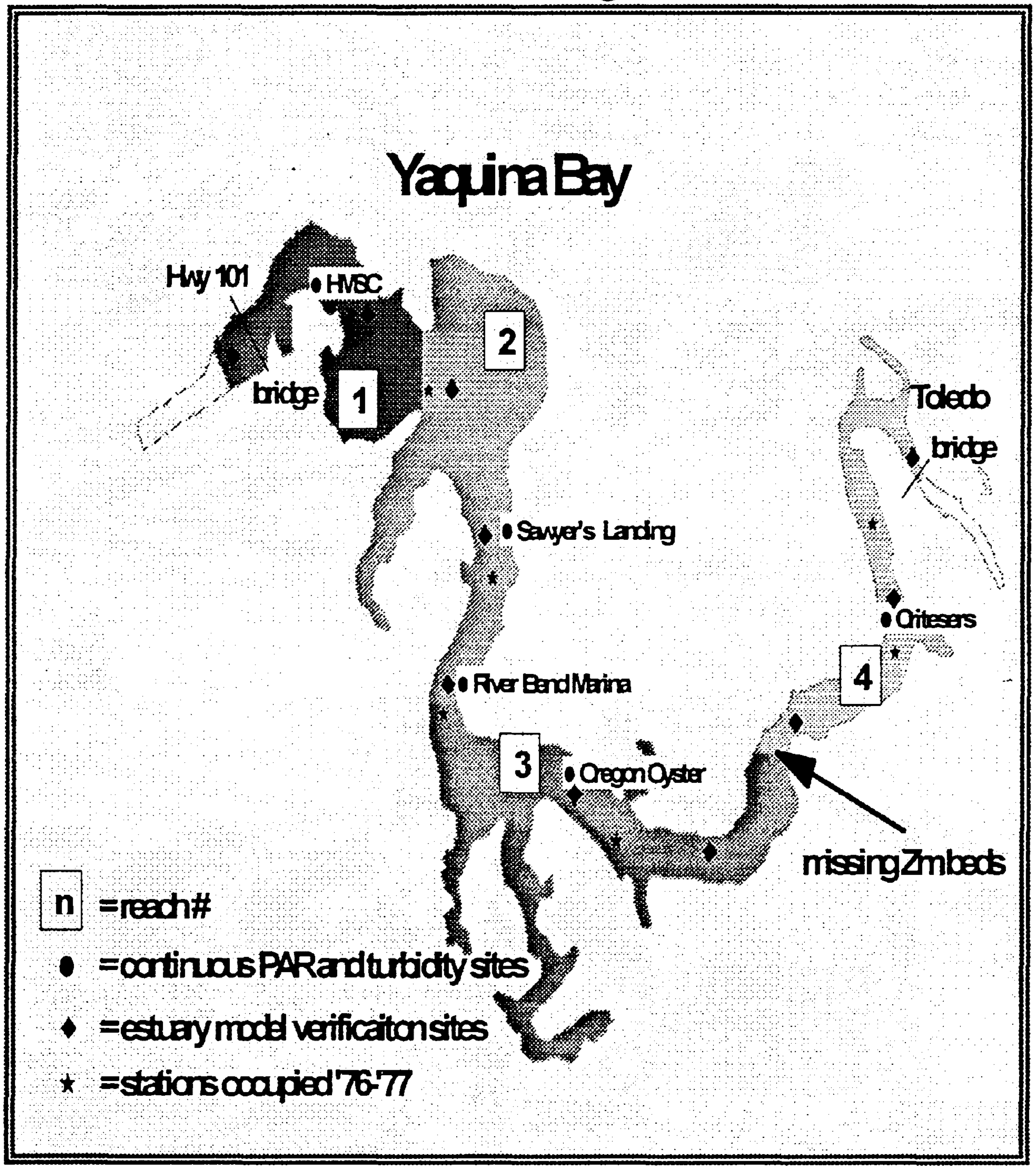
The utility of using water quality to define sea grass habitat requirements is that the total maximum daily loads (TMDLs) of the identified water quality parameters could be calculated. TMDLs for various tributaries and reaches of an estuary could be established through waste load allocation modeling in comprehensive watershed plans either to maintain current SAV distributions or to attain more extensive distributions that were characteristic of an earlier, less impacted, time. Watershed models that are able to incorporate physics and geology, as well as, land-use are only now beginning to become available (Jay, personal communication). In calculating TMDLs the contributions of natural estuarine processes to the water quality parameters need to be assessed before waste loads are allocated to anthropogenic processes (Jaworski, 1981). For example, nitrogen and phosphorous flux continuously from sediment due to benthic respiration, with water column concentrations varying inversely with the tidal range, i.e. spring vs neap (D'Elia et al., 1981). Light attenuating particles can be present in the water column due to particle trapping by estuarine circulation (turbidity maximum), episodic bottom current shear and wind-induced resuspension (Briggs and Cronin, 1981). Unique to the west coast is the possible transport of nutrient-rich deep water into estuaries that was upwelled onto the shelf during periods of continuous northerly winds (usually during the summer). Each of these processes can contribute to nutrient and/or particle loading to estuaries and need to be assessed before land use changes in the watersheds are considered.

Objective 1a: The objective of this goal is to determine the vulnerability of Zm habitat in coastal Oregon estuaries to changes in water column conditions. The WQVs that are associated with healthy SAV beds in Chesapeake Bay may not be quantitatively applicable to other regions and may not be entirely applicable to the distribution of a single species e.g. *Zostera marina*, even in the Chesapeake system. However, according to Dennison et al. (1993), these WQVs represent the "absolute minimum water-quality characteristics necessary to sustain plants in shallow water.....exceeding any of the five water-quality characteristics seriously compromises the chances of SAV survival."

Each estuary will be stratified and sampling stations will be selected using probabalistic sampling protocols (Stevens, 1994). The length of each estuary will be determined from the mouth to a predominantly freshwater upstream location. Each will be segmented into 4, roughly equal (river mile) intertidal reaches that may correspond to areas of different salinities (if known), or merely distances from the mouth.

Each of the 4 salinity-based (DeBen et al., 1990) reaches of Yaquina Bay (Figure 1) was segregated into 3 depth intervals, (deeper than -6', channels), low (-6' to +2'), and medium (+3' to +5'). Sampling locations were selected from the depth intervals in eachreach using the Stevens (1994) protocols. The deepest (channel) locations would permit virtually unlimited sampling access but the tidal range of Oregon's estuaries (10'-14') would limit access of the shallowest intertidal locations to a few spring tidal cycles a year.

Figure 1


Yaquina Bay
Hwy 101
HMSC
bridge
1
2
Toledo
bridge
Sawyer's Landing
Critesers
4
River Bend Marina
3
Oregon Oyster
missing Zm beds
n =reach#
=continuous PAR and turbidity sites
=estuary model verificaiton sites
=stations occupied '76-'77

The specific null hypotheses to be tested are based on the following:

As a first approximation of this vulnerability, current water column conditions will be compared to the WQVs of Dennison et al. (Table 1) that were proposed to be sufficient to permit SAV to grow to a depth of –1m , MLW, equivalent to -1.7' MLLW in Yaquina Bay. This comparison is reflected in the general null hypothesis, H_1: the individual water quality parameter means are not significantly different from Dennison's WQVs. This would be tested by comparisons of estuary-wide and reach parameter means against the WQV. Student's t-test would be used. An interpretation of the null hypothesis generally being upheld, would be that water column conditions are near to those that could cause problems for Zm growth. Alternative hypothesis H_1a_1, where estuary-wide or reach mean values were generally less than the WQVs, would suggest that conditions for Zm growth to this depth were in good shape. Alternative hypothesis H_1a_2 , where the estuary-wide or reach mean values generally exceeded the WQVs would suggest that conditions for Zm growth were presently in poor shape. The inability to reject the null hypothesis, H_1 and the possibility of alternative hypothesis H_1a_2 would suggest that causes for elevated water column values should be sought (Goal 3).

During each sampling interval 3 samples from each depth interval per reach will be taken. Prior to testing H_1, the values from the three depth intervals within each reach will be compared to one another using ANOVA. This will allow testing of the horizontal homogeneity of these parameters (H_2). Three samples are sufficient to detect differences of 50% with an assumed depth interval SD of 15%. Although 9 samples will be taken within each reach, only 6 per reach per sampling time would be needed to detect differences (H_3) of 25% with an assumed reach SD of 15%. All these estimates are based on a power of 0.8 to detect significant differences at an alpha level of $\leq$0.05.

If no differences are found among depth intervals in any reach the parameter values from all depths will be used to represent the reach in comparisons among reaches, also using ANOVA. If among depth differences within a reach are found, comparisons of all reaches will be by common depth. There will be 3 sampling stations for each of the 3 depths and 4 reaches for a total of 36 stations. These stations will be sampled bi-monthly throughout the year.

Objective 1b: Establishing a relationship between Zm coverage and water column processes. The WQVs of Dennison et al. resulted from estimates of breakpoints in multidimensional renderings of measured water column concentrations and the presence, absence, or transience of SAV populations in Chesapeake Bay. The much larger tidal range on Oregon's coast compared to the Chesapeake system suggests that exposure could play a significant role in the vertical distribution of Zm in Oregon estuaries and needs to be considered. Probit analysis will be used to investigate the roles of light attenuation and desiccation in Zm distributions because the response variable, percent coverage, is reported categorically.

The percent coverage of sediment by Zm at the water column stations will be determined by other investigators. Their results will be reported as 0%, 50%, or 100% coverage and will come from the 3 depth strata. The predictor variables will be light attenuation (m^{-1}) and exposure (hours*day^{-1}) . The daily exposure to air will be calculated using the tidal model developed by Frick (1996) coupled with the bay's updated bathymetry (contracted to be done fall-98, D. Young, PO).

SCIENTIFIC MERIT OF OBJECTIVE 2: If the PNW-WQVs are found to be significantly different from the Chesapeake-derived WQVs it may not be due to biological differences between the Atlantic and Pacific Zm populations. Although carbon fixation rates by Zm have been shown to vary among habitats, possibly in response to variability of the light regime (Zimmerman, et al., 1994, and references within), little difference in minimal light requirements have been reported (21%±3% of surface incident, mean±SE) among specimens from the Atlantic and Pacific Oceans and the Baltic (Dennison et al., 1993). Little is known about the light requirements of eelgrass in the Pacific Northwest (Olson and Doyle, 1995); it is known that Zm has not been found deeper than about -6.6 m MLLW in Puget Sound (Phillips, 1984) and in Yaquina Bay it was found in the 1997 survey between -2 m and +0.5 m of MLLW. What is very different is the much greater summer tidal range of PNW estuaries, 3 m vs <1 m in the Chesapeake system. The excursion of highest and lowest tides through the daylight hours of each lunar month is an imposition of additional variability (Dring and Lüning, 1994; Zimmerman et al., 1994) on the flux of photosynthetically active radiation (PAR, 400 to 700 nm). Continuous light measurements are the best way to document the consequences of this varying light field.

Continuous PAR measurements have shown that the *in situ* light regime in estuaries can be much more dynamic than expected from sidereal considerations (Zimmerman et al., 1994) and that production estimates based on average monthly or seasonal attenuation coefficients can miss periods of elevated attenuation during the spring that are critical for Zm survival (Moore et al., 1996; Bulthuis, 1997). Although the expected vegetative growth of Zm in this locale is the Apr-Oct interval (Kentula and McIntire, 1986) continuous instrument deployments covering the estuary will be throughout the year. Seasonal Zm production will be computed using continuous PAR measurements with Chesapeake-derived and Pacific coastal estuary-derived mean attenuation coefficients. Photosynthesis vs irradiance (P vs I) parameters will be taken from the literature (Zimmerman et al. 1994) for these calculations. Shoot growth (from the proposed studies of Boese and DeWitt) will be correlated with nearby continuous PAR measurements (this study) in Yaquina Bay as an independent estimate of the above-ground fraction of the seasonal production. The acquisition by the Branch of a mesocosm facililty for manipulating seagrass would facilitate the determination of P vs I and other physiological parameters that may be specific to the race of Zm, or other

SAVs present in Yaquina Bay or PNW estuaries, in general.

Objective 2a: The objective of this goal is to evaluate the applicability of periodic monitoring in delineating the submarine irradiance of PAR in coastal Oregon estuaries. Failure of Zm to survive from one season to another has been attributed to short periods (2-4 weeks) of elevated light attenuation during its early growth period. The occasional late spring freshet on the Oregon coast coupled with the early onset of Zm's growing season (April) are a combination of circumstances that could be inimical to Zm survival. Continuous monitoring of PAR irradiance would be ideal in identifying such events (if they occur) and quantifying their consequences. However, the unit cost of continuous monitoring of PAR is high and deployment sites are limited because of security considerations. Our interest in outer coastal Oregon estuaries, some of which have no docks for deployment, compels us to evaluate how representative are temporally discrete measurements made at easily accessed locations (channels) and, can inexpensive light meters that could be continuously deployed give us PAR-equivalent data. To address these questions continuous deployments of PAR sensors and the evaluation of a light intensity meter are components (Goal 2b) of this research project.

Continuous deployments of PAR sensor arrays will be placed on accessible docks or Coast Guard daymarks within each reach of Yaquina Bay (Figure 1). Reach 2 will have 2 arrays deployed for a total of 5 arrays, bay wide. Each array will consist of 2 PAR sensors that will obtain 4 to 12 measurements per daylight hour. Four of the arrays will have the sensors a fixed distance apart (1.0 m) at a fixed vertical position. The Hatfield Marine Science Center (HMSC) array will consist of a near-surface floating sensor and a deeper, fixed-position sensor. The geometry and structure of these arrays has not been designed at this time and each may be site specific. Attenuation coefficients will be calculated from the fixed- distance paired PAR units directly and this distance will be calculated for the HMSC site from the Frick tide model that is most accurate (time and amplitude) at the HSMC location. The HMSC floating sensor will be used in concert with a roof mounted PAR sensor (EPA building ~200 m south). The submarine and air sensors will be used to document the loss of light through the water-air interface. This information would be used to evaluate the utility of PAR measurements obtained from continuously monitoring air-mounted units in simulating the submarine light field obtained from simultaneously determined submarine irradiance measures at the 5 sites around the bay.

The specific null hypotheses to be tested are based on the following:

The following null hypotheses will test the representativeness of temporally discrete light attenuation coefficients in Yaquina Bay. H_4: the discrete attenuation coefficients for a reach are not significantly different from those from the continuously monitored sites. Daily averaged coefficients from the moored sensors for the time interval of interest (e.g. monthly, n=30) will be compared to the coefficients obtained discretely on the same time interval. Student's t-tests and will be performed on data from each reach. The discrete sites will be the channel sites from the random selection process sampled bi-monthly during the midday highs of the neap and spring tides. H_5: the discretely obtained light attenuation coefficients are positively correlated with the results from the moored sensors. The general expectation is that light attenuation would be positively related to reach number, with reach 4 receiving the river load of suspended solids. This expectation should result in a range of attenuation coefficients. Mean discrete and continuous values from each reach for each and all sampling periods would be tested for significant correlations. The expectation is that the short-term variability in attenuation coefficients from the moored arrays would be greatest in reach 4 as the consequences of storms as elevated suspended material in the river pass by the arrays. Longer term variability could result from the growth of phytoplankton in response to river-borne nutrients (sewage and watershed) or upwelling-injected nutrients from the ocean. Temporal plots of attenuation coefficients from the reaches would give qualitative information about the duration of light attenuating events or processes. Tests of equality of variances at different times within a reach and among reaches would permit statistically based confirmation of general statements about variability. Differences between and among variances will be looked for using tests of homogeneity of variance (H_5) such as F-tests for 2 comparisons, or F-max or Bartlett's tests for several.

Independent of the comparisons of discretely determined attenuation coefficients among reaches (H_3) would be those obtained by comparisons of attenuation coefficients among reaches from the moored arrays (H_6) using ANOVA.

The following null hypothesis will test the utility of the roof-obtained PAR measurements. H_7: the total submarine PAR irradiance at a fixed depth obtained from continuous submarine monitoring is not significantly different from the irradiance computed from the in-air PAR unit. These irradiance values will be computed on a daily basis and compared over weekly, monthly, and growth season (March-Sept) intervals. The comparisons will be by reach; the daily computed irradiance will utilize the daily air-to-water loss value coupled with the reach-averaged attenuation coefficient from the discrete water column sampling. Student's t-tests will be used.

Objective 2b: Instrumentation for continuous, *in situ*, PAR monitoring is expensive, less expensive alternatives are needed. Full spectrum (350 to 1100 nm) light intensity meters (HOBO StowAway LI, Onset Computer Corp., Bourne, MA) will be deployed at the same depths as the LI-COR spherical meters (see IMPLEMENTATION section) to evaluate the feasibility of using Onset meters in future, more extensive data collections at more remote sites. Intercalibration between the LI-COR and Onset sensors will use *in situ* measurements of the spectral irradiance at the sites using a LI-COR spectroradiometer because the Onset sensor is strongly dependent on the spectral characteristics of the incident light and *in situ* light attenuation is strongly wavelength dependent above 700 nm (Jerlov, 1968). This attenuation of radiation at longer wavelengths than the PAR waveband suggests that the Onset sensors may be integrating radiation essentially in the PAR range at depths exceeding 1 m (Jerlov, 1968). Integration of spectral radiance will be used to calibrate the Onset meters.

The range of the Onset meters is into the infrared and they are twice as sensitive to 900 nm radiation as 650 nm radiation making comparisons to PAR (400-700 nm) sensors in direct or diffuse sunlight meaningless. However, pure water strongly attenuates light greater than 800 nm to the extent that, for example, below 3 m in a Nebraska lake no radiation >750 nm or <375 nm was detected. Using a spectroradiometer that yields irradiance per nm of radiation we will determine the depth below which only radiation in the PAR range penetrates. This will be done in each reach and the maximum depth of penetration of radiation exceeding 700 nm will be determined. This depth would represent the minimum depth of water that could be above a deployed Onset sensor for it to be comparable to PAR sensors.

The specific null hypotheses to be tested are based on the following:

Response factors to PAR for individual meters will be determined (significant inter unit variability, Dr. Annette Olson, personal communication). An array accommodating several Onset sensors, a spherical PAR unit and a spectroradiometer will be deployed at the HMSC dock at a fixed depth during a tidal cycle. The fixed depth will be determined by the expected tidal range and the PAR-only penetration criterion. The predictably changing water depth provides the varying irradiance to which each sensor would respond. Under these calibration conditions the following null hypothesis will be tested H_8: there is a positive correlation between the Onset sensor output and the PAR radiation determined by the spherical sensor and spectroradiometer. If this positive correlation is found, individual Onset sensors will be attached to the moored PAR arrays and longer-term correlations between sensors will be ascertained before they would be considered for solo deployment.

SCIENTIFIC MERIT OF OBJECTIVE 3. If any one of the WQVs are close to being exceeded, or are exceeded in locations within the estuary, identification of the cause(s) of the excedence(s) would be needed for rational management of the watershed for optimum SAV growth. If nutrient WQVs were exceeded, direct sewage sources would need to be sampled and septic drain field contributions would need to be measured or estimated from adjacent land uses (Valiela, et al., 1992; Short and Burdick, 1996), and the contribution of benthic flux would need to be measured or estimated. Yaquina Bay has municipal sewage (secondary treatment) from Toledo discharged into it. If light attenuation was found to be excessive, partitioning of the TSS into inorganic and organic fractions would be needed. The organic components could be assigned to autochthonous sources (algae or fecal pellets containing algae), or allochthonous sources (originating from terrestrial plants and/or sewage). This partitioning could be done by determining the carbon and nitrogen isotope ratios in the TSS in combination with an analysis of the lignin and chlorophyll a content. Particles from different sources and, the water itself, contribute to light attenuation in ways that have been modeled (Gallegos, 1994; Gallegos and Kenworthy, 1996). Knowing which sources are contributing directly or indirectly to the shading of SAV by TSS would aid managers in making decisions for current or future land use practices.

