Appendix D

MONITORING PLAN

THE S.O.N.G.S. WETLAND MITIGATION PROGRAM

CALIFORNIA COASTAL COMMISSION

45 FREMONT, SUITE 2000

SAN FRANCISCO, CA 94105

DRAFT 9/20/05

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EXECUTIVE SUMMARY

Through its 1991 and 1997 coastal permit actions (permit # 6-81-330 and 6-81-330) the California Coastal Commission (CCC) adopted permit conditions that require Southern California Edison (SCE) and its partners to create or substantially restore 150 acres of tidal wetlands at an approved location within the Southern California Bight. A revised preliminary plan for wetland restoration at the San Dieguito River Valley, submitted to the CCC by SCE on November 3, 1997, calls for the excavation of approximately 115 acres of upland to create tidal wetland and the enhancement of 35 acres of existing tidal wetland through the continuous maintenance of a tidal inlet in perpetuity. Created and restored habitats in this plan include subtidal basins and channels, and intertidal mudflats and marsh.

Condition A (Wetland Mitigation) of the CCC coastal development permit for SONGS (CDP) requires that monitoring of the wetland restoration be done over the full operating life of SONGS Units 2 & 3. This monitoring will be done to measure compliance of the mitigation project with the performance standards specified in the SONGS CDP. In accordance with Condition D (Administrative Structure) of the CDP, scientists retained by the Executive Director of the CCC shall develop the Monitoring Plan to guide the monitoring work and will oversee the monitoring studies outlined in the Plan. The SONGS CDP provides a description of the performance standards and monitoring required for the wetland mitigation project and this Monitoring Plan closely adheres to the monitoring requirements of the permit.

The performance standards in the SONGS CDP fall into two categories. The first category includes long-term physical standards relating to topography (erosion, sedimentation), water quality (e.g., oxygen concentration), tidal prism, and habitat areas. The second category includes biological performance standards relating to biological communities (e.g., fish, invertebrates, and birds), marsh vegetation, *Spartina* canopy architecture, reproductive success of marsh plants, food chain support functions, and exotic species.

Changes in topography involving excessive erosion or sediment deposition that impedes tidal flow or a structural weakening of berms or associated structures will trigger maintenance operations. To monitor topography, a visual survey will be done semiannually throughout the San Dieguito wetland. Additional surveys may be done following extreme climatic events. Reference points will also be established along the 4.5' NGVD elevation line and other points of reference needed to evaluate the total area of restored tidal wetland. The reference locations will be independently monitored annually by CCC staff.

Water quality variables shall be similar in the restored wetland to reference wetlands**.** To monitor water quality, water temperature, salinity, turbidity, and dissolved oxygen and nutrient concentrations will be measured in San Dieguito Lagoon and the reference wetlands using dataloggers and biweekly water samples. Measurements will be taken at the current sampling stations and at selected locations in the restored wetland where water quality is most likely to be problematic. Dissolved oxygen concentration also will be compared once per year between the San Dieguito Lagoon restoration and the reference wetlands in a comprehensive survey.

The tidal prism shall be maintained. Tidal prism of the restored lagoon will be calculated on completion of construction and used as the standard of comparison during subsequent monitoring. Tidal prism during spring tides within the restored lagoon should be maintained within 10% of this initial value. Topographical data from the as built drawings and hourly water level data taken from tide gauges and environmental dataloggers will be used to calculate the tidal prism following the methods of Elwany et al. (1994). Briefly, this involves: (1) determining the maximum and minimum water levels for each tidal cycle, (2) calculating the surface area of the tidally inundated portion of the wetland as a function of elevation, and (3) integrating the surface area from minimum to maximum tidal levels to give the tidal prism. Topographical data from the entire wetland will be updated annually using a Total Station or equivalent.

Conditions that would cause a greater than 10% reduction in tidal prism and trigger the need for maintenance dredging include 1) a bottom elevation of the sill at the inlet of greater than 0.5 feet NGVD or 2) dawn dissolved oxygen in the lagoon basins of less than or equal to 3 parts per million. Bottom elevation of the sill can be monitored by measuring the surface water elevation at Jimmy Durante Bridge. A minimum water surface elevation under the Jimmy Durante Bridge that exceeds 0.5 feet NGVD (+3.06 feet MLLW) during spring tides will trigger maintenance dredging.

Limited ground surveys will be combined with low-level aerial photographs to determine whether the areas of wetland habitats have changed more than 10 % from the as-built elevations. Wetland habitats to be evaluated in this standard are: (1) subtidal (<-0.9 feet NGVD), (2) intertidal mudflat (-0.9 to 1.3 feet NGVD), and (3) intertidal vegetated salt marsh (1.3 to 4.5 feet NGVD).

Within 4 years of construction, the total densities and number of species of fish, macroinvertebrates, and birds must be similar to the densities and number of species in similar habitats in the reference wetlands. To monitor fish, enclosure traps will be used in shallow water (<1.5 m deep), beach seines will be used in areas that are <1.5 m deep and within 30 m of shore, and purse seines will be used in areas that are >1.5 m deep or >30 m from shore. Habitats that will be sampled are main channels, tidal creeks, and a basin in the restored wetland only. Sampling stations will be widely spaced within each habitat and, if possible, a minimum of 10 stations within each wetland will be sampled by each method. Fish will be sampled twice, approximately 1 month apart, between June through August at San Dieguito Lagoon and the three reference wetlands.

To monitor macroinvertebrates, quadrats will be used to sample larger epifauna, large cores will be used to sample deeper living infauna, and small cores will be used to sample small, shallow living infauna. Data on the larger epifaunal species obtained from the enclosure traps and seines used to sample fish will also be used. Invertebrate sampling will be done in main channel, tidal creek, and basin habitats in conjunction with enclosure sampling for fish. If possible, 10 stations will be sampled per wetland with a minimum of 2 widely spaced replicate samples per station. For the small core samples, each sample may consist of a composite of 4 to 5 cores. Invertebrates will be sampled once per year in conjunction with fish sampling.

To monitor birds, all individuals within replicate rectangular plots of known size will be visually identified and counted. Sampling effort will be standardized for each plot. Bird sampling will be done in tidal creek, main channel, mud flat, open water, and vegetated marsh habitats. Aerial photographs together with ground surveys will be used to select standard size plots within each of these habitat types. Bird sampling will be standardized by tide cycle and tidal elevation and all wetlands will be sampled simultaneously to control for the potential effects of weather and tidal height on bird activity. Initially, sampling observations will be made during the winter, and the spring and fall migrations. Six surveys will be made in each wetland during each period and six spatial replicates will be taken on each survey. Bird sampling effort may be adjusted after the first year, depending on spatial and temporal variability in estimates of bird species richness and density.

The proportion of total vegetation cover and open space in the restored wetland must be similar to that in the reference wetlands. To monitor vegetation cover, the proportion of vegetation cover and open space in the restored wetland will be compared with that in the reference wetlands using data obtained from low-level multi-spectral aerial photography taken once per year in late spring to early summer. The percent cover of algae must be similar to the percent cover found in the reference sites. To monitor macroalgae, qualitative observations for the presence of algal mats will be made during routine water quality monitoring. Estimates of the areas of algal mats will also be made from the aerial images. The restored wetland must have a canopy architecture of *Spartina foliosa* that is similar in distribution to the reference sites, with an equivalent proportion of stems over 3 feet tall. To monitor the canopy architecture of *Spartina,* the mean proportion of stems > 3 feet tall will be determined in stands of this plant and compared between the restored wetland and Tijuana River Estuary.

The reproductive success of salt marsh plants shall be similar in the restored wetland to that in the reference wetlands in at least one out of every three years. To monitor the reproductive success of salt marsh plants, flowering and seed viability will be measured for at least three species that occur in the intertidal habitat of the restored and reference wetlands. Sampling to quantify flowering will be done in the early summer. Flowering will be measured as the proportion of randomly selected stems with flowers (inflorescences) or individual plants with flower stalks. Sampling for seed set and viability will be done in late summer-early fall. Seed set will be determined by the presence of seeds in sampled flowering stems and flower stalks. Seed viability will be tested for randomly selected samples of seeds.

The food chain support provided to birds must be similar to that provided by the reference sites. To monitor food chain support, measurements of the feeding activity of large shorebirds and terns that forage in open habitats will be conducted during the same time of year as bird sampling. Feeding observations will be conducted on replicate birds and per capita successful feeding attempts compared between restored and reference wetlands.

The important functions of the wetland must not be impaired by exotic species. To monitor exotic species, data collected for fish, invertebrates, birds, and plants will be evaluated relative to this standard, and a special survey for presence of exotic species also will be conducted once a year.

To determine compliance with the conditions of the CDP, the performance standards for topography, tidal prism and habitat areas will be evaluated in comparison to "fixed" values. The performance standards for water quality, biological communities, salt marsh vascular plants, algae, *Spartina* canopy, plant reproductive success, and food chain support are "relative" and require that values of these attributes in the restored wetland be similar to values in the reference wetlands. Reference wetlands for this restoration project are Tijuana River Estuary, Mugu Lagoon, and Carpinteria Salt Marsh.

There is no single best approach for determining similarity in the relative performance standards between the restored and reference wetlands. At a minimum, average values in the restored wetland must be within the range of values observed contemporaneously at the 3 reference wetlands. This provisional criterion will be re-evaluated during the initial post-restoration monitoring. Multivariate statistical procedures may be used to gain insight into the performance of the restored wetland (e.g., Nonparametric Multidimensional Scaling), but they will not be used to evaluate whether the restored wetland has met the performance criteria.

Management issues relevant to the SONGS wetland mitigation requirement are inlet maintenance, excessive changes in topography, and exotic species. SCE has a plan for managing the inlet in perpetuity to ensure uninterrupted tidal flushing of the restored wetland. This plan provides conditions that would trigger the need for additional maintenance dredging at the inlet (SCE 2004). Topographic degradation of the wetland and berms is likely to occur over time as a result of sedimentation and scour. If topographic surveys and aerial photographs indicate major topographic degradation has occurred, then the appropriate corrective action (e.g., dredging) will be taken to reconfigure the wetland to its "as designed" condition. Exotic species may invade restored habitats. If invasive exotic species are found in the restored wetland, and these species could adversely affect the success of the restoration, experts working in this field will be consulted and a program to control the spread of these species will be developed.

1. INTRODUCTION

Through its 1991 and 1997 coastal permit actions (permit # 6-81-330-A, formerly 6-83-73) the California Coastal Commission (CCC) adopted permit conditions that require Southern California Edison (SCE) and its partners to create or substantially restore 150 acres of tidal wetlands at an approved location within the Southern California Bight. The purpose of this condition is to serve as out-of-kind mitigation that compensates for past, present and future impacts to fish caused by the operation of San Onofre Nuclear Generating Station (SONGS) Units 2 & 3.

On June 11, 1992, the CCC approved SCE's choice of the San Dieguito River Valley as the restoration site that meets the minimum standards identified in the permit and best meets the objectives of the wetland mitigation requirement. On April 9, 1997, the CCC reaffirmed its prior determination that San Dieguito River Valley is the restoration site and determined that SCE can propose an additional site for restoration only if achieving all 150 acres of restoration at San Dieguito River Valley becomes infeasible due to hydrology or other engineering concerns. The CCC also determined that up to 35 acres of enhancement credit could be obtained for inlet maintenance if wetland restoration is done at San Dieguito.

A preliminary plan for wetland restoration at the San Dieguito River Valley was submitted to the CCC by SCE on September 30, 1997. A revised plan was submitted on November 3, 1997. The revised plan calls for the excavation of approximately 115 acres of upland to create tidal wetland and the enhancement of 35 acres of existing tidal wetland through the continuous maintenance of a tidal inlet in perpetuity. Created and restored habitats in this plan include subtidal basins and channels, and intertidal mudflats and marsh. In addition, the plan includes the construction of nesting habitats, flood control devices such as berms, and the creation of non-tidal salt marsh, coastal sage scrub and grassland habitats.

Condition A (Wetland Mitigation) of the CCC coastal development permit (CDP) for SONGS requires that monitoring, management (including maintenance) and remediation of the wetland restoration be done over the full operating life of SONGS Units 2 & 3. The full operating life of SONGS includes past and future years of operation of SONGS Units 2 and 3 and the decommissioning period to the extent there are continuing discharges. The number of past operating years at the time the wetland is ultimately constructed, will be added to the number of future operating years and decommission period, to determine the length of the monitoring, management and remediation requirement.

Monitoring will commence upon completion of construction of the wetland. Initially, limited monitoring will be conducted in SDL and perhaps the reference wetlands. The purpose of this limited monitoring will be to estimate the trajectory of biological development, assess tidal functioning, and assess damage to existing wetland habitat within SDL following restoration. This limited monitoring will provide necessary information for adaptive management of the restored wetland. As the restored wetland develops, and when it appears that some of the performance standards may be met, monitoring will be scaled up in the restored portion of SDL and in the reference wetlands to measure the success of the mitigation project in achieving stated restoration goals (as specified in the final restoration plan) and in the achieving performance

standards specified in the SONGS coastal development permit (and listed below). Limited monitoring will continue in the non-restored portion of SDL to monitor any changes to existing wetland habitat within SDL following restoration. SCE and its partners are fully responsible for any failure to meet these goals and standards for the full operating life of SONGS Units 2 and 3. Condition D of the CDP establishes a strategy to reduce the level of monitoring when the performance standards have been met for three successive years. Specifically, the permit states that: "The mitigation projects will be successful when all performance standards have been met each year for a three-year period. If the CCC determines that the performance standards have been met and the project is successful, the monitoring program will be scaled down. The CCC's work program shall reflect the lower level of monitoring required. If subsequent monitoring shows that a standard is no longer being met, monitoring may be increased to previous levels, as determined necessary by the Executive Director."

In accordance with Condition D (Administrative Structure) of the CDP, the monitoring of the wetland restoration will be done independently of SCE and its partners. Scientists retained by the Executive Director of the CCC shall develop the Monitoring Plan, in consultation with SCE and appropriate lead agencies, and will oversee the monitoring studies outlined in the Plan. The present document serves as the Monitoring Plan for the SONGS' wetland mitigation requirement and provides a general framework to guide the monitoring work.

It should be noted that the SONGS CDP provides a description of the monitoring required for the wetland mitigation project. Specifically, the permit describes the duration of monitoring, the performance standards to be used for judging the success of the restoration, the use of reference sites as a standard of comparison, and the parties responsible for monitoring and evaluating the restoration project. This Monitoring Plan closely adheres to the conditions of the permit and includes a description of the performance standards that will be used to evaluate the success of the restoration and the sampling methods that will be used to make this evaluation. The focus of the CCC Monitoring Plan is on assessing compliance with the performance standards stated in the permit. Thus, there are a number of issues related to management of the restored wetland that are not included in this document, such as monitoring and maintenance of least tern nesting sites, removal of trash, control and enforcement of public access, mosquito control and development in the watershed. In addition, the CCC or other agencies may add monitoring requirements as part of their regulatory oversight of the wetland restoration project, and this Monitoring Plan does not consider these possible requirements.

2. MONITORING

The performance standards in the SONGS permit fall into two categories. The first category includes long-term physical standards to be maintained for the operating life of SONGS. These include standards relating to topography (erosion, sedimentation), water quality (e.g., oxygen concentration), tidal prism, and habitat areas. The second category includes biological performance standards relating to biological communities (e.g., fish, invertebrates, and birds), vegetation, *Spartina* canopy architecture, reproductive success of marsh plants, food chain support functions, and exotic species.

Listed below are the physical and biological performance standards on which the success of the restoration will be evaluated. Included with each standard is a discussion of the recommended sampling methods for collecting the information needed to evaluate that standard. In accordance with Condition D of the SONGS CDP, final determination of the sampling design (e.g., number and distribution of sampling stations, samples per station) and specific details of the monitoring methods will be presented in biennial work plans.

2.1. Physical Performance Standards

1. Topography –*The wetland shall not undergo major topographic degradation (such as excessive erosion or sedimentation) (Special Condition 3.4.a.1).*

   Methods: Following construction, visual surveys will be done semiannually throughout the restored San Dieguito wetland to monitor for any sign of substantial erosion or sediment deposition (e.g., bank failures, bar formation) that could impede tidal flow in the wetland. Additional surveys may be done following extreme climatic events. Constructed berms (and associated structures, e.g. culverts and weirs) are a special topographical feature of the restoration. These features also will be visually inspected during the surveys. Furthermore, any major topographic changes observed during sampling to evaluate the other performance standards (described below) will be noted and used to evaluate this standard. Since the success of the restoration depends critically on tidal exchange and the proper functioning of the berms and associated structures, excessive erosion or sediment deposition that impedes tidal flow or a structural weakening of berms will trigger maintenance operations.

   CCC staff has defined 4.5' NGVD as the upper limit of tidally influenced habitat for the calculation of acreage credit for this restoration project. Because of this, the location of 4.5' contour is an important benchmark that will be checked annually to evaluate compliance of SCE with the acreage requirement. Reference points will be established along the 4.5' NGVD elevation line and independently monitored by CCC staff. The 4.5' NGVD contour will be overlaid on aerial photographs and used to evaluate the total area of restored tidal wetland. Reference points will be established along the contour using an electronic Total Station or equivalent, which measures elevation with 3 cm accuracy. The location of the 4.5' NGVD contour will be checked annually.

2. Water quality - *Water quality variables shall be similar to reference wetlands (Special Condition 3.4.a.2).*

   Methods: Water temperature, salinity, dissolved oxygen, nutrient concentrations, and turbidity will be measured in San Dieguito Lagoon and the reference wetlands. These parameters are commonly used measures of wetland water circulation and mixing activity (MEC 1993). Salinity and dissolved oxygen concentration are currently used to trigger inlet opening activities at the San Dieguito Lagoon (Elwany et al. 1994). These measures can vary over a 24-hour period, and change rapidly with inlet closure. Measurements of water temperature, salinity, and dissolved oxygen will be made using continuously recording environmental dataloggers deployed in each wetland. Data loggers will be deployed in the

main channel and tidal creeks of San Dieguito Lagoon and the reference wetlands and in the constructed basin of San Dieguito Lagoon. CCC staff scientists have also measured these variables in water samples taken biweekly at the surface and the bottom at five stations in San Dieguito Lagoon since October 1994 (Appendix 1). In order to achieve adequate spatial coverage, these measurements will continue during the post-restoration monitoring period at the five current sampling stations and at selected locations in the restored wetland where water quality is most likely to be problematic (e.g. tidal creeks, back of basins). Since restoration activities could influence nutrient delivery to the lagoon, with effects on macrophyte growth and water quality, water samples for dissolved nutrients (nitrate, nitrite, ammonium, and orthophosphate) and turbidity will also be taken at these sampling stations. In addition, because of its importance to wetland health, dissolved oxygen concentration will be measured once per year at dawn in comprehensive surveys at the San Dieguito Lagoon restoration and the reference wetlands and these data will be compared among wetlands.

3. Tidal prism - *The designed tidal prism shall be maintained, and tidal flushing shall not be interrupted" (Special Condition 3.4.a.3)*

Methods: The tidal prism is the net volume of water exchanged within a wetland due to the tides. Numerical modeling by Jenkins and Wasyl (1998) suggested that after restoration, the potential tidal prism in the lagoon could increase by approximately 135 to 150% during monthly spring and daily mean tides, respectively. However, predictions of tidal prism from this modeling are likely to differ from actual values for the final as-built wetland since they do not include the effects of friction, which could result in significantly smaller tidal prisms. Therefore, tidal prism of the restored lagoon will be calculated on completion of construction and used as the standard of comparison during subsequent monitoring. Tidal prism within the restored lagoon should be maintained within 10% of this initial value during spring tides taking into consideration that the tidal prism of the lagoon depends on the ocean tide range which differs from month to month and from year to year.

Topographical data from the as built drawings and hourly water level data taken from tide gauges and environmental dataloggers will be used to calculate the monthly spring tidal prism following the methods of Elwany et al. (1994). Briefly, this involves: (1) determining the maximum and minimum water levels for each tidal cycle, (2) calculating the surface area of the tidally inundated portion of the wetland as a function of elevation, and (3) integrating the surface area from minimum to maximum tidal levels to give the tidal prism. Topographical data from the entire wetland will be updated annually using a Total Station or equivalent.

Conditions that would cause a greater than 10% reduction in tidal prism and trigger the need for maintenance dredging include 1) a bottom elevation of the sill at the inlet of greater than 0.5' NGVD or 2) dissolved oxygen in the lagoon basins measured at dawn of less than or equal to 3 parts per million (ppm) except during time periods of red tides when the ocean and lagoon dissolved oxygen levels may be less than 2 ppm. Bottom elevation of the sill can be monitored by measuring the surface water elevation at Jimmy Durante Bridge. A minimum water surface elevation under the Jimmy Durante Bridge that exceeds 0.5' NGVD (+3.06'

MLLW) during spring tides will trigger maintenance dredging. The areas to be dredged will be determined by comparing topographical survey data to the design configuration.

To maintain tidal flushing, SCE's restoration plan calls for a one-time restorative dredging of the inlet channel and lagoon channels followed by subsequent routine maintenance dredging every eight months to maintain the inlet channel at the designed configuration. The plan calls for an inlet channel depth of -2.0 to -4.0' NGVD and a channel width of 150' at MHHW east of Highway 101. The inlet channel west of Highway 101 will be narrower with a depth of -2.0' NVGD and a width of approximately 130'. Tidal flushing will be uninterrupted if these channel depths and widths are maintained. Initially, topographic surveys of the inlet will be done monthly by SCE to monitor changes in the depth of the inlet channel (Elwany 1992-2004). Subsequently, depending on the results of 1-2 years monitoring, the frequency of surveys may be reduced.

4. Habitat areas - *"The area of different habitats shall not vary by more than 10% from the areas indicated in the final restoration plan".*

   Methods: Limited ground surveys to check elevations at established reference points will be combined with low-level aerial photographs to determine whether the areas of wetland habitats have changed more than 10 % from the as-built elevations measured on completion of construction. Wetland habitats to be evaluated in this standard are: (1) subtidal (<-0.9' NGVD), (2) intertidal mudflat (-0.9 to 1.3' NGVD), and (3) intertidal vegetated salt marsh (1.3 to 4.5' NGVD). Reference points for evaluating compliance with this standard will be established at these habitat boundaries following completion of construction and in conjunction with the establishment of reference points for the 4.5' NGVD elevation (see 2.1.1 above).

2.2. Biological performance standards

1. Fish - *"Within 4 years of construction, the total densities and number of species of fish shall be similar to the densities and number of species in similar habitats in the reference wetlands" (Special Condition 3.4.b.1).*

   Methods: Three methods will be used to sample fish: enclosure traps, beach seines, and purse seines. Enclosure traps will be used in shallow water (<1 m deep) to sample primarily gobies (family Gobiidae), which are small, abundant fishes that are poorly sampled by other methods. Beach seines in combination with blocking nets will be used to sample larger fish in areas that are <1.5 m deep and within 30 m of shore. Purse seines will be used in areas that are >1.5 m deep or >30 m from shore. The rationale for the use of these methods, and recommended gear configuration and sampling protocol, are provided in Appendices 2-4. Fish captured by all 3 methods will be identified and counted in the field and returned to the water alive. In cases where species identification is uncertain, voucher specimens will be retained for later identification in the laboratory.

   Habitats to be sampled during fish monitoring are main channels, tidal creeks, and basin. Basins of the type planned for the San Dieguito Lagoon Restoration do not occur naturally in

existing southern California wetlands that could serve as reference sites. Consequently, basin habitats in the San Dieguito Lagoon Restoration will be sampled using the same methods employed in channel habitats (e.g. a combination of enclosures, beach seines, and purse seines).

Because estuarine fish are patchily distributed, samples must be spaced widely across these habitats to obtain representative estimates of fish species richness and density (Appendix 4). The pattern of fish distribution dictates that individual tidal creeks, stretches of main channel separated by at least 100 m, and areas within basins separated by at least 100 m will be the fundamental units of replication (stations) within the restored and reference wetlands. Samples spaced <100 m apart within these stations are not independent, although they can be useful for improving the estimate of richness and density at the station level.

For all three fish sampling methods, the number of stations sampled within a wetland will be maximized within logistical constraints. If possible, a minimum of 10 stations within each wetland will be sampled by each method and stations will be roughly evenly spread (stratified random placement) across the wetland (Appendix 4). For enclosure traps, 2-4 replicate samples spaced at least 40 m apart will be taken within each station. For beach and purse seines, only one replicate will be taken per station and extra replication will occur at the station level.

Fish density and species richness also varies seasonally. Density is predictably highest in southern California wetlands during June and July and species richness is normally highest from June through October (Horn and Allen 1985, Brooks 1999, Merkel & Associates, Inc. 2002). Sampling effort will be kept to modest levels by sampling fish from June through August. Because periods of peak density and species richness may not be perfectly synchronous at different wetlands in southern California, fish will be sampled twice during this period at San Dieguito Lagoon and the three reference wetlands. Sampling episodes will be spaced roughly 1 month apart. In the event that there are large differences between the restored and reference wetlands during the sampling period, additional surveys may be conducted to rule out geographical differences in the timing of peak density and richness as the cause for differences among wetlands.

In addition to seasonal variation, fish species richness and density vary over shorter time scales (e.g., hourly, weekly, daily, and diurnally). Because weekly and daily variation is modest relative to spatial variation (Appendix 4), it is unnecessary to repeat fish sampling on a daily or weekly basis. Within-day variation occurs mainly in response to tidal fluctuations. Thus, sampling will be restricted to periods of similar tides (e.g., high tides within a certain range of height). Diurnal variation in estimates of fish density and richness (e.g., Merkel & Associates, Inc. 2002) are likely driven by reduced net avoidance during dark hours, not actual differences in density and richness. Differences in estimates of density and richness between light and dark periods, however, are not dramatic and patterns of diurnal variation are tightly correlated between samples taken during the two time periods (Merkel & Associates, Inc. 2002). Given the logistical difficulties of sampling at night and the tight correlation between daytime and nighttime samples (Appendix 4), fish will be sampled only during daytime.

2. Macroinvertebrates - "*Within 4 years of construction, the total densities and number of species of macroinvertebrates shall be similar to the densities and number of species in similar habitats in the reference wetlands" (Special Condition 3.4.b.1).*

Methods: Five methods will be used to sample larger macroinvertebrates. Epifauna (e.g., California Horn Snail, *Cerithidea californica*) will be sampled by counting individuals on the sediment surface within 0.5 x 0.5 m quadrats. Deep living infauna (e.g., Jackknife Clam, *Tagelus californianus*, Ghost Shrimp *Neotrypaea californiensis*) will be sampled using a 10 cm diameter core pushed into the sediment to a depth of 50 cm. The contents of the 10 cm core will be sieved through a 3-mm mesh screen in the field. Animals retained by the 3-mm mesh will be identified and counted in the field and returned to the habitat. Although the cores sample epifauna as well as infauna, the area of bottom that they sample is small relative to the sizes of some epifaunal species of interest (e.g., sea hare, *Aplysia californica*). To address this problem, densities of the larger epifaunal species will also be estimated using enclosure traps and seines while sampling fish (see Section 2.2.1, Fish). Epifauna captured during fish sampling will be identified and counted in the field.

Smaller invertebrates (e.g., most annelids) will be sampled using a 4.8-cm diameter core pushed into the sediment to a depth of 6 cm. The smaller core samples will be preserved on site in 10% buffered formalin and returned to the laboratory for processing. Details of methods to be used for processing these samples in the laboratory are provided in Appendix 5. Specimens will be identified and counted under the microscope and archived in ethanol. Invertebrates will be identified to genus or family for smaller specimens (e.g., polychaetes, amphipods) and species for larger specimens (e.g., bivalves, decapod crustaceans) (see Appendix 5).

Invertebrate sampling will be done in main channel, tidal creek, and basin habitats in conjunction with enclosure sampling for fish and thus will be conducted in water depths of < 1 m. The scale of spatial patchiness in invertebrate density is not predictable, though where patches are evident, they are <30 m in extent (Appendix 5). Therefore, sample locations (i.e., quadrats and cores) for measurements of species richness and total density within a station (tidal creek, section of basin) will not be any closer than 30 m apart. Similar to fish, significant differences in invertebrate density occur at the "station" level. Optimization analyses indicate that few samples are needed per "station" (Appendix 5). Taken together, the analyses suggest that from 5 to 10 stations should be sampled per wetland to maximize power to detect differences between the restored wetland and reference wetlands. A minimum of 2 widely spaced replicate samples will be taken per station. Each sample may consist of a composite of 4 to 5 cores, which will give a better estimate of the mean per station than 2 or 3 single cores. Composite sampling will not be done for invertebrate samples retained on the 3-mm mesh because there is no cost savings for these samples (Appendix 5). Sampling will be conducted annually in the summer, following spring recruitment, on two surveys done in conjunction with fish sampling (see Section 2.2.1 above). In the event that there are large differences between the restored and reference wetlands during the summer survey, additional surveys may be conducted to rule out

geographical differences in the timing of invertebrate recruitment as the cause for differences among wetlands.

3. Birds - "*Within 4 years of construction, the total densities and number of species of birds shall be similar to the densities and number of species in similar habitats in the reference wetlands" (Special Condition 3.4.b.1).*

   Methods: Birds will be sampled by walking near or within replicate rectangular plots of known size and visually identifying and counting all individuals sighted within each plot. Specifying the time spent identifying and counting birds within each plot will standardize sampling effort. The following habitats will be sampled: tidal creek, main channel, mud flat, basin, and vegetated marsh. Aerial photographs together with ground surveys will be used to select standard size plots within each of these habitat types. At the restored and reference wetlands, at least 5 plots will be randomly placed within each habitat type (Appendix 6). A GPS unit will be used to record the specific location of each plot. A laser-range finder will be used to determine observer-bird distance to aid in delineating plot boundaries.

   Weather conditions will be evaluated at the beginning of each sampling period. Sampling will not be conducted when weather conditions affect either bird behavior or the visual acuity of the observer. In general, sampling will not be conducted under the following conditions: (1) precipitation or heavy fog, (2) winds exceeding 15 mph, or (3) temperatures below 40° F. Nor will sampling be conducted when disturbances affect the movement or behavior of birds.

   Bird sampling will be standardized by tide cycle and tidal elevation. Sampling of tidal channel, main channel, and mudflat habitat will be conducted only during falling and low tide. Timing of sampling in open-water habitats is more flexible but this habitat will not be sampled when vegetated habitat is covered by water, which allows waterfowl to move out of the embayment. All sampling will be conducted during periods of sufficient light to allow for visual detection and accurate identification of birds. In addition, if variability in density estimates is noted with distance from the inlet, it may be necessary to "nest" sampling within inlet, central, and back sections of the wetlands. All wetlands will be sampled simultaneously to control for the potential effects of weather and tidal height on bird activity.

   Bird assemblages in coastal wetlands of southern California exhibit strong seasonal patterns in species richness and density that are driven by the movement of migratory birds (Appendix 6). Initially, sampling observations will be made during three periods: winter, spring, and fall. These periods have high bird densities and distinct species composition. Based on analyses of existing wetland bird data, at least six surveys will be made in each wetland during each period and six spatial replicates will be taken on each survey (Appendix 6). Sampling effort may be adjusted after the first year, depending on spatial and temporal variability in estimates of bird species richness and density.

4. Salt marsh vascular plants - "*The proportion of total vegetation cover and open space in the marsh shall be similar to those proportions found in the reference sites" (Special Condition 3.4.b.2).*

Methods: The proportions of total vegetation cover and open space in the restored and reference wetlands will be estimated using low-level multi-spectral aerial photography acquired during low spring tides. Because the ability to classify ground cover type based on spectral data varies with weather conditions (Appendix 7), ground truthing will be conducted during each aerial survey. To ground truth the photographs, transect lines will be established at selected stations in the restored and reference wetlands that contain a mixture of open space and native vegetation. Cover of open space and vegetation will be estimated within replicate 30 x 30 cm quadrats to match the resolution of pixels in the aerial photographs (Appendix 7). Aerial photographs will be taken once per year in late spring to early summer (April - June), which is the period of maximum growth of marsh plants. This period also coincides with maximum flowering of some exotic annual species (e.g., mustard) and will maximize the ability to distinguish between native and nonnative vegetation (Section 2.2.9, Exotic species).

5. Algae - *"The percent cover of algae shall be similar to the percent cover found in the reference sites" (Special Condition 3.4.b.2).*

Methods: This performance standard is designed to monitor the development of unusually dense mats of filamentous green macroalgae in the restoration site that have the potential to interfere with wetland structure and function. Mats of filamentous green algae can occur on mudflats, in channels, and on the surface of open water. Qualitative observations for the presence of algal mats will be made during routine water quality monitoring (see Section 2.1.2). Estimates of the areas of algal mats will also be made from the aerial images gathered to monitor the cover of salt marsh vegetation and open space (Section 2.2.4). These images will be acquired during the low spring tides. Should excessive algal growth be observed at the restoration site relative to the reference sites, more quantitative comparisons may be made (e.g., by measuring mat thickness or biomass density) at these sites using line intercept or quadrat sampling.

6. *Spartina* canopy architecture - *"The restored wetland shall have a canopy architecture that is similar in distribution to the reference sites, with an equivalent proportion of stems over 3 feet tall" (Special Condition 3.4.b.3).*

Methods: Canopy architecture of the cordgrass *Spartina foliosa,* if present, is described by the height of stems, and will be determined in quadrats selected in a stratified-random manner in stands of cordgrass. Sampling of cordgrass will be done concurrently with the monitoring of salt marsh vascular plants (see Section 2.2.4). From these data the mean proportion of stems > 3 feet tall will be determined. These values will constitute replicates for analyses to compare canopy architecture in the restored wetland with that of the reference wetlands.

7. Reproductive success of salt marsh plants - *"Certain plant species, as specified in the work program, shall have demonstrated reproduction (i.e. seed set) at least once in three years" (Special Condition 3.4.b.4).*

Methods: The reproductive success of salt marsh plants will be evaluated by measuring

flowering and seed viability for at least three species that occur in the intertidal habitat of the restored and reference wetlands. Candidate species for evaluating plant reproductive success include *Arthrocnemum subterminale*, *Salicornia virginica, Frankenia salina,* and *Limonium californicum*. Sampling to quantify flowering will be done in the early summer when the presence of flowers is expected to be greatest. Flowering will be measured as the proportion of randomly selected stems with flowers (inflorescences) (e.g., *A. subterminale*, *S. virginica, F. salina*) or individual plants with flower stalks (e.g., *L. californicum*). Flowering will be quantified along transect lines in areas where the targeted species are abundant. Sampling for seed set and viability will be done in late summer-fall when seed set is expected to be greatest. Seed set will be determined by the presence of seeds in sampled flowering stems and flower stalks. Seed viability will be tested for randomly selected samples of seeds. Seeds obtained from randomly collected flowering stems or flower stalks will be placed in greenhouse conditions suitable for germination of the targeted species. Viability will be evaluated as the percentage of these seeds that germinate. Compliance of this performance standard will be based on whether the proportion of plants with flowers and seed viability of the targeted species in the restored wetland is similar to that in the reference wetlands in at least one out of every three years.

8. Food chain support - ***"****The food chain support provided to birds shall be similar to that provided by the reference sites, as determined by feeding activity of the birds" (Special Condition 3.4.b.5).*

   Methods: Measurements of the feeding activity of birds will be conducted during the same seasons as bird sampling (see Section 2.2.3, Birds) and on bird species that are present in both restored and reference wetlands. Only large shorebirds (e.g., willet, marbled godwit) and terns that forage in open habitats (embayment or mudflat) during daylight hours will be used to evaluate this performance standard because these are the only types of birds for which it is practical to determine whether foraging attempts are successful. Feeding activity will be measured from focal observations on feeding birds over a known time interval (e.g., 2 minutes). Feeding observations will be conducted on replicate birds. The total number of successful feeding attempts will divided by the number of observed birds to estimate a per capita feeding rate for comparison between restored and reference wetlands. Observations of feeding activity will be conducted during similar tide conditions across wetlands to account for the known influence of tide height on bird feeding activity (Appendix 6).

   Since weather conditions affect foraging rates by affecting prey behavior and visibility, feeding observations will be standardized with regard to these environmental variables in the same manner as with the bird sampling (Section 2.2.3). Weather conditions will be recorded each half hour during sampling and sampling will not be conducted during adverse weather conditions.

9. Exotic species - *"The important functions of the wetland shall not be impaired by exotic species" (Special Condition 3.4.b.6).*

   Methods: Exotic species can cause compositional and functional changes in estuarine ecosystems. Such changes can occur, for example, through the alteration of food webs or the

physical structure of habitats (e.g., burrowing activities that affect the stability of tidal channel banks). Monitoring data collected for fish (Section 2.2.1), invertebrates (Section 2.2.2), birds (Sections 2.2.3 and 2.2.8), and plants (Sections 2.2.4, 2.2.5, 2.2.6, and 2.2.7) will be evaluated relative to this standard. In addition, a special survey that covers as much of the wetland as possible that looks for exotic species will be conducted once a year. A list of exotic species that might occur in the wetlands of southern California is provided in Appendix 8.

3. ANALYTICAL METHODS FOR DETERMINING CONDITION COMPLIANCE

3.1 FIXED STANDARDS

The performance standards for topography, tidal prism and habitat areas will be evaluated in comparison to "fixed" values. The performance standard for tidal prism (Section 2.1.3) specifies that the designed tidal prism shall be maintained and tidal flushing not interrupted. For monitoring, a reduction in the monthly spring tidal prism of greater than 10% in comparison with as the designed tidal prism will trigger analysis to determine appropriate maintenance and remediation measures. The performance standard for habitat areas (Section 2.1.4) specifies that the area of different habitats shall not vary by more than 10% from the areas indicated in the final restoration plan. Limited ground surveys to check elevations will be combined with low-level aerial photographs to determine whether the areas of wetland habitats have changed more than 10 % from the as-built elevations measured on completion of construction.

3.2 RELATIVE STANDARDS

In contrast to the fixed performance standards, the performance standards for water quality, fish, invertebrates, birds, salt marsh vascular plants, algae, *Spartina* canopy, plant reproductive success, and food chain support are "relative". The SONGS CDP requires that these wetland attributes have values similar to those of natural wetlands within the region.

3.2.1 REFERENCE SITES

The SONGS CDP specifies that successful achievement of the performance standards will (in most cases) be measured relative to reference sites. The wetlands chosen for reference are required to be relatively undisturbed, natural tidal wetlands within the Southern California Bight. Relatively undisturbed wetlands have minimal human disturbance to habitats (e.g., trampling of vegetation, boating, fishing). Natural wetlands are not constructed or substantially restored. Tidal wetlands are continuously open to the ocean and receive regular tidal inundation. The Southern California Bight extends from Pt Conception to the US/Mexico border. The wetlands chosen as reference sites for this restoration project are Tijuana River Estuary, Mugu Lagoon, and Carpinteria Salt Marsh.

The rationale for requiring that the value of a resource in the restored wetland be similar to that of natural undisturbed wetlands is based on the belief that to be successful the restored

wetland must provide the types and amounts of resources that occur in natural wetlands. Resources in natural wetlands, however, vary tremendously in space and time. Differences in physical characteristics of a wetland (e.g., soil, topography, flood regime, tidal hydrology) can cause plant and animal assemblages to differ greatly among tidal wetlands while seasonal and inter-annual differences in weather, nutrient loading, and oceanographic conditions can cause the biological assemblages within tidal wetlands to fluctuate greatly over time. Ideally, the biological assemblages in a successfully restored wetland should vary in a manner similar to those in the natural wetlands used for reference. Temporal variability, especially of the sort associated with changes in the weather or oceanographic conditions can be accounted for by sampling the restored and natural reference wetlands concurrently. Concurrent monitoring of the natural wetlands will help ensure that regional changes in weather and oceanographic conditions affecting the restored wetland will be reflected in the performance standards, since nearby reference wetlands will be subjected to similar changes in the weather and oceanographic conditions.

3.1.2 METHODS OF EVALUATING SIMILARITY

An important feature of the relative performance standards is that they do not require that the restoration and references sites be identical (e.g., have identical species assemblages or that each species occur in the same abundance). Such a requirement would be unrealistic as attributes vary even among natural undisturbed wetlands. The CCC required only that attributes be similar, in part to avoid making the performance standards too difficult for the restored wetland to achieve. If similarity is defined too stringently, then the restored wetland might be considered a failure even if it is providing high resource values. On the other hand, if similarity is defined too loosely, then the restored wetland could be considered successful even if it failed to substantially increase resource values at the mitigation site.

Judging whether the restored wetland complies with relative standards involves analyses that compare values at the restored wetland to those at the reference wetlands. Unfortunately, there is no single best approach for determining similarity in wetland attributes between the restored and reference sites. In preliminary analyses, Reed and Schroeter (2004) found a high correspondence among 6 methods for determining similarity between artificial (=restored) and reference kelp reefs. One of these methods evaluated whether the average value for a given variable measured at the restored reefs was within the range of the average values for the reference reefs. Based on the correspondence among the different analyses, the "within-the-range" test will be used to determine permit compliance for SCE's kelp reef mitigation program. At present, data are not available with which to conduct similar analyses for the wetland project, but at a minimum, average values in the restored wetland must be within the range of values observed contemporaneously at the 3 reference wetlands. This provisional criterion will be re-evaluated during the initial post-restoration monitoring.

In addition to the "within-the-range" test, a variety of statistical procedures may be used to gain insight into the performance of the restored wetland, including those that incorporate several variables. For example, multivariate analyses (e.g., Nonparametric Multidimensional Scaling, Canonical Discriminant Function Analysis) may be used to assess overall performance of the restoration and to serve as a diagnostic tool to aid in possible remedial

action and adaptive management. A similar approach has been taken to assess convergence of community structure on the experimental and natural control reefs for SCE's kelp reef mitigation program (Reed and Schroeter, 2004). While these analyses may be useful for understanding the nature of the similarity between communities in the restored and reference wetlands, they will not be used to evaluate whether the restored wetland has met the performance criteria.

4. MANAGEMENT OF THE MITIGATION SITE

The SONGS wetland restoration project at San Dieguito Lagoon is only part (albeit an important part) of a larger master plan to restore and enhance the San Dieguito River Valley (JPA, 2000). Restoration of non-tidal wetlands and upland habitat, and provisions for public access and viewing are to be included in the Park Master Plan. Many tasks and programs typically listed in such management plans (e.g., public outreach, watershed management, future land acquisition) are beyond the scope of the SONGS mitigation project, while other tasks (e.g., response to catastrophic events, routine removal of trash and debris, mosquito control) require managed coordinated efforts throughout the entire river park, which is in itself a task that is typically included in the management plans of most ecological reserves. Here, we discuss only those management issues relevant to the SONGS mitigation requirement of creating or substantially restoring 150 acres of tidal wetland that is similar in structure and function to natural undisturbed wetlands in the Southern California Bight.

4.1. INLET MANAGEMENT

SCE has a plan for managing the inlet in perpetuity (SCE 2004). The plan calls for regular dredging of the inlet channel to ensure uninterrupted tidal flushing of the restored wetland and provides conditions that would trigger the need for additional maintenance dredging condition (see Section 2.1.3. Tidal Prism). Data on wetland topography and water level collected as part of the CCC's post-construction monitoring program will be used to determine whether the inlet channel and tidal prism are maintained in an "as designed" condition. If these data indicate substantial sedimentation has occurred in the inlet channel, then maintenance dredging will be implemented to reconfigure the channel to its "as designed" condition.

4.2. TOPOGRAPHY

Topographic degradation of the wetland and levees is likely to occur over time as a result of sedimentation and scour. Topographic data and aerial photographs collected as part of the CCC's post-restoration monitoring program will be used to determine the extent to which the topography of the restored wetland has changed. If these data indicate major topographic degradation has occurred, then the appropriate corrective action (i.e. dredging or sediment deposition) will be done to reconfigure the channel to its "as designed" condition.

4.3. CONTROL OF WEEDS AND OTHER INVASIVE EXOTIC SPECIES

There is a potential for weeds to colonize restored marsh habitats and impede the establishment of desirable marsh species, particularly in areas at high elevations where tidal inundation is less frequent. If in the best professional judgment of CCC staff, invasive exotic species compromise wetland standards and functions, these species will be removed at a frequency that is necessary for marsh plants to become established.

The possibility also exists that exotic marine species will invade lower intertidal and subtidal habitats and usurp resources or destroy sensitive marsh habitat typically used by native species. Examples of such species include the green crab (*Carcinus maenas*), Asian mussel (*Musculista senhousia*), isopod (*Sphaeroma quoyanum*), and a green macroalga (*Caulerpa prolifera*), which have invaded several coastal wetlands in California. Unfortunately, controlling the spread of exotic marine species is extremely problematic. The topic of invasive species control in marine environments has received considerable attention in recent years and currently is the subject of several ongoing research programs in California and elsewhere. If exotic marine species are found in the restored wetland, then experts working in this field will be consulted and a program to control the spread of these species will be developed using the most current information.

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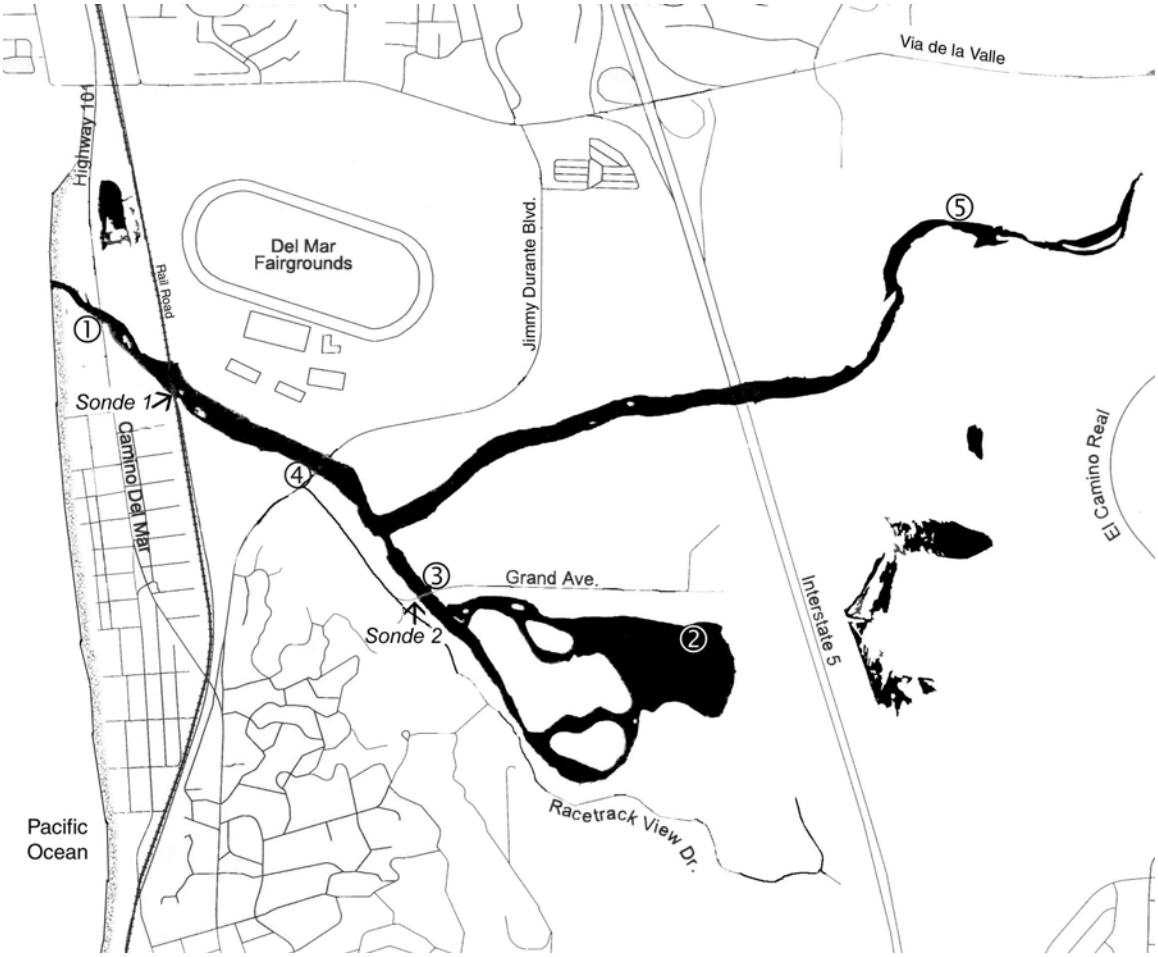
Appendix 1

Locations of water quality monitoring stations in San Dieguito Lagoon. Numbers 1-5 indicate sites at which bi-weekly dawn measurements of water temperature, salinity, and dissolved oxygen have been taken since October 2, 1994. Sonde 1 and Sonde 2 indicate stations at which water temperature, salinity, dissolved oxygen and water surface level have been measured every 15 minutes since March 24, 2000 (Sonde 1) or since June 8, 2001 (Sonde 2).



Appendix 2

Methods for sampling gobies with enclosure traps in southern California wetlands

Mark Steele, Steve Schroeter, Mark Page, and Dan Reed

Marine Science Institute
University of California
Santa Barbara, CA 93106

Background

Southern California Edison is required by its coastal development permit for Units 2 and 3 of the San Onofre Nuclear Generation Station (SONGS) to create or substantially restore a minimum of 150 acres of southern California wetland. San Dieguito Lagoon was selected as the site that best meets the permit's requirements and objectives. The success of the restoration of San Dieguito Lagoon will be judged relative to several performance standards. Many of these performance standards require that various attributes of the restored wetland be similar to those of 3 to 4 reference wetlands in southern California that are tidally influenced and relatively undisturbed. One of the attributes of the restored wetland that must be similar to reference wetlands is the abundance of fish (section 3.4b.1 of the SONGS permit). Another attribute is wetland function, a key component of which is food chain support.

Gobies (family Gobiidae) are often the most abundant of fishes in southern California wetlands and they are key players in estuarine food chains, linking secondary consumers (e.g., small benthic and planktonic invertebrates) with higher trophic levels, such as larger fish (e.g., California halibut) and birds (e.g., egrets). Because of their relatively small size and propensity for burrowing, gobies are difficult to sample with the gear most commonly used to sample fish in southern California (e.g., beach seines, trawls, and purse seines). Based on data gathered for previous monitoring programs in San Dieguito Lagoon and from work in Batiquitos Lagoon, these methods may underestimate goby density by one or two orders of magnitude, or more (MEC Analytical Systems, Inc. 1993, Merkel & Associates, Inc. 1999).

The goal of the work presented here was to develop a method for sampling gobies that provides relatively accurate and representative estimates of density. Additionally we strove to develop a cost effective sampling method that minimizes negative impacts to the wetland (e.g., mortality of fish and invertebrates, trampling of vegetation, damage to banks).

A review of the literature and communications with wetland fish biologists identified enclosure "traps" as the sampling gear most likely to meet our goals. The rest of this report focuses on these sampling devices. The term "enclosure traps" refers to a variety of sampling devices variously know as drop traps, throw traps, drop samplers, and enclosure traps (Kushlan 1974, 1981, Chick et al. 1992, Rozas and Minello 1997). The unifying characteristic of these devices is that they rapidly enclose a known volume of water, trapping any fish (or other mobile animals) within them. The trapped fish can then be removed from the enclosed area with any of a variety of nets at the leisure of the investigator, without them escaping. Enclosure traps should not be confused with passive traps, which capture animals by attracting them from surrounding areas with bait (typically food or conspecifics). Minnow traps are an example of such passive traps and they are commonly used to sample fish in southern California wetlands (e.g. Talley 2000). While very useful for specific purposes, passive traps that rely on attraction to capture animals are not well suited to estimating densities, because the "area of attraction" is not usually known, it may change with environmental conditions, and it varies among species. Moreover, passive traps typically work well for only a very limited number of species.

In southern California, work with enclosure traps has focused on collecting accurate samples of gobies, all of which are bottom dwellers that will use borrows in the substrate for shelter and as nest sites. This benthic lifestyle makes gobies less accessible to nets than mid-water species are. Consequently, most workers in southern California have applied the ichthyocide rotenone (e.g., Merkel & Associates, Inc. 1999, Allen et al. 2002) or an anesthetic such as quinaldine (e.g., Horn and Allen 1985) to the water within the enclosure to drive gobies from their burrows. Use of such chemicals complicates sampling and can negative affect wetlands. Rotenone, for example, kills the fish sampled and kills many invertebrates (Cushing and Olive 1957, Anderson 1970). Moreover it is toxic to humans, especially if inhaled in power form, and it is a regulated pesticide in many places, including California. One of the key components of this study was to test whether gobies could be adequately sampled without the use of chemicals.

Methods, Materials, and Results

In order to develop an effective and efficient sampling method with minimal impacts to wetlands, we conducted a series of experiments to answer the following questions:

- Does enclosure size affect estimates of density?
- Do gobies avoid capture by sheltering in burrows?
- How much sampling effort must be expended per enclosure sample to obtain a representative estimate of density?
- What is the catch efficiency of enclosure traps? (In other words, what fraction of the fish within an enclosed area is captured?)

Does enclosure size affect estimates of density?

Methods and Materials

We evaluated the influence of enclosure size on the accuracy and the precision of estimates of goby density. The *accuracy* of the density estimate could be affected if the probability of either a fish avoiding an enclosure as it is deployed or escaping from the enclosed area is a function of enclosure size. The *precision* of the estimate (or sample variation) is influenced by spatial and temporal patterns of abundance. For example, if the enclosure is smaller than the average patch size, then samples will tend to fall either inside or outside of patches of fish, generating high levels of variability among samples. In contrast, if the enclosure size is larger than patch size, then areas of high and low density (in and out of patches, respectively) will be averaged within each enclosure, reducing variability among samples.

The size of the enclosure could also influence the shape of the distribution of data. For example, if fish distribution is patchy and enclosure size is smaller than patch size, or if densities are uniformly low, data sets generated by sampling with small enclosures will be dominated by zeros. This is an undesirable feature for statistical analysis because it makes statistically powerful and flexible parametric tests inappropriate.

For the reasons just described, large enclosures should be preferred over small ones. These reasons, however, are largely statistical and in practice must be balanced with logistical constraints. The main logistical constraints are that large enclosures are unwieldy and difficult to

use, and when fish are abundant, large enclosures may capture so many fish that sample-processing time becomes limiting.

To evaluate the effects of enclosure size, we conducted a field experiment in late May and early June 2002. For this experiment we sampled fish using enclosures of 3 sizes: 0.25 m^2, 0.5 m^2, 1.0 m^2 area sampled. These 3 sizes spanned the range of potentially useful sizes that could be deployed by a single person. At the small end of the scale, it seemed possible that the 0.25-m^2 enclosure would sample such a small area that data sets generated from it might be dominated by zeroes. On the other extreme, the 1.0 m^2 enclosure was the largest size that a single person could deploy – and then sometimes with difficulty and ineffectively.

All enclosures were 0.9 m high, cylindrical in shape, and built of 3-mm thick sheets of translucent (white) polypropylene plastic (see Table 1 for a summary of dimensions and specifications and Fig. 1 for an example). Depending on the size of the enclosure, one or two sheets of plastic were formed into a cylinder with seams that overlapped by 10 cm, which were sealed by 5 pairs of evenly spaced, stainless steel bolts and wingnuts. These fasteners were removable and allowed the enclosures to be transported and stored as flat sheets, saving space. When sealed with fasteners in cylindrical form, the enclosures were essentially impermeable to water.

We used polypropylene because it has the necessary combination of flexibility (needed to form it into a cylinder), rigidity (needed to support itself as a cylinder), durability, and affordability. It is, however, less dense than water, which causes it to float – an undesirable characteristic for a device that must remain on the bottom while sampling is conducted. We compensated for this characteristic by bolting a pair of lead weights onto opposite sides of each enclosure, about 10 cm above the bottom of the cylinder. These weights kept the enclosures on the bottom while sampling and, by lowering the center of gravity of the enclosures, improved their stability. Placing the weights 10 cm above the bottom of the enclosure kept them from interfering with the "sealing" of the enclosure, which was accomplished by pushing the bottom edge about 5-10 cm into the substrate. This technique was intended to keep fish from escaping through gaps between the bottom of the enclosure and the substrate. Enclosures were deployed by throwing them about 1 – 2 m from the investigator, who then followed and pushed the bottom edge of the trap into the sediment to seal it.

In this experiment, fish were removed from each enclosure with 2 types of nets: first with a BINCKE net (Anderson and Carr 1998) and later with a long handled dipnet. The BINCKE net has a rectangular, hinged frame that folds in the middle. We built our nets with frames that when folded were 1/2 the inner diameter of the enclosure. Thus, when they were unfolded, their width was the same as the diameter of the enclosure. They could therefore be run along the sides of the enclosure, minimizing net avoidance by trapped fish. Nevertheless, it sometimes took several sweeps to catch all the fish that could be caught with these nets.

