A Management and Monitoring Plan for Quino Checkerspot Butterfly (*Euphydryas editha quino*) and its Habitats in San Diego County

Advisory Report to the County of San Diego

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Introduction

The Quino checkerspot butterfly has vexed biologists since its discovery nearly a century ago in southern California. It has proven to be a difficult species to study and is proving to be a difficult target for conservation efforts. Its very taxonomy was confused until recently. The butterfly had been known by a misnomer; referred to as *Euphydryas editha wrightii*, not as it is correctly referred to now, *Euphydryas editha quino*. The correct subspecies name had been inappropriately assigned to a closely related checkerspot butterfly. Through much of the 1980s and into the next decade the Quino checkerspot was thought to have disappeared from all areas north of the Mexican border. And, although we now know that the butterfly likely disappeared from Orange County thirty years ago, it was rediscovered in Riverside County in the early 1990s, and in San Diego County at several formerly occupied sites soon after. Those disappearances and reappearances were a lesson for biologists; the butterflies can survive environmentally challenging circumstances, for years sustaining in diapause as caterpillars waiting to pupate, fly, and reproduce as adults. We now understand that the Quino checkerspot butterfly experiences dramatic fluctuations in abundance; has local populations that frequently are extirpated, and less frequently reestablished; and has complex resource needs that are met at few locations.

The life history and behavior of the Quino checkerspot butterfly may challenge planners, but some important information will provide a basis for a focused conservation strategy. This subspecies, once one of the most abundant butterflies in southern California, has been lost from nearly all of its historical range (Mattoni *et al.* 1997). It is now restricted to a small portion of western Riverside County and an even smaller area of San Diego County at its southern border (Mattoni *et al.* 1997; Pratt *et al.* 2001; U.S. Fish and Wildlife Service 2000). Virtually all of its best habitat is gone; lost first to agriculture and grazing (Burcham 1957; Mooney *et al.* 1986), then to explosive suburbanization of its coastal and near-inland grassland and sage scrub habitats, and accompanying degradation from air pollution, off-road vehicles, frequent fires, and other byproducts of human use (DeSimone 1995; O'Leary 1990; O'Leary *et al.* 1994; Westman

1981). Also known is that remaining habitats have been diminished in their capacity to support the Quino checkerspot. Its native larval hostplants and nectar resources have been replaced by a rapid expansion of non-native plants that now occupy most of southern California's grass- and forblands (Freudenberger *et al*. 1987; Minnich and Dezzani 1998). These facts were recognized with the listing of the Quino checkerspot butterfly as endangered under the federal Endangered Species Act.

One might expect to look to the U. S. Fish and Wildlife Service's draft recovery plan for management and monitoring guidance for the species; however, while describing the geographic habitat needed to support species recovery, the plan offers little to inform implementation future on-the-ground conservation actions (U.S. Fish and Wildlife Service 2000). Contributors to the plan, including two of the authors of this report, could not agree on an empirical definition of habitat for the species. However, they did describe the resources used by the Quino checkerspot butterfly, and a number of the landscape and site characteristics associated with butterfly presence. A comprehensive quantitative description of the environmental correlates of habitat suitability is still lacking. Similarly, the draft recovery plan, as well as the current survey guidelines for the species that are issued by the Service, lack a clear articulation of the procedures necessary to assess population sizes at levels of resolution that can assist in directing management actions. Uncertainties compromising the Quino checkerspot butterfly's conservation might fairly be described as overwhelming when one considers that no established techniques exist for managing, rehabilitating, or restoring habitats of varying condition.

The purpose of this presentation is to provide the framework for an adaptive management program that can reduce these uncertainties. We propose several data gathering exercises, including field experimentation, and a conceptual model to guide ongoing efforts to generate reliable data about the Quino checkerspot butterfly and its habitats. This document is organized into three sections — first is a description of resource and other habitat needs of the butterfly and the environmental threats that put it at risk; second is an articulation of an experimental approach to better management and restoration of habitat for the subspecies; and third is a contribution to the development of a monitoring scheme that can be used to assess the status and trends of the Quino checkerspot butterfly and its habitats, and can guide effective and efficient management responses.

Life History of the Butterfly and an Envirogram

The Quino checkerspot butterfly, *Euphydryas editha quino* (Behr) (Lepidoptera: Nymphalidae), is native to coastal sage scrub, chaparral, and valley grassland plant communities of cismontane southern California to northern Baja California, Mexico. Due to substantial habitat loss and declining population densities, the Quino checkerspot was listed as federally endangered in 1997 (62 Federal Register 2313). This subspecies is generally defined by its geographic distribution, although there is at least some evidence of genetic differences between it and related subspecies (Baughman *et al.* 1990; Mattoni *et al.* 1997).

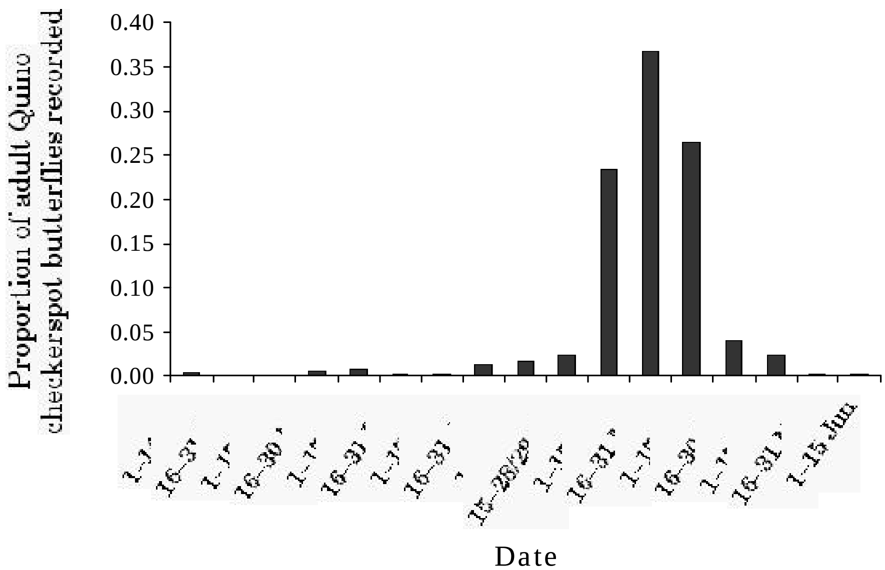


Figure 1. Proportion of adult Quino checkerspot butterflies recorded in USFWS database by date, 1958–2000 (n=958) (figure from Zonneveld et al. 2003).

Eggs of the Quino checkerspot butterfly are laid on its primary larval hostplant, *Plantago erecta*, or less frequently on related species, usually from mid-February to April (Emmel and Emmel 1973; Mattoni *et al.* 1997; Murphy and White 1984; Orsak 1978; Pratt *et al.* 2001). As larval hostplants senesce the larvae cease feeding and enter diapause (Mattoni *et al.* 1997; Osborne and Redak 2000). Winter rains correspond with the cessation of the diapause and induce *Plantago erecta* to germinate; postdiapause larvae resume feeding and develop to pupation. Adults emerge from the pupae after approximately two weeks, again dependent on winter rains, but usually from mid-February through March with extreme records from December through May