Terrestrial plants fix carbon through the C_3- carbon fixation pathway resulting in tissue carbon containing less ^{13}C ($\delta^{13}C$ approximately -25‰) than the C_4- carbon fixation pathway that yields $\delta\ ^{13}C$ values around approximately -12‰ (Lajtha and Michener, 1994). The del (δ) notation in isotope research refers to the following relationship:

$$\delta\ (‰) = \frac{R_{sa} - R_{std}}{R_{std}} \times 1000 \quad \text{or} \quad (R_{sa}/R_{std} - 1) \times 1000$$

where R_{sa} is the ratio of the heavier to lighter isotope of the sample and R_{std} the ratio in the standard material.

Marine plants tend to have $\delta^{13}C$ signatures intermediate between the terrestrial values and can be species-specific (Lajtha and Michener, 1994, Chapter 7). The use of $\delta^{13}C$ values alone to distinguish most carbon sources to sub-estuaries of Hood Canal in Puget Sound, WA was found to be insufficient (Simenstad and Wissmar, 1985). The concomitant use of $\delta^{13}C$ and $\delta^{15}N$ measures have been shown to provide better resolution in tracing sources of estuarine organic matter (Peterson et al. 1985), with terrestrial sources containing $\delta^{15}N$ values of approximately -4‰ and estuarine detritus approximately +5‰ (Craft et al., 1988). Particulate nitrogen originating from sewage treatment plants would tend to be more enriched in ^{15}N than the original nitrogen source material (ie. food) because degradative processes tend to leave behind the heavier isotopes while producing ^{15}N-depleted dissolved products, such as urea and ammonia. For example, dissolved nitrogen species from sewage sources (ammonia depleted in ^{15}N) may be the dominant nitrogen source for up-estuary plankton in the early summer. It is likely that the salt wedge containing dissolved nitrogen species from upwelled water (nitrate enriched in ^{15}N, Lajtha and Michener, 1994, Chapter 7) reaches the up-estuary area later in the summer following spring tides. The $\delta^{15}N$ signature of planktonic algae in the upper reach of the estuary may be expected to become more positive in response to the changing sources of nitrogen as the growing season progresses. The lignin content of TSS would further elucidate the source of these solids because the plankton and macro algae, including Zm (but not *Spartina alterniflora*), contain virtually no lignin (Hedges et al., 1979; Haddad and Martens, 1987; Ciefuentes, 1991; Goni and Hedges, 1995). In addition, from the lignin parameters, the plant source of terrestrial material, gymnosperm (conifers) or angiosperm (broad leafed) could be elucidated (Hedges et al., 1979).

Objective 3: The object of this goal is the determination of the sources of suspended matter and nutrients entering the water column of these estuaries. The sources of these water quality impacting materials are varied and the goal of such a determination would be to allocate the load among the identified sources. The approach to be taken in this study is to determine unique characteristics or combinations of characteristics of the possible sources and looking at the position of samples along mixing curves of the sources. For suspended solids, stable isotopes of carbon ^{13}C and nitrogen ^{15}N, chlorophyll a, total lignin content, and the distribution of lignin decomposition products will be used to characterize sources of suspended organic matter. Source solids (endmembers) that would be considered include river borne material, settled fines from river backwaters, leaves, needles and wood of vegetation inhabiting the riparian zones of the river, soil litter from riparian zones of the river, undiluted sewage, axenic cultures of local phytoplankton, fecal pellet algae concentrates from the reaches of the estuary (herbivores concentrate phytoplankton but are relatively inefficient consumers), fine grained surficial sediment from different reaches of the estuary, and various macrophytes of the estuary, including Zm. Sources of nutrients that would be sampled include river water, sewage, septic field seeps to high intertidal channels, upwelled oceanic water, and sediment interstitial water. To help elucidate the sources of dissolve inorganic nitrogen species the $^{15}N/^{14}/N$ ratios would be determined in the nitrate and ammonium components using the methods of Sigman, et al., (1997) and Holmes, et al., (1998), respectively. Estimates of source-specific loads will be made using mass budget calculations based on linear mixing models. Collaboration with watershed modelers will be sought to help asses TMDLs for the estuary.

SCIENTIFIC MERIT OF OBJECTIVE 4: Segments of coastal Oregon estuaries generally fall into one of two categories (1a or 1b) in the estuarine classification scheme of Hansen and Rattray (1966). Category 1a is well-mixed with slight salinity stratification, generally near the estuary mouth, while category 1b has appreciable stratification at the head of the estuary, closest to the river input. Given the hydrodynamic similarities of these estuaries and our desire to link changes in watershed land use to estuarine processes that control particle formation and/or distribution, the generalized estuarine model of Hopkinson and Vallino (1995) is a good choice to evaluate. This one-dimensional, advection-dispersion reaction model incorporates the generalized hydrodynamics of an estuarine system at steady-state, using river input, estuary geometry, and dispersion coefficients from the literature. These physical process parameters are joined with coupled nutrient uptake and release, and organic matter production/oxidation reactions.

The model is based on the premise that water column community metabolism is primarily controlled by three forcing functions affecting nutrient availability, and the flushing of food web components from the estuary. The first is residence time, which influences the time for uptake of dissolved inorganic nitrogen (DIN), the remineralization of allochthonous organic matter and the flushing of phytoplankton and zooplankton from the estuary. The second is the DIN to organic nitrogen loading ratio. DIN is readily available to the algae autotrophs whereas the organic form requires a remineralization step. If the rate of the remineralization reaction is on the order of the flushing rate, it would result in much of this nitrogen not being "fixed" in the estuary, but being lost to the ocean. The third is the lability and nitrogen content of the organic matter entering the estuary. Sewage and forest litter would be at opposite extremes of lability for nitrogen, and both are entering many Pacific coastal estuaries. These physical and biological processes of the Hopkinson and Vallino model lead to predictions of steady-state distributions of salt, algae, and nutrients which can be measured throughout the estuary to assess the validity of the model in general, as well as the specific choices made for the various rates, yields, mortalities, and parametric ratios.

Although this model is based on assumptions such as steady-state for physical and biological processes that may be approximately valid only for limited times, the functions included and the predicted endpoints are appropriate for simulating some responses to different loading scenarios that would result from changes in watershed use. As written, the model does not explicitly address light attenuation which is directly relevant to SAV. Incorporation of the solids-specific (algae, clays) and water-specific (DOC and color) attenuation coefficients of Gallegos (1994) and a time-varying water column depth by adding a tidal subroutine (e.g. Vorosmarty and Loder, 1994) would increase the ecological realism of the model, would increase its utility, and the predicted light attenuation could be validated by our *in situ* PAR measurements (Objective 1a). The authors of the model are in the process of modifying it, and to the extent possible, we will work with them in making any changes.

Objective 4a: The object of this goal is the determination of estuarine water column responses to changes in the anthropogenic sources of suspended solids and nutrients. The model that we will evaluate for these purposes is that of Hopkinson and Vallino (1995). This model is a 1-dimensional advection diffusion model with biological components responding to carbon and nitrogen inputs, only. If we find that its simulated distributions closely mimic measured distributions of phytoplankton, dissolved inorganic nitrogen and salt we will use it as a backbone to add explicit algorithms to simulate inorganic particle distributions and tides.

The current model will be run using estuary-specific parameters such as river flow, dispersion coefficients, estuary length, and those from the literature, such as various uptake and growth rates. Although dissolved nutrient input from the river is solely discharge rate dependent the ability of moving water to support TSS is a power function of this rate and can be watershed dependent (Jay, personal communication). Boundary conditions (endmember concentrations) will be determined or estimated in the ocean and river waters entering the estuaries. These will include: autotrophs (as chlorophyll a), heterotrophs, dissolved inorganic nitrogen (DIN), labile organic material (OM), refractory OM (each in mg parameter m^{-3}) and salt (‰). Model accuracy will be evaluated through comparisons to concentrations determined at 10 evenly distributed channel locations along the length of the estuary Figure 1 (Jassby et al., 1997).

The accuracy of the simulations of the conservative parameter salt would be a test of the basic advection-dispersion elements of the model and is critical to all others. Salinity is the most easily determined parameter in this model as it would come directly from our CTD instruments. Physical parameter adjustment/selection to obtain the best match for this parameter will anchor these critical parameters. Repeated runs with random-normal distributions (Abramowitz and Stegun, 1965) of biological process parameters will be used in the evaluation phase using the literature to establish mean values and their uncertainties. ANOVA will be used to detect the parameters to which this model is most sensitive by comparing at several single locations the model outputs for, say, 25 runs for each parameter. The effects of the ranges of parameters available in the literature will be evaluated with those parameter combinations yielding the smallest deviation from the measured parameters selected as the likely, estuary-specific "true"values. Measurements of autotroph concentration as chlorophyll a and dissolved inorganic nitrogen species concentrations will be made for comparisons to model run outputs and those parameters affecting these concentrations will be manipulated for "best fit" to narrow the range of possible values. At this time, outputs: refractory and labile organic matter, and heterotrophic concentrations are not measured directly. Dissolved organic carbon, and particulate organic carbon and nitrogen will be determined and will put bounds upon these components and ratios but they are not explicit model outputs.

Ojective 4b: Once parameter selection has resulted in a minimum overall difference between model and measurements, estimates of precision at any location can be made by compiling the range of results obtainable for reasonable ranges in parameters including the variation in the measured endmember concentrations. The use of runs with combinations of random-normal parameter distributions would be equivalent to a propagation of errors analysis and would yield uncertainties of simulated output parameter means.

IMPLEMENTATION

Objective 1a, Probabilistic Station Selection: We will work with staff of the Regional Ecology Branch, who are familiar with the EMAP probabalistic sampling protocols (Stevens 1994) in selecting stations in the Yaquina estuary. In general, we will break an estuary into three depth zones that are continuous throughout. These stations will be sampled during optimum sun angles (local solar noon ± 2-3 h, Miller and McPherson, 1993) with a minimum of 5 feet of water over the bottom to obtain a profile at least 1 meter in length. This would result in sampling these sites during a 4-6 hour period with a sampling water depth of at least 6.5 feet. The number of stations in each zone will likely be in the range of 30 to 50. Since all the required samples will take more than one 4-hour interval, approximately an equal number of samples from each zone will be sampled during each interval over up to four 4-6-hour intervals. These stations will be sampled every other month starting in May 1998. Supplemental stations will be selected that will provide supportive data to other researchers involved in Zm out-plantings and productivity measurements (DeWitt and Boese projects)

Objective 1b, Water Quality Parameter Methodology: The stations selected above will be sampled using a SeaCat Profiler with an attached inlet to a shipboard pump. In general, instrumental data (CTD, fluorescence, PAR, turbidity, DO) will be collected through the water column and discrete water samples from the pump will be collected from mid-depth, but not to exceed 0 ft MMLW. The water subsamples will be quantitatively filtered for TSS (gravimetric collection) and chlorophyll a. Filtered water will be analyzed for water nutrients (ammonia, nitrate, nitrite, orthophosphate, and silicate), water color, and DOC. Unfiltered water will be analyzed for water nutrients and chlorophyll a. Analytical methods are listed in Table 2. Turbidity and fluorescence responses from the SeaCat will be calibrated using the measured TSS and chlorophyll a concentrations.

Objective 2a, Continuous PAR Methodology: We will obtain a continuous *in situ* record of scalar PAR irradiance and attenuation coefficients at sites along the estuary axis using 4π sensors (LI-193SA with LI-1400 datalogger, LI-COR, Lincoln, NB) at two fixed depths (-1m to -2m MLLW). Turbidity, temperature and salinity will also be monitored continuously at a near-surface depth. Spherical sensors are used because Zm blades are able to intercept light from all angles (Morris and Tomasko, 1993). Five relatively secure sites have been chosen for continuous deployments of the PAR arrays. The locations are the Hatfield Marine Science Center dock complex, Sawyer's Landing marina, River Bend marina, Oregon Oyster dock, and Critesers Marina (Figure 1). Negotiations for the use of Coast Guard day marks for these deployments are ongoing. A floating and fixed PAR array at the Hatfield Marine Science Center dock will permit the calculation of the attenuation coefficient of the entire water column minus the top 1 m. A 2π PAR sensor will be continuously mounted on the roof of the EPA building to provide in-air irradiance.

Computation of productivity from integration of the continuous PAR records will allow us to compute carbon fixation during the growing season and compare productivity from literature P vs I relationships to those obtained at different, or the same, sites in the estuary using other methods such as leaf growth (see Boese Research Project). The need for continued continuous deployments of paired PAR sensors and the utility of PAR profiles will be assessed from the. variability of daily integrated PAR and attenuation coefficients.

Objective 2b, Alternative to PAR Methodology: The LiCor arrays are expensive

(~$2000) and with their surface dataloggers they are especially vulnerable to interference by the public. Relatively inexpensive, full spectrum (350 to 1100 nm) light intensity meters (HOBO StowAway LI, Onset Computer Corp., Bourne, MA) will be deployed at the same depths as the LI-COR spherical meters to evaluate the feasibility of using these meters in future, more extensive data collections at more remote sites. Intercalibration between the LI-COR and Onset sensors will use *in situ* measurements of the spectral irradiance at the sites using a LI-COR spectroradiometer because the Onset sensor is strongly dependent on the spectral characteristics of the incident light and *in situ* light attenuation is strongly wavelength dependent above 700 nm (Jerlov, 1968).

Objective 3, Anthropogenic Sources of Water Quality Parameters: From a second subsample of the water pumped from selected stations a sufficient mass of particulate material will be collected for the analysis of TOC/N, lignin, carbon and nitrogen isotopes. These results will be combined with the analyses of materials that would be considered pure end members of a two or more source mixing problem. End member candidate materials for excess nutrients might be sewage effluent, estuarine fecal material, decayed Zm and leaves of local deciduous trees. Analysis of water from estuary side channels, septic seeps, sewage out falls, and benthic flux chambers would contribute to the segregation of anthropogenic from "natural" contributions of nutrients.

Objective 4a, Model Implementation and Verification. The model of Hopkinson and Vallino (1995) was developed at the Ecosystem Center of the Marine Biological Laboratory in Woods Hole, MA and represents the synthesis of their thoughts on linking runoff and water column metabolism. This model was in part funded by the Land Margin Ecosystem Research (LMER) program of the National Science Foundation and was validated using parameters and values from Parker River/Plum Island Sound, MA, a LMER site. We have the model up and running and have reproduced some of the published distributions.

a) Discrete water sample concentrations and SeaCat instrument values will be used for boundary (end member) concentrations of salt, nutrients, etc. Initially, default model parameters, Yaquina Bay geometry and diffusion coefficients (Callaway and Specht, 1982) will be used for model runs. The need to add a second or third dimension to our modelling considerations will be made in consultation with EPA collaborator Pete Eldridge and consultant Dick Callaway. In addition, coupling of this bay model to a shelf-regional model may be necessary to capture the dynamics of upwelling and net tidal flux of model components. This will be accomplished through collaboration with new offshore oceanographic-meteorologic program that is to be administered through Oregon State University. Boundary concentrations will be determined during maximum and minimum river flow periods and periods of upwelling. In addition, hourly sampling of physical water column characteristics will be sampled for 24 hrs during neap and spring tides during the winter and summer seasons.

b) To assess the accuracy of the model in computing concentrations of algae, nutrients, etc. (Table 3) down the length of the estuary we will deploy the SeaCat Profiler at evenly-spaced stations (Jassby et al., 1997) at midchannel with discrete water samples from selected depths at each station.

Objective 4b, Model Modification, Light and Tides. a) We will incorporate light attenuation linked to inorganic particles and phytoplankton through PAR absorption and scattering relationships (Gallegos, 1994). This would result in a length-of-estuary distribution of attenuation coefficients that, through a P vs I relationship, would delineate the light environments sufficient to sustain SAV growth. We could use the P vs I algorithms presented by Zimmerman, et al.(1994), and others. For the most accurate, locally-applied model, growth dynamics of the local race of Zm will ultimately need to be determined (Objective 1 III , above).
b) We will incorporate algorithms to represent tides. With the modeled light attenuation this would generate a more realistic *in situ* light environment as the tide-induced water column depth changes exceed the minimum depth and leaf extension of many Zm beds in Yaquina Bay.

c) Repeated model runs with random-normal distributions (Abramowitz and Stegun, 1965) of the newly created parameters will be used to evaluate the relative sensitivity of the modified model.

EXPECTED RESULTS AND BENEFITS: The major products from this project will be an evaluation of the current susceptibility of eelgrass beds in Yaquina Bay and a model that could be used to provide results for different future development scenarios. Estimates of the composition of the suspended material that is controlling light attenuation will be given. With these results, the effectiveness of different mitigation efforts to enhance water quality related to eelgrass habitat could be evaluated.

Table 2. Parameters needed for calculating Water Quality Values of Dennison et al. (1993).

Description	Analysis
light attenuation coefficient	photosyn. active radiation (PAR) 2 depths/station
total suspended solids	mass collection on preweighed membrane filters
chlorophyll a	acetone/water extract with fluorometer quantitation
dissolved inorganic nitrogen	AutoAnalyzer
dissolved inorganic phosphorus	AutoAnalyzer
salinity distribution	CTD

Table 3. Parameters needed for Hopkinson and Vallino (1995) estuarine ecosystem model.

Description	Analysis
dissolved inorganic nitrogen	NH4, NO3, NO2 AutoAnalyzer
C:N ratios of allochthonous solids	TOC/N
particulate organic C and N	TOC/N (filters and sediment)
dissolved organic carbon	DOC
total suspended solids	mass collection on preweighed membrane filters
flocculation of DOC to POC	DOC, TOC/N on glass fiber filters
water residence time	river gauge data with estuary geometry
river and ocean concentrations	endmembers for the hydrodynamic model
estuary geometry	tidal analysis, GIS
dispersion coefficients	literature values
river flow	river gauges (USGS)

Project C3. Nutrient Processes: Watershed versus Oceanic Inputs to PNW Estuaries

Principal Investigator: Anne C. Sigleo

Goal: The goal of the proposed work is to define annual dissolved nitrogen and suspended nitrogen budgets for two estuaries in the northwestern part of the U.S. under various scenarios for watershed rainfall/run off conditions, oceanic inputs and anthropogenic activities in the area. A further goal is to establish predictive relationships between readily measured nutrient characteristics and to use these data as indicators of ecosystem change, or condition, in PNW estuaries by linking predictive estuarine circulation and watershed models.

RATIONALE: Willapa Bay, Washington is a highly productive estuary that supports over 50% of the commercial oyster production on the west coast. In addition, the bay serves as a nursery for Dungeness crab and English sole, and as a spawning area for chinook, chum and coho salmon. It provides extensive clamming opportunities and is home to shrimp, salmon, and steelhead and cuthroat trout.

Water quality, including nutrient load, is of concern for many of the stakeholders in Pacific Northwest estuaries, particularly those involved with aquaculture, who are dependent on water quality for a marketable product. To capitalize on the nutrient data base developed from research over the past three years, work will continue in Willapa Bay, Washington. Data from Yaquina Bay, Oregon will be used to provide a comparative framework, thus strengthening the potential applicability of the results to multiple PNW estuaries. These two estuaries encompass a size range of 17 km^2 in Yaquina Bay, to 320 km^2 in Willapa Bay, one of the larger PNW estuaries. The respective watersheds for these estuaries vary from 655 km^2 in the Yaquina to over 2000 km^2 (1,865-2,849 km^2 reported range) in Willapa Bay (Baker et al., 1995). Both estuaries support aquaculture (oysters, hardshell clams), fishing, and migratory waterfowl. The Willapa watershed is dominated by timber plantations, along with some agriculture, beef ranching and dairy farming. Urbanization is minimal, with the exception of Long Beach Peninsula and the lower Willapa river. The Yaquina estuary lies at the mouth of the Yaquina River and appears more urbanized with a population of 9,785 (July '96) located in the city of Newport and Toledo (3,200). The population of the Willapa area (6,884 (1996)) is distributed around the bay at Long Beach (1,400), Ilwaco (864), South Bend (1,660) and Raymond (2,960).

