

**Pre-spill Assessments of Coastal Habitat Resources:**

Volume I:  
Development of Protocols

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## **Executive Summary**

Marine resources along the coast of California are vulnerable to impacts from spilled oil. Assessing the damages to natural resources from oil spills requires some assessment of the resource before it is impacted by the spill. Baseline data that could be used for injury assessment after an oil spill have not been gathered for much of the California coastline. As an alternative, pre-spill data could be collected in the hours before oil arrives on shore after an offshore oil spill.

Despite the value of assessing the existing condition of marine resources before a spill comes ashore, there is no established procedure for such an assessment. The goal of this coastal habitats quick-response “go-kit” project was to develop standard sets of procedures for California Department of Fish and Game’s (DFG) Office of Spill Prevention and Response (OSPR) that could be used to rapidly assess injury to biological communities immediately preceding the arrival on shore of oil from an offshore spill.

Specific protocols were developed for three coastal California habitats: sandy beaches, rocky intertidal and wetlands. The aim was to produce a set of protocols for each of these habitats that are supported by response trustees, along with detailed methods and equipment for biologists to employ in a quick response pre-spill situation. The protocols developed represent an attempt to balance the collection of relevant, scientifically rigorous data against the limited time available for sampling.

The results of the project are presented in two volumes. This volume (Volume 1) provides background information and a summary of the considerations and decisions made when developing the protocols. The actual protocols are not in this volume, but are included in the second volume: “Volume 2: Quick-Response Protocols.”

### **Sandy beaches**

Sandy beaches comprise three-quarters of the world's shorelines (Bascom 1980), including much of the California coast (Smith et al. 1976). Exposed sandy beaches compose 43% 74% and 93% of the mainland coasts of San Luis Obispo, Santa Barbara and Ventura Counties, respectively (Dugan et al. 1998a). Sandy beaches are thus likely to receive the majority of contamination from oil spills and other impacts associated with human activities. Despite their importance as a major component of the coast, recipients of ocean and land-based pollutants, and ecological, recreational and economic resources, beaches are the least understood and studied intertidal habitat on the California coast.

The sandy beach habitat provided more of a challenge than the other two habitats. The lack of regular monitoring programs and the highly mobile nature of the biota of beaches and of the habitat itself leads to inherently high variability in estimates of abundance and distribution. This makes it challenging to provide information that could potentially be used directly for before and after spill comparisons. In addition, the pre-spill data on sandy beaches needs to be collected very rapidly, likely the first habitat impacted by oil, whereas wetland habitat may have several hours until impact as the tide rises.

The protocols for the sandy beach habitat are not as fully developed in this report as the rocky intertidal and wetland habitats are. With more research the protocols can be developed further for a more robust characterization of the habitat and before and after comparisons. Given the research and monitoring that has been done on the coast of California (Dugan et al. 2003, Barnes and Wenner 1968, Cox and Dudley 1968, Dugan et al. 2000, Farallones Marine Sanctuary Assoc. 2002, USFWS 2006), and the methods that were used, a short set of protocols is suggested for the sandy beach habitat that provides a quick and basic data collection until more research can be done.

The suggested sandy beach protocols include a brief general log and beach characterization, including 360° pan photographs, GPS coordinates, beach widths, and general description, such as groomed or un-groomed and beach backing. In addition, a wrack and tar survey will be performed consisting of using a line-intercept method for wrack and a band transect method for tar for an estimate of percent cover and wrack composition. Finally, sand crabs (*Emerita analoga*) will be for collected chemical analysis.

### **Rocky intertidal**

The rocky intertidal zone supports a diverse and conspicuous assemblage of invertebrates and macroalgae. Impacts to this habitat are felt directly among the intertidal organisms and to the other connected surrounding communities and populations. Assessing rocky intertidal habitats before impacts occur is critical for determining natural resource damages. Most current rocky intertidal habitat assessments have been too time-intensive to be used before an impact. Often, the variables of time and available resources work against researchers in comprehensive damage assessments. The challenge is to develop a protocol that can be applied during a limited period of time and yet produce scientifically defensible data. For rocky intertidal habitats, our goal was to develop a sampling design that is rapid, repeatable, and returns quantifiable data for sampling of the rocky intertidal. We conducted field tests specifically to assess the statistical power that could be obtained using different sampling designs. The final protocol provides a standardized sampling procedure valid for detecting impacts to multiple types of rocky intertidal habitats in a quick and comprehensive manner for natural resource damage assessments.

The final rocky intertidal protocol consists of five basic sets of procedures, starting with completing a general log, taking 360° pan photographs, and identifying species present and recording their relative abundance and condition. A timed search of abalone or sea stars will be performed at the same location, recording abundance within size classes. Mussels will be collected for tissue analysis of petroleum hydrocarbons at every other general log sampling location. In addition, photo transects will be performed at which photographs of 110 quadrats will be taken for scoring in the lab to determine percent cover.

## **Wetland**

California wetlands are highly productive, unique ecosystems that support diverse floral and faunal communities. Southern California has only 25 to 30 small, isolated salt marshes, with a total area of less than 12,500 acres, along an approximately 160 kilometer length of coast, (Zedler 2001, Zedler 1982). Although relatively few species can handle the saline conditions of coastal wetlands, wetland communities are highly variable, both among wetlands depending on the size of the wetlands, the history of tidal influence and disturbances in nearby areas, and also within wetlands along an elevation gradient or along an exposure gradient to tidal flushing (Zedler 1982). The diversity of these rare ecosystems should be taken into consideration when estimating impacts to coastal California wetlands. We have included collection and analysis of a variety of different wetland resources in our sampling protocol, rather than only surveying the vegetation, to provide a more complete assessment of damage to a wetland ecosystem.

The development of the wetland protocols included reviewing injury assessment documents produced as part of the Natural Resource Damage Assessment (NRDA) process at oil spill sites at which wetland areas were impacted. Specifically we looked for assessment documents with explanations of what parameters were sampled and what methods were used to gather injury assessment information. We spoke with agency personnel from OSPR, NOAA, USFWS to get their feedback and input regarding the types of information they would find useful and important to collect in a rapid pre-spill situation. Also, a general literature search was performed to discover what methods and sampling equipment had been used at other spills. An initial set of protocols were developed and then tested in the field to determine how long they took to execute and to determine the logistics of sampling. Vegetation photo transects, pan photographs, snail photos, benthic invertebrate collection, soil collection – for both characteristics and chemical analysis – and bivalve collection were field tested at Mugu Lagoon salt marshes.

The wetland protocol consists of a “core” protocol and additional protocols. The core protocol was designed to maximize the amount of information gathered in a short period of time. The core protocol can be repeated at multiple locations in a large wetland if time permits, and at one or two locations in a small wetland. Five locations or sites are recommended to be sampled in a large wetland before moving on to the additional protocols in order to cover a substantial portion of the wetland. The core protocol consists of completing a general log data sheet of basic site information, taking 360° pan photographs, 100 close-range (0.75m x 0.5m) photos of vegetation and 50 close-range photos of snails. Vegetation photos will be scored in the lab for estimates of percent cover.

Benthic macroinvertebrates will be collected for population density estimates using a 10.5 cm diameter core to a depth of 5cm in replicates of 20 per site sampled. Two sets of sediment cores will be collected at each site, one for the soil characteristics of grain size, salinity and organic matter and the other for the analysis of petroleum hydrocarbons. Ten replicates of each sediment core type will be collected at each sample site. Bivalve cores will be collected for a density estimate and tissue collection for

polycyclic aromatic hydrocarbon (PAH) analysis. Each sample will be from a composite of three cores. Snails will be collected in replicates of ten with 40 snails per sample.

Fish seining will be employed for an abundance estimate or a presence/absence survey if appropriate. Also, fish can be collected for analysis of petroleum hydrocarbon metabolites in bile. This protocol is less developed than the other protocols due to a lack of field testing. A bird survey will also be conducted in mudflats and on edges of wetlands. Two of the possible protocols, crabs and insects, require a 24-hour time period to complete. Although it may be unlikely to have that much time available for sampling, these overnight protocols are included because they are important to the wetland ecosystem and are also taxa that would likely be impacted if a spill occurred. Yellow sticky traps will be set out in the vegetation and mudflats to collect insects.

The protocols and datasheets for the three habitats are described in detail in Volume 2: Quick-Response Protocols.

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In addition to the co-authors of this report, two other individuals made substantial contributions and are co-authors on individual chapters. Jenny Dugan at UC Santa Barbara provided her expertise and insight into sandy beach habitats to help develop the sandy beach protocols. These protocols are quite simplified at this point because of a lack of information on many of the important characteristics of sandy beaches and the methods needed to characterize them fully; future research will be needed to develop the protocols further. Minerals Management Service provided additional funding to UCSB to support the development of the sandy beach protocols. Forrest Vanderbilt, a graduate student at UCLA, conducted the work developing the rocky intertidal protocols.



## **I. Introduction**

Marine resources along the coast of California are vulnerable to various impacts from oil and gas activities, perhaps most conspicuously from spilled oil. Assessing the damages to natural resources from oil spills requires some assessment of the resource before it is impacted by the spill. Baseline data that could be used for injury assessment after an oil spill have not been gathered for much of the California coastline. As an alternative, pre-spill data could be collected in the hours before oil arrives on shore after an offshore oil spill. Such a pre-spill assessment could provide information that would be useful for a science-based determination of pre-spill condition.

Despite the value of assessing the existing condition of marine resources before a spill comes ashore, there is no established procedure for such an assessment. The goal of this coastal habitats quick-response “go-kit” protocol was to develop standard sets of procedures for California Department of Fish and Game’s (DFG) Office of Spill Prevention and Response (OSPR) that could be used to rapidly assess injury to biological communities immediately preceding the arrival on shore of oil from an offshore spill.

Specific protocols were developed for three coastal California habitats: sandy beaches, rocky intertidal and wetlands. The aim was to produce a set of protocols for each of these habitats that are supported by response trustees, along with detailed methods and equipment for biologists to employ in a quick response pre-spill situation. The focus was on developing protocols that would result in data that would quantify the loss of resources and could help determine the cause of the loss (e.g., showing that oil was the cause of a change rather than seasonal fluctuations). Inevitably, however, the limited time available for assessing living resources before a spill could impact an area means that the extent and intensity of sampling must be limited. The protocols developed in this project represent an attempt to balance the collection of relevant, scientifically rigorous data against the limited time available for sampling.

The final report for this project is presented in two volumes: “Volume 1: Development of Protocols,” and “Volume 2: Quick-Response Protocols.” In this first volume, we provide background information on previous work done in each of the three habitats that is relevant to pre-spill assessment protocols. The amount of previous work differs significantly for the different habitats, so the structure of this discussion also differs for the different habitats. From this previous work and our knowledge of these systems in California, we developed potential protocols for each habitat. For rocky intertidal and wetland habitats, the protocols were modified considerably based on preliminary field tests of the initial draft protocols, and these tests are described in this volume. Additional field tests with agency personnel, including OSPR, NOAA and USFWS, have also been conducted, with some additional modifications resulting. The resulting protocols, with instructions on how to implement them, model data sheets, and other related material, are presented in Volume 2.

Note that these protocols are likely to continue to be modified as additional information is developed and additional testing identifies areas that could be improved. For example, during the final stage of this project there was a substantial oil spill in San Francisco Bay,

and the draft version of the rocky intertidal protocol was implemented in some early post-spill assessments. Some initial lessons from that application have been incorporated into the protocols presented here, but undoubtedly there are other modifications that could improve these further.

## **II. Sandy Beach Habitat**

Principal authors: Jenny Dugan  
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### *Introduction*

Sandy beaches comprise three-quarters of the world's shorelines on average (Bascom 1980), including much of the California coast (Smith et al. 1976).. Sandy beaches are thus likely to receive the majority of contamination from oil spills and other impacts associated with human activities. Primary sites affected by a number of recent significant oil spills in central and southern California have been sandy beaches (Avila Beach, Guadalupe Dunes, Surf Beach and Huntington Beach). Despite their importance as a major component of the coast, recipients of ocean and land-based pollutants, and ecological, recreational and economic resources, beaches are the least understood and studied intertidal habitat on the California coast. No monitoring program for sandy beach biota exists for the state.

The lack of regular monitoring programs and the highly mobile nature of the biota of beaches, including shorebirds, and of the habitat itself leads to high variability in estimates of abundance and distribution. This makes it challenging to provide information that could potentially be used directly for before and after spill comparisons.

Intertidal zonation on exposed sandy beaches is extremely dynamic due to the highly mobile nature of the sandy substrate, the intertidal animals and the resources on which these animals depend (McLachlan and Jaramillo 1995, Brown and McLachlan 1990). In general, two to three different intertidal zones inhabited by distinct groups of mobile animals are present on most exposed sandy beaches (McLachlan and Jaramillo 1995). These zones generally correspond to the relatively dry sand/substrate of the upper intertidal at and above the drift line, the damp sand of the middle intertidal, and the wet or saturated sand of the lower intertidal zone (Figure 1). In addition, a supralittoral or coastal strand zone exists at the extreme high water level on many beaches (Figure 1). Unlike rocky shores, the location of these zones and of the diversity of organisms that inhabit them changes with the tides, wave conditions, and the seasons.

Macrophyte wrack is a key resource that provides food and habitat to many beach invertebrates. An average of 37% (14% -55%) of the invertebrate species (richness) present on beaches of Ventura and Santa Barbara counties were wrack-associated forms (Dugan et al 2003). The species richness and abundance of these elements of the invertebrate community are positively correlated with the abundance of wrack for beaches that possess all zones (dry, damp and saturated sand) present and are not groomed, raked etc. Wrack abundance could be potentially used as a proxy for species richness and abundance of certain taxa including talitrid amphipods, tyliid and oniscoid isopods, and insects for ungroomed beaches and those without coastal armoring or heavy vehicle use.

Macrofauna species in the swash zone of exposed sandy beaches consist primarily of mobile burrowing invertebrates including crustaceans, including crabs, amphipods and isopods, polychaete worms, and bivalve molluscs. The sand crab, *Emerita analoga*, comprised an average of 40% (7-94%) and 64% (22-99%) of the macrofaunal abundance and biomass respectively on exposed sandy beaches of Ventura County and southern Santa Barbara County (Dugan and Hubbard 1996, Dugan et al. 2003). Sand crabs can also be a major component of shorebird prey, as suggested by the gut contents of Semipalmated Plovers, Snowy Plovers, Western Sandpipers, and Sanderlings collected from a sandy beach at Point Mugu (Reeder 1951). Sand crabs occur on almost all beach types in southern and central California but may be more restricted on beaches in northern California.

Sand crabs, *Emerita analoga*, may be capable of rapid uptake of petroleum over the gill surface. These small decapods thus have potential for use as a biological indicator of pollutants, and may be especially useful on sandy coasts where mussels are never found. Populations of *E. analoga* have been used as bioindicators (Siegel and Wenner 1984, Wenner 1988) and are known bioaccumulators of metals, pesticides and hydrocarbons (Burnett 1971, Rossi et al. 1978, Wenner 1988, Dugan et al. 2005). High concentrations of petroleum hydrocarbons have been reported in *E. analoga* from selected southern California beaches (Rossi et al. 1978, Dugan et al. 2005, DFG unpublished data, Entrix 1996) and recent studies have indicated that sand crabs can accumulate significant concentrations of total hydrocarbons and PAHs in their tissues and eggs on beaches in central and southern California (Dugan et al. 2004, 2005). Toxicity of petroleum to sand crabs has been demonstrated, indicating a similar response to that of mysids (Barron et al. 1999 a, b).

Beaches are important components of coastal food webs. Many species of migratory, wintering and breeding shorebirds utilize California's sandy beaches. The distribution and abundance of shorebirds can vary on a variety of spatial and temporal scales on California beaches ((Dugan et al 2004. McCrary and Pierson 1998, Shuford et al. 1989, Webster et al. 1980). Peak numbers of shorebirds are observed during fall, winter and spring and consist of migrant and wintering birds. Shorebirds often feed opportunistically in a variety of coastal habitats, but several species of shorebirds, including Sanderlings, feed primarily on sandy beaches during the nonbreeding season. Exposed sandy beaches, even relatively narrow bluff-backed beaches, may be increasingly important as sources of prey for shorebirds during migration and wintering due to the loss of coastal wetlands and alternative feeding habitats (Hubbard and Dugan 2003). No monitoring program for beach shorebirds, other than the western snowy plover, exists for the state.

### *Research Needs*

With the exception of selected beaches on Santa Rosa Island in Channel Islands National Park, no established biological monitoring system is in place for sandy beaches in California. This means far more research needs to be done before a final set of procedures for a Rapid Response Protocol can be developed. Field testing and research are needed to evaluate some of the questions arising from the inherent variability of

sandy beach biota and habitats. Some of these research needs are discussed in this section.

The number of replicate transects needed for each kilometer of coastline to produce a good estimate of beach habitat characteristics and zones needs to be established through vigorous field testing in different beach and tide conditions. A determination of precision or error is needed for different beach types through field trials. It will also need to be determined whether transects should be randomly or uniformly selected across a kilometer of shoreline. Other constraints such as stream mouths or access paths intercepting the beach shoreline need to be considered in transect arrangement. Other questions include how much information on habitat will be available before the spill and how much time is needed to lay out transects and characterize beach zones.

For estimating the abundance of macrophyte wrack, a key resource for many invertebrates on sandy beaches, the number of across-shore transects needed to provide good estimates of wrack availability and composition needs to be determined with field testing. Also, an evaluation of whether the standing crop of wrack serves as a good proxy for species richness and abundance for selected taxa on beaches that do not possess all the zones and habitats or are raked or groomed is needed. Importantly, an estimate of day-to-day and tidal variability in wrack abundance needs to be developed to enable the use of these results with more confidence. An oil spill is not expected to affect the total abundance of wrack, however, a spill could potentially affect the abundance of unoiled wrack. On the other hand, if wrack is removed by spill response and cleanup operations, an accurate pre “cleanup” estimate of wrack availability would potentially provide important information for estimating these impacts.

Regarding the sampling of sand crabs, the number of crabs needed per sample for tissue analysis varies with the size of the animals available. For large crabs (CL= 20 mm or more) 8-10 animals per sample is sufficient. For small animals 4-10 mm, much larger numbers of individuals are needed per sample to make up approximately 10-20 grams of wet tissue. If only small megalopa and juvenile crabs are available, this will require hundreds of individuals. Additional research needs to be done to evaluate the number of replicates needed for sufficient statistical power for petroleum hydrocarbon analyses in this species. The spatial arrangement of sampling efforts and the number of replicates necessary for laboratory quality assurance/quality control (QA/QC) analyses also needs to be determined.

Field testing of protocols and variance structure for mobile invertebrates as a potential sample parameter is necessary to determine the time required as well as the accuracy associated with different observers. An assessment of the variability in measures of presence/absence is also needed for evaluating the potential use of species checklists and possible estimates of relative abundance for key species. A variety of different standard survey methods, such as coring, sieving, pitfall traps, and sticky traps are available for this type of testing.

Considering bird surveys for potential sampling, field testing is needed to evaluate variability in bird numbers and species composition using replicate counts on successive days. The effect of segment size, human activities, spill response activities, species interactions (e.g., raptors), time of day and tide constraints on shorebird results also need to be determined.

### *Final Protocol*

Even with the research needs presented in the previous section, several potential protocols can be suggested for further development for a more robust characterization of the beach habitat and before and after comparisons. Given the research and monitoring that has been done on the coast of California (Dugan et al. 2003, Barnes and Wenner 1968, Cox and Dudley 1968, Dugan et al. 2000, Farallones Marine Sanctuary Assoc. 2002, USFWS 2006), and the methods that were used, a short set of protocols is suggested for the sandy beach habitat that provide a quick and basic data collection until more research can be done. The suggested sandy beach protocols include: a general log, a few measurements of general beach characteristics, 360° pan photographs, a wrack and tar survey, and collection of sand crabs for tissue analyses.

For beach characteristics, a general log will be completed with basic habitat information. A minimum of two 360° pan photographs will be taken per site area, ideally with photos linked to GPS coordinates (See Appendix 3). Beach characteristics will be recorded such as the water table outcrop, wrack line position (which wrack line and how to choose needs to be delineated), beach backing and whether the beach is groomed or ungroomed. Many beach measurements that could be taken, such as beach slope, will not be taken due the time constraints involved in a rapid pre-spill survey.

A wrack and tar survey can be completed by using a line-intercept method for wrack and a band transect method for tar for an estimate of percent cover and wrack composition. A distance measuring wheel will be used while walking shore normal transects. The abundance of wrack within width categories will be recorded for every clump of wrack that intersects the edge of the measuring wheel track. All tar within one meter of the wheel transect will be recorded. In addition, wrack estimates will be made along the upper wrack line along sections of beach. The approximate width, % cover and depth of wrack will be estimated within a specified section of beach for a general estimate of wrack percent cover along the upper wrack line. Although these methods can give a reasonable estimate of wrack and tar standing stock, they cannot indicate the pre-spill rate of wrack or tar input to the beach.