BINKE net frames were made of two stainless steel rods (8-mm diameter), each bent into the shape of one half of a rectangle. The two bent rods were connected with two sleeves of flexible plastic tubing that served as hinges, completing the rectangular frame. This frame was covered with knotless nylon netting (1.6-mm mesh). When sampling, the stainless steel net frames were

pushed a few cm into the surface of substrate, which probably helped capture burrowing gobies. Sediments brought up in the BINKE net were rinsed through the net before fish were removed.

For this study, we swept each enclosure with a BINCKE net until 6 consecutive passes captured no fish. To be certain that no fish had been missed by the BINCKE net, we swept the enclosure with a long-handled dipnet (≈ 1-m handle and 0.4 x 0.3 m frame) until 5 consecutive passes with the dipnet captured no fish.

We tested for differences among the 3 sizes of enclosures by deploying them simultaneously in blocks containing one enclosure of each size (i.e., an unreplicated, randomized block design). Within each block, the 3 enclosures of different sizes were spaced 1 m apart and their positions within the block were assigned randomly. This study was conducted at 3 lagoons in San Diego County: San Dieguito, Batiquitos, and Los Peñasquitos. Samples were taken at one or two stations in each lagoon, with 3 – 5 blocks of enclosures sampled at each station. Stations within lagoons were categorized as near or far from the ocean inlet. The sampling design is summarized in Table 2. Estimates of mean density were compared among enclosure sizes, lagoons, distance from inlet, and blocks with a mixed-model, nested analysis of variance (ANOVA design and results are summarized in Table 3).

Results

The smallest enclosures (0.25 m^2) significantly underestimated goby density relative to the two larger enclosures (0.5 and 1.0 m^2), but density estimates from the two larger enclosures did not differ significantly (Table 3, Fig. 2). Density estimates from the smallest enclosure were only about 64% of those from the two larger enclosures. Small enclosures also produced datasets composed of more zeros than larger enclosures: 31, 19, and 6% of all values were zeros for small, medium, and large enclosures, respectively. Similarly, the variability (CV) of abundance estimates declined with enclosure size, from 88 to 69 to 45% for small, medium, and large enclosures, respectively.

The largest size enclosure traps (1.0 m^2) produced data that were most suitable for parametric statistics, however, these traps were logistically very difficult to use. The most significant constraint was that it was difficult to seal the bottom of the enclosure in areas where the substrate was not extremely flat. This constraint limits the habitat types in which the large enclosure can be used. It was also difficult to deploy the large enclosure properly (e.g., it would fail to maintain its cylindrical shape). These two problems led to a higher failure rate per deployment and consequently the large enclosures often had to be re-deployed. It was also considerably more difficult to transport the large enclosure in the field, and processing time of samples was often high due to large catches.

Considering these logistical constraints, we concluded that an intermediate size enclosure offered an acceptable compromise between logistical and statistical considerations. Polypropylene sheets are readily available in 2.4 m lengths, but not longer. Consequently, we used enclosures with a circumference of 2.3 m (i.e., a single 2.4 m sheet with 0.1 m overlap on the seam encompassing a sample an area of 0.4 m^2) for subsequent studies.

Do gobies avoid capture by sheltering in burrows?

Methods and Materials

Since gobies in southern California wetlands associate closely with the substrate and will use burrows in it for shelter, there is a widespread belief that chemicals (e.g., rotenone or quinaldine) must be used to obtain accurate estimates of density because this poison drives gobies out of their burrows (Horn and Allen 1985, Merkel & Associates, Inc. 1999). As noted earlier, however, using such chemicals has several serious drawbacks. We tested whether our sampling missed gobies that were sheltering in burrows.

To determine whether significant numbers of gobies evaded capture by nets when chemicals were not used, we first sampled enclosures intensively with nets and then took sediment cores in the sampled area and searched these for gobies. For this work, we sampled in San Elijo Lagoon on August 1 and at San Dieguito Lagoon on August 13. Sixteen enclosures were sampled in each lagoon and these were arranged in 4 blocks of 4 replicates. Blocks were separated by 5 m, and within blocks, enclosures were placed 1 m apart in a line parallel to shore, at depths of about 50 to 70 cm. We used enclosures that sampled a footprint of 0.4 m^2 (Table 1).

We sampled each enclosure with a BINCKE net until 3 consecutive passes with no fish had been obtained and then sampled with a long handled dipnet until we had obtained 5 consecutive passes with no fish. At that point we took 8 benthic cores (10-cm diameter) in each enclosure to a depth of about 0.5 m to determine whether our netting had failed to capture any gobies hiding in burrows. The cores were dissected by hand to check for gobies in burrows and surface sediments were separated from deeper sediments containing burrows. All sediments were sieved through 3-mm mesh.

Results

We found little evidence that gobies evaded capture by hiding in burrows. We found only 2 gobies in the 256 cores that we took and both of these were on the surface of the sediment, not in burrows. Individually, our cores sampled a small surface area (0.008 m^2), but in total we sampled 2.1 m^2 of bottom surface area with them. Based on the average density of gobies captured in nets from all 32 enclosure traps, about 232 gobies would have been expected to be captured in an area the size of that sampled by our 256 cores. These figures suggest that our netting technique captured about 99.1% of all gobies present in the enclosures. This calculation assumes that any fish missed by the nets would have been captured in cores, which may not by true, but our goal in this study was to determine if gobies were evading capture by retreating to burrows. We found no evidence that they were. In a study described later, we evaluated capture efficiency with a mark-recapture technique.

<u>How much sampling effort must be expended per enclosure sample to obtain a representative estimate of density?</u>

Methods and Materials

The sampling technique in the preceding study was relatively time consuming because of the conventions we used to determine when to stop netting within enclosures. We sampled with a BINCKE net until we captured no fish in 3 consecutive hauls and then we sampled with a dipnet

until 5 consecutive hauls produced no more fish. On average, it took 8.2 (±0.5) BINCKE net hauls, 5.8 (±0.3) dipnet hauls, and about 30 minutes of work by two people to reach this stopping point.

To determine whether sampling could be streamlined without sacrificing accuracy or precision, we evaluated the effect of eliminating dipnetting and reducing the number of fishless BINCKE net hauls on the mean and variability of our density estimates and capture rate. Specifically we compared the mean and coefficient of variation (CV) of goby densities for the following stopping rules: (1) our original rule, stop after 3 consecutive BINCKE hauls with no fish + 5 consecutive dipnet hauls with no fish; and the following scenarios with no dipnetting; (2) stop after 3 *consecutive* BINCKE hauls with no fish; (3) stop after 3 BINCKE hauls with no fish (need not be consecutive hauls); (4) stop after 2 BINCKE hauls with no fish; (5) stop after 1 BINCKE haul with no fish; (6) make at least 3 BINCKE hauls regardless of whether fish are caught and stop when 2 hauls have produced no fish; and (7) make at least 3 BINCKE hauls and stop when 2 *consecutive* hauls have produced no fish.

Results

The various stopping rules had relatively little effect on the estimate of the mean and the CV (Fig. 3a). Eliminating dipnetting after BINCKE netting had a trivial effect, reducing the estimate of the mean by <0.8% and increasing the CV by 1.0% (Fig. 3a). Ceasing netting after the first fishless BINCKE haul had the greatest effect, reducing the estimate of density by 6.4% and increasing the CV from 122% to 132%. This rule had a large effect on netting effort, reducing the average number of BINCKE hauls from 8.2 BINCKE hauls to 4.3 (Fig. 3b).

Although terminating netting after the first fishless haul had relatively little effect on the estimate of goby density, it had a more pronounced effect on the capture rate of fish in individual enclosures. We calculated capture rate by using the number of fish captured by the combination of BINKE and dipnetting (our original stopping rule, #1 above) as our estimate of the true number of fish present and then calculated the percentage of those fish that would have been captured if the various stopping rules had been employed instead. This calculation was made for each replicate. By definition then, our rule #1 had a capture rate of 100% with a CV of 0%. For the stopping rule that required the least effort, "stop after the first fishless BINCKE haul" (rule 5 above), the average capture rate was 82% and quite variable (CV = 38%), with 0 – 100% of the catch obtained by the first fishless haul. All rules requiring at least 3 BINKE hauls (rules 2, 3, 6, & 7 above) performed similarly, with capture rates >90% and CV's around 20% (Fig. 3b).

One gets a different impression of the efficacy of the various stopping rules depending on whether density estimates or catch rates are compared: all rules provided similar estimates of mean density, but some produced distinctly lower and more variable catch rates. This difference was generally caused by mathematical phenomena. When goby densities were high (>35 per enclosure, $n = 9$ samples), the vast majority of fish (>95% in all cases) in each enclosure were captured before the first fishless haul. These densely populated samples had a disproportionately large effect on the estimate of mean density, but they were weighted equally to sparse samples in calculating mean catch rates. Due to the binomial nature of fish capture (caught or not), the first haul in an enclosure with few gobies was more likely to catch no fish than was the first haul in an

enclosure containing many gobies. Hence, capture rates of 0% at the first fishless haul were more common in areas with low densities of gobies than in high-density areas. Similarly, low-density samples were more likely to produce a fishless haul when one or more fish were still present in the enclosure. These missed fish had a relatively large effect on the proportion caught in low-density samples compared to high-density samples.

What is the catch efficiency of enclosure traps?

Methods and Materials

The results presented above indicated that the catch efficiency of enclosure traps was very high. This interpretation hinges on the assumption that gobies missed by nets were still in the enclosure and recoverable by cores (i.e., they were either in burrows or on the sediment surface and could not evade capture by cores). This assumption may not be true, but if it was, then about 99% of gobies in enclosures were captured by the combination of BINCKE and dip nets. If dipnetting was eliminated and BINCKE netting stopped after 3 hauls with no fish, then the catch rate was still about 98%. If gobies missed by nets were not recoverable by cores, however, then these catch rates are overestimates.

To estimate catch rates directly, we conducted a mark-recapture study. Gobies (*Clevelandia ios, Gillichthys mirabilis, Quietula y-cauda,* and *Acanthogobius flavimanus*) were tagged with subcutaneous injections of non-toxic acrylic paint in their dorsal musculature. These marks were visible through the skin. Ten to 23 tagged gobies were introduced into each enclosure (0.4 m^2). Enclosures were placed in undisturbed areas that were 27 – 57 cm deep. After sealing the bottom of the enclosure by forcing it into the substrate, the tagged gobies were released and given 5 – 10 minutes to redistribute themselves within the enclosure before netting began. Only BINCKE nets were used, and we ceased netting after 3 hauls produced no fish. Enclosures were sampled in San Elijo Lagoon and Los Peñasquitos Lagoon. There were 2 stations at San Elijo and 3 at Los Peñasquitos. At each station 4 – 6 enclosures were sampled (see Fig. 4 for numbers of replicates per station). Stations were chosen to represent a range of microhabitat types, from firm, sandy substrate with little live or dead vegetation to soft and muddy substrate with considerable quantities of live and dead vegetation. Sampling was conducted on 4 days in April and May 2003.

Results

Overall, recapture rate was high, averaging 92 ± 2% (mean ± 1 SE), and fairly consistent, ranging from 77 – 100% (CV = 8%). There was no significant difference in recapture rates among stations or wetlands (Table 4, Fig. 4), indicating that variation in habitat type had little influence on recapture rates. There was an indication that some species of gobies were sampled slightly better than others (Table 5), but except for *Clevelandia ios* sample sizes were small, making conclusions about differences among species unreliable.

Summary and Conclusions

The work described in this report demonstrates that enclosure traps are extremely effective for sampling gobies in southern California wetlands. Because gobies are such an important component of the fauna of these wetlands, they should be routinely sampled, but the most widely used methods for sampling wetland fish are inefficient at sampling gobies (e.g., see data

contained in MEC Analytical Systems, Inc. 1993). Although enclosure traps have been used in several studies in southern California, currently they are seldom used in wetland monitoring studies. This situation probably exists because other methods are less selective and sample larger areas per sample, and thus catch greater numbers of fish of a wider variety of species (e.g., Horn and Allen 1985). Furthermore, we suspect that the enclosure trapping that has been done has been relatively inefficient due to poor design of the sampling apparatus (enclosure and nets; e.g., see Horn and Allen 1985). This trap inefficiency combined with species selectivity and the widespread belief that chemicals (e.g., rotenone) must be used to sample enclosure efficiently has probably discouraged many workers from using enclosure traps.

Our work indicates that rotenone or other ichthyocides or anesthetics are not required to obtain accurate estimates of goby density from enclosure traps. Since they are not needed to obtain accurate estimates, yet they pose risks to the environment and personnel, require additional permitting, and complicate the logistics of field sampling, we recommend they not be used. Avoiding use of chemicals eliminates one of the impediments to using enclosure traps.

The design of the sampling apparatus is critical for it to sample effectively and at a reasonable cost. Key elements of the design include: (1) Material for the enclosure walls that is thin and flexible, yet rigid enough to cut into the substrate to seal the enclosure and prevent fish from escaping. (2) Material that is lightweight to ensure portability in the field. (3) Nets that fit snugly within the enclosure to minimize net avoidance. We know of no study in southern California that has included all of these design elements.

We found that the size of enclosure traps affected estimates of mean goby density and the variability of these estimates, as well portability and ease of use in the field. The largest enclosures we used (1.0 m^2) provided the highest and least variable estimates of goby abundance. These estimates, however, did not differ significantly from those from 0.5-m^2 enclosures, which were more portable and easier to deploy in the field. We recommend using 0.4-m^2 enclosures because they are only slightly smaller than 0.5-m^2 enclosures, yet easier to build and less expensive. We used enclosures that were 0.9-m high and recommend this size: much shorter or taller enclosures would be difficult to sample effectively. This height limits enclosure traps to relatively shallow waters, but these are the areas where gobies are most abundant.

In our study, the choice of convention on when to stop netting within enclosures had a considerable effect on the time and effort (judged by number of net hauls) needed to complete sampling of an enclosure. The choice of stopping rule, however, had relatively little effect on the estimate of mean density and its variability. Taken together, these findings suggest that the stopping rule requiring the least netting (stop after the first fishless haul) should be used. This suggestion must be tempered by the finding that goby density influenced the performance of the various stopping rules. At low densities, stopping at the first fishless haul caused serious underestimation of density and caused catch efficiency to become highly variable. Hence, we recommend either of two sampling strategies: use a conservative stopping rule (e.g., stop after 3 fishless hauls); or use stopping rules that are flexible and change in response to goby density. For example, based on our data, at densities above 25 gobies per 0.4-m^2 enclosure, terminating BINCKE netting when the first fishless haul will capture about 97% of gobies that can be captured. At densities below 25 fish per 0.4-m^2 enclosure, 3 fishless hauls must be made to

obtain a similar capture rate (95%). A flexible sampling strategy will use sampling time more efficiently than an inflexible one because it takes little time to make extra net hauls in areas where gobies are sparse and few extra hauls will be needed to obtain two more fishless hauls. By contrast, in densely populated areas, many extra hauls are often needed to obtain more fishless ones – yet these extra hauls have only a trivial effect on the density estimate because relatively few additional fish are caught in each one. Flexible rules, however, can be difficult to implement in the field, in which case a conservative stopping rule (3 zeros) should be used to provide relatively unbiased estimates across the full range of densities.

We estimated catch efficiency (% of fish present in an enclosure that were captured) in two ways: by assuming that extensive netting and coring would provide an accurate estimate of the total number of gobies present in an enclosure, and by marking and recapturing fish released into enclosures. Both methods indicated that catch efficiency was high, but the two estimates differed somewhat. The mark-recapture study recovered about 92% of gobies placed in enclosures, whereas the other study indicated that about 98% of all fish in an enclosure were recaptured. The difference is slight, but in an unexpected direction: we expected to have higher recovery rates of marked fish added to enclosures than of fish naturally present in the enclosures. We had this expectation because the tagged fish were not familiar with the locations of burrows in the enclosure and therefore seemed likely to be less able to escape our nets. This assumption may be false, but if it were, then our two estimates of catch efficiency should have been the same. We suspect that the true cause of the difference between the two estimates is that some gobies escape from the enclosures beneath the bottom edge because it does not seal perfectly. Such escapees would be noticed in the mark-recapture study, but not in the other study. Nevertheless, a catch efficiency of around 90% is more than adequate for the needs of the long-term monitoring of San Dieguito Lagoon. Overall, this study indicates that enclosure traps are excellent tools for sampling gobies in southern California wetlands.

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Table 1. Enclosure trap dimensions and specifications.

Area sampled (m^2)	Diameter (m)	Circumference (m)	Height (m)	Mass of lead weights (kg)	# of sheets of plastic used*
0.25	0.56	1.8	0.9	1.8	1
0.4	0.71	2.3	0.9	1.8	1
0.5	0.80	2.5	0.9	1.8	2
1.0	1.13	3.5	0.9	2.7	2

* Standard sheet size available is 2.4 x 1.2 m x 3 mm

Table 2. Summary of sampling design for test of effects of enclosure size.

Lagoon	Stations per lagoon	Blocks* per station	Enclosure sizes per block (n = 1 of each)
San Dieguito	near inlet (1)	3	0.25, 0.5, 1.0
	far from inlet (1)	3	0.25, 0.5, 1.0
Los Batiquitos	near inlet (1)	5	0.25, 0.5, 1.0
	far from inlet (1)	4	0.25, 0.5, 1.0
Los Peñasquitos	near inlet (1)	3	0.25, 0.5, 1.0

* There was no replication within blocks.

Table 3. Summary of analysis of variance (ANOVA) testing for effects of enclosure size on the estimate of mean goby density[†].

Factor	Effect type	SS	df numerator, denominator	MS	Error term	*F*	*P*
Enclosure size (S)	fixed	2.51	2, 4	1.25	S x L	26.7	**0.005***
Lagoon (L)	random	37.16	2, 10	18.58	B(L)	11.4	**0.003**
Inlet Distance (D)	fixed	1.67	1, 26	1.67	residual	1.6	0.215
Block(Lagoon) (B(L))	random (nested)	16.28	10, 26	1.63	residual	1.6	0.170
S x L	NA	0.19	4, 26	0.05	residual	<0.1	0.996
S x D	NA	2.62	2, 26	1.31	residual	1.3	0.298
residual		26.88	(26)	1.03			

[†] Goby density was transformed to ln(x+1) to satisfy the assumptions of homogeneity of variance and normality.

* Post hoc Tukey HSD tests revealed that 0.25-m^2 enclosures significantly underestimated density relative to 0.5-m^2 ($P = 0.015$) and 1.0-m^2 ($P = 0.005$) enclosures, but estimates from 0.5- and 1.0-m^2 enclosures did not differ ($P = 0.24$).

Table 4. Summary of ANOVA testing for differences in the recapture rate (%) of tagged gobies in enclosure traps among stations within two lagoons.

Factor	Effect type	SS	df numerator, denominator	MS	***F***	*P*
Lagoon*	random	110.0	1, 3	110.0	0.91	0.41
Station(Lagoon)	random (nested)	364.2	3, 19	121.4	2.44	0.10
residual	NA	943.6	(19)	49.7		

* Tests for 'Lagoon' effect use the nested 'Station(Lagoon)' term as the error term.

Table 5. Total numbers of tagged gobies released, recaptured, % recaptured, and range of sizes used in study of enclosure-trap catch efficiency.

Species	# Released	# Recaptured	% Recaptured	Range of sizes used (mm SL)
Clevelandia ios	264	245	92.8	15 – 40
Gillichthys mirabilis	18	16	88.9	27 – 104
Quietula y-cauda	14	10	71.4	23 – 46
Acanthogobius flavimanus	6	6	100.0	59 – 85
All species combined	302	277	91.7	15 – 104

Figure Legends

Fig. 1. Sampling with a BINCKE net inside a 0.4-m^2 enclosure trap.

Fig. 2. Mean densities (±1 SE) of gobies captured in enclosure traps of three different sizes (n = 16 for each bar).

Fig. 3. Effects of using different "stopping rules" when terminating netting within enclosure traps. Top panel shows differences in estimates of mean density of gobies (± 1 SE) and the coefficient of variation (CV) of those estimates for each stopping rule. Bottom panel shows for each stopping rule (1) the mean (-1 SE) % of the total number of gobies captured, calculated on a *per replicate basis*, (2) the CV of this mean, (3) the % of gobies captured when all data were *pooled*, and (4) the mean (± 1 SE) number of BINCKE net hauls required to reach each stopping point. All data points are based on n = 32 enclosure samples.

Fig. 4. Mean rates of recapture (% recovered ± 1 SE) of tagged gobies placed inside enclosure traps at 5 different stations in 2 wetlands: San Elijo Lagoon (SEL) and Los Peñasquitos Lagoon (LPL). Sample size is given above each bar.

Fig. 1

Fig. 2

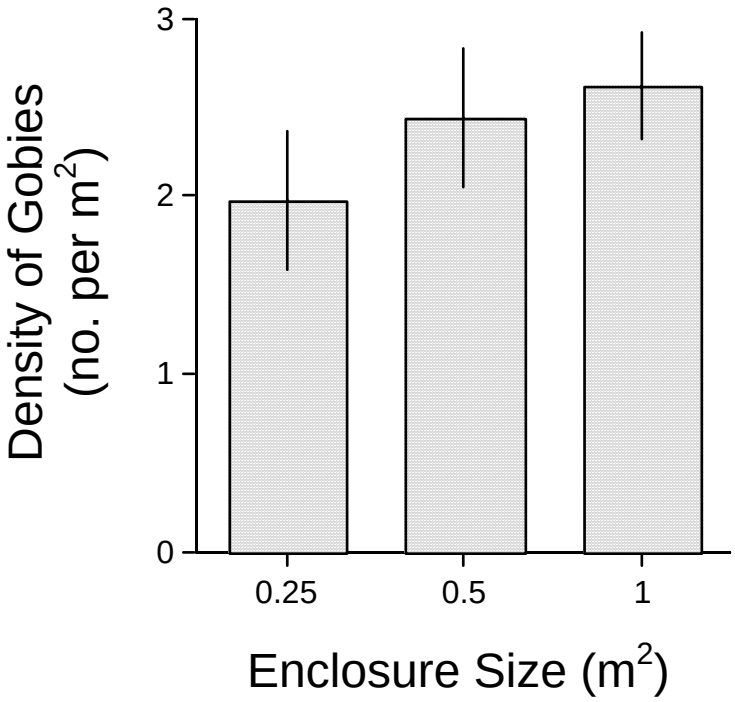


Fig. 3

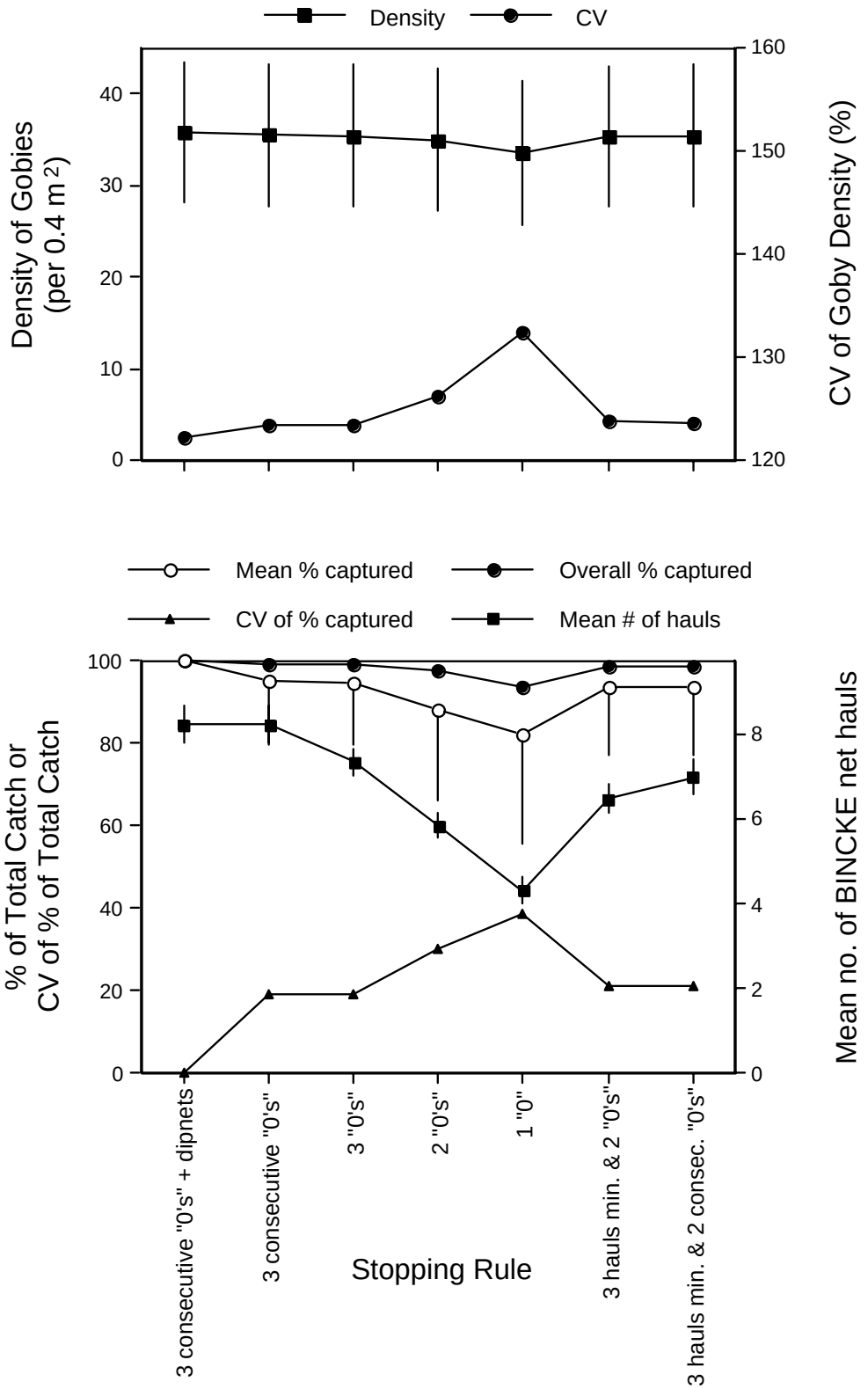
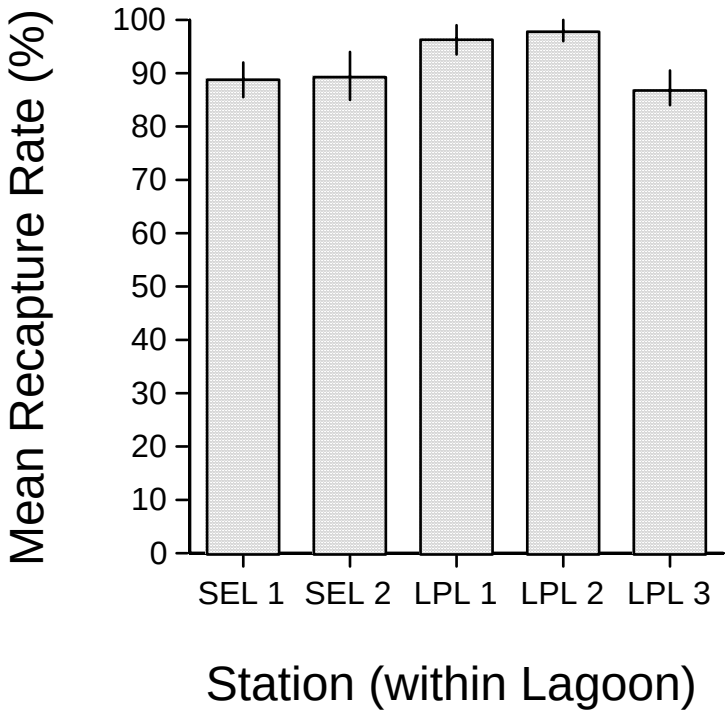

Density
CV
Density of Gobies (per 0.4 m 2)
CV of Goby Density (%)
0
10
20
30
40
120
130
140
150
160
Mean % captured
Overall % captured
CV of % captured
Mean # of hauls
% of Total Catch or CV of % of Total Catch
Mean no. of BINCKE net hauls
0
20
40
60
80
100
0
2
4
6
8
3 consecutive "0's" + dipnets
3 consecutive "0's"
3 "0's"
2 "0's"
1 "0"
3 hauls min. & 2 "0's"
3 hauls min. & 2 consec. "0's"
Stopping Rule

Fig. 4


Mean Recapture Rate (%)
100
90
80
70
60
50
40
30
20
10
0
SEL 1
SEL 2
LPL 1
LPL 2
LPL 3
Station (within Lagoon)

Appendix 3

Variation in estimates of species richness and density of wetlands fish caused by different methods of purse and beach seining

Mark Steele, Steve Schroeter, Mark Page, and Dan Reed

Marine Science Institute
University of California
Santa Barbara, CA 93106

DRAFT
May 16, 2005

Background and motivation for the study

The coastal development permit for SONGS Units 2 and 3 requires Southern California Edison to create or substantially restore a minimum of 150 acres of coastal wetland. The permit establishes biological performance standards that must be met by the restored wetland. One of these standards requires that within 4 years of construction, the total densities and number of species of fish be similar to reference wetlands (section 3.4b.1 of the SONGS permit).

A wide array of methods have been used to sample fish in southern California wetlands (Table 1; Horn and Allen 1985, Allen et al. 2002), yet no standardized sampling methodology exists. The same is true of wetlands in other areas (Rozas and Minello 1997). This situation likely exists because no single method is efficient and effective at sampling all species in all habitats found within wetlands (Horn and Allen 1985). Though no single method efficiently and effectively samples the entire assemblage of fishes found in southern California wetlands, a combination of methods may provide good estimates of species richness and density. Three methods in particular, when used in combination, should provide good estimates of richness and density: beach seines, purse seines, and enclosure traps. Beach and purse seines likely capture all fish species found in southern California wetlands, though their catch efficiency may not be high for all species. For estimating species richness, Merkel & Associates (2002) found that purse seines caught the most species of any method they used, though only slightly more species than either beach seines or otter trawls. Beam trawls and enclosure traps caught many fewer species. Moreover, they found that density estimates obtained with purse seines, beach seines, and enclosure traps were fairly similar and more than an order of magnitude higher than those obtained with beam and otter trawls.

Small beach seines attached to poles on either end and pulled with these poles by two persons ("two-person pole seines") are well suited to sampling relatively shallow areas (< 1.5-m depth) within 30 m of shore, which constitute much of the subtidal habitat in southern California wetlands. Small purse seines are best suited to areas at least 1-m deep (to allow deployment by boat) and they can be used to sample areas both deeper and farther from shore than pole seines. In combination, these two methods can sample most or all species of fish in the vast majority of habitats in southern California wetlands. Both, however, have a major shortcoming: they vastly underestimate the density of small, benthic species (mainly gobies, family Gobiidae; see data in Merkel & Associates 2002), which are a very abundant and important component of the fish fauna in these wetlands. This shortcoming of seines necessitates the use of enclosure traps to obtain accurate estimates of the density of small benthic fish and thus the total density of wetlands fish. Sampling characteristics and guidelines for construction and use of enclosure traps are provided in Appendix 2. Here we explore the sampling characteristics of small beach and purse seines.

Specifically, the objectives of this study were to 1) determine optimum size and configuration of purse and beach seines; 2) determine whether blocking nets are necessary for effective beach seining; 3) determine the optimum number of beach seine hauls within blocked areas to balance accuracy and effort; and 4) estimate catch efficiency of beach seines. We strove to develop cost effective methods that minimize negative impacts to wetlands (e.g., by minimizing trampling of

vegetation, damage to banks and subtidal substrates, and mortality of captured fish) while providing representative and precise estimates of species richness and density of fishes.

Methods, Materials, and Results

We conducted a series of experiments to answer the following questions:

- Does net length and configuration affect estimates of fish density and richness?
- Are blocking nets necessary to obtain accurate or representative estimates of fish density and richness with beach seines?
- How many hauls with a beach seine within a blocked area must be made to obtain a representative estimate of density and richness?
- What is the catch efficiency of beach seines? (Specifically, what fraction of fish within a blocked area is captured?)

Does net length and configuration affect estimates of fish density and richness?

Purse seines

Methods and Materials

Purse seines are long walls of netting with a buoyed top line (float line), a weighted bottom line (lead line), and a line of rings hanging off the lead line through which runs a rope (purse line). A purse seine is deployed (normally by boat) roughly in a circle, so the two ends meet. The purse line is then pulled taught, bunching or "pursing" the bottom of the net so that fish cannot escape out the bottom of the net. The rest of the net is then pulled in until fish in are bunched into a small portion from which they can be readily retrieved.

Purse seines have long been used in fisheries and many variants exist. Purse seines used for commercial fishing are often large (usually > 1 km long and 200-m deep), and commonly used to capture schooling pelagic fish (e.g., sardines, mackerel, and tuna). Hunter et al. (1966) modified the design of commercial fishing purse seines to make a much smaller net more appropriate for ecological sampling. Since then, many biologists have used small purse seines that are variants of the traditional commercial design to sample fishes. Some of these variants have been used to samples fish in southern California wetlands (e.g., Allen et al. 2002, Merkel & Associates 2002).

How variation in the design of purse seines affects estimates of density and species richness of fishes is relatively unexplored, but crucial to the interpretation of data gathered with them. Several conflicting factors must be balanced to produce a net that achieves the purpose of providing representative estimates of richness and density. For example, long nets that sample large areas are likely to produce more precise (i.e., less variable) estimates of density because they average out small-scale spatial patchiness in fish distribution; yet this comes at the cost of decreased spatial replication of samples due to long sample collecting and processing times. Similarly, building purse seines with netting of large mesh size produces nets with less material per unit area, making them easier to deploy and retrieve (due to reduced weight and resistance in the water). This gain in ease of use comes at the cost of increased escapement of small fish through the net, which reduces the accuracy of density estimates.

We compared species richness and density estimates obtained from two different sized purse seines (Table 2). Both used small-mesh (3.2 mm), knotless, nylon netting – the same size mesh as that used in standard beach seines in southern California wetlands – both to reduce escapement through the mesh and to facilitate comparison between samples taken with purse and beach seines. The lengths and depths of the nets were constrained by our need to sample relatively narrow (≥ 15 m) and shallow (≥ 1 m) bodies of water. The longest net we could use was about 36 m (roughly 12 m in diameter when set in a circle). Given these constraints we used one net that was 36.4-m long and sampled an area 105.2 m^2 when set in a circle; and a second net that was half that length (18.2 m) and thus sampled 26.3 m^2, an area 25% the size sampled by the larger net. The smaller net was close to the smallest size that would function properly when deployed from a small boat. The smaller net was 2.4-m deep, which is deeper than the deepest water we sampled with it. The longer net was 3.6-m deep, a depth that allowed it to purse properly. All other details of net configuration were the same for the two nets (Table 2). These purse seines, unlike many others, were intended to reach the bottom and drag the lead line across the bottom while being pursed, in order to capture demersal species.

If estimates of density and species richness of fish obtained with the two nets were similar, then the smaller net would be preferred for sampling because it would allow greater replication per until time. To determine if this was so, we compared estimates of density and species richness obtained with the two nets in an experiment conducted at San Dieguito Lagoon on 4 days in September 2004. Sampling was conducted on two consecutive days on two different occasions: September 2 and 3, and September 16 and 17. All sampling was done in a ~ 0.5 km stretch of the main channel that was approximately 30-m wide and no deeper than 2 m. On each day, 4 – 6 replicate samples were taken with the large net and 6 – 12 samples were taken with the small net. Samples were spaced relatively evenly along the 0.5-km stretch of channel. On each day, sampling was done over a period of 4 – 5 hours; during the first half of this period all sampling with one net was completed, and then sampling with the second net was completed during the second half of the period. We alternated which net was used first to avoid bias. A total of 21 and 38 replicate samples were taken with the large net and small net, respectively.

Sampling with purse seines requires some practice, attention to detail, and a systematic approach. The approach that worked best for us was as follows: First the net was carefully stacked into a small boat (4.2-m long) with a wide, flat deck, and low gunnels. The float line was stacked near the bow and the lead line, purse rings, and purse line were stacked near the stern. The net was stacked so that it would feed out freely and avoid tangling the purse line. A bucket, which acted as a sea anchor, was attached to all loose lines (float, lead, and purse) at the end of the net on top of the stack. The net was deployed by throwing the bucket over the port side of the boat while driving backwards in a clockwise circle, as the net was fed out. When a full circle was completed, the bucket attached to the starting end of the net was retrieved and attached to the boat. At this point the engine was turned off and tilted out of the water to avoid tangling the net. The purse line was then pulled tight (pulling from the free end of the net). Once pursed, the purse rings and lead line were pulled over the side of the boat by grabbing the purse line on either side of the bunched purse rings. This step completely sealed the bottom of the net. Next, the netting was pulled into the boat from the loose end, stacking the float line near the bow and the lead line and rings near the stern, until all captured fish were herded into a small pouch of net that remained in the water. Large fish (> 50 cm) were removed from the pouch by hand and then the pouch was lifted from the water and its contents placed in a cooler (~ 40 L) full of seawater.

All fish captured were then identified, counted, and released. The net was then checked carefully to be certain it was untangled and stacked correctly, at which point it was ready to be deployed again.

We used analysis of variance (ANOVA) to compare estimates of density obtained with the two purse seines. The ANOVA model included the factors Net Size (fixed), Week (random), Day (random and nested within week), and interactions among these factors. We compared estimates of species richness from the two nets graphically. The number of species captured was compared per sample, per equivalent effort, and per equivalent area sampled. To standardize by area sampled, we compared the number of species captured per one haul of the large net with that captured in four hauls of the small net (which sampled 25% of the area sampled by the large net). To standardize by effort, we compared the number of species captured per one haul of the large net with that captured in 2 hauls of the small net, because it took, on average, half as long to collect a sample with the small net as it did the large net.

Results

The large (36.4-m) purse seine produced estimates of fish density that were about 1.6 fold greater than those produced by the small (18.2-m) seine, a statistically significant difference (Fig. 1, Table 3). Although the difference between the estimates produced by the two nets was somewhat variable among the 4 days of the study (Fig. 1), this temporal variation was not statistically significant (i.e., interactions between net size and day or period were not significant: Table 3). In addition to producing larger estimates of density, the large net tended to produce less variable estimates, with average CV's of 56 (± 17) and 83 (± 23)% for the large and small net, respectively (mean CV ± 1 SE calculated from $n = 4$ days).

As expected given the four-fold larger area sampled by the large purse seine, the large seine produced estimates of species richness that were much higher on a per haul basis than those from the small net (Fig. 2). On a per area basis (4 small seine hauls combined vs. 1 large seine haul), however, estimates of species richness produced by the two nets were generally quite similar. On a per effort basis (2 small seine hauls combined vs. 1 large seine haul), however, the large seine consistently produced greater estimates of species richness than did the small net. Overall, 19 species were captured with the large net and 14 species were captured with the small net; 7 species were captured only in the large net and 2 were captured only in the small net. All 9 of the species unique to a given net size were rare: 6 were represented by only a single individual and the other 3 by only 3 – 4 individuals (Table 4).

Beach seines

Methods and Materials

To determine whether the size of beach (two-person pole) seines affected estimates of fish density and species richness in ways similar to those found for purse seines, we conducted an experiment comparing catches from beach seines that were 7.6-m and 15.2-m long. Beach seines of similar sizes have been widely used to collect fish in southern California wetlands (e.g., Allen 1982, Nordby and Zedler 1991, Saiki 1997, Brooks 1999, Ambrose and Meffert 1999,

Desmond et al. 2002, Merkel & Associates 2002). Nets much smaller or larger than those we used are likely to have characteristics undesirable for gear intended for generalized sampling in southern California wetlands. Seines much longer than 15.2 m become more difficult to set and retrieve; and they are likely to catch very large numbers of fish, which greatly increases sample-processing time, thus limiting replication of samples. The increased sample-processing time can also increase handling mortality. Beach seines much less than 7.6 m sample small areas and therefore are likely to produce highly variable estimates of density.

Both the 7.6-m and 15.2-m beach seines were 1.8-m deep, "heavy leaded" (one 28 g lead every 30 cm), and built of 3.2-mm-mesh knotless nylon netting (delta style), the same netting used for the purse seines and the same netting used to build most beach seines used to sample fish in southern California wetlands. The 7.6-m seine was a standard beach seine, essentially just a wall of netting, whereas the 15.2-m seine had a 1.8 x 1.8 x 1.8 m bag in the center of it. Additional beach seines of various lengths were used to block off the segments of tidal creeks and main channels sampled. Aside from length, these seines were identical in design to the 7.6-m seine. The blocking nets were secured in place with wood stakes placed every 1 – 5 m (distance depending on conditions) along the nets to keep them in place, upright, and the lead line in contact with the bottom.

To test how beach-seine size affected estimates of fish density and species richness, we took spatially paired samples in segments of tidal creeks and main channels. Samples within pairs were immediately adjacent to one another and taken within segments of creeks or channels blocked off with beach seines that ran across them (blocking nets). The purpose of these blocking nets was to keep fish in the area being sampled from escaping. Six pairs of samples were taken in 3 wetlands: 4 pairs in Carpinteria Salt Marsh (3 tidal creek pairs and 1 main channel pair); 1 pair in the main channel of San Elijo Lagoon; and 1 pair in the main channel of Los Peñasquitos Lagoon. For analysis, all data were pooled.

To estimate density and richness of fish species in each sample, 3-8 hauls of the primary beach seine (7.6 or 15.2 m) were made in each blocked area and then the blocking nets were hauled in. The number of hauls made with the primary seine, though variable among pairs of replicates, was always the same for the two samples within a pair. Fish caught in the blocking nets were also included in the estimates of density and richness. All fish captured were placed in large (~ 1 x 0.7 x 0.4-m high) bins full of water, identified, counted, and released. Differences in estimates of density and species richness produced by the two nets were tested with ANOVA.

Results

Net length did not affect the estimate of species richness: a total of 11 species were captured by the 7.6-m net and 10 by the 15.2-m net; and an average of about 6 species were captured per replicate with both nets (Table 5). Moreover, variability (CV) of the estimates of richness was nearly identical for the two nets.

The small net, however, produced estimates of total density that were about 1.6 fold greater than those produced by the large net, though this difference was not quite statistically significant (p = 0.06; Table 5). The extent to which the large net underestimated densities relative to the small net varied among groups of fish: estimates of density of midwater species – and the variability of

these estimates – did not differ between the two net sizes (Table 5). Net length, however, seriously affected estimates of the density of demersal species: the small net produced estimates of density of demersal species that were approximately twice as large as those produced by the large net (Table 5). This difference was consistent for two different groups of demersal species: gobies and all other demersal species. (Results are presented separately for these two groups of demersal species because gobies will be well sampled by enclosure traps, but other demersal species may be too sparse to sample effectively with enclosure traps.) In addition to being greater, estimates of the density of gobies were considerably more variable for the small net than the large net, but the same was not true for other demersal species.

Are blocking nets necessary to obtain accurate or representative estimates of fish density and species richness?

Methods and Materials

Blocking nets are widely used when sampling fish with beach seines in southern California wetlands (e.g., Nordby and Zedler 1991, Ambrose and Meffert 1999, Desmond et al. 2002). Their use is predicated on the belief that many fish evade capture by beach seines by fleeing the area being seined and that by blocking off this area with other nets, these fish can be retained and captured. We are aware of no data supporting this belief, but it appeals to intuition. Using blocking nets, however, considerably complicates logistics and increases sampling effort. Hence, even if samples taken without blocking nets underestimate actual levels of species richness and density, if those estimates are representative (i.e., predictive) of the true values, considerable sampling effort could be saved by beach seining without blocking nets to obtain indices of density and species richness.

We conducted an experiment to test whether accurate (predictive) indices of species richness and density could be obtained with beach seines used without blocking nets. Spatially paired samples were taken in blocked and unblocked areas in 3 wetlands on 4 days in October 2003 (Table 6), resulting in a total of 16 pairs of samples of blocked and contiguous unblocked areas. A 7.6 x 1.8 m beach seine (described above) was used to sample both areas within a pair. In unblocked areas, fish were captured with one haul of the net across a segment of a tidal creek or main channel. The same procedure was followed in blocked areas with the addition of pulling both blocking nets to shore. The unblocked area in each pair was sampled as the blocking nets were taken across the channel or creek. The segments of main channel or tidal creek sampled were 6-m long and 2.6 – 35 m across.

ANOVA was used to test for differences in estimates of species richness and density between blocked and unblocked areas. The model included the terms Blocked? (fixed), Wetland (random), Station (random, nested within wetland), and the Blocked? x Wetland interaction. Including the term Station accounts for the paired design of this study. Ordinary least squares regression was used to test whether density or species richness in blocked areas could be predicted from values of those variables in unblocked areas.

Results

Estimates of density and species richness of fish were much greater where blocking nets were used than where they were not (Fig. 3, Table 7). Overall, estimates of density and richness were more than 4 fold (1.09 vs. 0.28 m^{-2}) and 2 fold (2.94 vs. 1.44 per haul) greater in blocked areas than in unblocked areas, respectively. The differences in density and richness between blocked and unblocked areas were relatively consistent among wetlands (Fig. 3) as shown by non-significant interactions between wetland and blocking in ANOVA (Table 7). When gobies were excluded, density estimates did not differ significantly between blocked and unblocked areas (Table 7), though density was estimated to be more than twice as high in blocked areas than in unblocked areas (0.63 vs. 0.25 m^{-2}).

Estimates of density and species richness in unblocked areas were poor predictors of these values in blocked areas. Density in the unblocked member of a pair explained little of the variation in density in blocked areas (r^2 = 0.09 and 0.01, P = 0.26 and 0.72) for density of all species and density of species other than gobies, respectively. (Density was transformed to ln(x+0.1) for this analysis.) By contrast, species richness in the unblocked area significantly (P = 0.02) predicted richness in the blocked area, but the predictive power of the relationship was relatively low (r^2 = 0.35).

How many hauls with a beach seine within a blocked area must be made to obtain a representative estimate of density and richness?

Experiment 1: Single haul vs. 5 hauls

Methods and Materials

The preceding study demonstrated that representative estimates of species richness and density of wetlands fish cannot be obtained without the use of blocking nets; however, it provides no guidance as to how many hauls must be used within blocked areas to obtain representative estimates of these variables. To address this question we conducted an experiment that compared catches from blocked areas that were seined just once (plus the catch of the blocking nets) with catches from blocked areas that were seined 5 times before retrieving the blocking nets. We sampled spatially paired 6-m segments of tidal creeks and main channels with 7.6-m beach seines (described above). These segments were 5 – 28.5 m wide, thus areas of 30 – 171 m^2 were sampled. Eight sets of paired replicates were sampled in Carpinteria Salt Marsh on 15 and 16 December 2003 (3 pairs in tidal creeks, and 5 pairs in main channels). Sampling was conducted during relatively flat tides of middle height, during which tidal currents were weak. ANOVA was used to test for differences in estimates of species richness and density between the two treatments. The factors Treatment (1 or 5 hauls), Habitat (tidal creek or main channel), Station (a unique identification for each pair of samples; nested within habitats), and the interaction between Treatment and Habitat were included in the model.

Even if single hauls in blocked areas underestimate actual levels of species richness and density relative to 5 hauls, if those estimates predict the values obtained with 5 hauls, then representative samples could be obtained and sampling effort could be reduced by seining only once within blocked areas rather than 5 times. We used ordinary least squares regression to test whether

density or species richness in blocked areas seined 5 times could be predicted from values of those variables obtained in blocked areas seined only once.

Results

Estimates of certain variables differed significantly between the 1 and 5 haul treatments, whereas others did not. Estimates of species richness did not differ significantly between samples obtained with 1 versus 5 hauls within the blocked area: 2.88 vs. 3.25 species were captured per haul and a total of 7 vs. 8 species were captured with 1 vs. 5 hauls, respectively (Table 8). Similarly, estimates of the density of midwater species did not differ between the two treatments. In contrast, there were large differences between the two treatments in estimates of density of demersal species and of all species combined. Five hauls produced significantly greater (1.5 fold for total density and >2 fold for demersal species) estimates of density than did 1 haul (Table 8). Precision of the estimates of richness and density ranged from similar to somewhat better for 5 hauls than 1 haul (Table 8). There was no indication that the relative efficacy of the two sampling treatments differed between tidal creek and main channels, as interactions between Treatment and Habitat in ANOVA were not statistically significant (Table 8).

Estimates of species richness and density obtained in blocked areas seined only once significantly predicted the values of these variables obtained in blocked areas seined 5 times. The predictive power of this relationship was moderate for species richness ($r^2 = 0.46$, $P = 0.04$) and total density ($r^2 = 0.46$, $P = 0.04$; data transformed to ln(x+0.1)), and somewhat higher for midwater and demersal species when analyzed separately ($r^2 = 0.61$ and 0.71, $P = 0.01$ and 0.006; data transformed to ln(x+0.01) and ln(x+0.1), respectively).

Experiment 2: Catch versus hauls in blocked areas seined 10 times

Methods and Materials

The preceding study indicates that hauling a beach seine 5 times through a blocked area before retrieving the blocking nets provided considerably higher estimates of density for demersal species than did a single haul, but it does not evaluate the adequacy of 5 hauls in providing representative estimates of density. In other words, is even 5 hauls enough? To explore this question, we sampled blocked areas with 10 beach-seine hauls before retrieving the blocking nets. We used these data to evaluate how estimates of density and species richness changed as a function of the number of seine hauls. Ideally, seining within a blocked area would be stopped at the point at which estimates of density and species richness change little with each additional haul. This approach, however, may be impractical if many hauls must be made before estimates of density and richness stabilize.

We conducted this study in 2 wetlands, Carpinteria Salt Marsh (April 22 and 23, 2004) and San Dieguito Lagoon (April 26 and 27, 2004), in 3 habitats (tidal creek, main channel, and basin). A total of 19 samples were taken: the main channel habitat was sampled in both wetlands ($n = 6$ and 5 in Carpinteria and San Dieguito, respectively), tidal creeks were only present Carpinteria (n = 6 samples), and a basin was only present in San Dieguito (n = 2 samples). Comparisons between wetlands were restricted to samples from the main channel habitat; comparisons

between main channel and tidal creek habitats were restricted to Carpinteria; and comparisons between main channel and basin habitats were restricted to San Dieguito.

We sampled 7-m-long segments of tidal creeks, main channels, and basin shoreline with standard 7.6-m beach seines (described above). These segments ranged in width from 3.9 – 24.5 m, resulting in areas of 27.3 – 171.5 m^2. All fish captured were identified, counted, and released. Sampling was conducted during relatively flat tides of middle height, during which tidal currents were weak.

Results

Estimates of species richness and total fish density climbed rapidly over the first 5 hauls within blocked areas and then the rate of increase slowed, especially for species richness (Fig. 4a). After 5 hauls 90 ± 3% (mean ± 1 SE) of all species that were captured after 10 hauls plus blocking nets had been captured. After 5 hauls, the estimate of density was 67 ± 3% of the eventual total. There was, however, a major difference between functional groups of fish in the proportion of the total catch that had been obtained after 5 hauls. Almost all (95 ± 2%) midwater fishes were captured after 5 hauls, compared to only 51 ± 4% of demersal species (Fig. 4b). There was no evidence that the rate of capture differed between two types of demersal fishes: gobies and other species (Fig. 4b). As a result of the different patterns of depletion of midwater and demersal species (Fig. 4c), the proportion of the total catch composed of each group never stabilized, with demersal species becoming more dominant as the number of hauls increased (Fig, 4d).

For demersal species, 24% of the total catch was made with the blocking nets after 10 hauls of the main beach seine had been made and catches with that seine had fallen to low levels (Fig. 4b & c). By contrast, only 3% of the total catch of midwater species came from the blocking nets. A similar 4% of the estimate of species richness was obtained in the blocking nets. From these data, we estimate that 94, 98, and 75% of the estimates of total species richness and density of midwater and demersal species, respectively, could be obtained by making 5 hauls within blocked areas and then hauling the blocking nets. These estimates assume that similar proportions of the total catch would be obtained from the blocking nets when they are hauled after only 5 rather than 10 hauls of the primary beach seine. This assumption is conservative because a larger proportion of fish would remain uncaptured in blocked areas after only 5 hauls than after 10 hauls. Thus a greater proportion of all fish in blocked areas should be captured in blocking nets after 5 hauls than after 10 hauls.

Making only 5 hauls instead of 10 within blocked areas would save considerable time and effort. For this approach to be useful, it must be equally effective in different wetlands. We compared the efficacy of 5 hauls (% of total catch made after 5 hauls) in Carpinteria Salt Marsh and San Dieguito Lagoon with ANOVA. For all 3 estimates (species richness and densities of midwater and demersal species), there were no significant differences between wetlands in the percentage of the total catch made after 5 hauls (Table 9). There was, however, some evidence that the percentages of total catches obtained after 5 hauls differed between habitats within wetlands (Table 9).

What is the catch efficiency of beach seines?

Methods and Materials

During the experiment comparing estimates of species richness and density obtained by blocked and unblocked beach seining, we also estimated catch efficiency of the standard 7.6-m beach seine. Catch efficiency was estimated from the recovery rate of tagged fish released into blocked segments of tidal creeks and main channels. Since these blocked areas were only seined once before retrieving blocking nets, this study is expected to underestimate catch efficiency from blocked areas seined multiple times.

We released tagged fish (total numbers released are presented in Table 10) into 12 blocked areas: 7 in Carpinteria Salt Marsh (4 tidal creek and 3 main channel samples), 2 in San Elijo Lagoon (main channel only), and 3 in Los Peñasquitos Lagoon (main channel only). Fish were captured in nearby areas, tagged with a subcutaneous injection of non-toxic acrylic paint – visible through the skin – and released into the blocked areas. Tagged fish were released throughout each blocked area and allowed to redistribute themselves for at least 10 minutes before the area was seined. Catch efficiency (proportion of tagged fish recovered) was compared among species, functional groups of species, wetlands (restricted to main channel habitat only because tidal creeks were only sampled in Carpinteria Salt Marsh), and between habitats (main channel vs. tidal creek; comparison restricted to Carpinteria Salt Marsh).

Results

Catch efficiency varied among taxa (Table 10). There appeared to be two major groups of fish for which catch efficiency differed widely. Midwater fishes (mainly topsmelt and California killifish) were recaptured at high rates, somewhat over 70%, whereas demersal fishes (gobies and other less abundant bottom dwellers) were recaptured at much lower rates, around 30%.

There were no significant differences in catch efficiency among wetlands for either of the two functional groups, though samples sizes were small (Table 11). There were, however, large and significant differences in catch efficiency between the two habitats studied in Carpinteria Salt Marsh. Both midwater and demersal fish were recaptured at much higher rates in the main channel habitat than in the tidal creek habitat (Table 11).

Discussion and Recommendations

Beach and purse seines are widely used to sample fish in southern California wetlands, but their sampling characteristics are relatively poorly known. We focused on these two gear types because they (and enclosure traps; Appendix 2) are the methods most likely to provide representative estimates of density and species richness of wetlands fish while minimizing avoidable impacts to the wetlands sampled (Table 1). Other gear types have clear shortcomings for estimating fish density and species richness. For example it is difficult, if not impossible, to estimate densities from samples taken with gillnets and traps. Moreover, fish taken in gillnets often die, an impact to the biota of wetlands that ideally should be avoided in routine monitoring. Trawls (e.g., beam and otter) typically vastly underestimate wetlands fish densities (e.g., Merkel & Associates 2002), probably due to gear avoidance.

Beach seines are the gear most widely used for sampling wetlands fishes in southern California, yet methods have not been standardized. Some workers use "traditional" beach seines that are set parallel to shore and hauled in with lines (e.g., Allen et al. 2002), others use a virtually identical net but attach poles to either end and walk it in to shore (Merkel & Associates 2002), and still others use smaller pole seines hauled along shore (e.g., Brooks 1999). Some workers use relatively large (13 – 16 m x 1.8 – 2.1 m) beach seines with a square bag (~ 1.8 x 1.8 m) (Allen et al. 2002, Merkel & Associates 2002, Desmond et al. 2002), others use smaller (5-10 m) nets with (Saiki 1997) or without a bag (Brooks 1999). Some workers use beach seines with relatively large mesh (6 – 12 mm; e.g., Allen et al. 2002) and other use smaller mesh (3.2 mm; e.g., Nordby and Zedler 1991). Some workers use beach seines in blocked areas (e.g., Nordby and Zedler 1991, Ambrose and Meffert 1999), whereas others use no blocking nets (e.g., Allen 1982, Saiki 1997, Brooks 1999, Allen et al. 2002, Merkel & Associates 2002). The results of this study indicate that some of these variations among beach seines will dramatically affect estimates of fish density and species richness, making comparisons among studies conducted with different beach seining gear or methods extremely difficult.