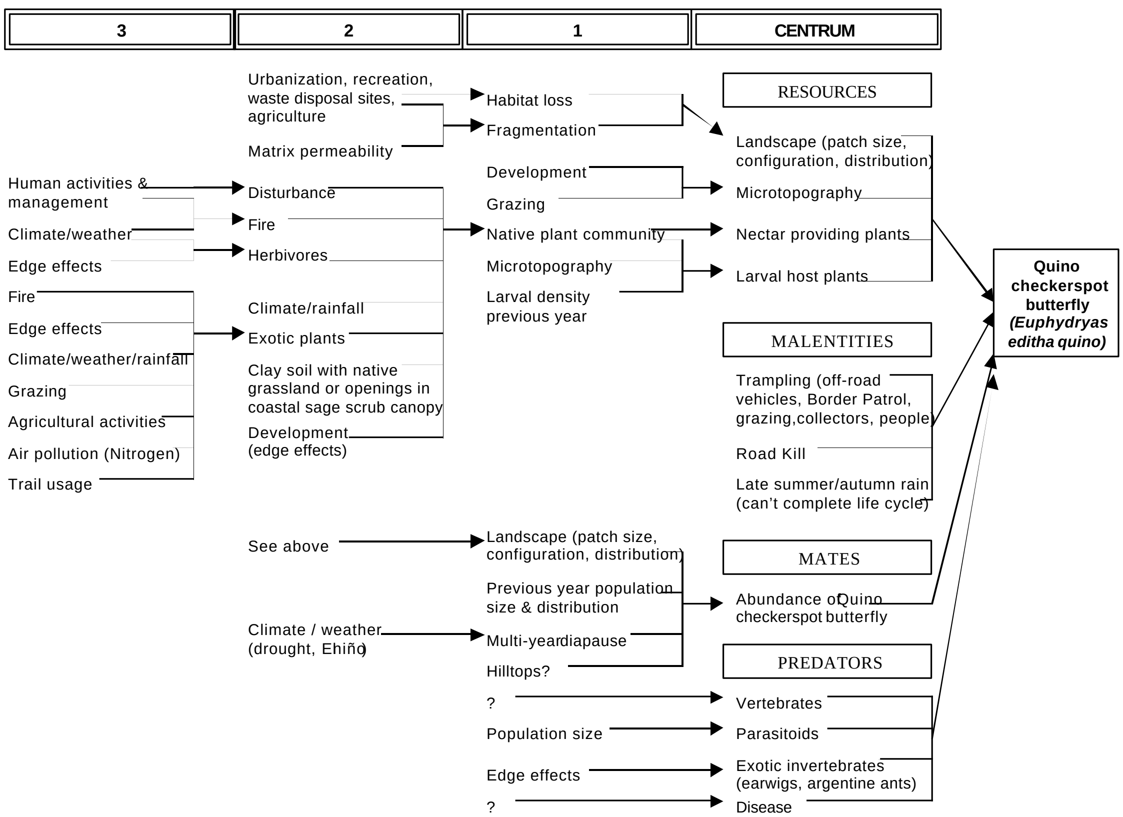
(Figure 1). Adult male Quino checkerspots patrol across habitat areas for females, perching intermittently on the ground or vegetation. Males also appear to engage in "hilltopping" activity (see Shields 1967), where they guard hilltops or ridges against other males. Females flying into these elevated "territories" are pursued by males. If females land and are receptive, mating commences. Although larvae are found almost exclusively on *Plantago erecta*, alternative larval hosts may include other species of *Plantago, Castilleja exserta*, and rarely *Antirrhinum coulterianum* or *Cordylanthus rigidus* (Mattoni *et al.* 1997; Murphy and White 1984; Pratt *et al.* 2001; Scott 1986). The relative importance of these alternate hostplants to larval survival and maintenance of Quino checkerspot populations is not known, but may be locally important. Adult individuals are dependent on a wide variety of native wildflowers, which are visited for nectar (Emmel and Emmel 1973; Mattoni *et al.* 1997; Murphy and White 1984; Orsak 1978).

In an effort to organize this life history and other available information describing the relationship between the Quino checkerspot butterfly and its environment in San Diego County, we have developed an envirogram following the approach of Andewartha and Birch (1984). Factors that affect the status of populations of the Quino checkerspot are presented in four categories – absence or reduced availability of critical resources, "malentities" (direct causes of mortality of individuals), availability of mates, and impacts from predators and parasites (Figure 2). This model identifies the processes and mechanisms that result in changes to any of the factors identified in the species "centrum," which encompasses the proximate determinants of population dynamics (Andrewartha and Birch 1984). While significant uncertainty remains relative to these causal relationships, these factors present hypotheses about butterfly-environment interactions that can be tested through experimentation.

Critical environmental factors that threaten the species include those physical and biotic phenomena that can result in population extirpations from genetic, demographic, and environmental causes. Management and monitoring prescriptions will benefit from a clear description of the causes of risk to populations and presumed metapopulations, and by a ranking of those causes, so that limited resources are appropriately directed to conservation actions.

As illustrated in the envirogram, a wide array of environmental features, biotic phenomena, and species-specific demographic circumstances affect Quino checkerspot populations. The dominant causes of population declines and disappearances, however, result from reductions and losses of the immediate resources critical to the species. All inputs leading to the "resources" centrum directly and indirectly affect butterfly status and must be considered in conservation planning. Accordingly, reserve design, management strategies, and monitoring schemes should focus on these key environmental features. Although this advisory document does not make explicit recommendations regarding reserve design under San Diego County's multiple species habitat conservation plan, the species' recovery plan and the envirogram presented here underscore that the size, configuration, and distribution of habitat patches are primary determinants of the likelihood of species persistence.

Figure 2. Envirogram for Quino checkerspot butterfly.



A wide range of human activities have directly and indirectly reduced the extent, and connectivity of habitat patches, and altered the composition of vegetation communities that those patches support. That reduction has limited the availability of native larval host plants (in San Diego County primarily *Plantago erecta* and *Castilleja exserta*), as well as important nectar resources used by adult butterflies. Reserve design must attempt to assemble constellations of habitat patches that are as large and close together as possible, are interconnected as natural open spaces where available, and include patches that support the best available native vegetation. Furthermore, the envirogram recognizes that prime habitat has diverse microtopography, which can buffer local populations against year-to-year variation in precipitation by providing habitat refugia during times of drought and deluge.

Securing a reserve system of multiple patches distributed across the planning area is necessary, but not adequate in itself, to ensure persistence of Quino checkerspot butterfly populations. Native plant communities that include the butterfly's critical resources are threatened by invasive non-native plant species that have replaced, or threaten to replace, native plants across the planning area. So complete has been this invasion that as much as 99 percent of the native grassland community in southern California has been replaced, and all remnant patches of native grasslands now harbor non-natives that might complete the process.

It is necessary to make a statement about what constitutes habitat for the Quino checkerspot butterfly. We do not include all of the possible permutations of habitat that might be necessary from a regulatory context to identify potential sites for surveys, but identify the most typical habitats that have supported the species. We recognize that in other parts of the subspecies range habitat attributes may vary, and that our statement does not encompass the full range of possible conditions, even within San Diego County. An explicit statement of our assumptions about habitat is, however, necessary to formulate a useful monitoring and management effort.

The draft recovery plan for Quino checkerspot butterfly describes "habitat requirements and limiting factors," listing resources used by the butterfly and a number of population dynamic characteristics of the subspecies drawn from qualitative studies and natural history observations (U.S. Fish and Wildlife Service 2000). Although plant taxa indicative of Quino checkersput butterfly habitat have not been identified, the butterfly has been associated with vegetation communities that support its two most frequently used host plants, *Plantago erecta* and *Castelleja exserta*. Commonly occurring with these plant species are *Lepidium nitidum, Layia platyglossa, Lasthe-*

nia californica, Dichlostemma capitatum, Linanthus dianthoflorus, as well as *Allium, Muilla, Cryptantha, Plagiobothrys,* and *Amsinckia* species, several of which are used as nectar sources by adult butterflies. *Dudleya multicaulis* and *Dudleya varigata* are found in many clay lens situations with the butterfly, as are lichen species in the genus *Acaraspora*, which are dominant elements in cryptogamic soil crusts (Pratt undated). These plant species tend in to be found in a mosaic of exposed soils interspersed with shrub cover. The suitability of these forb patches is determined by an as yet unidentified combination of larval hostplant density, nectar resource availability, topographic diversity, and contemporary patterns of precipitation.

For purposes of this presentation habitat for Quino checkerspot butterflies can be defined in simple terms — extensive collections of patches of primary larval hostplants, *Plantago erecta*, distributed in grassland- and coastal sage scrub-dominated open spaces. At least some of that *Plantago* should and may occur with other native forbs that can be used by the butterfly as secondary larval hostplants and adult nectar resources. Little undeveloped land in San Diego County currently meets these basic criteria.

Habitat Management

Having identified critical components of Quino checkerspot butterfly habitat in cis-montane San Diego County, it is readily evident that key resources have been lost by a combination of urban and rural development, various other human-caused and -exacerbated disturbances, and an expanding invasion of non-native species that threatens to transform native grasslands and open sage scrub. Reestablishing or enhancing native vegetation over large areas of degraded land will need to accompany the preservation of geographically appropriate configurations of land for Quino checkerspot butterfly conservation to succeed. Even in the absence of explicit information on Quino checkerspot butterfly status or trend, the establishment of large-scale experiments is required within an adaptive management framework to identify methods that can facilitate recovery of severely degraded lands no longer occupied by the butterfly and inform ongoing management of occupied lands.

The dominant cause of habitat decline in areas that still support Quino checkerspot butterfly populations is a combination of severe overgrazing and invasion of non-native annual plants. Overgrazing by cattle in California coastal sage and perennial grassland communities leads to at least two serious impacts — removal of larval hostplants and adult nectar sources, and accelerated invasion of non-native annual plants (Orsak 1978; U.S. Fish and Wildlife Service 2000). Non-native plant invasion can lead to vegetation type conversion, and only active management approaches can restore maintain these lands in their approximate pre-grazed state (Allen *et al.* 2000; Bartolome and Gemmill 1981; Heady 1988; Stylinski and Allen 1999; Whelan 1989; White 1967). Much of the current land designated as Quino checkerspot butterfly habitat is undergoing a type conversion to non-native annual grasslands, either from perennial grasslands supporting a substantial proportion of forbs, or from coastal sage scrub. The causes of this type conversion are many and complex (Allen *et al.* 2000; Klopatek *et al.* 1979; Minnich and Dezzani 1998; Pavlik *et al.* 1993; Zedler *et al.* 1983), but regardless of the mechanism of the conversion, strategies must be developed to maintain open stands of coastal sage scrub or perennial grasslands with extensive patches of native forbs for Quino checkerspot butterfly habitat.