To provide information on the background level of petroleum hydrocarbon in beach biota using sand crabs, samples of crabs can be collected at each beach area using clean techniques and frozen for later tissue analyses. The sand crabs can be collected using a stainless steel shovel. To collect sufficient biomass in a short time, sampling will be opportunistic. Shovel samples can be quickly assessed for presence of sand crabs by tossing the shovel contents to spread the sand, with any sand crabs collected by hand using gloves. They will be collected along shore-normal transects from just above to just below the swash zone. Strategically spaced samples can be used to identify the zone with

high crab density, with subsequent efforts centered on this zone. If there is a sand crab aggregation in the swash zone, cleaned mesh dive bag can be used, with sand shoveled into the mesh bag rapidly and then the bag rinsed after ten shovels; this procedure requires two people for optimum efficiency. It is very important not to walk around in the active swash before sampling because all the sand crabs will leave the area.

Several other protocols have been considered, but were not included in the final protocols reported here. They need more development or were deemed too lengthy for this initial rapid sampling. These protocols are described in Appendix 2 “Potential Protocols.”

### **III. Rocky Intertidal Habitat**

Principal authors:   Forrest Vanderbilt  
                              Richard F. Ambrose

#### *Introduction*

The rocky intertidal zone supports a diverse and conspicuous assemblage of invertebrates and macroalgae that produce more organic material than almost any other intertidal habitat. Impacts to this habitat are felt directly among the intertidal organisms and to the other connected surrounding communities and populations. Coastal ecosystems provide important functions to both terrestrial and marine systems, and replacing lost ecosystem function has become a priority for government environmental entities. Assessing rocky intertidal habitats before impacts occur is critical for determining natural resource damages.

Many different methods for assessing rocky intertidal habitats have been developed (e.g., Gonor and Kemp 1978, Richards and Davis 1988, Engle 2005; see Murray et al. 2006). Since these assessment methods have been developed for a variety of purposes, many are not suitable for a pre-spill assessment. In particular, most current rocky intertidal habitat assessments have been too time-intensive to be used before an impact. These sampling procedures typically require multiple teams of researchers spanning the length of at least one low tide with more equipment than two people could reasonably carry. Often, the variables of time and available resources work against researchers in comprehensive damage assessments. There is often little to no warning that a spill is going to occur, the location of the spill is remote, and researchers have varying degrees of expertise and equipment resources (EVOSTC 2004). Time or the lack thereof is a critical resource in sampling the rocky intertidal; however, the lack of time cannot be used as an excuse for collecting inaccurate data. The challenge, then, is to develop a protocol that can be applied during a limited period of time and yet produce scientifically defensible data.

For rocky intertidal habitats, our goal was to develop a sampling design that is rapid, repeatable, and returns quantifiable data for sampling of the rocky intertidal. We conducted field tests specifically to assess the statistical power that could be obtained using different sampling designs. The final protocol provides a standardized sampling procedure valid for detecting impacts to multiple types of rocky intertidal habitats in a quick and comprehensive manner for natural resource damage assessments.

The next few sections describe the studies done to develop the rocky intertidal protocol; the final section provides a summary of the final protocol.



## *Methods*

### 1. Field

A total of six study sites, three rocky benches and three cobble beaches, were sampled with 110 photographs, and results for detecting an impact were compared between three methods.

The six study sites chosen were typical of southern California rocky bench and cobble beach habitats. White's Point, Point Fermin, and Arroyo Hondo were the rocky bench habitats, and Leo Carrillo, Little Dume, and Paradise Cove were the cobble beach habitats (Figure 2). The inclusion of both intertidal habitat types allowed for comparison of protocol effectiveness in the more variable cobble beach habitat. Areas with 30 meters of along-shore contiguous habitat and visibly displayed zonation were chosen. The rocky benches were chosen for their accessibility, variety of representative intertidal species, and association with the Multi-Agency Rocky Intertidal Network's (MARINE) study sites. The cobble beaches were chosen due to their stability, variety of species, ease of access, and as a comparison to the rocky benches. Although much of the research and monitoring in rocky intertidal habitats has focused on rocky benches, cobble beaches comprise a large fraction of the rocky intertidal habitat in many regions, so we tested the protocol in this habitat, too.

For estimating cover of sessile invertebrates and algae, a 30 meter base transect was established parallel to the ocean, with sampling transects extended toward the ocean at three-meter intervals. Each sampling transect was divided into ten equally proportioned distances based on total transect length. For each site, there was a total of eleven sampling transects with ten photographs per transect for a total of 110 photographs. All photographs were taken with a 5 megapixel Canon PowerShot S2IS on the highest resolution (2592 x 1944). Photographs were framed using a 0.5 meter x 0.75 meter PVC quadrat. In order to maintain the spatial relationship of quadrats at each study site, the location along the corresponding sampling transects and base transect of each photograph was recorded. Sampling for each study location was completed during one tide sequence to prevent temporal variation, and was conducted during January and February 2006.

### 2. Lab

A point-contact method was used to analyze the photographs. Each photograph was uploaded onto a computer and overlaid with a grid of 100 equally spaced dots in Adobe® Photoshop 7.0. The grid could be free-transformed to insure proper spatial arrangement within the 0.5 meter x 0.75 meter quadrat. By layering in Adobe® Photoshop 7.0, the dot grid can be removed to reveal the organism underneath a particular dot. In addition to adding flexibility and ensuring a high degree of accuracy to point-contact sampling, this scoring method allows the researcher more time in the field to take photographs.

Using the Multi-Agency Rocky Intertidal Network's (MARINE) photoplot scoring protocol, the taxonomic identification was done to the lowest practicable level, genus or

species, except for those organisms not easily identified from photographs (Engle 2005). Percent cover was calculated for all species, including bare rock, based on the number of point contacts at each study site.

### 3. Power Analysis

The goal of the rapid response protocol is to collect data in a short period of time before a spill occurs at a site, but to be maximally useful the data must be statistically rigorous. One concern about samples taken in a spatially heterogenous area such as the rocky intertidal habitat is that the samples may not have sufficient statistical power to detect important changes. Therefore, we performed a power analysis on a subset of the species. Our goal was to be able to detect a 50 percent change in a species population with 80 percent certainty that the change was related to an impact and not natural variation. The levels were set based on accepted practice.

Because some species may be in such low numbers or spatially oriented in certain intertidal zones, not all species were used for the power analysis. A subset of species referred to as representative taxa were selected to represent the species most often associated with the high, mid, and low intertidal zones.

A prospective power analysis relies on two main parameters: variance and sample size. Since we set our sample size to 110 photographs based on the number of photoplots sampled at each site, the only parameter we could attempt to modify after the initial sampling was variance. The rocky intertidal shows strong zonation; therefore we attempted to reduce our variance by stratifying our data. We stratified the samples using either the length along a segment or the intertidal height. In addition, we evaluated the effect of using a paired analysis, pairing data from the same quadrats before and after a simulated impact, on sample variance.

Length zonation required dividing the distance along the sampling transect into three zones. The results for a given species were categorized into high, mid, and low intertidal zones. The height stratification method required a re-visitation to the study sites to take height measurements. The total height was divided into thirds and species results were categorized into associated height groups. The methods were compared based on the required time in the field and lab, the equipment needed, and statistical results.

To further refine our procedure, a paired power analysis was applied. Due to our repeatable sampling procedure, each photoplot can be re-photographed at a later date at the same area with reasonable accuracy. Therefore, a before-after comparison can be made within each quadrat rather than simply comparing the mean and standard error of the before data with the mean of the after sample. Data taken for a given representative taxon were manipulated to simulate an impact dropping the population on average 50 percent of its original number. We used a random numbers generator to produce a list of coefficients whose average was 0.5. Those coefficients were then multiplied by the number of individuals of a given species within each quadrat. Those new numbers, the impacted individuals, were then compared to the original set of individuals from each

quadrat. The paired results were then analyzed to indicate if this technique could result in a greater probability of accurately identifying an impact.

### *Results*

Photographic sampling took on average 80 minutes to set up the site and take 110 photographs. To score the photographs and enter the results in a database, an average of 11 hours was needed per site. Species percent cover estimates were recorded in 16 different taxonomic categories because some individuals could not be easily identified to the species level from the photographs. Table 1 shows the percent cover data separated by general habitat type and study site.

Spatial variability in the abundance of species as well as overall abundance can influence the statistical power of a test to detect changes in abundance. To see how abundance may have affected statistical power, we assigned rank abundances to each species at each site based on its percent cover data. Power to detect a 50% change in abundance was generally low for species with low abundance, and high for the most abundant species (Figure 3).

We chose the following species for our representative taxa: *Silvetia compressa*, *Chondracanthus canaliculatus*, *Endocladia muricata*, *Anthopleura elegantissima/solis*, *Chthamalus spp/Balanus glandula*, *Mytilus spp*. These species spanned the abundance scale, were indicators of specific intertidal zones, and could be easily identifiable in the field and on photographs. However for the analysis *Silvetia compressa* was not observed at Leo Carrillo and White's Point and *Chondracanthus canaliculatus* was not observed at Paradise Cove and White's Point. Our analysis of the six species at the six sites thus had 32 data points.

Although some species had relatively high power to detect a 50% change in abundance at some sites (Figure 3), power was low for many species-site combinations. We stratified the data using two approaches, by transect length and tidal elevation, to see if this would improved statistical power. Both methods of stratification yielded similar results (Figure 4), but height stratification required an additional 80 minutes in the field and additional equipment to acquire the needed height information. Points fall close to the line of equal power, and the power values were not significantly different from each other ( $\alpha = 0.05$ ). Since the two approaches to stratification yielded similar results but height stratification was more time-consuming, subsequent analyses were based on length stratification.

Stratification did increase the number of taxa-site combinations that could be sampled with reasonable statistical power (Table 2). Without stratification, there were no taxon-site combinations with sufficient statistical power (80% power to detect a 50% change in abundance) with 27 photographs and only 11 taxon-site combinations with 110 photographs. With length stratification, there were five taxon-site combinations with sufficient power with 27 photographs and 16 taxon-site combinations with 110 photographs. However, even with stratification, there was sufficient power for only half of the taxon-site combinations with 110 photographs, which was the maximum feasible number of photographs that could be taken given the time constraints for sampling.

The relatively poor power to detect changes at the sites stems from the high variability inherent in rocky intertidal communities, even with length stratification. However, these calculations assumed a random sample of the site before and after an oil spill. In fact, because the baseline transect can be marked and the spatial location of each photograph can be reconstructed, it would be possible to take the post-spill photographs at practically the same location as the pre-spill photographs. Pairing photographs this way would greatly reduce the amount of variability in the samples, and thus increase the power to detect a change in abundance. With both length stratification and pairing photographs, 19 taxon-site combinations had sufficient statistical power with 27 photographs, and 27 taxon-site combinations had sufficient power with 110 photographs, a substantial improvement over stratification alone.

These analyses indicate that fewer than 110 photographs could be taken at each site and a reasonable level of statistical power could be maintained. Although we feel that 110 photographs is a reasonable effort for the pre-spill protocol, these analyses show that fewer photographs could be taken if there was not enough time to take 110 photographs.

The methods described provide rapid, repeatable, quantitative data using relatively few resources in the field and in the lab for standard baseline intertidal sampling. The method required 80 minutes to sample a 30 meter wide swath of rocky shore with minimal equipment. By recording the exact location for each quadrat a second pair of samplers could return to the location and with reasonable certainty re-sample each quadrat location. The use of the digital format allows for verification of results between observers without sacrificing time in the field. Since the information will be collected and stored before analyzed, multiple experts can analyze the data and compare results for quality control.

### *Complications*

Three main issues arose with scoring of the photographs. First, the high degree of topographic variability meant the camera was not directly perpendicular to the quadrat frame, creating parallax error. However, the low profile of many intertidal species makes this error inconsequential in percent cover estimates, but could be greater if the rugosity of the study site is very high (Meese and Tomich 1992). Second, lighting at times can be low in some photographs, reducing the color and clarity of the image. There were on occasion points which were deemed unscorable because low light levels prevented an identification of what was below the dot. An addition of a separate strobe would have decreased the number of unscorable points. Although not found during this study, very bright light can also cause problems. During monitoring studies, we use an umbrella to soften direct sunlight, which otherwise causes extremes in contrast that make it difficult to score all sections of the photographs. However, the rapid surveys probably cannot use this method because it takes one person just to hold the umbrella. Finally, some algal species are difficult to identify by looking only at photographs. An individual with prior knowledge of the types of algae present along a given shore might be able to provide a lower level of taxonomic identification.

Because algal cover is based on a single photograph, it is not possible to record any understory algae, if these are present. In some assemblages, this makes little difference, but it prevents the assessment of the full species composition and abundance in complex multi-layered assemblages. This compromise is necessary in order to capture a large enough quantitative sample of the rocky intertidal community in a short time, and is commonly made in intertidal studies. It does not reduce the ability of the protocol to detect an impact to the rocky intertidal, but it does mean that not all impacts of an oil spill may be recorded.

### *Final Protocol*

The final rocky intertidal protocol consists of five basic sets of procedures, starting with completing a general log, taking 360° pan photographs and filling out a species log. A timed search of abalone or sea stars will be performed at the same location, and these protocols will be performed a minimum of every 200 meters within the entire rocky intertidal potential spill area of impact. Mussels will be collected for tissue analysis at every other general log sampling location. In addition, photo transects will be performed in which photographs of 110 quadrats will be taken for scoring in the lab to determine percent cover. Pan photographs and quadrat photos can be linked to GPS coordinates as discussed in Appendix 3.

To aid and accelerate the overall sampling process, flags can be marked and set out after completing the species log to indicate where the timed search, mussel collection and photo transects will be done. A second, third or fourth sampler can then go behind the initial sampler and complete the last three protocols.

## IV. Wetland Habitat

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### *Introduction*

California wetlands are highly productive, unique ecosystems that support diverse floral and faunal communities. Southern California has only 25 to 30 small, isolated salt marshes, with a total area of less than 12,500 acres, along an approximately 160 kilometer length of coast, (Zedler 2001, Zedler 1982). Although relatively few species can handle the saline conditions of coastal wetlands, wetland communities are highly variable, both among wetlands depending on the size of the wetlands, the history of tidal influence and disturbances in nearby areas, and also within wetlands along an elevation gradient or along an exposure gradient to tidal flushing (Zedler 1982). Even algal communities can be diverse within a wetland. Zedler's study of algal mats at Tijuana Estuary found 7 species of bluegreen algae, 2 green algae and 74 diatom species (Zedler 1982). Because coastal wetlands are unique systems with fluctuating salinities, animals occurring there are well adapted and are often limited to those areas (Long and Mason 1983). For example, the endangered light-footed clapper rail, *Rallus longirostris levipes*, and the Belding's savannah sparrow, *Passerculus sandwichensis beldingi*, are limited to California coastal marshes. The diversity of these rare ecosystems should be taken into consideration when estimating impacts to coastal California wetlands.

According to the Natural Resource Damage Assessment (NRDA) injury assessment documents that we reviewed (ENTRIX 2002, Llansó and Vølstad 2001, Peterson 2002, Osman 2001, Michel et al. 2002, Aquatic Resources Subgroup 2002, Finley et al. 1995, NOAA et al. 2002, Ashbrook and Doty 2000, NOAA et al. 2003, LOSCO et al. 1997, LOSCO et al. 2001, Showers 1988, CDPR et al. 1993, NOAA et al. 2005, FDEP 1997, NPS et al. 2002, American Trader Trustee Council 2001), the data that have been collected after oil spills in wetlands to assess injury to wetland ecosystems mainly includes the amount of vegetation and mudflat area impacted, as well as measurements of damage specifically to vegetation. Other wetland resources or biological communities within the wetlands, such as benthic invertebrates, bivalves, epifauna, and sediment (which in turn affects the biological communities), did not seem to be taken into consideration the way that fish, crabs, and invertebrates were in damage assessments for subtidal or intertidal areas. The qualitative estimates of abundance and observations of behavior, as well as presence/absence assessments, for assessing marsh fauna differ markedly from the more detailed quantitative assessment methods included for subtidal and intertidal habitats (NOAA 1996a). We have included collection and analysis of a variety of different wetland resources in our sampling protocol, rather than only surveying the vegetation, to provide a more complete assessment of damage to a wetland ecosystem.

Vegetation parameters such as percent cover, stem counts, and height measurements may not vary much between before and immediately after a spill, it could take months for those factors to change (Vicki Lake, personal communication).

However, if the spill is large enough and extensive enough for the vegetation to be extremely heavily oiled, then NRDA personnel may not be able to sample these vegetation parameters. A large spill does not always result in a proportionally large impact, so it is difficult to predict whether the vegetation will be heavily impacted. Therefore, it is still beneficial to collect this and other vegetation information, such as species richness and live/dead estimates, for pre-spill baseline data.

Benthic invertebrates are highly sensitive to the environment and can be good indicators of habitat quality and environmental change (Llansó and Vølstad 2001). Most benthic invertebrates are relatively sedentary and thus cannot avoid pollution in their environment. In certain taxonomic groups, a change in species composition and abundance can be seen in direct response to pollutants (Kingston et al. 1995, Gray et al. 1990, Bilyard 1987, Dauer 1993). For example, amphipods and harpacticoids show a decrease in abundance while polychaetes, oligochaetes and nematodes increase in abundance due to organic enrichment of the soil after an oil spill (Kingston et al. 1995, Peterson et al. 1996, Swartz et al. 1986, Gray et al. 1990, Llansó and Vølstad 2001). This change in population density makes them desirable to sample to show effects of oil impact. In addition, changes in abundance may directly affect higher trophic levels, such as fish and birds that feed on them.

Sediment characteristics such as grain size, salinity and organic content are critical aspects of the wetland ecosystem that affect the biological communities, the vegetation and marsh fauna. The presence of invertebrate species can vary with soil grain size, organic matter and salinity (Peterson 2002, Levin et al. 1998, Warwick 1988); it is important to know if changes in infauna abundance are due to natural variation or to oil spill effects. Soil salinities can vary seasonally in a coastal salt marsh, for example, becoming brackish during a heavy rainy season and hypersaline during a dry season if a coastal lagoon has been closed to tidal flushing (Zedler 1982). Determining the concentrations of petroleum hydrocarbons present in the soil before the spill is also important, particularly in areas of southern California where natural oil seepages occur.

Bivalves can accumulate petroleum hydrocarbons in their tissue because they lack the ability to metabolize them, unlike vertebrates and most other invertebrates (Lee et al. 1976, NOAA 1996a). Thus bivalves are useful to collect for infauna tissue analysis. It is important also to have a population density estimate because bivalves are often important to the ecosystem, are fairly uncommon throughout the wetland, and are likely to be killed in a significant oil spill event. Gastropods are highly sensitive to oil pollution and can also bioaccumulate petroleum hydrocarbons in their tissue (Rostad and Pereira 1987, NOAA 1996a). After a spill, snails can be collected for the epifauna tissue analysis.

For fish, mortality counts and models are usually used at spill sites during the NRDA process to quantify the injury. Fish kills are uncommon at most spills and fish are often not as impacted as other organisms because they can leave the area before injury occurs. The annual variability of many fish species is so large that only severe impacts would be able to be measured at statistically significant levels, but factors can be taken into consideration to decide whether fish abundance data would be worth collecting, such as the potential severity of the spill, which may warrant collecting the pre-incident data,

or recent weather conditions that could complicate abundance estimates (NOAA 1996a). If an abundance survey is deemed to be impractical or not representative, a species presence/absence survey can be done with less effort. Also, petroleum hydrocarbon metabolites in bile can be analyzed to show exposure to oil.

Although mortality counts and models have been used to assess injury to the bird populations in many NRDA processes, additional information gathered before a spill can be added to certain models for increased robustness. The number of dead birds found before oil impact can be taken into consideration post-spill so that a more accurate number of dead birds can be attributed to the spill. In the case of the well failure in Dixon Bay, LA in 1995, it was thought that some of the dead birds found post-spill were dead prior to the spill and had been oiled afterward (Finley et al. 1995). Including only freshly-dead birds in post-spill counts could also reduce the potential error of counting birds that were dead before the spill occurred.

Insects are an important part of wetland ecosystems. They feed on vegetation and algae, provide food for birds and herpetofauna, pollinate wetland plants and burrow and aerate soils (Zedler 1982). The endangered salt marsh bird's beak, *Cordylanthus maritimus maritimus*, relies on bees for pollination (Zedler 1982). While there are a variety of insect trapping methods, such as pitfall traps, light traps, funnel, Malaise, nests, water and sticky traps (Southwood 1966), we recommend using flat sticky traps because of the minimal equipment involved, the ease and rapidity of setting out the traps and the return on insect numbers in a 24-hour period.

### *Development*

We began the development of the pre-spill assessment protocol with a review of damage and injury assessment documents produced as part of the Natural Resource Damage Assessment (NRDA) process at oil spill sites at which wetland areas were impacted. Specifically we looked for assessment documents with explanations of what parameters were sampled and what methods were used to gather injury assessment information. In addition, we spoke with a dozen agency personnel from OSPR, NOAA, and USFWS to get their feedback and input regarding the types of information they would find useful and important to collect in a pre-spill situation. Finally, a general literature search was performed to discover what methods and sampling equipment had been used at other spills.