We found that estimates of density and species richness were significantly affected by three differences in beach seine configuration and methods: seine size, the use of blocking nets, and the number of hauls made within blocked areas. Beach seine size had no effect on estimates of species richness, but greatly affected estimates of density. Use of blocking nets significantly increased estimates of both species richness and density. Furthermore, estimates of density and species richness increased rapidly with the number of hauls made within blocked areas until about 5 hauls had been made, at which point increases in these estimates slowed considerably.

We suspect the reason that density estimates obtained with the 7.6-m-long beach seine were higher than those from 15.2-m-long net is that it was easier to keep the lead line of the smaller net in contact with the substrate. We frequently observed demersal species (the group for which density was underestimated with the larger net) swimming under or being passed over by the lead line when it came off the bottom. While estimates of the density of demersal species were higher using the 7.6-m seine compared to the 15.2-m seine (a good sampling characteristic) they were also more variable (generally a bad characteristic). We suspect the increased variability of the density estimates from the smaller net actually reflects the true patterns of variation in abundance in nature, which were better measured by the more effective 7.6-m net. In addition to the difference in length (7.6 vs. 15.2 m), the two nets differed in configuration: the larger net had a 1.8 x 1.8 x 1.8 m bag in the center of it. Bag seines are thought to outperform traditional beach seines without bags because the bag is meant to serve as a holding area for captured fish as the net is drawn through the water. Hence, fish in the bag should be less likely to escape the net. If this function of the bag occurred in our study, it was outweighed by the increased efficiency of the smaller net. It also seems likely that any benefits of a bag seine would be reduced by the use blocking nets, which keep fish from escaping the area seined.

Use of blocking nets in conjunction with beach seines produced estimates of species richness and density that were much higher than estimates produced by beach seines used in unblocked areas. Our observations indicate that fish evaded capture in unblocked areas by swimming out of the area being seined. This observation raises the question of whether fish also flee the area being blocked when the blocking nets are deployed, thus biasing estimates of density and potentially species richness. Field observations lead us to believe that any such bias would be small: fish

were observed to avoid persons walking blocking nets out, but they seemed equally like to swim into the area being blocked as out of it. Also, blocking nets can be deployed much more rapidly than a seine can be hauled while capturing fish, thus reducing the opportunity for fish to escape from the area being blocked. It is possible to test whether avoidance of blocking nets biases estimates of density and species richness obtained by seining in blocked areas. This could be done, for example, by blocking a very long segment of creek or channel, releasing tagged fish into this area, and then blocking and seining small segments within the larger area. Estimates of density and species richness of tagged fish obtained from the small blocked segments would then be compared the known density and richness of tagged fish released into the larger area. Such a study would be time consuming, and we suspect, based our field observations, likely to show little evidence of bias in estimates of density and species richness caused by avoidance of blocking nets; particularly of bias among wetlands, which would be of concern to the wetland restoration monitoring project.

The number of seine hauls within blocked areas has not been standardized among studies that have used this method, though it has been similar among studies. Nordby and Zedler (1991) and Desmond et al. (2002) ceased sampling in blocked areas when catches "declined to near zero, usually 4-5 hauls." Ambrose and Meffert (1999) used a fixed number of hauls: 5 in each blocked area. Our work indicates that the "5-haul rule" is a good one for balancing accuracy with effort. We recommend it over the "cease seining when catches approach zero" rule, which is somewhat vague and therefore more difficult to implement in the field. For example, Nordby and Zedler (1991) provided data on the efficiency of their rule. In their example (one blocked sample), they ceased sampling after 5 hauls. In that haul they captured about 100 fish. Those fish constituted <20% of the total captured, but certainly the catch was not very near zero. In our study 0 – 75 fish were captured in the 5th haul, and only 7 of 190 hauls (in 4 of 19 replicates) produced no fish. Overall, we found that 5 seine hauls plus the catch of the blocking nets captured 98, 75, and 94% of the total catch of midwater fish, demersal fish, and species of fish captured with 10 hauls plus blocking nets.

Our results indicate that beach seines will underestimate the abundance of demersal species relative to midwater species. This agrees with our observations that demersal species are adept at escaping seines by swimming under or remaining on the substrate under the lead line. It may be important to know the extent to which the density of demersal species is underestimated relative to midwater species when total density of fish is compared among wetlands. If in certain wetlands demersal species are more abundant relative to midwater species, then total density in those wetlands will be underestimated relative to wetlands in which midwater species are proportionally more abundant. Such underestimates could be corrected for if the extent to which demersal species are underestimated relative to midwater species is known.

To determine the extent to which density is underestimated by any sampling technique, one must know the catch efficiency of it. There are two components to catch efficiency, gear avoidance and escapement. Our data on catch efficiency are not definitive, but they provide some guidance on the extent to which density was underestimated in blocked areas seined 5 times before blocking nets were retrieved. Our data are not definitive for two reasons. First, we have no quantitative data on gear avoidance (i.e., the proportion of fish that leave an area as it is being blocked) and whether it differs among species or groups of species. Second, taken alone, our study with 10 seine hauls in blocked areas cannot be used to estimate catch efficiency (i.e., the

percentage of fish within blocked areas that are captured) because there is no way to estimate escapement. We did, however, estimate escapement using mark-recapture in blocked areas, but these areas were seined only once before the blocking nets were retrieved.

Nevertheless, comparison between our 10-haul study and our mark-recapture study is informative and allows us to estimate catch efficiency (and escapement) within blocked areas hauled 5 times. In the mark-recapture study, about 74% of tagged midwater fish were recaptured with one seine haul and both blocking nets (Table 10). In the 10-haul study, a very similar percentage of midwater fish were captured in the first haul plus the blocking nets (Fig. 4b). This similarity between the two studies implies that escapement of midwater fish from blocked areas was negligible. Thus, assuming no gear avoidance when setting blocking nets, we estimate that about 98% of the individuals of midwater species are captured in blocked areas hauled 5 times.

The situation is quite different for demersal fish. Results of our mark-recapture study indicate that about 30% of the individuals of demersal species were recaptured with one haul and blocking nets (Table 10). Results of the 10-hauls study indicate that about 42% of all demersal fishes that will be captured with 10 hauls plus blocking nets will be caught in the first haul and the blocking nets (Fig. 4b). The difference between these two studies implies that the 10-haul study overestimates that catch efficiency by 42/30, or about 1.4 fold, due to unaccounted for escapement from the blocked areas. Thus, our estimate that 5 hauls plus blocking nets will catch 75% of all demersal fish that will be captured in 10 hauls and blocking nets, should be divided by 1.4 to arrive at an estimate of catch efficiency for demersal species in blocked areas hauled 5 times. Hence, assuming gear avoidance is minimal when deploying blocking nets, we estimate that about 54% of demersal fish present will be captured in blocked areas hauled 5 times. Thus, to make the estimate of density of demersal fishes comparable to that obtained for midwater species it should be doubled. Further work directly measuring rates of gear avoidance and escapement from blocked areas hauled 5 times would be useful, though time consuming.

A note of caution regarding our estimates of catch efficiency and escapement: our findings do not apply to very small fish that can pass through the 3.2-mm mesh of the nets we used. For our mark-recapture work, we only tagged fish that were captured with 3.2-mm mesh. Density of larvae and very small juveniles (especially gobies) will be underestimated with nets using 3.2-mm or larger mesh, but nets with smaller mesh are impractical for use because of their much greater drag through the water. For small, abundant fish (e.g., gobies), use of enclosure traps with nets constructed of smaller mesh can partly overcome this shortcoming of beach seines.

We found no compelling evidence that the efficacy of beach seining varied among wetlands (e.g., Fig. 3, Tables 7, 9, 11). This important finding indicates that comparisons of density and species richness among wetlands will not be confounded with methodological biases unique to each wetland.

We did, however, find evidence that the efficacy of beach seining varied among habitats (Tables 9, 11). The evidence is difficult to interpret, however, because it varies between studies. The mark-recapture study indicated that escapement of both midwater and demersal species was higher in tidal creeks than in main channels; whereas the results the 10-haul study imply that escapement was higher in main channels than tidal creeks. We have no logical explanation for these conflicting results. Nevertheless, the implication is that differences in the efficacy of beach

seines do exist among habitats, and consequently, comparisons among habitats should be avoided unless estimates of density can be corrected by habitat-specific multipliers obtained from new studies.

Given our findings regarding beach seines, when the goal of sampling is to obtain representative estimates of species richness and density, we make the following recommendations:

1. Blocking nets should be used to obtain representative estimates of species richness and density.

2. Small beach seines (~ 7.6 x 1.8 m) should be used rather than larger ones (~ 15.2 x 1.8 m) because they provide more accurate estimates of density and equivalent estimates of species richness, while allowing greater replication of samples due to reduced sample gathering and processing time.

3. Beach seines should be hauled 5 times within blocked areas before retrieving blocking nets. This procedure will capture the vast majority of fish that can be captured in the blocked area, and provides good (but see 4 below) estimates of species richness and density.

4. To compare total fish density among sites that differ in the relative abundance of demersal and midwater species, densities of demersal species should be multiplied by a correction factor that takes into account higher rates of escapement by these species. We estimate this correction factor to be 2.

5. Comparisons among habitats should be avoided because catch efficiencies appear to differ among habitats.

In addition, our experiences in the field using beach seines also lead us to make the following recommendations:

6. Sampling with beach seines and blocking nets should be done during periods of low water flow, typically periods with little change of tidal height. When flow is rapid, it is difficult to use blocking nets effectively. Extended periods of low water flow typically occur at low to mid-tidal heights.

7. Stakes should be placed every 1 – 5 m (distance depending on conditions) along blocking nets to secure them in place when any current is present.

Purse seines have been less widely used in southern California's wetlands (see Allen et al. 2002 and Merkel & Associates 2002), but they produce among the highest estimates of fish density species richness of any method used in these systems (Merkel & Associates 2002). Moreover, they are well suited to sampling areas that are deeper or more distant from shore than beach seines used with blocking nets can sample.

We found that the size of purse seines had a large effect on estimates of fish density. Our larger net (36.4 x 3.6 m) produced higher estimates of density than did the smaller net (18.2 x 2.4 m). Based on our field observations, this difference between the two nets was mainly caused by

different levels of gear avoidance. Large numbers of midwater species were regularly observed swimming out of the area being sampled before it could be completely encircled by the small net. We did not observe such gear avoidance with the larger net. The shorter net was also not as deep as the longer net (2.4 vs. 3.6 m) and this difference in design may have allowed increased escapement out of the bottom of the small net before it was completely pursed.

On a per effort basis, the larger purse seine also produced much higher estimates of species richness. Moreover, the total number of species captured by the larger net was somewhat greater than that captured by the smaller net (19 vs. 14). Hence, the larger purse seine allowed more time-efficient and accurate sampling of wetland fishes than the smaller net. Therefore we recommend that purse seines of the same or very similar size and configuration be used to measure species diversity and density of wetlands fishes in areas too deep or far from shore to be sampled with blocked beach seines as described above (with the caveats noted below).

We cannot determine with certainty exactly how accurate estimates of species richness and density obtained with our purse seines were, but accuracy could be measured (see e.g., Charles-Dominique 1989). Based on our field observations and our data on the proportion of fish captured with one beach seine haul and blocking nets, we suspect that estimates of density and species richness from the larger purse seine are quite accurate for midwater species. Densities of demersal species are probably vastly underestimated by the purse seines we used (Table 4). We can think of no practical way to improve their ability to catch demersal species. Hence, though we recommend purse seines for sampling areas that are deeper or farther from shore than can be sampled with beach seines, we caution that estimates of density (and possibly species richness) of demersal species obtained with them should be used with caution. Furthermore, though data on density and species richness of midwater species obtained with both blocked beach seines and purse seines (of the larger design we used) are probably quite comparable, comparisons of estimates of density and richness of demersal species between these two different methods should be avoided. Additionally, estimates of density and species richness obtained with different sampling methods (e.g., purse seines, beach seines, and enclosure traps) should only be pooled if the areas sampled by each method are consistent among sampling stations, otherwise biases among stations will be introduced.

One species of fish, the striped mullet (*Mugil cephalus*), was obviously undersampled by beach and purse seines. Most mullet trapped within blocked areas or encircled in purse seines leapt out, and they were regularly observed swimming out of areas as blocking nets were deployed. Because of their large size and relative abundance, mullet can dominate fish biomass in southern California wetlands (Horn and Allen 1985). They are readily caught with gillnets, but other methods greatly undersample them (Horn and Allen 1985). We view gillnets as an unacceptable method for routine monitoring work in wetlands because of the high mortality rates of fish captured in them and the difficulty in obtaining density estimates from them. We recommend that mullet density be quantified in two ways: by recording the number of fish that leap out of blocked areas when beach seining and encircled areas when purse seining; and during periods when the water is clear, by counting the number of mullet that can be seen in defined areas. It may also be worthwhile to make these sorts of visual estimates of density and presence for large, sparse species, like leopard sharks (*Triakis semifasciata*) and grey smooth-hounds (*Mustelus californicus*).

In conclusion, variation in net dimensions and netting techniques (e.g., use of blocking nets, number of hauls within blocked areas) can dramatically affect estimates of density and species richness of estuarine fishes. Hence, it is important to standardize the methods used within a monitoring program. Standardization among programs would also facilitate comparisons among wetlands that are monitored by different groups. To obtain adequate estimates of density and species richness of wetland fish, as required by the coastal development permit for SONGS units 2 and 3, we recommend that fish be sampled with enclosure traps, blocked beach seines, purse seines, and visually.

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Figure Legends

Fig. 1. Density estimates from purse seines of two sizes. Means and 1 SE are shown.

Fig. 2. Estimates of species richness from purse seines of two different sizes. Means and 1 SE are shown.

Fig. 3. Comparison of mean density and species richness of fish measured with beach seines in blocked and unblocked sections of tidal creeks and main channels in 3 wetlands. Error bars are ±1 SE.

Fig. 4. Catch versus hauls in 19 blocked areas seined 10 times with a 7.6-m beach seine before retrieving 2 blocking nets. Shown are a) the percentages of total estimates of species richness and density obtained after each haul; b) percentages of total estimates of density of midwater and demersal species; c) mean densities and numbers of species obtained in each haul; and 4) composition (midwater and demersal species, %) of the cumulative catch after each haul. Values are means ±1 SE. Error bars are omitted in some instances for clarity. For midwater and demersal fishes the mean % captured was only calculated from replicates in which at least 10 individuals were captured.

Table 1. Summary of characteristics of gear used for sampling estuarine fishes in southern California wetlands.

Gear type	Advantages	Disadvantages	Effort per sample	Species sampled	Provides density estimate?
beach seine with blocking nets	• standard technique that allows direct comparison with many other studies • catch efficiency can be measured	• disturbs shoreline and sediments by trampling	very high	most/all	yes
purse seine	• little disturbance of habitat (above or below water)	• difficult (but possible) to estimate catch efficiency • requires a boat	high	most/all	yes
otter trawl	• little disturbance of banks & above water vegetation	• low catch efficiency • difficult to measure catch efficiency • catch efficiency strongly influenced by habitat type & water conditions (e.g., by influencing net avoidance) • somewhat selective • disturbs substrate • requires a boat	high	most	yes
beam trawl	• little disturbance of banks & above water vegetation	• low catch efficiency • difficult to measure catch efficiency precisely • catch efficiency strongly influenced by habitat type & water conditions • somewhat selective • disturbs substrate • requires a boat	high	many	yes

Table 1 continued

channel/fyke net	• relatively little disturbance to habitat	• tide dependent • tidally biased • area sampled not easily defined if channel/creek doesn't drain completely • costly to replicate (requires an extra net for every extra replicate)	moderate*	many	maybe
gill net	• effective for large open-water fish that are difficult to sample with other gear (e.g., mullet) • low disturbance to habitat	• fish sampled are killed • very selective • difficult/impossible to determine area sampled • catch efficiency difficult to measure & may vary with conditions	moderate	many	no
enclosure	• relatively easy to measure catch efficiency • very effective for gobies & other small, abundant species	• not effective for large, active fish • poor for estimating species richness because small area sampled misses rare species	low	few	yes
traps (e.g., minnow)	• little disturbance of habitat	• very selective • area sampled is unknown • area sampled likely varies among habitats, tidal conditions, etc.	very low	very few	no

* Requires moderate effort if sampling stations are permanent. Requires high effort if new sampling stations are selected for each survey.

Table 2. Purse seine dimensions and specifications.

Length (m)	Depth (m)	Area Sampled (m^2)	Mesh Size
18.2	2.4	26.3	3.2 mm
36.4	3.6	105.2	3.2 mm

Notes on the design of both nets:

- Nets were built of knotless nylon netting (delta style; 16 kg breaking strength), with a green, plastic coating.
- Float lines had 156-g-buoyancy floats every foot.
- Lead lines had 76.5-g leads every foot.
- Stainless steel purse rings with a 0.45-m drop (from lead line) were spaced 0.6-m apart.

Table 3. Results of ANOVA testing for differences in estimates of mean fish density between purse seines of two sizes.

	SS	df (num., denom.)	MS	*F*	*P*
Net Size	1.28	1, 1	1.28	2929.18	**0.01**
Wee	2.12	1, 2	2.12	1.73	0.32
Day(Week), D(W)	2.45	2, 51	1.23	4.40	**0.02**
	<0.01	1, 2	<0.01	<0.01	0.98
S x D	1.03	2, 51	0.52	1.85	0.17
	14.21	51	0.28		

* Densities were transformed to ln(x+1) to satisfy the assumption of normality.

Table 4. Total numbers of fish captured with purse seines of two lengths: 36.4 and 18.2 m. N = 21 and 38 samples with the large and small net, respectively.

			Number caught	
Family	Common Name	Scientific Name	36.4-m purse seine	18.2-m purse seine
Midwater species				
Engraulididae	deepbody anchovy	*Anchoa compressa*	2	1
Clupeidae	Pacific sardine	*Sardinops sagax*	0	4
Atherinidae	topsmelt	*Atherinops affinis*	8811	2195
	grunion	*Leuresthes tenuis*	many*	48
Sphyraenidae	California barracuda	*Sphyraena argentea*	9	3
Belonidae	California needlefish	*Strongylura exilis*	3	0
Structure associated species				
Haemulidae	sargo	*Anisotremus davidsonii*	114	10
	salema	*Xenistius californiensis*	86	116
Sciaenidae	queenfish	*Seriphus politus*	218	50
Fundulidae	California killifish	*Fundulus parvipinnis*	1	0
	bluefin killifish	*Lucania goodei*	0	1
Serranidae	kelp bass	*Paralabrax clathratus*	192	63
	spotted sand bass	*Paralabrax maculatofasciatus*	64	23
Syngnathidae	barred pipefish	*Syngnathus auliscus*	8	3
Bottom dwelling species				
Blenniidae	bay blenny	*Hypsoblennius gentilis*	1	0
Gobiidae	arrow goby	*Clevelandia ios*	1	0
	cheekspot goby	*Ilypnus gilberti*	1	0
	shadow goby	*Quietula y-cauda*	27	2
Pleuronectidae	diamond turbot	*Hypsopsetta guttulata*	3	0
Paralichthyidae	California halibut	*Paralichthys californicus*	1	2
Myliobatidae	bat ray	*Myliobatus californica*	1	0
		Total Number Captured	9543	2521
		Total Species Captured	19	14

* Grunion captured with the 36.4-m net were pooled with topsmelt because these species are fairly difficult to distinguish and doing so greatly slowed down counting of the very abundant topsmelt. Grunion were recorded as present or absent in samples from the 36.4-m net.

Table 5. Comparison of estimates of species richness and density obtained with 7.6-m and 15.2-m beach seines.

	7.6-m seine		15.2-m seine			Results of ANOVA[1]	
Variable	mean ± SE	(CV)	mean ± SE	(CV)	N	$F_{1,5}$	P
Number of Species	6.00 ± 0.63	(26)	6.33 ± 0.72	(28)	6	0.35	0.58
Density							
Gobies[2]	3.65 ± 1.50	(101)	1.84 ± 0.52	(69)	6	6.45	**0.05**
Demersal nongobies[3]	0.47 ± 0.17	(89)	0.28 ± 0.10	(87)	6	19.83	**0.007**
Demersal Species[4]	4.12 ± 1.45	(86)	2.12 ± 0.53	(61)	6	8.07	**0.04**
Midwater Species[5]	0.69 ± 0.37	(131)	0.85 ± 0.46	(132)	6	0.20	0.67
All Species	4.81 ± 1.79	(91)	2.96 ± 0.87	(72)	6	6.00	0.06

[1] Analysis of variance included the factors net length and station (which of 6 pairs of samples). All densities were transformed to ln(x + 0.1) to satisfy the assumption of normality.
[2] Family Gobiidae; includes *Clevelandia ios*, *Ilypnus gilberti*, *Quietula y-cauda*, and *Gillichthys mirabilis*.
[3] Includes *Leptocottus armatus*, *Hypsopsetta guttulata*, and *Paralichthys californicus*.
[4] Includes gobies and demersal non-gobies.
[5] Includes *Atherinops affinis*, *Fundulus parvipinnis*, *Cymatogaster aggregata*, and *Mugil cephalus*.

Table 6. Experimental design of study testing whether blocking nets are necessary to measure density and species richness of wetlands fish. A pair consisted of a blocked and a contiguous unblocked segment.

Date	Wetland Sampled	N of paired samples
October 3, 2003	Carpinteria	3
October 4, 2003	Carpinteria	8
October 16, 2003	San Elijo	2
October 17, 2003	Los Peñasquitos	3

Table 7. Results of ANOVA testing for differences in estimates of fish density[1] and species richness obtained in blocked versus unblocked areas (stations) in 3 wetlands.

Source	SS	df num., denom.	MS	*F*	*P*
		Density of all species combined			
Blocked? (B)	8.15	1, 15	8.15	12.85	**0.003**
Wetland[2] (W)	0.63	2, 13	0.31	0.24	0.79
B x W[3]	1.24	2, 13	0.62	0.97	0.40
Station(Wetland)	16.75	13, 15	1.29	2.03	0.10
error	8.27/9.51	13/15	0.63/0.63		
		Density excluding gobies[4]			
Blocked? (B)	0.48	1, 13	0.48	0.42	0.53
Wetland[2] (W)	0.88	1, 12	0.88	0.79	0.39
B x W[3]	0.01	1, 12	0.01	0.01	0.91
Station(Wetland)	13.33	12, 13	1.11	0.96	0.52
error	15.01/15.02	12/13	1.25/1.16		
		Species Richness			
Blocked? (B)	18.00	1, 15	18.00	22.50	**0.0002**
Wetland[2] (W)	8.09	2, 13	4.04	1.60	0.24
B x W[3]	0.39	2, 13	0.20	0.22	0.80
Station(Wetland)	32.79	13, 15	2.52	3.15	**0.02**
error	11.61/12.00	13/15	0.89/0.80		

[1] Density was transformed to ln(x+0.1) to satisfy the assumption of normality.
[2] Differences among wetlands were tested using "Station(Wetland)" as the error term.
[3] The non-significant B x W interaction term was pooled in the error term (following Winer et al. 1991) to produce more powerful tests of blocking and station. Values for the error term are given with and without pooling.
[4] Data from San Elijo Lagoon were excluded because only gobies were captured there.

Table 8. Comparison of estimates of species richness and density obtained with 7.6-m beach seines hauled either 1 or 5 times prior to retrieving blocking nets in 6-m stretches of tidal creeks and main channels in Carpinteria Salt Marsh on December15 and 16, 2003.

A) Means and Variation

	1 haul of seine		5 hauls of seine		
Variable	mean ± SE	(CV%)	mean ± SE	(CV%)	N
Number of Species	2.88 ± 0.55	(54)	3.25 ± 0.41	(36)	8
Density					
Gobies[1]	0.26 ± 0.10	(111)	0.63 ± 0.22	(99)	8
Demersal nongobies[2]	0.06 ± 0.02	(112)	0.15 ± 0.06	(112)	8
Demersal Species[3]	0.32 ± 0.10	(85)	0.78 ± 0.24	(87)	8
Midwater Species[4]	0.36 ± 0.31	(244)	0.23 ± 0.19	(235)	8
All Species[5]	0.68 ± 0.33	(135)	1.01 ± 0.28	(78)	8

B) ANOVA results[6]

	Treatment (1 v 5 hauls)		Habitat (creek v channel)		Treatment x Habitat		Station (w/in Habitat)	
Independent Variable	$F_{1,6}$	P	$F_{1,6}$	P	$F_{1,6}$	P	$F_{6,6}$	P
Number of Species	0.8	0.41	11.0	**0.02**	<0.1	0.94	2.0	0.21
Density								
Gobies	26.2	**0.002**	0.8	0.40	2.6	0.16	17.2	**0.002**
Demersal nongobies	7.7	**0.03**	0.2	0.71	0.8	0.39	2.8	0.12
Demersal Species	34.0	**0.001**	0.7	0.42	4.1	0.09	17.2	**0.002**
Midwater Species	0.3	0.62	2.5	0.16	0.5	0.50	7.4	**0.01**
All Species	7.5	**0.03**	<0.1	0.98	2.7	0.15	9.2	**0.008**

[1] Family Gobiidae; includes *Clevelandia ios*, *Ilypnus gilberti*, and *Quietula y-cauda*. Transformed to ln(x + 0.1) to satisfy the assumption of normality for ANOVA.
[2] Includes *Leptocottus armatus*, *Hypsopsetta guttulata*, and *Paralichthys californicus*. Transformed to ln(x + 0.01) to satisfy the assumption of normality for ANOVA.
[3] Includes gobies and demersal non-gobies. Transformed to ln(x + 0.1) to satisfy the assumption of normality for ANOVA.
[4] Includes *Atherinops affinis* and *Fundulus parvipinnis*. Transformed to ln(x + 0.01) to satisfy the assumption of normality for ANOVA.

[5] Transformed to ln(x + 0.1) to satisfy the assumption of normality for ANOVA.
[6] Effects of treatment, the interaction of treatment with habitat, and station(habitat) were tested using the residual as the error term; whereas effects of habitat were tested using station(habitat) as the error term.

Table 9. Percent[1] of total catch made after 5 hauls in blocked areas that were seined 10 times before hauling blocking nets.

Habitat	Species Richness[2] Mean% ± SE (*n*)	Midwater species[2] (density) Mean% ± SE (*n*)	Demersal species (density) Mean% ± SE (*n*)
	Carpinteria Salt Marsh		
Tidal Creek	97 ± 3 (6)	96 ± 3 (4)	56 ± 4 (6)
Main Channel	90 ± 3 (6)	99 ± 1 (5)	40 ± 2 (6)
	San Dieguito Lagoon		
Main Channel	81 ± 10 (5)	96 ± 1 (4)	50 ± 10 (5)
Basin	94 ± 6 (2)	80 ± 11 (2)	75 ± 10 (2)
	Results of ANOVA		
Differences between Wetlands[3]	$F_{1,9} = 0.24$, $P = 0.63$	$F_{1,7} = 4.61$, $P = 0.07$	$F_{1,9} = 1.03$, $P = 0.34$
Tidal Creek vs. Main Channel[4]	$F_{1,10} = 3.22$, $P = 0.10$	$F_{1,7} = 1.08$, $P = 0.33$	$F_{1,10} = 12.3$, $P <$ **0.01**
Main Channel vs. Basin[5]	$F_{1,5} = 0.40$, $P = 0.56$	$F_{1,4} = 5.61$, $P = 0.08$	$F_{1,5} = 1.96$, $P = 0.22$

[1] Percentages of total numbers of fish captured (midwater and demersal) were only calculated for samples in which a total of at least 10 individuals of that group were captured to avoid having percentages based on small numbers unduly influence estimates.
[2] For ANOVA these data were transformed to arcsine√X to satisfy the assumption of normality.
[3] Comparison restricted to samples taken in the main channel habitat.
[4] Comparison restricted to samples taken in Carpinteria Salt Marsh.
[5] Comparison restricted to samples taken in San Dieguito Lagoon.

Table 10. Summary of mark-recapture study estimating catch efficiency of one haul of a 7.6 x 1.8 m beach seine and blocking nets.

Species or Group	Total Number Released	Total Number Recaptured	% of Total Recaptured	Average % Recaptured* Mean ± SE (N)
Atherinops affinis	290	222	76.6	75.9 ± 8.3 (8)
Fundulus parvipinnis	496	351	70.8	71.4 ± 5.3 (9)
Genyonemus lineatus	1	1	100	(0)
Syngnathus auliscus	1	0	0	(0)
Leptocottus armatus	3	0	0	(0)
Hypsoblennius gentilis	1	0	0	(0)
Porichthys notatus	1	0	0	(0)
Paralichthys californicus	11	4	36.4	(0)
Hypsopsetta guttulata	20	6	30.0	(0)
Pleuronichthys ritteri	1	0	0	(0)
Clevelandia ios	285	93	32.6	30.3 ± 6.2 (9)
Ilypnus gilberti	35	16	45.7	57.7 (1)
Quietula y-cauda	9	0	0	(0)
Gillichthys mirabilis	7	3	42.9	(0)
Midwater	787	574	72.9	73.9 ± 5.5 (12)
Demersal	374	122	32.6	30.6 ± 5.2 (11)
Demersal (excluding gobies)	38	10	26.3	(0)
Gobies	336	112	33.3	30.3 ± 6.4 (9)
All species	1161	696	59.9	61.3 ± 4.9 (12)

* Replicates in which < 10 tagged individuals of the species or group were released are excluded from these calculations.

Table 11. Catch efficiency of one beach seine haul and blocking nets in 3 wetlands[1] and 2 habitats[2].

	Catch Efficiency (% Recapture Rate)	
	Midwater Species[3]	Demersal Species
	Mean ± SE (N)	Mean ± SE (N)
Wetland		
Carpinteria Salt Marsh	80.7 ± 0.8 (3)	47.7 ± 7.7 (3)
San Elijo Lagoon	98.0 ± 2.0 (2)	30.0 ± 17.0 (2)
Los Peñasquitos Lagoon	67.7 ± 16.4 (3)	26.0 ± 7.8 (3)
ANOVA results:	$F_{2,5} = 3.21$, $P = 0.13$	$F_{2,5} = 1.49$, $P = 0.31$
Habitat		
Main channel	80.7 ± 0.8 (3)	47.7 ± 7.7 (3)
Tidal creek	61.5 ± 5.6 (4)	18.3 ± 7.3 (3)
ANOVA results:	$F_{1,5} = 8.61$, $P =$ **0.03**	$F_{1,4} = 7.64$, $P =$ **0.05**

[1] Comparison among wetlands restricted to main channel habitat as tidal creeks were only sampled in Carpinteria Salt Marsh.
[2] Comparison between habitats restricted to Carpinteria Salt Marsh, see note above.
[3] Recapture rates of midwater species transformed to arcsine√X to satisfy the assumption of normality.

Fig. 1.

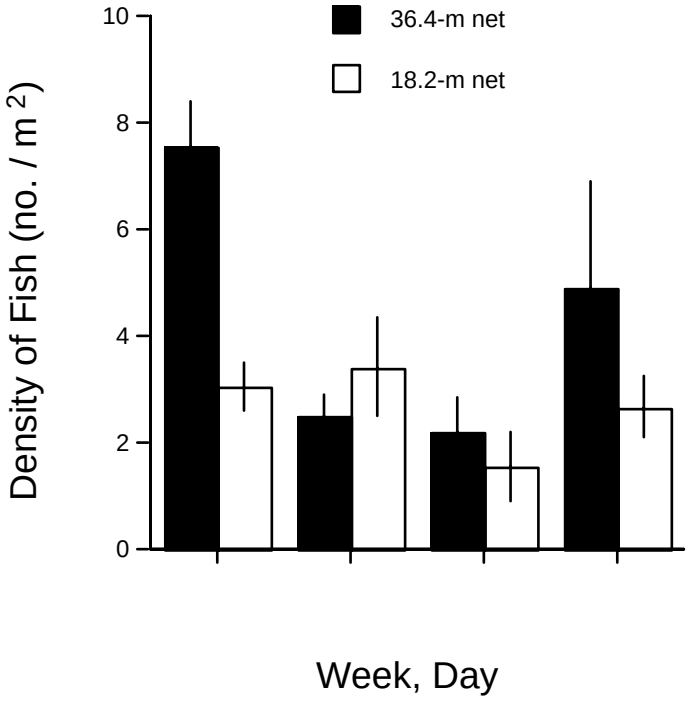


Fig. 2.

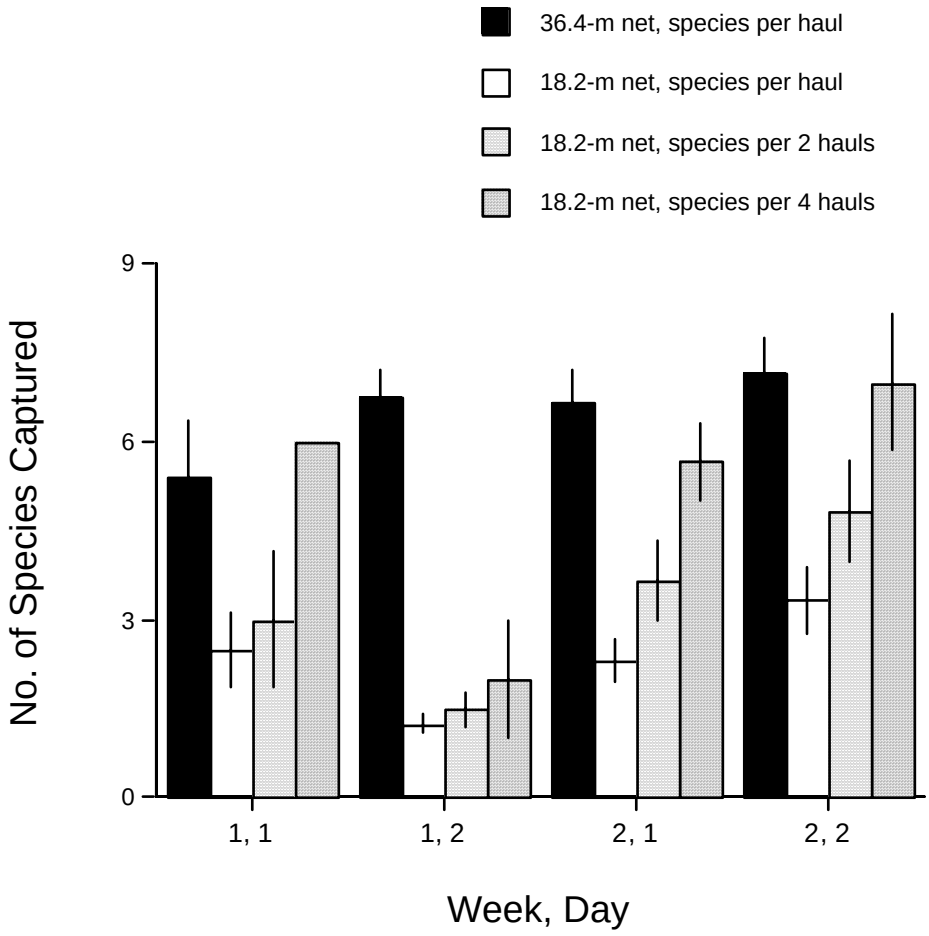

36.4-m net, species per haul
18.2-m net, species per haul
18.2-m net, species per 2 hauls
18.2-m net, species per 4 hauls
No. of Species Captured
9
6
3
0
1, 1
1, 2
2, 1
2, 2
Week, Day

Fig. 3.

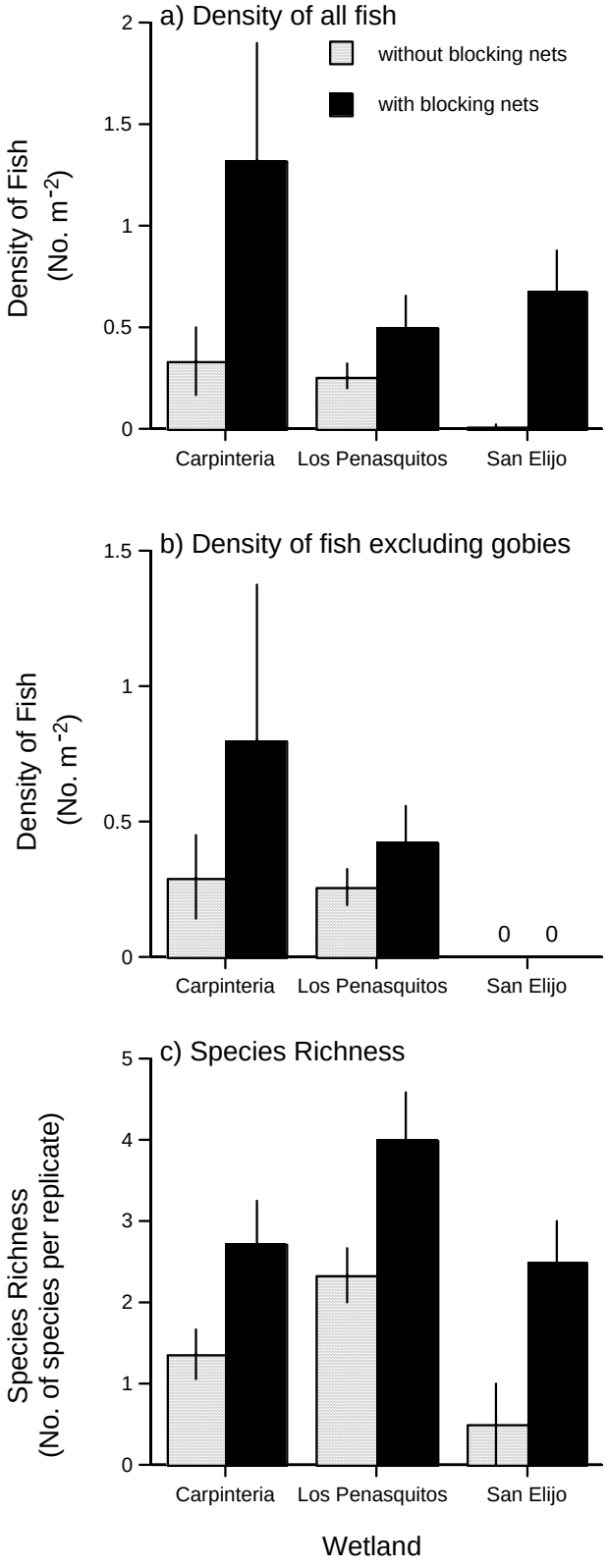
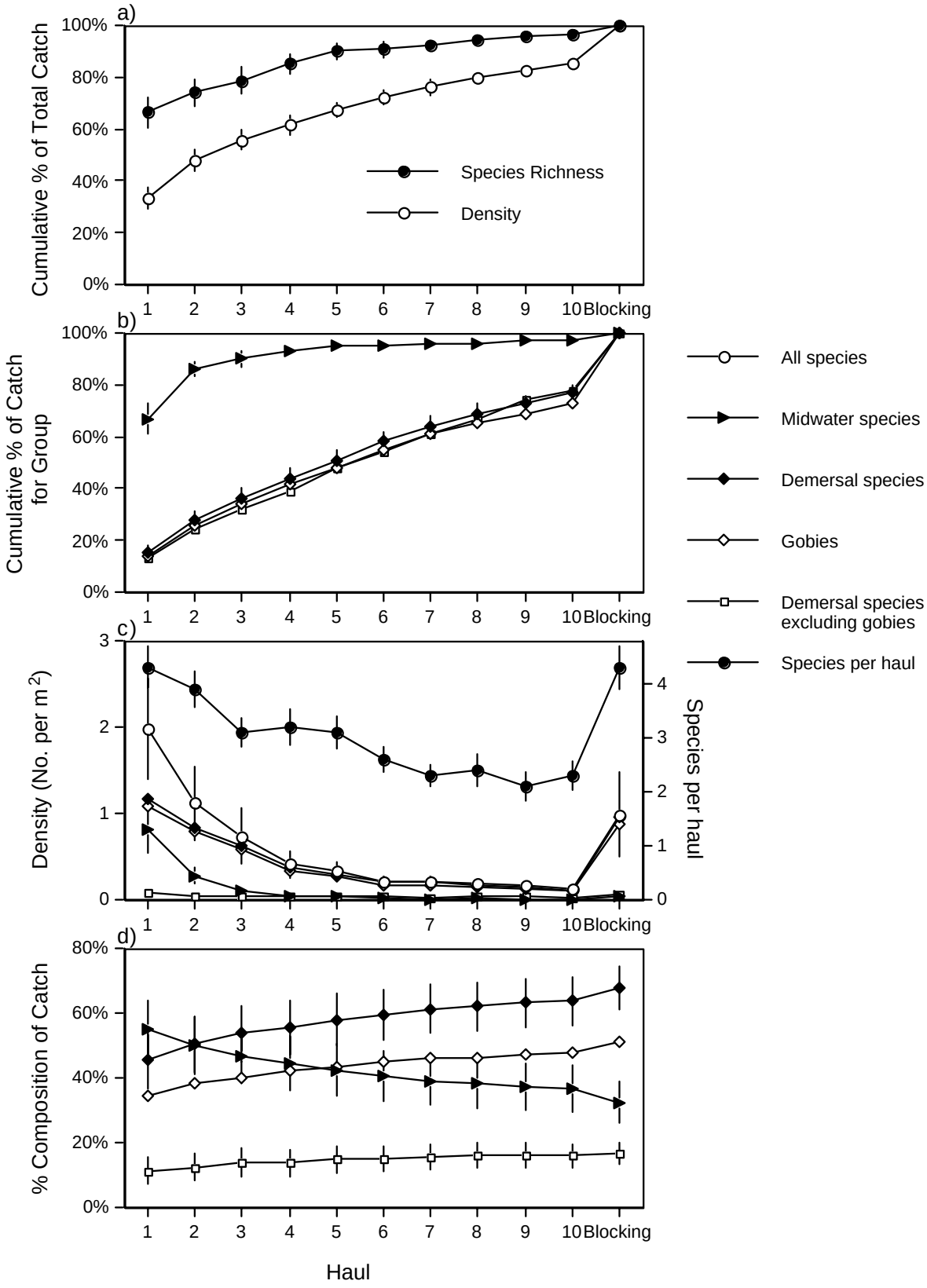

a) Density of all fish
without blocking nets
with blocking nets
Density of Fish
(No. m-2)
2
1.5
1
0.5
0
Carpinteria
Los Penasquitos
San Elijo
b) Density of fish excluding gobies
Density of Fish
(No. m-2)
1.5
1
0.5
0
0
0
Carpinteria
Los Penasquitos
San Elijo
c) Species Richness
Species Richness
(No. of species per replicate)
5
4
3
2
1
0
Carpinteria
Los Penasquitos
San Elijo
Wetland

Fig 4.


a)
b)
c)
d)
Cumulative % of Total Catch
Species Richness
Density
Cumulative % of Catch for Group
All species
Midwater species
Demersal species
Gobies
Demersal species excluding gobies
Species per haul
Density (No. per m 2)
Species per haul
% Composition of Catch
Haul
Blocking

Appendix 4

Spatial and temporal variation in fish assemblages in southern California coastal wetlands: implications for post-restoration monitoring at San Dieguito Lagoon and reference sites

Mark Steele, Steve Schroeter, Mark Page, and Dan Reed

Marine Science Institute

University of California

Santa Barbara, California, 93106

DRAFT

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Background and motivation for the study

The coastal development permit for SONGS Units 2 and 3 requires Southern California Edison to create or substantially restore a minimum of 150 acres of coastal wetland. The permit establishes biological performance standards that must be met by the restored wetland. One of these standards requires that within 4 years of construction, the total densities and number of species of fish in the restored wetland be similar to reference wetlands (section 3.4b.1 of the SONGS permit).

To determine whether the performance standards for fish have been met, estimates of density and species richness must be obtained in the restored and reference wetlands. These estimates must accurately reflect the wetland-wide values of those two variables. It can be difficult to obtain such representative estimates if there is wide spatial variation in density or species richness within wetlands or if there is strong temporal variation in these variables. Typically, both situations exist in southern California wetlands (Desmond et al. 2002). Effective sampling programs can be developed in the face of such spatio-temporal variation, but only if the magnitude and patterns of variation are relatively well understood. Such an understanding not only enables measurement of the variables of interest, it can also help to maximize the efficiency of sampling programs.

In this paper, we measure spatial and temporal variation in density and species richness of fish assemblages in a series of studies in southern California wetlands and use this information and that from published studies to provide guidelines for sampling designs that optimize effort, and thus maximize efficiency.

Methods, Materials, and Results

Patch size

Methods and Materials

To determine whether there is a characteristic spatial scale of patchiness in fish density, we sampled fish with enclosure traps (Appendix 2) in tidal creeks and main channels in Carpinteria Salt Marsh and Mugu Lagoon. At Carpinteria we sampled one section of the main channel (on 13 September 2002) and one tidal creek (on 14 September 2002). Each section was 100-m long and samples were taken every 2 m. In the main channel, because fish densities were relatively low along the 100-m transect, which was placed near the center of the channel, we took an additional 30 samples spaced 2-m apart along a 60-m line approximately 5 m towards shore and parallel to the 100-m line of samples. Fish densities were higher along this shorter transect. At Mugu Lagoon, two tidal creeks and one section of the main channel were sampled on 29 and 30 October 2002, respectively. In the two tidal creeks, samples were taken every 2 m along 160-m-long transects (n = 80 in each creek). In the main channel, samples were taken along a 280-m transect. Over the first 98 m, samples were taken every 2 m and over the remainder of the transect, samples were taken every 4 m. In total, 95 samples were taken along the 280-m transect. Enclosure traps (cylindrical, 0.4 m^2 x 0.9-m high) were sampled with BINCKE nets with 1.6-mm mesh until 3 hauls had produced no fish (details in Appendix 2). Fish captured were identified, counted, and released.

The data on fish density were analyzed for spatial autocorrelation using variogram and correlogram analysis (Rossi et al. 1992; details provided in Appendix 5). Variograms

(semivariance) and correlograms (Moran's I) were constructed using GS+ geostatistical software (Gamma Design Software). To ensure robust results, lag class intervals were set to distances that ensured that at least 30 pairs of samples were used to estimate semivariance and correlation for each lag class; and lag distance was set to one half the length of each transect (Rossi et al. 1992). Because abundance data are typically log-normally disturbed, data were transformed to ln(x+1). We did not attempt to fit any of the standard variogram functions to our data because these functions clearly did not fit our data.

Results

Variograms revealed two general patterns in fish density: an initial increase in variability as samples became more widely spaced, followed by a drop, and then a leveling off; and, more commonly, cyclic fluctuation about a set value of variation (Fig. 1). The first pattern was found only on the long (280 m) transect in the main channel of Mugu Lagoon. On this transect, densities in closely spaced samples were similar but became increasingly different as the separation distance increased to about 70 m, at which point they became somewhat more similar until they were separated by about 100 m. Variation among samples separated by 100 – 140 m was intermediate and fairly constant. Viewed as spatial autocorrelation rather than semivariance, samples on the Mugu main channel transect were highly correlated when close together but this correlation declined until they were uncorrelated when about 40 m apart, and then they became increasingly negatively correlated to about 70 m. From there, they became less negatively correlated until separated by about 100 m, and from that point on, they were uncorrelated. At this site then, samples were not independent until they were separated by at least 100 m.

The second pattern found to varying degrees along the other 5 transects was cycling about a set value of semivariance or correlation (Fig. 1). The amplitude and wavelength of the cycle varied among the 5 transects. Cycles indicate a repeated spatial pattern in density. For example, in the tidal creek sampled at Carpinteria Salt Marsh, fish densities tended to be similar to those within a few m and to those 20 and 40 m away, while differing from those 10 and 30 m away. In other words, this cycle had a wavelength roughly 20-m long. Wavelengths of the other cycles in fish density appeared to vary from about 40 to 80 m.

Measuring spatial variation at hierarchical scales

Methods and Materials

Analysis of variograms and correlograms gives insight into patch size and the spacing of samples necessary to ensure independence of samples, but it offers little insight into how many samples are needed to obtain precise estimates of density and species richness and how those samples should be distributed among spatial scales. Hierarchical sampling analyzed with nested analysis of variance (ANOVA) can be used to estimate variance of at each nested scale, and these estimates, combined with cost estimates, can be used to maximize the precision of the wetland-wide estimates of fish density and species richness for a given level of sampling effort (Sokal and Rohlf 2001). It is the precision of these wetland-wide estimates that will set the power of the tests comparing the restored wetland with reference wetlands. We used this hierarchical sampling approach to help evaluate how best to sample fish density and species richness.

Replication of sampling in wetlands can occur at various natural and imposed scales. For example, each tidal creek could be viewed as a replicate within a wetland, and then several

widely spaced "blocks" containing closely spaced replicate samples could be taken within each creek. Such a sampling scheme would be spatially nested (hierarchical), with replicates nested within blocks, which are nested within creeks, which are nested within wetlands.

We used such spatially nested designs to sample fish density and species richness with 3 methods: enclosure traps, beach seines, and purse seines. We used these three methods because together they provide good estimates of fish density and species richness (Appendix 3). For this work, it was not necessary to study each method concurrently, so to simplify logistics, we studied each method separately. With each method, we used the same general sampling design, described below (and summarized in Table 1).

Enclosure traps:

We sampled fish with enclosure traps in 2 wetlands in autumn of 2002. In Carpinteria Salt Marsh, we sampled two habitats: main channel (29 September) and tidal creek (30 September). In Mugu Lagoon, we sampled three habitats: main channel (13 October), tidal creek (14 October), and basin (12 October).

Within each habitat, we sampled 4 stations. Stations were separate tidal creeks or widely spaced areas in main channel and basin habitats. A segment 140-m long constituted a station in tidal creek habitat, whereas stations were 260-m-long segments in main channel and basin habitats. The greater extent of stations in main channel and basin habitats relative to tidal creeks reflects the greater extent of these habitats in nature. In tidal creeks, stations were separated by at least 100 m along waterways, or 50 – 250 m straight-line distance. Stations in main channel and basin habitats were separated by 350 – 550 m along waterways.

At each station, we sampled 4 blocks that contained 4 replicate samples. (There was one exception to this: we sampled only 3 blocks in one of the main channel stations at Mugu Lagoon because sampling in the 4th block would have disturbed federally protected marine mammals, harbor seals.) The 4 replicates within each block were spread over a 20-m stretch. One sample was taken at a random position within in each of the four 5-m-long segments within each 20-m-long block. The spacing of blocks differed among habitats, reflecting natural differences in the extent of different habitats. In tidal creeks, blocks were 20-m apart, whereas they were 60-m apart in the main channel and basin habitats.

Enclosure traps (cylindrical, 0.4 m^2 x 0.9-m high) were sampled with BINCKE nets with 1.6-mm mesh (details in Appendix 2). For each replicate, BINCKE net hauls were made until 3 hauls had produced no fish. Fish captured were identified, counted, and released.

Beach seines:

We sampled fish with beach seines in blocked areas in Carpinteria Salt Marsh on 6 – 8 October 2004. Two habitats were sampled: tidal creek and main channel. We sampled 6 stations in each of the two habitats and took 2 replicate samples at each station. As defined above for the enclosure trap study, stations were separate creeks in the tidal creek habitat and widely spaced segments of the main channel habitat. The two replicate samples at each station were separated by 50 m. Their spacing was thus similar to that of blocks in the enclosure trap study. Stations in tidal creeks were at least 100 m apart along waterways (50 – 250 m apart by line of sight). Main channel stations were 200 – 350 m apart (by line of sight or along waterways).

Samples of fish were obtained by seining blocked 6-m-long segments. The 6-m segments ranged in width from 3 – 12 m for tidal creeks and 10 – 26 m for main channels. A beach ("pole") seine

7.6-m long x 1.8-m deep was used to seine the blocked segments. This net was hauled through the blocked area 5 times before both blocking nets were hauled in. A sample was composed of the catch of all 7 hauls. All three nets used were built of 3-mm mesh, knotless, nylon netting. (Details of seine design and methods are provided in Appendix 3.) Seining was conducted during periods with low water flow, i.e., on relatively flat tides ranging from low to middle heights. All fish captured were identified, counted, and released.

Purse seines:

We used purse seines to sample fish in two wetlands, San Dieguito Lagoon and Tijuana Estuary, on 29 and 30 September 2004, respectively. Only the main channel habitat was sampled because this was the only habitat present in these two wetlands that was broad and deep enough to sample with purse seines. Within this habitat, the sampling design was essentially the same as that used with beach seines: 2 replicate samples were taken at each of 6 stations. Replicates were spaced 50 m apart and stations were 300 – 450 m from the next nearest station.

We used a purse seine that was 36.4-m long x 3.6-m deep, built of 3-mm knotless, nylon netting. Other details of purse seine design and methods can be found in Appendix 3. Samples were taken in water 1 – 3 m deep during mid to high tides. All fish captured were identified, counted, and released.

Analysis

Nested analysis of variance (ANOVA) was used to estimate the variance in both fish density and species richness at the various hierarchical spatial scales sampled. Separate analyses were conducted for each habitat and for fish density and species richness. The ANOVA model for enclosure trap samples included the terms Wetland, Station nested within Wetland, and Block nested within Station (replicates, then, were nested within Blocks). Since only one wetland was sampled with beach seines and there were no "blocks" in this design, those models included only Station. The models for purse seine samples included Wetland and Station nested within Wetland.

Variance at each hierarchical level was calculated using standard methods (see e.g., Winer et al. 1991, Sokal and Rohlf 2001). Nested ANOVA assumes that variance is additive from small nested scales to larger scales. Put another way, the underlying statistical assumption is that variance at a higher nested level must be greater than that at a lower level. In practice, with real data, this assumption does not always hold. When that happens, nested ANOVA produces negative estimates of variance at higher hierarchical levels, which is logically impossible. In such cases, we followed the procedures described by Fletcher and Underwood (2002) to estimate variance. In short, the term with the largest negative estimate of variance is dropped from the ANOVA model and its variance set to zero. The new reduced model is then run and if it produces yet another negative estimate of variance, that term is then dropped from the model and its variance set to zero. This procedure is followed until there are no negative estimates of variance, which can produce a model with only an error term in it. In such cases, variance at the lowest nested scale (the scale of replicates) equals the variance of all replicate samples pooled, and variance at all higher hierarchical scales is estimated as zero.

Once variance estimates have been obtained, they can be used to optimize sampling effort at each hierarchical scale to produce an estimate of the dependent variable (fish density or species richness) that is the most precise possible for a given level of sampling effort. Alternately, a

desired level of precision (variance) of the estimate can be set and the necessary sampling effort can be calculated. Either approach relies on the same basic procedure: minimizing the sum of the products of variance and cost at each hierarchical level of sampling. Using our study with enclosure traps as an example, the goal then is to minimize

$$C_{n(c)}S^2_{n(c)} + C_{c(b)}S^2_{c(b)} + C_{b(a)}S^2_{b(a)} \quad (1)$$

Where C_i is the cost (in time, money, or any other appropriate metric) at each level, i and S^2_i is the variance at each level, i. Thus, $C_{n(c)}$ is the cost of sampling an extra replicate, $C_{c(b)}$ is the cost per extra block, $C_{b(a)}$ is cost per extra station; $S^2_{n(c)}$ is the variance at the replicate level, $S^2_{c(b)}$ is the variance at the block level, and $S^2_{b(a)}$ is the variance at the station level.

The total cost of sampling in one wetland is given by the formula

$$C = bcnC_{n(c)} + bcC_{c(b)} + bC_{b(a)} \quad (2)$$

Where b is the number of replicates at the first nested level (stations in our study), c is the number of replicates at the second nested level (blocks in our study), n is the number of replicates at the lowest nested level (replicates within blocks in our study).

To minimize the sum of the products of cost and variance at each hierarchical level, first, $C_{n(c)}S^2_{n(c)}$ is minimized by solving the following equation for the optimal n:

$$n = \sqrt{(C_{c(b)}S^2_{n(c)}/C_{n(c)}S^2_{c(b)})} \quad (3)$$

Then $C_{c(b)}S^2_{c(b)}$ is minimized by solving the following equation for the optimal c:

$$c = \sqrt{(C_{b(a)}S^2_{c(b)}/C_{c(b)}S^2_{b(a)})} \quad (4)$$

The optimal replication of b can then be obtained by setting C (i.e., deciding how much effort will be spent per wetland) and solving equation 2 for b:

$$b = C/(cnC_{n(c)} + cC_{c(b)} + C_{b(a)}) \quad (5)$$

Or setting the desired level of variance about the wetland-wide estimate of the mean (S^2_a) and solving the following equation, which gives the expected value for S^2_a, for b:

$$S^2_a = S^2_e/ncb + S^2_{c(b)}/cb + S^2_{b(a)}/b \quad (6)$$

Which gives

$$b = (S^2_e/nc + S^2_{c(b)}/c + S^2_{b(a)})/S^2_a \quad (7)$$

In this study, however, we did not solve for optimal b because that requires somewhat arbitrary decisions to be made about how much total effort should be spend sampling or what level of variance is acceptable. Instead, we used the values of n and c produced by equations 3 and 4 with a range of possible values of b in equation 6 to evaluate graphically how the overall value of within-wetland variance was expected to change with replication at the station level.