Failure to manage existing Quino checkerspot butterfly habitat will result in continued habitat degradation for the species. Evidence, some of it experimental, has shown that native grasslands and shrublands in the geographic range of the butterfly are subject to a wide variety of disturbances that are leading to conversion of species-rich indigenous perennial plant communities into non-native annual plant communities with low native species richness (Allen *et al.* 2000). Native shrub species are uncommon on disturbed sites, despite nearby seed sources. Fire in many locations has led to vegetation type conversions, suggesting that altered stable states occur when native plant communities are forced beyond a threshold of resilience. Furthermore, deposition of atmospheric nitrogen (largely from automobile exhaust) appears to favor non-native annual plants; and certain rare annual forbs seem to be declining due to competition from those same annuals (Cione *et al.* 2002; Padgett and Allen 1999; Padgett *et al.* 1999; Weiss 1999). The most pervasive disturbances of southern California's open landscapes are having deleterious effects on resources required by the Quino checkerspot butterfly.

We note that the 2003 fires may have produced conditions that could support *Plantago erecta* as an early succession community element. The fires may have also killed Quino checkerspot butterflies. While not incorporated as a specific research question in this plan, an assessment of the recovery of vegetation in fire areas would provide additional information that would help make management decisions. In one scenario, successional habitat with *Plantago erecta* could support Quino checkerspot butterflies, given a nearby source area (Mattoni et al. 1997). Alternatively, if weeds dominate post-burn patches, they will have little such potential as the now-familiar type conversion to annual grassland progresses.

Research To Develop Large Scale Habitat Enhancement Techniques

It would be reassuring to look to techniques developed for the restoration of coastal sage scrub and perennial grasslands for the tools to enhance Quino checkerspot butterfly habitat. Unfortunately such techniques are not available. Of the relevant habitats, most effort has been expended investigating techniques to restore the shrub component of coastal sage scrub with little attention or research into establishment of forbaceous annual and perrenial species. The focus of restoration research and regulation is almost exclusively on shrub establishment (Cione *et al.* 2002; Eliason and Allen 1997), with few studies concerning the native herbaceous understory (see discussions in Allen *et al.* 2000; Bowler 1993). Even less focus has been placed on reestablishment of cryptobiotic soil communities (Bowler and Belnap 2000). However, some techniques not yet published in the peer-reviewed literature have been proposed for restoration of native forbs in a coastal sage scrub mosaic, such as dethatching and extensive hand weeding (Dodero and Hanson 2002). It is evident that most coastal sage scrub restorations do not reestablish a diverse understory of native forbs and grasses, and that efforts to do so are expensive and of limited size. The continued existence of the Quino checkerspot butterfly will depend on the development of techniques that can efficiently reestablish native forbs and grasses on large scales.

Techniques for large-scale enhancement or establishment of diverse communities of annual forb species within coastal sage scrub, grassland, and other vegetation types is an essential research priority. The major impediment to native forb recovery is the presence of invasive annual grasses and forbs, which are in turn promoted by frequent fire and nitrogen deposition. We propose a multifactorial experiment to investigate techniques of forb understory restoration. This experiment would optimally be conducted in an area with degraded coastal sage scrub, where a sparse shrub community is present, but the understory is dominated by non-native grasses.

A minimum of ten 1.0 ha (50 m × 200 m) sites are to be selected within the experimental area(s). Sites should be selected to be similar in shrub species composition and density. Each hectare site is to be considered a block of plots, consisting of four 50 m × 50 m (2,500 m^2) treatments. Each of the four plots within each site are to be randomly assigned to one of the following treatments: 1) annual grass herbicide + forb understory restoration, 2) annual grass herbicide + no forb restoration, 3) no herbicide application + forb understory restoration, 4) no herbicide application + no restoration. Because the treatments will include herbicides, the sites should not have a significant native grass community. Also, because of the uncertainty of the influence of herbicide on native insects (most herbicides contain trace levels of pesticides), research sites should not be occupied by listed or sensitive insect species.

Herbicide treatments are proposed to consist of applications of fusilade (fluazifop-p-butyl) at full label concentrations (0.17 kg AI/ha or current maximum label rate) during the early to mid-growing season for non-native annual grasses (mid-winter after emergence but prior to seed formation). Multiple herbicide applications may be needed to be made annually to achieve maximum grass removal; however, in no case should label recommendations be violated. Herbicide applications are to be made for at least the first 3 years of the study.

Forb understory plant community restoration treatments should consist of broadcast seeding the appropriate number and species of seed to achieve the desired target community. *Plantago erecta* and nectar resource species should be included in the seed mixture. Restoration from seed provides the necessary forb understory for Quino checkerspot habitat, and is not prohibitively expensive as would be restoring from plants in containers. Seed should be collected from nearby areas to maximize the possibility of maintaining regional and local plant genetic diversity (Montalvo and Ellstrand 2000).

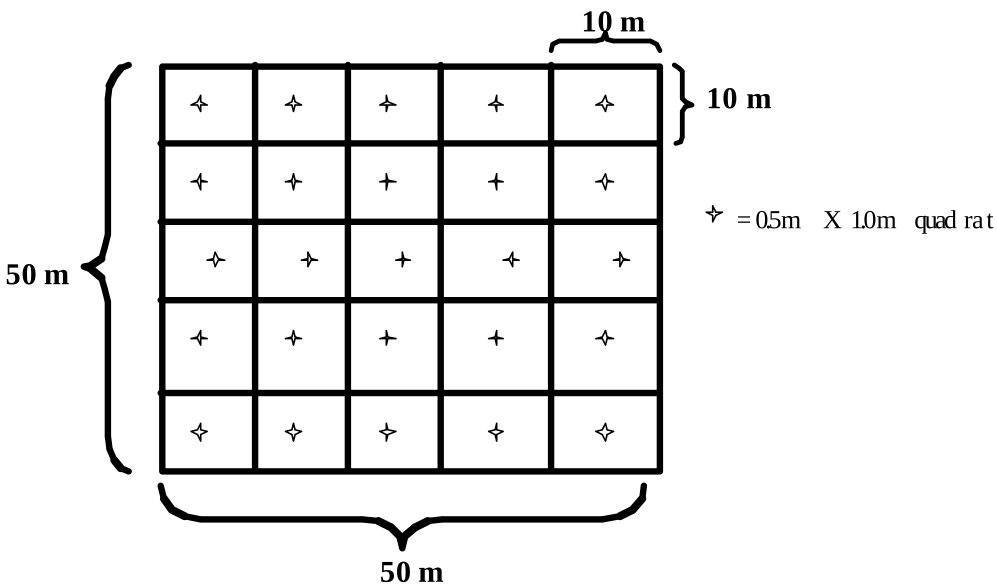


Figure 3. Quadrat sampling design to estimate treatment effects. Each star represents a placement of a single quadrat frame. Note there will be 4, 50 m x 50 m plots within each block (=1 ha site).

The effectiveness of treatment applications should be evaluated by monitoring the vegetation response of the forb communities within each experimental 1 ha plot. Percent cover estimates of forbs (including *Plantago erecta*, other larval hostplants, and adult nectar resources), grasses, and bare ground are to be made in year one following seeding with 1.0 m × 0.5 m Daubenmire quadrat frames. Initially, a minimum of 25 plant cover estimates should be taken within each 50 m × 50 m treatment plot. A stratified sampling design should be utilized with quadrats being placed within each 10 m × 10 m section of the plot (Figure 3). Cover estimates should be made in early spring, following winter rains, during the time when most plants are growing.

The effects of re-seeding and herbicide treatments can be analyzed using a repeated measures two-way analysis of variance incorporating a randomized block design. The main effect treatments (seeding and herbicide) each consist of 2 levels: (1) re-seeded versus no re-seeding and (2) herbicide versus no herbicide. Individual 50 m x 50 m plots within each site are randomly assigned to a specific treatment combination. Each treatment is replicated a minimum of 10 times (10, 1 ha sites = blocks). Time of sampling (year 1, 2, 3, …N) represents the repeated measures factor. The dependent variables of interest are at a minimum the percent cover of forbs and grasses. These variables can be expanded to individual species. Using a similar approach, repeated measures multivariate analysis of variance can be employed to assess the effects of treatment on the entire understory plant community, as opposed to the above univariate approach. If the experiments are allowed to run long enough, a variety of statistical techniques are available to determine any long-term patterns that may develop with respect to the understory plant community composition during succession.