In spite of their importance, sufficient data on nutrients and energy flow through the primary producers for resource assessments and future planning are lacking for both estuaries. Hydrographic input to Pacific Northwest estuaries is related to seasonal variations in rainfall (Peterson et al., 1982 and 1984). During winter months of peak river discharge, terrestrial material is transported downriver into the estuaries. During the summer months, however, beach sand from the continental shelf is transported into the estuaries by tidal currents (Scheidegger et al., 1971; Peterson et al., 1984; Schwatrz et al., 1985). Nutrient inputs to

PNW estuaries are thought to be similarly seasonal, although only sporadic data are available to support this theory.

Sufficient levels of nutrients are required for organism growth, health and reproduction. Above the optimum levels, however, high nutrient concentrations can alter the ecosystem by favoring species that flourish in high nutrient conditions. The recent toxic bloom of *Pfiesteria* sp. in subestuaries of Chesapeake Bay was the result of excessive nutrient loading (Milot, 1997). The reoccurrence of toxic algal blooms also is a major concern in Pacific coastal estuaries (Sayce and Horner, 1996; Horner et al., 1997). The initiator or stimulus for the organisms to produce the toxin is organism stress, particularly that due to eutrophic levels of nutrients (Pan et al, 1996a and 1996b; Sivonen, 1996; Kotaki et al., 1996; Bates et al., 1996; Paerl, 1996; Paerl and Millie, 1996; Christoffersen, 1996). In Willapa Bay, Yaquina Bay and the adjacent ocean coastal zones, toxins above a legal threshold result in closure of shellfish beds and the sale of oysters is prohibited. This results in a substantial loss of income for the oyster growers as well as a loss of food source for members of the Shoalwater tribe in Willapa bay.

In addition to harmful algal blooms, the consequences of high nutrient loading include oxygen depletion as a secondary effect due to oxygen uptake by microorganisms during the breakdown of large amounts of algal biomass. Thus, a nutrient budget, including nutrient sources, would benefit planners considering environmental impacts such as sewage disposal from increasing populations. A method of quantifying and stating nutrient loadings are TMDLs (total maximum daily loadings). The data for mass balance goals also provide fundamental data for supporting TMDLs.

The results of analyses to date provide data on nutrients affecting the primary producers at the base of the aquatic food web for resource assessment and balanced resource management for Willapa Bay. Nutrient concentrations, phytoplankton biomass concentrations and phytoplankton species abundances were determined in samples from at least five stations for over 34 months in collaboration with K. Sayce, Shoalwater Botanical Laboratory, Nahcotta, WA. The results indicate that high loadings of nitrate (up to 1.2 mg/L) were entering the estuary from coastal waters, rather than the rivers of the Willapa watershed. The 1995, 1996 and 1997 water years were unusually cool and wet with significantly higher rainfall, nitrate nitrogen concentrations (up to 1 mg/L) and settled plankton biomass (up to 150 ml/L phytoplankton + zooplankton). Dissolved nitrate values decreased steadily throughout the spring and summer, but did not disappear entirely. Ammonium and phosphorous were not detected in the main channels of the estuary, although traces of ammonium were measured in the Willapa and Palix Rivers. Phytoplankton, dominated by diatoms, also continued to bloom steadily throughout spring and summer. The 1995 nitrate values are comparable to those reported by the USGS for water years 1969 and 1970, also wet years with similarly high runoff. The results provided nutrient data of sufficient resolution to detect at least two primary nitrogen nutrient sources for Willapa Bay. Future work will continue to document the quantitative input from each of the sources. The water column baseline data on nutrient concentrations and variability can be utilized in circulation modeling and oyster condition index studies.

OBJECTIVES: The purpose of this research project is to assess the magnitude of each component of the nitrogen biogeochemical cycle, along with supporting information on phosphorous, carbon and silica, to evaluate potential nutrient limitations or sufficiencies for phytoplankton productivity and species composition of the above elements via Redfield ratios. Availability of nutrients is a major factor controlling phytoplankton primary productivity and species composition (Golterman et al, 1983), including species that can produce toxic blooms (Pan, et al. 1996a and b).

The specific objectives of this work are:

1) to determine the net fluxes of nitrate, nitrite, ammonium, dissolved organic nitrogen (DON), reactive phosphate, and dissolved silica between estuarine, river and ocean waters.
2) to determine whether PNW estuaries act as net sources or sinks of inorganic nutrients at the current levels of nutrient loading in Willapa and Yaquina estuaries.
3) to examine source-sink relationships within intertidal communities and estimate net fluxes of nutrients across the sediment-water interface.
4) to examine wet depositional inputs of nutrients. This is not a likely PNW source (Weathers et al., 1988), however, some measurements are required to prove or disprove the idea.

Scientific Approach: 1) To determine nutrient fluxes, samples will be collected weekly. Phytoplankton in estuaries respond rapidly to light and changes in water column nutrients so that weekly samplings are the longest time interval appropriate to capture the effects of nutrients on plankton biomass and productivity. We have previously supplemented the weekly sampling scheme with hourly to two-hourly diel cycle samplings over three day periods to follow individual water masses (Sigleo et al, 1997) and were able to document an upwelling event. A similar approach is proposed again, along with the additional data collection via a continuous (every 15 minutes) nitrate monitor. The five stations used for weekly sampling are representative of the major parts of Willapa bay. The stations are located at 1) Naselle Channel, representative of the Naselle River outflow; 2) Nahcotta Channel, representative of the central-southern bay; 3) Palix River BCM dock for the Palix River that is available at all times to Bay Center Mariculture personnel; 4) Riddle Spit Channel between Riddle Spit and Nemah Spit, representative the northern center of the bay; and 5) Stackpole Slough west of Nahcotta Channel is located over oyster flats and represents oyster finishing beds (Fig. 1). Samples were collected at high slack tide for phytoplankton species, salinity, temperature and turbidity during 1993 and 1994. For data continuity, samples were collected for nutrient analyses (1.5 m depth) simultaneously with samples for phytoplankton species identifications and counts from the same five stations in 1995, 1996, and 1997. In 1997, additional stations were added at 1) the Pacific Ocean to quantify the nutrient content of water masses entering and exiting Willapa Bay, 2) Toke Point at the northern end of Willapa Bay in the North River and Willapa River mixing zone, 3) Naselle hatchery, 4) Willapa hatchery, 5) Loomis Lake as a measure of long term atmospheric input, and 6) Island Lake, a recreational lake surrounded by homes, to study the septic tank input. The hatchery samples (collected above the hatchery water inflow) are representative of the river nutrient inflow and are adjacent to the USGS river gaging stations above the head of tide. The Yaquina Bay sampling station is at the terminus of the OSU seawater intake dock and has the advantage that nutrients can be analyzed within minutes of collection. All samples are collected at high slack tide, except for specific experiments or where otherwise noted.

To determine the input of nitrate nitrogen in PNW estuaries, and particularly the net input flux of oceanic nitrogen requires continuous measurement over seasonal cycles. Because the oceanic water enters and exits twice daily on tidal cycles the method of estimating the amount entering, utilized within the estuary and leaving the system requires continuous measurements to model the appropriate functions from the acquired data. To accomplish this goal, we propose using an Ocean Systems Ltd nutrient monitor that can measure nitrate hourly up to 30 days. After discussing the problem with numerous oceanographers and oceanographic modelers, it was concluded that continuous monitoring of the major nitrogen species, namely nitrate, over all tidal cycles, seasons, and rainfall/runoff conditions was a reasonable means for obtaining the required data. To determine the amount of seawater in a water mixture, the element bromide is being used as the indicator of seawater (ie, percent seawater). Bromide can be measured very precisely and accurately by ion chromatography in our laboratory, along with the nitrate from manually collected samples. By measuring nitrate at a specific point just inside the mouth of Yaquina Bay continuously, the amount of nitrate can be approximated using Newport's available tide model by Walter Frick. This tide model is able to predict velocity under the Yaquina bridge to within 90% accuracy (Frick, pers. com.). The Yaquina calculations are simplified due to the very confined channelization of the water by jetties. From the simplest perspective, tide flat circulation modeling can wait until additional resources become available, and refinements to data collection and calculations can be made.

The instruments for more detailed modeling include acoustic Doppler profilers (ADPs) for 3-D current profiling on the tidal flats and in the channels. Drogues used in conjunction with the current profilers will provide actual water paths at desired depths to refine models for both surface currents and deeper ones. For example, unknown circular loops or eddies concentrating nutrients, suspended particulate material and biota within a bay have been identified in other estuaries this way (Science, v281:196, 1998). The tide flats themselves are a known source and sink of nitrogen species, and to address that issue, an NRC postdoc will begin working in April of FY99.

In order to calculate a flux, concentrations are typically measured at a single point (ie, a discrete point). One requires a fixed point to make any sense of the data. To relieve concerns that the station chosen for deployment of the NAS nitrate analyzer may not be representative of the Bay entrance, we plan several cruises at different seasons and tidal cycles to measure nitrate around the analyzer location at different depths, and under the Yaquina bridge to the jetty entrance. A second nitrate analyzer on a navigation buoy 15 or 20 miles outside the mouth of the Yaquina would improve the data set substantially.

Nitrogen fluxes are equal to the nutrient concentration times the mass water flow and the mass balance of an analyte is equal to the flux times time. In Willapa Bay, we propose mounting the nitrate monitor on a navigation buoy between Riddle Spit and Nemah Spit. Although the bay entrance would be ideal, we have yet to devise a workable mooring because the entrance is continuously shifting. In Yaquina Bay, the nitrate monitor is mounted on the terminus of the OSU dock at the mouth of the bay.

The nutrients studied are listed in Table A.2.3-1 and include nitrate, phosphate, and ammonium, all of which may be quantified reliably by ion chromatography using EPA Test Method 300.0 or spectrophotometric methods (Pfaff et al., 1991; Fong et al, 1995; Strickland and Parsons, 1977). Bromide, measured with the nutrients by ion chromatography, is used as an indicator of the oceanic component in a water mass. The assessment of nutrients also will include silica (Pan et al., 1996a).

During winter months of peak river discharge, terrestrial material is transported downriver into the estuaries. To quantify the river sediment loads and the nutrients contained therein, suspended sediments will be sampled intensively over the first and subsequent rainfall/high river flow events of the fall and winter (between October 1, 1998 and March 1, 1999) at the Willapa River station near Willapa, the Naselle River station near Naselle and the Chitwood station on the Yaquina River. Samples will be collected according to procedures outlined in USGS Techniques of Water Resource Investigations (TWRI) publication "Field Methods for Measurement of Fluvial Sediment". Samples shall be analyzed for suspended sediment concentration by weight, and relative grain-size fractions. Filtered water samples (60 ml) will be collected for dissolved nutrient analysis and frozen as soon as possible. These data will be combined with the hydrograph data obtained at the gaging stations by the USGS (Tabel 2) to produce estimates of suspended sediment loadings to the estuaries.

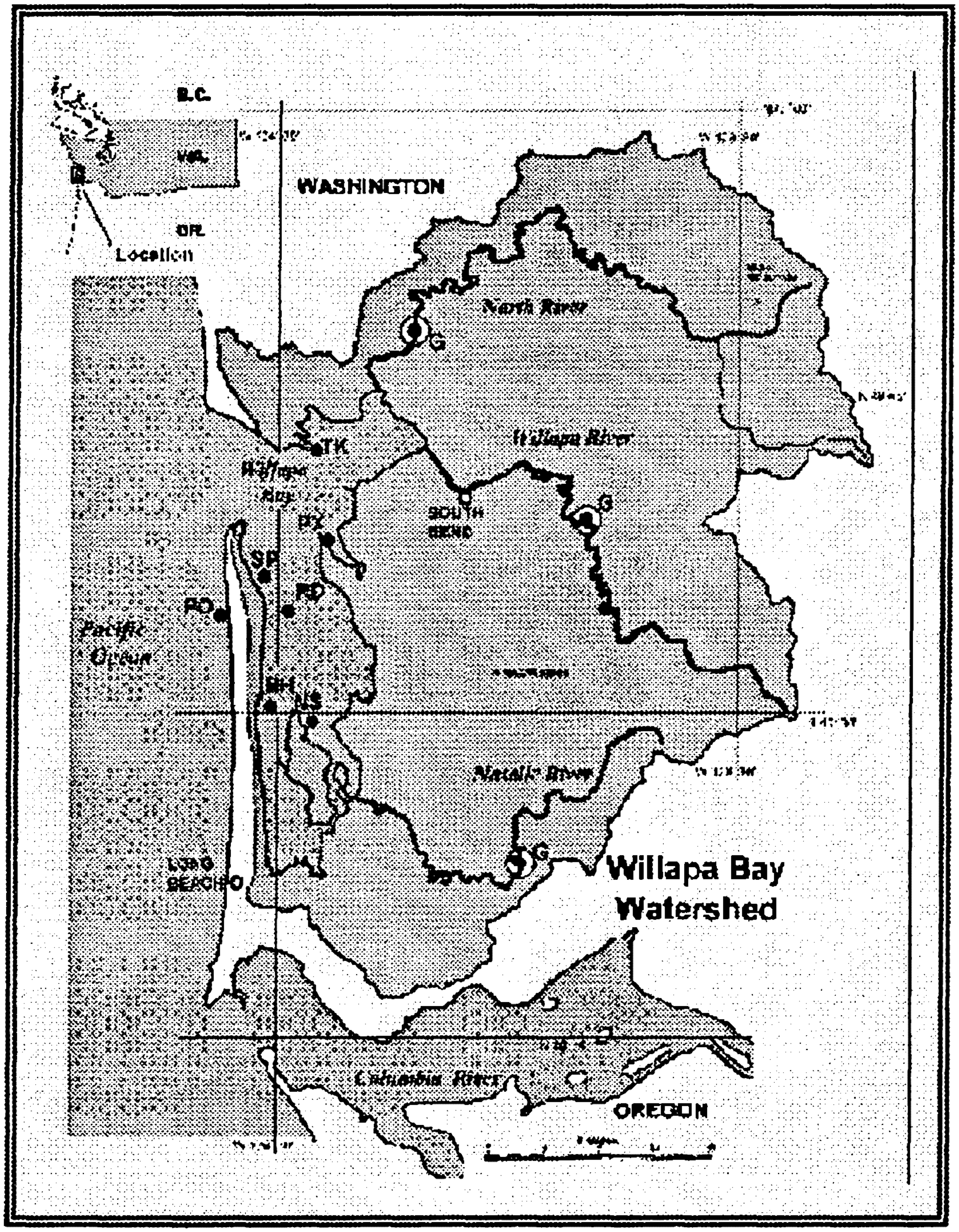


Figure 1. Willapa Bay sample collection locations at Naselle Channel (NS), Nahcotta Channel (NH), Palix River (PR), Riddle Spit Channel (RS), Stackpole Slough(SP), Toke Point (TK), and Pacific Ocean (PO). The USGS water flow measurement stations are labeled with a G.

Table 1. Water Sample Parameters

Sample type	Parameter measured	How measured or obtained	Percent of samples
Nutrient	N, P, Si, NH_3 HNO_3	Ion chromatograph Spectrophotometer Nitrogen monitor	100 20 80
Temperature	Degrees C	Thermometer	100
Turbidity	Depth	Secchi disc	50
Salinity	Density	Hydrometer	75
Phytoplankton group	Pigments	HPLC	10
Ocean vs River source indicators	Stable isotopes Bromide	Isotope mass spec (P.O., Coop, etc) Ion chromatograph	15 (contract) 100

Table 2. Willapa Watershed and Yaquina River Hydrographic Data

Name:	Gage Number	Drainage Area	Data Period	Notes:
North River	12017000 12016600	219 mi^2 188 mi^2	1927-1977, 1995-1997 1965	 Sed data 1965
Willapa River	12013500	130 mi^2	1947-present	Chemical data 1965-1986
Naselle River	12010000	54.8 mi^2	1929-present	Chemical data 1965-1980
Yaquina River	14306030	71.0 mi^2	1973-present	Sed data 73-74

2) To determine whether PNW estuaries act as net sources or sinks, the source(s) of water column nutrients will be further investigated during summer sampling using diagnostic indicators such as stable isotopes of carbon and nitrogen whose abundance can be indicative of unique sources or processes. In particular the oceanic vs river source of nitrate nitrogen will be examined using nitrogen isotopes (Sigleo and Macko, 1985; Bilby et al., 1996). We found in other studies that the nitrogen source differed seasonally, with winter inputs coming from terrestrial runoff of nitrate, whereas the summer source of nitrogen was remineralized benthic ammonium (Sigleo and Macko, 1985). Suspended sediments for nutrient studies will be collected via sediment traps (Sigleo and Shultz, 1993) at 2 m depth, timed to collect over 6-hour tidal cycles. Previously in the Potomac estuary we employed duplicate vertical arrays of three (surface, pycnocline, and near bottom). In Pacific coastal estuaries, the water column is generally well mixed vertically. Since vertical features such as stratification are uncommon, single traps at one intermediate depth are proposed initially.

3)Benthic fluxes, particularly benthic ammonium release from the Pacific intertidal and surf zones (Lewin, 1977; Sigleo et al., 1997), will be monitored by benthic chambers for four hour deployments (Boynton et al, 1980; Sigleo, 1985; Cowan and Boynton, 1996) using the benthic chamber design of Davis and McIntire (1983) or others. The oceanic input and exit for an individual parcel of water will be further examined using drogues (Power, 1996). This technique could be combined with larval recruitment studies for the estuarine fish research of Power, since larvae and oceanic phytoplankton are transported into estuaries in incoming water masses, as are oceanic nutrients from upwelling.

A major sink for fixed aquatic nitrogen in many coastal estuaries is denitrification (Seitzinger et al. 1984), or the conversion of organic nitrogen to N_2 by denitrifying bacteria. Seitzinger et al. (1984) found that the annual N_2 production for Narraganestt Bay, R.I., was equal to approximately 50% of the fixed inorganic N loading to the bay. Denitrification accounted for about 35% of the organic nitrogen mineralized and removed from the sediments as N_2. Denitrification is commonly measured using a ^{15}N tracer technique to quantify in situ denitrification rates (Xue et al., in press). Gas samples collected over discrete time intervals are analyzed on an isotope mass spectrometer to quantify the rate of N_2 production (Xue et al., in press).

4) Nutrient input from wet deposition will be measured with water from the rain gages at the Nahatta, Cranberry center and ridge weather stations. Cloudwater for the Northwest has the lowest ion content measured (Weathers et al, 1988), suggesting that rain is not a source of nutrients.

Expected Results and Benefits: The data produced already are being used to predict oyster growth and condition for the existing scenario for marketing purposes by the Willapa Bay Oyster Growers Commission, Share Bank Enterprises, Inc., and Taylor United. The Washington State Department of Fish and Wildlife both funds and uses our phytoplankton data to monitor for toxic blooms of *Pseudo-nitzschia*. The results ideally will include a model

(HSPF) that can predict environmental impacts based on changes in nutrient inputs (Lumb, 1994), possibly via a postdoc or graduate student.

The results for 1995 through 1997 indicated that winter storms bring nutrients, particularly phosphorous, downstream. During the spring and summer, however, the primary source of nutrients is oceanic upwelling. Because oceanic upwelling is a natural process with a wide range of variability, both the process and the natural range of nutrient supply must be known to assess anthropogenic effects. It is clear that there is a threshold effect, above which anthropogenic perturbations would have a significant effect. In other words, anthropogenic inputs must exceed a nominal threshold to adversely affect the estuary. Determining the threshold above upwelled nutrients by measuring the nutrients from upwelling and bioconcentration is fundamental to understanding nutrient processes in Pacific Northwestern estuaries.

Cross Linkages: Tide models for tidal exchange were developed for the Pacific coast from Baja to Alaska by Frick, Chapman and Clinton in EPA and are available. A circulation model developed for Puget Sound by Walters at the USGS in Tacoma can be developed for Willapa Bay via a 34K IAG and be verified using Walt Frick's Acoustic Doppler Current Profiler data and Barbara Hickey's current data (supported by Sea Grant and EPA, Newport). The circulation model is critical to predicting the tidal range of estuarine parameters such as salinity and turbidity. With respect to circulation modeling, the only way of organizing and making deductions, including forecasting, with large quantities of data is with a comprehensive circulation model. Salinity zones within the bays, for example, change drastically with 1) the tidal input or output, and 2) the rainfall/runoff parameters. Storm forcing also affects the salinity from the seaward side. To achieve prediction capability, physical parameters such as the channels that seawater enters versus exits (no, they're not the same usually, except in confined cases), along with speeds and direction are required. Then there is the chemistry and sediment transport. Fortunately, Athens considers sediment transport algorithms a priority item and may provide help with a "test" estuary. Chemical and watershed models such as HSPF exist and can be combined into the circulation model.