The NRDA document and sample methods search began with an internet search on OSPR's website. We were looking for spills that had impacted wetland areas and for which there had been an NRDA injury assessment document describing the area of impact, the samples that were collected, the equipment that was used and the specific methods involved in gathering that information. We contacted agency officials for more specific information regarding oil spills for which documents were not available.

We also performed a search on NOAA's website, on which there was an abundance of information, so much so that it became slightly difficult to navigate. A large amount of information was gathered from this site after many hours of searching.



NOAA personnel were also contacted for more information, and we were led to several guidance documents (NOAA 1996a, NOAA 1996b) that proved extremely helpful as a basic guideline, particularly for chemical analysis information. The U.S. Fish and Wildlife Service's (USFWS) and Environmental Protection Agency's (EPA) sampling guidance documents also were very useful for chemical analysis sample collection methodology (USFWS 2006, EPA 2001).

Tables were constructed with the information gathered from the search that included the samples collected for 11 spills that resulted in impacts to wetlands (Table 3, Table 4). A table was also constructed to compile the various sampling methods used by USFWS, NOAA and EPA for sediment, bivalve tissue and macrofauna collection (Table 5).

A basic list of suggested samples to collect was constructed after the document and literature searches and discussions with agency personnel were completed. The list was sent out to several wetland specialists for feedback and comment and revised again before a meeting with OSPR staff.

The next steps involved developing the methods and data sheets and field testing the potential protocols.

### *Methods and Results*

#### **1. Field Testing**

After our first meeting with OSPR to discuss the protocol progress and get feedback regarding the sample list and data sheets, the protocols were tested in the field to determine how long they took to execute and to determine the logistics of sampling. Vegetation photo transects, pan photographs, snail photos, benthic invertebrate collection, soil collection – for both characteristics and chemical analysis – and bivalve collection were field tested at Mugu Lagoon salt marshes.

##### a) Vegetation photos

The initial concept of the vegetation photo protocol called for setting out transects and taking photos of quadrats, moving the quadrat and sampling transects as needed throughout the sample area. The transects were to establish consistency between samplers, repeatability before and after the spill and impartiality while laying out the quadrats. One base transect was laid out and the sampling transect was moved perpendicular to the base transect; this protocol paralleled the rocky intertidal protocol for photoplots. The quadrat was laid over the vegetation flush to the ground as much as possible. The camera view was zoomed in to the extent of the quadrat and photos were taken from above and from the side to get a view of the height of the vegetation. Each set of photos was timed and 20 quadrats were photographed during this initial field test. After field-testing the protocol, the vegetation photo protocol was estimated to take two and a half hours to complete. Even if there was this time available for sampling in a pre-spill situation, two or three other protocols or sites would have to be abandoned to sample vegetation at one site, and vegetation photos could not be taken unless there was an

almost three hour timeframe available. Therefore, the original protocol was deemed too time-consuming for a pre-spill assessment.

To reduce the time needed to sample vegetation, we retained the idea of using photoplots (which, as described for the rocky intertidal protocol, have the advantage of relatively little field time needed, since identification and scoring for cover occurs in the laboratory) while revising the rest of the protocol to reduce time. Two major aspects were revised. First, we developed a camera “monopod” on which to mount the camera to face downward at a specified height so that the area within the viewfinder was the same as that of a quadrat on the ground. This maintained the sample area so quantitative aspects of cover could be derived from the photographs, but it substantially reduced the time to set up and take the photographs. Second, we abandoned the idea of laying out the baseline and sampling transects. Distances were estimated by pacing (measured before walking out to the site) and roughly the same area as the transect protocol (30m x 10m) was covered. Photos were also taken in the same general pattern as the transect protocol (five rows of ten quadrats across for 50 quadrats) for a total of 50 photos. By using an estimated distance rather than transect lines, the revised protocol may have slightly less repeatability (especially among samplers with naturally different pace distances), but the difference seems slight.

A second field test was conducted and timed using the revised protocol. The result was a vegetation protocol that took **eight minutes** instead of two and a half hours. The photos were still taken in an unbiased manner; the monopod extended out approximately ¼ meter from the sampler, so the photo area was not “chosen.” Instead, the sampler walks and stops after a certain number of paces, sets the monopod in front of his/her feet, sets the timer and levels the monopod, not looking in the viewfinder. The area photographed is the same in every photo due to the fixed height of the monopod. In the initial protocol, it was virtually impossible to set the quadrat completely flat on the ground if vegetation was tall, which resulted in laying the quadrat on top of some of the vegetation (level), standing over and zooming in to the quadrat area, resulting in different ground areas being sampled (while still containing the same 0.5 x 0.5m horizontal top plane of vegetation photographed). The protocol is still highly repeatable; the same 30m x 10m area will be sampled and GPS waypoints taken, and with a compass the directions walked can be recorded. The before-after comparison will not contain exactly the same photos, but percent cover taken from them will be averaged across the site<sup>1</sup>. In addition, even with the transects carefully laid out, there is the likelihood of not placing the quadrat in *exactly* the same place during successive sampling events unless each transect end is marked, which would be even more time-consuming. Also, there is less trampling involved than in the previous protocol because the sampler will walk through the vegetation area only once, while laying out transects takes one pass, photographing down the line takes another and positioning the quadrat and oneself over the quadrat takes more

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<sup>1</sup> Because there are fewer common species in salt marshes than in rocky intertidal habitats, and because spatial variation on the transect scale is generally much lower, achieving sufficient statistical power is not as difficult (see Section 2, below, on Sample Sizes). Therefore, it is not necessary to pair quadrats before and after the spill, as proposed for the rocky intertidal protocol.

stepping as well. Trampling of salt marsh vegetation could be a significant impact, so the revised protocol is environmentally beneficial as well as being much more rapid.

Feedback from a final field trial with OSPR, NOAA, USFWS, MMS and NPS indicated that we should extend the sampling area farther into the vegetation from the tidal creek. Thus, the sampling area will be extended to approximately 30m x 30m with 100 vegetation photographs taken in 10 rows of 10 photographs. In addition, the quadrat area photographed for vegetation and snails will be 0.75m x 0.5m (the area that is in view with a 35mm camera or digital camera equivalent) instead of 0.5m x 0.5m.

The camera monopod was constructed to be lightweight and inexpensive. It was made of two PVC pipes joined at a 90° angle, with a metal flange at one end with screws to attach the camera. A bullseye level was secured on top of the PVC pipe near the joint (close to the sampler). The height was measured so as to include in the camera viewfinder exactly the frame of the quadrat on the ground<sup>2</sup>. The horizontal distance was measured to be far enough away from a sampler to not include their feet, but not so far as to make the PVC pipe drop with the weight of the camera. Specifications and photos of the monopod are included in Appendix 1 of Volume 2.

#### b) Snail photos

In the initial protocol, snails were to be counted within quadrats along transects in the vegetation and mudflat for abundance data. This form of sampling would also take hours to complete. The protocol was changed to consist of taking photos of snail quadrats in the mudflat only. Counts of snail abundance would be made in the lab from the photos. Although photographs take much less time than actual counts in the field, we were concerned about the accuracy of photograph samples since mud snails can burrow in the mud, hence being invisible from the surface. A comparison was made to test the accuracy of counting snails from photographs in the lab. Snail counts in the field were compared to counts taken from photographs of the same quadrats during the first field test. Ten quadrats were analyzed. The margin of error was at the most  $\pm 2$  snails. Of the ten comparisons made, five were exactly the same, one differed by +1 snail, and four differed by  $\pm 2$  snails. Of the comparisons that varied, the total field counts ranged from 8 to 31. This level of precision was judged adequate for the pre-spill assessment.

During the initial field test, distances between quadrats were determined using the base transect for reference. 50 quadrats in the mudflat were sampled in 15 minutes. However, walking in the mudflat is an issue in an oil spill situation because deep holes will be made. Mudders™ are an option to prevent sinking in the mud, but they take some familiarization, take time to put on over boots, require extra equipment to carry, and still disturb the mud surface. As a result of this complication, instead of snail photos being taken in a similar pattern as the vegetation, they will be taken at the edge of the mudflat, spaced one meter apart in one row only for a 75 meter section. Because the photographs will be taken at the edge of the mudflat, the sampler can avoid stepping into the mudflat.

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<sup>2</sup> Note that this is going to differ for different cameras depending on lens focal length. Monopod specifications can easily be modified to fit specific cameras, or in some cases a different lens zoom setting could be used to ensure the photograph includes exactly the quadrat area.

The final change to the initial protocol was to take photographs using a monopod rather than laying out a quadrat for each photograph. The same monopod used for vegetation sampling was used for the snail samples. In the lab, a “framer” can be used to exclude the area on the edges of the photo, showing only the 0.5 x 0.5m area of a quadrat that would be included in the count of snails, as demonstrated in the vegetation and snail lab protocol in Volume 2. The quadrat area was subsequently changed to 0.75m x 0.5m (the area that is in view with a 35mm camera or digital camera equivalent) after discussion at the final field trial in December. The number of snail photographs will remain at 50.

As a result of the modifications to the initial protocol, the 50 photos used to sample mudflat snails can be taken in less than ten minutes. This is a substantial reduction in sampling time for the initial protocol, which would have taken more than one hour.

#### c) Sediment cores

During the first field test, sediment cores for chemical analysis and sediment cores for characteristics were sampled separately. All ten replicates for chemical analysis were collected along the 30 meter transect, then all ten samples for characteristics analysis were collected. Each set of cores took one hour to complete. During a subsequent field test, both sets of cores were collected at the same point along the base transect, with one set of cores collected every three meters for the 30 meters. Collecting both types of cores while moving along the base transect took half as much time as collecting each type separately, for a total sampling time of one hour.

#### d) Bivalve samples

To have sufficient material for chemical analyses, 10 grams (wet weight) of bivalve tissue is recommended. To determine the minimum number of bivalves needed to accumulate the 10 grams needed, bivalves were collected in the field and weighed in the lab to calculate a shell width/weight ratio. Only four bivalves were collected in the field testing location. The shell widths ranged from 15mm to 30mm and the tissue weights from 0.3g to 2.4g. A regression analysis was run and the resulting regression coefficient was 0.989 and  $p$  value was 0.0053 (Figure 5). Ten individual bivalves per tissue sample are recommended for collection.

The shell width to wet tissue weight ratio will vary depending on the species of bivalve. The recommended 10 individuals is a rough estimate based on the species collected during our field tests. With different species, the necessary weight might be obtained with fewer or more individuals, so the number of individuals collected must be adjusted accordingly.

Although the collection of bivalve tissue is an important component of the wetland protocol, the small number of bivalves we could collect for this analysis illustrates a limitation of this protocol. Some wetlands may have no bivalves at all, but even wetlands with sufficient bivalve populations may not have bivalves at the locations chosen for the pre-assessment sampling.

## 2. Sample sizes

The number of replicates for each wetland sample parameter was determined using data previously collected in Point Mugu salt marshes. The data included snail abundance, invertebrate density, invertebrate taxa richness, vegetation percent cover, vegetation species richness, crab abundance, and sediment grain size, organic matter and salinity content. We used four different analyses on the data: plotting the sample size versus the standard error divided by the mean, using a formula to input a desired level of precision ( $p$ ), using a formula inputting the allowable error in terms of confidence limits, and plotting a species accumulation curve. (See Murray et al. 2006, Chapter 4, for more discussion of these approaches.) These methods and the raw data that were analyzed are included in further detail in Appendix 1.

Synthesizing the results from the four methods, the suggested sample sizes are: snails = 50, invertebrates = 20, vegetation cover = 50, crabs = 50, sediment = 10.

The number of snails needed for each chemical analysis sample was determined from data collected at Mugu Lagoon in 2003. The chemical analysis sample should consist of around 10 grams wet weight of animal tissue. Whole snails were collected in the field, dissected in the lab, and the wet tissue of each snail was weighed. Based on the weights of 600 snails, a total of approximately 40 snails are needed to constitute a 10 gram sample.

### *Research Needs*

One issue that may be pursued further, which involves more research to increase confidence in accuracy of benthic invertebrate abundance estimates, is the sieve size used to sieve the organisms in the lab. Of the studies that we reviewed, the 0.5mm sieve size was most commonly used for macroinvertebrates (Michel et al. 2002, Osman 2001, Llansó and Vølstad 2001, Warwick 1988, CDFG 2003, Kingston et al. 1995, Warwick et al. 1990, Dauer 1993). Several researchers have contested whether the 0.5mm screen size is small and accurate enough for macroinvertebrate estimates. Levin et al. (1998), in their study of benthic invertebrates in a southern California *Spartina* salt marsh, showed that densities found on 1.0mm and 0.5mm screens were approximately 21 percent and 58 percent, respectively, of densities from a 0.3mm screen. They also found that screen size did not affect the relative proportions of annelids, molluscs and crustaceans, but that it did matter when looking at lower taxonomic levels. In Reish's (1959) review of the importance of screen size when sieving macroinvertebrates, after using a series of 11 screens from 0.15mm to 4.7mm openings, including 0.27, 0.35, 0.5 and 1.0mm, his results showed that over 90 percent of biomass could be retained on a screen size of 1.4mm, over 90 percent of the number of species on a 0.85mm screen, and over 90 percent of the number of individuals on a 0.27mm screen. However, the purpose of the analysis is to make a before and after spill comparison, not to just characterize the benthic infaunal community, so it may not be necessary to sample with a small enough screen to retain all species. In fact, many infauna studies do not use the smaller sieve sizes because of the extra time needed to process the samples. Luckily, sieve size does not have to be determined in the field, since the samples are processed in the laboratory. Thus, a

decision about greater accuracy in number of species sampled at a higher cost can be determined individually for each situation.

In addition to sieve size, the level of taxonomic identification has a large influence on the time (and cost) of processing infauna samples. Like sieve size, taxonomic resolution can be determined after the samples have been taken. Several researchers have found changes in macrofauna abundance to be detectable at higher taxonomic levels (Gray et al. 1990, Warwick 1988, Peterson et al. 1996). Gray et al. (1990) found the same patterns of macrofauna abundance response to oil pollution when grouping to family levels, to phyla, and even when including only abundances of the four major phyla, compared with species level classification. Warwick (1988) used five data sets of species abundance data and found the same results when grouping to the family level. Grouping to the phylum level did not produce similar results, however (Warwick 1988). Peterson et al. (1996) suggest the responses do not appear to be the same in phyla because of confounding factors not teased out in the multivariate analyses; the two different groups of phyla are responding to two different factors of pollution, with the annelids increasing in abundance in response to the organic enrichment and the arthropods decreasing in abundance due to toxicity. They did, however, also find the same responses in macrofauna abundance at the family level compared to the species level, but the species level was more sensitive (Peterson et al. 1996). If macroinvertebrates could be identified to higher taxonomic levels only, days or weeks of processing time could be saved.

It has not been determined whether both gastropods and bivalves, or both, are needed for tissue analyses. Bivalves have often been used in the past for chemical analysis of tissue samples for NRDA, but this may be because they are collected in subtidal areas and because mussels are easy to collect in the intertidal, rather than because their tissues bioaccumulate more hydrocarbons than gastropods. There seems to be a gap in the literature regarding a comparison between concentration levels of hydrocarbons in bivalves and gastropods, and thus whether one is “better” for analysis of hydrocarbon accumulation than the other. For now, we are including both in the protocol, as snails are easy to collect in wetland habitats.

The number of replicates of fish samples for analysis of petroleum hydrocarbon metabolites in bile needs to be established. Also the number of insect traps that should be used in a response protocol is unknown in terms of the number required to detect a change given a certain level of statistical accuracy. Further research should be done to determine the appropriate number of replicates to include in the protocol.

In addition, gravid female crabs (*Pachygrapsus*) could potentially be collected for measurement of egg viability and hatchability as an estimate of injury (NOAA 1996a). Also, observations of species behavior can be made such as their response to physical stimuli and their righting ability (NOAA, 1996a). We do not, however, have information on established methods for these measurements.

Another potential protocol is a mudflat burrows survey. This survey would consist of a count of distinctive burrow openings of different species, such as gaper

clams, ghost shrimp, fat innkeepers, tiger beetles. This type of sampling can take specialized knowledge, however; it may not be hard to do, but not many people may be familiar enough with the burrows to identify them accurately quickly. This survey could be done if someone on the team has the knowledge and capability to do so. We did not develop this protocol, but it could be developed in the future.

### *Final Protocol*

The wetland protocol consists of a “core” protocol and additional protocols. The core protocol was designed to maximize the amount of information gathered in a short period of time. The core can be repeated at multiple locations in a large wetland if time permits, and at one or two locations in a small wetland. Five locations or sites are recommended to be sampled in a large wetland before moving on to the additional protocols in order to cover a substantial portion of the wetland. Covering more of the wetland with the core protocol will increase the likelihood that spill impacts will occur at an area that has been pre-sampled with some basic information gathered, rather than risk spending hours at one location completing all protocols, and then have that site be unaffected by the oil.

The core protocol consists of (1) completing a general log data sheet of basic site information, and (2) taking 360° pan photographs, 100 close-range (0.75m x 0.5m) photos of vegetation and 50 close-range photos of snails. Vegetation photos will be scored in the lab for estimates of percent cover and possibly species richness, depending on the quality of the photos and the familiarity to the area and to the vegetation of the person scoring the photos. Snail photos will be scored in the lab for number of mud snails. All photographs can be linked to GPS coordinates as discussed in Appendix 3.

The additional, more extensive protocols consist of collection of benthic macroinvertebrates for population density estimates; sediment collection for grain size, salinity and organic content measures as well as chemical analysis; bivalve tissue samples for chemical analysis; bivalve population density survey; snail tissue collection for chemical analysis; snail proportion of live/dead estimate; fish seining for abundance or presence/absence survey and/or tissue sample collection for metabolites in bile; bird survey; and if a 24-hour time period is available, a crab survey and insect survey for population density and species richness estimates.

Most of the protocols are designed around a “base transect” of 30 meters, with the exception of the bivalve, fish and bird surveys. If the core protocol only is to be done at a location and not any of the additional protocols, to save time the transect tape will not be laid out.

Benthic macroinvertebrates will be collected for population density estimates using a 10.5 cm diameter core to a depth of 5cm in replicates of 20 per site sampled. Levin et al. (1998) found 78-89 percent of macrofauna in the top 2cm of sediment. Two sets of sediment cores will be collected at each site, one for the soil characteristics of grain size, salinity and organic matter and the other for the analysis of petroleum hydrocarbons. Ten replicates of each sediment core type will be collected at each sample site. Cores for characteristics will be inserted to a depth of 10cm, with a composite of

three cores, and cores for chemical analysis will be inserted to a depth of 5cm, with a composite of two cores.

Bivalve samples will be collected for a density estimate and tissue collection for polycyclic aromatic hydrocarbon (PAH) analysis. Each sample will be from a composite of three shovels. Snails will be collected in replicates of ten with 40 snails per sample. They will be frozen and analyzed in the lab for an estimate of the proportion of live to dead individuals. Each shell found with tissue will be assumed to have been alive at collection. Tissue will be sent to an analytical lab for PAH analysis.

Fish seining will be employed for an abundance estimate or a presence/absence survey if appropriate. Also, fish can be collected for analysis of petroleum hydrocarbon metabolites in bile. This protocol is less developed than the other protocols due to a lack of field testing. However, in its simplest form it could simply involve two samplers walking a short beach seine throughout potential fish habitat and recording the identities of all species collected.

A bird survey will be conducted in mudflats and on edges of wetlands.

Two of the possible protocols, crabs and insects, require a 24-hour time period to complete. Although it may be unlikely to have that much time available for sampling, these overnight protocols are included because they are important to the wetland ecosystem and target taxa that would likely be impacted if a spill occurred. Yellow sticky traps will be set out in the vegetation and mudflats to collect insects. In a comparison of yellow versus blue insect traps in a Nebraska salt marsh, Hoback et al. (1999) found 20 percent more insect families on yellow traps than on blue traps. Relative estimates are calculated per sampling method, in our case per trap, rather than per unit area (Southwood 1966).

The protocols and datasheets are described in detail in Volume 2. Supplemental pages, such as decision-making information and documents (e.g., sampling matrices, tidal influence charts, etc.) are also included and will be in the Go-Kit along with the protocol instructions and data sheets.

A sample numbering convention should be established before implementing the protocols and collecting data so that each sample has a unique identification number. If a scheme for establishing unique ID numbers exists, it should be used. Otherwise, a suggested numbering scheme is a three letter spill name, eight-digit date, one letter sample type, numerical site, and two-digit replicate number. For example: BAL051907A110.

#### *Quality Assurance/Quality Control (QA/QC)*

Several QA/QC measures can be put into place to minimize the sampling error and to increase consistency between samplers.