We measured the cost in time. $C_{n(c)}$, then, was our estimate of the time it took to sample a single enclosure trap and $C_{c(b)}$ and $C_{b(a)}$ were our estimates of the time it took to move from one block to another and from one station to another, respectively (Table 1).

The procedure described above for optimizing sampling effort cannot be used in cases where variance at lower nested levels is estimated to be zero. (E.g., equation 3 cannot be solved if the estimate of $S^2_{c(b)}$ is 0.) In such cases, we used the relative magnitude of variation at each level

and other aspects of experimental design to make conclusions about how best to allocate effort among hierarchical sampling scales.

Results

Nested ANOVA on data from enclosure traps revealed that the greatest variability in both density and species richness within wetlands was found at the replicate scale (samples spaced 1-9 m apart) in all 3 habitats sampled (Table 2). In tidal creeks, there was also considerable variance in both density and species richness at the station level, but relatively little variation among blocks (Table 2, Figs. 2-5). By contrast, in the main channel habitat there was greater variance at the block scale than the station scale. Another difference between tidal creek and main channel habitats was that densities and species richness differed between wetlands in the main channel habitat, but not the tidal creek habitat (Table 2, Figs. 2-5). The basin habitat was sampled only in Mugu Lagoon and here the only non-zero estimate of variance was at the replicate scale.

Analysis of data from beach seines revealed that for both density and species richness in tidal creeks and density in the main channel habitat, variance was greater at the station scale – places 100's of m apart – than at the replicate scale – samples 50-m apart (Table 2, Fig. 6). For species richness in the main channel habitat, however, there was no measurable variance at the station scale and all variance was assigned to the replicate scale.

Analysis of purse seine data revealed different partitioning of variance between the two response variables, density and species richness (Table 2, Fig. 7). For density, variance was greater at the station scale than the replicate scale; whereas for species richness, variance was greater at the replicate scale than the station scale. Density and species richness did not differ between the two wetlands sampled (Table 2).

The optimization procedure to maximize the precision of estimates of fish density and species richness per unit effort produced fairly consistent recommendations for sampling done with both beach and purse seines. In 5 of the 6 combinations of habitat studied (tidal creek and main channel) and variable measured (density or species richness), the optimization procedure indicated that only one replicate seine sample should be taken per station (Table 3). In the 6^{th} case, species richness measured with beach seines in the main channel habitat, it was not possible to solve the optimization equations because the estimate of variance at the station scale was zero.

The solutions for optimizing the efficiency of sampling with enclosure traps were more variable than those for sampling with seines. In 3 of the 4 cases where optimization was possible at the replicate scale (samples separated by 1 – 9 m), the optimal number of replicates per block was 1; and in the other case it was 2 (Table 3). In the basin habitat we did not attempt to optimize replication for measuring species richness because the data were not appropriate (see Table 3); and it was not possible to optimize sampling of density because estimates of variance were zero at all levels except the replicate scale.

Using the full ANOVA models for the enclosure data, it was only possible to calculate optimal replication at the scale of blocks (areas separated by 20 or 60 m) in 3 of 5 cases. In 2 of those 3 cases, the optimal number of blocks per station was 1; in the other case it was 3 (Table 3). To gain more insight into variability at the scale of blocks, we also estimated variance at this and larger scales by using nested ANOVA on the same data sets, but in these data sets, replicates within blocks were summed to create an aggregate sample. In other words, blocks became the

lowest spatial scale of replication, making the spatial structure of the enclosure study nearly identical to that of the beach and purse seine studies. These ANOVAs (not shown) produced non-zero estimates of variance at the block and station scales in 5 of 6 cases, thus permitting calculation of optimal replication at the block scale for these cases. In 3 of 5 cases, the optimal number of blocks (composed of 4 pooled samples) was 1; in another case it was 4; and in another 5 (Table 3).

For all 3 methods used to sample fish (enclosure traps, beach seines, and purse seines), the expected precision of wetland-wide estimates of density and species richness improved rapidly as the number of sampling stations per wetland increased up to 5 – 10 stations (Fig. 8). Beyond this level of replication, precision improved at a much slower and decelerating rate, while the cost of added replication increased at a constant rate (Fig. 9).

Estimating total species richness

Species richness can be compared among wetlands in two general ways: the average number of species per sample (as above) or the total number of species present in each wetland. To determine the number of samples needed to obtain an adequate estimate of total species richness, we analyzed data collected with beach and purse seines in the studies described above. With beach seine data (all collected in Carpinteria Salt Marsh), we conducted separate analyses for tidal creeks and main channels. With purse seine data (all collected in the main channel habitat), we conducted separate analyses with data collected in San Dieguito Lagoon and Tijuana Estuary. For each of these 4 analyses, 12 samples had been taken in the field. We evaluated how the total number of species captured changed as a function of the number of samples taken. For each possible number of samples taken (1-12), we calculated the mean number of species captured by randomly selecting the appropriate number of samples from the pool of 12 samples. This procedure was repeated 100 times for each number of samples (except 12, which could only take one value – the actual value found in the field).

Although there was some variation in the pattern of accumulation of species with samples, in all cases the rate of species addition slowed as more samples were taken and appeared to reach an asymptote after about 10-11 samples had been taken (Figs. 10 & 11). Based on these data, taking more than 10-11 samples would have little impact on the estimate of total species richness in a particular habitat in a wetland.

Tidal variation and effects of depth

Methods and Materials

We evaluated the influence of tidal variation by studying the effects of elevation and water depth across a range of tidal heights. We sampled fish density with enclosure traps in 3 wetlands on days with large tidal amplitude (1.7 – 2.5 m predicted on the coast) to capture the full range of tidal variation. Tidal variation within the wetlands we sampled (San Elijo Lagoon, Los Peñasquitos Lagoon, and Carpinteria Salt Marsh) was less than that on the coast because tides are muted in most wetlands in southern California. In each wetland, over the course of the tidal cycle, we estimated fish density at 3 different elevations at 2 or 3 depths.

We sampled on 4 days (Table 4) on which the tide was high in the morning and fell throughout the day until reaching a low in late afternoon. We sampled throughout this period, sampling the highest elevations and deepest depths first, during the high tide, and the lowest elevations and shallowest depths last, during the low tide.

The range of elevations sampled was much less than that expected on the coast (< 1 m; Table 4) because tidal amplitude was low in the wetlands studied. We sampled in water 23 to 76-cm deep because this range brackets the depths that we could sample effectively with enclosure traps. It was not possible to sample all elevations at all depths because of the limited tidal amplitude. For those elevation-depth combinations sampled, the numbers of replicate samples taken is given in Table 4. Adjacent replicate samples within each elevation-depth combination were separated by 10 m (except San Elijo in November, where spacing ranged from 10 – 40 m) and samples taken at the same elevation but different depths were offset by 5 m to avoid sampling already disturbed areas.

The elevation of areas sampled was estimated from the predicted height of the high tide. For example, for an area that was under 50 cm of water at high tide when the predicted height of that tide was 2 m, the estimated elevation of that area would be 1.5 m. We marked tide staffs with the height of the high tide and then used these staffs to measure all elevations throughout the rest of the day. All samples within an elevation category varied in elevation by less than 10 cm. Variation within depth categories was also < 10 cm. Depths were measured with meter sticks after placing the enclosure trap around the area to be sampled. We used the methods described in previous sections for sampling fish with enclosure traps.

We used ANOVA to test for differences in density estimates among elevations and depths. Both factors were treated as fixed in ANOVA models because they were drawn from a limited range of possible values of depth and elevation. The interaction between depth and elevation could not be evaluated with ANOVA because it was not possible for us to sample all combinations of the two factors. We did not evaluate the influences of elevation or depth on species richness because too few species were caught to justify such analysis.

Results

Two species of fish made up 98.7% of the fish caught in the 4 studies, the arrow goby, *Clevelandia ios* (93.5% of the total) and the shadow goby, *Quietula y-cauda* (5.2% of the total). We restricted our analyses to these species because all others were too rare to support analyses. The two species appeared to respond somewhat differently to elevation and water depth, so we analyzed them separately. Numbers of arrow gobies were sufficient in all four studies to justify statistical analysis, but this was true of shadow gobies in only one study (though we present graphical results for this species in a second study).

Estimates of arrow goby density tended to be higher when sampling was done at a water depth of 45 cm than at deeper or shallower depths (Fig. 12). This pattern was only statistically significant in one study, however (Table 5). In contrast to arrow gobies, estimates of shadow goby density did not appear to be affected much by water depth (Fig. 13; ANOVA results from Los Peñasquitos Lagoon: $F_{2,25} = 1.04$, $P = 0.37$).

The elevation at which samples were taken strongly influenced estimates of shadow goby density, and sometimes influenced arrow goby density, but did so inconsistently among wetlands. Shadow gobies were most abundant at lower elevations (Fig. 13; ANOVA results

from Los Peñasquitos Lagoon: $F_{2,25} = 12.6$, $P = 0.0002$). The effect of elevation on arrow goby density was only statistically significant in Carpinteria Salt Marsh, where densities increased from high to low elevations (Fig. 12, Table 5), the same pattern seen in shadow gobies. Elevation had no obvious effect on arrow goby densities in Los Peñasquitos Lagoon, but densities of this species tended to increase with elevation in San Elijo Lagoon (Fig. 12, Table 5).

Daily and weekly variation

Methods and Materials

We measured daily and weekly variation in fish density and species richness in 3 studies, one each with enclosure traps, beach seines, and purse seines. The sampling design was similar for all 3 studies. One or two areas within one wetland were sampled on 4 days: 2 consecutive days in each of two periods separated by 2-3 weeks. By spacing the two periods by 2-3 weeks, we matched tidal conditions quite closely, thus minimizing this potentially confounding influence.

The study with enclosure traps was conducted in the main channel of San Elijo Lagoon on 16 and 17 June and 10 and 11 July 2003. Two 50-m-long areas ("stations") separated by several hundred m were repeatedly sampled on all 4 days. At each station on each day, 20 samples were taken. These were grouped into 10 pairs. One member of the pair was close to shore and the other was 1 – 15 m towards the center of the channel. A pair of samples was taken every 5 m along the 50-m stretch of channel at each station. The catches from the two members of a pair were pooled and treated as one replicate for analysis. On the second of consecutive days, the location of each sample was shifted by 2.5 m relative to the prior day to avoid resampling areas that had been disturbed on the previous day. Samples were taken in water 11-85 (mean = 41) cm deep. Enclosures (0.4 m^2 area sampled) were sampled with a BINKE net until 3 hauls containing no fish had been obtained. All fish captured were identified, counted, and released.

The study with beach seines was conducted in Los Peñasquitos Lagoon on 8, 9, 22, and 23 September 2004. Five replicate samples were taken in the same ~ 200-m-long stretch of main channel on each of the 4 days. Each sample consisted of the catch from an area 6-m long extending 18-32 m across the channel. Each area sampled was blocked with two blocking nets – beach seines with 3-mm mesh that were 30-35 m long and 1.8 m deep. The blocked area was sampled 5 times with a beach ("pole") seine that was 7.6 m long x 1.8 m deep built of 3-mm mesh, knotless, nylon netting. The 2 blocking nets were then hauled in and fish caught in them were included in the catch for that sample. (Details of seine design and methods are provided in Appendix 3). Replicate samples were separated by ~ 50 m. On the second of consecutive days, the location of each sample was shifted by ~ 10 m to avoid areas where the substrate had been disturbed the day before. All fish captured were identified, counted, and released.

The study with purse seines was conducted in San Dieguito Lagoon on 2, 3, 16, and 17 September 2004 and is described in detail in Appendix 3. For this exploration of temporal variation in fish density and species richness, we restricted our analysis to catches from the larger purse seine (36.4 x 3.6 m; 3-mm mesh) because of its superior sampling characteristics relative to the smaller net (Appendix 3). To briefly summarize the sampling, on each day we took 4 – 6 replicate samples in a ~ 0.5 km stretch of the main channel; an area roughly 30-m wide and 0-2 m deep. Samples were spaced approximately evenly along the 0.5-km stretch of channel. All fish captured were identified, counted, and released.

We used nested ANOVA to compare the relative magnitudes of daily and weekly variation in density and species richness. Days were nested within weekly periods and both were treated as random variables. The same optimization procedure described above in the section on spatial variation was used to determine how best to allocate effort between replication at the weekly, daily, and within day scales.

To a certain extent, our sampling designs confounded temporal and spatial variation. Where possible, we statistically eliminated the influence of spatial variation on the calculation of temporal variance in fish density and species richness. In the enclosure trap study, a spatial component was explicitly included in the design: two stations were sampled on each day in each weekly period. We filtered out this spatial variation by including the term Station in the ANOVA model. A more complicated confounding occurred at the level of replicates (within days), because while these samples were taken over the course of a day (and thus estimate within-day temporal variation) they were also distributed over space because it was impractical to sample a single spot repeatedly within a day. Fish originally located in this spot would flee after being captured and released, and other fish would avoid it due to the disturbance caused by sampling it. Hence, our replicate samples were taken at different spots, 5 – 50 m apart depending on the study. There can be considerable spatial variation at these small spatial scales (see results above). We minimized the influence of the small-scale spatial variation by including in the ANOVA models the term Area, which corresponded to particular locations 10 – 20 m in extent in which samples were taken. For example, in the study of temporal variation with beach seines, 5 Areas were sampled on each day. The Area term was excluded from ANOVA models if including it had little effect on the estimate of temporal variance at the Day or Week levels.

Results

Estimates of fish density varied much more among the 4 days of each study than did estimates of species richness (Fig. 14). Variance in both density and species richness, however, was always greater within days than among days or weeks (Table 6). The relative magnitude of variance among days versus among weeks, however, was not the same among all sampling methods and response variables. For enclosure samples, variance among weeks was greater than among days; and this was also true for species richness measured with beach seines. Density estimates from both beach and purse seines revealed greater variance among days than among weeks. For species richness measured with purse seines, estimates of variance among days and weeks were zero.

The optimization procedure to maximize the precision of estimates of fish density and species richness per unit effort was able to produce recommendations for replication at the within-day level for density as measured by all 3 methods, but only for species richness measured by enclosure traps (Table 7). Optimal replication within days ranged from 6 – 47 samples per day. In the 3 cases where optimal replication of daily sampling could be calculated, the optimal number of days of sampling was 1, 1, and 2. Where both could be calculated (3 cases), predicted optimal replication of samples within days was much higher than replication of daily samples; ratios ranged from 3:1 to 47:1.

Predicted precision of the estimates of density and species richness for the period sampled improved rapidly as the number of weeks sampled increased from 1 to 5, but precision improved little beyond 5 weekly sampling episodes (Fig. 15). Each extra weekly sampling period, however, added a great deal of extra effort (Fig. 15; Table 7). Yet even at low levels of weekly

sampling (e.g., 1-2 weeks), estimates of density and species richness from beach and purse seines were predicted to be quite precise (CV = 6-10% for density and 5-15% for species richness).

Discussion and Recommendations

An efficient and effective monitoring program must deal appropriately with spatial and temporal variation in the response variables being monitored. In regards to spatial variation, our studies indicate that the primary aim of a program monitoring wetland fishes should be to sample many (at least 5-10) widely spaced stations within each habitat in each wetland. If the goal of the program is to measure total species richness, then at least 10 beach or purse seine samples should be taken within each habitat. Results of variogram and correlogram analyses indicate that stations should be spaced at least 100 m apart to ensure statistical independence. These sampling stations are the fundamental units of replication within wetlands and thus have the greatest potential to improve precision of the estimates of mean density and mean species richness per sample or area (Fig. 9). Moreover, as the fundamental units of replication, the number of sampling stations will determine the degrees of freedom in the denominator of *F* tests of differences among wetlands (Underwood 1981). Greater df in the denominator of *F* tests produces greater test power. Thus, increasing the number of sampling stations will increase the power of tests by both improving precision and increasing df. To ensure that sampling provides representative estimates, stations should be selected in a stratified or stratified-random manner (Legendre et al. 2004). For a small wetland like Carpinteria Salt Marsh, all possible stations (e.g., all tidal creeks) may have to be sampled to obtain adequate sample sizes.

The precision of wetland-wide estimates of fish density and species richness can be further improved by replicating samples within stations. For both purse and beach seines, samples within stations should only be replicated if all possible stations can be sampled within the time available and extra time is anticipated. Because enclosure traps sample small areas (0.4 m^2) and it takes relatively little time to collect a sample, it is worthwhile and costs little to take more than one sample per station. Optimal sample sizes per station range from 1-5. We recommend at least 2 samples spaced by at least 20-60 m be taken per station.

Fish density and species richness may also vary temporally and effective monitoring must deal with such variation. In this study, we evaluated variation among weeks, days, and within days. Variation may also occur diurnally, seasonally, and annually. Our results indicate that among weeks, days, and within days, most variation occurs within days, so there should substantial replication of samples within days (Table 7). There should be little if any replication of days of sampling within weekly periods. For beach and purse seines, acceptable levels of precision can be achieved by sampling during only one or two weekly periods. For density, sampling with enclosure traps on only one day in each of two weekly periods is also predicted to produce an acceptable level of precision: a CV of 15%. For species richness, however, the same quantity of sampling with enclosure traps is expected to produce a less precise estimate: a CV of 35%. We view this as acceptable because enclosure traps, by virtue of the limited range of taxa collected by them and small area sampled, are not well suited to estimating mean species richness, which should instead be estimated with beach and purse seines. Total (rather than mean) species richness should be determined by the combination of all three methods. Overall, we recommend for all 3 methods that sampling be conducted during 2 weekly periods, with samples taken on only one day within each period. (Although the optimal number of sample days within weeks

was 2 for purse seine sampling – Table 7 – it would be more efficient to double the within day sample size than to repeat sampling on a second day. This approach, rather than taking 6 samples on each of 2 days, is predicted to have a trivial effect on the precision of estimates of density and species richness: an increase in CV of about 1% or less.) We recommend that a minimum of 10 beach and purse seine samples be taken per day in each habitat (Table 7). These samples should be distributed in space as described above. We recommend that at least 12 enclosure trap samples be taken in at least 6 widely spaced stations in each habitat.

We also recommend that the two sampling periods occur during summer and be spaced about a month apart. Sampling should be conducted during summer to capture predictable annual peaks in density and species richness of southern California wetland fish (Allen 1982, Horn and Allen 1985, Brooks 1999, Desmond et al. 2002, Merkel & Associates, Inc. 2002). Because periods of peak density and species richness may not be perfectly synchronous in different wetlands in southern California, sampling fish twice during the summer at the restored and reference wetlands may help to avoid erroneous inferences caused by hitting the peaks in certain wetlands but not others.

Desmond et al. (2002) found significant seasonal variation in fish abundance and interpreted this finding as indicating that wetland fish should be monitored at least 4 times a year, during different seasons. If the goal is to measure seasonal patterns, this interpretation is correct. If, instead, the goal is to compare among wetlands or years, a more efficient sampling design is to monitor fish in only one season and restrict all comparisons to this season. That is the approach we recommend.

There can also be significant interannual variation in fish density and richness (Brooks 1999, Allen et al. 2002, Desmond et al. 2002, Merkel & Associates, Inc. 2002). It is important, therefore, to restrict comparisons among wetlands to the same year or set of years.

Wetland fish density and species richness can also vary over time scales shorter than years, seasons, weeks, or days. Our work documented substantial within-day variation (Table 6). Because our samples also had to be distributed over space, to some extent our results confound within-day variation with spatial variation. Nevertheless, it is reasonable to expect that some of the variation we measured is temporal, and our study on the influence of tidal variation provides evidence of one type of within-day variation. Tidal variation can be dealt with by restricting sampling to certain tidal conditions, which can severely limit time available for sampling, or sampling can be done across a range of tidal conditions. Provided that sampling occurs over the same range of conditions in each wetland, there should be no bias in estimates of density or richness among wetlands. The results of our study on tidal variation imply that to the extent possible, samples should be taken at similar elevations and water depth. Another type of within day variation is caused by the normal movement patterns of fish, particularly schooling species, which can be observed transiting areas. Samples taken just seconds apart could conceivable capture or completely miss such mobile schools. While movement of schools of fish contributes to variability among samples, if enough samples are taken, estimates of density and species richness should be reasonably precise.

There is also evidence that estimates of density and species richness of wetland fish vary diurnally (Merkel & Associates, Inc. 2002). Estimates tend to be higher at night than during the day. This pattern however, probably does not reflect actual differences in density and species richness between night and day. Instead, the differences in catches of fish are probably driven by

reduced net avoidance during dark hours. Differences in estimates of density and richness between light and dark periods, however, are not dramatic and patterns of seasonal and spatial variation are tightly correlated between samples taken during the two time periods (Merkel & Associates, Inc. 2002). Given the logistical difficulties of sampling at night and the tight correlation between daytime and nighttime samples, we recommend that fish only be sampled during daytime.

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Figure Legends

Fig. 1. Spatial patterns of fish density along transects (1st column) and resulting variograms (2nd column) and correlograms (3rd column) in two habitats (main channel and tidal creek) in two wetlands (Mugu Lagoon and Carpinteria Salt Marsh). Data are from enclosure traps (0.4-m^2 area sampled). Note different scales on x and y axes.

Fig. 2. Spatial variation in fish density in two habitats (tidal creek and main channel) in Carpinteria Salt Marsh as measured with enclosure traps (0.4-m^2 area sampled). In each habitat, 4 stations were sampled, and within these 4 blocks of 4 replicate samples were taken. Bars show the mean of the 4 replicates and error bars represent ±1 SE.

Fig. 3. Spatial variation in fish density in three habitats (tidal creek, main channel, and basin) in Mugu Lagoon as measured with enclosure traps (0.4-m^2 area sampled). In each habitat, 4 stations were sampled (except station 4 in the main channel habitat, shown by ND), and within these 4 blocks of 4 replicate samples were taken. Bars show the mean of the 4 replicates and error bars represent ±1 SE.

Fig. 4. Spatial variation in species richness of fish in two habitats (tidal creek and main channel) in Carpinteria Salt Marsh as measured with enclosure traps (0.4-m^2 area sampled). In each habitat, 4 stations were sampled, and within these 4 blocks of 4 replicate samples were taken. Bars show the mean of the 4 replicates and error bars represent ±1 SE; where error bars are absent, all replicates captured only one species of fish.

Fig. 5. Spatial variation in species richness of fish in three habitats (tidal creek, main channel, and basin) in Mugu Lagoon as measured with enclosure traps (0.4-m^2 area sampled). In each habitat, 4 stations were sampled (except station 4 in the main channel habitat, shown by ND), and within these 4 blocks of 4 replicate samples were taken. Bars show the mean of the 4 replicates and error bars represent ±1 SE.

Fig. 6. Spatial variation in density and species richness of fish in two habitats (tidal creek and main channel) in Carpinteria Salt Marsh as measured with beach seines. In each habitat, 2 replicate samples were taken at each of 6 stations. Bars show the mean of the 2 replicates and error bars represent ±1 SE; where error bars are absent, the replicates captured the same number of species of fish.

Fig. 7. Spatial variation in density and species richness of fish in the main channel habitat in two wetlands (San Dieguito Lagoon and Tijuana Estuary) as measured with purse seines. Two replicate samples were taken at each of 6 stations. Bars show the mean of the 2 replicates and error bars represent ±1 SE; where error bars are absent, the replicates captured the same number of species of fish.

Fig. 8. Predicted changes in the precision (measured as the coefficient of variation, CV) of the wetland-wide estimates of density and species richness of fish as a function of the number of stations sampled per wetland. Solid lines represent density and broken lines represent species richness (not calculated for the basin habitat in Mugu Lagoon). Replication within stations is based on optimal values in Table 3, or set to one in cases where those values could not be calculated.

Fig. 9. Predicted changes in the precision (CV) of the wetland-wide estimates of density and effort needed to measure density as a function of the number of replicates sampled per wetland.

Calculated from variance estimates from enclosure trap samples taken in tidal creeks in Carpinteria Salt Marsh. Replication set to 1 at all other levels.

Fig. 10. Estimates of total species richness as a function of the number of beach seine samples collected in two habitats in Carpinteria Salt Marsh. Each point (except for 12 samples) represents the mean of 100 random samples.

Fig. 11. Estimates of total species richness as a function of the number of purse seine samples collected in the main channel habitat in San Dieguito Lagoon and Tijuana Estuary. Each point (except for 12 samples) represents the mean of 100 random samples.

Fig. 12. Mean density (±1 SE) of arrow gobies at various combinations of elevation and water depth in 3 wetlands. Combinations of elevation and water depth not sampled are shown with "ND". Gobies were sampled with 0.4-m^2 enclosure traps.

Fig. 13. Mean density (±1 SE) of shadow gobies at various combinations of elevation and water depth in 2 wetlands. Combinations of elevation and water depth not sampled are shown with "ND". Gobies were sampled with 0.4-m^2 enclosure traps.

Fig. 14. Temporal variation in density and species richness of fish measured with enclosure traps (0.4 -m^2), beach seines, and purse seines. In each study, data were collected on 2 consecutive days in each of two periods separated by 2 – 3 weeks. For enclosure traps, each bar represents the mean (±1 SE) of 20 samples composed of two pooled enclosures; means (±1 SE) of 5 samples per day are shown for beach seines; and means (±1 SE) of 4 - 6 samples per day are shown for purse seines. Enclosure trap data were collected in San Elijo Lagoon, beach seine data in Los Peñasquitos Lagoon, and purse seine data in San Dieguito Lagoon.

Fig. 15. Predicted changes in the precision (measured as the coefficient of variation, CV) of estimates of density and species richness of fish within a sampling period and the cost to obtain those estimates as a function of the number of weeks sampled. Curved lines represent the coefficient of variation and straight lines that increase to the right represent cost, measured in minutes of time spend by a crew of 4 people. Solid lines correspond to density and broken lines to species richness. Replication within weeks is based on optimal values in Table 7, or set to one in cases where those values could not be calculated.

Table 1. Sampling design of spatially hierarchical sampling studies.

Factor (Level)	Spatial Scale (separation between units)	Replication in pilot studies	Cost per unit (minutes with 2-4* people)
	Enclosure Traps		
Wetland	10's of km	2	NA
Station (within Wetland)	100's of m	4	15
Block (within Station)	10's of m	4	5
Replicates (within Blocks)	meters	4	15
Total number of samples taken per habitat[1] per wetland = 64			
	Beach Seines[2]		
Station (within Wetland)	100's of m	6	15
Replicates (within Station)	10's of m	2	60
Total number of samples taken per habitat[2] per wetland = 12			
	Purse Seines[3]		
Wetland	10's of km	2	NA
Station (within Wetland)	100's of m	6	5
Replicates (within Station)	10's of m	2	20
Total number of samples taken per wetland[3] = 12			

* Cost estimates are approximate average times to complete the work with 2 people (enclosure traps) or 4 people (beach and purse seines). Times given for blocks and stations are the extra time needed to move to and set up new blocks or stations, excluding the time needed to samples replicates within the blocks or stations.

[1] Three habitats were sampled with enclosure traps: tidal creeks, main channels and a basin at Mugu Lagoon. These habitats were analyzed separately.

[2] Two habitats were sampled with beach seines, tidal creeks and main channels, and these were analyzed separately.

[3] All samples taken with purse seines were in the main channel habitat.

Table 2. Results of nested ANOVA testing for differences in estimates of density and species richness among wetlands, sampling stations within wetlands, and blocks within sampling stations.

	SS	df (num., denom.)	MS	*F*	*P*	Variance Component[1]
		Enclosure trap[2]				
		Tidal Creeks				
		Density				
Wetland	2.56	1, 6	2.56	0.28	0.62	0
Station(Wetland)	54.80	6, 24	9.13	8.55	<0.001	0.504
Block(Station)	25.64	24, 96	1.07	1.79	0.03	0.113
error	57.35	96	0.60			0.618
		Species Richness				
Wetland	0.18	1, 6	0.18	0.28	0.61	0
Station(Wetland)	3.88	6, 24	0.65	4.81	0.002	0.032
Block(Station)	3.22	24, 96	0.13	1.31	0.18	0.008
error	9.82	96	0.10			0.103
		Main Channels				
		Density				
Wetland	82.17	1, 6	82.17	49.03	<0.001	1.274
Station(Wetland)	10.06	6, 23	1.68	0.95	0.48	0
Block(Station)	40.47	23, 93	1.76	3.27	<0.001	0.288
error	50.10	93	0.54			0.608
		Species Richness				
Wetland	4.98	1, 6	4.98	14.53	0.009	0.072
Station(Wetland)	2.06	6, 23	0.34	1.77	0.15	0.009
Block(Station)	4.46	23, 93	0.19	2.48	0.001	0.029
error	7.26	93	0.08			0.078
		Basin				
		Density				
Station(Wetland)	0.65	3, 12	0.22	0.94	0.45	0
Block(Station)	2.77	12, 48	0.23	0.91	0.54	0
error	12.23	48	0.26			0.252
		Species Richness				
		Analysis not conducted[3]				
		Beach seine[4]				
		Tidal Creeks				
		Density				
Station(Wetland)	4.094	5, 6	0.819	4.55	0.046	0.319
	1.079	6	0.180			0.180
		Species Richness				
Station(Wetland)	2.006	5, 6	0.401	7.53	0.01	0.174
	0.320	6	0.053			0.053
		Main Channels				

		Density				
Station(Wetland)	24.253	5, 6	4.851	10.52	0.006	2.195
	2.767	6	0.461			0.461
		Species Richness				
Station(Wetland)	0.142	5, 6	0.028	0.31	0.88	0
	0.546	6	0.091			0.063
		Purse seine[5]				
		Density				
W	0.075	1, 10	0.075	0.03	0.86	0
Station(Wetland)	24.201	10, 12	2.42	10.03	0.0002	1.096
	2.896	12	0.241			0.229
		Species Richness				
W	0.040	1, 10	0.040	0.29	0.60	0
Station(Wetland)	1.367	10, 12	0.137	1.25	0.35	0.016
	1.309	12	0.109			0.104

[1] Calculated following procedures described in Fletcher and Underwood (2002) and Sokal and Rohlf (2001).
[2] To satisfy the assumptions of normality and homoscedasticity, density and species richness per enclosure trap sample (0.4 m^2) were transformed to ln(x+1).
[3] Data on species richness from enclosure trap samples from the basin habitat were not well suited to analysis because all values of richness were either 0 or 1. Similarly, the majority of density estimates from enclosure traps in the basin habitat were zero, and hence, results of ANOVA above and optimization of replication (Table 3) should be viewed with caution.
[4] For beach seine samples, densities per m^2 and species richness per sample were transformed to ln(10x) and ln(x), respectively to satisfy the assumptions of normality and homoscedasticity.
[5] For purse seine samples, densities per sample (105 m^2) and species richness per sample were transformed to ln(x) to satisfy the assumptions of normality and homoscedasticity.

Table 3. Summary of analyses determining optimal replication at hierarchical spatial scales in different habitats and for different methods of sampling fish.

		Optimal Replication at Hierarchical Spatial Scales		
Variable	Habitat	Replicates w/in Areas	Areas[1] w/in Stations	Stations w/in wetlands
Enclosure Trap				
Density	Tidal Creek	1	1	set by effort allocated
	Main Channel	1	**, 1†	set by effort allocated
	Basin	*	**, 4†	set by effort allocated
Species Richness	Tidal Creek	2	1†	set by effort allocated
	Main Channel	1	3, 5†	set by effort allocated
	Basin	Not calculated[2]	Not calculated[2], **†	cannot be calculated
Beach Seine				
Density	Tidal Creek	NA	1	set by effort allocated
	Main Channel	NA	1	set by effort allocated
Species Richness	Tidal Creek	NA	1	set by effort allocated
	Main Channel	NA	**	cannot be calculated
Purse Seine				
Density	Main Channel	NA	1	set by effort allocated
Species Richness	Main Channel	NA	1	set by effort allocated

[1] Areas are places within stations that are separated by 10's of m: blocks in the enclosure trap study and replicates in the beach and purse seine studies.

[2] ANOVA was inappropriate for this dataset (thus making the optimization procedure impossible) because all values of richness in the basin were 0 or 1.

† To calculate optimal replication at the Area (= Block) scale with data from enclosure traps, two approaches were used: each enclosure trap sample was treated as a replicate (within an Area) or all 4 replicates within an Area were pooled and treated as a replicate. This second approach made the nesting structure of the analyses similar to those for beach and purse seines and it eliminated the vast majority of data points with values of zero, thus improving the distribution of the data. The entry presented in the Areas column with the "†" symbol is the result of optimization using replicates pooled within an area. Where only one value is presented, this value was generated by both optimization analyses.

* Analytical optimization was impossible because estimates of variance were zero at the Block scale.

** Analytical optimization was impossible because estimates of variance were zero at the Station scale.

Table 4. Designs of studies on effects of tidal variation and depth on estimates of fish density.

Site	Date (2002)	Elevations (m)	Depths (cm)	*n**; *N*	Length of area sampled (m)
San Elijo	October 7	1.2, 0.9, 0.5	45, 76	4; 20	30
Los Peñasquitos	October 21	1.3, 1.0, 0.7	23, 45, 76	6; 30	50
San Elijo	November 4	1.2, 0.8, 0.5	45, 76	6-12; 41	120
Carpinteria	November 18	1.2, 0.9, 0.4	45, 76	8; 40	70

* Number of samples per elevation-depth combination.

Table 5. Summary of results of ANOVA testing for differences among estimates of density* (arrow gobies only) obtained at different elevations and water depths.

	Elevation			Depth		
Site	*F*	df	*P*	*F*	df	*P*
San Elijo (Oct.)	1.41	2,16	0.055	0.54	2,16	0.26
Los Peñasquitos	1.23	2,25	0.31	6.22	2,25	**0.006**
San Elijo (Nov.)	2.51	2,37	0.10	3.25	1,37	0.08
Carpinteria	6.04	2,36	**0.005**	2.01	1,36	0.16

* Density was transformed to ln(x+1) to satisfy the assumptions of normality and homogeneity of variance.

Table 6. Results of nested ANOVA testing for differences in estimates of density and species richness among days and weeks.

Source	SS	df (num., denom.)	MS	*F*	*P*	Variance Component[1]
Enclosure Trap[2]						
Density						
Station	5.183	1, 75	5.183	7.13	0.009	NA
Week	4.261	1, 2	4.261	3.03	0.22	0.572
Day(Week)	2.804	2, 75	1.402	1.93	0.15	0.068
Error	54.512	75	0.727			0.727
Species Richness						
Station	3.089	1, 75	3.089	20.38	<0.001	NA
Week	0.543	1, 2	0.543	3.38	0.21	0.076
Day(Week)	0.322	2, 75	0.161	1.06	0.35	0.0009
Error	11.368	75	0.152			0.152
Beach Seine[2]						
Density						
Area	23.350	4, 12	5.838	3.56	0.04	NA
Week	0.123	1, 2	0.123	0.07	0.81	0
Day(Week)	3.413	2, 12	1.707	1.04	0.38	0.037
Error	19.670	12	1.639			1.522
Species Richness						
Area	1.894	4, 12	0.473	2.50	0.10	NA
Week	0.130	1, 2	0.130	52.02	0.02	0.052
Day(Week)	0.005	2, 12	0.002	0.01	0.99	0
Error	2.276	12	0.190			0.163
Purse Seine[2]						
Density						
Week	1.931	1, 2	1.931	1.22	0.38	0.132
Day(Week)	3.169	2, 17	1.584	2.32	0.13	0.172
Error	11.629	17	0.684			0.684
Species Richness						
Week	0.750	1, 2	0.750	0.56	0.53	0
Day(Week)	2.689	2, 17	1.344	0.66	0.53	0
Error	34.867	17	2.051			1.940

[1] Calculated following procedures described in Fletcher and Underwood (2002) and Sokal and Rohlf (2001).
[2] To satisfy the assumptions of normality and homoscedasticity, data were transformed as follows: enclosure trap samples: ln(x) for both density and species richness (per 0.8 m^2 sample); beach seine samples: ln(100x) for density per m^2 and ln(x) for species richness per sample; purse seine samples: ln(x) for density (per 105 m^2 sample), and species richness per sample was not transformed.

Table 7. Summary of cost estimates and analyses determining optimal replication at hierarchical temporal scales for different methods of sampling fish.

	Samples w/in Days	Days w/in Weeks	Weeks w/in Seasons
Estimated Costs† (Time (minutes) needed by 4 people to complete sampling)			
Enclosure Trap	15	200	340
Beach Seine	60	200	340
Purse Seine	25	200	340
Optimal Replication at Hierarchical Spatial Scales			
Enclosure Trap			
Density	12	1	set by effort allocated
Species Richness	47	1	set by effort allocated
Beach Seine			
Density	12	*	set by effort allocated
Species Richness	*	*	set by effort allocated
Purse Seine			
Density	6	2	set by effort allocated
Species Richness	*	*	set by effort allocated

† Cost estimates are approximate average times to complete the work with 4 people. Times given for days are exclusive of the time needed to collect sample and include the extra time needed to travel to and from a wetland from a staging area, set up, beak down, and cleanup time. Times given for weeks include the time necessary to travel to and from the office to a staging area near a wetland, retrieve and replace gear in storage, and thoroughly clean gear prior to storage.

* Analytical optimization was impossible because estimates of variance at the Day or Week level were zero.

Fig. 1

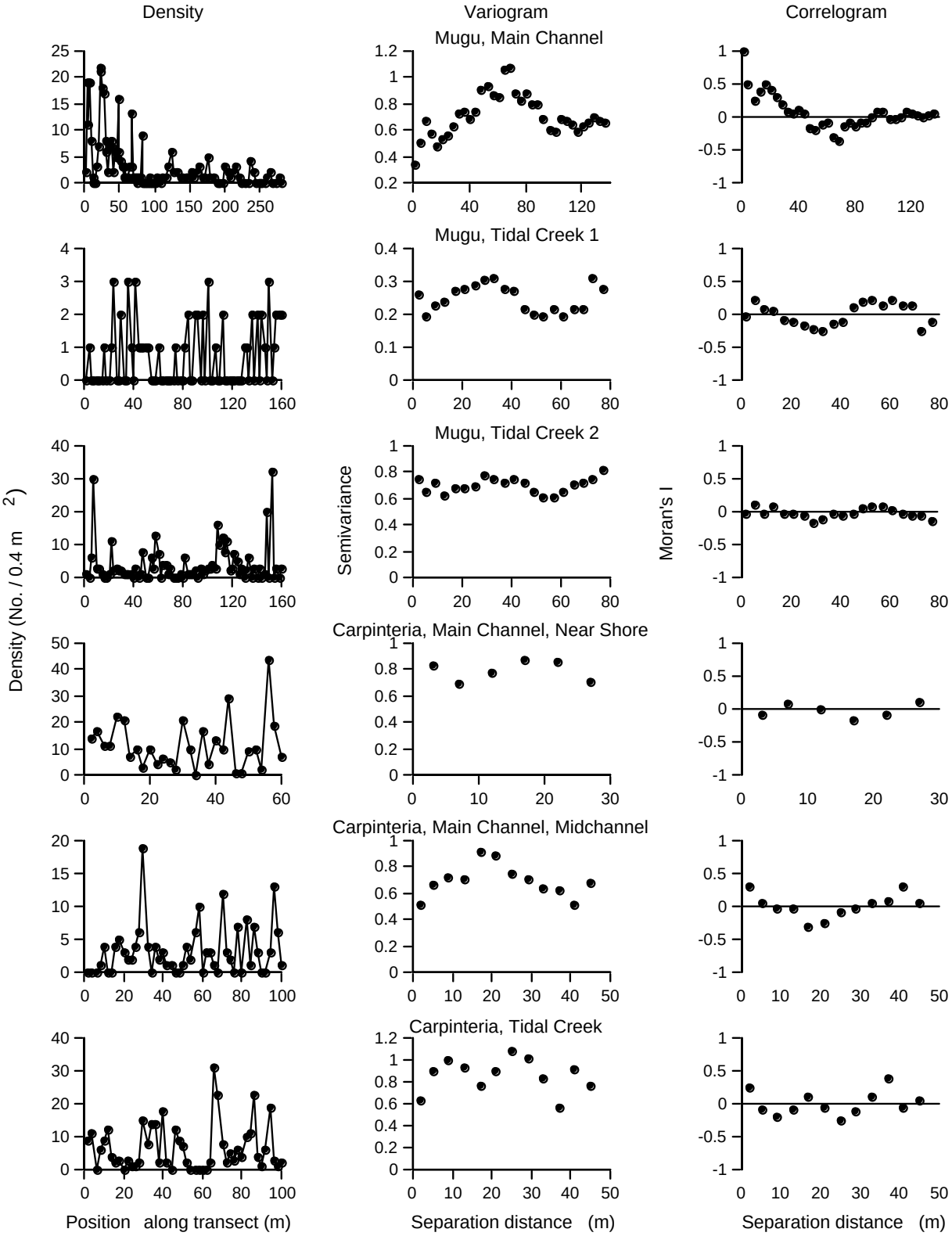

Density
Variogram
Correlogram
Mugu, Main Channel
Mugu, Tidal Creek 1
Mugu, Tidal Creek 2
Carpinteria, Main Channel, Near Shore
Carpinteria, Main Channel, Midchannel
Carpinteria, Tidal Creek
Density (No. / 0.4 m 2)
Semivariance
Moran's I
Position along transect (m)
Separation distance (m)
Separation distance (m)

Fig. 2

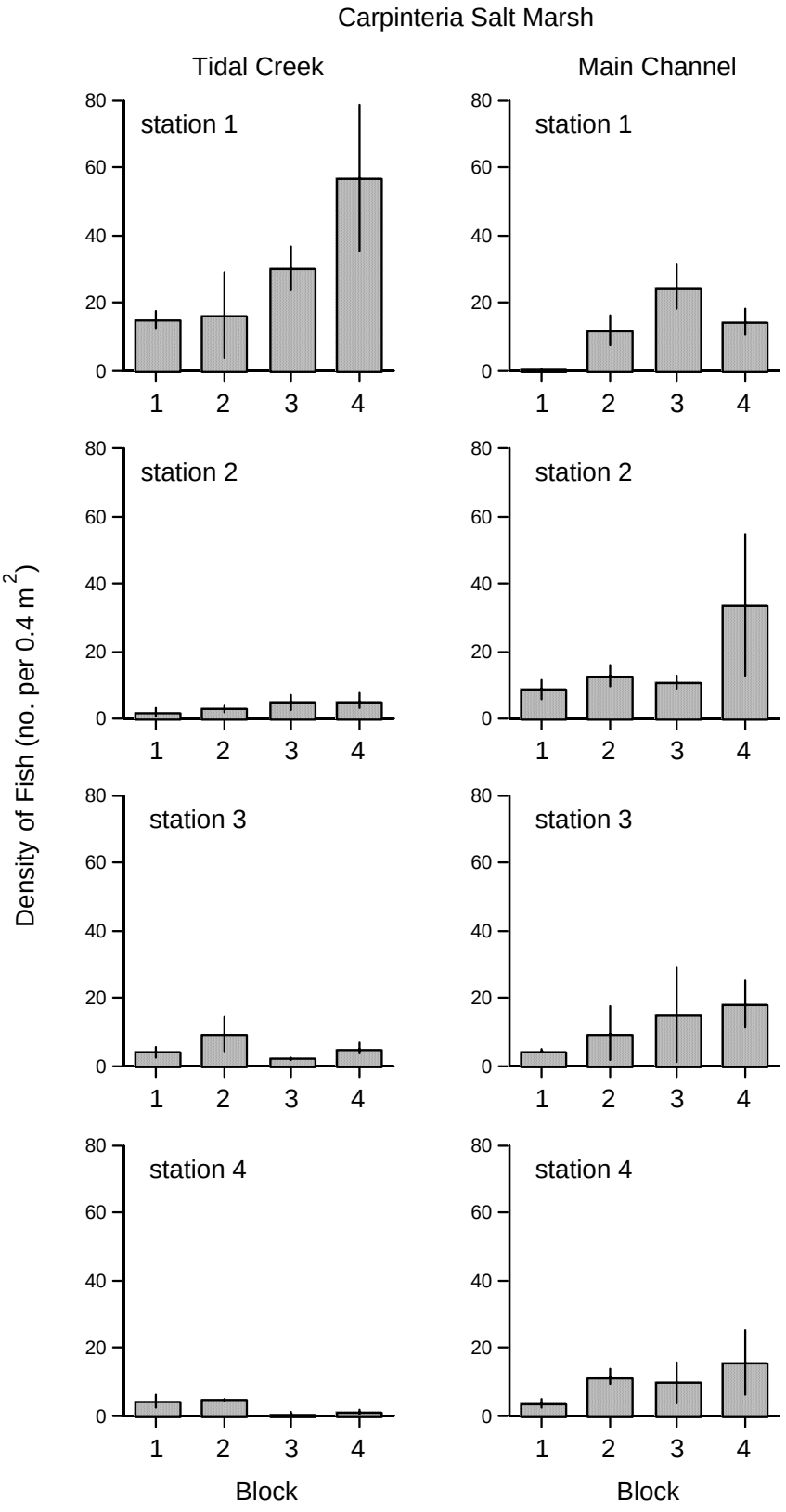

Carpinteria Salt Marsh
Tidal Creek
Main Channel
station 1
station 2
station 3
station 4
Density of Fish (no. per 0.4 m^2)
Block
0
20
40
60
80
1
2
3
4

Fig. 3

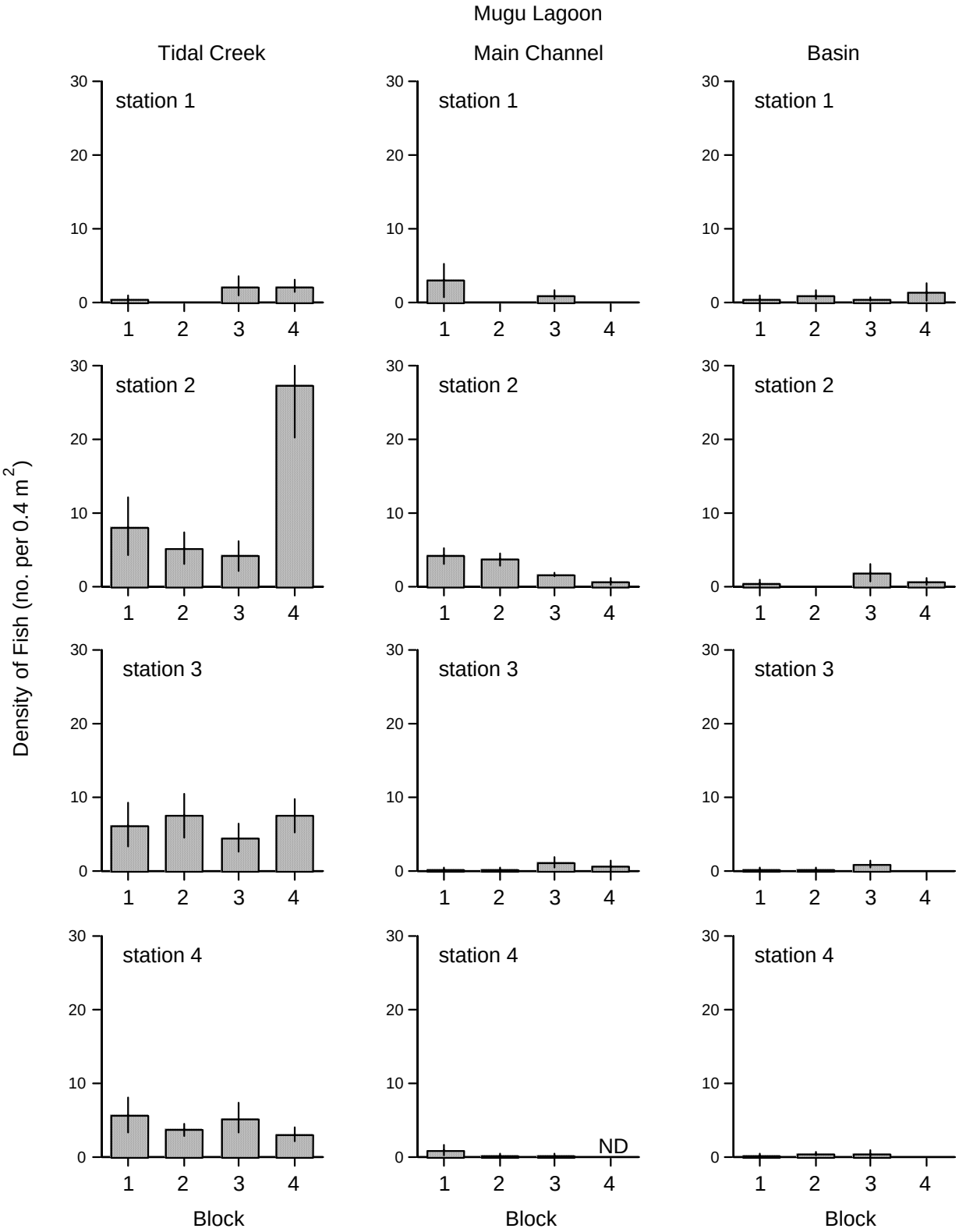

Mugu Lagoon
Tidal Creek
Main Channel
Basin
station 1
station 2
station 3
station 4
ND
Density of Fish (no. per 0.4 m2)
Block
0
10
20
30
1
2
3
4

Fig. 4

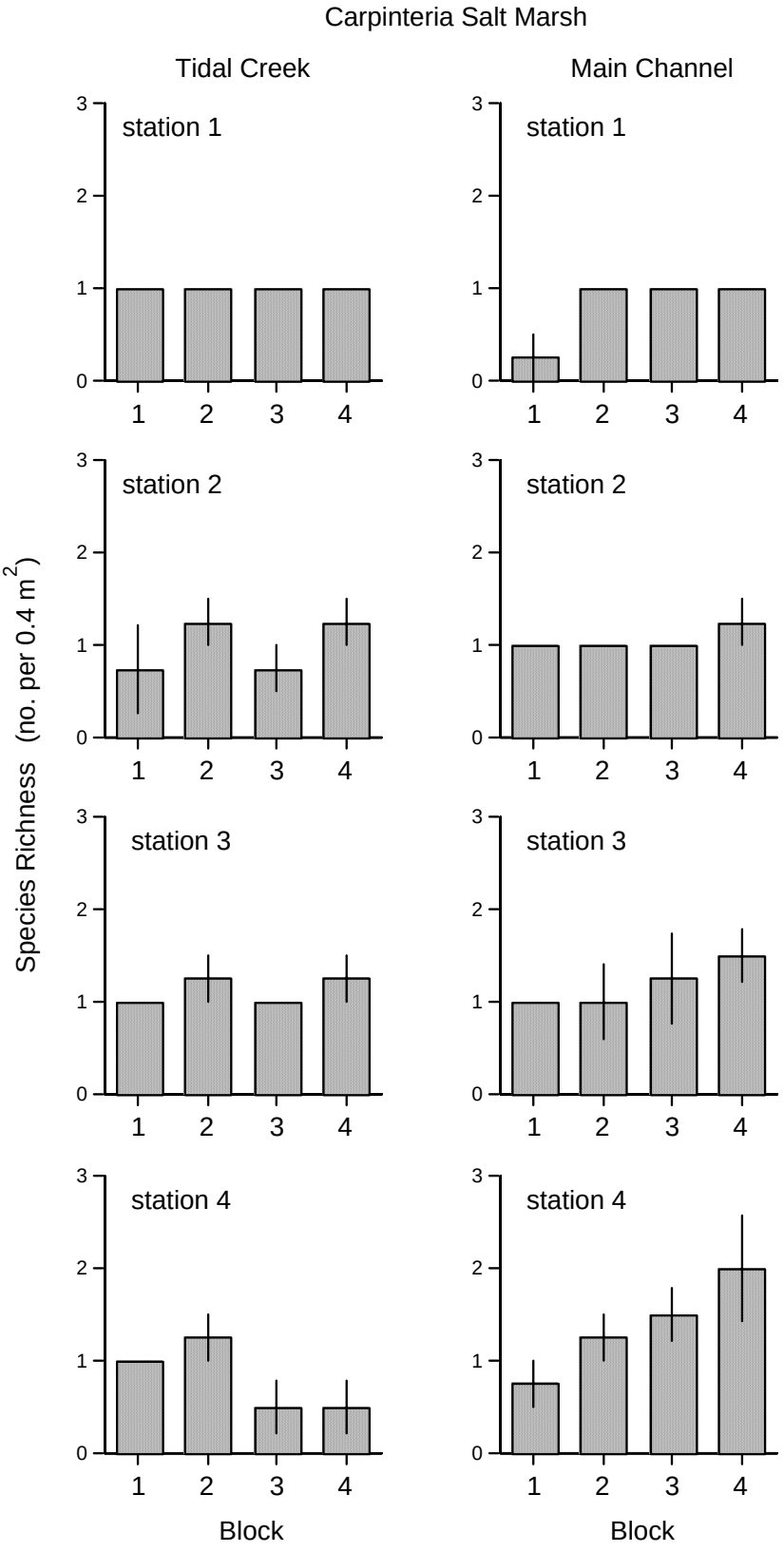

Carpinteria Salt Marsh
Tidal Creek
Main Channel
station 1
station 2
station 3
station 4
Species Richness (no. per 0.4 m2)
Block
0
1
2
3
4

Fig. 5

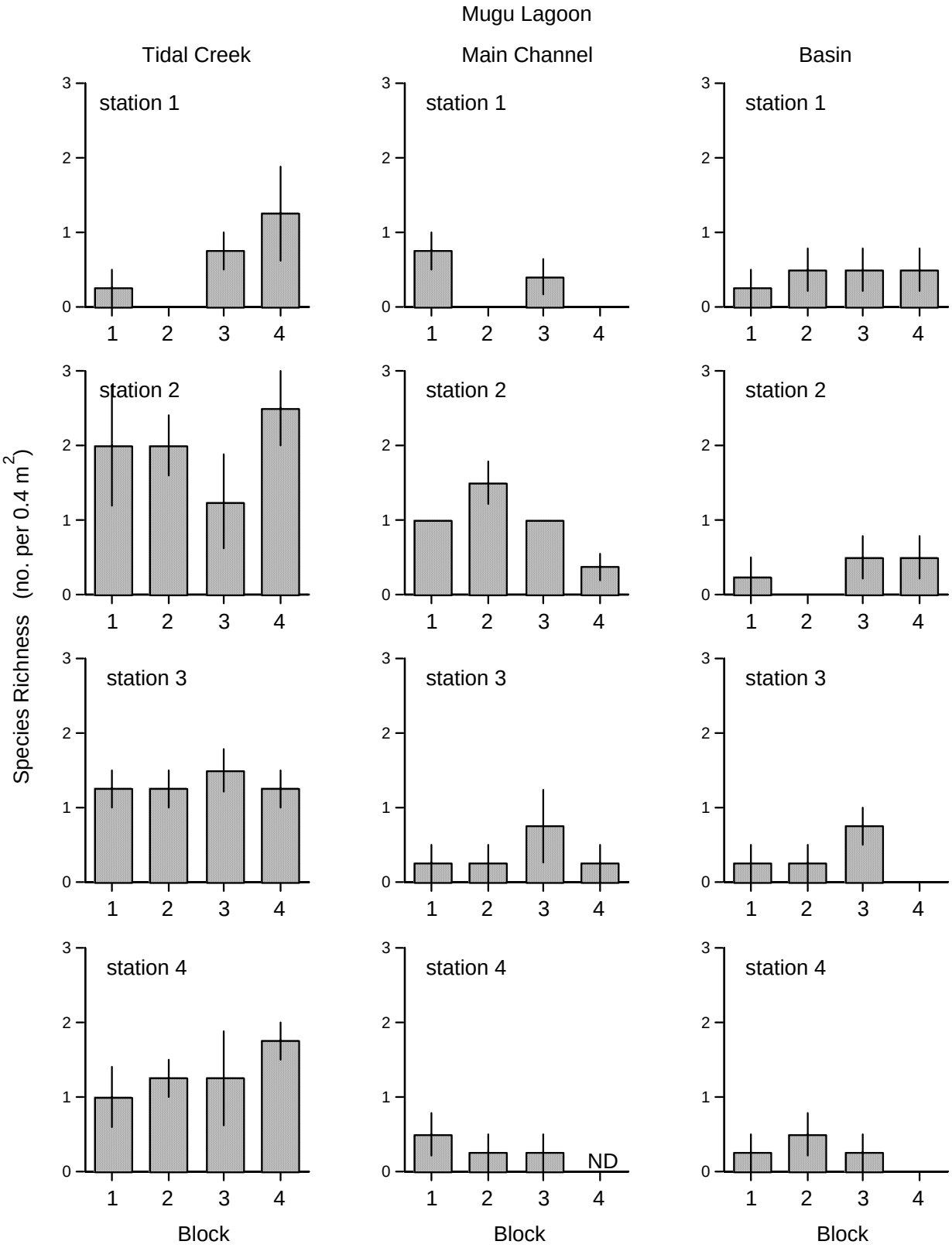

Mugu Lagoon
Tidal Creek
Main Channel
Basin
station 1
station 2
station 3
station 4
Species Richness (no. per 0.4 m2)
Block
ND