Determining terrestrial inputs into the estuaries from upland watersheds can be modeled to organize and analyze present and historical data, and to simulate or project effects of future changes. One widely utilized program in both EPA and the USGS is HSPF (Hydrological simulation program -Fortran) and auxiliary prediction programs such as HSPEXP (Dinicola, 1990; Lumb et al, 1994). The HSPF watershed model could be accomplished as a student thesis.

In Yaquina Bay and Coos Bay, nutrients, larval recruitment of crabs, barnacles and several other estuarine organisms are presently being studied at OIMB (Linda Shapiro, director). Offshore currents, nutrients and oceanographic processes are being studied by several faculty (Barth and Smith, Wheeler) in the Department of Oceanic and Atmospheric Sciences at OSU from Yaquina Head to Cape Blanco. Bob Emmett of NOAA, NMFS is studying the anchovy population from Grays Harbor to Tillamook Bay, and we are coordinating our sampling at Willapa Bay during the summer of 1998 to coincide with his oceanic sampling off of Willapa Bay.

Actual data exist that have not been utilized. These data include detailed current surveys by the USGS of Willapa Bay in 1976 and 1977, U. Washington current surveys in 1995 and 1996, and extensive ADP collections on 3 navigation buoys for 1998 by a contractor for Washington Department of Transportation.

5.0 Research Linkages, Users and Participants

5.1 Research Group Linkages

The individual projects and themes of the proposed CEB research plan are interlinked in many ways. DeWitt's work on interactions between burrowing shrimp and eelgrass habitats will complement that of Boese who is examining the effects of additional types of physical and biological disturbance on eelgrass, and the data sets should be strongly complimentary. Specht will make measurements in beds of the exotic seagrass species *Zostera japonica* which will parallel the types of measurements being made by DeWitt in beds of the native *Zostera marina*. Specht's studies will require bathymetric contouring and he will collaborate with Young to attain digital terrain elevation models of the estuarine intertidal and subtidal zones.

The Boese and DeWitt benthic studies also share sample locations with the habitat-faunal relationship studies of Ferraro and Cole. The Boese experiments will provide insight into how habitat-faunal relationships change as the eelgrass habitat is disturbed, while Dewitt's work will examine the effects of additional stressors on eelgrass and burrowing shrimp habitats. Both studies thus provides complementary information to Ferraro and Cole. Part of the work proposed by Ferraro and Cole seeks to develop optimal sampling designs for estuarine habitats, which may potentially benefit all other benthic research components.

The landscape scale research of Robbins is particularly aligned with research proposed by DeWitt, Ozretich, Sigleo, and Young. Research proposed by other CEB scientists concerned with habitat use and faunal distribution (Ferraro, Cole, and Power) can also use the landscape ecology framework as a vehicle for making predictions concerning the distribution of target organisms. More specific research questions, such as those asked by Boese and Specht, can also take advantage of the information developed by landscape pattern analysis. Results of landscape scale research within the estuary can also be interpreted in the context of the watershed research findings conducted by WED scientists in the Regional Ecology Branch. The integration of watershed processes will facilitate the understanding of the linkages between terrestrial and aquatic landscapes.

Basic physical and geochemical data for the water column and surficial sediment components of the ecosystem will be generated by the research of Ozretich, Sigleo, Young, and Specht. An accurate digital map of the Submerged Aquatic Vegetative habitats, and detailed digital maps of estuarine bathymetry, also will be available to support CEB research efforts.

The spatially explicit modeling effort involving Lee, Bodeen and DeWitt can directly interact with the population level development of ecosystems indicators proposed by

Power. Power's work deals with spatial variability of stressor effects on juvenile fish growth rates, and is thus similar in concept to the modeling effort. Field data collected by Power on fish, and DeWitt on burrowing shrimp populations, will serve as potential inputs to the spatially explicit modeling effort. Conversely, the modeling effort will serve as an analytical tool to help understand the results of the population level studies of stressor effects.

5.2 Graduate and Postdoctoral Research Collaborators:

CEB is greatly expanding its committment to the support of graduate and postdoctoral research positions to allow additional research questions related to the general research direction to be addressed. At present, there is one National Network for Environmental Management Studies (NNEMS) graduate research fellow beginning work at CEB. Ms. Kelly Chapin will interact with Ferraro's work on habitat-biota relationships with a study of comparative habitat utilization of native and exotic brachyuran crabs in Yaquina Bay. A National Research Council (NRC) Postdoctoral Fellow, Dr. Gail Dethloff will also collaborate with Ferraro in a project examining intertidal habitat use by fish and decapod species is Pacific Northwest estuaries.

In addition to Chapin's work, NRC fellow Dr. Kama Almasi will conduct a comparative study involving an examination of range expansion and community dynamics of the native (*Z. marina*) and non-native (*Z. japonica*) eelgrass species in Yaquina Bay. NHEERL Postdoctoral Fellow Dr. Scott Larned will examine the interactions between a native burrowing shrimp and the introduced *Z. japonica* to determine their effects on sediment and nutrient dynamics. Alamsi and Larned's work will interact with that of Specht, DeWitt, Sigleo and Young.

5.3 Users and Participants:

The principal expected users of the results of the CEB research effort are the many organizations involved in conducting habitat-based ecorisk assessments and futures analyses on PNW estuaries. These include ecosystem risk assessors, estuarine ecologists, and local, state, and federal agencies with estuarine resource management responsibilities. Local planners, particularly the Willapa Alliance have already begun using nutrient and phytoplankton data collected by CEB in Willapa Bay, and other important local contacts with groups such as the Central Oregon Coast Watershed Council are being developed. At the regional level, EPA Regions IX and X, the Washington State Department of Fish and Wildlife (collaborators 1995 - 1997), Washington Department of Ecology, and the Pacific Northwest District, US Geological Survey, Water Resources Division are all potential users of the research data. At the national level, an important client is the Office of Water of EPA.

There are numerous potential collaborators in the research program both regionally and nationally. Potential agency collaborators on-site at HSMC include the Oregon Department of Fish and Wildlife and National Marine Fisheries Service. CEB is funding one National Network for Environmental Management Studies (NNEMS) Fellow and two National Research Council Research Associates to conduct independent research projects in areas related to CEB research interests during FY 98. The Tillamook Bay National Estuary Program is conducting research on eelgrass-burrowing shrimp-oyster interactions in Tillamook Bay which are of interest to the CEB program. CEB is a collaborator in the Pacific Northwest Coastal Ecosystem Research Study (PNCERS) led by David Armstrong and Julia Parrish (Univ. Washington) and will exchange data with this large group of researchers. CEB has also provided facilities in support of PNCERS research through the guest worker program.

Additional scientific interactions will be sought with federal and academic experts for various components of the research plan. For seagrass these may include Mark Fonseca (NOAA/NMFS), Paul Harrison (Univ. British Columbia), Steve Rumrill (South Slough National Estuarine Research Reserve), Fred Short (Univ. New Hampshire), Ron Thom (Battelle Marine Sciences Laboratory), and Sandy Wyllie-Echeverria (Univ. Washington), and for burrowing shrimp may include David Armstrong (Univ. Washington), Brett Dumbauld (Washington Department of Fisheries), and Kris Feldman (Univ. Washington). Important interactions to expand CEB expertise on acoustic remote sensing methods may include Whitlow Au (Univ. Hawaii), Nick Chotiros (Univ. Texas), Darrell Jackson (Univ. Hawaii), and Bruce Sobal (US Army Corps of Engineers, Waterways Experiment Station). Potential participants in defining estuarine biota- habitat relationships include the Oregon Department of Fish and Wildlife (ODF&W: Bob Buckman, Dan Bottom) and National Marine Fisheries Service (NMFS: Bob Emmett).

Interactions for the physical-chemical process investigations may include the principal investigators (McManus et al.) on an EPA/NSF grant to study Tillamook Bay, the Georgia Pacific Corporation, the Sanitation Districts of Toledo, Siletz, Walport and Tillamook, as well as, the State Department of Environmental Quality and Lincoln and Tillamook County Health Division in providing current and historical waste loadings, including septic tanks, to the four estuaries and their major rivers. All local and state land use planners and shell fish producers would be potential users for the project products.

The spatially explicit modeling effort will interact with EPA Region IX personal to help define the key questions, exposure scenarios, and to find data sources. Particular contacts include Janet Hashimoto of the Water Division, who has the responsibility for regulating or monitoring sewage discharges and dredge disposal in California, and GIS personnel. As the models that are developed move to the field verification stage, interactions will occur with the major regional research organizations in San Francisco Bay (San Francisco Estuarine Institute) and Southern California (Southern California

Coastal Water Research Program).

One potential group of users of the spatially explicit modeling research results will be the Headquarters and Region 9 risk managers who have regulatory responsibility for sewage discharges (e.g., 301H), industrial discharges (NPDES permits) and dredge material disposal.

6.0 Project Management and Quality Assurance

The research program is an integrated effort representing contributions from all scientific research staff of the Coastal Ecology Branch. The overall research program is subdivided into thematic elements representing groups of related individual Principal Investigator research projects. Each thematic element will be administered by a Project Coordinator who will hold weekly meetings with PI's to insure integration of research within the thematic element. Project coordinators will be responsible for integration of research among the different research themes, and every other week all branch research groups will meet together to achieve among-group integration. The Branch Chief will insure that overall programmatic research goals are being met.

All research data will be collected according to the Quality Assurance standards of the Western Ecology Division of NHEERL. Quality Assurance Project Plans will be prepared for each research project and approved by the WED Quality Assurance officer. Existing CEB Standard Operating Procedures (SOP's) for chemical and biological analyses will be followed, and new SOP's will be prepared by principal investigators as required to document new research activities.

All research work will be performed under the general safety guidelines provided by the CEB Employee Health and Safety Manual, and the CEB Chemical Hygiene Plan, with additional guidance provided by the Health and Safety Handbook of the Western Ecology Division. All field work in support of the research effort will be conducted under the "Health and Safety Plan for Field Research Concerning the Ecology of Pacific Northwest Estuaries" (approved 4/27/98).

All research projects will undergo review for environmental compliance issues and will operate under an approved environmental compliance document.

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8.0 Appendices

8.1 Appendix A. Technical Details of Experiments for Project A4

Experiment 1.B. *Comparison of Acoustic, Photographic, and Videographic Remote-Sensing Methods to Identify and Map Estuarine Submerged Aquatic Vegetation*: The acoustic remote sensing method will be "SAVEWS" (Submersed Aquatic Vegetation Early Warning System), which is a 420-KHz hydroacoustic echolocation system used to map *Zostera marina* and other rooted vegetation in shallow waters (Sabol and Melton 1995; Sabol et al. 1997). SAVEWS will be operated by Dr. Bruce Sabol (USACE Waterways Experiment Station) who is collaborating on this project. If equipment is available, we may also evaluate other acoustic remote sensing systems, such as sidescan sonar and the RoxAnn system (Stenmar Group; Aberdeen, Scotland). The videographic remote sensing method will consist of an underwater video camera, linked to a GPS unit and depth sensor, which will be towed along a systematic series of transects across the study sites. The videography will be provided by Dr. Brad Robbins (CEB), a collaborator on this project. Hydroacoustic and videographic sampling will occur along transects located 10-m apart that will traverse each patch at high tide. An orthogonal set of hydroacoustic and videographic transects also will be made across each site. Thus, hydroacoustic and videographic data will be collected in a grid of 10-m width on each site. Ideally, the hydroacoustic and videographic data will be collected simultaneously, providing nearly 100% overlap of data. Both data sets will be interpolated across each study site using an inverse weighted interpolation algorithm. The photographic remote sensing method will be high-resolution, aerial infrared or natural color photography (either 1:2000 or 1:3600 scale) which will be provided by Dr. David Young (CEB), a collaborator on this project. These data will be digitized and photointerpreted across the six study sites. To minimize any potential temporal bias, aerial photographs will be taken at low tide on the earliest available date contemporary with the acoustic, videographic and ground-truth sampling. The success of the aerial photography is constrained by the availability of equipment and a pilot, and a "photographic window" unobscured by clouds. For these reasons, the study will be conducted in latesummer when the risk of cloudy weather is reduced. Ground-truth data will be obtained by sampling the study sites using a grid on 1-m^2 centers. Field staff will traverse thirty-three 3m wide transects recording the presence/absence of SAV by species as well as the presence/absence of burrowing shrimp (*i.e.* burrows) at each grid point.

The videographic remote sensing method will consist of an underwater video camera, linked to a GPS unit and depth sensor, which will be towed along a systematic series of transects across the study sites. The videography will be provided by Dr. Brad Robbins (CEB), a third collaborator on this project. Hydroacoustic and videographic sampling will occur along 1-m-wide transects located 10-m apart that will traverse each patch at high tide. An orthogonal set of hydroacoustic and videographic transects also will be made across each site. Thus, hydroacoustic and videographic data will be collected in a grid of 10-m width on each site. Ideally, the hydroacoustic and videographic data will be collected simultaneously, providing nearly 100% overlap of data. Both data sets will be interpolated across each study site using an inverse weighted interpolation algorithm.

The photographic remote sensing method will be high-resolution, aerial infrared or natural color photography (either 1:2000 or 1:3600 scale) which will be provided by Dr. David Young (CEB) who is also collaborating on this project. These data will be digitized and photointerpreted across the six study sites. To minimize any potential temporal bias, aerial photographs will be taken at low tide on the earliest available date contemporary with the acoustic, videographic and ground-truth sampling. The success of the aerial photography is constrained by the availability of equipment and a pilot, and a "photographic window" unobscured by clouds. For these reasons, the study will be conducted in July, August, or September when the risk of poor weather is reduced.

Ground-truth data will be obtained by sampling the study sites using a grid on 1-m^2 centers. Field staff will traverse (33) 3m wide transects recording the presence/absence of SAV by species as well as the presence/absence of burrowing shrimp (*i.e.* burrows) at each grid point.

Experiment 1.C. *Evaluation of Remote Sensing Methods to Detect, Identify, and Quantify Burrowing Shrimp:* Acoustic remote sensing methods will include a variety of echo-sounder methods (using different transducer frequencies, electronics, or software to sample or interpret signals) and possibly side-scan sonar. Interest in participating in this project has been expressed by Drs. Whitlow Au (Univ. of Hawaii), Nicholas Chotiros (Univ. Texas), Darrell Jackson (Univ. Washington), Bruce Sobal (USACE Waterways Experiment Station) and sonar development companies, such as BioSonics (Seattle, WA) and Marine Sonics (White Marsh, VA). Underwater videography will consist of a GPS-coupled video system towed behind a boat, and will be provided by Dr. Brad Robbins (CEB) who is collaborating on this project. Each video frame will image approximately 1-m^2 of the seafloor. Post-processing involves a trained individual classifying and quantifying objects on each video frame.
production (Table A.1). While processing the *Zostera* shoots, the number of leaf scars will be counted and recorded to reconstruct plant demography and leaf and rhizome production (Duarte et al. 1994) (Table A.1). The technique developed by Duarte et al. assumes that the PI is a continuous, linear process. Kentula and McIntire (1986) reported that PI changes throughout the growing season for

Prior to sampling for burrowing shrimp at the test sites (described below) each investigator will be allowed to spend at least one day testing their system over burrowing shrimp beds in Yaquina estuary. This preliminary trial period will provide an opportunity to "fine tune" the systems so that they have the best opportunity to be successful in the experiment.

Each remote sensing method will sample the same patches of intertidal sediments (study sites), some of which will be used to calibrate the signal, and the others to test the accuracy of the signal interpretation (i.e., habitat classification). All sites will be located in the intertidal zone of the central portion of Yaquina estuary in unconsolidated sediments inhabited by *Neotrypea* or *Upogebia*. Field staff will pre-classify sites at low tide by identifying the shrimp (i.e., by the configuration of the burrow openings; qualitative core samples can also be collected to verify the species identity of shrimp) and by measuring the density of burrow openings (i.e., number of burrow holes per 0.5-m^2). If uninhabited sediment patches cannot be located within or adjacent to burrowing shrimp habitats, 2-m x 2-m patches of shrimp beds will be defaunated by smothering as per Thrush et al. (1996). Each site will be marked with a numbered float to guide the boat pilot there at high tide.

Minimally, eight sites of each type will be identified; three sites will be used for calibration of the classification method, and the remaining sites will be used for testing the accuracy of the classification. Ideally a higher replication rate (such as 10 per patch-type) will be used, but that will be determined by the logistics of ground-truth sampling, the time required to sample sites acoustically, and the time to travel between sites. All crew on-board the boat from which sampling is conducted will be "blind" to the identity of each site. Since the acoustic and videographic methods can only be used at high tide, concealing the identity of the sites will not be difficult. The boat pilot will maneuver and anchor the boat such that the acoustic transponder or video camera is positioned directly over a site, and the investigator will be allowed to sample the site for a fixed of period time (nominally, two minutes).

Experiment 2.A. *Population Characteristics of Eelgrass and Burrowing Shrimp Along the Dominant Salinity-Temperature Gradient in Yaquina Estuary, Oregon*: This study is designed to provide a baseline of eelgrass and burrowing shrimp population dynamics, and to evaluate population-level response variables that may be used for subsequent work. Despite the fact that *Zostera marina* L. (eelgrass) is the most common and abundant seagrass in the northern temperate Pacific (Phillips 1984), information on the distribution and growth of eelgrass is limited, especially for Oregon. The distribution of *Zostera* in Oregon was reported by Proctor et al. (1980) and aspects of its growth were reported by Bayer (1979) for Yaquina Bay and by Stout (1976) and Kentula and McIntire (1986) for Netarts Bay. Although there is a relatively detailed database on the autecology of *Zostera* for Netarts Bay, the ability to extrapolate that information to other estuaries in Oregon may be limited. Netarts does not have a major river flowing into it, and, therefore, does not have freshwater inputs and the related temperature and salinity gradients similar to other Oregon estuaries. Although low salinity limits the up-river distribution of eelgrass world-wide, very little is known about changes in the autecology and demography of *Z. marina* along salinity-temperature gradients of Pacific Northwest estuaries (Phillips 1984, Thom 1990). Bird (1982), Posey (1986a, 1987) and Dumbauld et al. (1996) conducted limited population studies on *Neotrypea* in the mesohaline portions of various Pacific Northwest estuaries (including sites in Yaquina estuary studied by Bird). Dumbauld (1994) also studied the population biology of mud shrimp, *Upogebia*, in Willapa Bay, Washington, but it is uncertain whether the information from that study can be extrapolated to Oregon estuaries. Posey (1987) demonstrated that low salinity can be lethal to both *Neotrypea* and *Upogebia*, but no study has examined whether the population structure of either species changes along the natural, salinity-temperature stressor gradient common to Pacific Northwest estuaries.

Intensive Sampling Approach: Intensive-sampling study sites will be sampled monthly during day-time low tides year-round for burrowing shrimp and from May through September for *Zostera*. Sampling will be limited to times when the sites are exposed long enough to be sampled. From October through April, the sites will be visited as weather and the tides permit to determine what sampling can be done for *Zostera*. At minimum, descriptive notes and pictures of the sites will be taken any time a site is visited. Physical and chemical data will be collected in conjunction with the biophysical model-testing study (Expt. 2.D.) described below. In addition, we will attempt to measure the tidal elevation of the places where samples are taken within each site in conjunction with the bathymetric mapping work proposed by Dr. David Young (CEB).

Ten permanent transects will be equally spaced across each site and will run parallel to the tidal gradient, i.e., perpendicular to the shore (Figure A.1). The first and tenth transects will be located 5 m from the edge of the *Zostera* bed. A grid of permanent transects will be used as the sample design to facilitate finding marked shoots of *Zostera* on the sample date following marking. (See below for details on shoot marking.) Burrowing shrimp populations will be sampled along these transects also.