Regarding the wetland core protocol, the sampler should measure his or her pace before estimating the distances while taking the vegetation photos. Also, the sampler



should take a picture of a 0.75m x 0.5m quadrat flush on the ground before taking the vegetation photos, both to make sure the camera is zoomed to the correct distance or the camera monopod is the correct height and to later be used to analyze the vegetation photos in the lab by framing the photos. Note that many digital cameras reset the zoom to full wide angle once turned off, so the zoom may need to be reset to the appropriate quadrat size every time the camera is turned on. See the vegetation and snails lab protocol in Volume 2.

The lab analysis of vegetation and snail photos can consist of a mandatory recount of each photo and a second analyzer. A standard could be put into place with a specified acceptable amount of error. For example, if the total number of snails is less than 20, the two analyzer's counts cannot differ by more than 1; if the total is 20-100, the counts cannot differ by more than 5; and if greater than 100, cannot differ by more than 10. In addition, duplicate analyses can be conducted during laboratory procedures for soil characteristics analyses.

The laboratory procedures are described in Volume 2: Quick Response Protocols.

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## Tables

**Table 1.** Percent cover of dominant species, including rock and other substrate, found at 6 locations in Southern California.

'Other' species consisted of 6 species at Point Fermin, White's Point, and Leo Carrillo, 8 species at Arroyo Hondo and Little Dume, and 5 species at Paradise Cove.

Taxon	Cover (%)					
	Bench			Cobble		
	Arroyo Hondo	Point Fermin	White's Point	Leo Carrillo	Little Dume	Paradise Cove
<i>Ulva/Enteromorpha</i>	0.65	0.03	0.34	0.18	1.36	0.23
<i>Egrecia menziesii</i>	0.20	0.30	0.00	2.20	0.08	0.41
<i>Non-Coralline Crusts</i>	0.59	1.26	0.12	0.37	1.57	4.60
<i>Mytilus spp</i>	14.55	2.50	26.39	0.14	0.03	0.03
<i>Phyllospadix scouleri/torreyi</i>	6.81	0.66	0.00	1.80	1.37	3.17
<i>Silvetia compressa</i>	1.20	10.95	0.00	0.00	5.34	13.69
<i>Anthopleura elegantissima/solis</i>	9.82	1.26	1.56	0.96	4.04	1.24
<i>Articulated Corallines</i>	3.05	6.81	4.55	2.58	2.23	4.21
<i>Endocladia muricata</i>	0.42	14.25	5.90	3.12	9.95	9.78
<i>Chthamalus spp/Bal glandula</i>	5.20	14.19	10.58	10.31	2.15	5.64
Other	3.89(8)	1.71(6)	3.50(6)	0.36(6)	5.78(8)	0.76(5)
Substrate and unscorable	53.61	46.07	47.06	77.97	66.10	56.25
Total species	18	16	16	16	18	15

**Table 2.** Comparison of the additional number of taxon-site combinations that could achieve 80 percent power with the respective number of photographs for three analysis methods.

*Silvetia* was not observed at Leo Carrillo and White's point. *Chondracanthus* was not observed at Paradise Cove and White's point. Thus, the total number of possible taxon-site combinations was 32 (6 taxa x 6 sites minus 4 taxon-site combinations).

<u>Analysis method</u>	Number of photographs for 80% power				
	<u>0-27</u>	<u>28-55</u>	<u>56-83</u>	<u>83-110</u>	<u>&gt;110</u>
Entire study area, no stratification	<b>0</b>	<b>1</b>	<b>5</b>	<b>5</b>	<b>21</b>
Length stratified	<b>5</b>	<b>3</b>	<b>4</b>	<b>4</b>	<b>16</b>
Length stratified and paired quadrats	<b>19</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>5</b>

**Table 3.** Summary of sample parameters and methods of damage and injury assessment conducted as part of Natural Resource Damage Assessment at several U.S. oil spill locations resulting in impacts to wetlands.

Gallons	Spill	Parameters sampled	Surveys conducted	Notes
4,500	Humboldt/Kure, CA 1997	bird survey	noted amount of oiling, use of habitat to estimate degree of oiling & injury	small spill, only light oiling (per Steve Hampton)
12,600	North Pass, LA, 2002	Marsh flora/fauna, water column, benthic organisms, & habitats	surveys indicated minimal injuries - consisted of helicopter overflights, ground surveys, & on-water surveys, where no evidence (mortality of wildlife, birds, or fish) of injury was observed	
		Estimated: 1) area of injury (120 acres) through overflights, on-water surveys, photos, GPS, 2) initial service loss, 3) time to recovery – based on observations from past similar incidents (like Dixon Bay) Used Habitat Equivalency Analysis to quantify losses from oil impact as discounted service acre-years (56.2), given 3 parameters above.		*Trustees & RPs chose not to refine injury estimate b/c cost of studies would be > potential information gained & assumed restoration would not change as result of more detailed assessment
70,000	Suisun Marsh, CA 2004	vegetation	20 transects set perpendicular to sloughs - spp. id, richness, %cover, avg. & max. ht., leafcurling, chlorosis, evidence of exposure - wicking up stem or odor, multi-spectra camera to capture chlorophyll activity used as measure of plant function to define habitat injury (Vicki Lake, pers. comm.)	
		sediment	samples collected at different depths throughout marsh	(Damage assessment documents unavailable b/c recent & not released to public yet) (Julie Yamamoto & Vicki Lake, pers. comm.)
		wildlife	salt marsh harvest mouse noted	
		Invertebrates, fish, & water sampled too		
87,150	McGrath, CA 1993	vegetation	"measured loss"	Final Restoration Plan - (CDFG & USFWS)
		wildlife	birds, amphibians, fish, invertebrates injured	
		birds	mortality counts	
		sediment	"contaminated" (measured?)	cleanup involved removal
126,000	Chalk Point, MD 2000	vegetation	Quadrats: spp.% cover, stem count, stem ht, oiling, flowering/seed condition, chlorosis, photos	
		sediment	Collected samples to assess concentration of petroleum hydrocarbons in same veg quadrats	
		benthic invertebrates	Took PVC cores, 1' depth, sieved in field, 44 samples, in same veg quadrats & in intertidal	
		fish	Identified/counted/measured, injury noted, tissue collected -PHC(petroleum hydrocarbon) analysis	
		crabs	Tissue collection for PHC analysis	subtidal
		bivalves	Tissue collection for PHC analysis	subtidal
		birds	identified, counted, note degree of oiling, nest survey too	
		water	assess concentration of PHCs	Ichthyoplankton survey too
236,000	Whatcom Ck, WA 1999		aerial & ground photos, video	Fire occurred after spill too
	~3 miles of stream affected	vegetation	studies conducted to evaluate pre-incident conditions of plants, historic & on-site info. collected to compare baseline plant communities to incident injuries to scale restoration	

Gallons	Spill	Parameters sampled	Surveys conducted	Notes
		fish & invertebrates	Samples of "biota" (assuming fish) collected for PHC fingerprint analysis to compare to pipeline gasoline	gasoline from pipeline also analyzed to find out/predict toxicity, rate of degradation, fates, persistence
			mortality assessment. - 5 teams of 3-6 people collecting & recording dead & injured fish; "survey correction factor" used to take into acct likely dead fish but not found; Over 100,000 fish & invertebrates found dead, including 8,842 salmonids	
			"periodic" surveys of macroinvertebrates conducted to evaluate health, diversity & recovery rates	macroinvertebrate populations eliminated in >3 miles of stream
		sediment	Samples collected for PHC fingerprint analysis	
			sediment & groundwater contamination surveys (>115 subsurface explorations) - large scale - b/w pipeline & creek, repeated monitoring	
		water	Samples collected for PHC fingerprint analysis	temperature data monitored too & compared to pre-incident data found (but mainly b/c of lack of canopy cover from fire)
			repeated sampling at permanent water stations (8 along Creek & 12 w/in Bay) for extent & level of PHC exposure & later gasoline presence & rate of degradation	
		wildlife (riparian & terrestrial)	mortality assessment. - field survey recording of dead & injured wildlife; "survey correction factor" used to take into acct likely dead wildlife but not found; no final estimate - too difficult	
			also risk analysis conducted to assess risk to biota from contaminated sediments & water. Also lit. search for effects of oil toxicity on aquatic biota - invertebrates & fish - & lit search on fate/effects of similar spills	
			Used model (SIMAP) to estimate physical fate & biological effects of oil, and thus potential for injury	
			Long-term assessment. studies were considered, but b/c of cost & time delay for restoration, & no guarantee that further studies would result in change in restoration scale/plan, used time & money for restoration planning & implementation.	
275,562	Lake Barre, LA 1997	marsh / vegetation	field study conducted - oiling of vegetation, veg "status" (health/condition), photo documentation, invertebrate use of area, comparisons made to unoiled reference marsh, & to other spills in similar environments	4327 acres marsh impacted, ultimate injury estimate = "75.6 discounted service acre-years of lost marsh services" includ. 162 acres heavily oiled where near-total loss of above-ground biomass (measured?)
			increase in chlorosis & potential reductions in primary productivity (measured?)	
		sediment	injury to marsh & intertidal sediments already included in marsh injury estimate, no separate field survey done/necessary	subtidal sediment chemistry analysis indicated no significant injury occurred
		aquatic fauna	injury quantification began w/ mortality estimate, final injury as biomass (kg) lost	field effort to quantify injuries to aquatic biota

Gallons	Spill	Parameters sampled	Surveys conducted	Notes
			small dead fish & inverts observed, juvenile crabs found dead in traps set while adult crabs & fish were still alive, dead brown shrimp found in local catch	expensive & given natural regional variability difficult to detect magnitude of injuries thought to be present; so used modeling which took into acct mortality & future loss of growth/biomass during recovery time
		water	samples collected near pipeline break indicated PAH levels known to be toxic to aquatic organisms	(pre-spill PAH levels not required)
		birds	injury quantification began w/ estimate of bird mortality - 2 dead birds found, a # & variety of birds observed oiled	extensive field survey of birds unlikely to produce accurate results given large area in which birds would be hard to find, so used modeling which estimated mortality
		(human services considered too)		
		*Trustees & Texaco independent estimates of bird & aquatic faunal injury differed, could not reach consensus, but didn't matter b/c Texaco offer for restoration was sufficient to compensate for the loss. no need for Trustees to incur additional expense once agreement for restoration was reached		
362,000	Tampa Bay, FL 1993	Preassessment included:	documenting oil trajectory, pathways to exposure, & shoreline areas oiled; also sampling spilled oil ( <i>fingerprint?</i> ) & oiled areas	
		salt marsh	0.85 acres oiled, 0.75 acres considered injured, final injury = total loss of ecological services provided by 0.75 acres salt marsh for 1 year	
			ground surveys & aerial photos of oiled shoreline vegetation to determine extent & severity of injury	No additional surveys/sampling b/c of cost & small area impacted & indications of rapid recovery
		birds	Used record of captured rehabilitated birds as representing 50% of birds injured, so total injured = rehab # (366) X 2 = 732 birds	Did not perform additional studies to refine injury estimate b/c # estimated as injured was small compared to overall population, so those injuries would be difficult to detect as an overall decline in pop. #, also cost greater than information gained
		"bottom sediments"	including exposed mudflats - estimated area exposed to injury, & oil effects on biota based on literature	amt of damage in monetary terms = exposure area (58,540 ft <sup>2</sup> ) X sediment restoration costs per ft <sup>2</sup> (\$0.90 / ft <sup>2</sup> ) = \$5,268.60
			final injury = # of acres of <i>subtidal</i> sediment exposed to oiling enough to cause injury to subtidal benthic biota (assumes biota in contact w/ oil will die)	they had a hard time defining the area of sediment exposed using visual observations, divers, and SONAR, but decided on 58,540 ft <sup>2</sup> based on Coast Guard data during initial response
		"water column"	including fish & plankton - information collected & applied to models	

Gallons	Spill	Parameters sampled	Surveys conducted	Notes
			final injury = projected loss in fishery stocks caused by oil exposure using model = \$ value to determine scale of restoration	3 sub-models calculate: physical fate of the oil, biological injury it causes, the value of that injury
			overflights to determine location & extent of floating oil	
			water column sampling for hydrocarbons - 23 samples from 20 sites	
			plankton sampling for presence of larval fish & invertebrates in waters - for comparison w/ existing baseline data & models	
			there was an ongoing study of fish & relationship to infauna in sand beaches in the area, so sites were surveyed after the spill to compare	seine nets & small mesh nets for juveniles used
			also sand beach, seagrasses, shellfish beds, sea turtles, mangroves, & recreation loss	
400,000	Martinez, CA 1988	vegetation	Extent of oiling, plant species. covered by oiled, plant community present, state-listed plant search	("Initial Assessment of Plant communities" – CDFG document)
		Others?		
550,000	Westchester, LA 2000	wetland habitat	100 acres delta marsh vegetation impacted; also <1 acre fresh marsh, <1 acre freshwater edge of slough, 15 acres mudflat -- all 3 considered freshwater along river -- impacted	
			"information gathered during site visits" -- trajectory & extent of oiling from overflights, on-water surveys, & SCAT	ultimate injury estimate quantified as acre-years of services lost = 2 discounted service acre-years (DSAYs) of delta marsh
			conservative estimate made using HEA to give max. likely amt of injury (ecological services lost) that could have occurred as result of incident	*result of Habitat Equivalency Analysis was small amt of injury, so no further refined estimate pursued
		birds	injury quantification began w/ mortality estimate, ultimate injury estimate determined through modeling = 582 birds	results of extensive field survey of birds unlikely to be proportionate to time & expense required, and given large area in which birds would be hard to find along w/ many birds having been carried downriver or underwater, used modeling which estimated mortality & assumed all oiled birds would die
			<b>*bird spp composition &amp; abundance data used as inputs into model were from the survey conducted on one day - 2 days after spill</b> - to detect dead & oiled birds, of 1,680 birds observed, 15 dead & 9 oiled	
		aquatic fauna	injury quantification began w/ mortality estimate (no reports of mortalities observed), final injury expressed in biomass (19,400 kg) lost, ultimate injury estimate determined through modeling	including fish, blue crabs, shrimp, other invertebrates

Gallons	Spill	Parameters sampled	Surveys conducted	Notes
		water	samples collected indicated PAH levels known to be toxic to aquatic organisms	(pre-spill PAH levels not required)
		mammals	evidence of injury not sufficient to justify further investigation	
		*Trustees & RPs did not seek to refine injury estimates b/c costs to do so would increase overall costs too much compared to performing the restoration required based on preliminary injury estimates.		
?	Dixon Bay, LA 1995	marsh ~200 acres oiled	field surveys w/ video & field notes for the degree of oiling & width of oil banding in different segments	
			used Habitat Equivalency Analysis, w/ inputs for degree of injury (% lost marsh services), recovery time, & acreage oiled, to get final estimate of losses in ecological services & amt. of restoration needed to compensate	*Did not refine estimates through additional field surveys b/c changing inputs to HEA using very conservative estimates did not significantly change restoration output.
		vegetation	dominant species present, degree of oiling, oiled wrack noted	
		sediment - marsh	little oil observed, none noted having seeped below surface	
		birds/wildlife	oiled & dead found during shoreline survey of ~0.25 miles - documented in videos, photos & field notes, mammal tracks found w/ dead birds	no other sections of oiled shoreline suitable for surveys b/c of thick marsh veg & soft sediments (could not walk through & impact)
			considered use of models to estimate bird injuries, but could not b/c no reliable cost-effective method was available; so did not come up w/ final injury estimate	<b>*they think some of dead birds found may have died before the spill &amp; were oiled after</b>
		benthos (assuming subtidal)	15 sample stations established (+ 2 for reference), 6 rep's from ea. station collected for abundance & community structure	samples processed in phases, 1st 13 stations w/ 4 rep's / station analyzed, found no impacts to benthic community so did not analyze rest of samples; PHC levels in sediments not found to be at levels expected to impact benthic organisms
			sediment (subtidal) samples collected for PHC concentrations, one at ea. of same 15 stations	
		water column	overflight estimated 25 miles <sup>2</sup> covered w/ visible oil slicks on surface	
		*Trustees thought water column biota were injured (severe weather increasing dispersion), but could not use any models b/c dif. estimates of oil volume varied by 2 orders of magnitude, and no model accurately reflected topography/habitats; so no final estimate, but agreed upon wetlands creation project as compensation.		

**Table 4.** Summary of sample parameters and methods of damage and injury assessment conducted as part of Natural Resource Damage Assessments listed by parameter.

Parameters sampled	Spill	Go-Kit Wetland Sampling Protocol	NRDA Surveys conducted/ Notes
salt marsh	Tampa Bay, FL	Photo documentation of vegetation in 50 photos in 30m x 10m area. Two or more 360° pan photos per site, 50 snail photos for counts, 20 benthic invert. cores (abund.), 10 sed. cores ea. for characteristics & chem. analysis, bivalves for density & chem. analysis, fish survey & analysis of PHC metabolites in bile, bird survey, crab abund. – 50 traps, insect trap survey.	ground surveys & aerial photos of oiled shoreline vegetation to determine extent & severity of injury; 0.85 acres oiled, 0.75 acres considered injured, final injury = total loss of ecological services provided by 0.75 acres salt marsh for 1 year; No additional surveys/sampling b/c of cost & small area impacted & indications of rapid recovery
wetland habitat	Westchester, LA 2000	Photo documentation of vegetation in 50 photos in 30m x 10m area. Two or more 360° pan photos per site, 50 snail photos for counts, 20 benthic invert. cores (abund.), 10 sed. cores ea. for characteristics & chem. analysis, bivalves for density & chem. analysis, fish survey & analysis of PHC metabolites in bile, bird survey, crab abund. – 50 traps, insect trap survey.	"information gathered during site visits" -- trajectory & extent of oiling from overflights, on-water surveys, & SCAT; 100 acres delta marsh vegetation & 15 acres mudflat along river – impacted; ultimate injury estimate = 2 discounted service acre-years (DSAYs) of delta marsh, *result of Habitat Equivalency Analysis was small amt of injury, so no refinement of estimate pursued
marsh ~200 acres oiled	Dixon Bay, LA 1995	Photo documentation of vegetation in 50 photos in 30m x 10m area. Two or more 360° pan photos per site, 50 snail photos for counts, 20 benthic invert. cores (abund.), 10 sed. cores ea. for characteristics & chem. analysis, bivalves for density & chem. analysis, fish survey & analysis of PHC metabolites in bile, bird survey, crab abund. – 50 traps, insect trap survey.	field surveys w/ video & field notes on the degree of oiling & width of oil banding in different segments; used Habitat Equivalency Analysis, w/ inputs for degree of injury (% lost marsh services), recovery time, & acreage oiled, to get final estimate of losses in ecological services & amt. of restoration needed to compensate; *Did not refine estimates through additional field surveys b/c changing inputs to HEA using very conservative estimates did not significantly change restoration output.
marsh / vegetation, 4327 acres marsh impacted	Lake Barre, LA	Photo documentation of vegetation in 50 photos in 30m x 10m area. Two or more 360° pan photos per site, 50 snail photos for counts, 20 benthic invert. cores (abund.), 10 sed. cores ea. for characteristics & chem. analysis, bivalves for density & chem. analysis, fish survey & analysis of PHC metabolites in bile, bird survey, crab abund. – 50 traps, insect trap survey.	field study conducted - oiling of vegetation, veg "status" (health/condition), increase in chlorosis & potential reductions in primary productivity (measured?); photo documentation, invertebrate use of area, comparisons made to unoiled reference marsh & to other spills in similar environments; ultimate injury estimate = "75.6 discounted service acre-years of lost marsh services," includ. 162 acres heavily oiled where near-total loss of above-ground biomass (measured?)
vegetation	Suisun Marsh, CA	% cover and species richness taken from 50 photos of vegetation in 30m x 10m area.	20 transects set perpendicular to sloughs - spp. id, richness, %cover, avg. & max. ht., leafcurling, chlorosis, evidence of exposure (wicking up stem or odor), multi-spectra camera to capture chlorophyll activity used as measure of plant function (per Vicki Lake)