Fig. 6

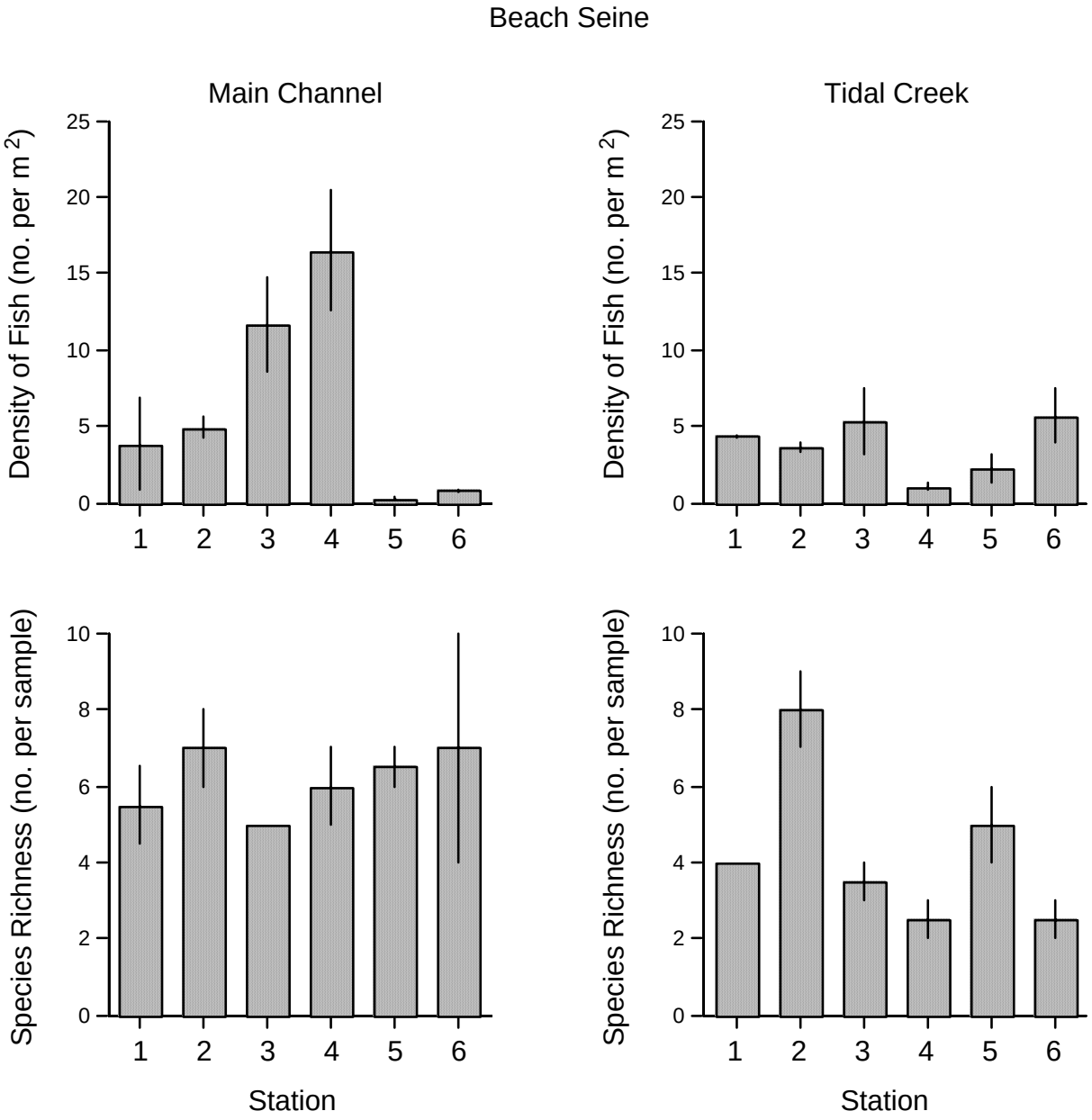

Beach Seine
Main Channel
Tidal Creek
Density of Fish (no. per m 2)
Species Richness (no. per sample)
Station
0
5
10
15
20
25
2
4
6
8
1
2
3
4
5
6

Fig. 7

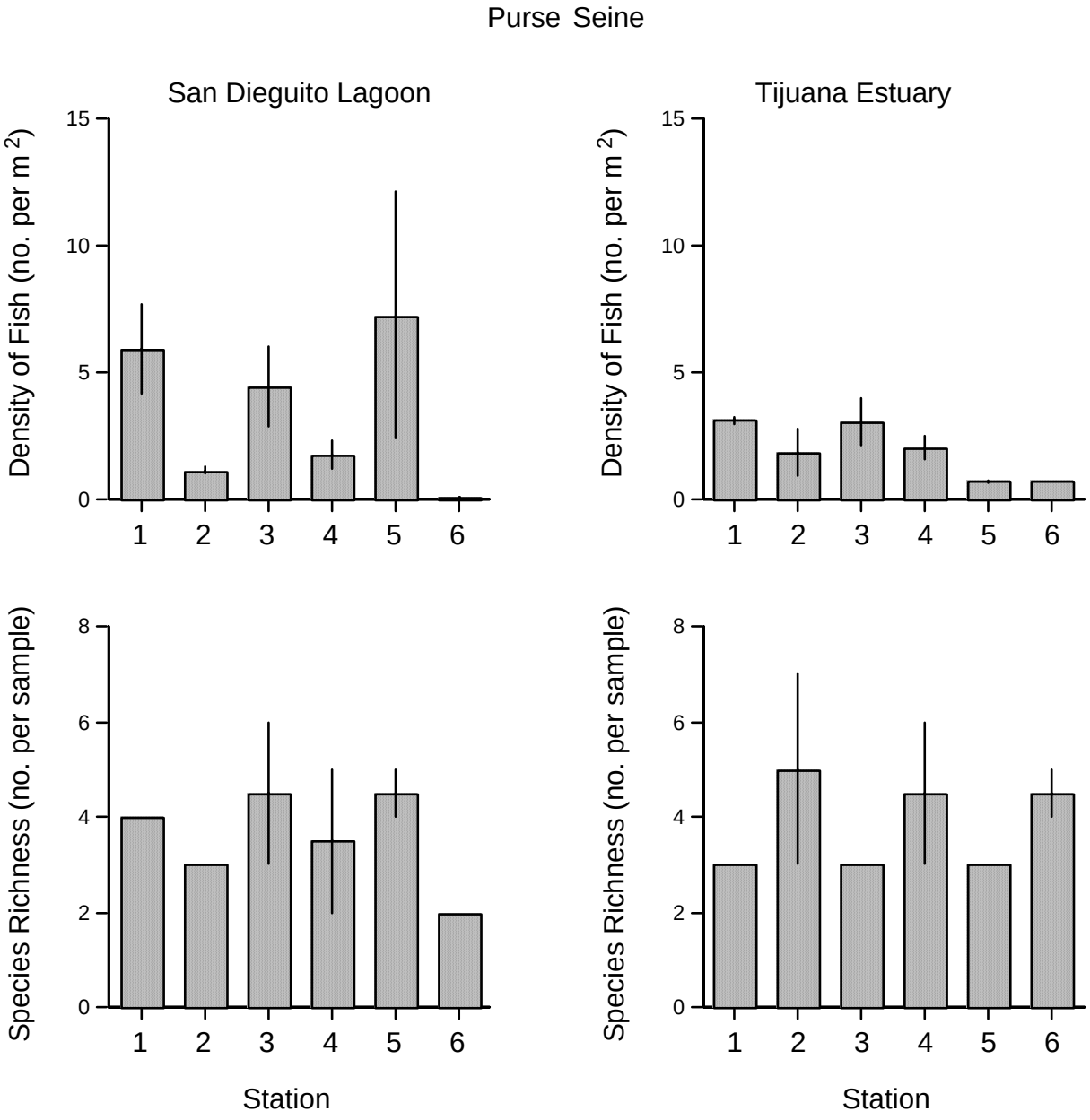

Purse Seine
San Dieguito Lagoon
Tijuana Estuary
Density of Fish (no. per m 2)
Species Richness (no. per sample)
Station
0
5
10
15
2
4
6
8
1
2
3
4
5
6

Fig. 8

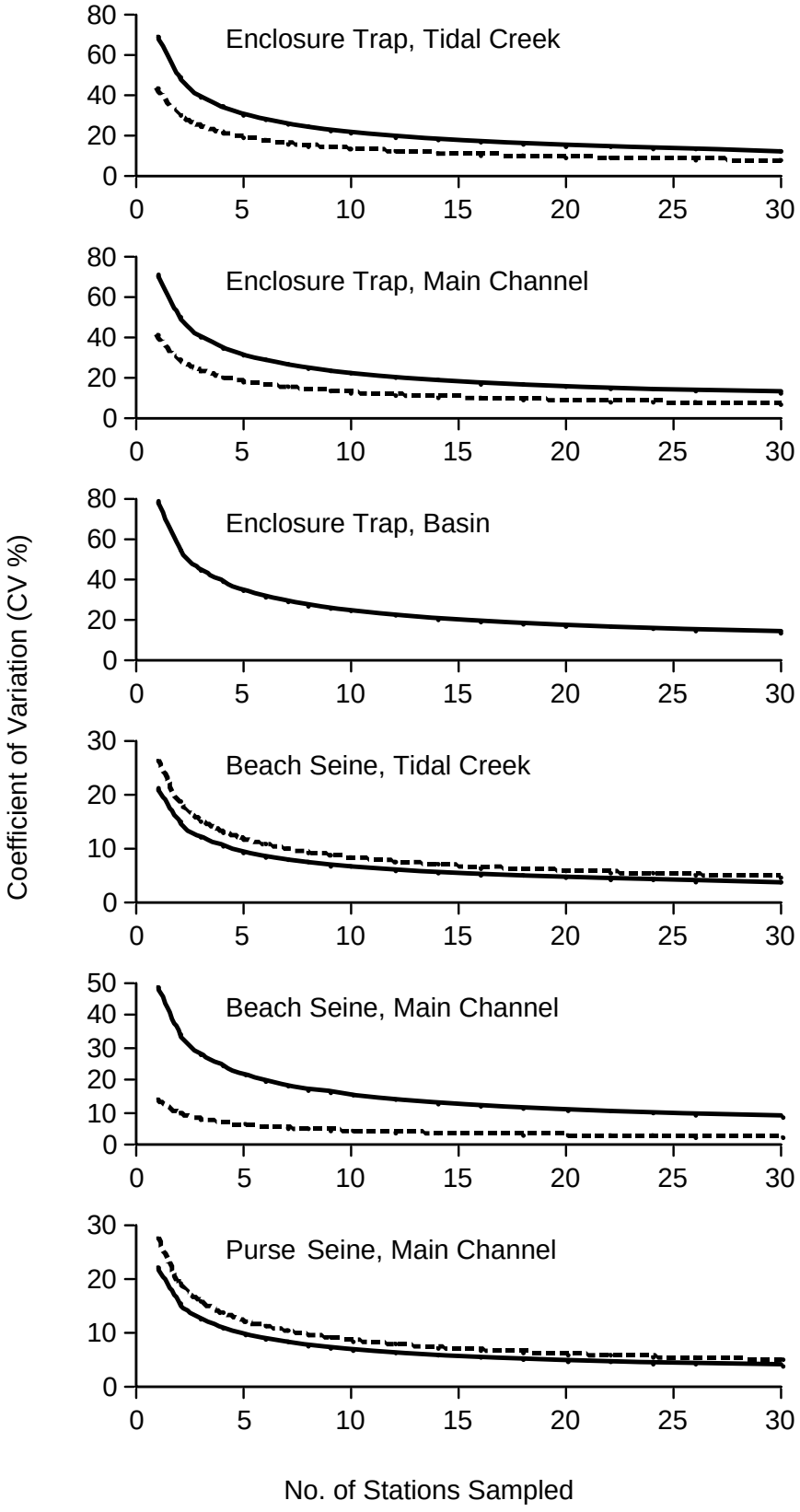

Enclosure Trap, Tidal Creek
Enclosure Trap, Main Channel
Enclosure Trap, Basin
Beach Seine, Tidal Creek
Beach Seine, Main Channel
Purse Seine, Main Channel
Coefficient of Variation (CV %)
No. of Stations Sampled

Fig. 9

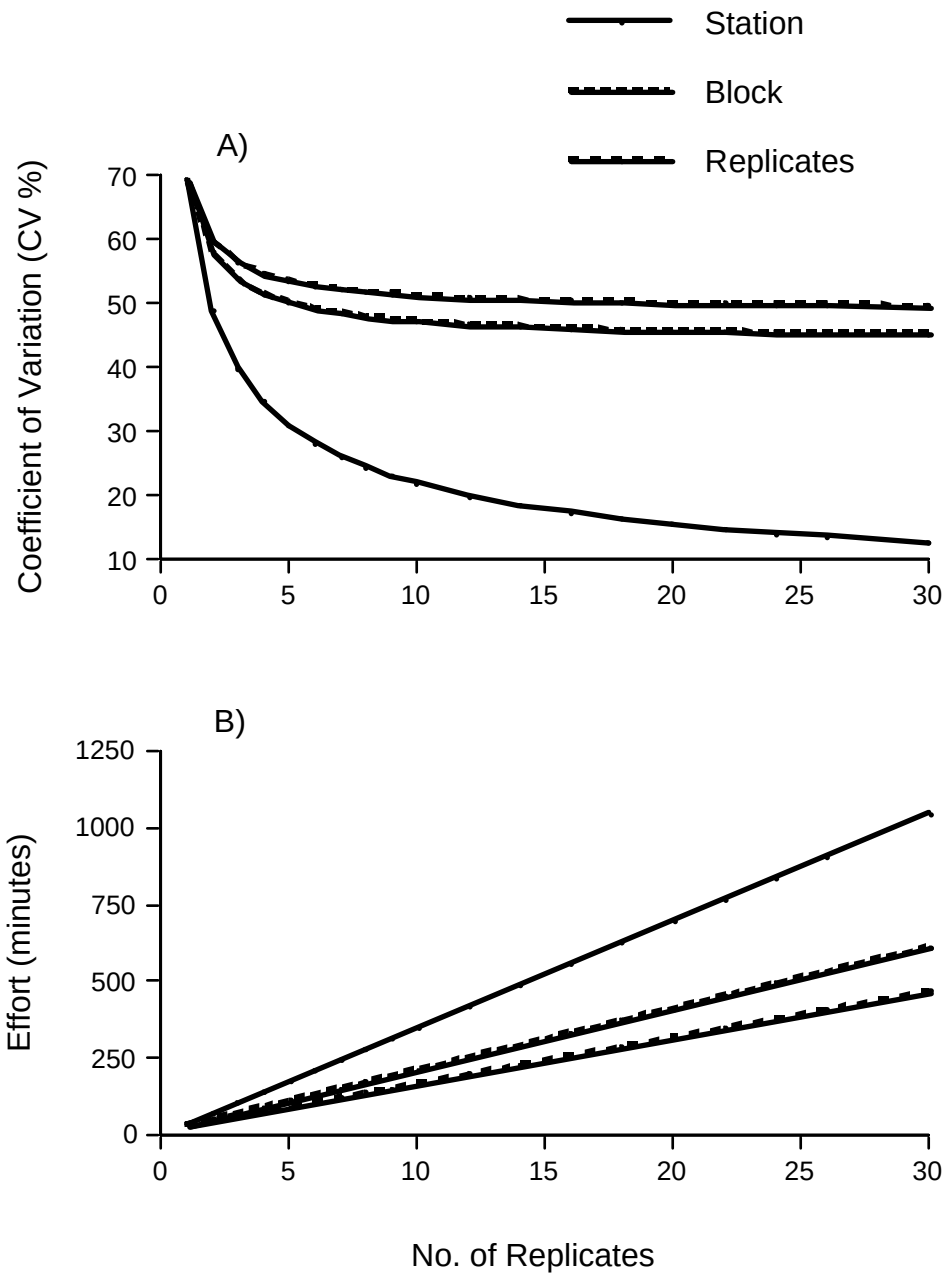

Station
Block
Replicates
A)
Coefficient of Variation (CV %)
70
60
50
40
30
20
10
0
5
10
15
20
25
30
B)
Effort (minutes)
1250
1000
750
500
250
0
No. of Replicates

Fig. 10

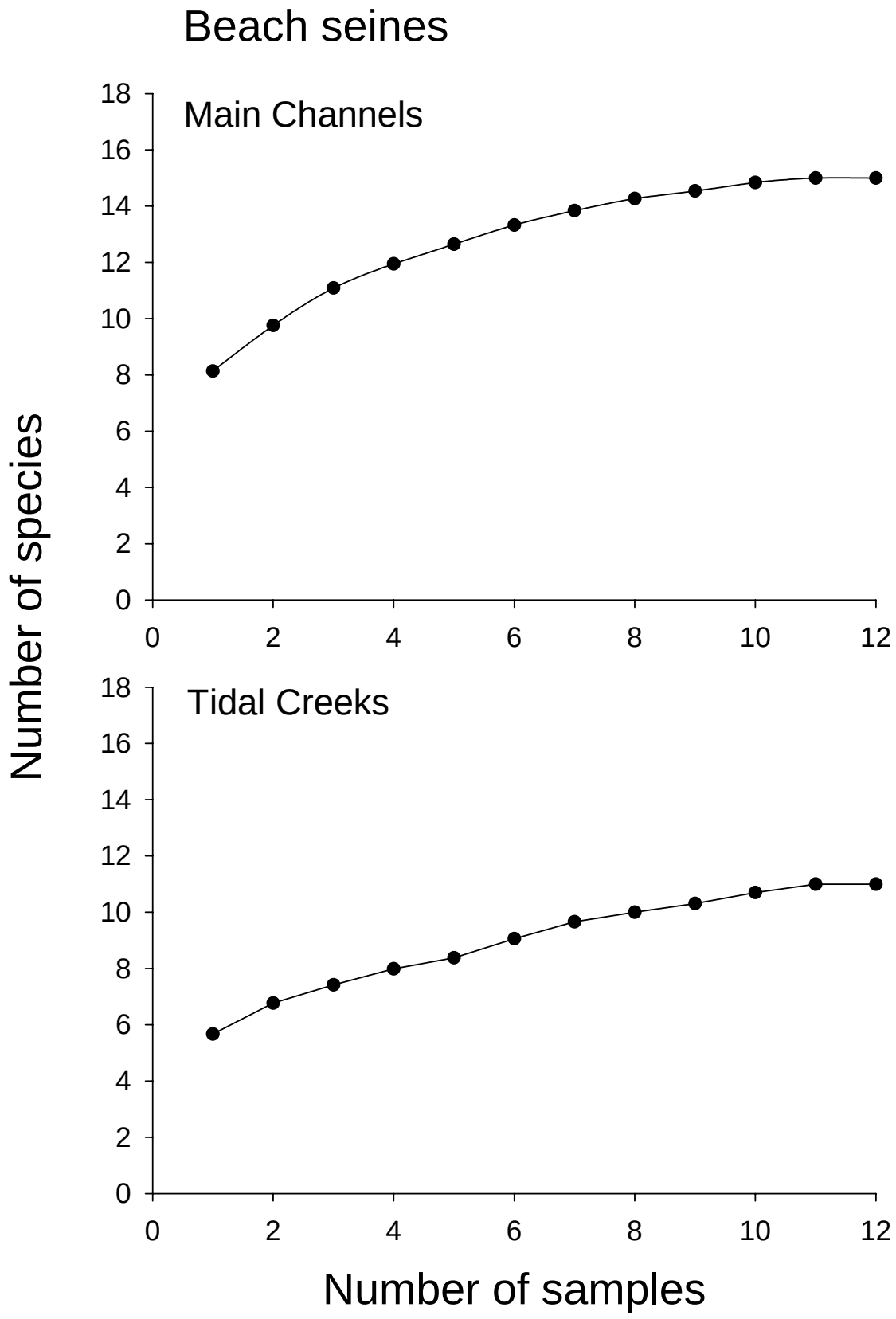

Beach seines
Main Channels
Tidal Creeks
Number of species
Number of samples
0
2
4
6
8
10
12
14
16
18

Fig. 11

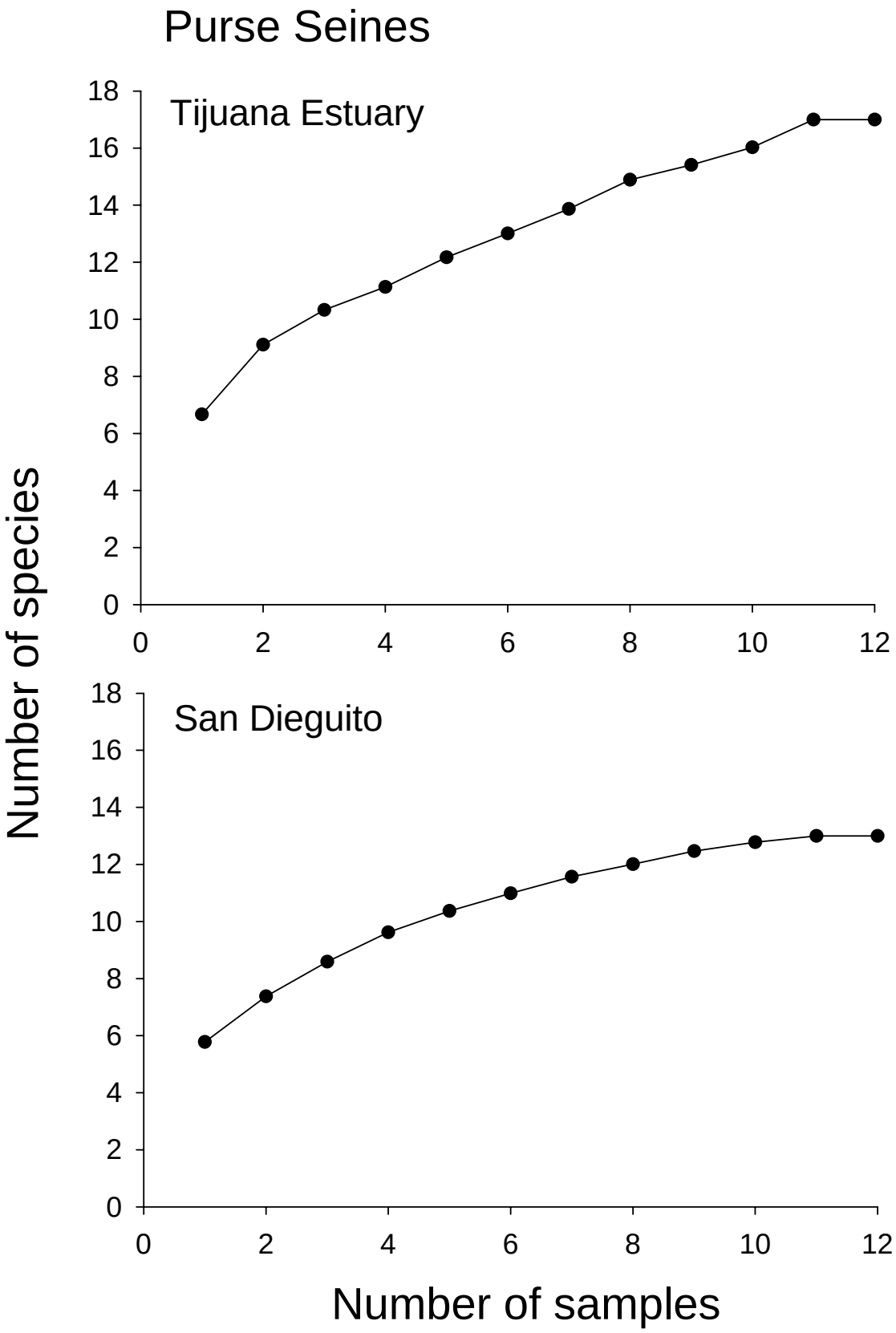

Purse Seines
Tijuana Estuary
San Dieguito
Number of species
Number of samples
0
2
4
6
8
10
12
14
16
18

Fig. 12

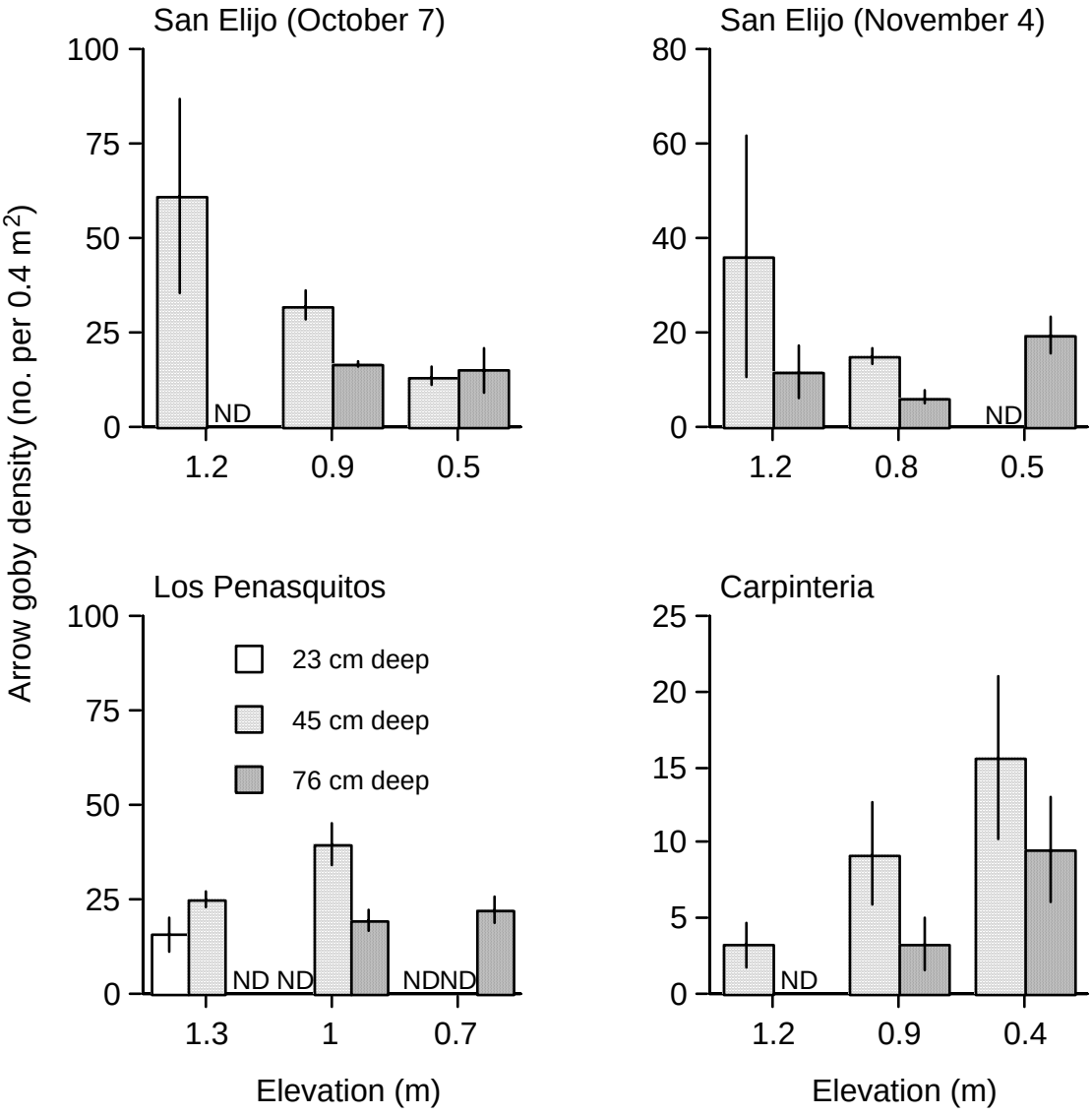

San Elijo (October 7)
San Elijo (November 4)
Los Penasquitos
Carpinteria
Arrow goby density (no. per 0.4 m2)
23 cm deep
45 cm deep
76 cm deep
ND
ND ND
NDND
1.2
0.9
0.5
0.8
1.3
1
0.7
0.4
Elevation (m)

Fig. 13

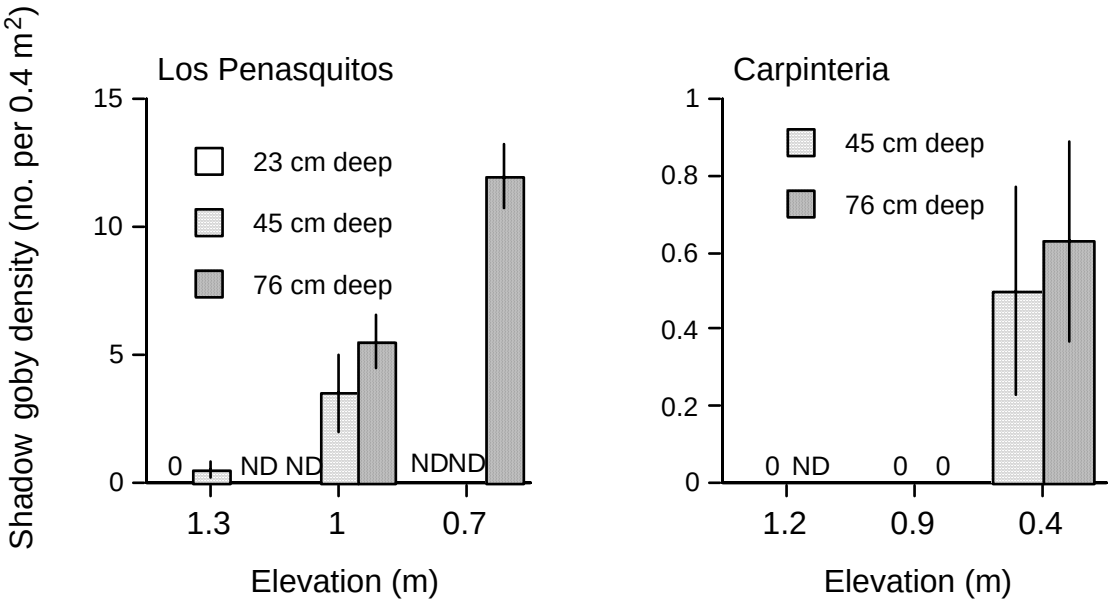

Shadow goby density (no. per 0.4 m2)
Los Penasquitos
23 cm deep
45 cm deep
76 cm deep
15
10
5
0
0
ND ND
NDND
1.3
1
0.7
Elevation (m)
Carpinteria
45 cm deep
76 cm deep
1
0.8
0.6
0.4
0.2
0
0 ND
0 0
1.2
0.9
0.4
Elevation (m)

Fig. 14

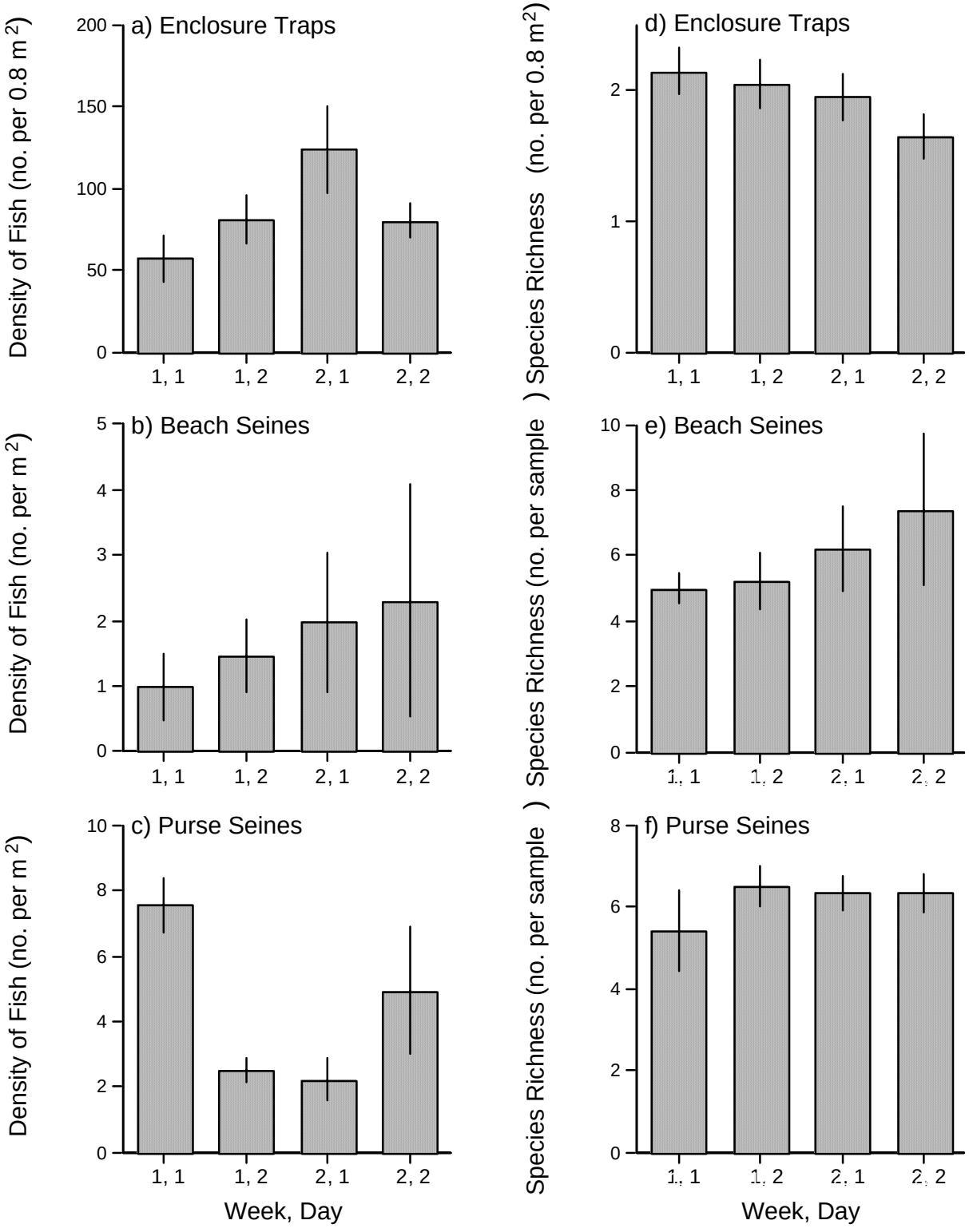
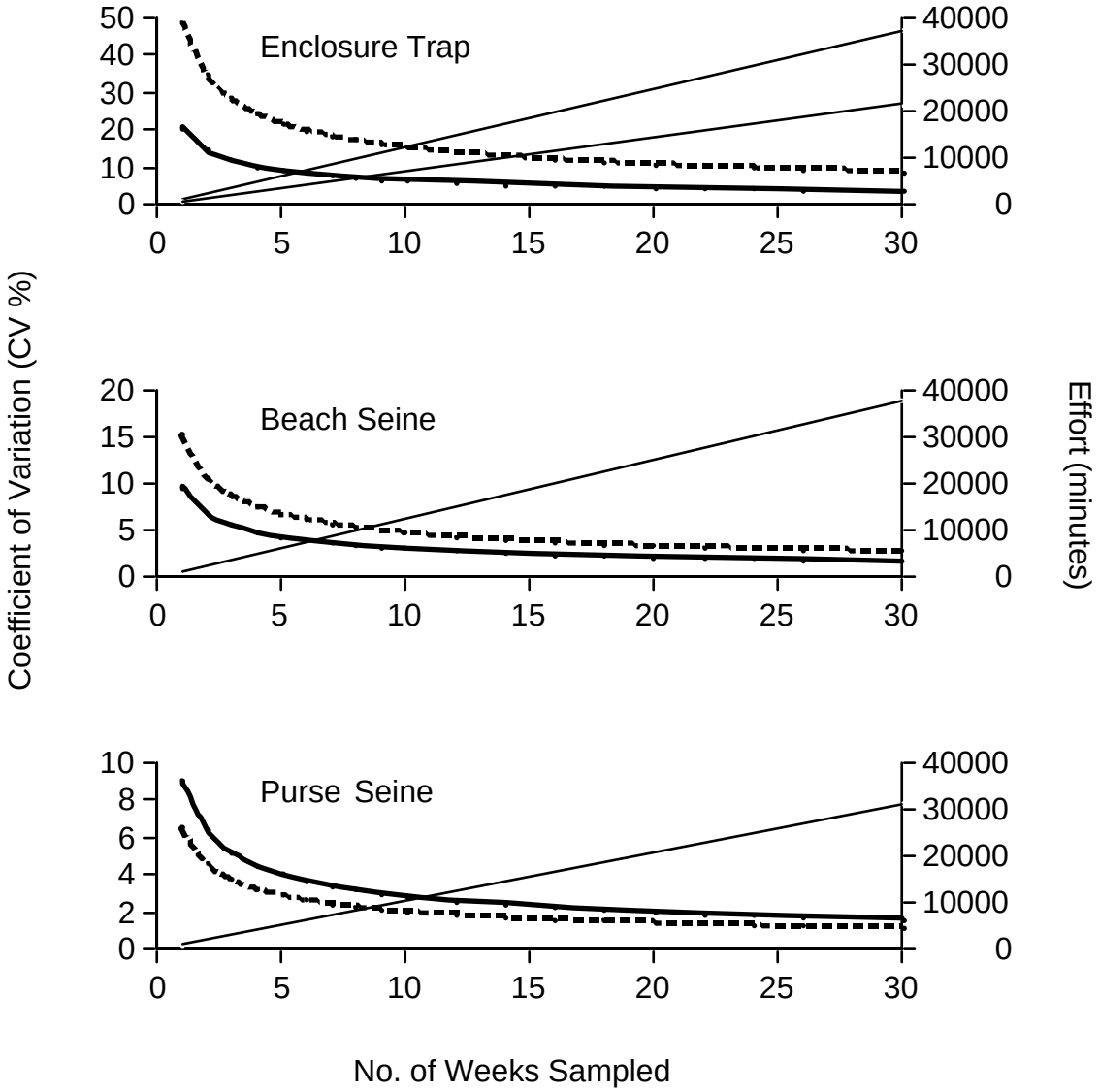

a) Enclosure Traps
b) Beach Seines
c) Purse Seines
d) Enclosure Traps
e) Beach Seines
f) Purse Seines
Density of Fish (no. per 0.8 m 2)
Density of Fish (no. per m 2)
Species Richness (no. per 0.8 m2)
Species Richness (no. per sample)
1, 1
1, 2
2, 1
2, 2
Week, Day

Fig. 15


Enclosure Trap
Beach Seine
Purse Seine
Coefficient of Variation (CV %)
Effort (minutes)
No. of Weeks Sampled
0
5
10
15
20
25
30
40000
30000
20000
10000

Appendix 5

Spatial variation in benthic macroinvertebrate density in southern California coastal wetlands: implications for post-restoration monitoring at San Dieguito Lagoon and reference sites

Mark Page, Steve Schroeter, Mark Steele, and Dan Reed
Marine Science Institute
University of California
Santa Barbara, Calfornia, 93106

DRAFT
May 16, 2005

Background

The coastal development permit for SONGS Units 2 and 3 requires Southern California Edison to create or substantially restore a minimum of 150 acres of coastal wetland. The permit establishes biological performance standards that must be met by the restored wetland. One of these standards requires that within 4 years of construction, the total densities and number of species of macroinvertebrates shall be similar to the densities and number of species of macroinvertebrates in similar habitats in the reference wetlands. The reference wetlands for comparison with the restoration at San Dieguito Lagoon are Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary.

The coastal wetlands of southern California contain tidal habitats that can be distinguished coarsely on the basis of topography and inundation regime (e.g., tidal channel versus main channel). Within each of these major habitats, variation in elevation, sediment characteristics, organic matter, algal coverage, and other physical and biological factors can lead to gradients and patchy distributions of benthic fauna (e.g., Thrush et al. 1989, Morrisey et al. 1992, Kendall and Widdicombe 1999). To effectively assess compliance with the performance standard for macroinvertebrates, sampling methods must account for spatial variation in the distribution and abundance of these animals. Unfortunately, the spatial scale of patchiness of benthic invertebrates is not known for the tidal habitats of southern California wetlands. In order to determine how far apart to space sample replicates and how many replicates to take, it is useful to have information on the spatial heterogeneity of invertebrates in the habitats of interest.

Because of spatial heterogeneity in invertebrate abundance, large numbers of samples may be required to obtain reasonably precise estimates of abundance. It may be possible to greatly reduce the costs of laboratory processing of these samples by combining several individual samples, mixing them well, and analyzing a subsample of the composite, which represents the average of the combined sample over the spatial scale at which they were collected. Compositing is a technique commonly used in many ecological systems (Bruchner et al. 2000, Carey and Keough 2002). Unfortunately, it is unknown how compositing will affect the accuracy of estimates of abundance and species richness for wetland invertebrates.

The objectives of this study were to 1) evaluate the patchiness of intertidal wetland benthic macroinvertebrates, 2) use this information to develop a sampling scheme that makes the most efficient use of sampling effort, and 3) explore the use of compositing as a cost saving approach to invertebrate sampling. The information derived from this study will be incorporated into the San Dieguito Lagoon Restoration Monitoring Plan.

Materials and Methods

Sample collection and processing

To identify scales of spatial patchiness in macroinvertebrates, we sampled 3 tidal creeks and 3 stretches of main channel habitat at Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary (main channel only). Macroinvertebrates were sampled in plots spaced 1 m apart along a 30 m transect line in each location. We sampled epifauna on the sediment surface using quadrats and infauna using cores of two sizes.

Epifauna (e.g., *Cerithidea californica*) were sampled by counting the number of individuals on the sediment surface within a 0.5 x 0.5 m quadrat placed in the center of each plot. Relatively large and deep living infauna were sampled using a 10 cm diameter core pushed into the sediment to a depth of 50 cm. The 10 cm core diameter is a standard used in benthic ecology (e.g., Morrisey et al. 1992) and sampling to a depth of 50 cm ensured the collection of the deeper living bivalves (e.g., *Tagelus californianus*, *Macoma secta*) and ghost shrimp (*Neotrypaea californiensis*). The contents of the 10 cm core were sieved through 3-mm mesh screen in the field. Animals retained by the 3-mm mesh were identified to species in the field and returned to the habitat.

Smaller invertebrates (e.g., most annelids) were sampled using a 4.8 cm diameter core pushed into the sediment to a depth of 6 cm (e.g., Levin and Talley 1999). The smaller core samples were preserved on site in 10% buffered formalin and returned to the laboratory for processing. In the laboratory, the formalin was decanted from each sample and the sample gently washed with fresh water through a 0.5 mm screen to remove the fine sediments. Invertebrates retained on the screen were stored in 70% ethanol with two drops of rose bengal dissolved in ethanol. The individual samples were later washed again, if needed, and poured into a petri dish for separation of animals from remaining sediment under a dissecting microscope. Specimens were identified and counted under the microscope and archived in ethanol. Invertebrates were identified to the most practical taxon, genus or family for smaller specimens (e.g., polychaetes, amphipods) and species for larger specimens (e.g., bivalves, decapod crustaceans).

Sediment characteristics

Spatial variation in the distribution and abundance of salt marsh macrofauna may be correlated with sediment grain size or with factors that covary with these characteristics such soil organic matter and oxygen concentration (review in Snelgrove and Butman 1992; Levin and Talley 1999). To examine relationships between sediment characteristics and macroinvertebrate densities, we measured the grain size and organic matter content of sediments in each plot. Samples for sediment analysis were taken in the center of each plot using a small (4.8 cm) core pushed to a depth of 6 cm and returned to the laboratory for processing.

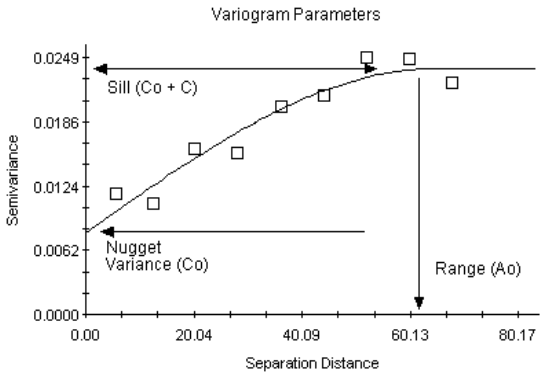
In the laboratory, grain size characteristics of sediments were determined using a modification of the hydrometer method (Bouyoucos 1962). Briefly, all twigs, shells, pebbles, and roots over 2 mm in greatest dimension were first removed from each sample. The sample was dried at 70°C to a constant weight. Fifty g of dry sediment sample was mixed with a solution of sodium

metaphosphate (100 ml of 50 g/l) and homogenized on a shaker for 24 hours at 125 rpm. The sediment solution was standardized to a 1-liter volume with distilled water and inverted several times to suspend the sediments thoroughly. Hydrometer and temperature measurements were made over a two-hour period on the sediment solution. Hydrometer measurements were corrected for temperature and a blank (5 g of sodium metaphosphate/1 liter of distilled water). The fraction of the sediment sample mass that consisted of sand (particles >62 μm diameter), silt (particles 2-62 μm diameter), and clay (particles <2 μm diameter) was calculated using equations in Bouyoucos (1962).

We measured soil organic content in a sample as percent weight loss following oxidation of organic matter in a muffle furnace at 450°C for 6 hours. Organic content was determined on subsamples of ~25 g dry weight. A mortar and pestle was used to grind apart any conglomerates in the dried sample prior to combustion. The organic fraction, expressed in percent, was calculated as the organic dry mass divided by the sample dry mass.

Statistical methods for autocorrelation analysis

The invertebrate sampling data were analyzed for spatial autocorrelation (lack of independence) using variogram analysis (Cressie 1993) (Fig. 1). A variogram is the plot of the semivariance (γ), in this case the average of half square difference between the values of the sampled variable (invertebrate density) at pairs of points separated by a certain distance, versus that distance. Variograms were constructed using GS+ geostatistical software (Gamma Design Software). A variogram function was then fit using GS+ to each empirical variogram to estimate of the scale of spatial autocorrelation in the sampled data. The two functions most frequently used were the spherical and linear functions. The spherical function provides an estimate of the scale of correlation in spatially autocorrelated data. A linear function with a positive slope suggests autocorrelation on a scale exceeding the area sampled, whereas a linear function with zero slope suggests a lack of autocorrelation. A hypothetical example of a variogram fit with a spherical function, and parameters that are estimated from the function are shown below. The nugget variance is a measure of unexplained random variation in the data. The sill, or model



asymptote, is a measure of the variation in the data among spatially uncorrelated samples. The range (A_0) is the distance at which spatial autocorrelation disappears. In some cases, the calculated value of A_0 exceeded the length of the transect (30 m). Because of uncertainty about the reliability of extrapolated estimates, these A_0 values are reported as exceeding the transect length.

Composite sampling

To determine the efficacy of compositing as a method to reduce the number of samples that need to be processed in the laboratory, we sampled the three tidal creeks in Carpinteria Salt Marsh used in the autocorrelation analysis (A, B, C: Fig. 1). We divided each creek into 5 equal length segments. Within each segment, we randomly selected a station that was sampled with a small and large core. Small core samples were preserved on site and returned to the laboratory. Large core samples were sieved in the field through 3-mm mesh and the animals transported to the laboratory.

In the laboratory, five small core samples from each creek were sieved through 0.5-mm mesh and combined to form one composited sample. Large core samples from each creek were similarly composited. The composited samples were mixed well, and split into 4 subsamples using a plankton splitter. We calculated total density and species richness for all combinations of 1, 2, and 3 subsamples from the composited sample for each creek and tested for differences in the percent deviation in the mean of the composite subsamples from the overall mean of the non-composited samples using ANOVA and post hoc tests.

Optimizing sampling effort among spatial scales

Without any prior knowledge of the scales at which spatial heterogeneity in abundance of invertebrates is greatest (see variogram analysis above), it is impossible to know how best to allocate sampling effort. Because of the natural topography of wetlands, replication of sampling can occur at various natural and imposed scales. For example, each tidal creek could be viewed as a replicate within a wetland, and then several "blocks" containing closely spaced replicate samples could be taken within each creek. Such a sampling scheme would be spatially nested (hierarchical), with replicates nested within blocks, which are nested within creeks, which are nested within wetlands. With relatively limited preliminary sampling, it is possible to optimize replication at each nested level with the goal of maximizing the precision of the estimate of invertebrate abundance for a predetermined level of sampling effort in each wetland (Sokal and Rohlf 2001). It is the precision of the estimate of abundance within each wetland that will set the power of the test comparing the restored wetland with reference wetlands.

We used two data sets to explore how best to allocate sampling effort among nested spatial scales. First, we used the data gathered in the sampling study described above (hereafter called Study 1). Because these data were not originally intended to explore the question of how best to allocate sampling effort among hierarchical spatial scales, they had some limitations for this application. Specifically, they provide little insight into "within site" variability in abundance at spatial scales of 10's to 100 m or so. Therefore, we also used data gathered by S. Schroeter and J. Boland on invertebrates in San Dieguito Lagoon during December 1997 (hereafter Study 2). These data were collected in a hierarchical design, over scales ranging from 10's to 100's of m (Table 9). While the sampling design differed between the two studies (Table 9), the methods used by Schroeter and Boland to collect invertebrates were similar to those used in the study above. They used 15-cm cores that collected samples to a depth of 25 cm and quantified only

invertebrates retained on 3-mm mesh. They collected samples at three tidal heights, low or subtidal (- 1' NGVD), medium (0' NGVD), and high (+1' NGVD). (The low height was roughly equivalent to that sampled in Study 1.) They only sampled, however, in the main channel habitat, since the tidal creek habitat is essentially absent in San Dieguito Lagoon. Methods used in Study 2 were otherwise identical to those used in Study 1.

To analyze the data gathered in Study 1 with nested analysis of variance (ANOVA), we divided each 30-m transect (containing 30 samples spaced 1 m apart) into five 6-m long "blocks" containing 6 replicate samples. We then used nested analysis of variance (random model) to calculate the variance in invertebrate abundance (all species combined) at each of the hierarchical levels: wetland, station (tidal creeks or main channel sections), block, and replicate, following Sokal and Rohlf (2001). These variance estimates were then used to calculate the optimal allocation of replication among hierarchical levels, following the procedures described in Sokal and Rohlf (2001) and Appendix 1. These calculations give the optimal numbers of replicates within blocks and the optimal number of blocks within stations. The optimal number of stations is set by both the amount of sampling effort that will be spent in each wetland and the optimal levels of replication at the block and replicate levels. For Study 1, these analyses were performed separately for the two habitats studied, tidal creeks and main channels, and separately for the samples retained on 0.5-mm mesh and 3-mm mesh. The data gathered by during Study 2 were analyzed in the same general manner. However, for this data set, separate analyses were performed for each of the three tidal heights sampled, and this data set only contained information on animals retained on 3-mm mesh.

Results

Invertebrates sampled

Invertebrate taxa sampled during this study are given in Table 1. Across all wetlands, taxonomic richness was highest for the Annelids (13 to 21), followed by Mollusks (15-18), and Arthropods (8-12). Overall richness in our samples was similar among wetlands: 51 at Carpinteria Salt Marsh, 49 at Mugu Lagoon, and 45 at Tijuana Estuary. As expected, larger and deeper living forms were more abundant in the 10 cm diameter than in the 4.8 cm diameter cores. These larger forms included bivalves (e.g., *Tagelus californianus*, *Protothaca staminea, Tresus nuttallii*), annelids (e.g., *Hemipodus borealis*, *Nephtys caecoides*, *Glycera dibranchiata*), and arthropods (e.g., *Neotrypaea californiensis*, *Hemigrapsus oregoniensis*, *Pachygrapsus crassipes*).

Spatial variability in the density of benthic macroinvertebrates

Quadrat samples

The epifauna sampled using quadrats consisted primarily (>99%) of the snail, *Cerithidea california*. The densities of *C. california* in quadrats of the main channel transects in Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary were low (≤1 individual/0.25 m^2) and variograms were not constructed. For the tidal creeks at Carpinteria, densities of *C. californica* increased in creeks with increasing distance from the inlet (from A to C), and within a creek with increasing distance from the main channel (Fig. 2). A spherical function best fit the data from each creek (Table 2). However, for distances of <30 m, the fit appears linear with a positive slope, which reflects the gradient of increase in *C. california* density away from the main channel.

The density of *C. california* at Mugu also increased in creeks with increasing distance from the inlet (Fig. 3). However, within the creeks, densities of *C. california* did not show a consistent spatial pattern. In creek A, *C. californica* densities were too low to have much confidence in the variogram analysis. In creek B there was no spatial autocorrelation in density over the 30 m distance, whereas in creek C, there was autocorrelation in density on a scale of 7 m (Table 2).

Core samples: invertebrates retained on 0.5-mm mesh

No single variogram model satisfactorily described the spatial structure of invertebrates along the tidal creek transects. Fits of the spherical model to data from the creeks closest to the inlet (A) suggested spatial autocorrelation on a scale of 10 m at Carpinteria and 23 m at Mugu, and on a scale of >30 m in Creek B at Carpinteria (Table 3, Figs. 4, 5). However, variograms of data from the other creeks sampled in this study in both wetlands did not show spatial pattern in density over a scale of up to 30 m.

Density data from the main channel transects at Carpinteria and Mugu also showed a mix of patchy and random distributions on a scale of 30 m. Fits of the spherical model to data of the main channel transects nearest the inlet (A) suggested spatial autocorrelation on a scale of 17 m at Carpinteria and of >30 m at Mugu (Table 2, Figs. 6, 7). However, variograms of data from other main channel transects, including one from Tijuana Estuary, suggested a lack of spatial autocorrelation in invertebrate density over a scale of up to 30 m (Table 2).

When the analysis was conducted on the grouped data from all main channel transects, the variogram from Carpinteria suggests a gradient in density up the channel (Fig. 8a). However, this pattern is driven by the low density of invertebrates in transect A. When data from this transect are excluded, the density distributions in transects C through D appear random (Fig. 8b). Similarly, the variogram of the grouped main channel data from Mugu also suggests a lack of spatial structure in density over a scale of 190 m (Fig. 8c).

Core samples: invertebrates retained on 3-mm mesh

For those invertebrates sampled using the 10 cm diameter core and retained on 3-mm mesh, variogram analysis suggested more spatial structure in invertebrate density in the tidal creeks than main channel (Table 3). For Carpinteria, the variograms suggest spatial autocorrelation on a

scale of 18 m for creeks A and C and >30 m for B (Fig. 9). In creek C, this pattern was driven by variation in the density of epifauna, consisting entirely of the snail, *Cerithidea californica*. Removal of *C. californica* from analysis removed the spatial autocorrelation present in the creek C data (Table 3).

Variograms of density data from Mugu tidal creeks suggested spatial autocorrelation on a scale of >30 m for creek A, reflecting a gradient of increasing density of both epifauna and infauna up the creek (Table 3, Fig. 10). No spatial structure was found in creek B. The spatial autocorrelation evident in creek C (A_0=7 m) was driven by a dense patch of *Cerithidea californica*. When the *C. californica* were excluded from the analysis, the variogram is similar to that of creek A (A_0>30 m) and reflected a gradient of increasing density of infauna up the creek (Table 3).

For the main channel transects, variograms of data from Carpinteria, Mugu, and Tijuana generally suggested a lack of spatial autocorrelation in density over a scale of 30 m (Figs. 11-13). Some spatial structure was suggested from the analysis of transect A at Carpinteria (A_0>30 m), but invertebrate densities were low (≤4 individuals/78.5 cm^2) and the variogram results are probably not meaningful. The density of larger invertebrates generally increased from transects A to D at Carpinteria and a linear fit to the variogram of the grouped data had a positive slope reflecting this pattern (Fig. 14a). Variograms of the main channel transect data from Mugu suggested autocorrelation on a scale of >30 m for all 3 transects (Table 3, Fig. 12). In contrast to the pattern at Carpinteria, however, invertebrate density decreased abruptly from transects A and B to C at Mugu and a spherical fit to the variogram of the grouped reflected this pattern (A_0=46 m) (Fig. 14b).

Relationships between grain size, organic content of sediments, and invertebrate density

Sediments in the tidal creeks at Carpinteria Salt Marsh ranged from sandy (<5% silt-clay) to muddy sand (5-20% silt-clay) (Fig. 15). The sediments of creek A, located nearest the inlet, tended to be more sandy than those of creeks B and C. There was little variability in the silt-clay content of sediments within these creek transects except for creek A at Mugu where there appeared to be patches of silt/clay spaced approximately 5 to 10 m apart. The sediment characteristics of creeks B and C at Mugu were similar to those at Carpinteria. However, there was more heterogeneity along creek C with the silt-clay content ranging from 10 to 70% (Fig. 15).