If herbicide is not successful in allowing understory growth of native forbs, then subsequent experiments should focus on additional techniques. Manual removal of grass biomass and hand weeding has been used with significant success for the establishment of rare native plants and annual plant communities on the scale of hectares (Dodero and Hanson 2002). This technique may be effective and cost effective if applied at a large scale.

This experimental design would be useful to investigate the effects of narrow spectrum herbicides on native arthropod communities. Pitfall trapping could be used to track arthropod community response to the restoration efforts, and to test whether herbicide use has adverse consequences for native communities.

Butterfly and Habitat Monitoring Protocols

Spatial Stratification of Monitoring Design

With limited information available to inform reserve design and management for the Quino checkerspot butterfly, an adaptive management plan supported by rigorous monitoring will be required. An experimental framework to maximize rapid information gain from assessment and monitoring activities must sample both habitat conditions and checkerspot butterfly population status at appropriate spatial scales across the occupied region — for this effort, the border zone from Otay Mesa east to Tecate, north to near Jamul. A nested sampling scheme is suggested (Figure 4).

We recommend a stratified sampling scheme within San Diego County. The four "regions" are presumed to be separate populations, at least in the short term. These include three areas around Otay Mountain — West Otay, including occupied areas east Otay Mesa and the lower western slopes of Otay Mountain; Otay Lakes, including mesa tops surrounding Otay River, lands surrounding the Otay Lakes north to Proctor Valley, east to Dulzura; and East Otay, including Marron Valley and vicinity, east to Tecate Peak — and a fourth area, Jamul, including the San Diego National Wildlife Refuge and adjacent occupied areas surrounding Sweetwater Reservoir. In each of these regions, multiple "macrosites" should be selected. Macrosites can range in size from a few hectares to hundred of hectares. They can be occupied by the Quino checkerspot butterfly, unoccupied, or have unknown status. A standard for macrosite selection should be the presence of *Plantago erecta*. We believe that macrosites can be identified using a site suitability model in a GIS, combined with remotely sensed imagery to document and assess *Plantago* and other vegetation variables important to the butterfly (e.g. shrub cover). Using known locations of checkerspot populations, remotely sensed imagery can be employed to identify presumptive macrosite boundaries. At each macrosite a number of covariates should be measured, including distance to coast, distance to known site of occupancy, years since butterflies were last observed, elevation, climate from climate surface model, slope and aspect, distance to roads or other urban land uses, fire history, and disturbance type and degree. These variables are hypothesized to influence presence and abundance of Quino checkerspot butterflies, as expressed in the envirogram above (Figure 2).

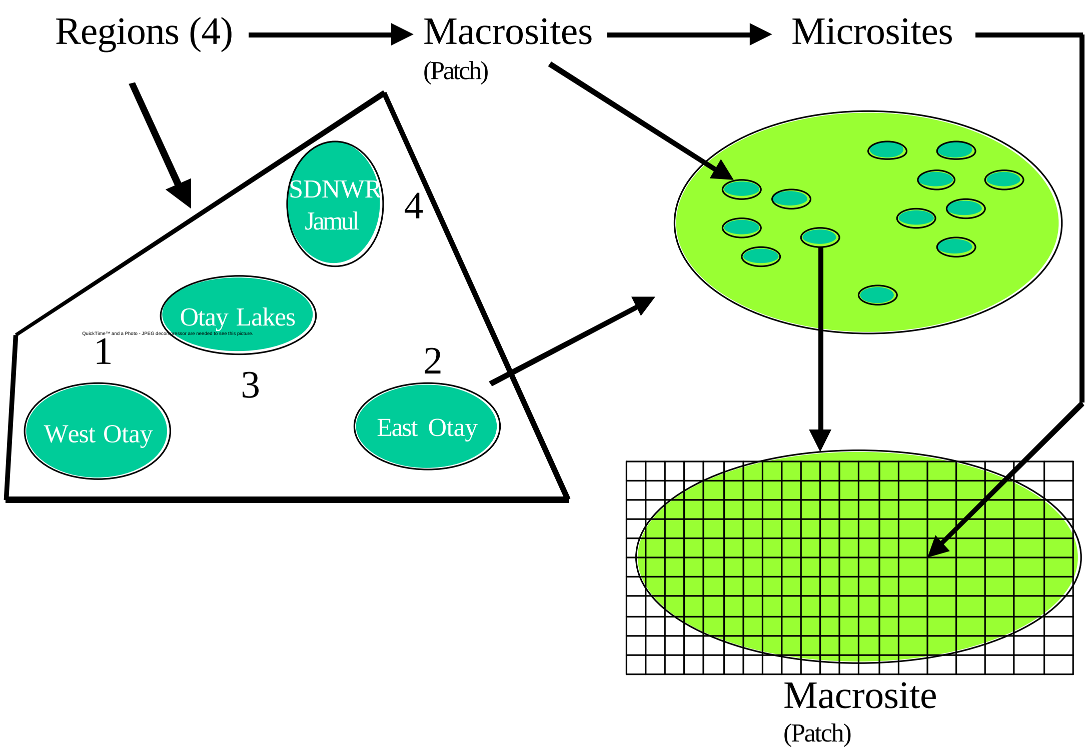


Figure 4. Monitoring scheme for Quino checkerspot butterfly habitat.

Within macrosites habitat variables must be measured at a local scale on multiple microsites. Microsites can be used to describe and compare macrosites. Measured at each microsite are variables that include vegetation cover; abundance of larval hostplants, nectar resource plants, and other plants that co-occur with the butterfly; bare ground; and other potential correlates of Quino checkerspot butterfly occupancy.

This spatially stratified description of Quino checkerspot butterfly's range in San Diego County provides the basis to investigate relationships between vegetation characteristics and Quino presence and to assess Quino checkerspot butterfly status and trend within the region. This will allow us to test the relationships in the centrum of the Quino checkerspot butterfly envirogram, i.e., what are the vegetational and environmental correlates of population size and stability? Furthermore, the spatially stratified scheme provides a framework to asses the variation in spatial distribution and density of Quino checkerspot butterflies through time. Initial monitoring efforts should balance the need to estimate status and trend with the need to answer fundamental questions about population dynamics. As more information becomes available, the monitoring program can be adjusted to become more accurate and efficient.

In the section below an investigation of the environmental correlates of habitat occupancy is proposed, which can guide Quino checkerspot butterfly management in San Diego County. We then turn to the actual measurement of Quino checkerspot butterfly status and trend, present a scheme that balances spatial and temporal resolution, and identify triggers that should initiate management actions.

Environmental Correlates of Habitat Occupancy

It is important to improve our understanding of the environmental correlates of Quino checkerspot butterfly population size and stability. These relationships are usually documented by surveying a large number of sites and recording the presence or absence of Quino checkerspot butterflies. A predictive model for the presence of Quino checkerspot butterfly can then be developed from the measured environmental variables using discriminant function analysis or Logistic Regression. The current rarity of Quino checkerspot butterfly will make estimating these relationships difficult because many (presumably) suitable sites are likely to be unoccupied. As a result, the statistical analyses are prone to bias (false negatives are common, false positives rare) and other weaknesses. Because of these constraints, the sample size needed to establish this relationship will be fairly large.

To reduce bias and improve the power of this study, a case-control design may be used. For this application, we define the few sites with Quino checkerspot butterfly present as the cases. Several control sites are defined as areas nearby where the status of Quino checkerspot butterfly is unknown (and likely absent). The strongest analysis for this type of design is a conditional logistic regression model, where each set of a case and its controls is considered to be a block. This pairing of cases to controls restricts the randomization for site selection. In doing so, fewer control sites are visited that are located far from any known populations. As a result, false negatives are minimized and power is maximized.

The design of this type of survey has two important considerations. First, the number and allocation of sites (cases and controls) needs to be determined. This must reflect the pattern of occupancy shown by Quino checkerspot butterfly on the landscape and the variability in the habitat-butterfly relationship. If occupied sites are very rare, then multiple controls per case will improve statistical power. If the environmental correlates are weak, many sets of cases and controls will be needed. A second issue is the design of the sampling protocol at each site. It is important that the presence of Quino checkerspot butterfly be determined accurately. In addition, relevant aspects of the habitat need to be measured, including presence and density of *Plantago*, the presence and density of nectar sources, and a description of the structure and composition of the plant community. The choice of relevant habitat features for analysis depends on our best understanding of the life history of the subspecies, and essentially allows for statistical testing of natural history observations.