Parameters sampled	Spill	Go-Kit Wetland Sampling Protocol	NRDA Surveys conducted/ Notes
vegetation	McGrath, CA	Photo documentation of vegetation and % cover and species richness taken from 50 photos of veg. in 30m x 10m area. Two or more 360° pan photos per site.	“measured loss”
vegetation	Chalk Point, MD	Photo documentation of vegetation and % cover and species richness taken from 50 photos of vegetation in 30m x 10m area. Two or more 360° pan photos per site.	Field surveys to assess extent of oiling, separated areas into categories of moderately & heavily oiled. 2 or 3 permanent 1m <sup>2</sup> quadrats (averaged) set for each category also divided by dominant veg for 37 quadrats, measured in ea quadrat: % cover, stem count, stem ht, amt of oiling, flowering/seed condition, chlorosis, noted presence & oiling of wrack & took 2 photos (1 closeup & 1 10-20 ft away); compared to reference areas (24 quadrats); sampled 3 times separated by 6 months
vegetation	Whatcom Ck, WA	Photo documentation of vegetation in 50 photos in 30m x 10m area. Two or more 360° pan photos per site.	~3 miles of stream affected; Took aerial & ground photos & video; studies conducted to evaluate pre-incident conditions of plants (oil caught on fire & killed vegetation), historic & on-site info. collected to compare baseline plant communities to incident injuries to scale restoration
vegetation	Martinez, CA 1988	Photo documentation of vegetation and % cover and species richness taken from 50 photos of vegetation in 30m x 10m area.	Extent of oiling, plant species covered by oiled, plant community present, state-listed plant search (“Initial Assessment of Plant communities” – CDFG document)
vegetation	Dixon Bay, LA	Photo documentation of vegetation and % cover and species richness taken from 50 photos of veg. in 30m x 10m area. Two or more 360° pan photos per site.	dominant species present, degree of oiling, oiled wrack noted
invertebrates	Suisun Marsh, CA	20 cores, 10cm diameter, to 5cm depth along 30m transect, for taxa richness and abundance data.	? (Damage assessment documents unavailable b/c recent & not released to public yet, Julie Yamamoto, pers. comm.)
benthic invertebrates	Chalk Point	20 cores, 10cm diameter, to 5cm depth along 30m transect, for taxa richness and abundance data.	Took PVC cores, 1’ depth, sieved in field, 44 samples total; collected from same (but fewer) quadrats as veg, in heavily oiled areas & reference areas only, species id & count (averaged), sampled 3 times, same time as vegetation sampled; also intertidal benthic invert cores collected (10 reps along 20m transect) & subtidal cores collected
invertebrates	Whatcom Ck, WA	20 cores, 10cm diameter, to 5cm depth along 30m transect, for taxa richness and abundance data.	"periodic" surveys of macroinvertebrates conducted to evaluate health, diversity & recovery rates; macroinvert populations eliminated in >3 miles of stream
invertebrates	Lake Barre, LA	20 cores, 10cm diameter, to 5cm depth along 30m transect, for taxa richness and abund. data; Crab abund. estimates from 50 traps; fish survey, pop. estimate or spp. presence, collect for PHC analysis of metabolites in bile..	small dead inverts observed; juvenile crabs found dead in traps set while adult crabs & fish were still alive, dead brown shrimp found in local catch; lumped with fish for final injury estimate to aquatic fauna, used model to determine biomass lost



Parameters sampled	Spill	Go-Kit Wetland Sampling Protocol	NRDA Surveys conducted/ Notes
benthic invertebrates (assuming subtidal)	Dixon Bay, LA	20 cores, 10cm diameter, to 5cm depth along 30m transect, for taxa richness and abundance data.	15 sample stations established <i>subtidal</i> (+ 2 for reference), 6 rep's from ea. station collected for abundance & community structure; samples processed in phases, 1st 13 stations w/ 4 rep's / station analyzed, found no impacts to benthic community so did not analyze rest of samples
bivalves	Chalk Point	15cm clam gun, 5 - 45cm depth, composite of 3 cores, 30g tissue sample for PAH analysis, 3+ rep.'s for chem. analysis, 6+ rep.'s for pop. density.	Collected clams & oysters using dredge, 30-60 bivalves per station, tissue collection for PHC analysis
crabs	Chalk Point	15cm clam gun, 5 - 45cm depth, composite of 3 cores, 30g tissue sample for PAH analysis, 3+ rep.'s for chem. analysis, 6+ rep.'s for pop. density.	Blue crabs collected in shallow water using standard crabbing techniques & 16 ft trawl. Tissue collection for PHC analysis. (probably for human contamination threat)
sediment	Suisun Marsh, CA	1" HDPE cores to 5cm depth, 2-core composite for chem. analysis, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	samples collected at different depths throughout marsh
sediment	McGrath, CA	1" HDPE cores to 5cm depth, 2-core composite for chem. analysis, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	"contaminated" (measured?), cleanup involved removal
sediment	Chalk Point	1" HDPE cores to 5cm depth, 2-core composite for PAH & fingerprint analyses, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	Collected samples from 23 of same quadrats as veg & inverts to assess concentration of petroleum hydrocarbons (PHC); replicates of 1-5 cores (not averaged) from ea quadrat sampled using pre-cleaned 4" diam core to 1' depth; sampled twice, 1 yr apart; also intertidal sed cores collected next to intertidal benthic cores for PHC analysis
sediment	Whatcom Ck, WA	1" HDPE cores to 5cm depth, 2-core composite for PAH & fingerprint analyses, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	Samples collected for PHC fingerprint analysis; sediment contamination surveys (>115 subsurface explorations) - large scale - b/w pipeline & creek, repeated monitoring
sediment	Lake Barre, LA	1" HDPE cores to 5cm depth, 2-core composite for chem. analysis, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	<i>subtidal</i> sediment chemistry analysis indicated no significant injury occurred; injury to marsh & intertidal sediments already included in marsh injury estimate, no separate field survey done/necessary
subtidal sediments	Tampa Bay, FL	1" HDPE cores to 5cm depth, 2-core composite for chem. analysis, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	including exposed mudflats - estimated area exposed to injury, & oil effects on biota based on literature; final injury = # of acres of <i>subtidal</i> sediment exposed to oiling enough to cause injury to subtidal benthic biota (assumes biota in contact w/ oil will die); they had a hard time defining the area of sediment exposed using visual observations, divers, and SONAR, but decided on 58,540 ft <sup>2</sup> based on CoastGuard data during initial response; amt of damage in monetary terms = exposure area X sediment restoration costs per ft <sup>2</sup> = \$5,268.60

Parameters sampled	Spill	Go-Kit Wetland Sampling Protocol	NRDA Surveys conducted/ Notes
sediment (subtidal)	Dixon Bay, LA	1" HDPE cores to 5cm depth, 2-core composite for PAH & fingerprint analyses, to 10cm depth, 3-core composite for soil characteristics; 10 rep.'s per site.	<i>subtidal</i> sediment samples collected for PHC concentrations, one at ea. of same 15 stations as benthic invertebrates; samples processed in phases, 1st 13 stations w/ 4 rep's / station analyzed; PHC levels in sediments not found to be at levels expected to impact benthic organisms; marsh sediments – did not collect or measure, little oil observed, none noted having seeped below surface
water	Suisun Marsh, CA	Not collecting water samples.	? (Damage assessment documents unavailable b/c recent & not released to public yet, J. Yamamoto, pers. comm.)
water	Chalk Point	Not collecting water samples.	samples collected to assess concentration of PHCs
water	Whatcom Ck, WA	Not collecting water samples.	samples collected for PHC fingerprint analysis; repeated sampling at permanent water stations (8 along Creek & 12 w/in Bay) for extent & level of PHC exposure & later gasoline presence & rate of degradation;
water	Lake Barre, LA	Not collecting water samples.	samples collected near pipeline break indicated PAH levels known to be toxic to aquatic organisms
water	Westchester, LA	Not collecting water samples.	samples collected indicated PAH levels known to be toxic to aquatic organisms
water column	Tampa Bay, FL	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	overflights to determine location & extent of floating oil; water column sampling for hydrocarbons - 23 samples from 20 sites; final injury = projected loss in fishery stocks caused by oil exposure using model = \$ value to determine scale of restoration, 3 sub-models calculate: physical fate of the oil, biological injury it causes, the value of that injury
water column	Dixon Bay, LA	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	overflight estimated 25 miles <sup>2</sup> covered w/ visible oil slicks on surface
birds	Humboldt/Kure, CA	Bird survey in mudflats and edge of vegetation	small spill, only light oiling; noted amount of oiling & use of habitat to estimate degree of oiling & injury; (per Steve Hampton)
birds	McGrath, CA	Bird survey in mudflats and edge of vegetation	mortality counts
birds	Chalk Point	Bird survey in mudflats and edge of vegetation	Used risk assessment where data collected - species, count, noted degree of oiling, & nest survey – used to estimate population, % of pop oiled, & data from literature used to estimate total mortality
birds	Lake Barre, LA	Bird survey in mudflats and edge of vegetation	Observations of bird mortality - 2 dead & a # & variety of birds oiled, then modeling to estimate mortality instead of extensive field survey
birds	Tampa Bay, FL	Bird survey in mudflats and edge of vegetation	Used record of captured rehabilitated birds as representing 50% of birds injured, total injured = rehab # X 2. No further studies to refine estimate-cost > information gained.

Parameters sampled	Spill	Go-Kit Wetland Sampling Protocol	NRDA Surveys conducted/ Notes
birds	Westchester, LA	Bird survey in mudflats and edge of vegetation	mortality estimate – survey conducted to detect dead & oiled birds, counted all observed (1,680 birds observed, 15 dead & 9 oiled), ultimate injury estimate determined through modeling = 582 birds; <b>abundance &amp; spp. composition data from the survey used as inputs into the model;</b> Additional extensive field survey results unlikely to outweigh time & expense required
birds/wildlife	Dixon Bay, LA	Bird survey in mudflats and edge of vegetation. Presence of other wildlife noted on General Log form.	shoreline survey of ~0.25 miles, found oiled & dead birds - documented in videos, photos & field notes, mammal tracks found w/ dead birds; no other sections of oiled shoreline suitable for surveys b/c of thick marsh veg & soft sediments (did not want to impact); considered use of models but no reliable cost-effective method was available; so did not come up w/ final injury estimate; <b>they think some of dead birds found may have died before the spill &amp; were oiled after</b>
wildlife	Suisun Marsh, CA	Presence of all wildlife noted on General Log form.	salt marsh harvest mouse noted
wildlife	McGrath, CA	Bird survey in mudflats and edge of vegetation	birds, amphibians, fish, invertebrates “injured” (measured?)
wildlife (riparian & terrestrial)	Whatcom Ck, WA	Presence of all wildlife noted on General Log form.	mortality assessmt. – field survey recording of dead & injured wildlife; "survey correction factor" used to take into acct likely dead wildlife but not found; no final estimate - too difficult
fish	Suisun Marsh, CA	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	? (Damage assessment documents unavailable b/c recent & not released to public yet, J. Yamamoto, pers. comm.)
fish	Chalk Point	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	Identified/counted/measured, injury noted, tissue collected for PHC analysis; and ichthyoplankton survey
fish	Whatcom Ck, WA	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	Samples of “biota” (assuming fish) collected for PHC fingerprint analysis; risk analysis conducted to assess risk to biota from contaminated sediments & water. Also lit. search for effects of oil toxicity on aquatic biota - invertebrates & fish - & lit search on fate/effects of similar spills; mortality assessmt. - 5 teams of 3-6 people collecting & recording dead & injured fish; "survey correction factor" used to take into acct likely dead fish but not found; Over 100,000 fish & invertebrates found dead, including 8,842 salmonids
aquatic fauna	Lake Barre, LA	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	injury quantification began w/ mortality estimate, small dead fish & inverts observed; field effort to quantify injuries to aquatic biota expensive & given difficulty detecting magnitude of injuries present used modeling taking into acct mortality & future loss of growth/biomass during recovery time, final injury as biomass (kg) lost

Parameters sampled	Spill	Go-Kit Wetland Sampling Protocol	NRDA Surveys conducted/ Notes
fish	Tampa Bay, FL	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	fish & plankton “information collected” & applied to models; plankton sampling for presence of larval fish & invertebrates in waters - for comparison w/ existing baseline data & models; also there was an ongoing study of fish & relationship to infauna in sand beaches in the area, so sites were surveyed after the spill to compare, used seine nets & small mesh nets for juveniles
aquatic fauna	Westchester, LA	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	including fish, blue crabs, shrimp, other invertebrates; injury began w/ mortality estimate (no reports of mortalities observed), final injury expressed in biomass (19,400 kg) lost, estimate determined through modeling
	Dixon Bay, LA	Fish population estimate or species presence and collection for analysis of PHC metabolites in bile, seining in tidal creeks.	*Trustees thought water column biota were injured (severe weather increasing dispersion), but could not use any models b/c dif. estimates of oil volume varied by 2 orders of magnitude, and no model accurately reflected topography/habitats; so no final estimate, but agreed upon wetlands creation project as compensation.
North Pass, LA	Marsh flora/fauna, water column, benthic organisms injured; surveys indicated minimal injuries - consisted of helicopter overflights, ground surveys, & on-water surveys, where no evidence (mortality of wildlife, birds, or fish) of injury was observed; Estimated: 1) area of injury (120 acres) through overflights, on-water surveys, photos, GPS, 2) initial service loss, 3) time to recovery – based on observations from past similar incidents (like Dixon Bay), Used Habitat Equivalency Analysis to quantify losses from oil impact as discounted service acre-years (56.2), given 3 parameters above. *Trustees & RPs chose not to refine injury estimate b/c cost of studies would be > potential information gained & assumed restoration would not change as result of more detailed assessment		
Westchester, LA	*Trustees & RPs did not refine injury estimates b/c costs to do so would increase overall costs too much compared to completing the restoration required based on preliminary injury estimates.		
Whatcom Ck, WA	Used model (SIMAP) to estimate physical fate & biological effects of oil, and thus potential for injury; Long-term assessmt. studies were considered, but b/c of cost & time delay for restoration, & no guarantee that further studies would result in change in restoration scale/plan, used time & money for restoration planning & implementation.		
Lake Barre, LA	*Trustees & Texaco independent estimates of bird & aquatic faunal injury differed, could not reach consensus, but didn't matter b/c Texaco offer for restoration was sufficient to compensate for the loss. no need for Trustees to incur additional expense once agreement for restoration was reached		

**Table 5.** Comparison of USFWS, RPI, NOAA, EPA and PERF methods for collection of sediment, bivalve tissue and macrofauna samples.

<b>Sediment - characteristics</b>				
	<b>USFWS &amp; RPI*</b>	<b>NOAA**</b>		<b>Go-Kit Methods</b>
Equipment	Ziploc bags	Ziploc bags		Ziploc bags, HDPE corer, wooden dowel
Replicates				Composite of 3 cores, 10 replicates per site
Depth		Top 10-15cm		10cm
Volume		50-200g		100 g
Methods	Refrigerate samples	Do not pour water out of top of core, could lose fine sediment / organic matter		Can pour water from top of core if substrate submerged – only negligible amount of sediment lost. Freeze samples for organic matter analysis.
<b>Sediment - chemical analysis</b>				
	<b>USFWS &amp; RPI</b>	<b>NOAA</b>	<b>EPA ***</b>	<b>Go-Kit Methods</b>
Equipment	Plastic tubes can be used, polycarbonate or polyethylene, 5cm (2in) diameter w/ 3mm wall thickness	All objects in contact w/ sample be made of glass, stainless steel, or PTFE (like Teflon)	All utensils in direct contact w/ sed should be made of inert materials (glass, Teflon, stainless steel or HDPE)	1” HDPE cores, Ziploc bags, polyethylene gloves
		Clean metal utensils <b>or wooden tongue depressors</b>	Avoid direct contact b/w sed samples & PVC, natural or neoprene rubber, nylon, talcum powder, polystyrene, galvanized metal, brass, copper, lead, other metal materials, soda glass, paper tissues, & painted surfaces	Wooden dowel
	Solvent-rinsed glass containers w/ Teflon or aluminum-lined lids	Pre-cleaned glass jars w/ teflon or aluminum-lined caps <b>or aluminum foil</b>	Borosilicate glass, (or HDPE, polycarbonate or fluorocarbon plastic containers) used to minimize leaching & sorption, with PTFE-lined cap (polytetrafluoroethylene)	Solvent-rinsed aluminum foil
	Use pre-cleaned disposable utensils (stainless steel blade, single-use core tubes, etc.). Only re-use shovel, rinsing b/w sites	New core tubes & sampling utensils used for ea sample	For samples contaminated w/ photoreactive compounds (PAHs), minimize exposure to light by using brown glass or clear glass wrapped in opaque clean aluminum foil	New core tubes & sampling utensils used for each sample
Replicates	Composite sample of 3+ subsamples, 10-15 samples / site	10-15 samples / site		Composite of 2 cores, 10 replicates per site
Depth	Usually top 2cm, unless want concentration by depth, then divide core into separate depths	2cm or 5cm	Top 2cm for recent deposition	5cm

Volume	500mL / 1pint / 16oz. for PAH by GC/MS-SIM method	100g	250mL	50+ grams, ~220mL
Methods		Do not pour water out of core	Do not dump off water from top of core	Can pour water from top of core if substrate submerged – only negligible amount of sediment lost.
	Record sample # on label & lid			Record sample # on label and place with sample bag into 2 <sup>nd</sup> Ziploc bag
		Composite subsamples	Can mix by rolling sediment on pre-cleaned aluminum foil. Homogenize subsamples before placing in containers (or can homogenize in lab)	Homogenize cores by mixing/rolling on solvent-rinsed aluminum foil
	Place all sed samples in cooler immediately. Keep @ 4°C, then freeze	Keep on ice at 4°C in dark	Put samples in cooler at 4°C, then freeze	Place samples in a cooler in the field, then freeze.
	Sed samples can be held frozen in the dark for several years w/o loss of integrity		For frozen samples, leave at least 10% of volume of container empty for expansion	
	Detection level should be 1 ppb for PAHs to support injury assessment		Carry extra containers on sampling trip	

<b>Bivalve Tissue</b>				
	<b>USFWS &amp; RPI</b>	<b>NOAA</b>	<b>PERF<sup>+</sup>*</b>	<b>Go-Kit Methods</b>
Equipment	Screen for sieving sediments		Ziploc bags	stainless steel shovel, PE sieve, Ziploc bags
	Gloves - change after each sample	All instruments used in handling samples must be non-contaminating material (such as glass, stainless steel, teflon, aluminum)	Aluminum foil; Wear protective gloves while rinsing specimens	Polyethylene gloves – change after each sample; Solvent-rinsed aluminum foil
	If oil is present, Clean sampling equipment b/w collections			Cannot solvent rinse equipment in field due to time constraints
Replicates	3 samples/site minimum; at least 6 stations sampled along exposure gradient		Typically minimum of 3 sites to make estimate of non-oil-related variability	Composite 3 cores. At least 3 replicates for chemical analysis, at least 6 for population density.
Depth				As deep as possible between 5 and 45cm
Volume	Detection level should be 1 ppb for PAHs to support injury assessment	10-15g / sample	Typically 30-50g wet weight	10g wet weight for chemical analysis, ~10 individuals
Methods	Collect live animals - shells intact & tightly closed. Don't open shells in field - risk of contamination		For bivalves, whole body samples are collected	Collect whole bivalves

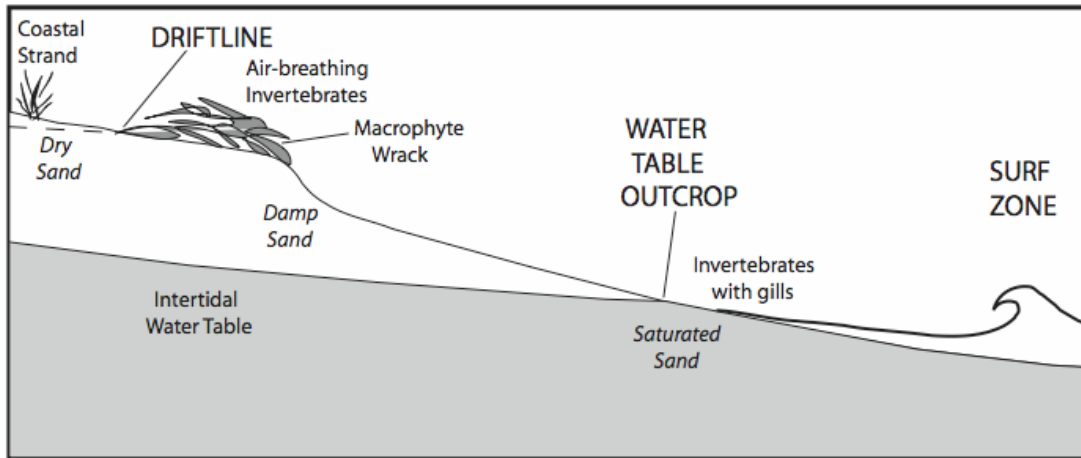
Methods	Individuals should be same approximate shell size if possible to reduce variability & Record shell size range. Use adults (>50mm) if possible b/c more tissue for analysis		All organisms should be of near-uniform size to reduce variability (if possible)	Collect individuals randomly, not size-biased
			Rinse ea organisms w/ site water or distilled water to remove loose sediment or oil	Rinse ea organisms w/ site water or distilled water to remove loose sediment or oil
	Wrap sets of whole organisms together in pre-cleaned aluminum foil	Organisms may be wrapped in solvent-rinsed aluminum foil, placing against the dull side	Wrap animals in solvent-rinsed aluminum foil & bag in labeled Ziploc plastic bags	Wrap bivalves in solvent-rinsed aluminum foil (facing dull side), place w/ label into double Ziploc bags
	Put all individuals of same sp from a site in a glass jar or double Ziploc bags			Choose individuals of the same species for each sample
	Put all samples in cooler immediately; keep at 4°C; Freeze asap	Keep at 4°C	Freeze in field	Place samples in a cooler in the field, then freeze.
	Once frozen, can be held for years w/o loss of integrity	Tissue sections may be taken in the laboratory from carefully preserved samples	Samples are transported to the analytical lab frozen	
	Also collect air & H2O temp, DO, salinity, & exposure level to tidal flushing		Store samples at -20°C in dark	
	Consider collecting sediment for analysis too			