Zostera will be sampled along each transect approximately 1m from the upper and lower edge of the bed, i.e., two sample points per transect (Figure A.1). Kentula and McIntire (1986) found that *Zostera* had distinct characteristics at the upper and lower limits of its distribution intertidally in Netarts. At each sample point, a 1-m^2 quadrat will be placed perpendicular to the transect and percent cover will be measured using both visual estimate and point count methods as was done by CEB scientists in the 1997 aerial photography study (Table A.1) (Young 1997). At the sample points on transects 2, 4, 6, 8 and 10, a circular quadrat of comparable size to that being used for the shrimp sampling will be placed in the lower left-hand quarter of the 1-m^2 quadrat (Figure A.2) and the number of vegetative shoots, number of flowering shoots, and number of seedlings counted (Table A.1). These measures will provide information on standing crop and sexual reproduction (timing, proportion of the shoots flowering, proportion of shoot growing from seed). To measure eelgrass growth dynamics and net primary production, three vegetative shoots typical of the plot will be chosen from the lower right-hand quarter of the 1-m^2 quadrat (Figure A.2) and marked according to methods developed by Kentula and McIntire (1986). Approximately four weeks later during the next tide suitable for sampling, one of the three marked shoots from each sample point will be harvested. Three shoots are marked to assure that at least one shoot will be found and in good condition, e.g., leaves intact. The rhizome for each shoot will be clipped from the shoot(s) growing in front of it and at the point where it originated (i.e., where either where the rhizome branches or another shoot is growing). This procedure will harvest enough of the rhizome to test the application of the methods of Duarte et al. (1994) which utilizes the patterns of leaf scars on seagrass rhizomes to estimate historical seagrass productivity from a one-time destructive sample. Shoots from each plot will be placed in a labeled plastic bag, transported on ice to the laboratory where they will be stored at 4°C until processing (within 96h).

Eelgrass leaf-area per shoot will be determined by measuring the width and length of each leaf. The methods of Kentula and McIntire (1986) will be used to determine plastochrone interval (PI) (the time interval between initiation of successive leaves on a shoot) (Patriquin 1973), export interval (EI) (the time between sloughing of two successive leaves on a shoot) (Kentula and McIntire 1986), and net primary production (Table A.1). While processing the *Zosterai* shoots, the number of leaf scars will be counted and recorded to reconstruct plant demography and leaf and rhizome production (Duarte et al. 1994) (Table A.1). The technique developed by Duarte et al. assumes that the PI is a continuous, linear process. Kentula and McIntire (1986) reported that PO changes throughout the growing season for *Zostera* in Oregon. Therefore, as recommended by Jensen et al. (1996), we will use the leaf marking data from the intensive sites in conjunction with the extensive sampling event in July (described below) to empirically establish the PI that will be used to generate *Zostera* demographic information (Duarte et al. 1994).

Figure A.1. Diagram of transect and quadrat placement to be used at each of the intensive study sites. Diagram is not to scale.

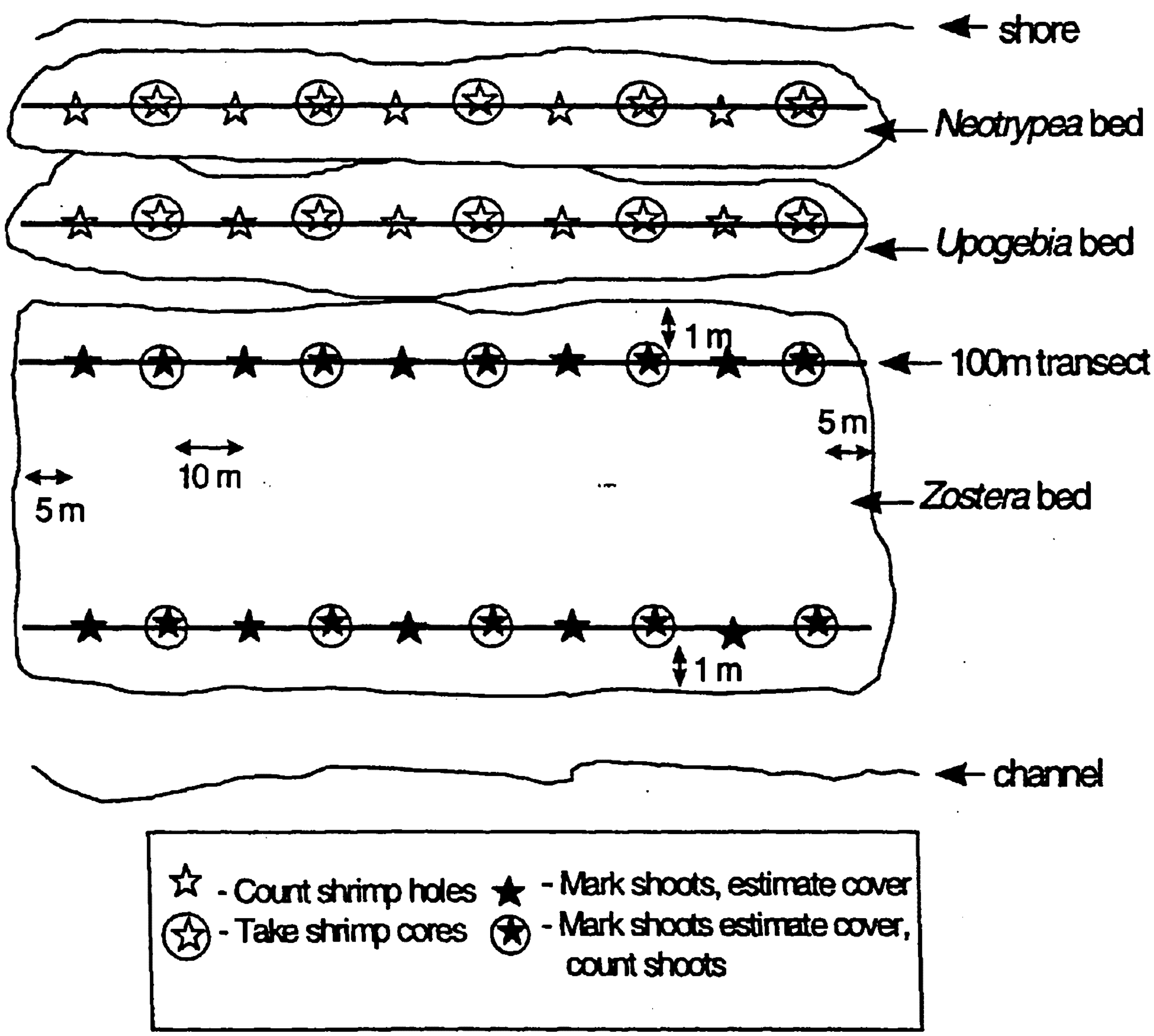
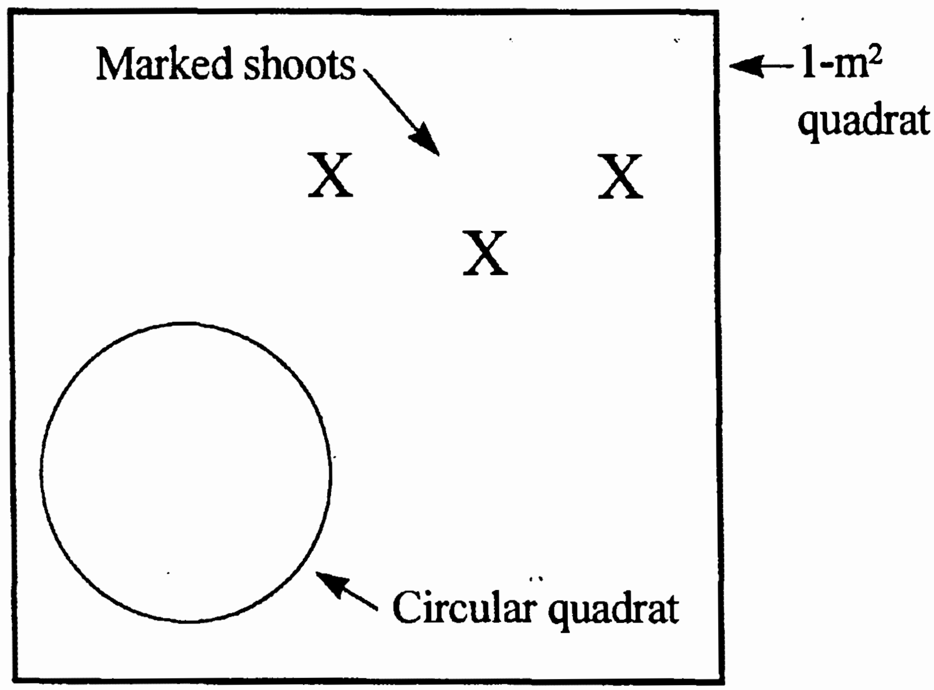


Table A.1. List of *Zostera* population variables, sample units, and sample sizes per intensive-study site per month. Zone refers to the upper and lower intertidal limits of the *Zostera* bed on the site. Three intensive-study sites will be sampled.

Variable Sampled	Sample Unit	Number of Samples per Zone	Number of Samples per Transect	Number of Samples per Site
% Cover	1-m^2 quadrat	10	2	20
Density vegetative shoots	circular quadrat	5	2	10
Density reproductive shoots	circular quadrat	5	2	10
Density of seedlings	circular quadrat	5	2	10
Density of eelgrass	circular quadrat	5	2	10
Leaf area	short shoot	10	2	20
Plastochrone interval	short shoot	10	2	20
Export interval	short shoot	10	2	20
Net primary production (above ground)	short shoot	10	2	20
Shoot demography (Duarte et al. 1994)	short shoot	10	2	20
Leaf production (Duarte et al. 1994)	short shoot	10	2	20
Rhizome growth (Duarte et al. 1994)	short shoot	10	2	20

Figure A.2. Diagram of placement of the 1-m^2 quadrat and the samples to be taken within it.



Neotrypea and *Upogebia* will be sampled along each of the 10 transects of the study grid. The center of the spatial distribution of each shrimp will be located by visual inspection of the distribution of burrow holes along each transect. *Neotrypea* burrows are readily identified by the cone-shaped deposit of sediment around the opening, whereas *Upogebia* burrows are identifiable by their larger diameter opening, lack of a sediment cone, and mucus lining (identified by touch). Burrow-hole density data (i.e., the number of burrow openings per m^2) will be obtained for both shrimp species on every transect using a 0.5-m^2 quadrat (Table A.2). These data will provide information on the spatial and temporal variability of shrimp density across each site. Mega-infaunal core samples and young-of-the-year (YOY) core samples will be collected along five transects and will be processed to provide data on the absolute density, size distribution, sex ratio, reproductive condition, and recruitment of each shrimp. Cores will be collected along every-other transect each month (i.e., even-numbered transects one month, odd-numbered transects the next month), and the location of core sites will alternate between the left and right side of the transect to avoid re-sampling the same patch of sediment. Along one transect, four mega-infauna cores will be collected using a 15-cm to 20-cm diameter x 1-m depth corer (method to be determined by Expt. I.A), sieved through a 3-mm mesh screen, and combined to form one sample; the material collected on the screen will be preserved in buffered formalin until picking, and each shrimp will be measured for carapace length, its gender determined, and reproductive condition of females assessed by the presence and developmental stage of eggs (as per Dumbauld 1994). Adjacent to the mega-infauna cores, four YOY core samples will be collected using a 15-cm diameter x 10-cm deep corer, sieved through a 0.5-mm mesh, and combined into one sample; the material collected on the screen will be preserved in buffered formalin containing rose bengal stain, then picked for small burrowing shrimp, which will be identified to species and counted. Thus, each month we will collect ten burrow-hole count samples, five mega-infauna core samples, and five YOY core samples at each site.

<u>Extensive-Sampling Approach</u>: Sample locations for the extensive sampling effort will be the same as those selected by Steve Ferraro and Faith Cole (CEB) within eelgrass, ghost shrimp, and mud shrimp habitats throughout Yaquina estuary. Ten sites within each habitat will be selected by a random-site generator which weights the distribution of sites by the spatial distribution of the habitats: more sites are located where the greatest proportion of habitat is present. This is the same random-sampling strategy employed by EMAP (USEPA 1992). We will use the mega-infauna core samples collected for Ferraro and Cole (using the same protocol described above) to quantify the demographic characteristics of *Neotrypea* and *Upogebia* at each site. Burrowing shrimp hole-count data also will be collected at each site. At sites within *Zostera* beds, four 15-cm diameter x 10-cm deep cores will be taken to collect samples of eelgrass. In addition, percent-cover of eelgrass will be measured by the point-intercept method and by visually estimating percent cover by species using 1-m^2 quadrats. To the extent possible, the *Zostera* core samples will be collected in July when

Table A.2. List of burrowing shrimp population variables, sample units, and sample sizes per intensive-study site per month. Three intensive-study sites will be sampled.

Variable Sampled	Sample Unit	Number of Samples per Transect	Number of Samples per Site
Neotrypea burrow-hole density	0.5-m^2 quadrat	1	10
Neotrypea abundance	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Neotrypea size-frequency distribution	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Neotrypea sex ratio	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Neotrypea female reproductive condition	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Neotrypea young-of-the - year abundance (recruitment)	composite of 25 5-cm diameter cores	0.5 (1 per 2 transects)	5
Upogebia burrow-hole density	0.5-m^2 quadrat	1	10
Upogebia abundance	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Upogebia size-frequency distribution	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Upogebia sex ratio	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Upogebia female reproductive condition	mega-infaunal core sample*	0.5 (1 per 2 transects)	5
Upogebia young-of-the - year abundance (recruitment)	composite of 25 5-cm diameter cores	0.5 (1 per 2 transects)	5

* coring method for burrowing shrimp will be determined in preliminary studies (Expt. 1.A)

Table A.3. Population variables and sample units for eelgrass and burrowing shrimp to be collected in July at each extensive-sampling study site. One sample will be collected per site. The samples will be collected from the 10 randomly selected sites used by Ferraro and Cole and the 3 sites used in the intensive-sampling study (i.e., a total of 13 sites).

Variable Sampled	Sample Unit
***Zostera* % cover**	1-m^2 quadrat
***Zostera:* density vegetative shoots**	circular quadrat
***Zostera:* density reproductive shoots**	circular quadrat
***Zostera:* density of seedlings**	circular quadrat
***Zostera:* total density**	circular quadrat
***Zostera:* above ground biomass**	circular quadrat
***Zostera:* below ground biomass**	circular quadrat
***Zostera:* total biomass**	circular quadrat
***Zostera* shoot demography (Duarte et al. 1994)**	circular quadrat
***Zostera* leaf production (Duarte et al. 1994)**	circular quadrat
***Zostera* rhizome growth (Duarte et al. 1994)**	circular quadrat
***Neotrypea* or *Upogebia* burrow-hole density**	0.5-m^2 quadrat
***Neotrypea* or *Upogebia* abundance**	mega-infaunal core sample*
***Neotrypea* or *Upogebia* size-frequency distribution**	mega-infaunal core sample*
***Neotrypea* or *Upogebia* sex ratio**	mega-infaunal core sample*
***Neotrypea* or *Upogebia* female reproductive condition**	mega-infaunal core sample*
***Neotrypea* or *Upogebia* young-of-the -year abundance (recruitment)**	composite of 25 5-cm diameter cores

* coring method for burrowing shrimp will be determined in preliminary studies (Expt. 1.A)

eelgrass primary productivity is expected to be maximum (Kentula and McIntire 1986). The sampling methods and intensities for each population variable are summarized in Tables A.2 and A.3.

In addition to the samples taken from the cores harvested by Ferraro and Cole, four 15-cm diameter x 10-cm deep cores will also be collected in July (i.e., when Ferraro and Cole sample their sites) in the upper and lower intertidal limits of the *Zostera* at each of the three intensive sites. The *Zostera* and associated sediment within the circular quadrat will be collected into a 3-mm mesh sieve. The sediment will be washed from the sieve and the plants placed into labeled plastic bags, transported on ice to the laboratory and refrigerated until processed (two weeks maximum). Eelgrass core samples will be processed to determine number of vegetative and flowering shoots and seedlings; and above and below ground biomass (Kentula and McIntire 1986). While sorting the shoots to determine biomass, information needed to determine *Zostera* demographics will be recorded, including number of leaf scars, attached and unattached shoots, and number of leaves per shoot (Duarte et al. 1994, Jensen et al. 1996). As stated above, these results will be compared with those obtained from the intensive sample to determine the best way to measure *Zostera*, *Neotrypea*, and *Upogenia* demographics in Oregon.

Experiment 2.B. *Development and Testing of Biophysical Population Models*: This effort will be conducted in collaboration with Dr. Bob Ozretich (CEB) and in consultation (and possibly collaboration) with other researchers studying eelgrass and burrowing shrimp ecology (such as, Drs. Mary Kentula [WED], Ron Thom [Battelle Marine Sciences Laboratory], Brett Dumbauld [Washington Department of Fisheries], and David Armstrong [Univ. Washington]). Dr. Ozretich will be testing a water quality-based model to predict the distribution of eelgrass, and we plan on combining our resources by selecting the same sampling sites and using the same water column data and eelgrass presence-absence data. Bob Ozretich and I plan to combine the best aspects of our two models in the future and to test the accuracy of the new model across Pacific Northwest estuaries. Environmental variables to be measured are listed in Table A.4.

One unresolved issue is how best to represent the temporal variability in water column parameters at each site. Clearly, water column parameters are likely be much more variable temporally (on tidal and seasonal time scales) than sediment-associated variables. Single samples at high tide collected once per season will not be sufficient to adequately characterize this variability. One approach is to report the extremes of the 95% confidence interval for each parameter at each site as the maximum and minimum values; however, we will consult with other estuarine scientists to identify other ways to integrate these temporally-variable data. We will also investigate linking measurements made at the sites to 1) water quality data collected continuously by moored CTD/PAR units (see proposal by David Specht, CEB) using regression techniques, and 2) to a circulation model for the Yaquina estuary, such as the 2-dimensional model used by the NOAA/PMEL Tsumami Project (Kamphaus 1998). These approaches could lead to allowing us the capability to accurately model temporal variability of water column parameters at each of the study sites.

At low tide, we will visit each site to sample eelgrass and burrowing shrimp (i.e., presence or absence within a 4-m^2 area) and to measure (or sample for) benthic habitat variables. The latter include porewater salinity (measured by a hand-held refractometer), sediment temperature (measured by a digital thermometer), and slope (measured using a 2-m long level and protractor or inclinometer). Bathymetric elevation will be obtained from the digital elevation map being developed by Dr. David Young (CEB). Water depth also will be recorded each time water quality variables are measured (i.e., three times per year), and bathymetric elevation for each site can be estimated by averaging the tide-corrected depth measurements. Sediment grain size (percent gravel, sand, silt-clay) and sediment total organic carbon (TOC) will be measured in the laboratory following current CEB SOPs.

<u>*Future Model and Experiment*</u>: The biophysical population model will be combined with the habitat suitability model being developed and tested by Bob Ozretich (CEB), and the new model will be tested in Yaquina estuary and one or more other Pacific Northwest estuaries. It is premature to speculate on the design of that study, but certain features are foreseen. First, it will be advantageous to conduct the study in estuaries in which other ecologists are studying the distribution and population biology of eelgrass and burrowing shrimp, and in which capability exists to measure water column parameters. Second, it will be logistically advantageous to form collaborations with the ecologists studying eelgrass and burrowing shrimp in those estuaries so that their staff may participate in the sampling effort. Candidate estuaries and researchers include: Tillamook Bay, OR (Roxanna Hinzman, Tillamook Bay National Estuary Project), Coos Bay, OR (Steve Rumrill, South Slough National Estuarine Research Reserve), Willapa Bay, WA (Brett Dumbauld, Washington Dept. of Fisheries; Si Simenstad or David Armstrong, Univ. Washington), and Padilla Bay, WA (Doug Bulthuis, Padilla Bay National Estuarine Research Reserve). Third, it will be important to include Yaquina estuary in the study in order to test whether the new model provides the same accuracy as its predecessor.