In the main channels at Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary, there was a general pattern of increase in % silt-clay with distances upstream (Fig. 16). Invertebrate data were missing from transects B and C at Tijuana Estuary because the sediments of transect C were too soft to sample and the high organic content of sediments from transect B lead to poor preservation of some samples.

As expected, there was a positive correlation between % silt-clay and % organic matter in the sediments for tidal creek and main channel sites in Carpinteria and Mugu (Fig. 17). At Mugu, where % silt-clay ranged from ~5 to ~80%, there was a significant difference in this relationship between tidal creek and main channel ($P<0.001$, ANCOVA); organic content was higher in tidal creek compared with main channel habitat for a given silt-clay content.

There was a significant positive correlation ($P<0.001$) between the density of invertebrates retained on 0.5-mm mesh and % silt-clay in both tidal creek and main channel habitat at Carpinteria Salt Marsh (Fig. 18). This pattern was driven by the low density of invertebrates in the very sandy sediments (generally <8% silt-clay) of transect A. In contrast, there was no correlation ($P>0.1$) between invertebrate density and % silt-clay in tidal creek or main channel transects at Mugu Lagoon. In addition, there was no correlation between invertebrate density and % silt-clay in the sandy sediments of transect C at Tijuana Estuary (data not shown).

Composite sampling

For the samples sieved with 0.5-mm mesh, there was a significantly greater deviation from the actual density of invertebrates (mean of all subsamples of the composite sample combined) for any 1 subsample of the composite (50% deviation) than for any 2 or 3 subsamples combined (<5% deviation) (Table 5, Fig. 19). A similar pattern was found for richness although the absolute deviation for any one subsample (~40%) was slightly closer to the deviation for any combination of 2 or 3 subsamples (10-15%) (Table 6, Fig. 19).

For the samples sieved with 3-mm mesh, there was significantly greater deviation from the actual density for 1 and 2 subsamples (30, 20%) than for 3 composited subsamples (10%) (Table 7, Fig. 20). The pattern differed somewhat for richness, for which deviations from actual richness where significantly greater for 1 composite subsample (25%) than for 2 and 3 subsamples combined (<10%) (Table 8, Fig. 20).

Optimizing sampling effort among spatial scales

In Study 1, there were significant differences in invertebrate density at the station level (Table 10). This was true of both tidal creek and main channel habitats and of invertebrates retained on both 0.5-mm and 3-mm meshes. There were also significant differences in the density of invertebrates retained on 3-mm mesh among blocks; no such differences were evident for invertebrates retained on 0.5-mm mesh. No significant differences in invertebrate density were detected between Carpinteria Salt Marsh and Pt. Mugu Lagoon. In Study 2, in which only invertebrates retained on 3-mm mesh were sampled, there were no significant differences among stations or blocks, though at the low tidal level (the same level sampled in Study 1), differences among stations were only marginally non-significant (Table 11).

Optimization analysis of Study 1 generated different allocation of replication for invertebrates retained on 0.5-mm mesh than for those retained on 3-mm mesh (Table 12). Whereas the analysis indicated that effort should be directly primarily towards sampling many "stations" (i.e. tidal creeks or main channels) with no replication of blocks or samples within blocks for the smaller invertebrates retained by the 0.5-mm mesh, it indicated that 2 blocks (with no replication within blocks) per site should be sampled for the larger invertebrates retained on the 3-mm mesh. This result should not be surprising, considering the statistically significant differences among

blocks detected for invertebrates retained by 3-mm mesh, which were absent for invertebrates retained by 0.5-mm mesh.

The results of the optimization analysis for Study 2 were consistent with those of Study 1 (Table 13). This analysis indicated that for invertebrates retained on 3-mm mesh at the low-tidal level equivalent to the habitat sampled in Study 1, 2 samples separated by about 15 m should be taken per station. Though this was the scale of separation of replicates in Study 2, it is roughly equivalent to the scale of separation of blocks in Study 1.

Taken as a whole, the results of the two studies indicate that the precision of the estimate of invertebrate abundance at each wetland can be maximized by sampling many stations within a wetland with little replication (1-2 replicate samples) within stations.

Summary and Conclusions

No one spatial autocorrelation model satisfactorily described the spatial patterns of macroinvertebrate density in tidal creeks and main channels (Table 15). Three patterns were evident: 1) no autocorrelation among plots spread 1m apart over 30 m (e.g., Carpinteria tidal creek C), 2) gradients in density over 30 m in some tidal creeks (e.g., Carpinteria tidal creek B) or >30 m in main channels (Carpinteria, Mugu), and 3) patches of invertebrates <30 m in extent at some locations (e.g., Mugu tidal creek A). In cases where no autocorrelation was evident at a scale of 30 m, it is possible that spatial structure in density on a scale >30 m may not have been detected in the analysis of 30 m segments.

Since the scale of patchiness was not predictable, the potential implication of our findings is that sample placement for measurements of total density within a length of habitat (tidal creek or main channel) should be randomly chosen and not be any closer than the minimum distance of spatial dependence. The range of A_0 values suggest that sample replicates taken up to at least 30 m apart may not be independent (Tables 2, 3, 4). To be conservative, replicates should be separated by at least 30 m to avoid sampling within the same patch of invertebrates.

There was a weak, but significant positive relationship between the % silt-clay of the sediments and the density of invertebrates retained on 0.5 mm and 3-mm mesh in both tidal creek and main channel habitats at Carpinteria. This pattern was driven by low invertebrate densities in sandy sediments of one tidal creek and one main channel transect at Carpinteria and was not found at Mugu or Tijuana. The implication of this finding for sampling design is that spatial autocorrelation may be more pronounced when sampling across a wide range of sediment characteristics.

Analyses of compositing indicated that for animals retained on 0.5-mm mesh, compositing can effectively reduce laboratory sampling effort by 60% with a very small decrease in accuracy (about 5%). By contrast, compositing is not as useful in reducing laboratory effort for animals retained on the 3-mm mesh. There are two reasons for this. First, compositing produces much smaller time savings because the larger animals can be effectively separated from sediments and identified in the field. Second, due to the larger sized animals retained on the 3-mm mesh, it is difficult to produce subsamples that are close to true aliquots. Based on our analyses, composite

sampling is recommended for invertebrate samples retained on the 0.5-mm, but not the 3-mm mesh.

ANOVA indicated that the largest differences in abundance occurred at the “station” level (creek or long stretch of main channel). However, optimization analysis indicates that few samples are needed per “staion”. Taken together, the analyses suggest that as many “stations” as possible should be sampled per wetland to maximize power to detect differences between the restored wetland and reference wetlands. In some wetlands, such as Carpinteria Salt Marsh, this protocol could end up sampling most of the creeks present. A minimum of 2 widely spaced replicate samples should be taken per station. Each sample could consist of a composite of 4 to 5 cores, which would tend to give a better estimate of the mean per stations than 2 or 3 single cores. Additional precision in the estimate of wetland-wide abundance could be obtained by taking more samples per stations, but such replication should not come at the expense of sampling fewer stations.

Table 1. List of benthic invertebrates sampled at Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary. Taxa in boldface were found primarily in samples sieved through 3-mm mesh.

Carpinteria Salt Marsh	**Mugu Lagoon**	**Tijuana Estuary**
Annelida	Annelida	Annelida
Apoprionospio pygmaea	*Apoprionospio pygmaea*	*Apoprionospio pygmaea*
Armandia brevis	*Armandia brevis*	*Armandia brevis*
Boccardiella hamata	*Boccardiella hamata*	**Capitellidae**
Boccardiella sp.	**Capitellidae**	Glyceridae
Cirratulidae	***Glycera dibranchiata***	*Hemipodus borealis*
Glyceridae	**Glyceridae**	**Nephtydae**
Goniadidae	***Hemipodus boreali***	***Nephtys caecoides***
Hemipodus borealis	**Lumbrineridae**	***Nereis***
Lumbrineris	Nephtydae	Phyllodocidae
Nephtydae	*Nephtys caecoides*	*Polydora* sp.
Nephtys caecoides	Oligochaete	Polydorid
Nereidae	Phyllodocidae	**Spionidae**
Oligochaete	*Polydora nuchalis*	*Streblospio benedicti*
Polydora nuchalis	Polydorid	
Polydora sp.	*Prionospio heterobranchia*	
Polydorid	*Prionospio* near cirrifera	
Prionospio heterobranchia	*Prionospio* sp.	
Prionospio *near cirrifera*	*Spio* sp.	
Spio filicornis	Spionidae	
Spionidae	*Streblospio benedicti*	
Syllidae		
Arthropoda	Arthropoda	Arthropoda
Amphipoda	**Amphipoda**	Copepoda
Caprellidae	Copepoda	Corophidae
Copepoda	Cumacea	Cumacea
Cumacea	***Hemigrapsis***	***Hemigrapsis***
Neotrypaea californiensis	insect/larvae	***Hemigrapsis oregoniensis***
Ostracoda	Megalopa	insect in casing
Pagurus hirsutiusculus	Ostracoda	Megalopa
Scleroplax granulata	***Pachygrapsis crassipes***	***Neotrypaea californiensis***
		Ostracoda
		Pachygrapsis crassipes
		Rocinela belliceps
		Scleroplax granulata
Echinodermata	Echinodermata	
hydroid polyp	hydroid polyp	
Mollusca	Mollusca	Mollusca
Acteocina inculta	***Acteocina inculta***	***Acteocina inculta***
Assiminea californica	*Assinimea californica*	*Assiminea californica*

Bulla gouldiana	***Bulla gouldiana***	***Bulla gouldiana***
Cerithidea californica	***Cerithidea californica***	***Cerithidea californica***
Cryptomya californica	***Chione***	***Chione***
Leprometis obesa	***Chione californiensis***	***Cryptomya californica***
Macoma nasuta	***Cumingia californica***	***Cumingia californica***
Macoma secta	***Laevicardium substriatum***	***Haminoea virescens***
***Mytilus* sp.**	***Leporimetus obesa***	***Laevicardium substriatum***
Nuttallia nuttallii	***Macoma nasuta***	*Littorina scutulata*
Protothaca staminea	***Macoma secta***	***Macoma nasuta***
Tagelus	***Melampus olivaceus***	***Melampus olivaceus***
Tagelus californianus	***Mytilus***	*Musculista senhousia*
Tellina carpenteri	***Protothaca staminea***	*Mytilus*
Tresus nuttallii	***Tagelus***	*Protothaca staminea*
	Tagelus californianus	***Tagelus***
	Tellina carpenteri	***Tagelus californiansus***
		Tellina carpenterii
Nematoda	Nematoda	
Nematoda	Nematoda	
Nemertea	Nemertea	Nemertea
Phorona		Phorona
Phoronida		Phoronis
Phoronis		
Platyhelminthes	Platyhelminthes	
Sipuncula		

Table 2. Results of variogram analysis of the epifaunal invertebrate, *Cerithidea californica*, sampled in tidal creeks in 0.5 x 0.5 m quadrats. A_0=range (distance at which density values become independent obtained from model fit).

Habitat	Location	Transect	Model fit	A_0	Scale of autocorrelation
Tidal creek	CSM	A	spherical	>30 m	>30 m
		B	spherical	>30 m	>30 m
		C	spherical	>30 m	>30 m
	ML	A	spherical	>30 m	>30 m
		B	linear	---	random
		C	spherical	7	<30 m

Table 3. Results of variogram analysis on all invertebrates (both infauna-I and epifauna-E) retained on 0.5-mm mesh.

Habitat	Location	Transect	Inverts	Model fit	A_0	Scale of autocorrelation
Main channel	CSM	A		spherical	17	<30 m
		B	I + E	linear	---	random
		C	I + E	linear	---	random
		D		spherical	28	<30 m
	ML	A	I + E	spherical	>30 m	>30 m
		B	I + E	linear	---	random
		C	I + E	linear	---	random
	TJ	C	I + E	linear	---	random
Tidal creek	CSM	A	I + E	spherical	10.	<30 m
		B	I + E	spherical	>30 m	>30 m
		C	I + E	linear	---	random
	ML	A	I + E	spherical	23	<30 m
		B	I + E	linear	---	random
		C	I + E	linear	---	random

Table 4. Results of variogram analysis of invertebrates retained on 3-mm mesh.

Habitat	Location	Transect	Inverts	Model fit	A_0	Scale of autocorrelation
Main channel	CSM	A	I + E	exponential	>30 m	>30 m
		B	I + E	linear	---	random
		C	I + E	linear	---	random
		D	I + E	linear	---	random
	ML	A	I + E	spherical	>30 m	>30 m
		B	I + E	spherical	>30 m	>30 m
		C	I + E	spherical	>30 m	>30 m
	TJ	C	I + E	exponential	>30 m	>30 m
Tidal creek	CSM	A	I + E	spherical	18	<30 m
		B	I + E	spherical	>30 m	>30 m
		C	I + E	spherical	18	<30 m
		A	I	spherical	18	<30 m
		B	I	spherical	>30 m	>30 m
		C	I	linear	---	random
	ML	A	I + E	spherical	>30 m	>30 m
		B	I + E	linear	---	random
		C	I + E	spherical	7	<30 m
		A	I	spherical	>30 m	>30 m
		B	I	linear	---	random
		C	I	spherical	>30 m	>30 m

Table 5. Results of ANOVA and post hoc tests comparing the deviation in mean density of 1, 2, and 3 subsamples taken from composited samples from the mean for all subsamples combined for invertebrates retained on 0.5 mesh.

Descriptive Statistics

No. Composites	Mean	Std Dev.	Std Err	N
1	50.000	5.354	1.545	12
2	4.805	3.472	0.818	18
3	2.805	2.038	0.588	12

Analysis of Variance for Y=|% deviation of whole sample mean|

Source	Type III SS	Df	Mean Sq.	F	Prob.
Model	18162.098	2	9081.049	625.826	0.000
Error	565.910	39	14.511		
Total	18728.007	41			

Post Hoc tests for Factor = No. Composites

Test	Group 1	Group 2	Mean Diff.	SE	q	Prob.
Student-Newman-Keuls	1	2	45.195	1.004	45.023	0.000
		3	47.195	1.100	42.919	0.000
	2	3	2.000	1.004	1.992	0.167

Table 6. Results of ANOVA and post hoc tests comparing the deviation in mean richness of 1, 2, and 3 subsamples, taken from composited samples, from the mean of all subsamples combined for invertebrates retained on 0.5 mesh

Descriptive Statistics

No. Composites	Mean	Std Dev.	Std Err	N
1	41.095	9.879	2.852	12
2	16.324	13.414	3.162	18
3	10.230	8.695	2.510	12

Analysis of Variance for Y=|% deviation of whole sample mean|

Source	Type III SS	Df	Mean Sq.	F	Prob.
Model	6613.186	2	3306.593	25.977	0.000
Error	4964.243	39	127.288		
Total	11577.429	41			

Post Hoc tests for Factor = No. Composites

Test	Group 1	Group 2	Mean Diff.	SE	q	Prob.
Student-Newman-Keuls	1	2	24.771	2.973	8.332	0.000
		3	30.866	3.257	9.477	0.000
	2	3	6.094	2.973	2.050	0.155

Table 7. Results of ANOVA and post hoc tests comparing the deviation in mean density of 1, 2, and 3 subsamples from composited samples from the mean of all subsamples combined for invertebrates retained on 3 mesh.

Descriptive Statistics

Group	Mean	Std Dev.	Std Err	N
1	29.692	29.023	8.378	12
2	19.795	12.992	3.062	18
3	9.897	9.674	2.793	12

Analysis of Variance for Y=|% deviation of whole sample mean|

Source	Type III SS	Df	Mean Sq.	F	Prob.
Model	2351.011	2	1175.506	3.482	0.041
Error	13164.696	39	337.556		
Total	15515.707	41			

Post Hoc tests for Factor = No. Composites

Test	Group 1	Group 2	Mean Diff.	SE	q	Prob.
Student-Newman-Keuls	1	2	9.897	4.842	2.044	0.156
		3	19.795	5.304	3.732	0.031
	2	3	9.897	4.842	2.044	0.156

Table 8. Results of ANOVA and post hoc tests comparing the deviation in mean richness of 1, 2, and 3 subsamples taken from composited samples from the mean of all subsamples combined for invertebrates retained on 3 mesh.

Descriptive Statistics

No. Composites	Mean	Std Dev.	Std Err	N
1	27.083	24.814	7.163	12
2	7.500	11.015	2.596	18
3	3.750	8.823	2.547	12

Analysis of Variance for Y=|% deviation of whole sample mean|

Source	Type III SS	Df	Mean Sq.	F	Prob.
Model	3911.310	2	1955.655	7.870	0.001
Error	9691.667	39	248.504		
Total	13602.976	41			

Post Hoc tests for Factor = No. Composites

Test	Group 1	Group 2	Mean Diff.	SE	q	Prob.
Student-Newman-Keuls	1	2	19.583	4.154	4.714	0.002
		3	23.333	4.551	5.127	0.002
	2	3	3.750	4.154	0.903	0.527

Table 9. Sampling design of Studies 1 and 2.

Factor (Level)	Spatial Scale (separation between units)	Replication in pilot studies
	Study 1	
Wetland	10's of km	2
Station (within Wetland)	100's of m	3
Block (within Station)	6 m	5
Replicates (within Stations)	1 m	6
Total number of cores taken per habitat* = 180		
	Study 2	
Station (within Wetland)	250 m	3
Block (within Station)	50 m	3
Replicates (within Blocks)	15 m	3
Total number of cores taken per tidal level** = 27		

* Two habitats were sampled, tidal creeks and main channels, and these were analyzed separately.
** Three tidal heights, high, medium, and low (subtidal), were sampled and these were analyzed separately.

Table 10. Summary of ANOVA testing for differences in density* of invertebrates between wetlands (Carpinteria and Pt. Mugu), and among stations and blocks within stations. Data are from Study 1.

Factor	SS	df	MS	*F*	*P*	Variance Component**
Invertebrates retained on 0.5-mm mesh						
Tidal Creeks						
Wetland	237.6	1	237.6	5.57	0.08	2.165
Station(Wetland)	170.7	4	427	38.28	<0.001	1.334
Block(Station)	13.4	12	1.1	0.40	0.96	0
residual	448.8	162	2.8			2.656
Main Channels						
Wetland	56.5	1	56.5	1.00	0.38	0
Station(Wetland)	227.0	4	56.8	16.90	<0.001	1.750
Block(Station)	40.3	12	3.4	0.80	0.65	0
residual	682.8	162	4.2			4.156
Invertebrates retained on 3-mm mesh						
Tidal Creeks						
Wetland	0.30	1	0.30	0.05	0.83	0
Station(Wetland)	24.52	4	6.13	5.06	0.01	0.156
Block(Station)	14.54	12	1.21	3.50	<0.001	0.070
residual	56.08	162	0.35			0.346
Main Channels						
Wetland	6.95	1	6.95	0.46	0.53	0
Station(Wetland)	59.88	4	14.97	6.24	0.01	0.407
Block(Station)	28.80	12	2.40	3.45	<0.001	0.141
residual	112.56	162	0.69			0.695

* Abundance (# per core) was transformed to square root (x + 0.5) to satisfy the assumptions of homogeneity of variance and normality.
** Calculated following procedures outlined in Fletcher and Underwood (2002) and Sokal and Rohlf (2001).

Table 11. Summary of ANOVA testing for differences in density of invertebrates among stations and blocks within sites in Study 2.

Factor	SS	df	MS	*F*	*P*	Variance Component**
Low Tidal Level (Subtidal)						
Station(Wetland)	389.4	2	194.7	4.79	0.057	8.556
Block(Station)	732	6	122.0	1.09	0.405	0.568
residual	6035.4	18	335.3			18.630
Medium Tidal Level						
Station(Wetland)	233.4	2	116.7	2.09	0.205	3.383
Block(Station)	1003.8	6	167.3	2.07	0.109	4.802
residual	4368.6	18	242.7			13.481
High Tidal Level						
Station(Wetland)	3.8	2	1.9	0.30	0.753	0
Block(Station)	112.2	6	18.7	0.45	0.833	0
residual	2219.4	18	123.3			6.852

Table 12. Optimal allocation of replication among hierarchical spatial scales based on Study 1.

		Tidal Creeks		Main Channels	
Factor (Level)	Scale (separation between units)	Optimal Replication, 0.5-mm mesh*	Optimal Replication, 3-mm mesh	Optimal Replication, 0.5-mm mesh*	Optimal Replication, 3-mm mesh
Station (within Wetland)	100's of m	Set by effort allocated	Set by effort allocated	Set by effort allocated	Set by effort allocated
Block (within Station)	6 m	1	2	1	2
Replicates (within Blocks)	1 m	1	1	1	1

* Because variance at the "Block" scale was estimated to be zero by ANOVA, optimization was done on a reduced model not containing the block term. This optimization indicated that one replicate sample should be taken per station, which is equivalent to one block with one replicate per block.

Table 13. Optimal allocation of replication among hierarchical spatial scales based on Study 2.

Factor (Level)	Scale (separation between units)	Low Intertidal	Medium Intertidal	High Intertidal
Station (within Wetland)	250 m	Set by effort allocated	Set by effort allocated	Set by effort allocated
Block (within Station)	50 m	1	2	*
Replicates (within Blocks)	15 m	2	1	*

* Not possible to optimize allocation because the only non-zero variance estimate was at replicate level.

Table 14. Comparison of costs (in minutes) for 5 cores from field collection through data entry with and without compositing. Separate estimates were made for samples sieved through 0.5-mm and 3-mm mesh.

	Time in minutes per sample				
	0.5mm cores		3mm cores		
	Composited		Composited		
Source	Yes	No	Yes	No	notes
Field Collection (5 samples)	4	4	4	4	per sample
Sieving	0	0	4	4	per sample
Field preservation of samples	1	1	1	1	per sample
Removal of formalin	10	10	8	0	total time
Compositing					
Mixing 5 samples	10	0	10	0	total time
Splitting	20	0	20	0	total time
Lab analysis	100	100	5	2	per sample
Field analysis	0	0	0	5	per sample
Data entry (for 2 splits)	2.5	2.5	2.5	2.5	per sample
Total time	255	547.5	71	92.5	for 5 samples

Table 15. Summary of spatial patterns of macroinvertebrate density in tidal creeks and main channels. *low density of invertebrates

Quadrat samples

		Patch size		
		< 30-m	≥ 30-m	Random
Tidal Creeks	CSM	0	3	0
	ML	1	1*	1
	Totals	**1**	**4**	**1**

Invertebrates retained on 0.5 mm mesh

		Patch Size		
		< 30-m	≥ 30-m	Random
Tidal Creeks	CSM	1	1	1
	ML	1	0	2
	Totals	**2**	**1**	**3**
Main Channels	CSM	2	0	2
	ML	0	1	2
	Totals	**2**	**1**	**4**
	CSM Combined (A, B, C, D)	0	1	0
	ML Combined	0	1	0
	Totals	**0**	**2**	**0**

Invertebrates retained on 3 mm mesh

		Patch Size		
		< 30-m	≥ 30-m	Random
Tidal Creeks	CSM	2	1	0
	ML	1	1	1
	Totals	**3**	**2**	**1**
Main Channels	CSM	0	1	3
	ML	0	3	0
	TJ	0	1	0
	Totals	**0**	**4**	**0**
	CSM combined	0	1	
	ML Combined	0	1	0
	Totals	**0**	**2**	**0**

Figure 1. Location of sampling stations in Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana River Estuary.

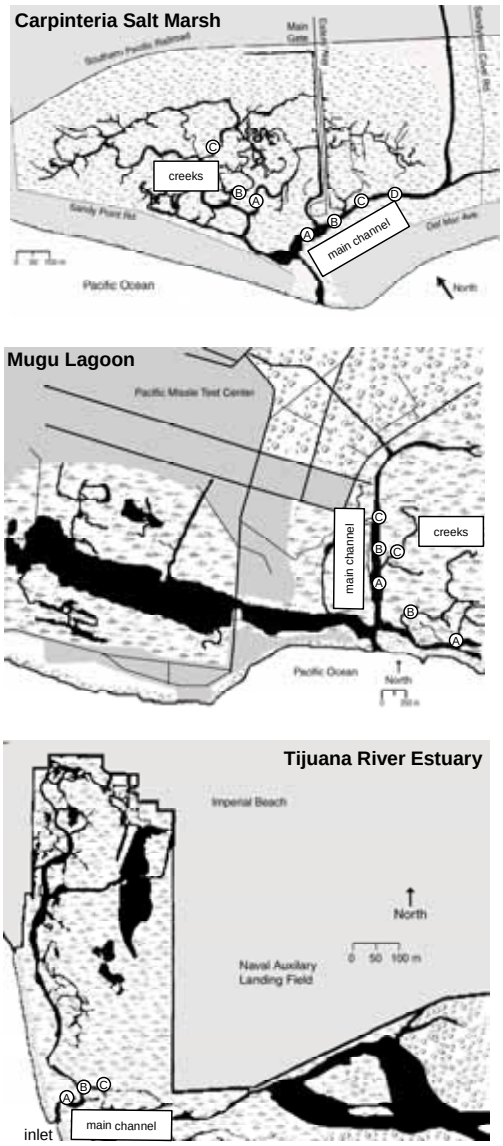


Figure 2. Densities and variograms for horn snails (*Cerithidea californica*) sampled in Carpintera Salt Marsh tidal creeks (0.25 m^2 quadrats). Note the variable scales on the y-axis in this and subsequent figures.

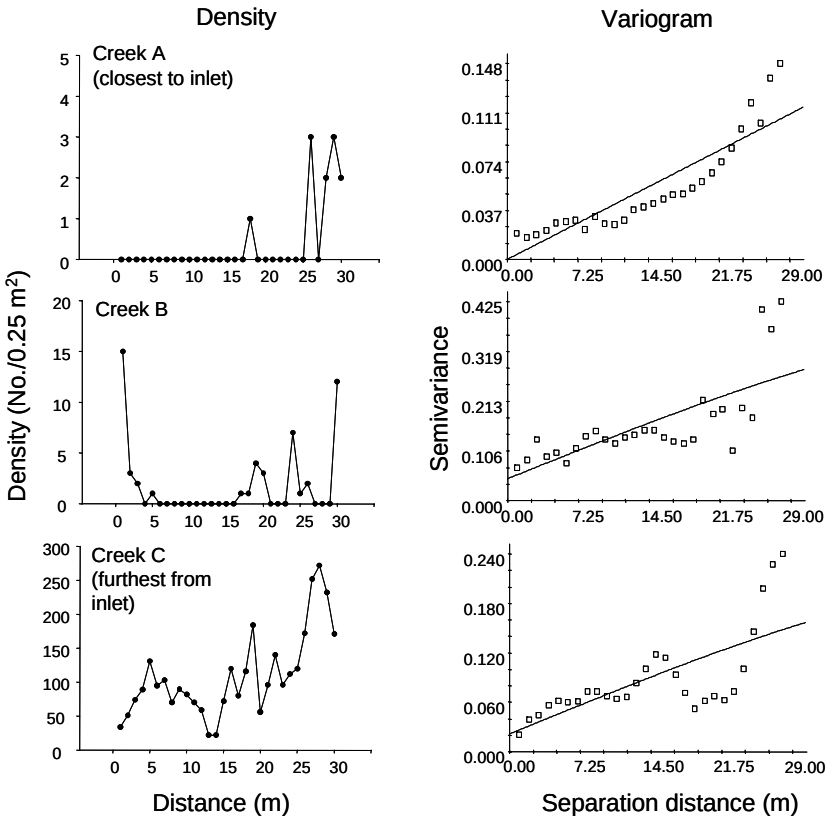


Figure 3. Densities and variograms for horn snails (*Cerithidea californica*) sampled in Mugu Lagoon tidal creeks (0.25 m^2 quadrats).

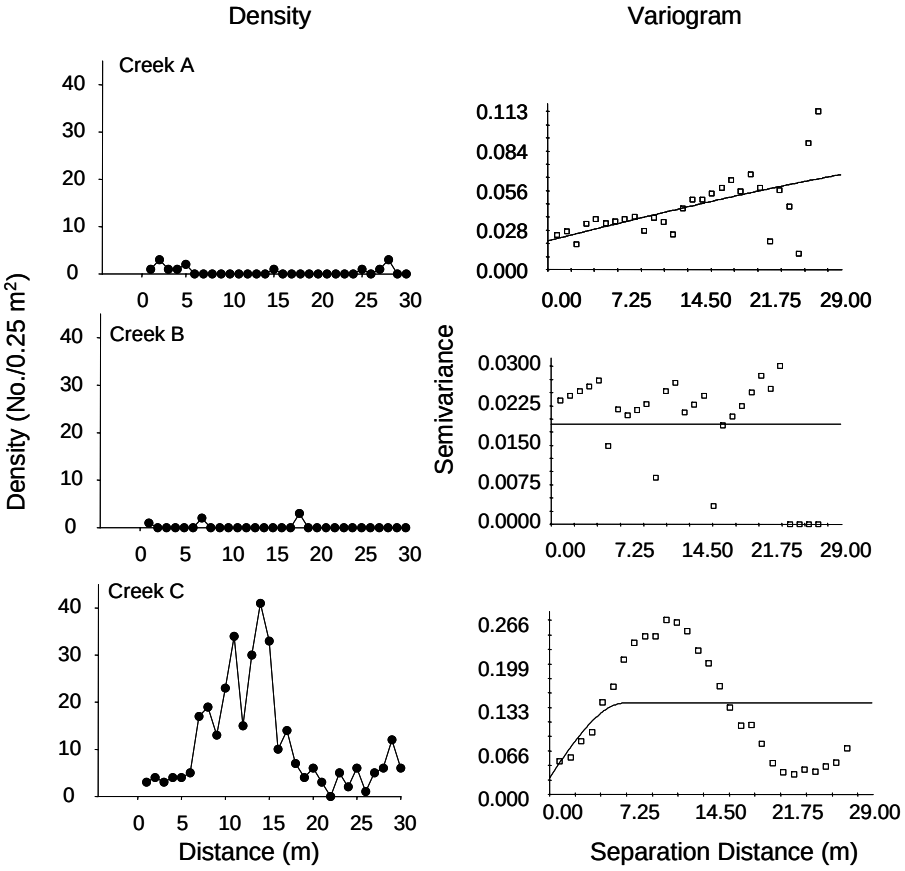


Figure 4. Densities and variograms for invertebrate macrofauna sampled in Carpinteria Salt Marsh tidal creeks (cores seived through 0.5-mm mesh).

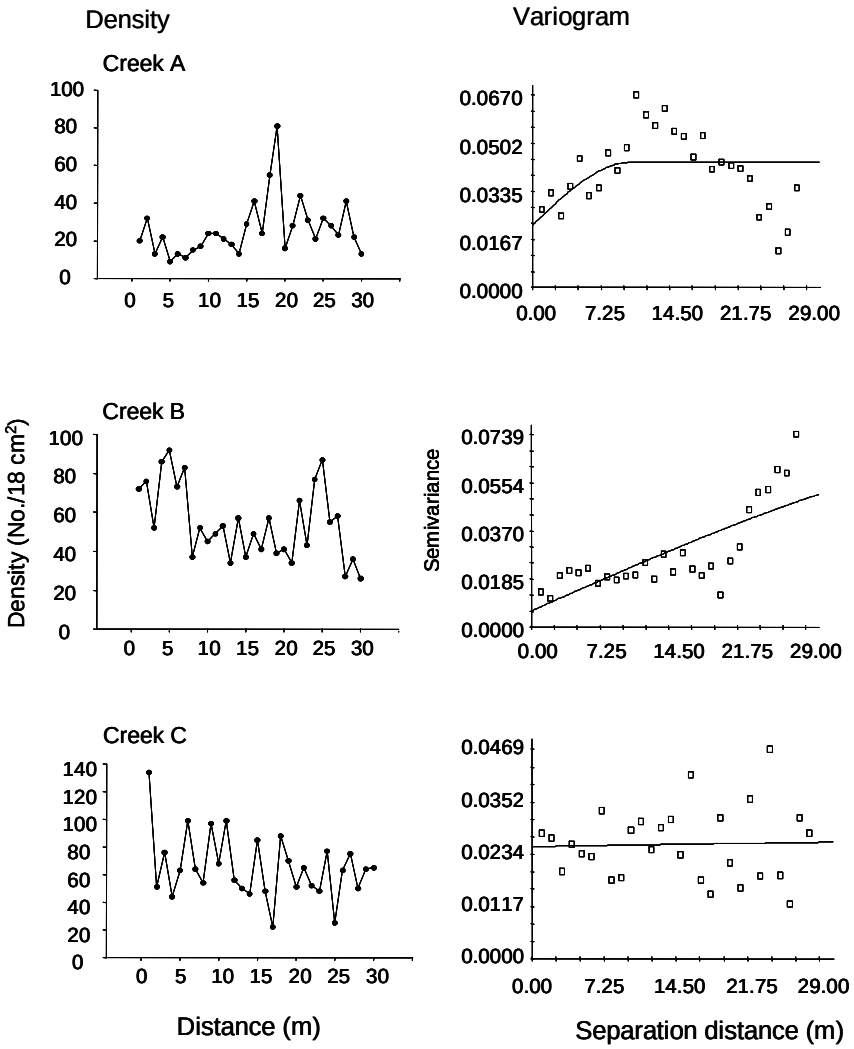


Figure 5. Densities and variograms for invertebrate macrofauna sampled in Mugu Lagoon tidal creeks (cores seived through 0.5-mm mesh).

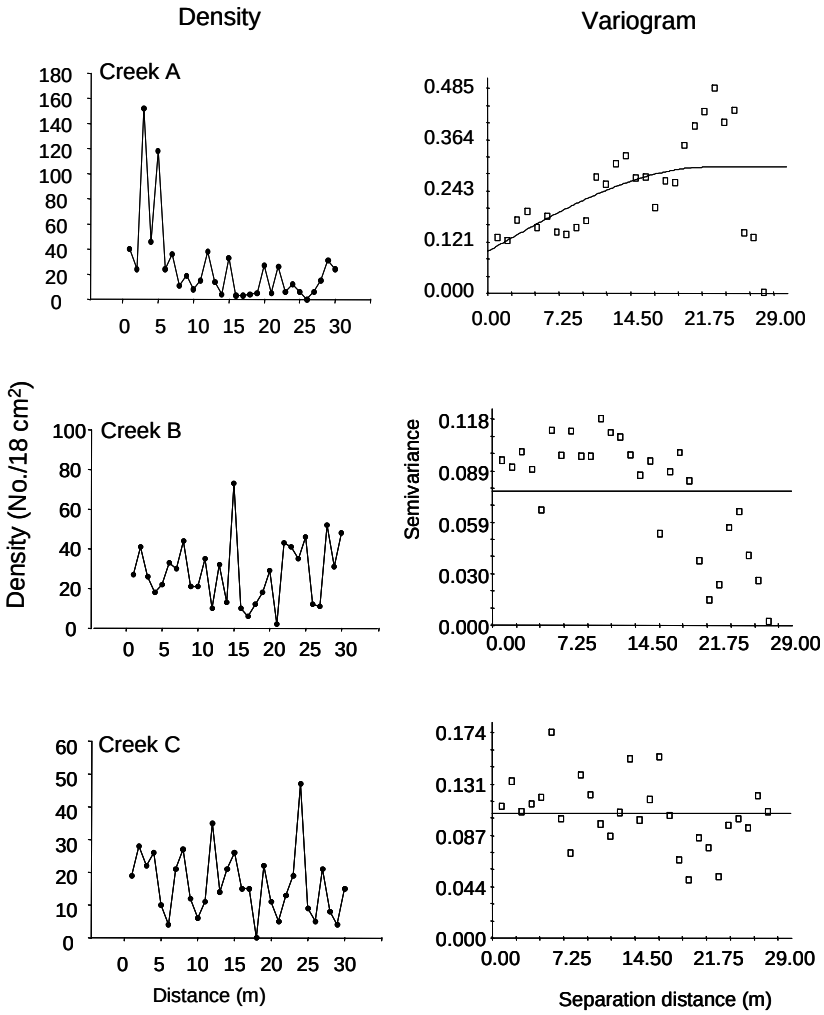


Figure 6. Densities and variograms for invertebrate macrofauna sampled in Carpinteria Salt Marsh main channel (cores seived through 0.5-mm mesh).

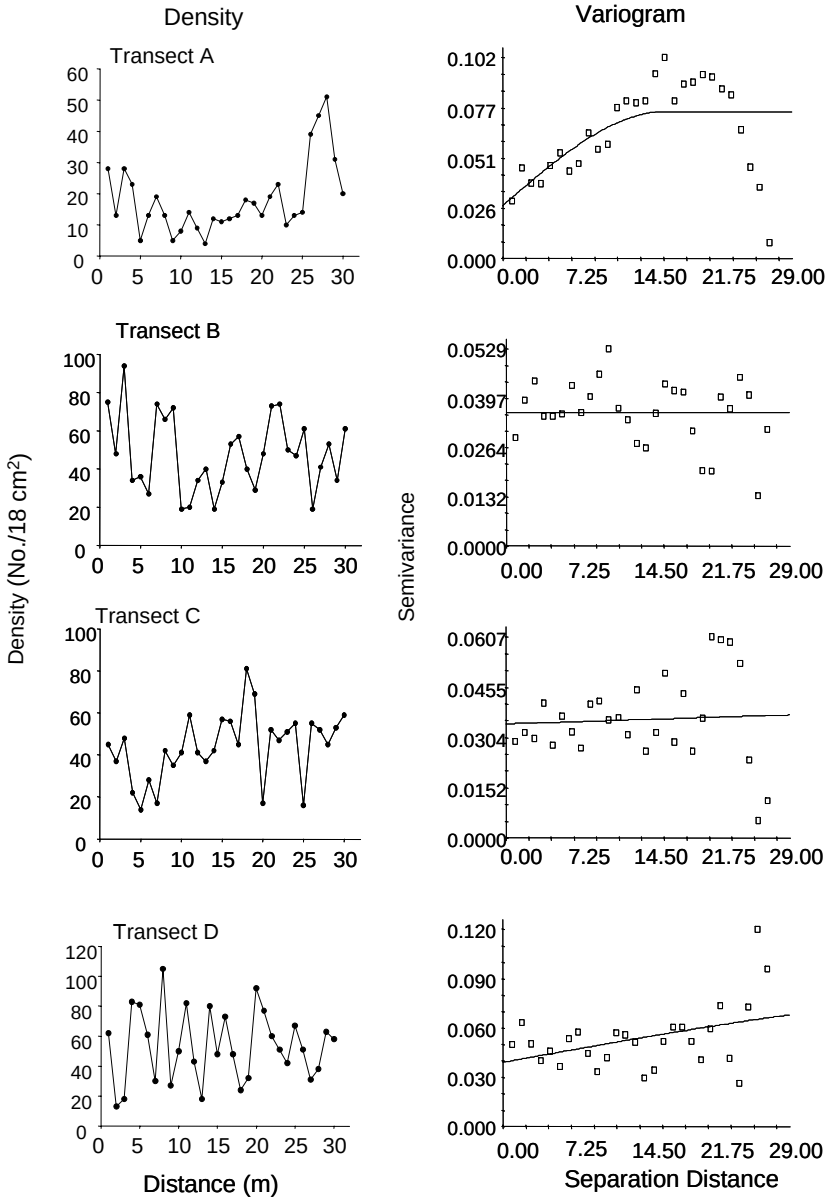


Figure 7. Densities and variograms for invertebrate macrofauna sampled in Mugu Lagoon main channel (cores seived through 0.5-mm mesh).

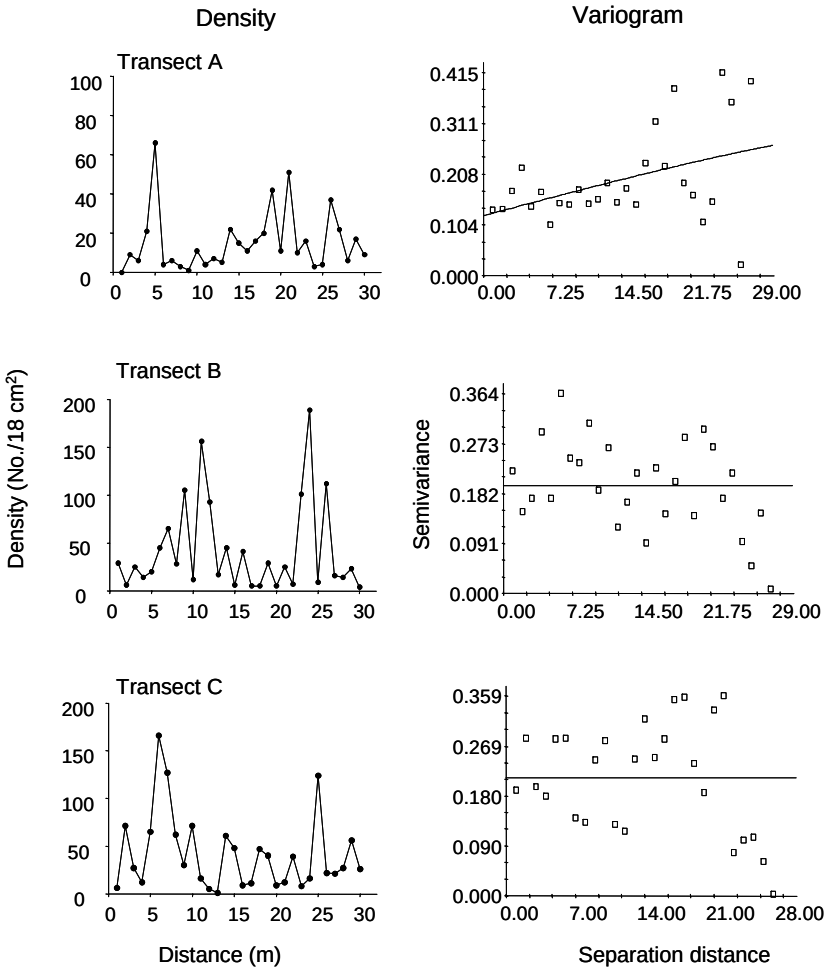


Figure 8. Combined main channel 0.5 mm data for a) Carpinteria Salt Marsh, b) Mugu Lagoon, and c) Tijuana Estuary.

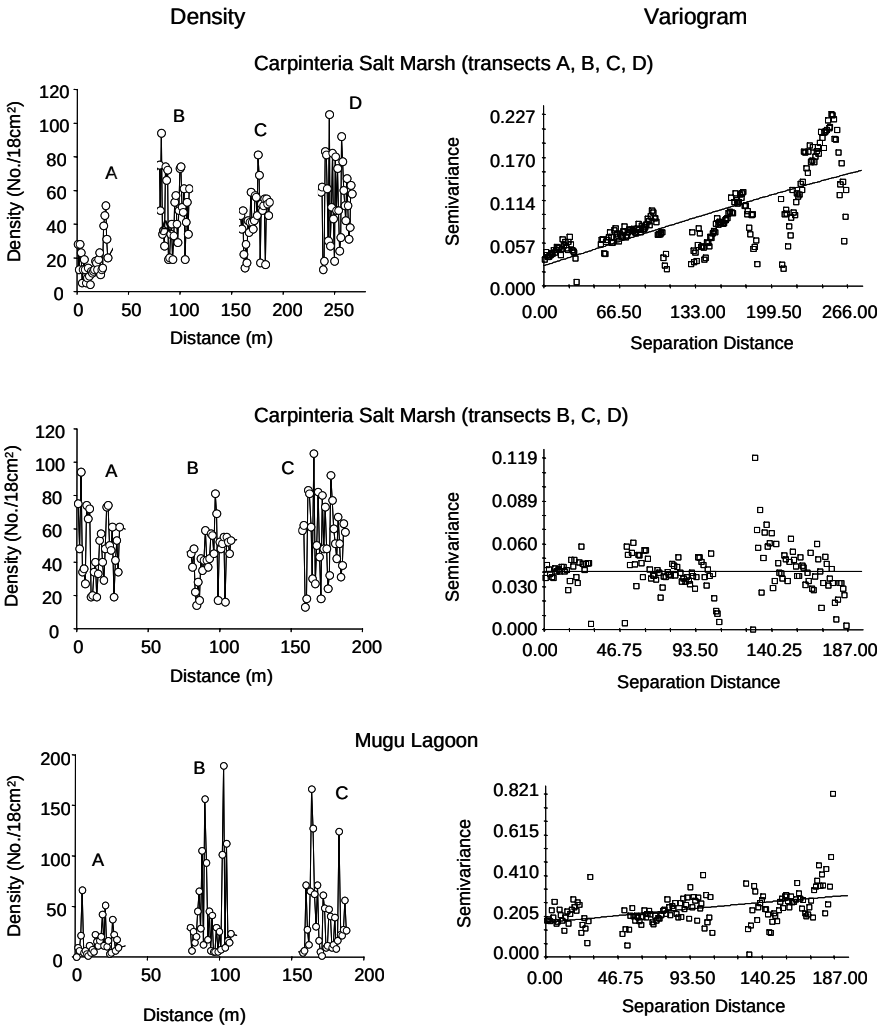


Figure 9. Densities and variograms for invertebrate macrofauna sampled in Carpinteria Salt Marsh tidal creeks (cores seived through 3-mm mesh).

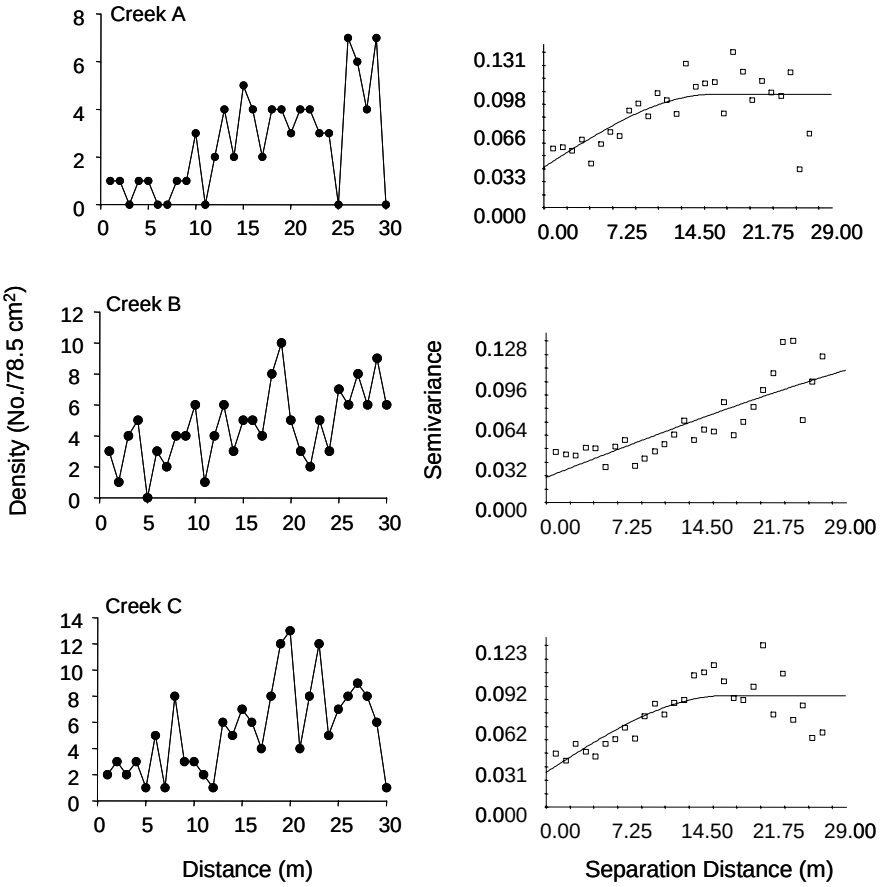


Figure 10. Densities and variograms for invertebrate macrofauna sampled in Mugu Lagoon tidal creeks (cores seived through 3-mm mesh).

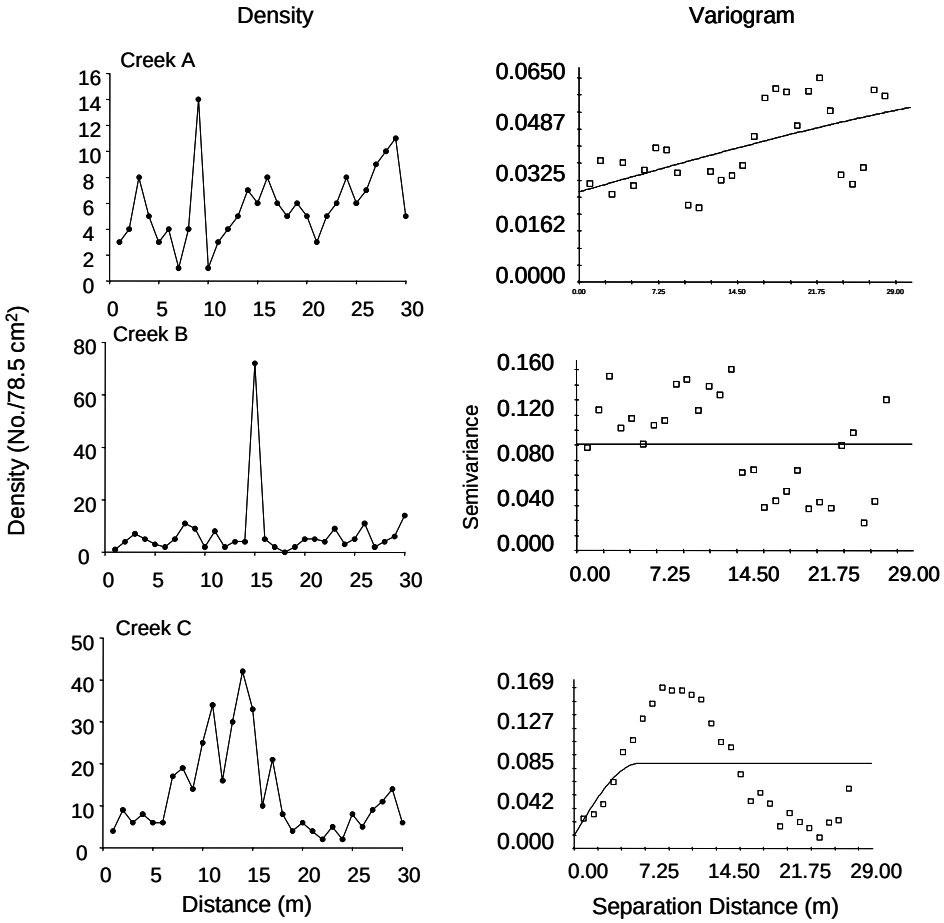


Figure 11. Densities and variograms for invertebrate macrofauna sampled in Carpinteria Salt Marsh main channel (cores seived through 3-mm mesh).

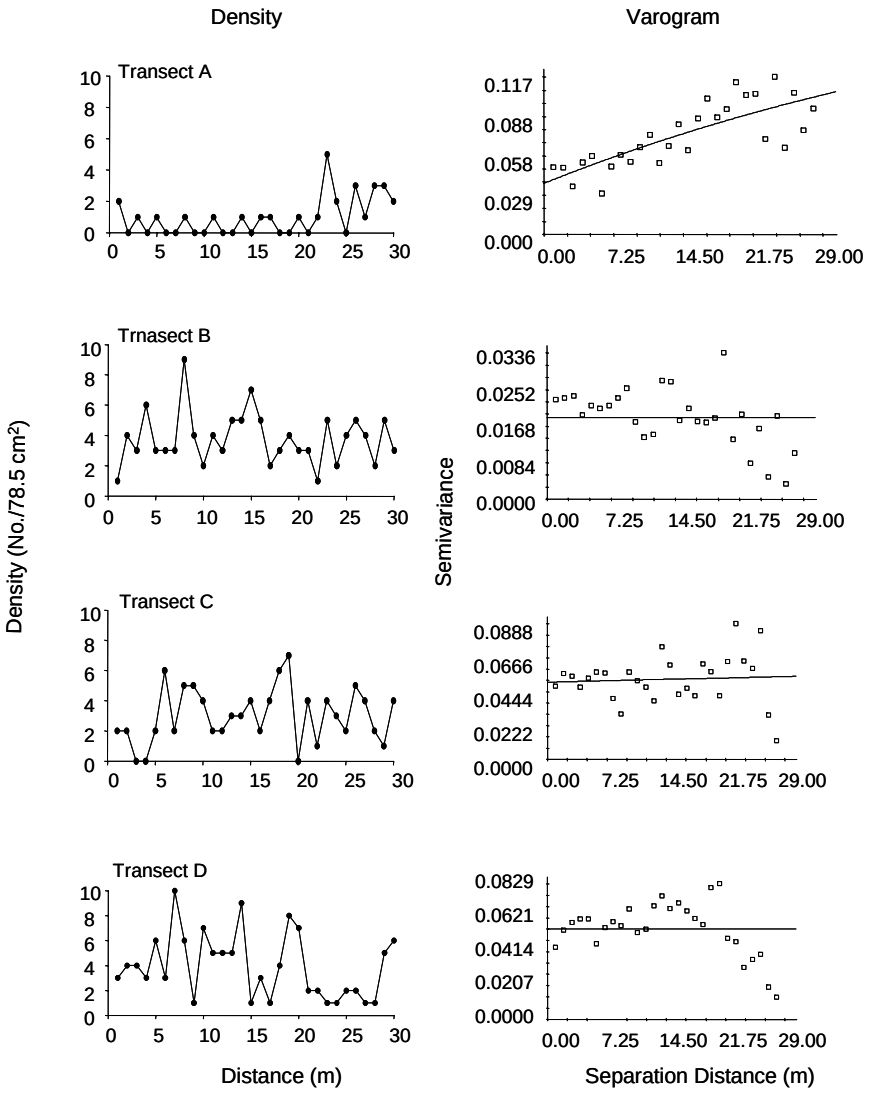


Figure 12. Densities and variograms for invertebrate macrofauna sampled in Mugu Lagoon main channel (cores seived through 3-mm mesh).

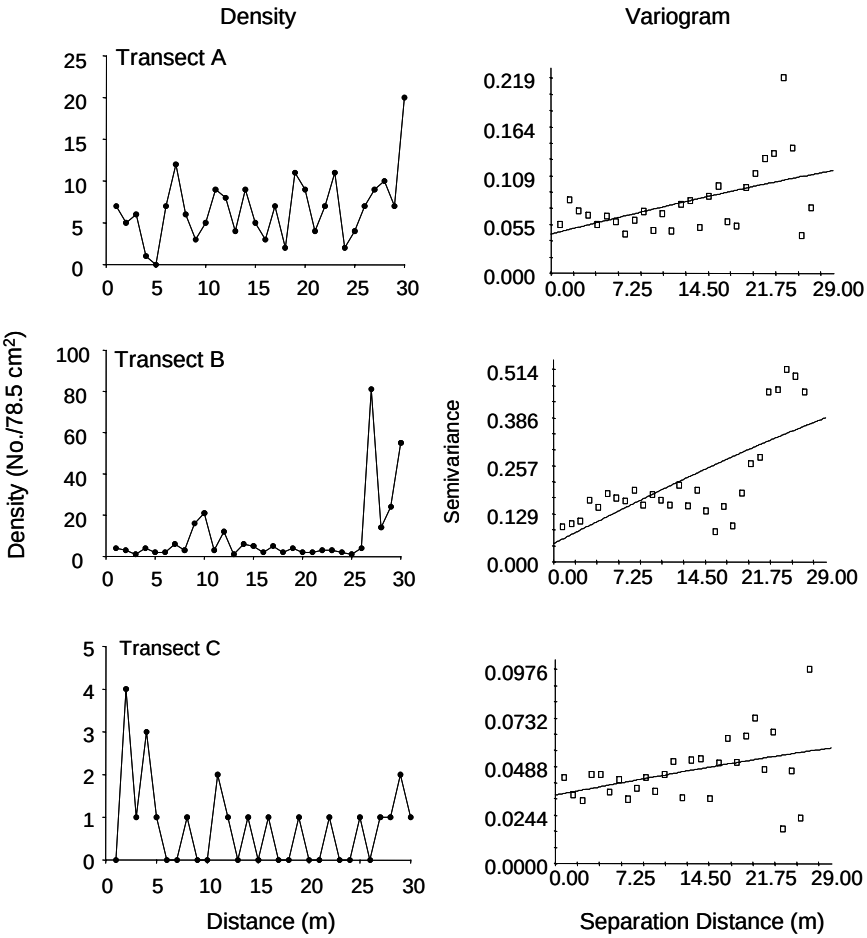


Figure 13. Densities and variograms for invertebrate macrofauna sampled in Tijuana Estuary main channel retained on 0.5 mm and 3-mm mesh.