<b>Macroinfauna</b>			
	<b>USFWS &amp; RPI</b>		<b>Go-Kit Methods</b>
Equipment	0.01m <sup>2</sup> cylindrical hand corer w/ small hole in top		10cm diameter corer w/ small hole in top
	Sample bags or jars		Ziploc bags
	0.5mm sieve		0.5mm sieve (in lab)
	10% buffered formalin		10% buffered formalin
Replicates	3-5+ / tidal elevation (for intertidal) / segment		20 replicates
Depth	Top 15cm (or less), mark depths on outside of corer		5cm, depth marked on outside of corer
Methods	After inserting into sediment, place finger over hole in top to create vacuum and pull up		After inserting into sediment, place finger over hole in top to create vacuum and pull up
	If core can't be extracted whole, slide metal plate or other object underneath core and then remove		If core can't be extracted whole, slide gloved or bagged hand underneath core and then remove

Methods	Empty into labeled container		Empty core into Ziploc bag, Place label and 1 <sup>st</sup> sample bag into 2 <sup>nd</sup> Ziploc bag
	Preserve w/ 10% buffered formalin		Preserve w/ 10% buffered formalin
	Place label inside sample container		Transfer from Ziploc bags to 1L plastic jars at vehicle. Attach label to outside of jar w/ clear packaging tape. Label lid w/ sample ID #
	Ship samples preserved in formalin as dangerous goods		
* USFWS 2006; RPI 2002			
** NOAA 1992, NOAA 1996			
***EPA 2001			
+* Robertson 1999			



## Figures

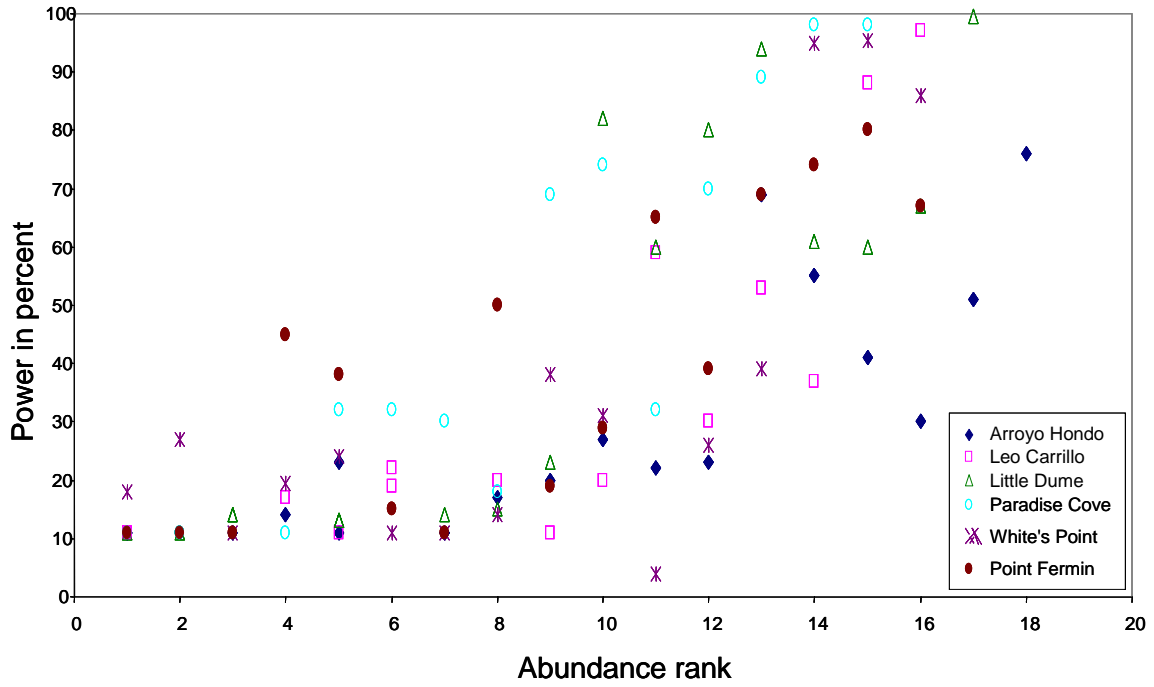


**Figure 1.** Profile of an exposed sandy beach showing the intertidal and supralittoral zones.

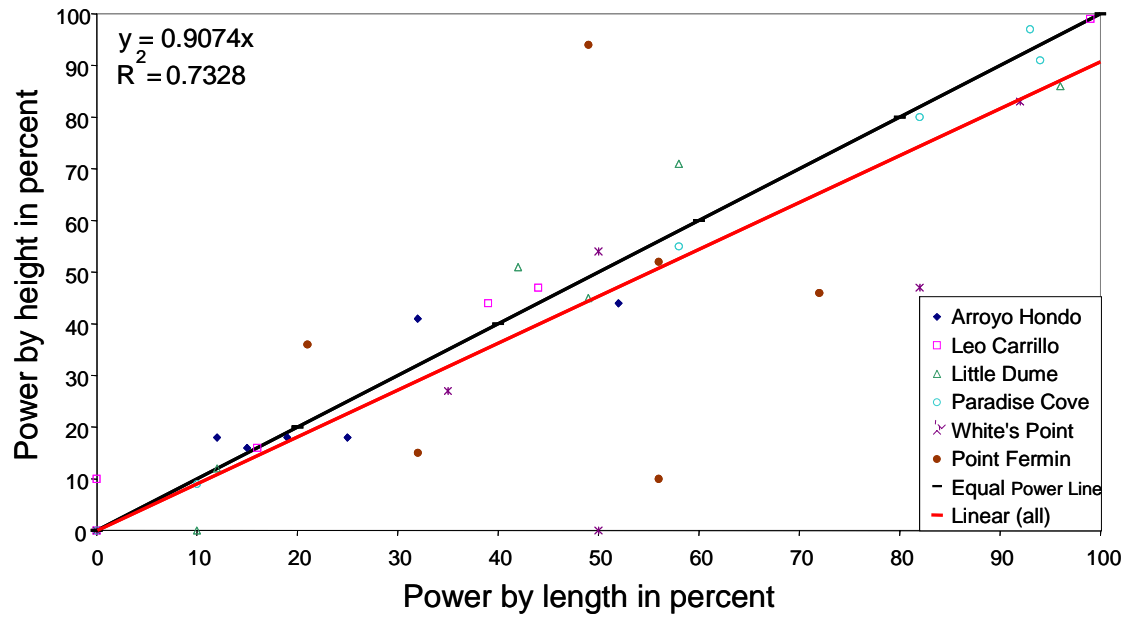
The relative locations of major invertebrate types, accumulations of macrophyte wrack and ephemeral coastal strand vegetation are indicated. Air-breathing invertebrates can include talitrid amphipods, oniscoidean isopods, insects and arachnids. Invertebrates of the damp and saturated sand zones can include hippid crabs, isopods, amphipods, bivalves, gastropods, and polychaetes. (From Dugan and Hubbard 2006)



**Figure 2.** Location of the six rocky intertidal study sites within Los Angeles and Santa Barbara Counties.

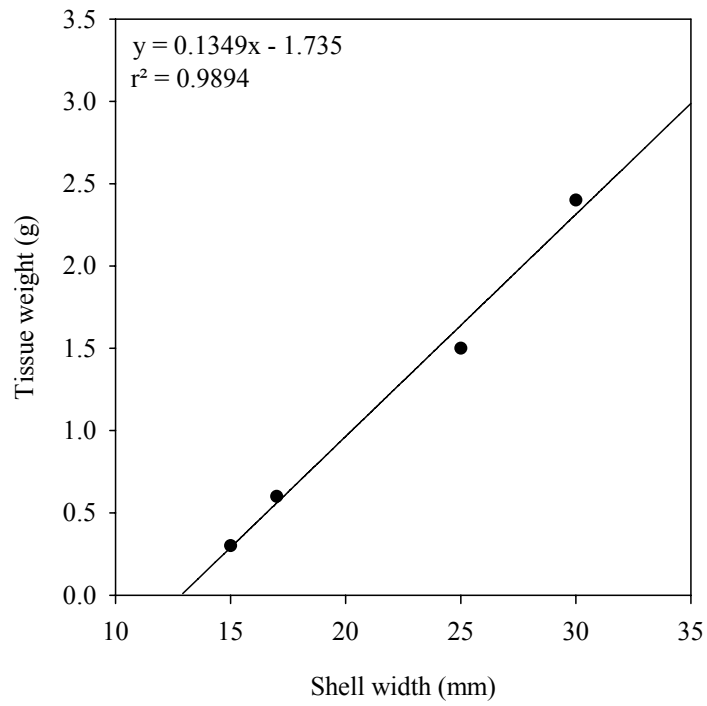


**Figure 3.** Plot of taxa abundance ranks versus the associated power to detect a fifty percent change in their population.



**Figure 4.** Plot of power values of length versus height stratification for the rocky intertidal representative taxa, 36 taxa for six sites.

The black line indicates equal power by both methods; the red line is the actual regression line.



**Figure 5.** Regression analysis of bivalve shell width to tissue wet weight..

## **APPENDICES**

## **Appendix 1. Sample Sizes**

Analyses were performed on data collected in 2005 in Point Mugu salt marshes in order to determine the number of replicates to collect for each sample parameter. Data that were analyzed include snail abundance, invertebrate density, invertebrate taxa richness, vegetation percent cover, vegetation species richness, crab abundance and sediment characteristics. Snail abundance data were also collected during field tests at Point Mugu in February and March 2007.

Four different analyses were used on the data.

The data are presented below. For all SE/Mean graphs, the raw data were first randomly ordered using a random numbers generator. Only this one random permutation was considered, not every permutation. The same applies to the species richness data for the species accumulation curves. Numbers presented within graphs are the rounded numbers of replicates inferred from each method.

### ***Method 1***

The first analysis is conducted by plotting the sample size versus the cumulative standard error divided by the cumulative mean (Kingsford & Battershill 1998). The first analysis was used for snail abundance, vegetation cover, crab abundance, and sediment data. The cumulative SE/Mean was calculated for those data and was plotted against the number of quadrats, number of traps, and number of samples, respectively. The appropriate sample size is chosen based on where the curve generally levels off and where there is little improvement with the addition of more replicates.

For snails, there is a fairly large decline at 10 quadrats, another drop at 17 quadrats, but then an increase back up at 25 quadrats. The curve gradually lowers in increments of 5 quadrats and does not level off until around 47 quadrats are included. The level of precision at 50 quadrats is just below 0.4, so the number of replicates determined from this method is judged at over 50 quadrats.

Vegetation data differ depending on the site. In some cases the curve levels off at 15 or 25 quadrats, in the other cases not until 35 or greater than 50.

The crab data curve fluctuates and does not level off within 18 traps, so the determined number of replicates for crab traps using this method is greater than 18.

All sediment data curves using this analysis leveled off within 10 samples, some at 4 or 5 samples and most at well below a level of precision of 0.10. Given that many of the curves leveled off around 9 samples, the sample size for sediment was chosen as an even 10 samples.

### ***Method 2***

The second method involves using a formula to input a desired level of precision ( $p$ ),  $n = [SD / p \cdot \text{mean}]^2$ , where SD is the standard deviation (Andrew & Mapstone 1987). The second analysis performed on the snail, benthic invertebrate, vegetation, and crab data was the equation inputting the desired level of precision. Graphing many sets of snail abundance data showed varying numbers of replicates at a  $p$  value of 0.10, a reasonable level of precision. Forty-five was the number of quadrats chosen from this

method. For the benthic invertebrate density, at a precision of 0.10 twenty-five samples were determined from this analysis. Although 150 samples correspond to a more precise level of 0.05, 150 samples is not a feasible number of replicates to collect in the field. Vegetation percent cover data from four different sites were analyzed with this method. Fifty-five quadrats were chosen from this analysis as a conservative estimate. Using the  $p$  equation method on the crab abundance data resulted in around 50 or 75 traps at a 0.10 precision level. Again, 200 or 300 samples at a level of precision of 0.05 is completely infeasible.

### ***Method 3***

The third method uses a formula inputting an allowable error in terms of confidence limits,  $n = 4s^2 / L^2$ , where  $s^2$  is the sample variance and  $L^2$  is the size of the 95% confidence limits (Snedecor and Cochran 1980 in Kingsford & Battershill 1998). This method was also applied to the snail, benthic invertebrate, vegetation, and crab data. The allowable error that was input into the equation was dependent on the mean value of the data in terms of a reasonable standard error. For snail abundance data, the sample size of 50 was chosen because the number of quadrats inferred for two of the sites was 50 and above and below 50 for the other two sites. Similarly, 75 quadrats were chosen as the sample size for vegetation from the four sites analyzed. For the benthic invertebrate data, the number of replicates selected based on the size of the 95% confidence limits for both of the sites analyzed was 20.

### ***Method 4***

In the fourth method, a species accumulation curve is plotted; the number of replicates is plotted on the x-axis and the cumulative number of different species is plotted on the y-axis. The species accumulation curve was used for the benthic invertebrate and vegetation data, the two data sets that had species or taxa richness data to analyze. For each replicate the numbers of unique taxa were determined and the cumulative sum of unique taxa were plotted against the number of replicates. The number of replicates is determined based on where the curve levels off, where adding more replicates does not result in adding unique species to the data set. For benthic invertebrate richness, the appropriate sample size was greater than six. The conservative estimate of the number of vegetation quadrats was 35 based on the two species accumulation curves.

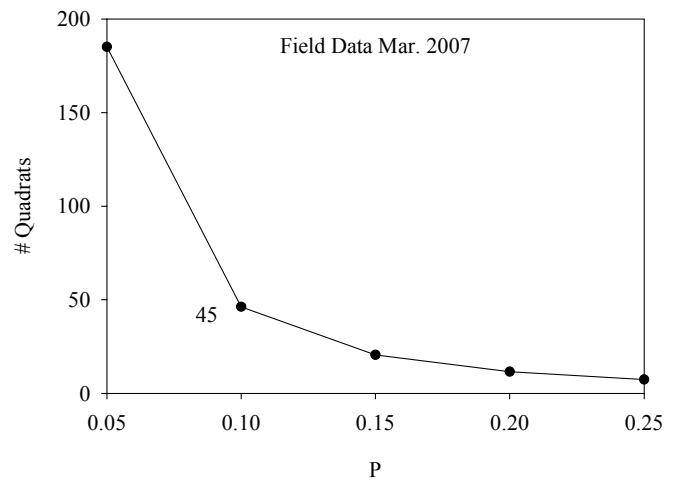
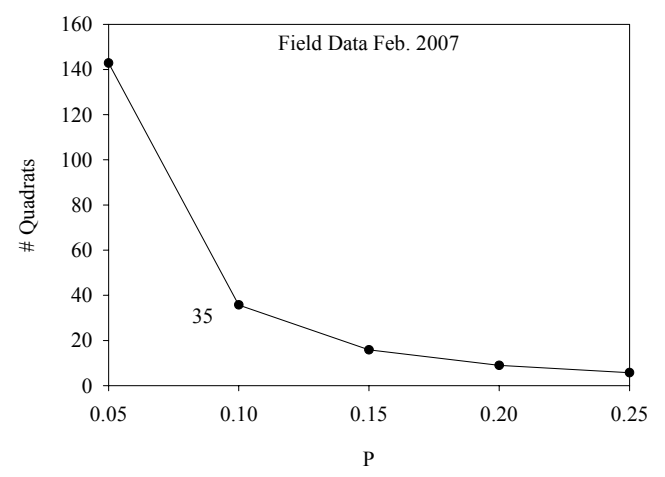
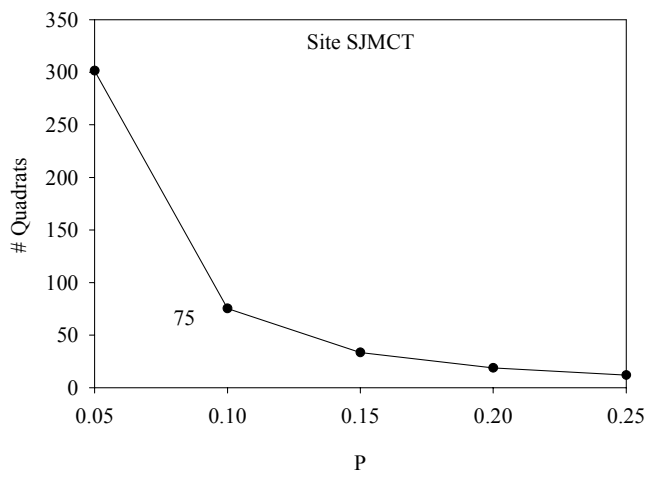
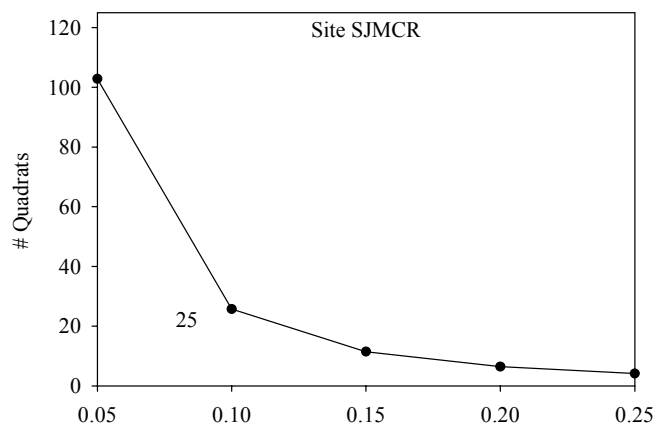
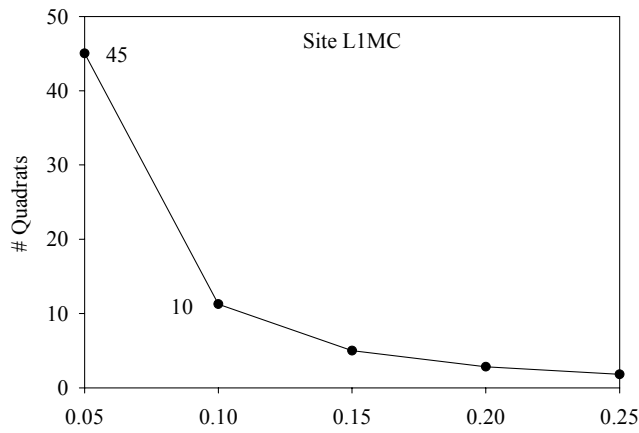
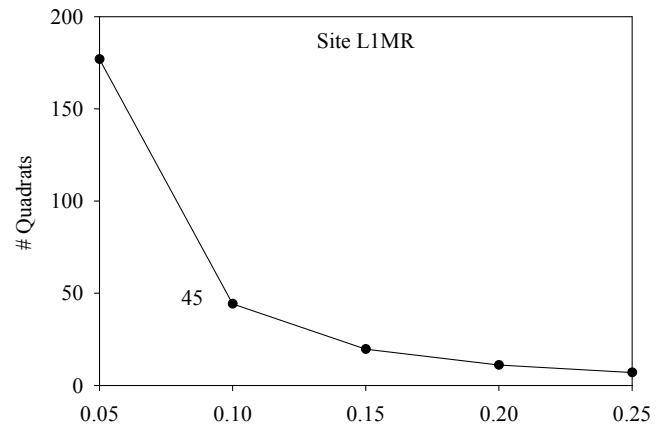
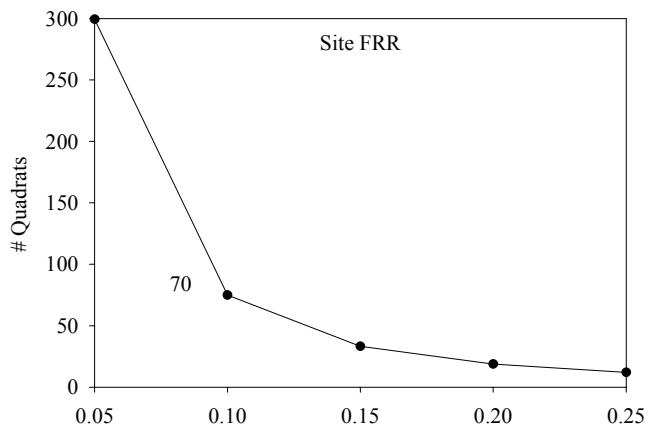
### ***Summary***

Considering the results from all four of these methods, the resultant sample sizes (number of replicates) were determined as follows: snails = 50, invertebrates = 20, vegetation cover = 50, crabs = 50, sediment = 10.