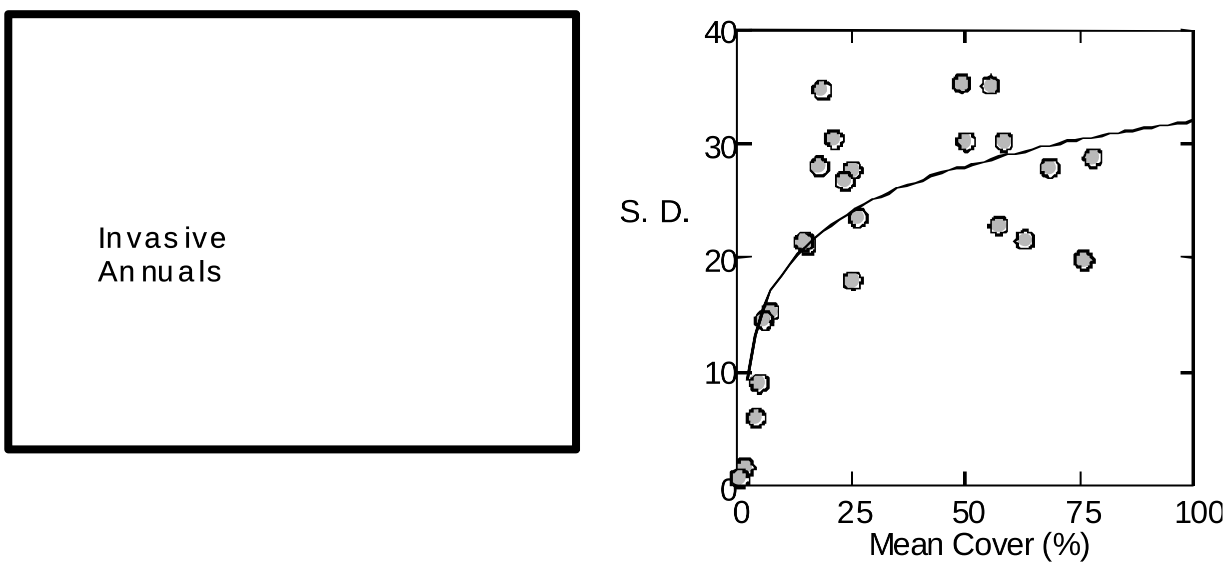


Figure 5. Variation in the coastal sage scrub habitat at Shipley Reserve, Riverside County, California. Variation within a site was a function of the mean density. The standard deviation of cover for dense sites was 30%. To achieve 80% power for differentiating two sites that differ in cover by 20%, 36 plots would be required at each site.

Plant communities in Southern California tend to be heterogeneous. Other work in coastal sage scrub communities suggests that local vegetation sampling requires a large effort. In work at

Shipley Reseve in Riverside County, 20 replicate 1 m $\times$ 0.5 m quadrats were sampled for each hectare of coastal sage scrub. Analysis of these data suggests that this intensive effort was necessary to reduce uncertainty associated with habitat patchiness (Figure 5). Depending on the assumption of the statistical model used, between 9 and 36 quadrats per site may be needed.

Table 1. Variation in coastal sage scrub at Rancho Jamul, San Diego County, California. Significant variation among transects within a site was observed for native shrubs and invasive grasses. Four transects appears to be adequate to achieve an estimate within 10%. To achieve 80% power for differentiating two sites that differ in cover by 20%, six transects would be required at each site.

	Artemisia californica	*Erigonum fasciculatum*	*Bromus diandrus*
Transect 1	25	35	20
Transect 2	25	50	33
Transect 3	16	40	50
Transect 4	4	30	29
Mean	17.5	38.8	33.0
SD	9.95	8.54	12.57

An alternative sampling strategy would be to use point-intercepts along several transects. In preliminary work at Rancho Jamul, San Diego County, four replicate 50 m transects were sampled for each hectare plot of coastal sage scrub (Table 1). Depending on the assumption of the statistical model used, between four and six transects per site may be needed.

It is possible that a mixed strategy of transects and quadrats would be useful. Transects provide good spatial coverage, but are harder to map with percent cover. Quadrats are more precise, but are time consuming to conduct on large scales. An example of a mixed strategy would be four transects with two to four quadrats placed along each transect. A mixed strategy like this does pose a challenge because the information from the two types of sampling is difficult to integrate into a single statistical analysis.

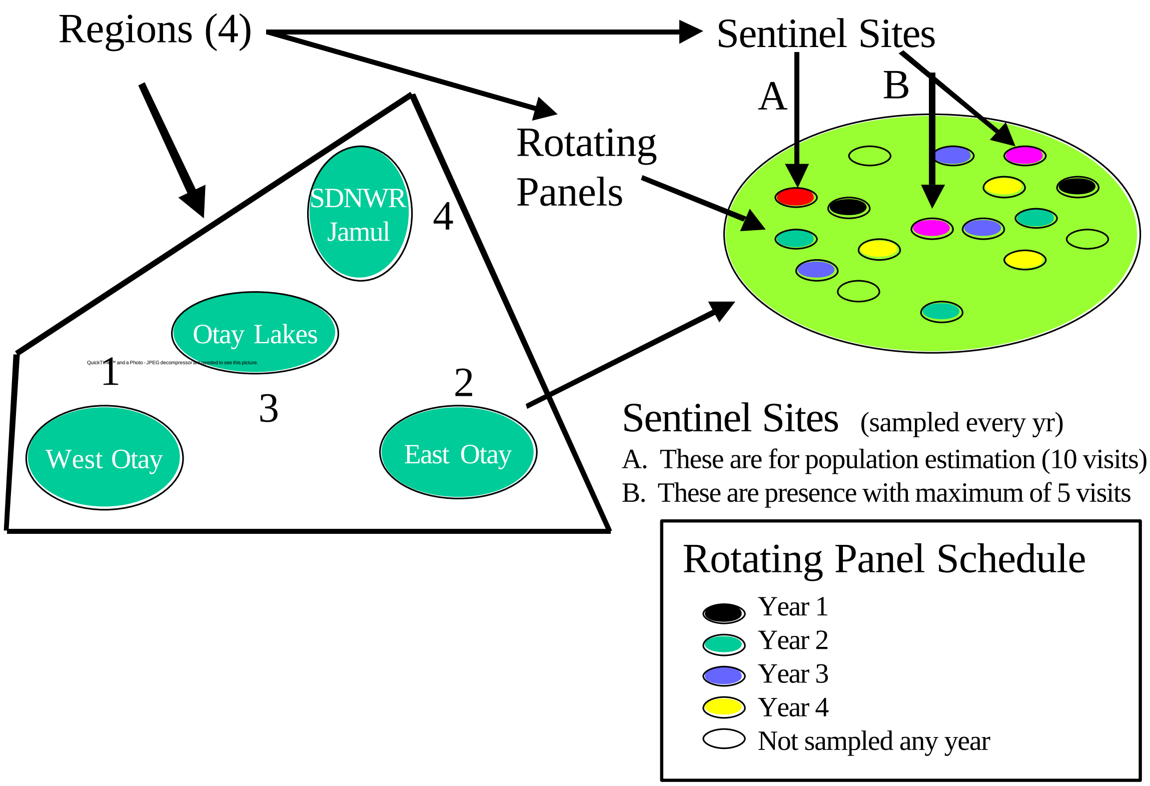


Figure 6. Monitoring protocol for the Quino checkerspot butterfly.

Monitoring Butterfly Status and Trend

The purpose of the butterfly monitoring scheme must be explicit at the outset or risk becoming an exercise in collecting data that does not further the objective of species conservation. The monitoring scheme should fulfill the information needs of managers, and should not only provide data, but identify when managers should initiate actions at regional and subregional scales. The potential responses to monitoring actions fall into two categories — manipulation of the species or manipulation of the habitat. Appropriate manipulation of the species can include such direct actions as captive rearing and release to supplement existing populations, translocation of wild or captive stock, or other actions that directly affect individuals of the species. Alternatively, monitoring results may trigger manipulation of habitat. These manipulations can include creation of habitat where it has been destroyed, enhancing existing unoccupied habitat, or enhancing existing occupied habitat.

The question remains, exactly how detailed must monitoring data be to inform management decisions. As discussed below, reliable butterfly population size estimates are time consuming to obtain, and expensive. If we take an approach of efficiency, an inventory for presence/absence of butterflies may provide information adequate to guide management decisions. Current consideration of metapopulation persistence is consistent with this approach — Hanksi's incidence model, informed by presence/absence data, calculates probability of extinction using the percent of occupied patches, not population size in each patch (Hanski 1999; Hanski *et al.* 1996). The commonly observed wide annual variation in butterfly population numbers in response to weather validates the choice of presence rather than abundance as a metric of population health (Pollard 1988). Nevertheless, for population status and trend information some estimate of population size is desirable. We therefore will consider a monitoring approach combining labor-intensive methods to obtain population estimates, and less labor-intensive methods that establish presence and probable absence.