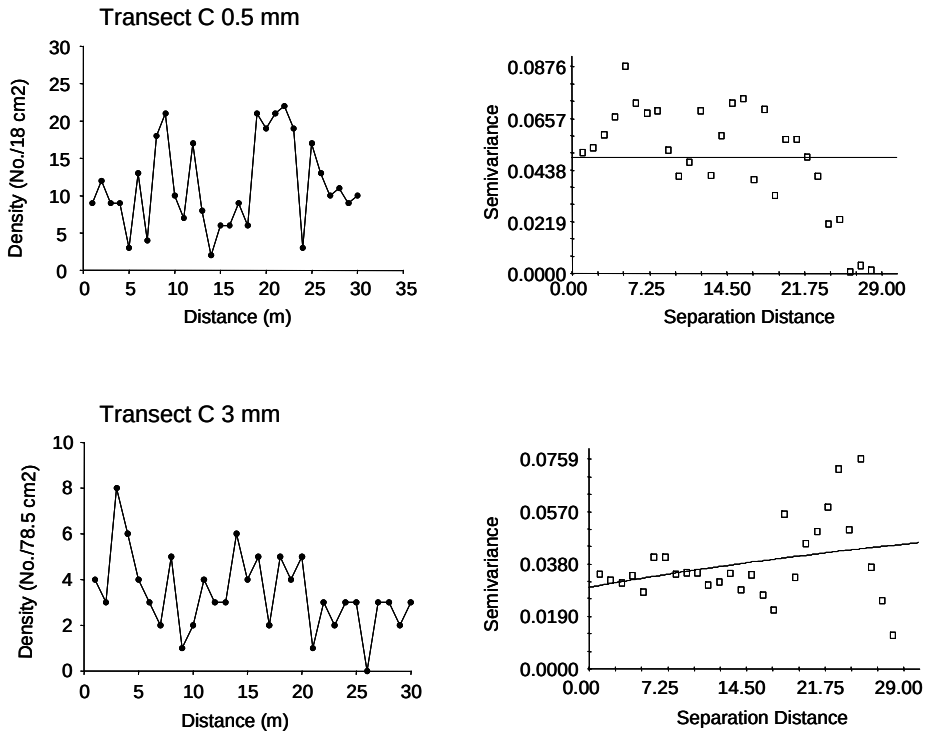


Figure 14. Combined main channel 3 mm data for a) Carpinteria Salt Marsh, b) Mugu Lagoon.

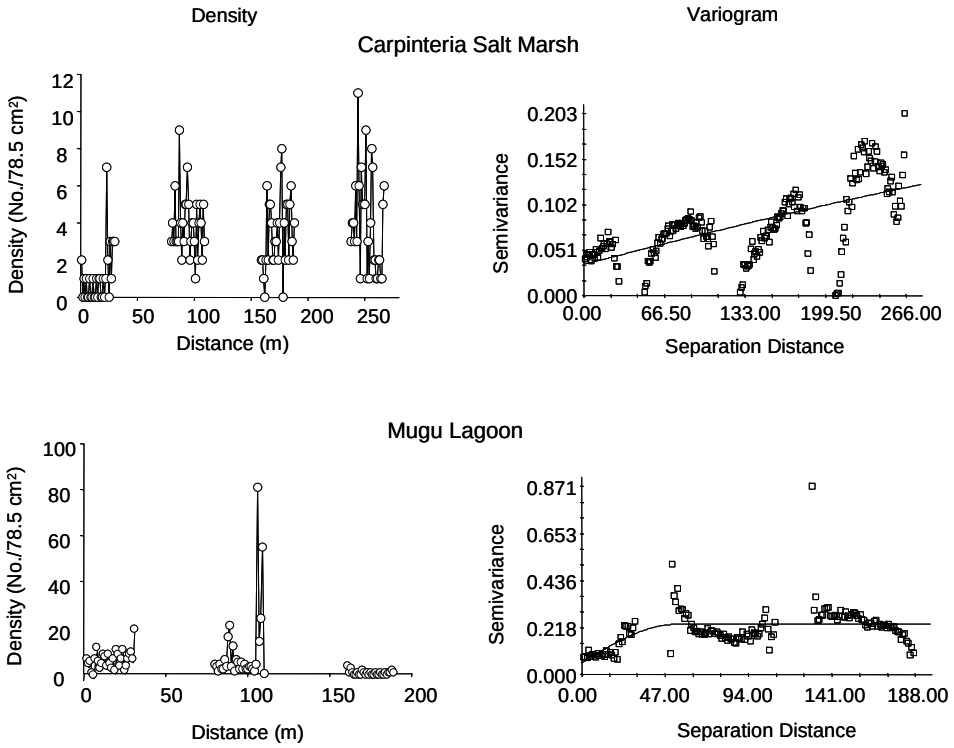


Figure 15. Variation in % silt-clay in sediments along tidal creek transects in Carpinteria Salt Marsh and Mugu Lagoon.

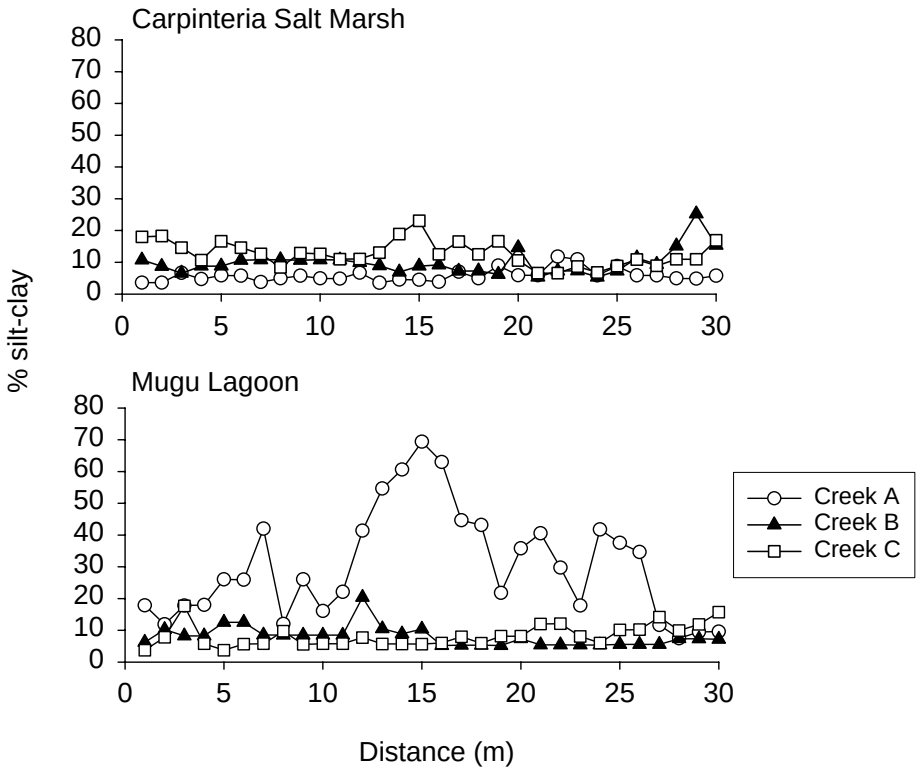


Figure 16. Variation in % silt-clay in sediments along the main channel transects in Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary.

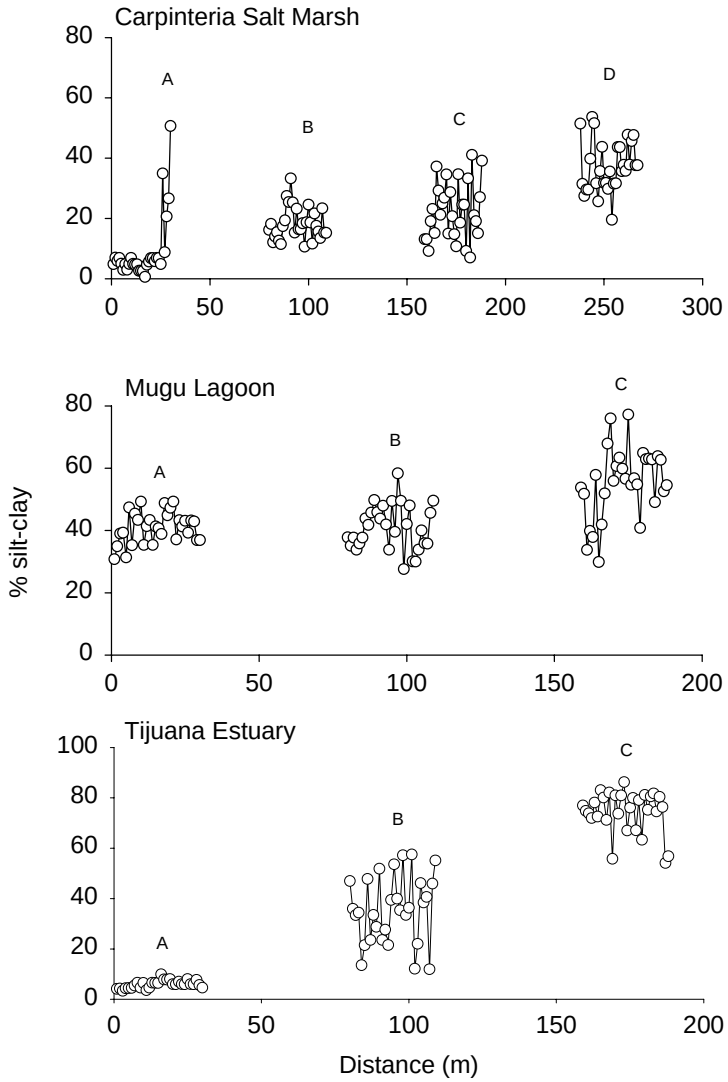


Figure 17. Relationship between % organic matter and % silt-clay in sediments in tidal creeks and main channel of Carpinteria Salt Marsh, Mugu Lagoon, and Tijuana Estuary.

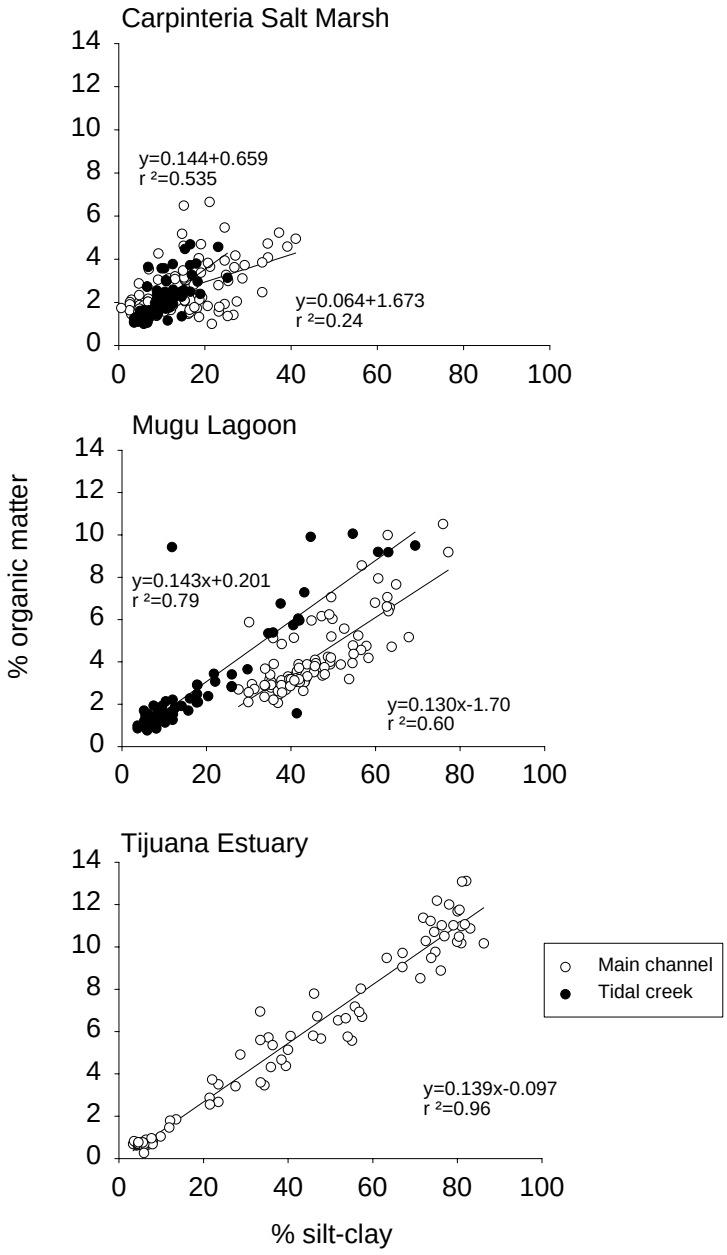


Figure 18. Relationship between % silt-clay in sediments and density of benthic invertebrates retained on a 0.5-mm mesh. Note different scales.

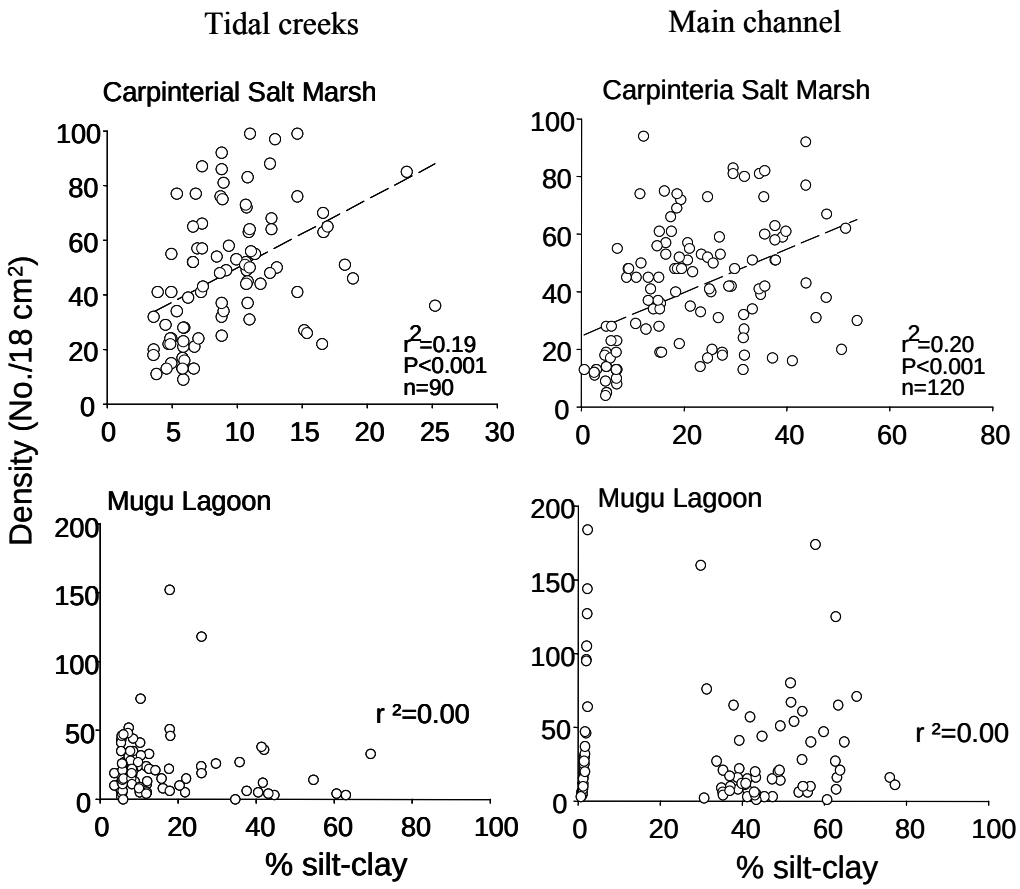


Figure 19. Deviation in mean density and richness of 1, 2, and 3 subsamples taken from composited samples from the mean of all subsamples for invertebrates retained on 0.5 mesh.

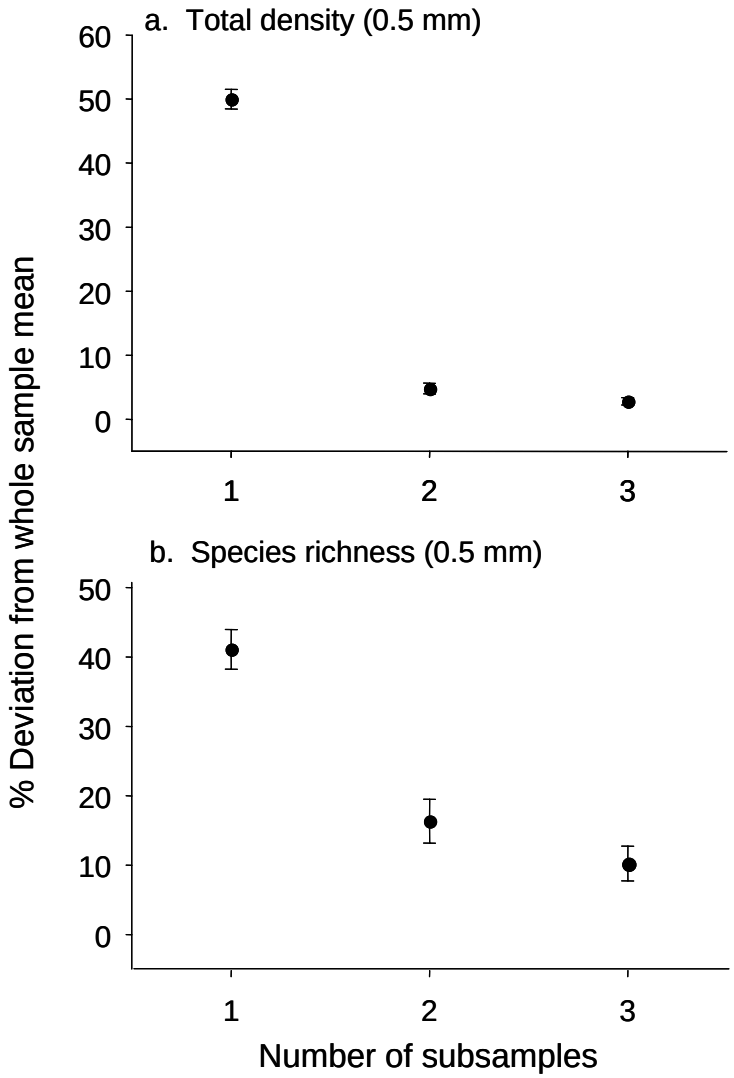
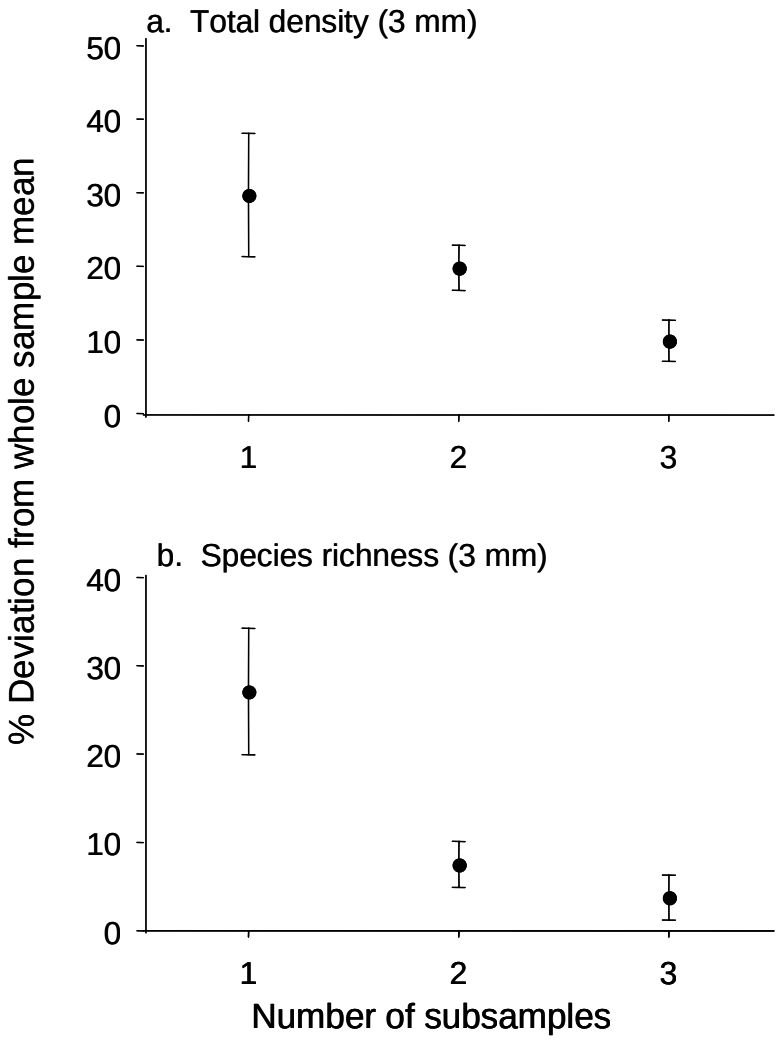


Figure 20. Deviation in mean density and richness of 1, 2, and 3 subsamples taken from composited samples from the mean of all subsamples for invertebrates retained on 3 mesh.



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Appendix 1. Hierarchical Sampling Optimization

The cost of invertebrate sampling in one wetland is given by the formula

$$C = bcnC_{n(c)} + bcC_{c(b)} + bC_{b(a)} \quad (1)$$

Where C is the cost (in time, money, or any other appropriate metric), *b* is the number of replicates at the first nested level (stations in our study), *c* is the number of replicates at the second nested level (blocks in our study), *n* is the number of replicates at the lowest nested level (replicates within blocks in our study), $C_{n(c)}$ is the cost per replicate, $C_{c(b)}$ is the extra cost per block, and $C_{b(a)}$ is extra cost per station. We measured the cost in time. $C_{n(c),}$ then, was our estimate of the time it took to take a core and completely process it, e.g., including field time to collect and process the sample and lab time for sorting, counting, and data entry. $C_{c(b)}$ and $C_{b(a)}$ were our estimates of the time it took to move from one block to another and from one station to another, respectively.

The goal of the optimization is to minimize the product of the cost and variance at each nested level:

$$C_{n(c)}S^2_{n(c)} + C_{c(b)}S^2_{c(b)} + C_{b(a)}S^2_{b(a)} \quad (2)$$

Where S^2_i is the variance at each level, *i*. First, $C_{n(c)}S^2_{n(c)}$ is minimized by solving the following equation for the optimal *n*:

$$n = \sqrt{(C_{c(b)}S^2_{n(c)}/C_{n(c)}S^2_{c(b)})} \quad (3)$$

Then $C_{c(b)}S^2_{c(b)}$ is minimized by solving the following equation for the optimal *c*:

$$c = \sqrt{(C_{b(a)}S^2_{c(b)}/C_{c(b)}S^2_{b(a)})} \quad (4)$$

Finally, $C_{b(a)}S^2_{b(a)}$ is minimized by setting C (i.e., deciding how much effort will be spent per wetland) and solving equation 1 for *b*, or

$$b = C/(cnC_{n(c)} + cC_{c(b)} + C_{b(a)}) \quad (5)$$

In this study, we did not solve for the optimal *b* because we did not intend to make judgments about how much time should be spent sampling each wetland for invertebrates.

Appendix 6

Estimating bird species richness and density in coastal wetlands of southern California: application to monitoring birds in the San Dieguito Lagoon Restoration project

Kathleen Whitney
Marine Science Institute
University of California Santa Barbara
Santa Barbara, CA

DRAFT 5/16/05

Background

The coastal development permit for SONGS Units 2 and 3 requires Southern California Edison to create or substantially restore a minimum of 150 acres of coastal wetland habitat. San Dieguito Lagoon was selected as the site for this restoration project. The permit establishes physical and biological performance standards that must be met by the restored wetland. One of these standards requires that total densities and species richness of birds in the restored wetland be comparable to those found in similar habitats of reference wetlands within four years of completion of wetland construction. The reference wetlands for comparison with the restored marsh at San Dieguito Lagoon are Carpinteria Salt Marsh, Mugu Lagoon and Tijuana Estuary.

Coastal wetlands of southern California contain habitats that are important for breeding, staging and over-wintering birds. Important habitats include mudflats, channels, vegetated marsh and open water embayments. Each of these habitats supports a unique assemblage of wetland birds (Table 1). Sampling birds in tidal habitats poses logistic challenges to the investigator. Access to sampling plots can be more limited than in other terrestrial habitats, thereby increasing traveling time/effort and the cost of conducting sampling. Furthermore, walking through these habitats during sampling causes some degree of damage to the vegetation and may create pathways that enhance access for mammalian predators. The remaining tidal wetlands in southern California are home to protected populations of rare birds, mammals, fish, insects, and plants. Every effort must be made to minimize impact to these rare species and their habitat during sampling. A list of endangered or threatened birds reported from San Dieguito Lagoon, Carpinteria Salt Marsh, Mugu Lagoon or Tijuana estuary is provided in Table 2.

Coastal wetland habitats: Because assemblages of birds differ among habitats within coastal wetlands, sampling must be stratified by habitat. Furthermore, the efficacy of sampling may differ among habitats. Differences in bird assemblages and density among habitats and factors that affect sampling efficacy in different habitats are reviewed below. The focus is on 4 main wetland habitats used by birds in southern California coastal wetlands: open-water embayments, mudflats, channels, and vegetated marsh.

Embayments: Lagoons and bays are open habitats where all birds that are present are (potentially) detectable by the observer. Embayments support mainly waterfowl and seabirds. When these birds are present, distributions are typically clumped within the habitat. Sampling may be conducted by an observer on foot or by boat. The use of laser-range finders to determine observer-bird distance and minimize edge effects (i.e., counting birds that are not in the plot or excluding birds that are) is recommended in this uniform habitat. There are strong seasonal patterns in bird species richness and abundance in this habitat and they are addressed in Section II.

Mudflats: Mudflats are also open habitats, but minor vertical stratification (depressions and intertidal channels) can reduce detection of some birds in this habitat. Mudflats are

used primarily by foraging shorebirds but other groups, including waders, gulls, and ducks, forage in areas of mudflat covered by shallow water. Both seabirds and shorebirds will form aggregations of resting individuals on mudflat, especially on substrate with coarse-grained sediment (K. Whitney per obs). Generally, the distribution of birds on mudflats is clumped, reflecting fine scale heterogeneity in sediment texture and infauna (food) density. Some of the most challenging species to identify are found on mudflats and observers must be skilled in the identification of shorebirds, sub-adult gulls and terns to accurately assess species richness in this habitat. An observer on foot or from a boat just offshore can sample mudflats, but mudflats with fine-grained sediment can be very difficult to traverse on foot. As in embayments, laser-range finders should be used to determine observer-bird distance and to minimize edge effects. Seasonal patterns in bird density and abundance are most marked in this habitat (Section II).

Channels: Channels are defined by vertical relief, which may obscure birds to an observer. Hence, it is important that sampling methods used in this habitat effectively deal with vertical relief. Channels vary in depth, width, hydrological energy, and edge vegetation; all of these factors contribute to variation in habitat use by birds at different points along a channel. Of the habitat types targeted for sampling in the SONGS project, channels are likely to show the highest spatial variation.

A diverse and variable bird assemblage uses channels. The great majority of bird species that use channels, however, also use other wetland habitats (Table 1). At Estero de Punta Banda, for instance, all but one species (97.7%) found in channels were also observed in mudflat or within the bay (Table 3). However, in wetlands that lack other open-water habitats (e.g., Carpinteria Salt Marsh), waterfowl may occur exclusively in larger channels. Channels can be sampled by walking along the vegetated edge or by boat (a flat-bottomed boat or kayak is recommended). Waterfowl in channels are likely to flush before the observer detects them, violating one of the assumptions of distance sampling methodology (see sampling methods below).

Vegetated Marsh: Although the vegetated habitats of tidal wetlands in southern California lack the structural complexity of most vegetated terrestrial habitats, there is sufficient vertical stratification to complicate sampling of birds within them. The majority of species that are found in vegetated portions of these coastal wetlands also occur in other habitats. A few species, however, are found almost exclusively within wetlands vegetation (Table 4). These “marsh-specialist” species (rails and bitterns) are secretive and density estimates will be difficult to obtain without targeted sampling effort. Vegetated habitat in southern California coastal wetlands is often dominated by pickleweed (*Salicornia virginica*) but may also include Pacific cordgrass (*Spartina foliosa*) at low elevation. Differences in the height and structure of these vegetation types affect habitat use by wetland birds. Bird species assemblages, abundance and density are sufficiently different in cordgrass and pickleweed to warrant their treatment as distinct habitat types.

Pacific cordgrass is the preferred nesting habitat for the endangered (federal listing) light-footed clapper rail (*Rallus longirostris levipes*) in southern California. At Estero de

Punta Banda in Baja California, Mexico, clapper rails were found exclusively in cordgrass-dominated habitat year-round (K. Whitney, unpublished data). In marshes that lack cordgrass, clapper rails use vegetation with similar structure (such as *Juncus* sp. or *Scirpus* sp.) or pickleweed marsh. Clapper rails establish nesting and foraging territories in cordgrass marsh and therefore occur at low densities in this habitat throughout the year (Eddleman and Conway 1998). Non-breeding rails (sora, Virginia rail, black rail) that over-winter in coastal wetlands of southern California also use cordgrass marsh (K. Whitney, per obs.). In general, bird species richness and density are low in this habitat. Substrates in cordgrass vegetation are often muddy and sampling this habitat can be difficult.

Pickleweed typically covers a more extensive area of the vegetated marsh in southern California than does cordgrass. Pickleweed is used by breeding and over-wintering rails (including clapper rails in southern California) and is the primary nesting habitat for the endangered (state listing) Belding's savannah sparrow (*Passerculus sandwichesis beldingi*) (Powell 1993). Shorebirds also roost in pickleweed marsh when low elevation foraging habitat is inundated and some investigators prefer to conduct counts at roosts.

Review of Sampling Methods

Methods for sampling birds vary in the type and quality of data they produce. This variation is influenced by the habitat sampled and the biology of the birds that occupy those habitats. To satisfy the permit requirements for the SDL restoration project, statistically comparable estimates of bird species richness and density for each of the four wetlands (San Dieguito Lagoon, Tijuana Estuary, Mugu Lagoon, and Carpinteria Salt Marsh) are required. As described above, bird assemblages are sufficiently different among habitats within wetlands to warrant the use of habitat-stratified sampling for monitoring birds. The following section reviews methods for sampling birds that are commonly used and describes the efficacy of each method in different habitats. This information is used to assess the suitability of each method for the SDL restoration project.

Censusing: A census is assumed to be a complete count of individuals within the study area. Historically, censusing has been used extensively for counting shorebirds and waterfowl in tidal wetlands**.** Area effects are likely to confound species richness and density estimates obtained from census data (Figure 1). Therefore, counting birds by census is not recommended for monitoring birds for the SDL restoration project.

Index Sampling Methods: The great majority (>90%) of terrestrial bird studies are conducted using index methods. Index sampling methods use incomplete counts to estimate abundance with the assumption that abundance is correlated to the estimate obtained from sampling. If incomplete detection is likely, as is the case for sampling birds in vegetated habitat, index methods will not provide an accurate estimate of density unless the relationship between the index and the true population data has been determined prior to sampling. To accomplish this, concurrent sampling needs to be

conducted to calibrate the relationship between the index (a partial count) and the target parameter (Thompson et al. 1998).

The methods discussed below are types of fixed-area sampling. They are summarized in Thompson et al (1998) and Bibby et al. (1997). Each sample consists of a count of birds within a fixed-area quadrat (hereafter referred to as plot) within the study area. Specifying count duration standardizes effort. Counts obtained from replicate plots are used to estimate average bird density within the study area.

All birds in a sampling plot are likely to be detected when the entire plot is visible to the observer from a single observation point or transect line. Failure to detect all birds within the sampling plot decreases the accuracy of species richness and (especially) density data collected in this manner. The condition of complete detectability is reasonably satisfied in open habitats of coastal wetlands (mudflat and open-water embayments) and fixed area sampling is therefore most effective for obtaining density estimates in these habitats.

Point Counts: Point count methods are the most widespread (>46%) in quantitative studies of terrestrial bird distribution and abundance. Point count methodology is summarized in Ralph (1995) and Bibby et al. (1992). Sampling is conducted by a stationary observer in the center of a circular plot of specified radius. Bird species richness and density estimates are obtained with replicated sampling in the study area. Replication is achieved by randomly selecting points within the study area or along systematically placed line transects. The accuracy of estimates of both species richness and density is affected by variation in the detectability of birds that are present in the sampling plot, caused by variation in habitat attributes or behavior.

Point counts could be used to sample breeding savannah sparrows in pickleweed marsh for the SDL restoration project as the method is best suited for sampling vocalizing birds in vegetated habitat. However, strip or line transects (described below) may provide better density estimates. Point counts are not optimal for sampling birds in mudflats or embayments because of need to traverse muddy sediments and disturbance to the habitat (mudflats), and the potential for disturbing birds while traveling to the observation area. Circular plots are also not recommended in channels, as vegetated habitats are almost certain to be included in the sampling plot.

Strip transects: Strip transect sampling is a less-widely used technique for assessing bird populations (~29% of terrestrial bird studies). This method, however, can be effective in open habitats if birds do not move out of the sampling plot in response to the presence of an observer. Sampling is conducted by an observer moving along a linear transect counting only birds that are detected within a specified perpendicular distance from the transect line (Merikallio 1958). The sampling design consists of a number of randomly positioned lines or a grid of systematically placed lines superimposed on the study area. The linear plot design has certain advantages over either circular or square plots including higher detection probability, a function of decreased observer-bird distance within a plot of equal area (Thompson et al. 1998). However, narrow transect strips are required when detection attenuates sharply with distance (as in vegetated habitats) and

sampling efficiency is reduced in terms of the area of habitat surveyed per unit effort. Species identification is also improved at shorter observer-bird distance, an important consideration for sampling species that pose identification challenges such as *Calidris* sp. and other sandpipers. If bird species are not correctly identified, species richness estimates are likely to be inaccurate.

Strip transect sampling has been used for sampling shorebirds on mudflat (Yates et al. 1996; Colwell and Sundeen 2000)**,** for aerial sampling of shorebirds and waterfowl (Page 1997; Prenzlow and Lovvorn 1997), and for sampling birds in *Spartina alterniflora* (Melvin and Webb 1998) and in pickleweed marsh (Burnell 1993). The methodology is ideally suited for sampling large areas of mudflat. Transects should be aligned along the direction of the tidal flow, a modification that improves counting efficacy by sampling birds that forage at different portions of the habitat relative to the tide-line. Strip-transects are also appropriate for sampling open-water areas (especially by boat) but edge effects should be considered when choosing the appropriate transect dimensions (Figure 2). Strip transect sampling may be used to sample birds in channels and tidal creeks if the channel banks are used to define the transect boundaries. To obtain density estimates in channels, sampling area can be calculated using Geographic Information Systems (GIS) software and shape files created from geo-rectified aerial photographs or satellite images. In vegetated marsh, both habitat characteristics and bird behavior contribute to difficulty in detecting birds as discussed above and distance-sampling methods are preferable for obtaining density estimates.

Spot (territory) mapping: Spot mapping is currently a widely used technique for bird population studies. For example it is currently used for the Monterey, San Diego, and Los Angeles Breeding Bird Atlas projects. For this method, the study area is divided into a grid of (usually) square sampling plots and each plot is sampled. The methodology is summarized in Bibby et al. (1997). The assumption of accuracy in mapping is important for density calculations. The method is ideally suited for mapping nesting territories; therefore it could be used for sampling Belding's savannah sparrows or clapper rails during the breeding season. Of all available methods, this is the most time consuming and costly for obtaining density data.

Distance Sampling Methods: Distance sampling methods were developed in an attempt to improve estimates of density when complete detection of individuals within the sampling area is not possible. Theory, application, and methodology are summarized in Buckland et al. (2001). For distance sampling, the observer documents every bird that is detected and records the distance from each bird to a point or transect line. A detection function that addresses the attenuation of detectability with distance is used for calculating density from the sampling data. DISTANCE, a computer program for analyzing distance sampling data is available online. A user's manual is also available (Laake et al. 1993). Distance sampling is relatively new to ornithology and is currently used less frequently (< 10% of terrestrial bird studies) than index sampling methods but that is changing rapidly. The methodology is more complicated than that of index sampling (with an associated increase in sampling time and cost), and there are

conflicting reports regarding the effectiveness of distance sampling relative to traditional sampling methods.

Measurement error and observer bias in distance estimates are a potential confounding factor in this method. Chen (1998) reports that measurement errors during line-transect sampling cause systematic bias which cannot be reduced by increased sampling replication. Norvell et al. (2003) found that distance sampling methods (variable circular plots in this study) were robust to violation of the assumption of accurate estimation of distance even given a spatially complex and multi-species setting. However, the same paper reports that standard errors are likely to be high when sampling species that are rare or exhibit clumped distributions. DeSante (1986) found that variable circular plot sampling required substantially less time to complete than spot-mapping and provided accurate estimates of species richness and territory density when sampling breeding sub-alpine birds (Table 5). Laser range-finders can be used to improve distance estimation during sampling. Distances from observer to bird within a 20-100 meter range can be obtained using laser technology with an accuracy of plus or minus 1 meter (Buckland et al. 2001).

Two sampling approaches are used to collect data using distance sampling. The first approach, variable circular plot sampling, is conducted by a stationary observer from a point in a circular plot of infinite radius. The sampling design consists of a series of randomly positioned points or a grid of equally spaced points along line transects within the study area. The observer records all birds that are detected during a specified time interval and estimates the radial distance of each bird from the sampling point. Variable circular plots could be used for sampling birds in vegetated marsh. However, fewer individuals will be detected using stationary counts than by using linear transects under most circumstances (see strip transects above).

The second approach, line transect sampling, is conducted by an observer traveling along a transect line of specified length within a plot of infinite width. A number of randomly positioned lines or a grid of systematically spaced lines randomly is superimposed on the study area. As with point transect sampling, the observer records the linear distance from the transect line of each bird that is detected.

Line transect sampling provides a larger sample size per unit effort than strip transect sampling (Buckland et al. 2001) (Figure 3). This could improve density estimates for secretive species (such as rails) in vegetated habitats. Line transect sampling is recommended for sampling non-breeding birds in vegetated habitats for the SDL Restoration project. Although the method would be effective in other coastal wetland habitats, index methods should provide equally robust estimates in mudflat and embayments where birds are more easily detected and typically occur at greater densities.

Estimation of Species Richness from Sampling

Estimates of species richness based on counts obtained by sampling over limited time periods often underestimate species richness (Cam et al. 2002). Whitney (unpublished data) quantified sampling error in estimates of species richness during bird community

studies in Estero de Punta Banda, a 700-hectare coastal wetland in Baja California, Mexico. In this study, species richness was consistently underestimated by sampling 23 replicates 4 times each. Sampling error was high, 15% in winter and 23% in summer. Rare species figured prominently in the disparity between richness estimates (46% and 75% of species not encountered during sampling in summer and winter respectively). The number of new species recorded per unit effort tends to decrease with increased sampling replication. The shape of this species-accumulation curve is likely to vary among habitats.

Standardizing sampling conditions

A number of factors including weather conditions and disturbances affect bird activity and behavior and, by extrapolation, detectability. Sampling should not be conducted during periods when weather conditions affect either bird behavior or the visual acuity of the observer. In general, sampling should not be conducted under the following conditions: (1) precipitation or heavy fog, (2) winds exceeding 15 mph, and (3) temperatures below 40 F. Nor should sampling be conducted when disturbances affect the movement or behavior of birds. Disturbances include (but are not limited to) people, dogs, avian or mammalian predators, and vehicles.

Weather conditions should be evaluated and measurements taken at the beginning of each sample. The following conditions should be recorded: (1) precipitation, (2) temperature, (3) wind speed, (4) wind direction, and (5) percent cloud-cover. The observer should also record any potential disturbances in the area (object and number) and estimate the distance from the source of disturbance to the transect line or sampling point.

Recommendations

Habitat-stratified random sampling is recommended for sampling birds for the SDL Restoration project. Minimally, sampling should be done in mudflat and open water habitats. Vegetated marsh should also be sampled if information on clapper rail or Belding's savannah sparrow is required to fulfill permit obligations regarding endangered species (section 1.3 i in the SONGS permit). Sampling vegetated habitat is also necessary if the CCC specifically requires density estimates for vegetated marsh. If vegetated marsh is sampled, cordgrass and pickleweed marsh should be treated as separate habitats (see coastal wetland habitats section). Sampling of channels is not required for obtaining species richness estimates but may be necessary for reasons outlined in the "coastal wetland habitats" section above. Sampling channels is also necessary if the CCC requires density estimates in channels specifically. Habitats that occur in marshes targeted for sampling in SONGS post-restoration monitoring are shown in Table 6. Precision analysis using data from Estero de Punta Banda (Whitney, unpublished) and Mugu Lagoon (Keeney, unpublished) suggests that from 4 to 6 spatial replicates will adequately characterize bird densities in the restored and reference wetlands (Figures 6, 8).

Sampling Methods: Index methods are recommended as the most cost-effective means to obtain estimates of bird species richness and density in mudflats, embayments and

channels. Rectangular (strip transect) plots aligned along the tidal ebb are recommended for sampling mudflat. Rectangular plots with a relatively low height/length ratio are recommended for sampling birds in open-water habitat. Channel transects must be defined by the vegetated edge; transect area calculation will require GIS software and a trained technician. Georectified images of Carpinteria Salt Marsh (CSM) and Mugu Lagoon (MUG) are available. Wetland habitat shape files (mudflat, channel, marsh, and pan) are also available for CSM; these can be obtained through Dr. Armand Kuris at UCSB. If georectified images and shape files of (minimally) channel, cordgrass and pickleweed marsh do not exist for MUG, San Dieguito and Tijuana Estuary, production of these files will add considerable cost to sampling channels and vegetation in these wetlands.

Distance-sampling is suggested for sampling non-breeding birds in vegetated habitat as the method will likely increase detections per unit effort and improve density and possibly species richness estimates. During the breeding season, territorial birds in vegetated marsh are conspicuous (vocal) and index methods (strip or point transect methods) are sufficient for obtaining density estimates during the nesting period. Methods recommendations are summarized in Table 7.

Timing of sampling effort

Temporal heterogeneity in wetland bird community composition and abundance is driven primarily by season and tidal influence. The following sections address these temporal patterns with specific reference to habitat types targeted for sampling in the SDL bird monitoring project.

Seasonal Patterns

Bird communities in the coastal wetlands of southern California exhibit strong seasonal patterns driven by the movement of migratory birds (Figure 4). There are four distinct periods in the annual cycle of wetland birds: 1) breeding, 2) fall migration, 3) over-wintering, and 4) spring migration. These periods correspond roughly to the four seasons, but there is considerable overlap of the relatively prolonged migratory periods of spring and fall migration with the more defined summer and winter periods (Table 8).

Over-wintering: The over-wintering phase of the annual cycle is a period of relatively stable bird community composition as well as high species richness and abundance in coastal wetlands. Migratory shorebirds, waterfowl and seabirds are present and abundant at this time and this is the optimal time to sample these diverse and numerically-dominant members of coastal wetland bird communities in southern California. Sampling of wintering shorebirds and waterfowl should focus on mudflat and open-water habitat respectively. As fall migration overlaps considerably with the beginning of the over-wintering period, I define the sampling period conservatively as December 1 – February 10. Delaying winter sampling until December allows sufficient time for waterfowl to arrive and prevents the inclusion of migratory individuals in sampling. Bird species

present during the over-wintering period are a sub-set of those that occur during migratory periods.

Migration: Bird abundance, density, and species composition are most dynamic during migratory periods and variation in estimates of species richness and density obtained from sampling is likely to be high at this time (Figure 5). These patterns during migration are driven primarily by changes in shorebird (spring and fall) and seabird (fall) assemblages in southern California wetlands. Although all habitats are affected, the most dramatic effects are observed on mudflats and open-water habitats.

- Spring migration is temporally compressed, beginning in late February and ending in May. As a result, bird population densities and bird species turnover at wetland staging areas during the spring tend to be higher than those that occur in the fall (Recher 1966). The spring shorebird migration peaks in March and this is the optimal time to sample staging shorebirds in southern California.
- Fall migration begins in July with the first wave of returning seabirds and shorebirds and continues into November. The fall migration typically has distinct peaks in abundance as different species (and age classes) of birds move through wetland staging areas. Shorebird migration in southern California peaks in August and September.

Breeding: The period between spring and fall migration is the shortest in the annual cycle in southern California coastal wetlands. Between June 1 and July 10 (approximately), only locally-breeding species (and possibly some over-summering shorebirds) are present. Bird species richness and abundance and are lowest (in most habitats) at this time. Unlike the rest of year, the vegetated marsh is now the primary habitat for bird activity and therefore sampling. Resident breeding species begin to establish territories in mid to late March and this is the best time to sample them. The exception is California least tern which does not arrive in southern California until May and should not be sampled until June.

Tidal Cycles

Tides also influence bird distribution and abundance in wetlands. Tide height and stage determine the extent of available foraging habitat for shorebirds and other wetland birds and also affect the availability of prey items in intertidal habitats. Shorebirds, in particular, show strong, tide-driven patterns in distribution and abundance in estuarine habitats (Long and Ralph 2001). The following sections summarize the effects of tides on variability in bird assemblages in coastal wetland habitats and suggest habitat-based sampling strategies for monitoring birds.

Embayments: Unlike other habitats in tidal wetlands, embayments remain inundated throughout the tide cycle; therefore, tidal influence on habitat use by birds in open-water habitats is less marked. However, with rising tides, birds that utilize open water habitats disperse into channels and inundated portions of the vegetated marsh and sampling these birds becomes increasingly difficult.

Mudflat**:** In general, bird density on mudflats is inversely correlated with tide height**.** Shorebirds, the numerically-dominant component of mudflat avifauna, occupy mudflats for the entire time that they are exposed but densities were highest shortly after low tide in several wetlands on the coast of New Jersey (Burger 1977). However, Burger (1983) found that shorebird abundance and species richness was more strongly correlated to tide stage (low, incoming, high, outgoing) on mudflats in New York than tide height.

Vegetated Marsh: Shorebirds and waders relocate to higher elevation habitat in the vegetated marsh and upland habitats when mudflats and beaches are inundated. Rails and other secretive species of the vegetated marsh are visible during high spring tides as they perch on plants and debris.

Channels: Bird assemblages in channels vary with tide height and degree of inundation. Shorebird abundance at EPB was highest in channels at tide heights < 1 meter when channel banks were exposed for foraging. Wader abundance, however, increased at tide heights >1.4 meters. Waterfowl were found in channels at all tide heights (K. Whitney, unpublished data).

Time of day

For the majority of wetland birds, activity patterns are structured around the tide cycle and timing of sampling is necessarily structured around tides. Breeding species, however, are often most vocal (conspicuous) at specific times during the day. Therefore, time of day must be considered for sampling breeding birds in vegetated marsh. Savannah sparrows and other breeding passerines in vegetated marsh are most vocal in the early morning hours. Activity drops considerably as the temperature rises and these species become increasingly difficult to detect during sampling. Ideally, sampling should not continue past 10:30 AM. Clapper rails vocalize in the morning and in the early evening and can be sampled at either time.

All sampling of non-breeding birds should be conducted during periods of sufficient light to allow for visual detection and accurate identification of birds.

Recommendations

The biological performance standard for the SONGS permit specifies that bird density and number of bird species in the restored marsh at San Dieguito Lagoon be similar to those found in similar habitats in the three reference marshes. Although wetland functional requirements for birds are not specified, the existence of three distinct species assemblages during breeding, migratory staging and over-wintering is probably sufficient justification to recommend sampling during each of these periods.

Minimally, sampling should be conducted during the over-wintering period specified in Table 8. At this time, a majority of species that use the wetland at any time of year are present and both density and species composition are relatively stable, allowing for resasonable estimates of both parameters. Bird species that over-winter in southern California coastal wetlands are a subset of the species that use the wetland throughout the

year. Therefore, species richness estimates obtained from sampling during the over-wintering period will underestimate total species richness. Sixty four species of birds were observed during the seven years at Carpinteria Salt Marsh (CSM) for which monthly count data exists. Of these 64 species, 55% (35 species) were observed between February and November but not during December or January (the months recommended for winter sampling) for at least one of these seven years. These species include 13 migratory species (3 fall migrants, 5 spring migrants and 5 that are observed during both fall and spring migration), 5 transient species (those that occupy nearby open-water and beach habitats), 8 species that are rare in CSM (recorded 5 or fewer times during the 10-year survey period), 5 resident species (those that occur in CSM year-round) and 4 winter species (those that regularly over-winter in CSM) (Figure 4). Of these 35 species, 8 (12.5%) were never observed during December or January at CSM. Five of these eight species are rare at CSM (Figure 5). To obtain a more accurate estimate of bird species richness, sampling will need to be conducted during migratory periods as well. Sampling during either spring or fall migration will require greater replication to achieve reasonable estimates of density or species richness as wetland bird assemblages are dynamic during these periods. Sampling during migration is recommended if assessment of the staging function of the restored marsh at San Dieguito is desired. Precision analysis using data collected from Estero de Punta Banda (Whitney, unpublished) suggests that from 4 to 6 replicate samples taken every 2 weeks can adequately estimate bird densities in the restored and reference wetlands (Figure 7).

Breeding-season monitoring may be required for special-status species that currently breed at San Dieguito Lagoon to evaluate compliance with the permit standard that specifies no impact to endangered species. These species include light-footed clapper rail and Belding's savannah sparrow. Breeding season monitoring of California least terns may also be necessary to evaluate the success of habitat restoration efforts targeting this species and compliance with the restoration goal of providing habitat for rare and endangered species (1.4h). If breeding season monitoring is not required, sampling during the breeding season is not recommended to minimize impact to these species.

All bird sampling for the SDL restoration project must be standardized by tide cycle. This is critical for sampling mudflat for reasons detailed above. The following data should be recorded for each sample taken: (1) date, (2) time of start and completion of sampling transect or point (3) tide stage (high, low, incoming or outgoing), (4) tide cycle (neap, spring), and (5) tide height.

Mudflat sampling should be conducted only during falling and low tide. Transects should not be sampled until they are entirely exposed by the falling tide. Timing of sampling in open-water habitats is more flexible but this habitat should not be sampled when vegetated habitat is sufficiently inundated to allow waterfowl to move out of the embayment. This is primarily a function of tide height rather than cycle and is likely to vary among marshes.

Evaluating Food Chain Support

The SONGS permit specifies that food chain support provided by the restored marsh at San Dieguito Lagoon be similar to that of the reference marshes. Birds are to be used as a model system to evaluate compliance with this biological standard. Feeding activity of wetland birds can be quantified in two ways: (1) direct measurement of foraging attempts or of prey captured (2) indirect measurement of feeding activity.

Methods

Direct Methods: Direct measures of foraging activity are counts of predation attempts or successful predation events (or both). Foraging individuals are targeted and observed for a specified time period. Foraging activity is defined as the number of predation attempts or successful predation events recorded by the observer within the time period specified. Direct methods are best suited for species that stalk or dive for prey items and for species that consume larger prey so that outcomes of foraging attempts are readily identified by the observer.

Indirect Methods: Indirect methods are preferable when it is difficult to define or determine the outcome of a foraging attempt. Foraging activity is measured by determining the time an individual spends foraging in a plot of specified size. Foraging period is defined as the time that it takes a foraging bird to move from one side of the plot to the opposite side. The underlying assumption is that foraging birds spend more time in profitable foraging plots. Quammen (1982) used 25 m^2 plots placed at 0.3 m MLLW to sample foraging shorebirds in Upper Newport Bay. Indirect methods are best suited for species that feed continuously by probing, scything or plucking prey items. Individuals that cease feeding while within the plot should be excluded from analysis.

Focal Species Requirements

Measurement of food chain support provided to birds should focus on species that occur in all four marshes. Only birds that forage in open habitats (embayments or mudflat) during daylight hours are suitable. Candidate species for foraging observations for the SDL restoration project are listed in Table 9. Large waders, including great blue heron, great egret and snowy egret and shorebirds such as long-billed curlew, marbled godwit and willet can be sampled using direct methods. Indirect methods are preferable for smaller shorebirds including dowitchers and western or least sandpiper. It may be advisable to select a few target species for foraging observations to assess different elements of the wetland food web.

Timing of sampling

Season: Bird abundance in southern California wetlands is greatest between September and April and sampling should take place within this period. Seasonal patterns in foraging activity have been reported for shorebirds. Increases in foraging duration or feeding rates are associated with increased energetic demands of thermoregulation (Puttick 1984) or migration (Myers, Morrison et al. 1987). Therefore, sampling of foraging shorebirds should be conducted within defined periods (wintering, migratory, or both) as specified in Table 8.

Tide: Terns, herons and egrets (with the exception of the nocturnally-foraging black-crowned night heron) can be observed foraging at virtually any time in coastal wetlands. Terns will forage over open-water areas or large channels. Herons and egrets forage within vegetated marsh, along channels and at the edge of embayments. Shorebirds forage during virtually all daylight hours when mudflat is exposed (Puttick 1984). Most studies conclude that shorebird abundance on mudflats peaks at low tide and many species follow the outgoing tide (Evans 1979; Goss-Custard 1984). Foraging rates for some species have been shown to be higher on incoming than on outgoing tides (Puttick 1979). Sampling design should therefore specify tide conditions and specify the range of tide heights during which sampling shall be conducted.

Environmental Factors: Weather conditions affect foraging rates by affecting prey behavior and visibility. The following factors have all been shown to affect foraging efficiency in shorebirds: (1) rainfall (Goss-Custard 1970; Pienkowski 1981), (2) substrate temperature (Goss-Custard, Jenyon et al. 1977; Pienkowski 1980), (3) wind (Feare 1966; Baker 1974) and (4) light-levels (Dugan 1981; Hulscher 1982). Sampling methodology must therefore be standardized with regards to these environmental variables. Weather conditions should be recorded for each half hour during sampling (see Standardizing sampling conditions above). Sampling should not be conducted under the following conditions: (1) precipitation, (2) winds exceeding 12 mph, or (3) temperatures below 45 F.

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Table 1. Bird species of coastal wetlands in southern California including status, seasonal patterns, and habitat affinities.