Experiment 2.C. *Effects of Multiple Abiotic Stressors on the Population Biology of Keystone Species - Methods for Mesocosm Experiments:* Using data from the mesocosm competition experiments (expt. 3.A. and 3.B.), power analysis will be employed to determine the replication rate that will allow us to measure changes in the responses in critical population variables, such as %cover, abundance, and growth or productivity. The levels and replication rates listed here are presented only to illustrate

possible experimental designs.

Table A.4. Environmental parameters to be measured for the biophysical population models.

Parameter	Analytical Method	Sampled Medium
light attenuation coefficient	photosynthetic active radiation (PAR) 2 depths/station	water column
overlying water salinity (conductivity)	CTD	water column
water temperature	CTD	water column
depth	CTD	water column
total suspended solids	mass collection on preweighed membrane filters	water column
dissolved inorganic nitrogen	AutoAnalyzer	water column
dissolved inorganic phosphorus	AutoAnalyzer	water column
Zostera or burrowing shrimp	presence or absence	sediment
porewater salinity	refractometer	sediment
sediment temperature	hand-held digital thermometer	sediment
sediment-surface slope	inclinometer	sediment
sediment grain size (% sand, silt, clay)	sieve method	sediment
sediment organic carbon and nitrogen	TOC/N analyzer	sediment
Bathymetric elevation	Digital elevation map or depth-sounder measurements	water column and sediment

Fiberglass or PVC cylinders (30.5 cm diameter x 91 cm height [= 12" x 36"]) will be filled with 76 cm (= 30") of fine-grained sediment, sieved to <1-mm, obtained from intertidal habitats adjacent to eelgrass and burrowing shrimp beds. Each cylinder will be placed within one of the 61 cm x 61 cm (= 2' x 2') compartments of a holding tank (Figure B1). *Zostera* will be planted uniformly across the sediment surface of each cylinder at densities of ~200 shoots m^{-2} (i.e., ~15 shoots per cylinder; this is a moderately high shoot density for Pacific Northwest populations [Phillips 1984]) and allowed to establish rooting during an acclimation period (duration of the acclimation period will be set based on advice from experts such as Drs. Fred Short [Univ. New Hampshire] and Ron Thom [Battelle Marine Science Laboratory]). Eelgrass will be harvested from Yaquina estuary or nearby estuaries, transported damp and shaded in coolers to the EPA laboratory, held in running seawater at ambient salinity and temperature at the laboratory, and planted within 48 hr of harvest. Salinity, light, or temperature will be changed gradually to allow acclimation to the experimental conditions. Similarly, burrowing shrimp will be harvested at least 1 week prior to the start of the experiment and acclimated to experimental conditions in seawater. *Neotrypea* and *Upogebia* will be added to the cylinders at densities of ~100 m^{-2}, which are moderately high densities for these species (Dumbauld 1994; unpubl. data). Shrimp used in the experiments will be sorted to uniform size, and sub-samples of shrimp will be measured for initial carapace length, wet weight, and dry weight.

The mesocosm experiments will be conducted for 6 to 20 weeks. The duration will depend on whether effects can be observed during the experiment; if effects are not apparent during non-destructive sampling, the experiment will be terminated after the longer period. Eelgrass or burrowing shrimp in the cylinders will be sampled non-destructively every two weeks and destructively at the end of the experiment. Population variables measured non-destructively for eelgrass will include percent cover, density of vegetative and reproductive shoots, and growth as indicated by the change in leaf surface area (for the longest leaf in each of 5 shoots) (Table 2). Four weeks before the end of the experiment, 5 shoots per cylinder will be marked for above-ground net primary productivity measurements using methods developed by Kentula and McIntire (1986). At the end of the experiment, eelgrass will be sampled destructively for above-ground net primary productivity, above and below ground biomass, plastichrone interval, and export interval (see expt. 2.A.). Non-destructively sampled population variables measured for *Neotrypea* and *Upogebia* include the density of burrow openings within a 20-cm diameter quadrat and the maximum height of the sediment-water interface; the latter will be measured as the distance from the rim of the cylinder to the top of the highest mound of sediment produced by the burrowing shrimp. At the end of the experiment, the sediment will be pumped out of each cylinder (using a venturi aspirator or a suction sampler such described by Miles and Whitlatch, 1997) and washed through a 3-mm mesh screen to capture the shrimp. Destructively-collected variables for the shrimp will include total abundance of shrimp, carapace length, sex and reproductive condition, and wet and dry weights (Table A.5).

Additional variables that could be studied in these factorial-design, multiple-stressor experiments include nutrient concentration (eelgrass), substrate-type, sedimentation, dissolved oxygen concentration, or persistent chemical contaminants. Selection of the variables to be used will be guided by the results of testing the biophysical models for each species (expt. 2.B.). Four-way multiple stressor-response experiments could be conducted also, but using central-composite experimental designs rather than fully-factorial experimental designs. Multi-factorial (i.e. 3-way or 4-way) analysis of variance will be used to analyze the data to test the hypotheses that 1) no significant interaction exists in the effects caused by multiple stressors (for all possible combinations of the stressors tested), 2) that individual stressors have no significant effect on the survival or growth of the test organism, and 3) that each stressor has the same magnitude of effect on the test organism.

Field Experiments: Eelgrass or shrimp will be harvested from nearby populations, sorted to uniform size and condition, and "planted" inside caged patches (40-cm diameter) at experimental sites. The patches will be created by excavating a 40-cm diameter x 60-cm depth core of sediment, lining the hole with 3-mm mesh plastic screen (i.e., underground fence) to a depth of 60 cm, and refilling the hole with the excavated sediment which will be sieved to <3-mm to remove burrowing shrimp and eelgrass. Population densities of each species will be similar to those used in the mesocosm experiments. A cage will be placed over each patch to prevent burrowing shrimp emigration or immigration, predator immigration, and to provide protection from scouring. After an acclimation period of a few days, the above-ground cage will be removed from all plots, but the below-ground fence will be retained to prevent burrowing shrimp from leaving or entering the plot by lateral burrowing.

The population-level response variables will be similar or identical to those used in previous mesocosm and field experiments (Table A.5). Non-destructive samples will be measured approximately every 4 weeks (depending on site accessibility due to tides and weather). The field experiment will be conducted for 6 to 20 weeks, depending on the rate at which responses are observed in mesocosm experiments and on when such responses are measurable in the field. Data from the experiments will be analyzed by analysis of variance to test the same hypotheses as in the analogous mesocosm experiment: 1) no significant interactions in effects of co-occurring stressors, 2) no effect of each stressor alone, and 3) all stressors have the same magnitude of effects. Beyond this, it is premature to design these experiments in further detail as they are not likely to be started until the above mesocosm and competition experiments are concluded (i.e., not until sometime in 2000).

Experiment 2.D. *Mapping Changes in Eelgrass and Burrowing Shrimp Populations Along Stressor Gradients*: Several issues will need to be addressed prior to testing hypotheses about population spatial changes in relation to stress. First, we need to know what spatial scale to map for each species. The mapping accuracy study (expt. 1.B.) will examine how accuracy changes with spatial scale for each of three remote-sensing methods. Results from that study will be balanced against the resolution of available data (to be acquired from other researchers or public agencies) and the cost of obtaining high-resolution spatial data. Second, we need to know the temporal scale at which to map population change. We anticipate that annual

Table A.5. List of population variables, sample units, and sampling frequencies for eelgrass and burrowing shrimp that may be collected in mesocosm and field stress-response experiments. One sample will be collected per replicate experimental chamber (cylinder in mesocosm, plot in field experiments).

Variable Sampled	Sample Unit	Sampling Frequency
***Zostera* % cover**	20 cm diam. quadrat	every 2-4 weeks*
***Zostera:* density of vegetative shoots**	20-cm diam. quadrat	every 2-4 weeks*
***Zostera:* density reproductive shoots**	20-cm diam. quadrat	every 2-4 weeks*
***Zostera:* total shoot density**	20-cm diam. quadrat	every 2-4 weeks*
***Zostera* leaf area**	5 shoots	every 2-4 weeks*
***Zostera* above-ground biomass**	20-cm diam. quadrat	start and end of expt.
***Zostera* below-ground biomass**	20-cm diam. quadrat	start and end of expt.
***Zostera* total biomass**	20-cm diam. quadrat	start and end of expt.
***Zostera* plastochrone interval**	20-cm diam. quadrat	start and end of expt.
***Zostera* export interval**	20-cm diam. quadrat	start and end of expt.
***Zostera* net primary productivity (above ground)**	5 shoots	start and end of expt.
***Neotrypea* or *Upogebia* burrow-hole density**	20-cm diam. quadrat	every 2-4 weeks*
***Neotrypea* or *Upogebia* maximum height of sediment deposit (mesocosm only)**	1 mound per replicate	every 2-4 weeks*
***Neotrypea* or *Upogebia* abundance**	all shrimp per replicate	start and end of expt.

Variable Sampled	Sample Unit	Sampling Frequency
***Neotrypea* or *Upogebia* size-frequency distribution**	all shrimp per replicate	start and end of expt.
***Neotrypea* or *Upogebia* wet and dry wt.**	all shrimp per replicate	start and end of expt.
***Neotrypea* or *Upogebia* sex ratio**	all shrimp per replicate	start and end of expt.
***Neotrypea* or *Upogebia* female reproductive condition**	all shrimp per replicate	start and end of expt.

* variables sampled every 2 wk in mesocosms, and approximately every 4 in the field (depending on accessibility to study sites due to tides and weather).

or biennial maps may be adequate for these purposes, but also recognize that eelgrass and burrowing shrimp distributions can change dramatically seasonally. One approach to resolving this question would be to generate population change maps for select sites on a seasonal basis for 2+ yr, measure the rate of population change over different temporal scales, and select the scale at which the rate of change reaches an asymptote. Third, we need to know whether natural rates of population change vary geographically across the Pacific Northwest. It seems likely that latitudinal gradients in day length, temperature, rainfall, or storm intensity could affect natural rates of population expansion and contraction. Underlying natural environmental gradients will have to be identified and their influence measured before we can confidently use population change maps to detect and measure the effects of anthropogenic stress.

Experiment 3.A. *Measuring Interspecific Competition Among Eelgrass and Burrowing Shrimp: Methods for Mesocosm Experiments:* The experiments will be very similar to those used for the multiple stressor experiment (Expt. 2.C). *Zostera* will be planted uniformly across the sediment surface of each cylinder at densities of 0 to 400 shoots m^{-2} (i.e., natural densities for the Pacific Northwest; Phillips 1984) and allowed to establish rooting during an acclimation period (this acclimation period will be set based on advice from experts such as Drs. Fred Short [Univ. New Hampshire] and Ron Thom [Battelle Marine Science Laboratory]). Burrowing shrimp will be harvested at least 1 week prior to the start of the experiment and acclimated to experimental conditions in seawater. *Neotrypea*, harvested and sorted to size ~1 wk prior to the experiment, will be added to the cylinders at densities ranging from 0 to 450 m^{-2}, whereas *Upogebia* will be added at densities ranging from 0 to 300 m^{-2}. The higher densities are equivalent to those measured recently in Yaquina estuary (unpubl. data). Sub-samples of shrimp will be measured for initial carapace length, wet weight, and dry weight.

The mesocosm competition experiments will be conducted for 8 to 16 weeks. The duration will depend on whether effects can be observed during the experiment; if effects are not apparent during non-destructive sampling, the experiment will be terminated after the longer period. Population variables for both species in each cylinder will be sampled non-destructively every two weeks and destructively at the end of the experiment, and will be the same as those described for the stressor-response experiments (Table A.5).

Field Experiment: The patches will be created by excavating a 40-cm diameter x 60-cm depth core of sediment, lining the hole with 3-mm mesh plastic screen "underground fence" to a depth of 60 cm, and refilling the hole with the excavated sediment which will be sieved to <3-mm to remove burrowing shrimp and eelgrass. The plots will covered with a 3-mm mesh cage to prevent immigration or emigration of conspecifics or competitors. After an acclimation period of a few days, all cages will be removed, and underground fences will be removed from some plots but retained in others. The replication rate will be based on results of a power analysis of key response variables (i.e., %cover and net primary productivity for *Zostera,* abundance for the shrimp) using variance estimates from field populations (i.e., Kentula and McIntire 1986, Dumbauld et al. 1996). The duration of the experiments could be extended for as long as 1-yr if there is no apparent effect of competition; in this case, a bi-monthly sampling frequency will be used. The long duration may be required for competition to manifest itself, although we expect the effects to be revealed within weeks, particularly between *Neotrypea* and *Zostera* because of the high rate of sediment turnover caused by ghost shrimp.

8.2 Appendix B Technical Details of Model Development for Project B1

Appendix Table 1: Model Parameters. The "Range in Values" is an approximation of the range in values that may be used in simulations without the addition of any stochastic variation. Note that the stressor-response parameters and the vital rates calculated from these values are listed as a function of age ("age-specific") even though at this stage we assume that all adult classes (≥ 1 year) have the same sensitivity (see text and Table 2).

PARAMETER	DEFINITION	UNITS	POTENTIAL RANGE	HOW IT WILL BE VARIED
$\alpha_q(x)$	age specific parameter determining level of anthropogenic-related increased mortality in logistic function	Dimensionless	-7 → +7	Deterministic
$\alpha_m(x)$	age specific parameter determining level of anthropogenic-related reduction in fecundity in logistic function	Dimensionless	-7 → +7	Deterministic
$\beta_q(x)$	age specific parameter determining slope of increased anthropogenic-related mortality in logistic function	Dimensionless	1 → 16	Deterministic
$\beta_m(x)$	age specific parameter determining slope of reduced anthropogenic-related fecundity in logistic function	Dimensionless	1 → 16	Deterministic
bs	lapse rate for stress function	Dimensionless	.0187 → .251	Deterministic
c	cell number	Dimensionless	0 → 31,416	Definition
d	distance from center of domain to a stress source	Dimensionless	0 → 100	Deterministic / Stochastic
dq(x,c)	anthropogenic-related increase in mortality for age x, cell c, time t	Dimensionless	0 → 1	Calculated
dm(x,c)	anthropogenic-related relative decrease in fecundity for age x, cell c, time t	Dimensionless	0 → 1	Calculated

eggs(c,t)	total eggs produced by all females in cell c at time t	eggs	$0 \rightarrow 2.84\ 10^{8}$	Calculated
$m_n(x)$	natural fecundity for age x	eggs / time	$0 \rightarrow 76485$	Stochastic
m(x,c)	total fecundity for age x,cell c, $(= m_n(x)\ [\ 1 - dm(x,c)\ ])$	eggs / time	$0 \rightarrow 76485$	Calculated
n(x,c,t)	number of individuals of age x in cell c at time t	individuals	$0 \rightarrow 100$	Calculated
$n_{0-2}(c,t)$ *	number of individuals in 0-2 month class in cell c at time t	individuals	$0 \rightarrow 10{,}000$	Calculated
$n_{3-12}(c,t)$*	number of individuals in 3-12 month class in cell c at time t	individuals	$0 \rightarrow 41.5$	Calculated
nc(r)	number of cells at distance r from a stress source	cells	$1 \rightarrow 628$	Calculated
ncells	total number of cells in the domain	cells	31,416	Definition
n_{st}	number of stress sources	cells	$1 \rightarrow 31{,}416$	Deterministic
$n_{tot}(c,t)$	total number of individuals cell c at time t $(= n_{0-2}(c,t) + \sum n(i,c,t), i=1,12)$	individuals	$0 \rightarrow 10{,}100$	Calculated
P	Barnthouse logistic function	Dimensionless	$0 \rightarrow 1$	Calculated
p(x,c)	total probability of survival from age x to age x+1 in cell c $(= p_n(x)\ [\ 1 - dq(x,c)\ ]\ =\ [\ 1 - q_n(x)\ ]\ [\ 1 - dq(x,c)\ ])$	Dimensionless	$0 \rightarrow 0.984$	Calculated
$p_n(x)$	natural probability of survival from age x to age x+1 $(= [\ 1 - q_n(x)\ ])$	Dimensionless	$0 \rightarrow 0.984$	Stochastic

p_p *	survival rate from the larval pool to the 0-2 month class	Dimensionless	0 → .004	Stochastic
pool(t)	total number of eggs produced by all cells at time t (= $\sum$ eggs(c,t))	eggs	0 → 1.78 10^{13}	Calculated
p_{peq}	probability of survival from the pool to the 3-12 month class (= "r_{seq}" in Brousseau's paper)	Dimensionless	.00001462	Stochastic
p_{0-2} *	probability of survival from 0-2 month class to 3-12 month class	Dimensionless	.004 → 1.0	Stochastic
p_{3-12} *	probability of survival from 3-12 month class to 2nd year	Dimensionless	0.177	Stochastic
q_p *	mortality from the pool to the 0-2 month class (= [1 - p_p])	Dimensionless	.996 → 1.0	Stochastic
$q_n(x)$	natural age specific mortality for age x	Dimensionless	.016 → 1.0	Stochastic
q(x,c)	total mortality for age x, cell c (= [1 - p(x,c)] = 1 - [1- $q_n(x)$] [1 dq(x,c)])	Dimensionless	.016 → 1.0	Calculated
ρ	distance from center of domain to a cell	Dimensionless	0 → 100	Calculated
r	distance from stress source to a cell	Dimensionless	0 → 200	Deterministic
R_{jp} *	ratio of probability of survival in the 0-2 month stage to the probability of survival in the pool (= p_{0-2} / p_p)	Dimensionless	1 → 68382	Deterministic
S©	normalized stress level in cell c	Dimensionless	1.6 10^{-12} → 10^{-2}	Calculated
S_o	stress level at stress source	Dimensionless	1.6 10^{-12} → 10^{-2}	Deterministic
S_{tot}	total stress from one source cell (= S_T / n_{st} = 1 / n_{st})	Dimensionless	3 10^{-5} → 1.000	Deterministic

S_T	total stress in the domain (= $\sum S_{tot}$ = 1.000)	Dimensionless	1.000	Definition
θ	angle between r and ρ	Dimensionless	$0 \rightarrow 2\pi$	Calculated
t	time	year or month	$1 \rightarrow 50$	Definition
x	age	year or month	$1 \rightarrow 12$ or $1 \rightarrow 144$ *	Definition
X	Barnthouse stress parameter = $\log_{10}$ [S/So]	Dimensionless	$-4 \rightarrow 0$	Calculated

DETERMINISTIC = Only varied deterministically during sensitivity analyses (no stochastic variability added)

STOCHASTIC = Stochastic variability added, may also be varied deterministically.

CALCULATED = Calculated in model (may have deterministic and/or stochastic components)

DEFINITION = Defined in model.

* = used only if time unit is one month rather than one year

Appendix Table 2: Key Assumptions in the Spatially Explicit Population Model. See Table 3 for a summary of the research phases and how specific assumptions will be relaxed.

ASSUMPTION	NOTES	WHEN ASSUMPTION RELAXED
HABITAT CHARACTERISTICS		
Benthic habitat spatially homogeneous.	Violated both due to within-habitat and among-habitat variability.	Phases IV, V, and VI.
Benthic habitat temporally homogeneous.	Likely to be violated though not apparent whether it is as important as spatial variation except in extreme cases.	Phase IV.
Water column habitat, and its affect on larval survival, temporally homogeneous.	Poor assumption - temporal variation in water column habitat quality is likely to be a major driver for variability in larval survival.	Phases IV and V.
Habitat types are discrete.	Discrete habitat types imposed as spatial structure in Phase IV. Effect of discrete habitats vs. gradients scale dependent.	Not addressed in this research.
STRESSOR CHARACTERISTICS		
Stressor decays exponentially with distance from source	Alternate models to be tested.	Phase III and VI.
Secondary ecological effects of stress are unimportant.	Use of field derived vital rates incorporates natural levels of interspecific interactions for that location and time. Potentially can have a large effect if food or key stone predator more sensitive than target species.	Not addressed during this research.
Stress level constant over time.	Should be approximated in short term for sewage discharges and sediment contaminants. Less valid over long term.	Not addressed during this research.
Logistic stressor function describes stressor effects on survival and fecundity with *Mya*.	General dose-response equation. Evaluated for mortality with fish with 77 compounds, not evaluated for fecundity. Should approximate any sigmoid response; questionable for biotic stressors.	Alternate models evaluated in Phase III and VI.
Stressor does not impact the larval (water column) stage.	Assumption good for sediment stressors. May be violated locally for effluents but likely to have negligible effect on larval pool.	Not addressed in this research.
Effects of natural stress and anthropogenic stress are independent	Synergetic effects on mortality not well documented in aquatic toxicology. Additivity of toxicity is a base assumption in many risk assessments.	Not addressed in this research.