A monitoring scheme for Quino checkerspot butterflies in San Diego County should use an experimental frame at region and macrosite scales similar to the frame used for vegetation monitoring. Within the four regions, sites to be assessed for butterfly populations are surveyed using one of several procedures. Sites visited for surveying butterfly populations are in two categories – sentinel sites, which are visited every year, and sites that are surveyed on a rotating schedule with visits occurring every three to five years, depending on panel size (Figure 6). Butterfly populations on select sentinel sites (Sentinel A) are subject to abundance surveys using transect survey techniques that require a minimum of ten visits during the flight season, and are described in detail below. Other sentinel sites (Sentinel B) are visited a maximum of five times in a given flight season to assess species presence. We suggest that one site in each region be identified as a Sentinel A site, and that approximately four other sites in each region are identified as Sentinel B sites. The sentinel sites (A and B) should be chosen at random from the universe of known occupied sites within each region to allow inference about population status within each region.

The general monitoring design outlined above provides a framework to monitor status and trend. Status describes the distribution of the species at one point in time, while trend describes changes in abundance and presence over time. Because resources are limited, any monitoring plan must balance the costs and benefits of these two measurements. Schemes that provide detailed data on status often have few resources remaining to establish trends. Likewise detailed data on trends may compromise the geographic coverage of a monitoring scheme. The number of sites covered in a monitoring scheme that is supported by limited resources will vary inversely with the frequency of return to each site — inevitably spatial and temporal resolution cannot both be high. (A more complete discussion of these issues is included in the appendix.)

Any scheme to establish status and trends for Quino checkerspot butterflies across a large area initially will require extensive sampling each year. Current data suggest that populations are rare and exhibit erratic changes in abundance. As a result, data from monitoring designs that revisit sites infrequently will be difficult to analyze and interpret. Instead, sites should be monitored annually so more information can be gained about population fluctuations. In addition, many areas have not been evaluated for the presence of Quino checkerspot butterfly. Because of this, visits to new potential sites, identified by topographic and vegetation characteristics, will be important to asses the status of the species.

Estimating the density of Quino checkerspot butterfly at each site is challenging because of variation in flight dates, demographic structure, and weather. Because of the large effort needed to establish regional status, there may be a trade-off between the number of survey visits to each site and the number of sites visited. Initially, the focus of the monitoring program should be on a broad scale.

Methods to obtain population information

Several methods have been developed to estimate abundance of butterflies. While eggs and pupae can be found and counted, nearly all population information for butterflies is acquired from either larval or adult surveys.

Larval surveys have been essential to many efforts to better understand the ecology of butterfly species (Murphy and Weiss 1988; Nicholls and Pullin 2000; Osborne and Redak 2000; Pratt

1987; Webb and Pullin 1996; Weiss *et al.* 1988). Success of larval surveys depends on the ability of observers to efficiently find and identify larvae in the field. Larval surveys have the advantage of providing evidence of reproduction in a specific area, while adult surveys may record individuals that have dispersed from a distant natal site; that is, larvae are excellent indicators of recruitment. Larval abundance may not be a good indicator of local effective population size because of mortality occurring during pupation. Opinions differ regarding the efficacy and efficiency of surveys for adult butterflies versus surveys for larvae. Murphy and Weiss (1988) describe larval monitoring techniques for the Bay checkerspot butterfly (*Euphydryas editha bayensis*) that not only provide reliable population estimates, but also allow inference regarding patterns of survivorship across habitat gradients, which can be useful for conservation planners. However, densities of Quino checkerspot butterfly larvae are lower than those of Bay checkerspot butterflies.

For Quino checkerspot butterflies, larvae can be found and enumerated most easily after diapause. At this stage, they are relatively large and conspicuous, and move from plant to plant while feeding. Webs of pre-diapause larvae can be difficult to locate, but experienced observers can find them. For example, Hein (unpublished) used distance sampling (see Buckland *et al.* 1993) of Quino checkerspot butterfly larvae to obtain density estimates. Pratt conducted surveys for Quino checkerspot butterfly presence in which he successfully located egg clusters, pre- and post-diapause larvae (Pratt *et al.* 2001). In light of the difficulty of locating larvae at low densities, and the scarcity of qualified and experienced observers, we concentrate the bulk of our proposal on observation of adults.

The most common method of counting butterflies is to observe adults. Most butterfly monitoring schemes, including long-term, large-scale surveys of British and Dutch butterflies, involve standardized surveys for adults(Moss and Pollard 1993; Pollard *et al.* 1995; Pollard *et al.* 1993). Population size estimates (as well as dimensionless indices) can be derived from adult observation or mark and recapture.

Mark-recapture methods provide detailed information about population parameters, and have been widely used (Arnold 1983; Ford 1957; Gall 1985; Watt *et al.* 1977). However, the handling necessary for this method has been criticized because of the resulting damage to some species

(Morton 1982; Murphy 1988; Singer and Wedlake 1981). As a practical matter, the protection afforded species listed under the Endangered Species Act essentially precludes the use of mark-recapture as a survey method. While it is often assumed that mark-recapture methods provide the most accurate estimate of population size, a comparison of methods in a population with a known number of butterflies showed that this method may provide the least accurate result (King 2000).

The other approach to obtain quantitative population data from surveys is counting adults along fixed transects. These methods have various permutations, but all involve counting butterflies along determined transects at regular intervals throughout the flight season. The method, described first by Pollard, is known as a Pollard Walk (Pollard 1977; Pollard *et al.* 1975). Subsequent refinements of the technique offer advice on transect layouts (Thomas 1983). Calculation of population indices from Pollard Walks is simple; the Pollard Index is the sum of the weekly average number of adults observed throughout the season.

Line transects provide an alternative to the Pollard Walk for collection and analysis of adult survey data. Line transect sampling provides unbiased estimates of population density that are comparable across sites. This comparability is important when population densities must be compared across sites where butterflies have different detectability (Brown and Boyce 1998). Distance of each butterfly from the observer is recorded, which allows for a statistical adjustment for detectability. Distance sampling is not likely to be feasible for Quino checkerspot butterfly, because the practical minimum number of observed butterflies necessary to model the detection function is sixty (Buckland *et al.* 1993), a population size that is unlikely to be realized at most locations. If populations of Quino checkerspot butterfly are found to have sufficient numbers to allow distance sampling to calculate detectability, then it should be used. It is possible to use the "pooling robustness" of line transect data to calculate a single detection probability. In this scenario distance information is collected at all sites, those data are pooled and a single detection probability is calculated from the pooled data. This approach is acceptable if the vegetation and other characteristics affecting detection are similar at all sites. Barring the use of distance sampling, double surveys can be used to estimate detectability as discussed below.

Even if transect counts are adjusted for search efficiency (i.e., detectability) they do not provide an estimate of total brood size without further manipulation. Because total brood size (i.e., adult population size) depends on the longevity of individuals, any index that does not account for longevity (e.g., the Pollard Index) has a more tenuous relationship to population size.

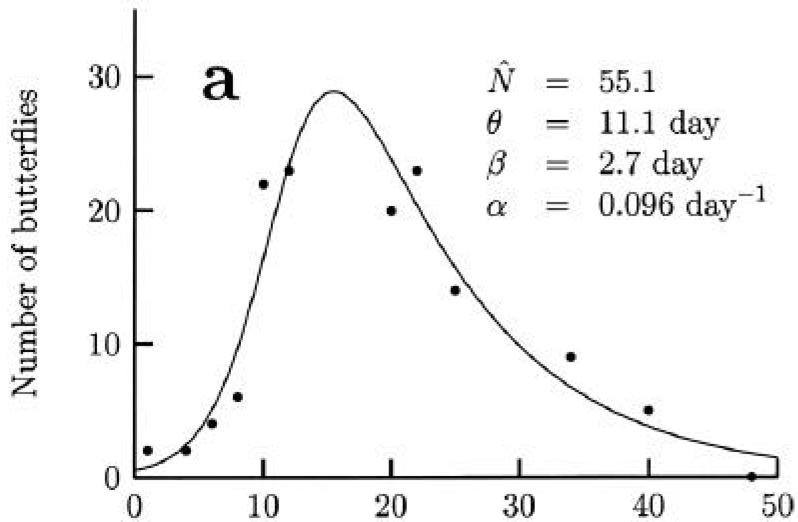


Figure 7. Description of abundance of *Glaucopsyche lygdamus palosverdesensis* in 1998 by Zonneveld model (Zonneveld *et al.* 2003).