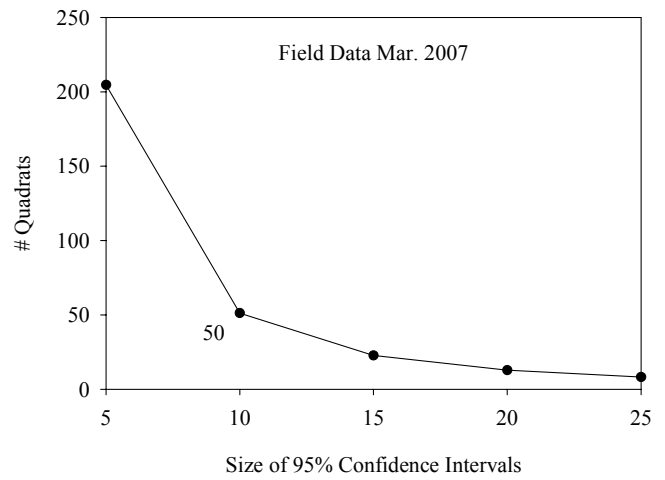
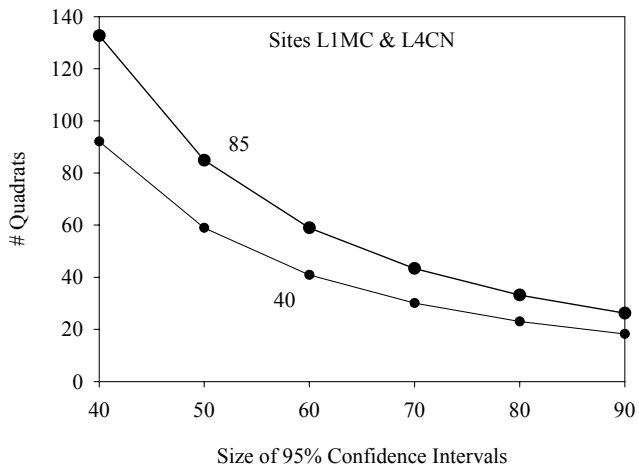
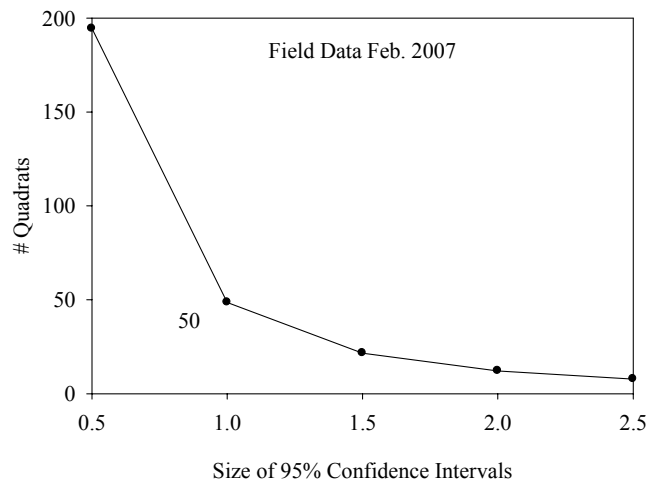
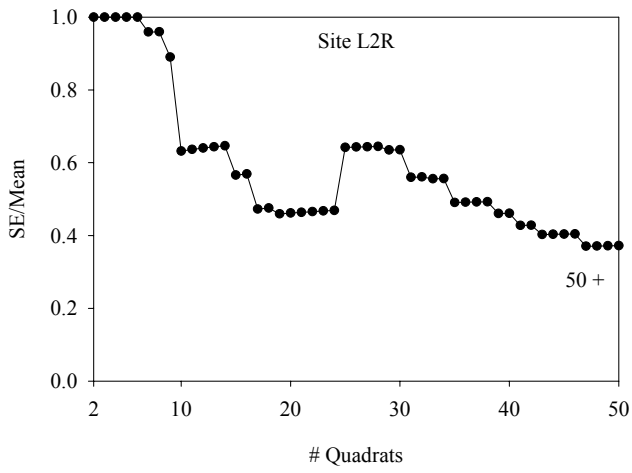


Snail (*Cerithidea californica*) abundance data.

Quadrat #	Site FRR	L2R	Site L1MR	Site L1MC	Site L4CN	Site SJMCR	Site SJMCT	2/07 Field Test	3/07 Field Test
1	196	108	1212	732	76	396	36	1	35
2	108	0	548	416	0	292	152	4	49
3	360	0	2640	776	12	128	32	9	105
4	284	0	584	276	4	180	16	12	37
5	360	0	804	592	116	208	32	3	34
6	280	0	1236	640	64	100	92	8	39
7	812	4			388			10	59
8	264	0			0			6	66
9	24	8			160			6	25
10	84	72			312			12	30
11	656	0			72			3	20
12	440	0			868			7	55
13	204	0			136			4	19
14	176	0			84			2	32
15	32	28			288			8	107
16	48	0			0			6	162
17	148	92			76			1	101
18	24	0			76			3	20
19	24	200			944				51
20	76	0			12				9
21	184	0			204				38
22	88	0			268				68
23	348	0			340				38
24	552	0			144				23
25	172	888			20				73
26	216	0			136				75
27	232	0			96				7
28	52	0			472				95
29	16	20			832				
30	208	0			152				
31	288	220			208				
32	368	0			112				
33	288	16			240				
34	356	0			12				
35	308	276			92				
36	340	0			24				
37	264	0			8				
38	84	0			216				
39	344	144			0				
40	168	0			8				
41	112	180			36				
42	184	0			48				
43	0	844			168				
44	12	0			48				
45	4	0			0				
46	8	0			592				
47	0	396							
48		0							
49		0							
50		0							
Mean	208	69.9	1171	572	177	217	60	5.8	52.6
SD	177	26	779	192	230	110	52	3.5	35.8



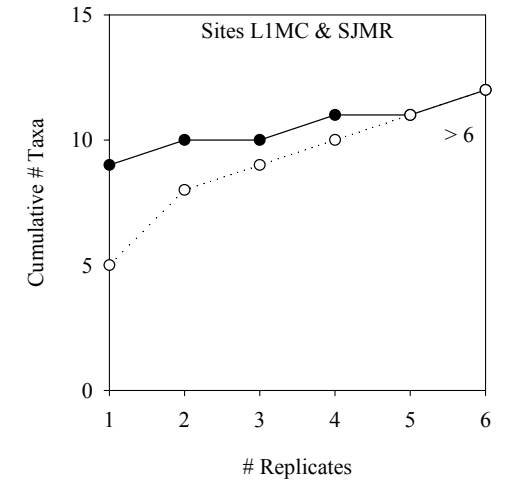
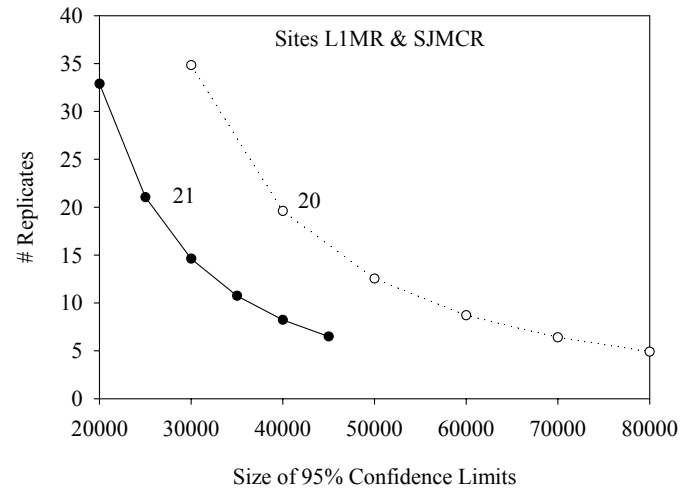
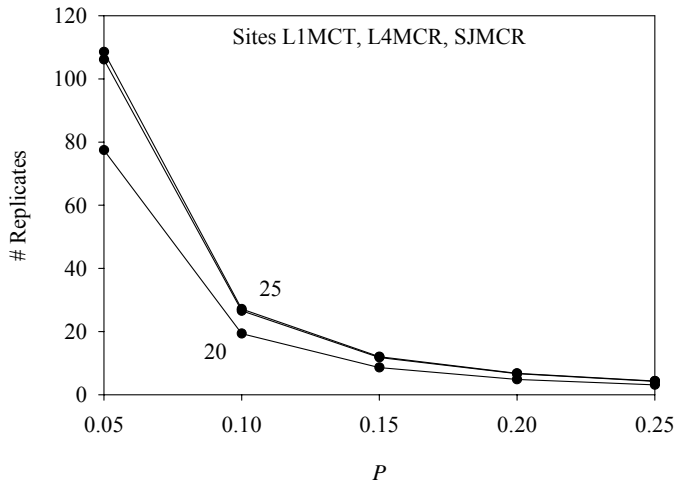
*Cerithidea* abundance. Method 2,  $p$  equation.



*Cerithidea* abundance. Methods 1 and 3, SE/Mean and confidence intervals equation.

Benthic invertebrate density and richness data.

Site:	L1MCT	L1MR	L4MCR	SJMCR	L1MC	L1MC # taxa unique from previous replicates	L1MC Cumulative Sum	SJMR	SJMR	SJMR
Replicate #	Density (/m <sup>3</sup> )	Density (/m <sup>3</sup> )	Density (/m <sup>3</sup> )	Density (/m <sup>3</sup> )	Total # taxa			Total # taxa	# unique taxa	Cumulative Sum
1	758850.93	20371.84	30557.76	183346.53	9	9	9	5	5	5
2	483831.13	30557.76	28011.28	109498.62	6	1	10	5	3	8
3	2500642.99	140056.38	28011.28	313216.99	5	0	10	5	1	9
4	1474411.70	84033.83	22918.32	84033.83	7	1	11	6	1	10
5	2205251.35	137509.90	63661.99	231729.65	6	0	11	5	1	11
6	2156868.24	147695.82	53476.07	109498.62	7	1	12	5	1	12
Mean	1596642.72	93370.92	37772.78	171887.37						
SD	831463.62	57341.99	16614.03	88531.84						
SE	339443.60	23409.77	6782.65	36142.97						



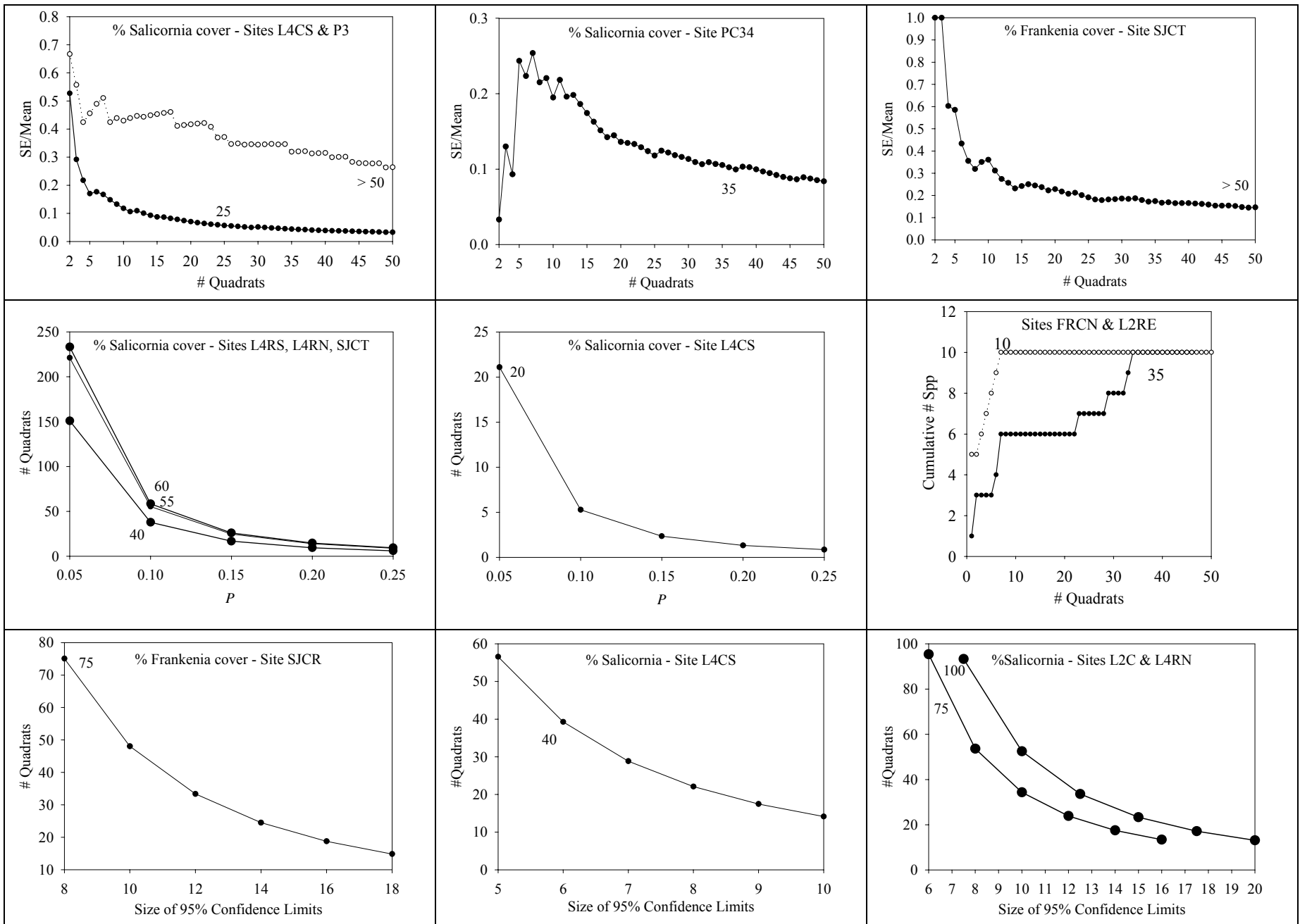
Benthic invertebrate density and richness. Methods 2, 3 and 4,  $p$  and confidence interval equations and species accumulation curve.

Vegetation percent cover data.

Site: Quadrat #	L2C Salicornia cover (%)	L4CS Salicornia cover (%)	L4RN Salicornia cover (%)	L4RS Salicornia cover (%)	P3 Salicornia cover (%)	PC34 Salicornia cover (%)	SJCT Salicornia cover (%)	SJCT Frankenia cover (%)	SJCR Frankenia cover (%)
1	12.24	85.71	2.04	0.00	10.20	95.92	100.00	0.00	30.61
2	100.00	26.53	10.20	0.00	2.04	89.80	16.33	46.94	0.00
3	0.00	69.39	0.00	67.35	26.53	61.22	0.00	0.00	26.53
4	0.00	93.88	67.35	0.00	38.78	79.59	0.00	30.61	46.94
5	75.51	91.84	71.43	0.00	2.04	6.12	8.16	4.08	6.12
6	34.69	36.73	0.00	36.73	0.00	40.82	48.98	44.90	0.00
7	22.45	42.86	81.63	46.94	0.00	10.20	73.47	26.53	36.73
8	93.88	95.92	0.00	89.80	46.94	69.39	28.57	16.33	44.90
9	95.92	95.92	14.29	93.88	0.00	18.37	0.00	97.96	6.12
10	85.71	81.63	32.65	0.00	4.08	85.71	42.86	2.04	0.00
11	10.20	77.55	44.90	61.22	0.00	0.00	16.33	69.39	16.33
12	36.73	36.73	61.22	40.82	0.00	65.31	38.78	57.14	0.00
13	24.49	71.43	44.90	73.47	2.04	16.33	0.00	100.00	61.22
14	95.92	85.71	12.24	18.37	0.00	40.82	40.82	59.18	0.00
15	14.29	91.84	53.06	93.88	0.00	97.96	100.00	0.00	10.20
16	0.00	46.94	71.43	69.39	0.00	53.06	91.84	0.00	16.33
17	0.00	97.96	55.10	55.10	0.00	69.39	46.94	16.33	0.00
18	22.45	97.96	71.43	73.47	55.10	55.10	59.18	20.41	65.31
19	18.37	89.80	65.31	65.31	0.00	16.33	8.16	38.78	67.35
20	97.96	83.67	61.22	44.90	0.00	73.47	83.67	0.00	67.35
21	63.27	93.88	2.04	85.71	0.00	26.53	61.22	30.61	75.51
22	93.88	89.80	44.90	79.59	0.00	26.53	40.82	30.61	22.45
23	40.82	91.84	87.76	0.00	6.12	100.00	91.84	0.00	95.92
24	87.76	97.96	69.39	71.43	24.49	93.88	53.06	40.82	0.00
25	95.92	97.96	8.16	95.92	0.00	75.51	61.22	36.73	2.04
26	36.73	100.00	2.04	91.84	63.27	0.00	55.10	44.90	57.14
27	22.45	69.39	40.82	14.29	0.00	34.69	55.10	16.33	100.00
28	0.00	87.76	22.45	83.67	4.08	42.86	89.80	2.04	14.29
29	100.00	75.51	0.00	26.53	0.00	34.69	55.10	4.08	4.08
30	4.08	42.86	55.10	0.00	2.04	38.78	0.00	0.00	100.00
31	83.67	85.71	65.31	75.51	0.00	81.63	16.33	12.24	8.16
32	2.04	95.92	67.35	44.90	0.00	42.86	81.63	0.00	100.00
33	87.76	85.71	53.06	89.80	2.04	8.16	30.61	69.39	26.53
34	75.51	91.84	53.06	69.39	0.00	38.78	55.10	40.82	0.00
35	8.16	87.76	79.59	79.59	38.78	30.61	97.96	0.00	0.00
36	34.69	93.88	79.59	24.49	0.00	48.98	20.41	61.22	87.76
37	55.10	89.80	57.14	38.78	0.00	83.67	0.00	0.00	0.00
38	100.00	93.88	12.24	0.00	8.16	0.00	28.57	26.53	83.67
39	65.31	75.51	87.76	0.00	0.00	24.49	24.49	6.12	100.00
40	59.18	73.47	12.24	87.76	0.00	81.63	20.41	6.12	44.90
41	63.27	95.92	53.06	32.65	65.31	67.35	20.41	16.33	32.65
42	0.00	59.18	51.02	81.63	0.00	44.90	2.04	97.96	24.49
43	75.51	100.00	4.08	73.47	0.00	65.31	28.57	22.45	100.00
44	40.82	95.92	18.37	71.43	36.73	67.35	22.45	75.51	30.61
45	95.92	83.67	34.69	67.35	6.12	46.94	85.71	6.12	8.16
46	8.16	97.96	2.04	59.18	2.04	97.96	91.84	6.12	46.94
47	34.69	91.84	24.49	48.98	2.04	0.00	18.37	18.37	8.16
48	48.98	91.84	0.00	61.22	0.00	48.98	0.00	65.31	51.02
49	87.76	91.84	40.82	46.94	51.02	55.10	32.65	24.49	48.98
50	93.88	65.31	0.00	93.88	0.00	46.94	97.96	0.00	79.59
Mean	50.12	81.88	38.98	52.53	10.00	50.00	42.86	27.84	37.10

Vegetation species richness data.