POPULATION CHARACTERISTICS		
Population is at equilibrium at time=0.	Often violated in short-term, approximated true in long-term. Violation has greatest effect on "larval" survival rate since rate was calculated assuming equilibrium population structure. Addressed by adding stochastic variation.	Phases IV and V.
Age-specific vital rates resemble those determined for *Mya*.	Used field derived rates for adults though likely to vary naturally with habitat and temporally. Addressed through sensitivity analysis and stochastic variation.	Phases III, IV, V.
Leslie matrix is suitable population model.	Leslie matrix is broadly accepted age-structured model. IBM approach might be preferred if modeling mobile species with strongly density-dependent migration.	Not addressed in this research.
Larvae are well mixed in water column to form a larval pool and are distributed equally to all cells.	Likely to be violated both because of habitat heterogeneity and stochastic processes.	Phase IV and V.
No migration of adult clams.	Good for *Mya* which is deep burrowing. Needs to be evaluated for other benthic species.	Not addressed in this research.
All adult ($\geq$1 year) age classes have same sensitivity.	Should be approximately true. Effects of increased sensitivity of juveniles (<1 year) will be evaluated.	Adults: Not addressed in this research. Juveniles: Phase III and IV.
DENSITY-DEPENDENT INTERACTIONS		
Adult-adult density-dependent interactions only affect fecundity and not adult survival	Based on literature, fecundity appears more sensitive than mortality. Effects on growth captured through changes in fecundity.	Not addressed in this research.
Adult-adult and adult-juvenile density-dependent interactions are predicted by a ramp function.	Experimental results for adult-adult approximate ramp function. Uncertain about adult-juvenile.	Phase III and IV.
Larval-larval density-dependent interactions predicted by Ricker or Beverton-Holt functions.	Common approach in fisheries models.	Phase V.

Appendix Table 3: Phases of the Research. Only changes in an approach are listed under a phase.

HABITAT	STRESSOR	POPULATION	DENSITY DEPENDENCE	TEMPORAL/ SPATIAL VARIABILITY	VALIDATION
PHASE I: Develop Preliminary One-Dimensional Spatially Explicitly Population Model for Benthic Clam					
1-Dimensional, homogeneous	Generalized stressor-response function (logistic) for post-metamorphic survival & fecundity	Develop Leslie matrix for *Mya* for equilibrium population	None.	None	Algorithm (inhouse program compared to RAMAS-GIS)
	Single source cell	Single larval pool w/equal recruitment in all cells			
	Exponential decay rate of stressor				
PHASE II: Develop Preliminary 2-D SEPM Incorporating Multiple Point Sources with Constant Total Stress					
2-dimensional circle, homo-geneous	Develop methods for multiple source cells w/overlapping stress	Use yearly time step (mortality in larval and 0-2 month classes not separated)	Derive functions for adult-adult; adult-juv.; larval-larval	None	Algorithm (functions compared to published results)
PHASE III: Conduct Simulations Using 2-D SEPM Incorporating Multiple Sources; Adult-Adult Density-Dependent Interactions; No Stochasticity					
	Simulate multiple source cells with different spatial configurations		Adult-adult effects on fecundity	Preliminary simulation of discrete habitat types	Sensitivity analyses on lapse rate, β, α

					Alternate stressor-response and decay sub-models

PHASE IV: Conduct Simulations Using 2-D SEPM Incorporating Multiple Sources; Density Dependence on Adult-Adult; Spatial & Temporal Stochasticity					
Simulate a range of discrete habitat types				Random spatial variation in habitat quality on fecundity and 0-year class survival	Sensitivity analysis vital rates
				Random temporal variation in habitat quality	
				Random density-independent temporal variation on larval survival (by varying P_{peq})	
PHASE V: Conduct Simulations Using 2-D SEPM Incorporating Multiple Sources; Density Dependence on Adult-Adult, Adult-Juvenile, Larval-Larval; Spatial & Temporal Stochasticity					
		Leslie matrix w/month time step (mortality in larval and 0-2 month classes separated)	Adult-Juvenile using ramp function	Random spatial variation in larval recruitment	Ho: Predicted temporal variation = observed in catch records
			Larval-Larval using Ricker and/or Beverton - Holt equations	Random density-independent & density-dependent temporal variation on larval survival (by varying P_{peq} and carrying capacity)	Compare alternate models for larval density dependence
PHASE VI: Simulate Geographical Site Using Multiple Sources; Density Dependence on Adult-Adult, Adult-Juvenile, and Larval-Larval Stages; Spatial & Temporal Stochasticity					
Simulate habitat quality based on field data	Empirical stressor distribution around S. Calif. outfalls.				Ho: Predicted 1-D stressor pattern = pattern observed in S. California
	Empirical dose-response based on pop. distributions around S. Calif. outfalls.				Ho: Predicted 1-D pop. distribution = distribution observed in S. Calif.

MATHEMATICAL DETAILS

A-I. LESLIE MATRIX APPLIED TO MYA

Brousseau et al. (1982) provided a life table for the clam *Mya arenaria* assuming an equilibrium population structure (Table A-1). As discussed in the text and below, this work did not separate larval and early juvenile mortality. Also as discussed in the text, we use the concept of a larval pool to sum the reproductive output from all the cells (subpopulations). The Leslie matrix for a species with a life span of twelve years, including the concept of a fully mixed larval pool, with a year time step, is:

$$\begin{bmatrix} eggs \\ n_1 \\ n_2 \\ n_3 \\ \cdots \\ n_{12} \end{bmatrix}_{c,t+1} = \begin{bmatrix} 0 & p_1m_2 & p_2m_3 & \cdots & p_{11}m_{12} \\ p_{peq} & & & & \\ & p_1 & & & \\ & & p_2 & & \\ & & & \cdots & \cdots \\ & & & & p_{11} \end{bmatrix} \times \begin{bmatrix} pool/ncells \\ n_1 \\ n_2 \\ n_3 \\ \cdots \\ n_{12} \end{bmatrix}_{c,t} \qquad (1)$$

or, for the eggs produced by cell c at time t :

$$eggs_{c,t+1} = p_{1,c}\ m_{2,c}\ n_{1,c,t} + p_{2,c}\ m_{3,c}\ n_{2,c,t} + \ldots + p_{11,c}\ m_{12,c}\ n_{11,c,t} \qquad (2)$$

The larval pool population is defined as

$$pool_{t+1} = \sum_c eggs_{c,t+1} \qquad (3)$$

and, for survival to the next generation:

$$\begin{aligned} n_{1,c,t+1} &= p_{peq}\ pool_t / ncells \\ n_{2,c,t+1} &= p_{1,c}\ n_{1,c,t} \\ &\cdots \\ n_{12,c,t+1} &= p_{11,c}\ n_{11,c,t} \end{aligned} \qquad (4)$$

where n_x = number of female individuals, x = age = 1,2,...12

p_x = probability of survival from age x to age x+1

p_{peq} = probability of survival from the larval pool to age 1

ncells = total number of cells in the domain

m_x = annual number of female eggs produced by a female of age x

c = cell number

t = time step (year)

Note that

a) both age-specific survival, p, and age-specific fecundity, m, may be modified by the anthropogenic stress

b) the presence of p in equation (2) implies reproduction ***after*** survival

	p	n	m	eggs
pool/ncells		2.84E+06	0	0
3-12 mo	P peq	41.51	0	0
2 yr	0.177	7.35	3744	27508
3	0.912	6.70	17170	115048
4	0.904	6.06	31159	188739
5	0.952	5.77	39957	230414
6	0.949	5.47	50341	275489
7	0.969	5.30	62460	331213
8	0.984	5.22	76465	398991
9	0.911	4.75	76465	363481
10	0.911	4.33	76465	331131
11	0.911	3.95	76465	301661
12	0.911	3.59	76465	274813
	Total Pop.		Total eggs	
	(excl pool)	100.00	=pool	2.84E+06

Table A-1: Life History Data for Mya arenaria. Data from Brousseau et al. (1982). Calculation of "larval" survival is based on an equilibrium population.

A-II. CONVERSION OF LESLIE MATRIX TO MONTH TIME STEP

The field technique used by Brousseau et al. (1982) did not sample juveniles less than 2mm in length, the 0-2 month old class. Thus, their estimate of "larval" survival includes both losses in the larval stage and in the early juvenile stage. Initially, we use a one year time step in the model, and use the life history data for the 3-12 month age class as representative for the first year class. Note that this approach does not change the total mortality but does allocate more of it to the larval stage and less to the post-metamorphic (benthic) stage.

A future step in the refinement of the model will be to treat the pool of eggs and each of the first twelve months of life in the benthos separately. The reasons are:

1. Brousseau's data starts with month 3 and the age step in the Leslie matrix should be the same for all ages;
2. Modeling adult-juvenile and larval-larval density dependence will require treating the pool and the first few months of the benthic stage, the recruits, individually.
3. With a month time step and the corresponding life history and stressor data, it would be possible to model seasonal variations in effects.

Therefore, for the entire life cycle, the model eventually will use a month as a time unit rather than a year. On a modern computer there is no noticeable difference between applying the Leslie equations 12 times or 144 times at each time step. The model will be designed to preserve certain properties of the Brousseau yearly life table. After the first year, probability of survival from one month to the next will be the Brousseau measured value raised to the 1/12 power so that after being applied 12 times the same probability of survival will obtained in each year and the equilibrium population distribution also will be maintained.

Similarly, in the ten months from three through twelve, the Brousseau survival ratio for the 3-12 month class to the second year will be taken to the 1/10 power and applied 10 times. Finally, the survival rate, p_{peq} from the pool to month three will be artificially split into a survival from pool to month one and the remaining survival to month three. The ratio of survival in the 0-2 month class to survival in the larval phase (Rjp) is used to partition the larval and juvenile survival. The survival from month one to month three will be raised to the ½ power and applied twice.

The result is that at the third month and during the first month of each year the month-based population calculation has the same value as the year-based calculation. Fecundity will be allowed for only one month each year, with the fecundity values being the same as those presented in Brousseau's annual data (Table A-1).

Using the fecundities and probabilities of survival from the life history characteristics (Table A-1) the probability, p_{peq}, of survival from the pool to the 3-12 month class for an unstressed population in equilibrium can be determined by simple algebraic steps which are equivalent to setting the principal Eigenvalue of the Leslie matrix = 1.0 .

Consider a single cell and Brousseau's life table. Eliminating **eggs** and **pool** between equations (2), (3), and (4) yields the required value of the probability of survival from the pool to the 3-12 month class (assuming no juvenile fecundity) as

$$p_{peq} = 1.0 \;/\; \left[p_1 \left(m_2 + \sum_{l=3}^{12} m_l \prod_{j=2}^{l-1} p_j \right) \right] \tag{5}$$

In the case of the Brousseau data p_{peq} = .00001462. This value will vary with different environmental conditions for the same species and among species.

A-III. DENSITY-DEPENDENT RAMP FUNCTION

A ramp function will be used to model adult-adult and adult-juvenile density-dependent interactions. Two parameters are needed to describe the relationship, a threshold density at which density-dependent interactions begin and a slope of the line (see Figure A-1). Both density and fecundity are expressed as "normalized" values which are the ratios of the predicted values to the equilibrium values. The normalized fecundity can then be calculated as:

If normalized adult density < threshold,
normalized fecundity = 1.0.

If normalized adult density ≥ threshold,
normalized fecundity =1- slope *(normalized adult density - threshold).

If normalized density > threshold + 1 / slope,
normalized fecundity = 0.

Similar calculations are used to predict effects on juvenile survival. Application of density-dependent effects on both fecundity and juvenile survival generate the net effect on recruitment success. Multiplication of these two linear functions can result in a quadratic (non-linear) effect on recruitment success over certain ranges. The implications of this observation will be explored.

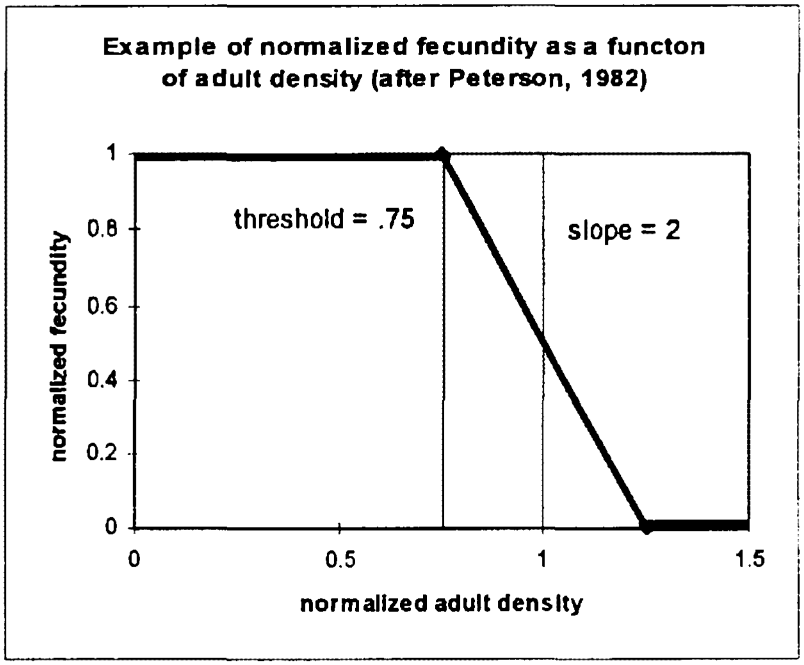


Figure A-1: Reduction in normalized fecundity as a function of adult density normalized to "natural" density.

IV. APPLICATION OF SINGLE STRESSOR SOURCE IN CIRCULAR DOMAIN

Consider a single source of stress located at the center of the circular domain (See Figure A-2).

The stress function used in this proposal is

$$S = S_o\, e^{-bs\, r} \tag{6}$$

For a single stress source a the center of the circular domain,

r_{max}

$$S_{tot} = S_o \int_{0} e^{-bs\,r}\, 2\,\pi\, r\, dr \qquad (7)$$

i.e. integrating rings of constant stress.

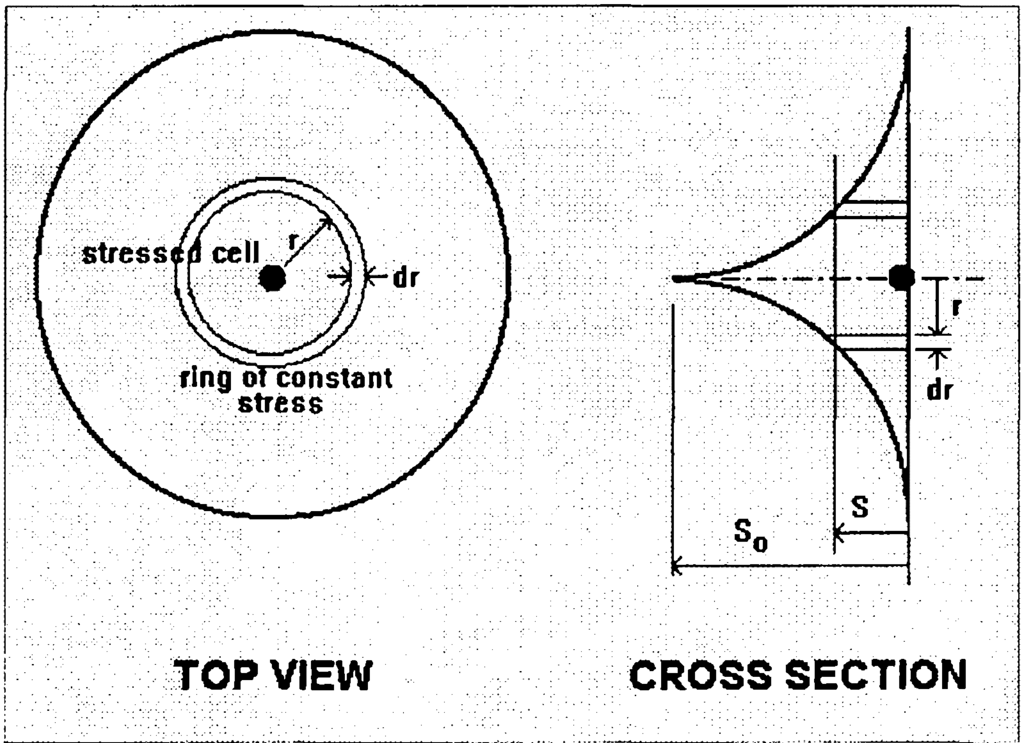


Figure A-2: Single source cell in circular domain.
where
r = the distance from the source,
r_{max} = radius of domain = 100,
bs = stress lapse rate,
S_o = stress level at the center (source cell),
S = stress level at r,
S_{tot} = total stress in the domain.

For comparing cases of different lapse rates, bs, we define the total stress in the system as

$$S_{tot} = 1.0 .$$

Then

$$S_o = bs^2 / [2 \pi \{e^{-100\,bs} (-100\,bs - 1) + 1\}] \qquad (8)$$

which shows that the lapse rate and S_o are intimately connected. To compare different stress profiles (e.g., dispersed vs. contained) it is convenient to select a set of S_o and examine the stress and accumulated stress as functions of radial distance from the source.

Equation (8) can be used to produce Table A-2. These results show that as dispersion increases (smaller bs), the stressor level increases in the source cell.

S_o	bs	r^* for $S / S_o = .15$
1.0 E-2	0.25066	8
1.0 E-3	0.07914	24
1.0 E-4	0.01874	100

Table A-2: Relationship between stress and lapse rate.

Here r^* is defined, as an example, as the point where the stress is approximately 15% of the stress at the origin.

$$r^* \cong -\ln(S/S_o) / bs = -\ln(.15)/bs = 1.897 / bs \tag{9}$$

Figure A-3 shows the raw stress as a function of r. This is the stress level associated with each cell at distance **r** from the source cell. By definition, the total stress introduced into the model

domain is a constant (1.0). This is illustrated in Figure A-4 which shows the integrals of each curve.