							HABITAT				
Common Name	**Scientific Name**	**Guild**	**Special status species**	**Typical density in southern California coastal wetlands**	**Detectability**	**Southern California Occurrence**	**Vegetated Marsh**	**Mudflat**	**Channel**	**Bay**	**Upland Edge**
Red-throated Loon	*Gavia stellata*	seabird			High	Winter				x	
Pacific Loon	*Gavia pacifica*	seabird			High	Winter				x	
Common Loon	*Gavia immer*	seabird	CSC		High	Winter				x	
Horned Grebe	*Podiceps auritus*	waterfowl			High	Winter				x	
Eared Grebe	*Podiceps nigricollis*	waterfowl			High	Winter			x	x	
Pied-billed Grebe	*Podilymbus podiceps*	waterfowl			High	Resident			x	x	
Western Grebe	*Aechmophorus occidentalis*	waterfowl			High	Local			x	x	
Clark's Grebe	*Aechmophorus clarkii*	waterfowl			High	Local			x	x	
American White Pelican	*Pelecanus erythrorhynchos*	seabird	CSC		High	Winter		x		x	
California Brown Pelican	***Pelecanus occidentalis californicus***	seabird	**FE, SE**		High	Local		x		x	
Double-crested Cormorant	*Phalacrocorax auritus*	seabird	CSC		High	Local		x	x	x	
American Bittern	*Botaurus lentiginosus*	wader		Low	Low	Migrant					
Great Blue Heron	*Ardea herodias*	wader			High	Resident	x	x	x		x
Great Egret	*Ardea alba*	wader			High	Winter	x	x	x		x
Snowy Egret	*Egretta thula*	wader			High	Winter	x	x	x		
Cattle Egret	*Bubulcus ibis*	wader		Low	High	Winter	x	x	x		
Green Heron	*Butorides virescens*	wader			Low	Resident	x		x		
Black-crowned Night-Heron	*Nycticorax nycticorax*	wader			High	Resident	x	x	x		
White-faced Ibis	***Plegadis chihi***	wader	**FSC, CSC**		High	Migrant		x			
Canada Goose	*Branta canadensis*	waterfowl			High	Migrant				x	x
Brant	*Branta bernicla*	waterfowl			High	Migrant				x	
Mallard	*Anas platyrhynchos*	waterfowl			High	Resident			x	x	
Gadwall	*Anas strepera*	waterfowl			High	Winter			x	x	

Northern Pintail	*Anas acuta*	waterfowl			High	Winter			x	x	
American Widgeon	*Anas americana*	waterfowl			High	Winter			x	x	
Canvasback	*Athya valisnera*	waterfowl			High	Winter				x	
Northern Shoveler	*Anas clypeata*	waterfowl			High	Winter			x	x	
Cinnamon Teal	*Anas cyanoptera*	waterfowl			High	Local			x	x	
Blue-winged Teal	*Anas discors*	waterfowl			High	Winter			x	x	
Green-winged Teal	*Anas crecca*	waterfowl			High	Winter			x	x	
Surf Scoter	*Melanitta perspicillata*	waterfowl		High	High	Winter				x	
White-winged Scoter	*Melanitta fusca*	waterfowl		Rare	High	Winter				x	
Ring-necked Duck	*Athya collaris*	waterfowl		Moderate	High	Winter				x	
Greater Scaup	*Athya marila*	waterfowl		Low	High	Winter				x	
Lesser Scaup	*Athya affinis*	waterfowl		Moderate	High	Winter				x	
Redhead	*Athya americana*	waterfowl			High	Winter					
Common Goldeneye	*Bucephala clangula*	waterfowl		Rare	High	Winter				x	
Bufflehead	*Bucephala albeola*	waterfowl		High	High	Winter			x	x	
Red-breasted Merganser	*Mergus serrator*	waterfowl		Low	High	Winter				x	
Ruddy Duck	*Oxyura jamaicensis*	waterfowl			High	Local				x	
Turkey Vulture	*Cathartes aura*	vulture		Low-moderate	High	Local					x
Northern Harrier	*Circus cyaneus*	raptor	CSC	Low	High	Resident					x
White-tailed Kite	*Elanus leucurus*	raptor	CSC	Low	High	Local					x
Red-tailed Hawk	*Buteo jamaicensis*	raptor		Low	High	Local					x
Osprey	*Pandion haliaetus*	raptor	CSC	Low	High	Local	x		x	x	
American Peregrine Falcon	*Falco peregrinus anatum*	raptor	SE, CSC[FP]	Low	High	Winter	x	x		x	
American Kestrel	*Falco sparverius*	raptor		Low	High	Local					x
American Coot	*Fulica americana*	waterfowl		High	High	Resident			x	x	
Common Moorhen	*Gallinula chloropus*	waterfowl		Low	Low	Winter				x	
Virginia Rail	*Rallus limicola*	wader		Low	Low	Resident?	x				
Sora Rail	*Porzana carolina*	wader		Low	Low	Resident	x				
Light-footed Clapper Rail	***Rallus longirostris levipes***	wader	FE, SE, CSC[FP]	Low	Moderate#	Resident	x				
Black-bellied Plover	*Pluvialis squatarola*	shorebird		Moderate-High	High	Winter		x			
Western Snowy Plover	***Charadrius alexandrinus nivosus***	shorebird	FT, CSC[FP]	Rare-moderate	Moderate	Resident		x			
Semipalmated Plover	*Charadrius semipalmatus*	shorebird			Moderate	Winter		x			

Killdeer	*Charadrius vociferus*	shorebird			High	Resident		x			
American Avocet	*Recurvirostra americana*	shorebird			High	Resident		x			
Black-necked Stilt	*Himantopus mexicanus*	shorebird			High	Resident		x			
Greater Yellowlegs	*Tringa melanoleuca*	shorebird		Low	Moderate	Winter		x	x		
Lesser Yellowlegs	*Tringa flavipes*	shorebird		Rare	Moderate	Winter		x	x		
Willet	*Catoptrophorus semipalmatus*	shorebird			High	Winter	x	x	x		
Spotted Sandpiper	*Actitis macularia*	shorebird		Low	Moderate	Winter			x		
Long-billed Curlew	***Numenius americanus***	shorebird	CSC	Moderate	High	Winter	x	x	x		x
Whimbrel	*Numenius phaeopus*	shorebird		Low-moderate	High	Winter	x	x	x		
Marbled Godwit	*Limosa fedoa*	shorebird		High	High	Winter	x	x	x		
Ruddy Turnstone	*Arenaria interpres*	shorebird				Winter		x			
Black Turnstone	*Arenaria melanocephala*	shorebird				Winter		x			
Red Knot	*Calidris canutus*	shorebird				Winter		x			
Pectoral Sandpiper	*Calidris melanotos*	shorebird				Winter		x			
Dunlin	*Calidris alpina*	shorebird				Winter		x			
Sanderling	*Calidris alba*	shorebird		Moderate	High	Winter		x			
Least Sandpiper	*Calidris minutilla*	shorebird		High	High	Winter		x	x		
Western Sandpiper	*Calidris mauri*	shorebird		HIgh	High	Winter		x	x		
Short-billed Dowitcher	*Limnodromus griseus*	shorebird		High	High	Winter		x			
Long-billed Dowitcher	*Limnodromus scolopaceus*	shorebird		High	High	Winter		x			
Common Snipe	*Gallinago gallinago*	shorebird		Low	Low	Winter	x		x		
Wilson's Phalarope	*Phalaropus tricolor*	shorebird		Low	High	Migrant				x	
Red-necked Phalarope	*Phalaropus lobatus*	shorebird		High	High	Migrant				x	
Glaucous-winged Gull	*Larus glaucescens*	seabird			High	Winter		x		x	
Western Gull	*Larus occidentalis*	seabird			High	Local		x		x	
Herring Gull	*Larus argentatus*	seabird			High	Winter		x		x	
California Gull	*Larus californicus*	seabird			High	Winter		x		x	
Thayer's Gull	*Larus thayeri*	seabird			High	Winter		x		x	
Ring-billed Gull	*Larus delawarensis*	seabird			High	Winter		x		x	
Mew Gull	*Larus canus*	seabird			High	Winter		x		x	
Heermann's Gull	*Larus heermanni*	seabird			High	Winter		x		x	
Bonaparte's Gull	*Larus philadelphia*	seabird			High	Winter		x		x	

California Least Tern	***Sterna antillarum browni***	seabird	FE, SE, CSC[FP]	Low-moderate	Moderate	Summer		x	x	x	
Forster's Tern	*Sterna forsteri*	seabird		High	High	Winter		x	x	x	
Common Tern	*Sterna hirundo*	seabird		Low	Low+	Winter		x	x	x	
Elegant Tern	*Sterna elegans*	seabird	FSC, CSC			Winter		x	x	x	
Royal Tern	*Sterna maxima*	seabird			High	Winter		x	x	x	
Caspian Tern	*Sterna caspia*	seabird			High	Winter		x	x	x	
Black Skimmer	*Rhynchops niger*	seabird				Winter*		x		x	
Short-eared Owl	*Asio flammeus*	raptor	CSC	Low	Low	Migrant	x				
Western Burrowing Owl	*Athene cunicularia hypugea*	raptor	FSC, CSC	Low	Low	Migrant	x				x
Belted Kingfisher	*Ceryle alcyon*	kingfisher		Low	High	Resident	x	x	x		
Black Phoebe	*Sayornis nigricans*	songbird		Low	High	Local	x				x
Say's Phoebe	*Sayornis saya*	songbird		Low	High	Winter	x				x
Loggerhead Shrike	***Lanius ludovicianus***	songbird	FSC, CSC	Low	High	Resident	x				x
Common Raven	*Corvus corax*	songbird			High	Local	x	x			x
American Crow	*Corvus brachyrhynchos*	songbird			High	Local	x	x			x
American Pipit	*Anthus rubescens*	songbird			Low	Migrant	x				x
Common Yellowthroat	*Geothlypis trichas*	songbird			High	Resident					x
Belding's Savannah Sparrow	***Passerculus sandwichensis beldingi***	songbird	FSC, SE	Moderate	Moderate#	Resident	x				
Song Sparrow	*Melospiza melodia*	songbird			High	Resident					x
Western Meadowlark	*Sturnella neglecta*	songbird			High#	Resident	x				x
Bird Species Richness							27	51	39	53	19

Status: FE (Federal Endangered), SE (State Endangered), FT (Federal Threatened), FSC (Federal Species of Special Concern), CSC[FP] (California Species of Special Concern: Fully Protected), CSC (California Species of Special Concern)

Occurrence: Resident = at least some members of population live year-round and breed in marsh habitats or adjacent upland; Local = breeds in different habitat but use of marsh for foraging may be year-round; Winter = overwinters only, breeds elsewhere and rare or absent during breeding months; Migrant = present during migration sometimes in great numbers but does not breed or over-winter

Table 2. Birds with protected status (state or federal) that occur in San Dieguito Lagoon or reference marshes for the SDL Restoration Project. Status and habitat affiliations are included.

		Status				**Habitat**					**Occurrence**			
Common Name	**Scientific Name**	Federal Endangered	Federal Threatened	State Endangered	State Threatened	embayment	channel	mudflat	cordgrass	pickleweed	Carpinteria Salt Marsh	Mugu Lagoon	San Dieguito Lagoon	Tijuana Estuary
California brown pelican	*Pelecanus occidentalis*	x		x		x	x	x			x	x	x	x
Light-footed clapper rail	*Rallus longirostris levipes*	x		x					x	x	r	b	b	b
Western snowy plover	*Charadrius alexandrinius nivosus*		x					x			**r**	**b**		**b**
California least tern	*Sterna antillarum browni*	x		x		x	x	x				b		b
Belding's savannah sparrow	*Passerculus sandwichensis beldingi*			*x*						x	b	b	b	b

b = currently breeding in this marsh (2004)
r = rarely reported from this marsh
x = currently occurs in this marsh (non-wetland breeding species)
b or **r** = San Dieguito Lagoon is included in the USFWS Critical Habitat designation for this species

Table 3. Bird species recorded from channels in Estero de Punta Banda, Baja, California, Mexico in 2002. Only one of these species was not encountered in other habitats.

	Channel		Other Habitat	
Species	Water	Edge	mudflat	embayments
American Pippit		**x**		
American Wigeon	x			x
Black-bellied Plover		x	x	
Black-crowned Night Heron	x	x	x	
Black Skimmer	x		x	x
Bufflehead	x			x
Caspian Tern	x		x	x
Cinnamon Teal	x	x		x
Clapper Rail		x		
Dowitcher sp.		x	x	
Eared Grebe	x			x
Forster's Tern	x		x	x
Great-Blue Heron	x	x	x	
Great Egret	x	x	x	
Greater Yellowlegs		x	x	
Green-winged Teal	x		x	x
Least Sandpiper		x	x	
Lesser Scaup	x			x
Long-billed Curlew	x	x	x	
Least Tern	x		x	x
Mallard	x	x		x
Marbled Godwit		x	x	
Osprey	x			x
Reddish Egret*		x	x	
Red-breasted Merganser	x			x
Ring-billed Gull	x		x	x
Savannah Sparrow		x		
Snowy Egret	x	x	x	
Sora		x		
Tri-colored Heron*		x	x	
Western Grebe	x			x
Western Gull	x		x	x
Whimbrel	x	x	x	
Willet		x	x	

Table 4. Habitat use at by birds at Estero de Punta Banda. Three of 16 species appeared to be habitat specialists and were not encountered in other available habitats.

	Vegetation		Other habitats	
Species	Cordgrass	Picklweed	Mudflat	Channel
American Bittern	**x**			
Black-bellied Plover		x	x	
Black-crowned Night Heron	x	x	x	x
Clapper Rail	**x**			x
Great-Blue Heron	x	x	x	x
Great Egret	x	x	x	x
Long-billed Curlew	x	x	x	x
Marbled Godwit	x	x	x	x
Northern Harrier		x		
Osprey		x		x
Reddish Egret*	x		x	x
Savannah Sparrow		x		x
Snowy Egret	x	x	x	x
Sora	**x**			
Whimbrel		x	x	x
Willet	x	x	x	x

* This species is at the northern limit of its Pacific coast range at EPB

Table 5. Field test of the variable circular plot sampling methods from DeSante (1986). "Actual" values determined by intensive spot-mapping. Negative values indicate underestimation. Census period 1VCP data from 12 stations each sampled 12 times. Census period 2 VCP data from 12 stations each sampled 4 times.

Parameter	"Actual"	VCP	% error
Census Period #1			
Total density of territories	135.8	126.9	-6.5
(48 hectares)	19	19	0
Species richness	8.39	7.8	-7
Species diversity			
Census period #2			
Total density of territories	132.1	110.1	-16.7
(48 hectares)	19	19*	0
Species richness	9.03	8.04	-11
Species diversity			

Table 6. Wetland habitats in marshes being sampled for bird monitoring in the SDL Restoration Project.

	Wetland			
Habitat	Carpinteria Salt Marsh	Mugu Lagoon	San Dieguito Lagoon	Tijuana Estuary
Spartina Marsh		x	x	x
Salicornia Marsh	x	x	x	x
Channels	x	x	x	x
Mudflat	x	x	x	x
Open Water		x	x	x

Table 7. Summary of sampling methods and suitability for use in coastal wetland habitats.

	Census*	Index Methods			Distance Sampling Methods	
		Spot Mapping	Point Count	Strip Transect	Line Transect	Variable Circular Plot
Variable						
Species Richness	yes	no	yes	yes	yes	yes
Abundance	yes	no	yes	yes	yes	yes
Density	yes	no	yes	yes	yes	yes
Spatial distribution in plot	no	yes	no	no	yes	yes
Logistics						
Relative Cost per Data Point	Expensive	Expensive	Moderate	Moderate	Expensive	Expensive
Observer Error Potential	Low	Low	Low	Low	Moderate	Moderate
Suitability of Method						
Embayment	Low	Low	Moderate	High	High	Moderate
Mudflat	Low	Low	Moderate	High	High	Moderate
Channel	Low	Low	Moderate	Highest	Low	Low
Cordgrass Marsh	Low	Low	Moderate	High	Highest	Moderate
Picklweed Marsh	Low	Low	Moderate	High	Highest	Moderate
Savannah Sparrow	Low	High	Moderate	High	High	Moderate
Clapper Rail	Low	High	Moderate	High	High	Moderate

Table 8. Phenology of wetland bird communities.

Season	Period	Duration	Bird Community Dynamics
summer	breeding	June 1 - July 10	stable
summer/fall	post-breeding migration	July 11 - November 30	unstable
winter	over-wintering	December 1 - February 14	stable
spring	breeding migration	February 15 - May 30	unstable

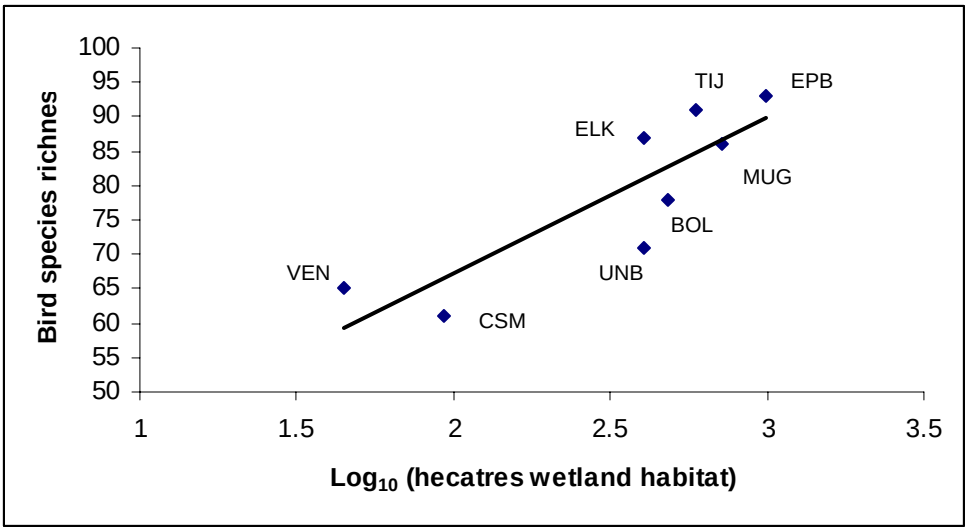


Figure 1. Species area relationship in some coastal wetlands of California and Baja California, Mexico (r^2 = .74). BOL=Bolsa Chica Lagoon, CSM = Carpinteria Salt Marsh, ELK = Elkhorn Slough, EPB=Estero de Punta Banda, MUG = Mugu Lagoon, TIJ = Tijuana Slough, UNB = Upper Newport Bay, VEN = Ventura River Estuary.

Note: Wetlands were chosen based on availability of accurate information on wetland habitat area and the existence of an updated bird species list that included the species status. Rare and accidental species were excluded from each species list to prevent artificial inflation of species richness as a consequence of observational bias (a result of the well-known propensity of bird watchers to seek out rare species in certain birding "hot-spots").

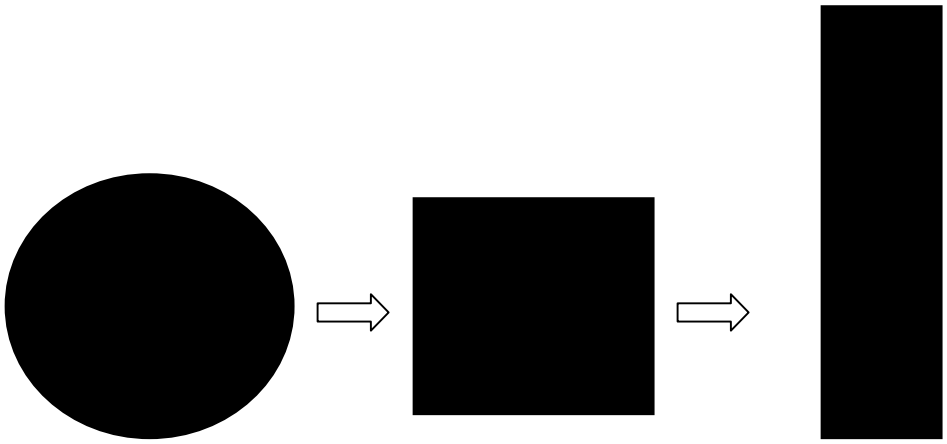


Figure 2. These three plot shapes contain the same area but differing perimeter to area ratios which is a measure of edge effect. The circular plot has a ratio of 2.36:1, the square plot 2.67:1 and the strip plot 3.33:1. A lower ratio signifies a lower potential for edge effect (reproduced from Thompson et al. 1998).

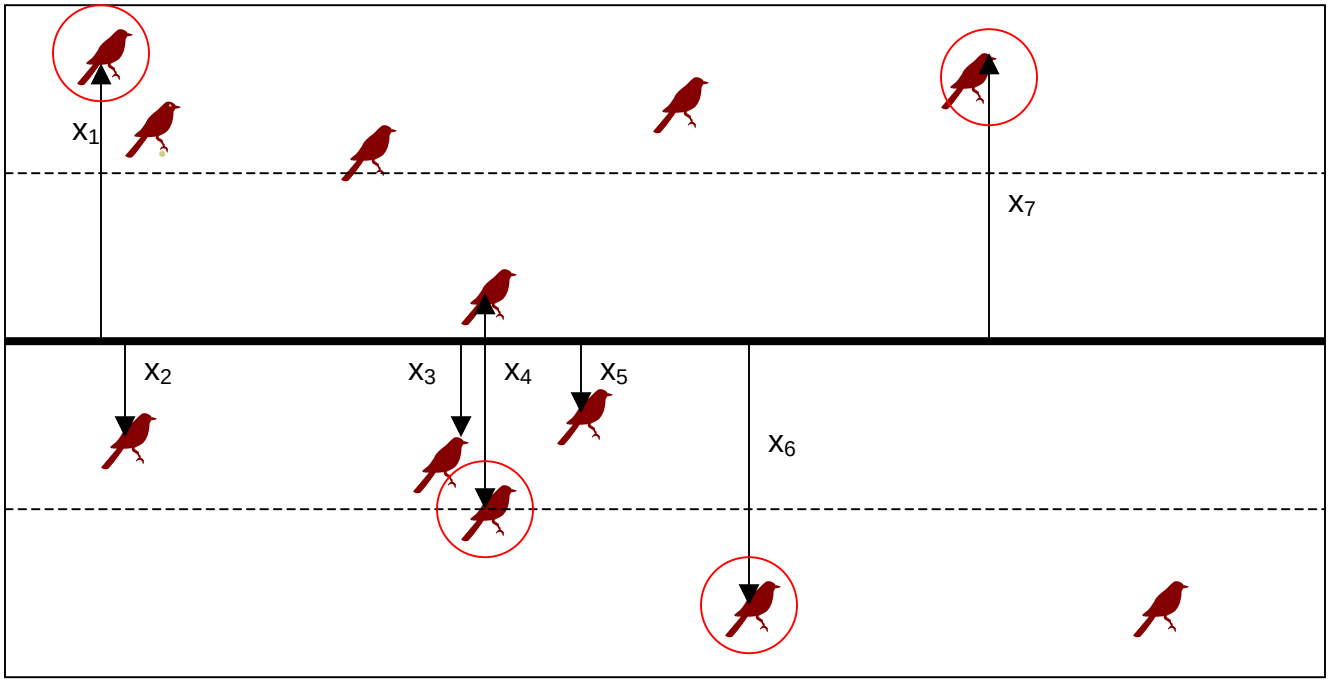
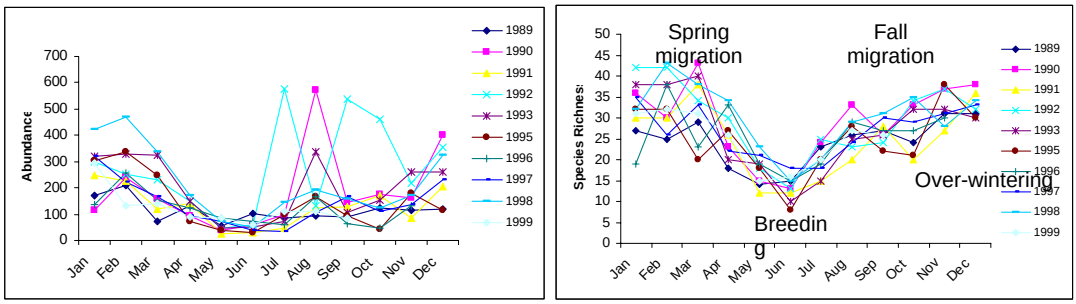
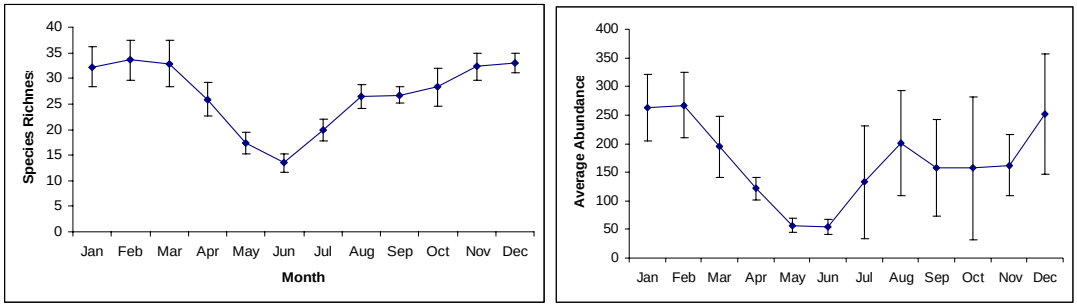


Figure 3. Linear transect sampling (distance sampling). Each bird that was detected by the observer is recorded as a point at an estimated distance (x_1, x_2, x_3…….x_n) from the transect line. Note that birds circled with red would not be included in a strip transect survey with plot dimensions specified by the dotted line.



Abundance **Species Richness**

Figure 4. Seasonal dynamics in patterns of bird abundance and species richness; data from monthly surveys at Carpinteria Salt Marsh, Carpinteria California (1989-1999). Carpinteria Salt Marsh surveys by Bob Hansen, Santa Barbara Audubon Society.



Species Richness **Abundance**

Figure 5. Monthly variation in bird species richness and abundance at Carpinteria Salt Marsh from surveys conducted between 1988-1999. Mean and 95% confidence interval are shown for each month.

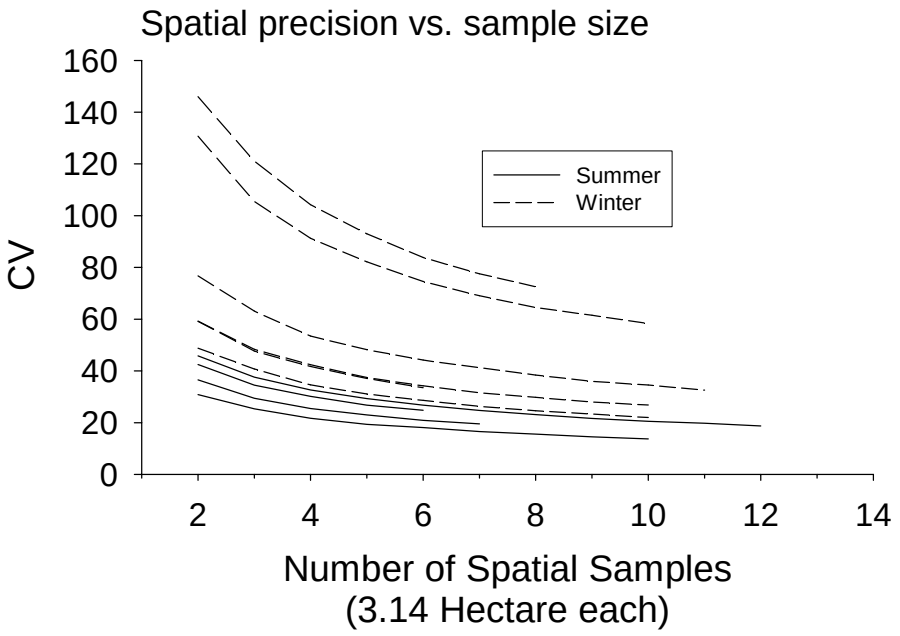


Figure 6. Spatial precision versus sample size for bird density from Estero de Punta Banda (data from Whitney, unpublished).

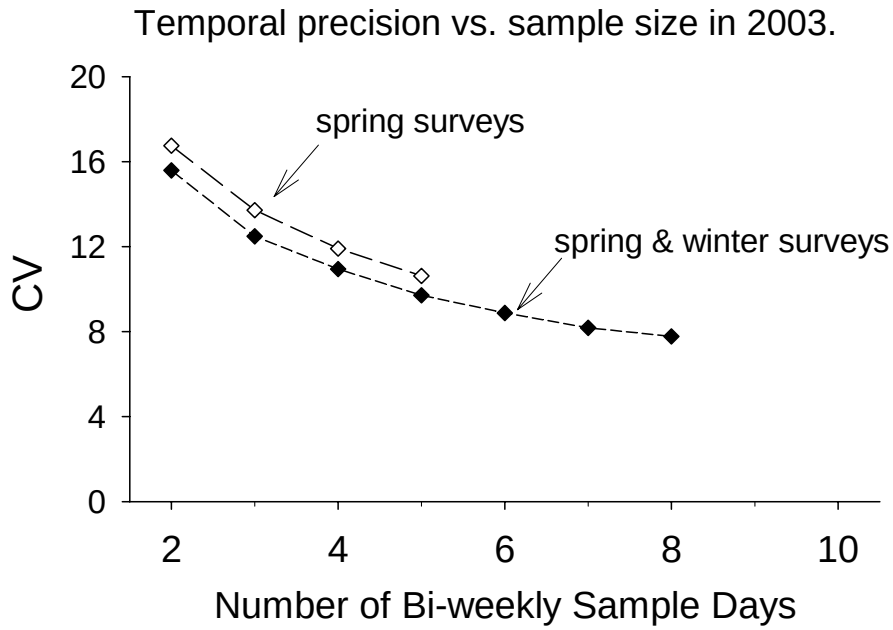


Figure 7. Temporal precision versus sample size for bird density from Estero de Punta Banda (data from Whitney, unpublished).

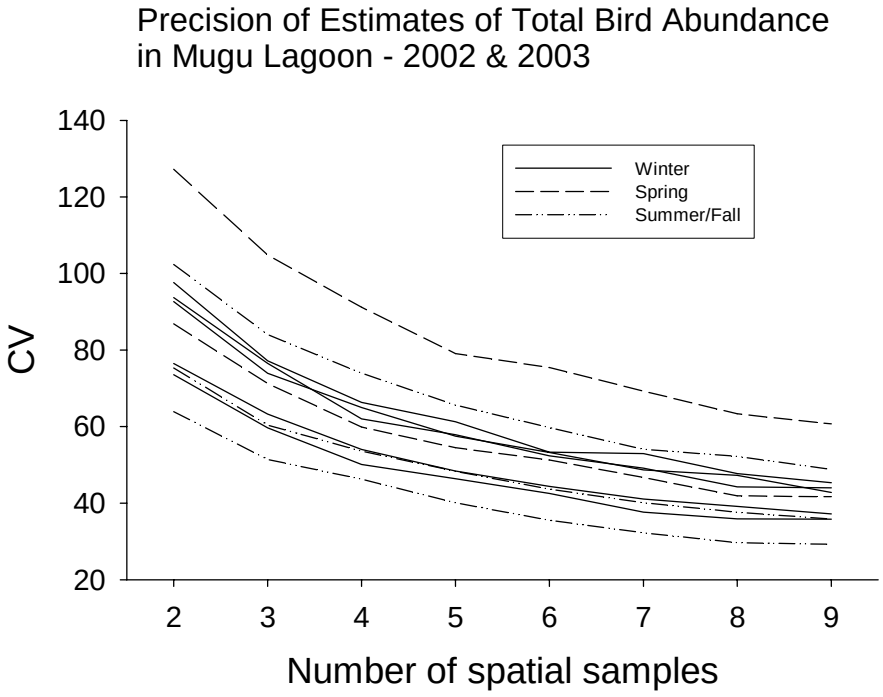


Figure 8. Spatial precision versus sample size for bird density from Mugu Lagoon (data from Keeney, unpublished).

Appendix 7

Estimating areas vegetative cover, open space, and selected wetland habitats southern California wetlands using multi-spectral aerial imagery

Steve Schroeter, Mark Page, Mark Steele, and Dan Reed

Marine Science Institute
University of California
Santa Barbara, CA 93106

1.0. INTRODUCTION

The coastal development permit for SONGS Units 2 and 3 requires Southern California Edison to create or substantially restore a minimum of 150 acres of coastal wetland. The permit states that upon completion of construction of the wetland monitoring will be conducted to measure the success of the wetland in meeting physical and biological performance standards. Here, we address methods for gathering data to address two performance standards: 1) the proportion tidally influenced area containing salt marsh vascular plants and open space and changes in these proportions over time, 2) the wetland-wide percent cover of algae, and 3) changes in habitat areas (e.g. channels, tidal creeks, open water, mud flats), which include non-vegetated as well as vegetated tidally influenced habitat. (SONGS 1997).

Typically, estimates of vegetative cover are made using quadrat or transect methods where the spatial scope of individual samples generally ranges from less than 1 m^2 to perhaps as large as 100's of m^2 (refs). Sufficiently accurate wetland-wide estimates using these methods rely on random or stratified random sampling designs, which require replication at levels are potentially laborious expensive. We have attempted to solve this logistical problem by developing synoptic estimates of vegetative cover and bare space using multi-spectral aerial images. The usefulness of this technique depends critically on verification of the accuracy of the aerial estimates with ground-based samples (i.e. ground-truthing).

2.0. METHODS

2.1. Estimates of cover from aerial imagery.

Data Acquisition. Image data are acquired using Ocean Imaging's (OI's) DMSC-MkII 4-channel aerial sensor made by SpecTerra Ltd., Australia. The DMSC's channels are fitted with 10nm bandwidth interference filters centered at 451, 551, 600 and 780nm. This wavelength combination was determined to be highly effective for plant type discrimination over marshes in the Southern California area during previous test flights. The imagery was acquired within 1 hour of solar noon to minimize shadow effects. Flight altitude was 1800', resulting in data spatial resolution of 26cm.

Data Processing. The image data are band-to-band realigned using SpecTerra's post-processing software. Although the DMSC is integrated with a DGPS (Differential Global Positioning System) unit which supplies each image frame with its center location, at sub-meter resolutions the data have to be manually re-referenced for best accuracy. This was done using SANDAG CIR imagery collected in 1998 (for areas west of I-5) and 2000 (for areas east of I-5) as a base layer. Some difficulties were encountered in this process, mostly in areas east of I-5 where the base 2000 data, were considerably different from the 2003 imagery. The final mosaic accuracy is estimated at RMS (root mean

square) of 1.25m. Prior to creating the final mosaic some of the image frames were radiometrically corrected to minimize atmospheric haze effects.

The 4-channel mosaic was classified for ground-cover type using TNT-Mips software. A 30-class unsupervised classification was generated and field-checked to determine which vegetation types. The ground-truth information was compiled and the classes were merged where necessary. It became evident that several areas in the lagoon demonstrated significant vegetation type overlaps between specific classes, i.e. the same class represented a different vegetation type in each region. For this reason, some areas of the lagoon were classified separately and merged into the final product. Although initial classification tests and field verifications showed the DMSC data contain sufficient spectral differences to separate, with good accuracy, the native marsh species (*Arthrocnemum*, *Frankenia, Salicornia*, etc.), significant class overlap existed in numerous parts of the lagoon. Therefore, the native marsh species were combined in to one "Salt Marsh Native" class. Other species that retained sufficient spectral uniqueness to allow accurate classification were kept as separate classes.

The resulting class raster was spot field-checked for accuracy and manually corrected in a few areas where discrepancies were found. The final class raster was exported out of the TNT-Mips software into an ArcGIS compatible (TIF format). The exported TIF file was converted to an ArcView shapefile using the Spatial Analyst extension in ArcGIS 8.3. The resulting shapefile was attributed to identify polygon classes and the areas of each of the polygons, allowing calculation of total area of each class (e.g. vegetative cover, bare space, open water) over an entire wetland.

The final DMSC imagery raster was exported out of TNT-Mips in two formats, both ArcGIS compatible. The TIF format has a set "true color" RGB enhancement, set in TNT-Mips, to allow for easier viewing in ArcGIS. The IMG format is the full 4-banded imagery without any set enhancements.

2.2. Estimates Of Cover From Ground-Based Methods

Cover of the various classes (e.g. salt marsh natives, bare space) were estimated on August 21, nine days after the aerial images were acquired, using line intercepts sites chosen from the aerial images for which there were identifiable landmarks to insure exact correspondence between the imaged areas and the ground-truth samples (Figure 1). Landmarks included natural features such as distinctive patches of bare space or clumps of bushes or 30 cm diameter orange bucket lids that were placed in the field prior to the overflight). At each site a transect was extended between points identifiable on the aerial photograph. Two additional transects were then laid out in parallel one meter on either side of the original transect. With the aid of the three transects and meter sticks, the identity of cover type (e.g. bare space, algae, native or non-native vascular plants) was noted on 50 cm x 50 cm grid-points throughout the area encompassed by the three transects, yielding 15 point estimates every 2 m wide x 1 m long segment of the area sampled (Figure 2). Data on layering were also be taken at each point, however

comparisons between field and aerial data were made using only the top-most layer sampled in the field. Plants and algae were identified to species, but were placed into categories corresponding to those used to classify for the aerial images (Table 1).

An additional experiment was done to detectable patch size of bare space by clearing three square patches measuring 30, 61, and 100 cm on a side in a dense combined stand of Jaumea carnosa and Distichlis spicata.

3.0 ANALYSIS

Estimates of area for the different classes ground cover present at each of the ground-truth sites based on analysis of aerial imagery and ground-based transects were compared using a chi-square test.

Images were inspected to see the size of the cleared patch that could be detected.

4.0 RESULTS

The comparisons between aerial and ground-based estimates of cover were made for a total of four classes: bare space, salt marsh native plants, Salt marsh invasive plants, and dead vegetation. The differences in estimates of bare space and salt marsh invasives between methods were on the order of 1% and there was no consistent pattern in the sign of the differences. The largest differences between methods occurred for the estimates of dead vegetation, which ranged from .1% to about 13%, with estimates based on aerial imagery consistently higher that those based transect estimates. The differences between methods for estimates of salt marsh natives were similar to those for dead vegetation, but the aerial estimates were consistently lower than those based on the transects (Table 1). Despite these differences, there was close correspondence between estimates of percent cover made from analysis of multi-spectral images and from ground-based transects, which was reflected in Chi-square analyses at each of the four ground-truth transects (Table 1).

5.0 DISCUSSION

The work summarized in this paper indicates that there is close agreement between estimates of areas of bare space and vegetative cover from the analysis of multi-spectral aerial images and ground-based estimates of cover using a 50 cm x 50 cm grid of points. Given this agreement, whole-wetland estimates of variates of interest derived from analysis of multi-spectral images will be used to compare the restoration at San Dieguito to values in the reference wetlands. These variates include: 1) the percent cover of native vegetation in tidally influenced habitats, 2) the area of tidally influenced habitat covered by native vegetation, 3) the area of bare space in tidally influenced habitat, 4) the percent cover of algal mats in tidally influenced habitats, 4) the area of algal mats in tidally influenced habitats, 5) the area of open water, 6) the area of tidal creeks, 7) the area of

main channels, and 8) the area of mud flats in tidally influenced habitat. Tidally influenced habitats are defined as those that occur at or below the tidal height of 4.5' NGVD (= 4.31' above mean sea level).

The answer will depend on the variate being measured. For certain variates the permit has fixed standards (e.g. restored habitat areas are specified and can vary by no more that 10%); for others the standards are relative (e.g. the total cover of vegetation) and compliance requires that they remain within the range of values at the reference wetlands. Any values of vegetated areas or bare space (II. A. Salt Marsh Vascular Plants) in the restored wetland that were outside the range of the reference wetlands (whether higher or lower) by more than 10% would represent non-compliance. This is because if the coverage (and area) of vegetation were above the range of the reference wetlands by more than 10%, then the coverage of the coverage of bare space would necessarily be below the range (and vice versa). It could be argued that for algae, values that were either above or below the range of the reference wetlands would also would also represent non-compliance, since algal mats can be beneficial at moderate abundances but are indicative of impaired wetland function at high abundances (Peters et al. 1985). For exotic species, values within or below the range would represent compliance and values above the range would represent non-compliance, assuming that such high values represented or were correlated with significant impairment of wetland function. For *Spartina* architecture, only those values below the range of the reference wetlands would represent non-compliance, since the standard is based on suitability of habitat for light-footed clapper rails (*Rallus longirostris levipes*), and values above the range would likely represent habitat enhancement.

The calibration shows close agreement between estimates of percent cover from aerial images and intensive ground-based methods, and in particular shows that the aerial images can distinguish between wetland and upland plant species and thus between wetland and upland habitats. This means that the aerial imaging can accurately measure areas of and temporal changes in restored habitats within the tolerances specified by the permit. The images can also aid in identifying topographical changes and in targeting ground-based estimates of such changes that are detected.

Recommendations:

1. **Re-analyze ground truth and aerial with re-sampling**
2. **Re-do ground-truthing, picking sites that are topographically more complex and that have a more even distribution of classes**

3. **Include transects on mudflats to ground-truth algal cover**

6.0 TABLES.

Table 1. Classification of categories for which areas and percent cover were estimated

Species or cover category	Class
Algae	Algae
Arthrocnemum subterminalis	Salt Marsh Native
Atriplex triangularis	Salt Marsh Native
Bare (Artificial)	Bare
Bare < 4.5 ft.	Bare
Bare > 4.5 to 6 ft.	Bare
Bare > 6 ft.	Bare
Bromus spp.	Invasive
Dead Plants / Dead Trees	Dead Vegetatioin
Distichlis spicata	Salt Marsh Native
Frankenia grandifolia	Salt Marsh Native
Golden Bush	Upland Native
Grass	Invasive
Ice Plant (*Carpobrotus spp.)*	Invasive
Jaumea carnosa	Salt Marsh Native
Juncus acutus	Salt Marsh Native
Mesembryanthemum crystalinum	Invasive
Mustard (*Brassica nigra*)	Invasive
Salicornia virginica	Salt Marsh Native
Tamarisk	Invasive
Trees	Invasive

Table 2. Comparison of cover estimates using analysis of multi-spectral digital images (aerial) and transect-based ground truth surveys (Ground-truth).

		% Cover Estimate		Chi-square	
Site	Category	Aerial	Ground-truth	Value	Prob.
Transect 1	Bare	0.14%	0.30%	0.92	0.63
Transect 1	Dead Vegetation	0.13%	0.00%		
Transect 1	Salt Marsh Natives	99.73%	99.70%		
Transect 2	Bare	29.42%	30.20%	0.84	0.66
Transect 2	Dead Vegetation	19.86%	6.00%		
Transect 2	Salt Marsh Natives	50.72%	63.80%		
Transect 3	Bare	0.00%	0.50%	0.94	0.63
Transect 3	Dead Vegetation	17.09%	7.80%		
Transect 3	Salt Marsh Natives	82.62%	91.70%		
Transect 4	Bare	1.45%	0.20%	0.78	0.99
Transect 4	Dead Vegetation	0.71%	< 0.01%		
Transect 4	Salt Marsh Natives	97.10%	99.60%		
Transect 4	Invasive species	0.74%	< 0.01%		

7.0 FIGURES

Figure 1. Map of ground-truth sites sampled in August 21, 2003.

Figure 1. Schematic map of ground-truth sampling grid at transect 2, August 21,2003. Orange line defines sample area (band transect).

Figure 3. Classification of cover types using multi-spectral images at San Dieguito wetland on August 21, 2003.

Figure 4. Image showing cleared patches in Jaumea / Distichlis matrix. 100 cm x 100 cm and 60 cm x 60 cm clearings can be seen as can a 30 cm diameter orange plastic bucket lid.

Figure 1. Numbers indicate transects referenced in Table 2.

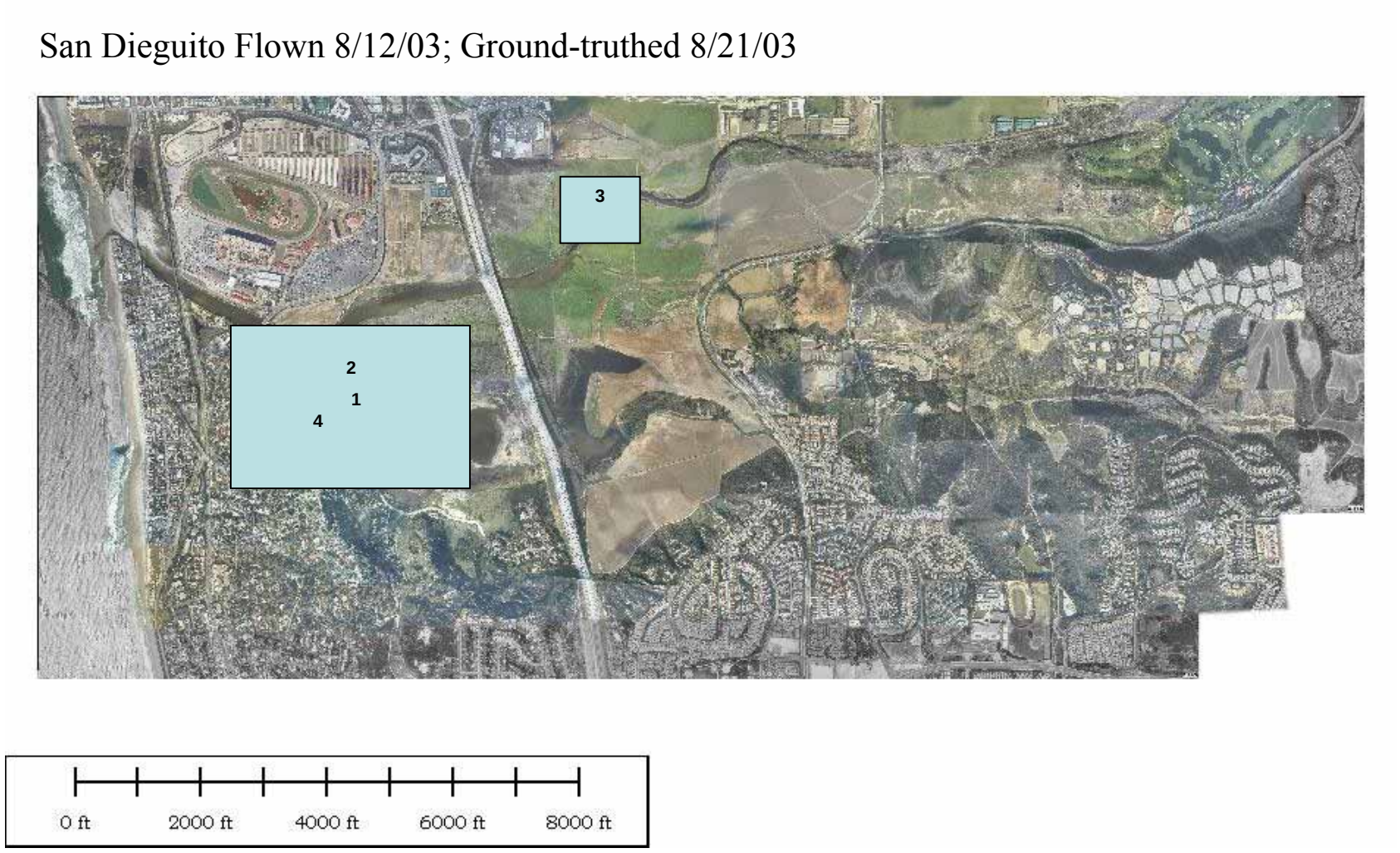


Figure 2.

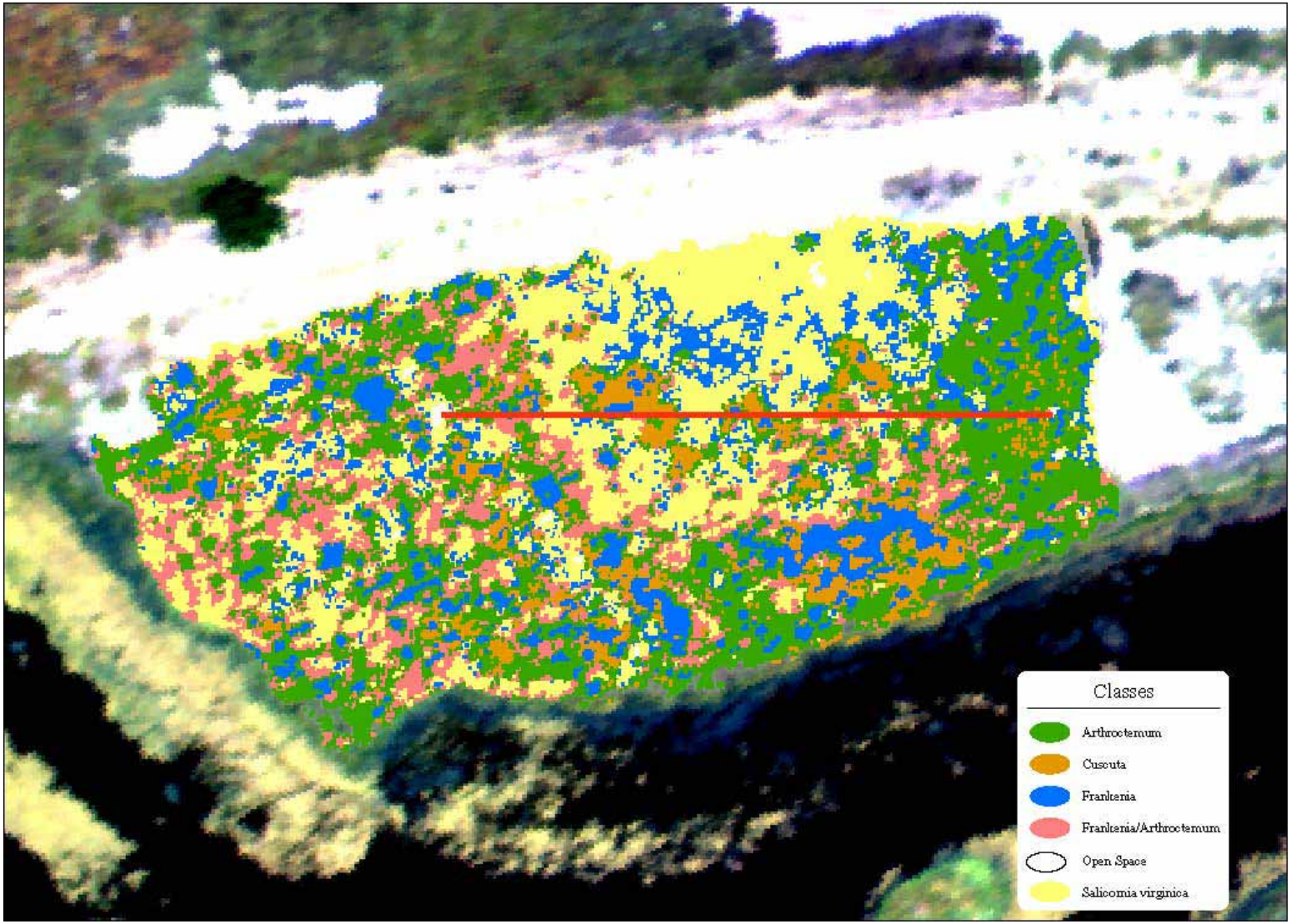

Classes
Arthrocternum
Cuscuta
Frankenia
Frankenia/Arthrocternum
Open Space
Salicornia virginica

Figure 3. Key: live plants wetland plants = green; dead plants = brown; bare space = white; missing data = gray.

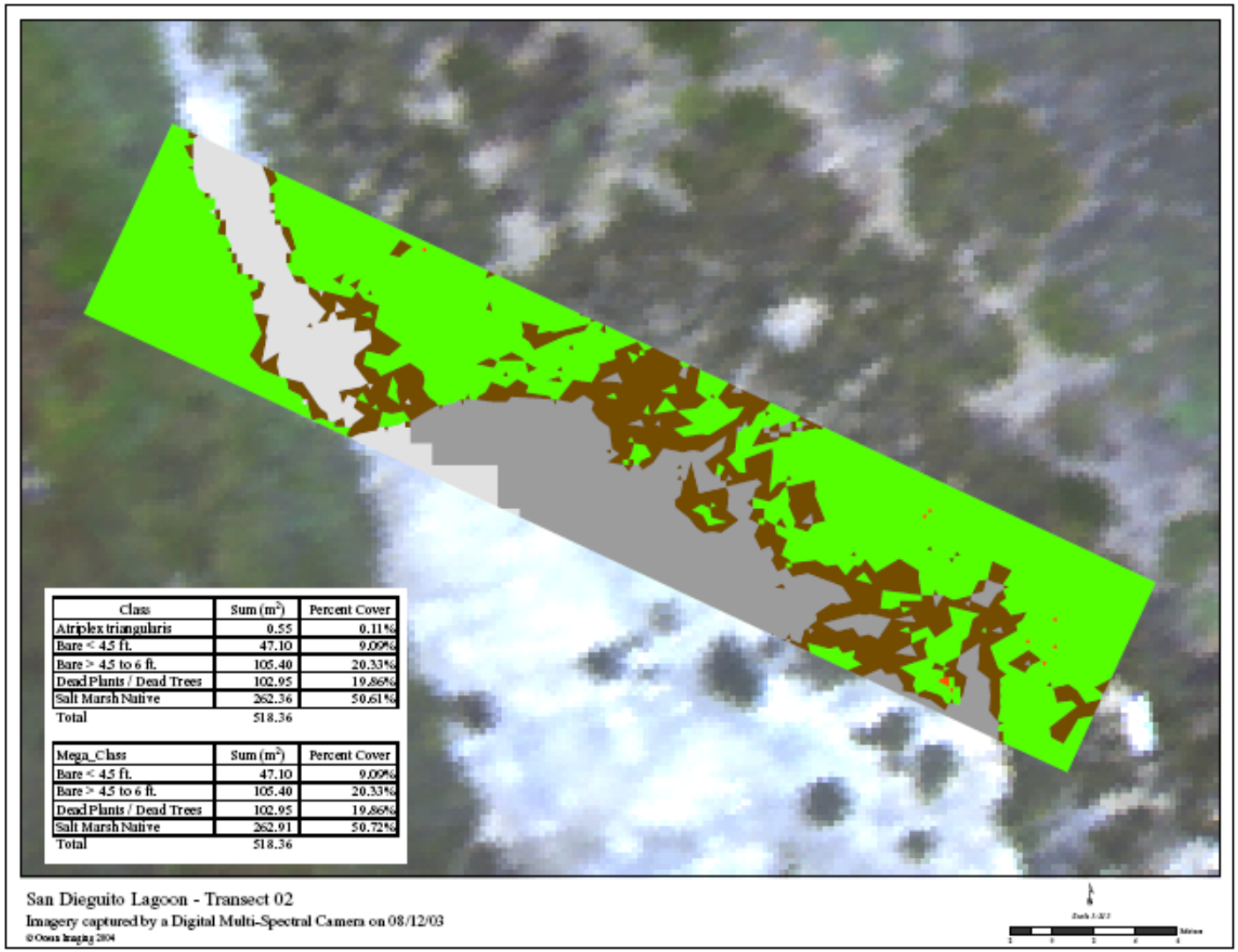


Figure 4.

8.0 REFERENCES

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Appendix 8

Exotic species reported from southern California that could become established in San Dieguito Lagoon. Species in boldface are already present.

Plants	
Acacia longifolia	Acacia
Anethum graveolens	Wild anise
Arundo donax	Giant reed
Atriplex semibaccata	Australian salt bush
Bassia hyssopifolia	Bassia
Beta vulgris	Wild beet
Brassica nigra	Black mustard
Bromis mollis	Soft chess
Bromus diandrus	Ripgut grass
Carpobrotus edulis	Iceplant
Centaurea melitensis	Star thistle
Chenopodium ambrosioides	Mexican tea
Chrysanthenum carinatum	Tricolor chrysanthemum
Conium maculatum	Hemlock
Cortaderia selloana	Pampusgrass
Cotula coronopifolia	Brass buttons
Datura stramonium	Jimson weed
Ehrharta erecta	Veldt grass
Erodium cicutarium	Red-stemmed filaree
Erodium cicutaruim	Herons bill
Hypochareris radicata	Rough cat's-ear
Iris pseudacorus	Yellow water iris
Limonium perezii	Statice
Limonium ramosissimum	Sea lavender
Lipidium latifolium	Perennial pepperweed
Lolium multiflorum	Italian ryegrass
Lythrum salicaria	Purple loosestrife
Malephora crocea	Ice plant
Marrubium vulgare	Horehound
Matthiola incana	Wild stock
Melilotus indicus	Sweet clover
Mesembryanthemum aequilaterace	Sea fig
Mesembryanthemum chilense	Wild sea fig
Mesembryanthemum rosea	Rosy ice plant
Mesembryanthenum crystallinum	Ice plant
Mesembryanthenum nodiflorum	Little ice-plant
Myoporum laetum	Myoporum
Nerium oleander	Oleander
Nicotiana glauca	Tree tobacco
Paragolis incurve	Rat-tailgrass
Phalaris aquatica	Harding grass
Phoenis dactyligfera	Date palm
Phyla nodiflora	Mat lippia
Polypogon monspeliensis	Rabbit's-footgrass
Raphanus sativus	Wild radish

Algae

Caulerpa taxifolia	Caulerpa
Sargassum muticum	
Undaria pinnatifida	
Lomentaria hakodatensis	

Invertebrates

Cnidaria: Anthozoa

Bunodeopsis sp.
Diadumene franciscana

Annelida: Polychaeta

Amblosyllis speciosa
Bispira sp.
Demonax
Ficopomatus enigmaticus
Hydroides dirampa
Hydroides elegans
Myrianida pachycera
Nicolea sp. A
Pseudopolydora paucibranchiata
Typosyllis nipponica

Mollusca: Bivalvia

Crassostrea gigas
Geukensia demissa
Musculista senhousia
Mytilus galloprovincialis
Teredo bartschi

Arthropoda: Pycnogonida
Ammothella hilgendorfi

Arthropoda: Crustacea: Cirripedia

Balanus amphitrite
Balanus eburneus

Arthropoda: Crustacea: Tanaidacea

?Sinelobus stanfordi

Arthropoda: Crustacea: Isopoda

Ianiropsis tridens

Limnoria tripunctata
Paranthura japonica
Sphaeroma quoyanum

Arthropoda: Crustacea: Amphipoda: Gammaridea

Ampithoe valida
Aoridea secunda
Chelura terebrans
Corophium acherusicum
Corophium insidiosum
Elasmopus rapax
Erichthonius brasiliensis
Grandidierella japonica
Jassa marmorata
Leucothoe alata
Liljeborgia sp.
Melita sp.
Metopella sp.
Paradexamine cf. *churinga*
Stenothoe valida

Arthropoda: Crustacea: Amphipoda: Caprellidea

Caprella mutica
Caprella simia

Arthropoda: Crustacea: Decapoda

Palaemon macrodactylus

Bryozoa: Chenostomata

Zoobotryon verticillatum

Bryozoa: Cheilostomata

Bugula flabellata
Bugula neritina
Cryptosula pallasiana
Watersipora arcuata
Watersipora ?subtorquata

Urochordata

Ascidia sp. A
Ascidia zara
Botrylloides violaceus
Botryllus firmus
Botryllus schlosseri
Botryllus sp. A
Ciona intestinalis

Ciona savignyi
Microcosmus squamiger
Molgula manhattensis
Polyandrocarpa zorritensis
Styela canopus
Styela clava
Styela plicata
Symplegma reptans

Fish

Gambusia affinis
Acanthogobius flavimanus
Lucania goodei

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