Zonneveld has developed an alternative method to analyze transect count data that calculates longevity and total brood size (Zonneveld 1991). The Zonneveld method recognizes that the changes in butterfly abundance over a flight season can be described by a mathematical model with several assumptions and parameters. The model assumes: 1) no net migration, 2) a constant death rate, and 3) emergence times that follow the logistic distribution. The following differential equation describes changes in insect density during a flight period:

$$\frac{d}{dt}x(t) = N\frac{b}{b(1+b)^2} - ax(t) \text{ with } b = \exp\{(t - q/b)\}$$

In this equation, *x(t)* represents instantaneous density of insects present at time *t*. Four parameters characterize presence: total number of pupae that eclose, *N*; peak emergence time, *q*; spread in emergence times, *b*; and death rate, *a*. This deterministic model must be modified to become a stochastic model. Zonneveld states that the area covered by the transect, *A*, times the actual density of insects in the area, *x(t)*, yields the number of insects in the transect. Each individual has a probability, *q*, of being observed. The observable number thus equals $\hat{x}(t) = qAx(t)$. The observable density presents the expected value, but due to chance processes the actual observation is likely to deviate from this; in response Zonneveld assumes that observations are Poisson distributed, with $\hat{x}(t)$ as the expected value (Zonneveld 1991). Despite the limited number of parameters, the model frequently fits actual transect counts (Longcore *et al.* 2003; Mattoni *et al.* 2001; Zonneveld 1991) (Figure 7). The solution of the model does, however, depend on an estimate of the death rate of adults. This parameter is strongly correlated with the estimate of population size; as a result, uncertainty around the death rate parameter can become entrained in the estimate of population size. For a butterfly dataset collected without consideration of model needs, the death rate of a population of *Glaucopsyche lygdamus* could not be estimated in 5 of 11 years sampled (Mattoni *et al.* 2001). Subsequent modification of the model has allowed prior information about death rate to be incorporated through a Bayesian statistical treatment, leading to model solution of previously intractable datasets.

Despite the relative mathematical simplicity of the Zonneveld model, solution of the differential equation is computationally complex. The model, therefore, has received very limited usage (see only Mattoni *et al.* 2001). However, a new software tool can analyze transect counts using the Zonneveld model. INsect Count Analyzer (INCA), is a freely available user-friendly software program for this purpose (Bruggeman and Zonneveld 2002; Longcore *et al.* 2003).

An additional question will arise in monitoring for rare species such as Quino checkerspot butterfly, of whether the species is present at all. Methods of surveying for presence of individual insect species have received little rigorous attention. More effort has been given to methods designed to determine a list of all species of a particular taxonomic group found in an area, e.g., bait trapping, malaise trapping, black lighting, pitfall trapping, sweep netting, vacuum sampling, and checklist surveying (Borror *et al.* 1989; Royer *et al.* 1998). For these surveys, considerable effort has been expended to estimate total number of species based on species accumulation rates (Hayek and Buzas 1997). However, virtually no guidance is available to estimate the probability of detection of single species. To remedy this situation, Zonneveld and colleagues have developed an extension of his model of flight period characteristics that calculates detection probabilities for various survey schemes (Zonneveld *et al.* 2003). They use the Quino checkerspot butterfly as a case study for the development of optimal detection schemes, and provide a scheme for the detection of Quino checkerspot butterfly with known uncertainty estimates. Detection probability depends more on the number of surveys than on the spacing of those surveys, so although the method gives recommendations about standard survey dates for each year, the more critical decision is to identify the number of surveys to be conducted (Figure 8). This method provides information about how to structure surveys to maximize detection probabilities. Actual search efficiency (the proportion of butterflies present that were observed) can be calculated from data collected on those surveys.

The Zonneveld model has a number of advantages over other methods of analyzing transect counts. First, it extracts information about four biologically relevant parameters (brood size, death rate, date of peak emergence, dispersion of emergence), rather than a single index number. In addition to population size, death rate, and the date and dispersion of peak emergence can be useful in understanding insect population dynamics. Second, the model provides estimates of statistical uncertainty for all parameters. While the level of uncertainty may be high (depending on the quality of the dataset), this knowledge has an advantage over the Pollard Index, which has no estimate of uncertainty. Line transect sampling alone does provide uncertainty estimates for the number of individuals observed on a single day, it does not describe total seasonal brood size, or provide any other ecologically meaningful information such as longevity. Third, by incorporating prior information into analyses in INCA, the method allows for increased accuracy over time. As more data are collected, the uncertainty of population parameter estimates decreases. This makes the model especially attractive for analysis of data in a long term monitoring effort, and allows data collected at one site to help interpret those collected elsewhere. Fourth, the method provides guidance on the probability of detection of populations at sites suspected of being occupied (although this information can be used independently of the model as a whole).

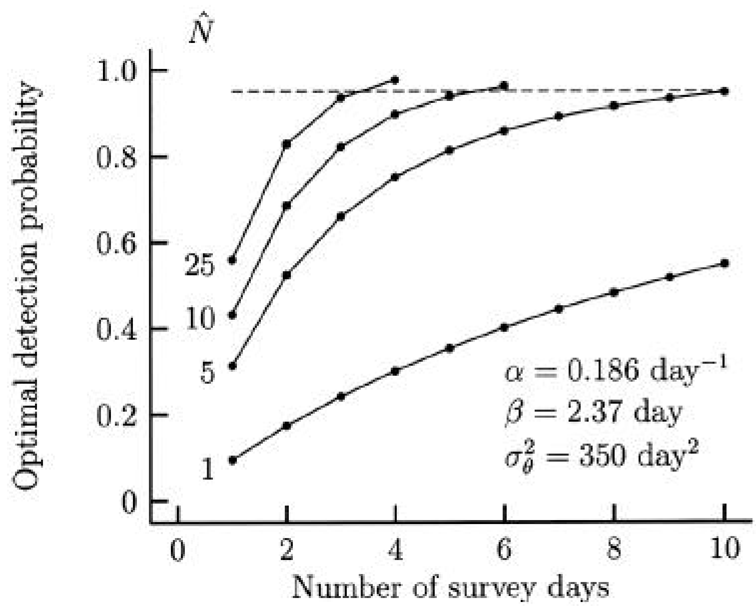


Figure 8. Optimal detection probability for Quino checkerspot butterfly as a function of the number of surveys. Each line indicates probabilities for a different observable population size. Average peak emergence is March 25. The dashed line indicates a detection probability of 0.95 (Zonneveld *et al.* 2003).

The model also has a number of disadvantages. First, as already discussed, estimates of death rate and population size are highly correlated, which poses problems for datasets that do not fit model assumptions cleanly. While this drawback can be addressed by the use of prior information, it remains a significant issue. Second, the Zonneveld model does not yet incorporate differences in detectibility (i.e. search efficiency) between sites, as does line sampling. Because *q* may differ from site to site, population sizes calculated using the Zonneveld model (or, indeed, the Pollard Index) are not completely comparable. Third, the assumption of no net migration from or into a population is frequently not true for the Quino checkerspot butterfly. In some years and during latter portions of flight seasons, adults disperse from natal areas. Individuals counted at the end of a flight season may be dispersers, rather than resident individuals. The phenomenon of hilltopping also complicates application of the model, especially for year-to-year comparisons (see Shields 1967). During low-density years, hilltops may be more likely to be utilized by males seeking mates (Baughman *et al.* 1988). Transect counts that include hilltops therefore may sample different geographic areas (occupied footprints) during different years. Like all methods of counting adult butterflies, the model depends on the ability of observers to count thoroughly resident adult butterflies — an observation that is reflected in the requirement that surveyors obtain permits from the U.S. Fish and Wildlife Service. Without skilled and experienced observers, butterflies that may appear similar and fly concurrently with Quino checkerspot butterfly will confound adult survey counts. Finally, the assumption that death rates remain constant throughout the flight period may be violated, as has been shown once, in a recent study (Schtickzelle *et al.* 2002).

Adult monitoring protocol

The monitoring scheme identifies sites as Sentinel A, Sentinel B, and Panel. Each of the Sentinel sites (A and B) should be surveyed each year. Panel sites should be surveyed on a four-year rotation. Sentinel A sites should be chosen randomly from known populations of Quino checkerspot butterfly, with at least one in each of the four subregions identified in San Diego County. Sentinel B sites, also chosen at random from known sites, should be surveyed less frequently, but in a manner designed to obtain reliable presence data. Panel sites should be chosen at random from remaining occupied sites, and also be surveyed on a rotating basis for presence. When environmental correlates determining Quino checkerspot butterfly presence have been identified early in the program, a set of panel sites of unknown occupancy will be surveyed based on the predictions derived from these results. We will call the two types of surveys "abundance surveys" and "presence surveys."

Abundance Surveys. To use the Zonneveld model, Mattoni et al. (2001) recommend that the peak count of butterflies on a transect exceed 25. However, they have subsequently shown that peak counts as low as five can be described by the model (Zonneveld *et al.* 2003). Of more importance is the completion of weekly surveys throughout the flight season, so that both early and late periods of presence are measured. These surveys should begin when adults are first observed, and continue until adults are not seen for two consecutive surveys. For planning purposes we assume that this involves weekly surveys from the last week of February to the first week of May each year (12 weeks). This period contains over 90% of the adult Quino checkerspot butterfly observations in the U.S. Fish and Wildlife Service database.

At each microsite to be subjected to abundance surveying, a Pollard transect will be established that follows Thomas' (1983) recommendations on survey placement to survey the entire microsite. The transect is not a sample of the microsite, but a survey of it. Only certain identifications of Quino checkerspot butterfly should be included, and sex of each observed individual recorded. Location (including distance from the trasect) and sex of each individual should be recorded on a topographic map of the site. To minimize variation in search efficiency, counts should only be conducted during appropriate weather conditions (>60 ºF, low winds), and those conditions recorded for each observation day.