Site	FRCN	FRCN	FRCN	L2RE	L2RE	L2RE
Quadrat #	# Spp	# Spp unique from previous quads	Cumulative Sum	# Spp	# Spp unique from previous quads	Cumulative Sum
1	5	5	5	1	1	1
2	3	0	5	3	2	3
3	3	1	6	2	0	3
4	4	1	7	1	0	3
5	5	1	8	3	0	3
6	5	1	9	2	1	4
7	6	1	10	3	2	6
8	6	0	10	2	0	6
9	6	0	10	1	0	6
10	6	0	10	1	0	6
11	5	0	10	1	0	6
12	1	0	10	1	0	6
13	6	0	10	2	0	6
14	2	0	10	1	0	6
15	6	0	10	1	0	6
16	4	0	10	2	0	6
17	4	0	10	2	0	6
18	2	0	10	1	0	6
19	5	0	10	1	0	6
20	2	0	10	1	0	6
21	4	0	10	1	0	6
22	2	0	10	1	0	6
23	4	0	10	3	1	7
24	4	0	10	1	0	7
25	2	0	10	1	0	7
26	4	0	10	3	0	7
27	5	0	10	2	0	7
28	1	0	10	1	0	7
29	2	0	10	2	1	8
30	3	0	10	1	0	8
31	5	0	10	1	0	8
32	1	0	10	1	0	8
33	5	0	10	2	1	9
34	5	0	10	3	1	10
35	1	0	10	1	0	10
36	3	0	10	2	0	10
37	2	0	10	1	0	10
38	6	0	10	1	0	10
39	6	0	10	3	0	10
40	7	0	10	1	0	10
41	4	0	10	2	0	10
42	7	0	10	1	0	10
43	5	0	10	1	0	10
44	7	0	10	2	0	10
45	2	0	10	1	0	10
46	1	0	10	1	0	10
47	4	0	10			
48	2	0	10			
49	2	0	10			
50	2	0	10			

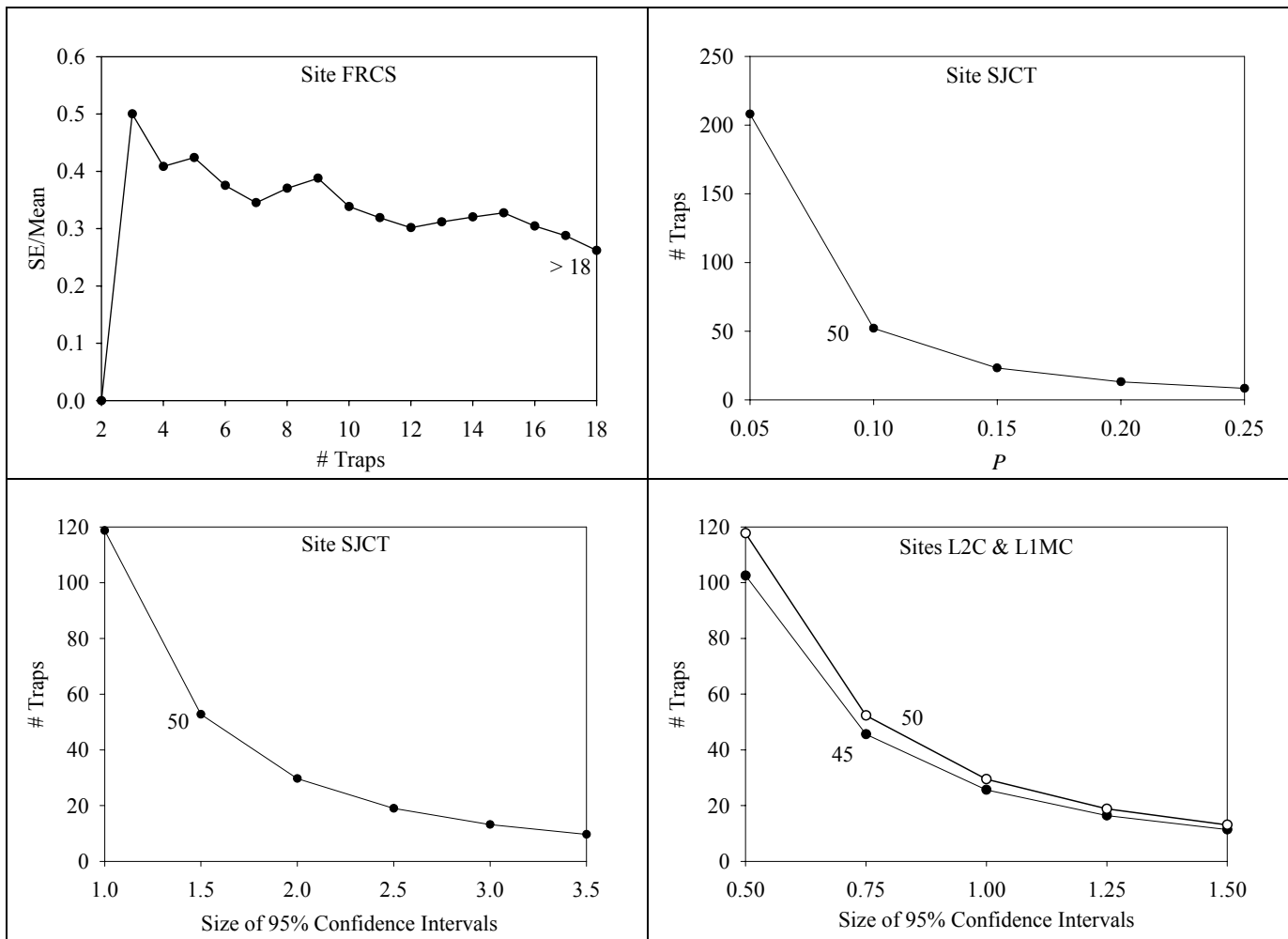


Vegetation % cover and species richness. Methods 1, 2, 3 and 4: SE/Mean,  $p$  and confidence interval equations and species accumulation curve.

Crab (*Pachygrapsus crassipes*) abundance data.

Site	FRCS	LIMC	L2C	L4CN	SJCT	FRCS	L4CN	SJCT	LIMC	L2C	SJCT			
Replicate #	Crab Abundance					SE / Mean	p	# Replicates	# Replicates	Confidence Intervals	# Replicates	# Replicates	Confidence Intervals	# Replicates
1	1	3	1	5	3		0.05	297.79	207.92	0.25	470.79	410.14	1	118.69
2	1	5	0	0	2	0	0.1	74.45	51.98	0.5	117.70	102.54	1.5	52.75
3	0	8	0	5	5	0.5	0.15	33.09	23.10	0.75	52.31	45.57	2	29.67
4	2	8	3	1	3	0.41	0.2	18.61	12.99	1	29.42	25.63	2.5	18.99
5	4	1	3	2	4	0.42	0.25	11.91	8.32	1.25	18.83	16.41	3	13.19
6	1	2	0	0	5	0.38				1.5	13.08	11.39	3.5	9.69
7	5	5	3	1	2	0.35								
8	0	1	2	0	6	0.37								
9	0	4	3	8	5	0.39								
10	2	8	9	5	14	0.34								
11	1	2	7	5	10	0.32								
12	1	2	6	1	12	0.30								
13	0		2	1	11	0.31								
14	0		1	2	0	0.32								
15	0		2	4	12	0.33								
16	5		6	3	20	0.30								
17	6		3	4	7	0.29								
18	4		2	1	15	0.26								
Mean	1.83	4.08	2.95	2.67	7.56									
SD	2.04	2.71	2.53	2.30	5.45									
SE	0.48	0.78	0.60	0.54	1.28									





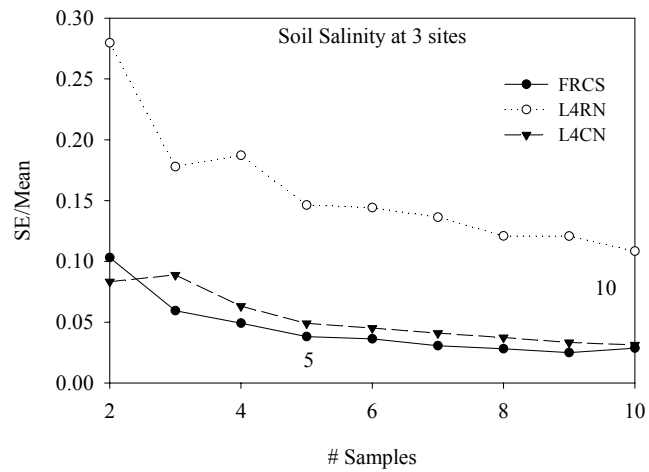
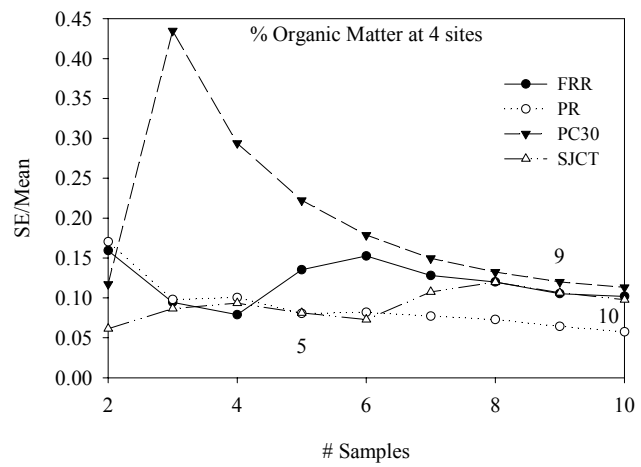
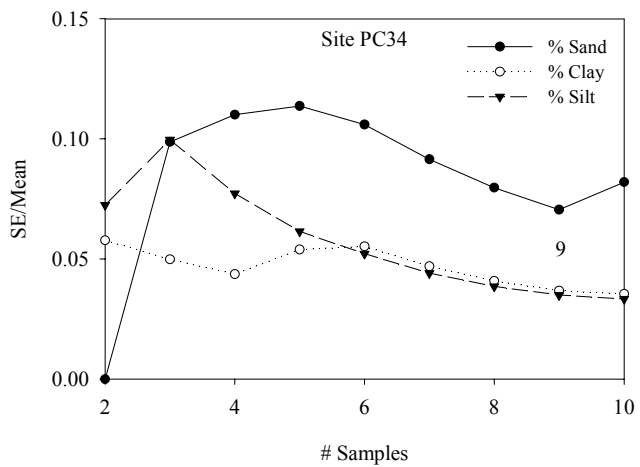
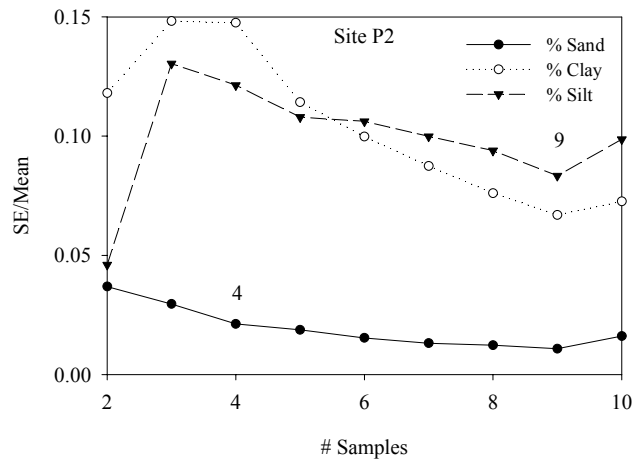
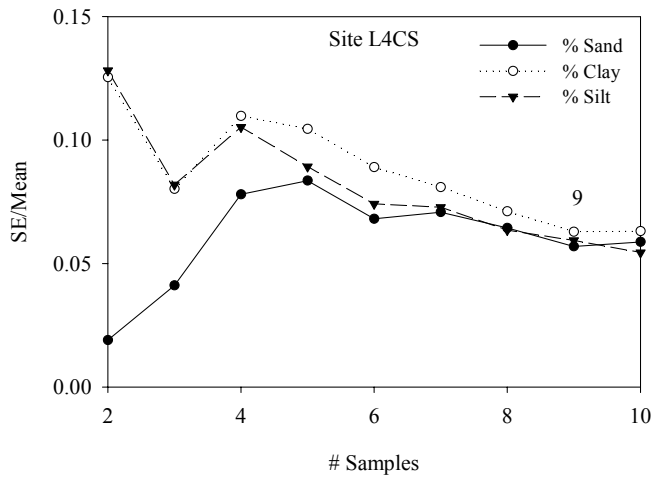
Crab (*Pachygrapsus crassipes*) abundance. Methods 1, 2 and 3: SE/Mean,  $p$  and confidence interval equations.

Sediment grain size data.

Site	Replicate #	%Sand	SE/Mean	%Clay	SE/Mean	%Silt	SE/Mean
L4CS	1	51.6		31.4		17	
L4CS	2	53.6	0.019	24.4	0.125	22	0.128
L4CS	3	46.6	0.041	31.4	0.080	22	0.082
L4CS	4	66.6	0.078	19.4	0.110	14	0.105
L4CS	5	40.6	0.084	36.4	0.105	23	0.089
L4CS	6	52.6	0.068	25.4	0.089	22	0.074
L4CS	7	38.6	0.071	35.4	0.081	26	0.073
L4CS	8	43.6	0.064	33.4	0.071	23	0.064
L4CS	9	52.6	0.057	29.4	0.063	18	0.059
L4CS	10	36.6	0.059	39.4	0.063	24	0.054
P2	1	49.13		22.4		28.47	
P2	2	45.63	0.037	28.4	0.118	25.97	0.046
P2	3	44.58	0.030	37.4	0.148	18.02	0.130
P2	4	47.19	0.021	19.4	0.148	33.41	0.121
P2	5	48.82	0.019	30.4	0.114	20.78	0.108
P2	6	46.75	0.015	35.4	0.100	17.85	0.106
P2	7	46.43	0.013	35.4	0.087	18.17	0.100
P2	8	48.73	0.012	33.4	0.076	17.87	0.094
P2	9	47.02	0.011	31.4	0.067	21.58	0.083
P2	10	41.20	0.016	19.4	0.073	39.40	0.099
PC34	1	22.25		40.75		37	
PC34	2	22.25	0.000	45.75	0.058	32	0.072
PC34	3	16.25	0.099	38.75	0.050	45	0.100
PC34	4	28.25	0.110	37.75	0.044	34	0.077
PC34	5	32.25	0.114	32.75	0.054	35	0.061
PC34	6	18.25	0.106	47.75	0.055	34	0.052
PC34	7	21.25	0.091	42.75	0.047	36	0.044
PC34	8	22.25	0.080	42.75	0.041	35	0.039
PC34	9	22.25	0.071	38.75	0.037	39	0.035
PC34	10	12.25	0.082	46.75	0.035	41	0.033

Sediment organic matter content and salinity data.

Site	Replicate #	% Organic Matter	Cumulative Mean	Cumulative SE	SE/Mean	Site	Replicate #	Salinity (ppt)	Cumulative Mean	Cumulative SE	SE/Mean
PR	1	2.38				FRCS	1	41.92			
PR	2	3.35	2.86	0.49	0.170	FRCS	2	51.56	46.74	4.82	0.103
PR	3	2.92	2.88	0.28	0.098	FRCS	3	47.33	46.94	2.79	0.059
PR	4	2.15	2.70	0.27	0.100	FRCS	4	42.51	45.83	2.26	0.049
PR	5	2.50	2.66	0.21	0.080	FRCS	5	46.12	45.89	1.75	0.038
PR	6	3.56	2.81	0.23	0.082	FRCS	6	41.11	45.09	1.64	0.036
PR	7	3.60	2.92	0.23	0.077	FRCS	7	46.04	45.23	1.39	0.031
PR	8	3.74	3.03	0.22	0.073	FRCS	8	48.78	45.67	1.28	0.028
PR	9	2.96	3.02	0.19	0.064	FRCS	9	47.51	45.88	1.15	0.025
PR	10	3.15	3.03	0.17	0.058	FRCS	10	38.05	45.09	1.29	0.029
PC30	1	9.17				L4RN	1	89.58			
PC30	2	11.61	10.39	1.22	0.117	L4RN	2	159.14	124.36	34.78	0.280
PC30	3	1.11	7.30	3.17	0.435	L4RN	3	105.67	118.13	21.02	0.178
PC30	4	9.99	7.97	2.34	0.294	L4RN	4	66.41	105.20	19.70	0.187
PC30	5	9.52	8.28	1.84	0.222	L4RN	5	101.27	104.41	15.28	0.146
PC30	6	9.77	8.53	1.52	0.179	L4RN	6	64.96	97.84	14.10	0.144
PC30	7	9.37	8.65	1.29	0.149	L4RN	7	66.75	93.40	12.72	0.136
PC30	8	7.59	8.52	1.13	0.132	L4RN	8	81.37	91.89	11.12	0.121
PC30	9	12.09	8.91	1.07	0.120	L4RN	9	153.00	98.68	11.93	0.121
PC30	10	13.50	9.37	1.06	0.113	L4RN	10	96.66	98.48	10.67	0.108
SJCT	1	12.18				L4CN	1	41.84			
SJCT	2	13.78	12.98	0.80	0.061	L4CN	2	49.44	45.64	3.80	0.083
SJCT	3	10.17	12.04	1.04	0.087	L4CN	3	36.42	42.56	3.78	0.089
SJCT	4	9.03	11.29	1.05	0.093	L4CN	4	44.56	43.06	2.72	0.063
SJCT	5	9.55	10.94	0.89	0.081	L4CN	5	42.63	42.98	2.11	0.049
SJCT	6	13.30	11.34	0.82	0.073	L4CN	6	48.90	43.96	1.98	0.045
SJCT	7	19.36	12.48	1.34	0.108	L4CN	7	49.09	44.70	1.83	0.041
SJCT	8	6.22	11.70	1.40	0.120	L4CN	8	40.89	44.22	1.65	0.037
SJCT	9	11.16	11.64	1.24	0.106	L4CN	9	46.42	44.46	1.48	0.033
SJCT	10	15.60	12.04	1.18	0.098	L4CN	10	49.14	44.93	1.40	0.031



Sediment grain size, organic matter and salinity. Method 1, SE/Mean.

Andrew, N.L. and B.C. Mapstone. 1987. Sampling and the description of spatial pattern in marine ecology. *Oceanogr. Mar. Biol., Ann. Rev.* 25:39-90.

Kingsford, M. and C. Battershill. 1998. *Studying temperate marine environments: a handbook for ecologists*. Canterbury University Press, Christchurch, New Zealand.

Methods described in:

Murray, S.N., R.F. Ambrose and M.N. Dethier. 2002. Methods for performing monitoring, impact, and ecological studies on rocky shores. U. S. Department of the Interior, Minerals Management Service, Pacific OCS Region. OCS Study, MMS 01-070. Camarillo, California.

## Appendix 2. Potential Protocols – Sandy Beach Habitat

A macrofauna invertebrate survey would be beneficial for characterizing the sandy beach habitat. However, this protocol would likely take too long to perform in the field for a rapid survey. Macrofauna invertebrates could be sampled along transects during a timed search by shore level. Shore levels would be divided into upper shore (dry sand and wrack line), mid beach (damp sand), and lower beach to swash zone. Potential macrofauna prey species selected for timed search are generally abundant and relatively large invertebrates such as the bloodworm, *Euzonus mucronata*, the isopod, *Exciorolana chiltoni*, the clams *Tivela stultorum* and *Donax gouldii*, and the sand crab, *Emerita analoga* as well as wrack-associated invertebrates. Most of these species occur in the mid to low intertidal zone and can be observed on a low tide of one foot or less in calm conditions. The macrofauna species, *Emerita*, *Tivela*, *Donax*, *Euzonus* and *Exciorolana* occur in the mid- to low intertidal zone and can be sampled with uniformly spaced cores taken along vertical transects in those zones. The beachhoppers, *Megalorchestia* sp. and the kelp flies, *Coelopa vanduzei* and *Fucellia costalis*, comprise the majority of the macrofaunal abundance and biomass on the upper beach. Burrows would be surveyed and wrack flipped to search for them. Upper beach fauna, such as beachhoppers and beetles, could also be sampled with cores on vertical transects. Flies could be sampled with an insect net.

For the bird survey, all shorebirds, gulls and other birds, including seabirds and terrestrial birds, should be identified and counted by a single observer using binoculars on one kilometer or shorter segments of beach habitat. Once established, the endpoints of the segments can be noted and their positions determined with GPS. The methodology of McCrary and Pierson (1998), and Dugan et al. (2004) can be adapted for this protocol. Care must be taken to not disturb birds or double count them. As they are counted, birds should be assigned to intertidal zones (upper intertidal, mid-intertidal, below WTO, swash zone) and their behavior (feeding, roosting, loafing) noted on a standard data form. For each one kilometer (or shorter) segment of beach, the date, observer name, starting and stopping time, weather conditions (cloud cover, wind and temperature) should be recorded. Human and dog use should be quantified during each shorebird survey. Flyovers by raptors and other disturbances should be noted. Any dead bird and mammals encountered should be recorded and tagged (if feasible); the focus should be on recently dead animals who could be mistakenly assumed to be killed by the oil spill in post-spill surveys.

### **Appendix 3. Photos Linked to GPS**

There are several programs that exist to link photographs to GPS coordinates. A few are discussed in this appendix; others may be worth researching.

OziPhotoTool is software that links digital photos to GPS coordinates and can be purchased for around \$25. OziExplorer (~\$85) is the software that downloads the GPS data. OziPhotoTool works only with OziExplorer. The software will work with any camera with a timestamp. It compares the time stamp from the photo metadata and matches it to the nearest GPS location based on the time it was taken. First, a picture is taken of the GPS unit showing the time. Then, a GPS track file is started, taking a position every so many seconds. After uploading the waypoints and photos the software corrects the time from the photos to match the GPS time, assigning the coordinate closest to the time each photo was taken. Photos show up as clickable icons in OziExplorer along the GPS “track.”

OziPhotoTool can load basic base maps to overlay and interact with GPS data that can be saved as a shapefile. It can also create a watermark for the photos with the coordinate, time, location, etc. onto the photo. A text file is created for the photos and waypoints with a list of photo names, Lat/Long, time information, etc.

GPS PhotoLink (~\$250) works the same way as OziPhotoTool, but a separate program to download the GPS waypoints (such as GPS Pathfinder) is still needed. GPS PhotoLink is slightly more developed, has different icons, and allows more control over watermarks – how they look, where they are placed on the photo, etc. – than OziPhotoTool. GPS PhotoLink also creates shapefiles that are compatible with ESRI ArcMap, while OziPhotoTool does not integrate with ESRI.

The other way to link photos to GPS involves high-end cameras that allow the GPS unit to attach directly to the camera. Then a program like “GPSi” is used to download the photos with the GPS information attached to the metadata. Ricoh has a camera with a built-in GPS, which is less expensive than buying both units separately and attaching, but a program to download the photos with the waypoint information is still needed.

In addition, an Access database can be used for storage and organization of the photos. For example, the NOAA HAZMAT group in Seattle has a database that they created to handle their photos, with site level information for each photo, and photos linked to the searchable database.