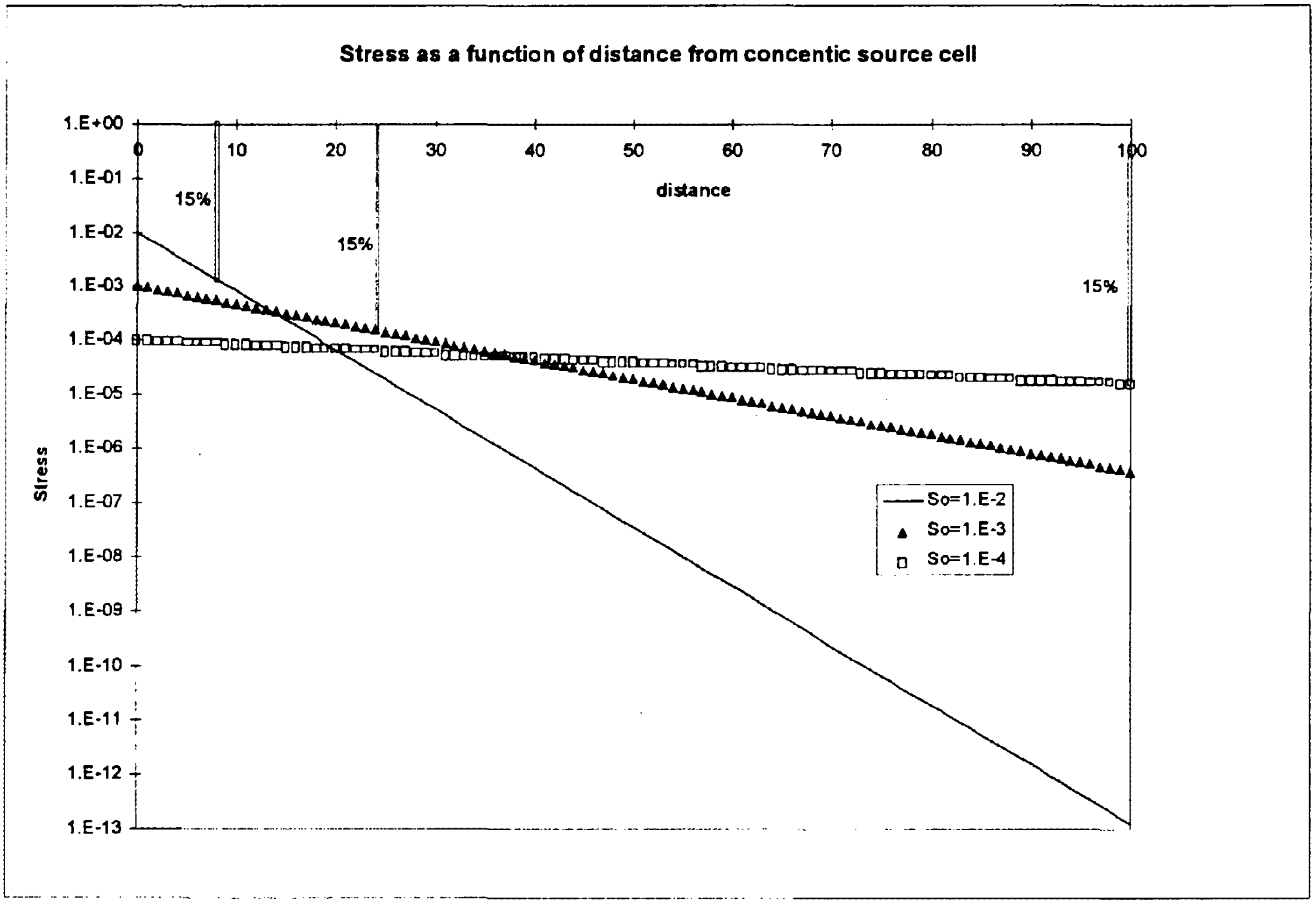


Figure A-3: Stress level as a function of distance from source cell for three different lapse rates in a circular domain. Lapse rates from Table A-2. "15%" indicates cell where stress level equals 15% of the source cell. Total stress in domain is 1.0 in all cases.

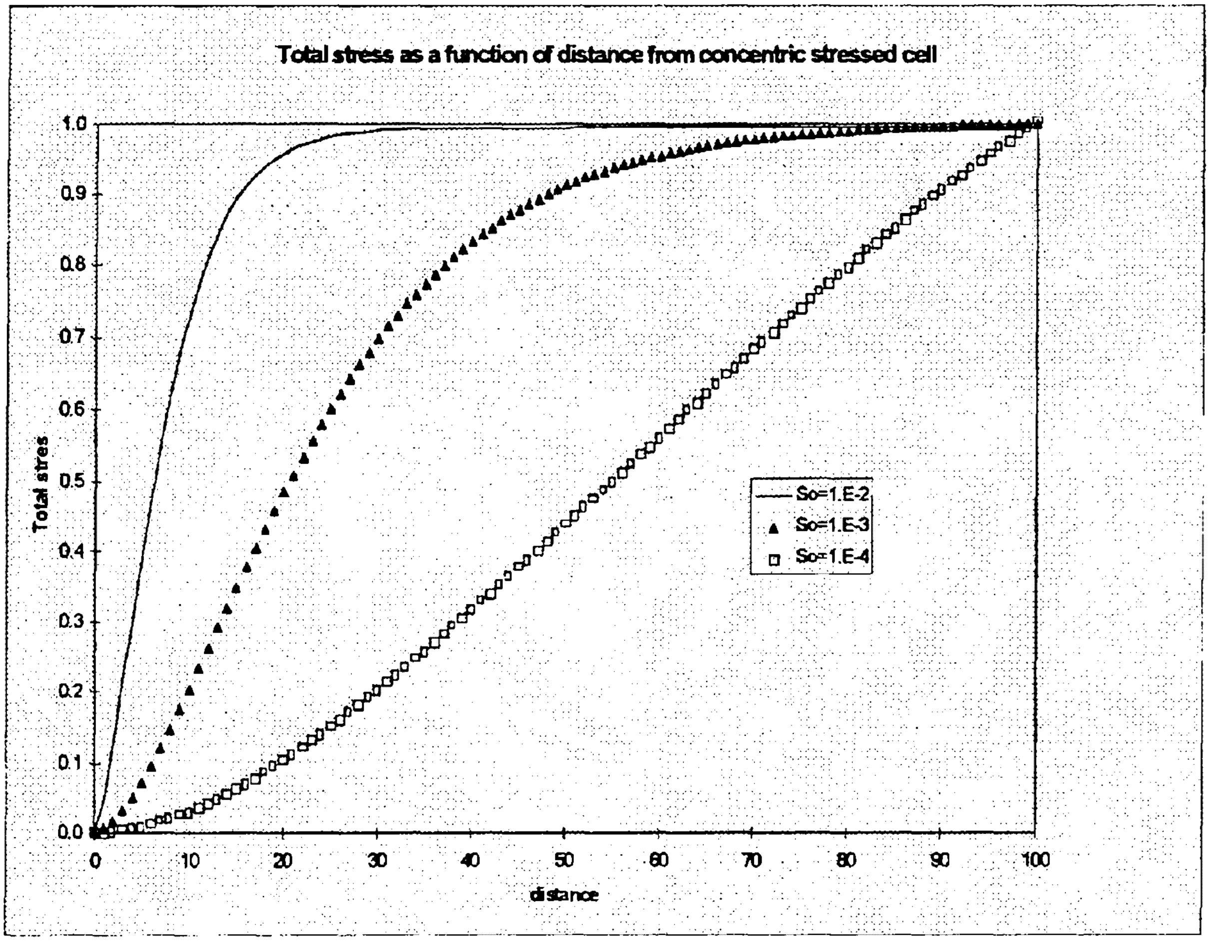


Figure A-4: Accumulated stress as a function of distance from the origin. Total stress = 1.0 for all cases.

V. NON-SYMMETRICAL AND MULTIPLE STRESSOR SOURCES

If the cell domain and the stressed cells are symmetrically located about the center of a circle, then all cells at the same distance from the center are affected equally by the stressor(s). This concept restricts the stressed region to circles or circular rings all concentric with the center of the circular domain of cells. Stresses can be calculated to the cells in each annular ring from the center to the edge and the Leslie matrix can be applied to what has essentially been reduced to a one-dimensional problem.

Since the cells are defined to have unit area, the number of cells at any radius, r, is

$$nc(r) = 2\,\pi\, r \tag{10}$$

and the total number of cells in the domain is

$$ncells = \pi\, r_{max}^{\;2} = \pi\, 100^{2} = 31{,}416 \tag{11}$$

In actual fact, the problem is solved by integrating the annular rings rather that each cell, so the potential problem of fractional cells in equations (10) and (11) vanishes.

If a stress source is not at the center of the domain (see Figure A-5), the stress still depends on the distance, r, from the source. Such a stress source has the potential of spreading stress to the limits of the grey circle in Figure A-5, but its effect will be limited to the area of the domain. The total stress from such an off-center source, S_{tot}, will still be defined as 1.00 (for a single source cell in the domain), but S_o and bs cannot be calculated from Equations (7) and (8), since they are based upon the stress source being located at the center of the domain. Stress can be numerically integrated over the grey circle, but S will be set to zero if $\rho > 100$, i.e. if the location of a cell is not in the domain.

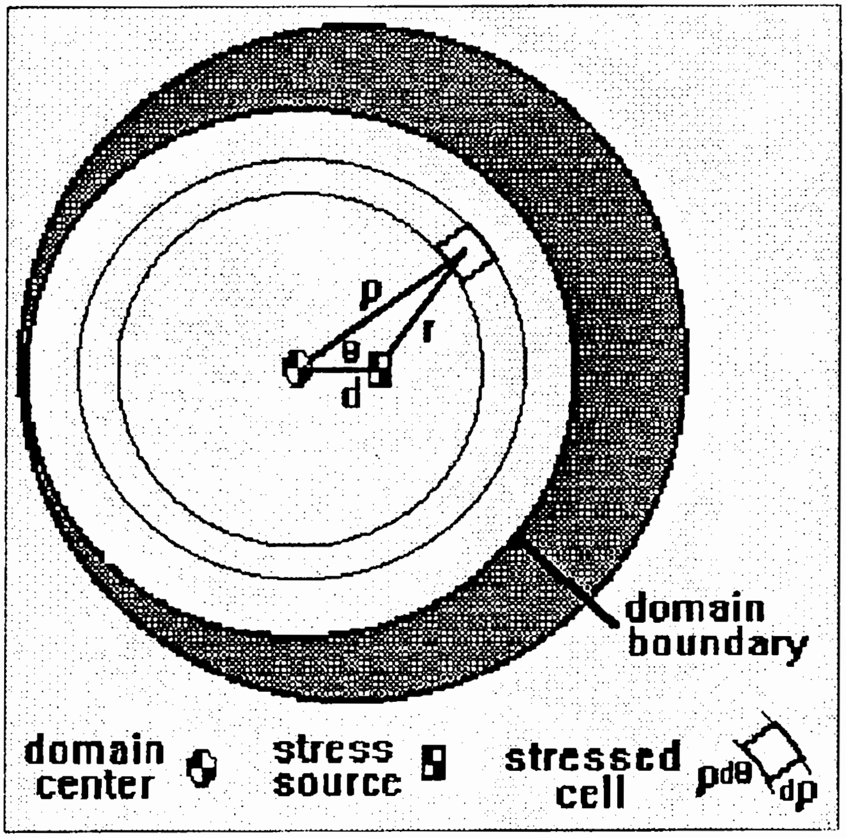


Figure A-5: Stressor source not at center of model domain.

Using the trigonometric "Law of Cosines"

$$r = \sqrt{d^2 + \rho^2 + 2\,d\,\rho\cos\theta} \tag{12}$$

and

$$S_{tot} = 2\,S_o \int_0^{100} \int_0^{\pi} e^{-bs\,r}\,\rho\,d\theta\,d\rho \tag{13}$$

or

$$S_o = .5 \Big/ \int_0^{100} \int_0^{\pi} e^{-bs\,r}\,\rho\,d\theta\,d\rho \tag{14}$$

If multiple stress sources are studied, the total stress from each will be accumulated and then each will be divided by the total number of stress cells, n_{st}, (since each source produces the same total stress) so that in comparative cases, the total stress to the domain will still be

$$S_T = \sum_{n_{st}} S_{tot} / n_{st} = 1.00.$$

VI. APPLICATION OF LOGISTIC STRESSOR-RESPONSE FUNCTION

Barnthouse et al. (1990, his equation #8) gives the fractional response, $\boldsymbol{P}$, to exposure concentration , X, as

$$\boldsymbol{P} = e^{(\alpha + \beta X)} / [1 + e^{(\alpha + \beta X)}] \qquad (15)$$

In Barnthouse's work the concentration term, X, is understood to be the $\log_{10}$ of a physical concentration (μg/L). We will use $X = \log_{10}$ **[S/S$_o$]** instead and then adjust the response to the stress by specifying increases in mortality and decreases in fecundity.

"β" determines the slope and curvature of the logistic function and "α ", a function only of **dm$_o$**
or **dq$_o$** , determines its level. The response function can be obtained by specifying either α and β or β and the response at a particular cell. We will specify β as β_q for increased mortality and β_m for reduction in fecundity and Barnthouse states that compared to other uncertainties there is no reason to try to use different β's for different age classes. In the earlier Phases we will use Barnthouse's nominal value of 6.0 for both β's and for all ages.

We replace Barnthouse's $\boldsymbol{P}$ with a notation of increased mortality due to anthropogenic stress, **dq**(x,c).

The α_q parameter can be calculated following Barnthouse. In general, for age x

$\alpha_q(x) = \ln [dq(x,c^*) / (1-dq(x,c^*)) - \beta_q(x) X_q^*]$

$X_q^* = \log_{10}$ (stress) for a fractional increase in mortality, dq(x,c*).

c* is the cell where we wish to perform the calculation of α_q.

We will define the response at the source cell where $X_q^* = \log_{10}(1.0) = 0$ since S/S_o=1.0 by definition of our normalised stress curve and

$$\alpha_q(x) = \ln [dq(x, c^*) / (1-dq(x, c^*))] \qquad (16)$$

Then for dq(x, c*) = .9 (say)

$$\alpha_q(x) = \ln(.9/.1) = 2.197$$

Now we have

$$dq_{x,c} = e^{(\alpha q(x) + \beta q(x)\ \log10[\ S/So])} / [1 + e^{(\alpha q(x) + \beta q(x)\ \log10[\ S/So])}] \quad (17)$$

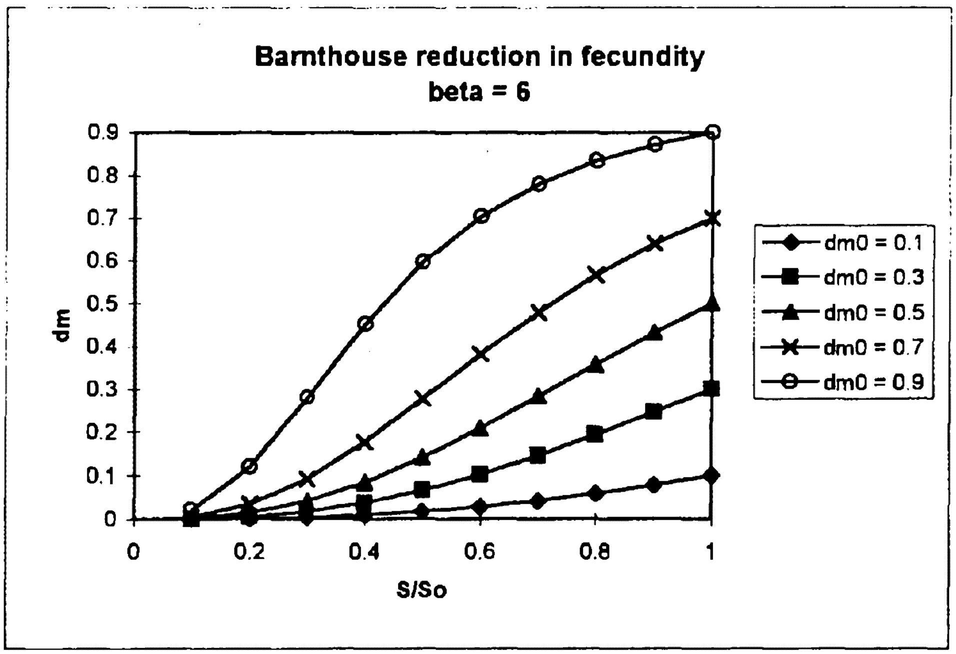
Similarly, for fecundity, (see Figure A-6 and Table A-3)

$$\alpha_m(x) = \ln[\ dm(x, {}_{c*}) / (1\text{-}dm(x, {}_{c*}))] \quad (18)$$

and

$$dm_{x,c} = e^{\{\alpha m(x) + \beta m(x)\ \log10[\ S/So)]\}} / [1 + e^{\{\alpha m(x) + \beta m(x)\ \log10[\ S/So]\}}] \quad (19)$$

The natural probability of survival, $p_x = [1\text{-}q_x]$, must be multiplied by the stress-related reduction in the probability of survival, $[1 - dq_{x,c}]$, to produce the total probability of survival. Natural fecundity, m_x, must be multiplied by the stress-related reduction in fecundity, $[1 - dm_{x,c}]$, to produce the total fecundity.



dm0	alpha
0.1	-2.1972
0.3	-0.8473
0.5	0.0000
0.7	0.8473
0.9	2.1972

Figure A-6: Change in reduction in fecundity, dm, as a function of the relative stress level.

Table A-3: Relationship between reduction in fecundity and $\alpha_m(x)$

The Leslie equations become

$$\begin{aligned} eggs_{c,t+1} = {} & p_{1,c}\ [1 - dq(1,c)]\ m_{2,c}\ [1 - dm(2,c)]\ n_{1,c,t} \\ & + p_{2,c}\ [1 - dq(2,c)]\ m_{3,c}\ [1 - dm(3,c)]\ n_{2,c,t} + \ldots \\ & + p_{11,c}\ [1 - dq(11,c)]\ m_{12,c}\ [1 - dm(12,c)]\ n_{11,c,t} \end{aligned} \tag{20}$$

The larval pool population is still defined as

$$pool_{t+1} = \sum eggs_{c,t+1} \tag{21}$$

c

and, for survival to the next generation:

$$n_{1,c,t+1} = p_{peq}\, pool_t \,/\, ncells$$
$$n_{2,c,t+1} = p_{1,c}\,[1 - dq(1,c)]\, n_{1,c,t} \qquad (22)$$
$$n_{3,c,t+1} = p_{2,c}\,[1 - dq(2,c)]\, n_{2,c,t}$$
$$\ldots$$
$$n_{12,c,t+1} = p_{11,c}\; n_{11,c,t}$$

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16. Abstract: The coastal Ecology branch (CEB), Western Ecology Division of the US Environmental Protection Agency will initiate a research program to evaluate the effects of alterations of estuarine habitats resulting from multiple stressor sources. The research will concentrate on stressor effects on the ecological resources of estuaries of the Pacific Northwest (PNW). The research program is designed to support the general mission of the US EPA which includes the safeguarding of the natural environment upon which the health and well being of the nation's population ultimately depends.
The goal of CEB research is to improve the ability to make key policy decisions on coastal environmental issues by defining key ecological processes and by developing models to predict stress-response relationships for ecological resources within Pacific Northwest estuaries at range of spatial and temporal scales. CEB research objectives are to 1) evaluate how specific estuarine habitats respond to a range of potential stressors which may lead to habitat alteration, 2) understand the influences of these stress factors at spatial scales from local to regional, and 3) develop indicators of ecological condition which may be used to evaluate estuarine status across multiple spatial scales.
The research effort will concentrate on two habitats 1) submerged aquatic vegetation (SAV) and 2) burrowing shrimp, with lesser effort on other types of estuarine habitats. SAV and shrimp are selected as focal research habitats and important assessment endpoints because in each case the characteristic species which define the habitat do so because of their "physical ecosystem engineering" activities.
To accomplish the objectives of the CEB research plan, research will be organized in three thematic elements: A. Indicators of Ecological condition for PNW estuaries; B. Stressor Response Modeling; C. Estuarine Physical-chemical stressors.

The research projects under Research theme A address the questions: 1) what are the biotic constituents of major estuarine benthic habitats of PNW estuaries, 2) what effects do various abiotic and biotic stressors have on the biotic composition of principal habitat types, 3) what role do biotic and abiotic stressors have in controlling the spatial extent and distribution patterns of major estuarine habitat types, and 4) what are appropriate indicators of ecological condition at the population, species, community, and landscape levels for PNW estuarine systems. Stressors that will be examined include anthropogenic physical disturbances such as clam and borrowing shrimp harvesting, and sedimentation, salinity, and water column light field alterations potentially generated by elevated runoff resulting from changes in landscape-use patterns. Biotic stressors that will be examined include disturbances such as the smothering of seagrass habitat by mat-forming algae potentially promoted by elevated nutrients, and biotic stress induced by competition between native and exotic seagrass species and between burrowing shrimp and seagrasses.

Projects under Research theme B will work at the population and community levels to develop modeling techniques to integrate the detailed studies of biological effects of estuarine stressors of theme A with the spatial-temporal stressor distribution studies of theme C. The principal current project is the development of spatially explicit modeling tools for estuarine benthic populations to allow predictions of population responses to the imposition of multiple stressors.

The research projects under Research Theme C will address the questions 1) what are the spatial and temporal distribution patterns of the primary physical and chemical factors determining estuarine habitat composition, 2) how are spatial variations in the physical anthropogenic alterations of watershed characteristics influence the

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