Even the most thorough survey will not locate all adult butterflies. To provide an adjustment for variation in search efficiency (i.e., "detectibility" in the bird survey literature) two counts in the same day by different observers should be made at each Sentinel A sites during the peak of the flight season. This method could work as a mark-recapture study without handling the butterflies — assuming that butterflies do not move large distances between surveys (for application of method to birds see Nichols *et al.* 2000; Thompson 2002). The first survey "marks" the butterflies by sex and location. The second survey constitutes recaptures. Because adult Quino checkerspot butterflies are mobile (especially males), the interpretation of recapture versus new observations will be difficult. This difficulty is more easily overcome in situations where more characteristics can be noted (e.g., brood size and age class when surveying for breeding birds, Gabor *et al.* 2000). The addition of new individuals that eclose between the two surveys poses another problem. Unlike applications of this method for breeding birds, adult individuals may be added to the population between surveys completed on a single day. Minimizing time between independent surveys will decrease this occurrence. No guidance is available in the literature for the implementation of repeated surveys by independent observers for butterflies, but this method offers the most promise for adjusting counts for search efficiency. Search efficiency calculated from double counts at the Sentinel A sites, if the technique is shown to be effective, can be used to adjust estimates for total seasonal brood size.

Abundance monitoring should be conducted for each Sentinel A site, and the number of Sentinel A sites should be increased as resources allow. If sites of known occupancy cannot be surveyed weekly throughout the season, five surveys spaced regularly (e.g., same as optimal spacing of presence surveys), will provide skeleton data that can be analyzed using INCA, with the use of information on death rate from other sites.

Presence surveys. If spaced optimally, five surveys for Quino checkerspot butterfly should detect, with 0.95 probability, populations with more than 10 observable individuals. (Observable individuals account for search efficiency; if search efficiency is 10%, a population of 100 butterflies will have 10 observable individuals. Results from the double count surveys will provide an estimate of search efficiency.) Such a survey protocol is used by the U.S. Fish and Wildlife Service and should be applied to sentinel and panel sites, for which presence of the butterfly is unknown. Presence surveys should be conducted as walking surveys, to cover the sample site completely. Zonneveld et al. suggest that the five presence surveys for Quino checkerspot butterfly should be completed on the last day of February, March 16, March 30, April 14, and May 1, amended to reflect weather circumstances (Zonneveld *et al.* 2003). Sites where adults have been observed for the first time should also be surveyed to locate pre-diapause larvae, which confirms recruitment.

While the Zonneveld et al. (2003) detection scheme gives direction on the timing of surveys to maximize the probability to detect individuals, it does not incorporate search efficiency to calculate the actual probability of detecting a population at any given site. This must be derived from survey data, because search efficiency cannot be known *a priori*, except for some special situations of extremely sedentary butterflies that do not apply here. New methods have been published to measure occupancy rates of habitat patches that account for variability in search efficiency that derives from changes in abundance over time (MacKenzie *et al.* 2003; Royle and Nichols 2003). These methods require the assumption that the surveyed organism is either present or absent for all surveys with each single year. This assumption can be reasonably met if surveys are timed appropriately (i.e., following the scheme suggested by Zonneveld et al. 1993 or another method to time surveys to match the flight season). Known seasonal changes in abundance can be used as covariates to explain search efficiency. This method has been applied to multiple visits within a series of days to survey butterflies, using temperature as a covariate affecting search efficiency (Casula and Nichols 2003). For long-term monitoring of quino checkerspot butterfly the methodology could be employed for multiple visits within a series of years, with daily population size calculated by the Zonneveld method from sentinel data as a covariate (Zonneveld 1991 as implemented in INCA). The program PRESENCE (http://www.proteus.co.nz) has recently been modified to allow such calculations. If implemented in San Diego County, this novel approach would represent the first attempt to incorporate this new methodology into a long-term butterfly monitoring scheme.

Comparison of larval and adult surveys

In some instances, Quino checkerspot butterfly may be efficiently surveyed by counting larvae. Larvae are closely tied to foodplant resources, show evidence of reproduction, and are slow moving. However, larval surveys pose the risk of habitat damage (perhaps slightly more than adult surveys), require correct identification of larvae, and measure the population before mortality occurs during pupation. A pilot study should be undertaken at a site occupied by Quino checkerspot butterflies to determine if larval surveys and adult surveys provide population estimates that vary congruently from year to year. Larvae should be counted on fixed transects on a weekly basis during post-diapause growth and development. INCA may also be able to analyze data collected on larvae; one substitutes emergence from diapause for eclosion, and pupation rate for death rate and the curve of abundance should be the same. The same site should then be monitored for adult Quino checkerspot butterfly population size following the recommended protocol. This effort should be continued for several years to determine the correlation between the two measurements. If the correlation is high, and the cost of larval surveys is lower, then revisions to the monitoring plan might be made to use this survey type more.

Analysis of Data and Triggers for Management Action

The monitoring program described here provides information to investigate basic questions of Quino checkerspot butterfly ecology in addition to supporting management decisions. This program is summarized in Table 2. A large effort during year one will provide the data to test for relationships between environmental conditions (at two scales) and butterfly occupancy. The macrosite and microsite data, combined with butterfly presence data from sentinel and panel sites provide the basis for a conditional logistic regression to test the relationships diagrammed in the conceptual model of the envirogram. Presence/absence data from several succeeding years can also be tested before the vegetation data are out of date.

The butterfly survey protocol will generate two important long-term datasets. These include abundance at Sentinel A sites, and an assessment of presence at the Sentinel B sites and a rotating set of Panel sites. These data may be used to establish status and trend of the species, and to trigger management actions. We suggest the following analyses to trigger management actions in a way that guides resources toward the actions with highest priority for conserving the species.

Trigger 1. The first trigger is a significantly negative odds ratio in a conditional logistic regression on the presence-absence data over six years. Essentially, this estimates whether statistically fewer sites are occupied than in the past. The analysis should be performed within regions and countywide. If a negative odds ratio is found, then the distribution of the sites should be considered to determine whether dispersal, habitat quality, or weather conditions are likely to be explanatory. These can be investigated by using appropriate dependent variables to test each explanation (e.g., distance to nearest patch, vegetation variables, and rainfall). If declines are uniform across the county, and can be attributable to low rainfall, then no action is triggered. If vegetation variables are explanatory, then enhancement action of sites where Quino checkerspot butterflies are extirpated is triggered. If dispersal seems to be the key, (i.e., sites with extirpation are statistically more distant from other sites), then analyze using Trigger 2.

Table 2. Summary of Quino checkerspot butterfly monitoring program.

Scale and Site	Description	Data Collected	Frequency of Data Collection
Regions	Four areas of butterfly occupation, East Otay, West Otay, Otay Lakes, and Rancho Jamul NWR	Conditions characterized by habitat and butterfly surveys	N/A
Vegetation			
Macrosites	Contiguous blocks of natural habitat from a few to 100s of hectares with presence of *Plantago erecta*.	Distance to coast, distance to nearest occupied macrosite, average elevation, topographic diversity, climate from climate surface model, slope, aspect, distance to roads, distance to urban land use, time since fire	Year 1, updated as land use and butterfly occupancy change
Microsites	Points within macrosites, corresponding with butterfly survey sites	Vegetation cover, abundance of larval hostplants, nectar resource plants, bare ground, cryptogamic soils, etc.	Year 1, then once every four years in staggered rotation
Butterfly			
Sentinel A	1 occupied site per region	Weekly transect counts to estimate population parameters	Yearly
Sentinel B	3–4 sites per region	Five visits to estimate probability of presence	Yearly
Panel Sites	Up to 20 sites per region each year, either occupied at low density, unoccupied, or with unknown status but habitat present. Some must be located at microsites near known occupied sites to serve as control sites in habitat analysis.	Five visits to estimate probability of presence	Once every four years in staggered rotation

This trigger may be replaced by results of the program PRESENCE, which can calculate a rate of change in occupied patches that incorporates search efficiency as influenced by population size.

The variable lambda (λ) expresses the rate of change in occupancy for each site, similar to a population growth rate (MacKenzie *et al.* 2003). If and when this methodology is employed, a substitute trigger to identify negative trends should be adopted.

Trigger 2. If a site has experienced a population extirpation without butterflies returning for three years during which the population size at sentinel A sites was equal to or greater than the mean population size (e.g, three "good" years), then consider one of two actions at the site. If the percent cover of larval hostplants and nectar sources at the site have diminished since the site was last occupied, then enhance. If not, then reintroduce the butterfly.

Trigger 3. If population and presence absence-data are stable, then revegetation of unoccupied sites is triggered. In this manner resources are directed to creation of new habitat only when declines in existing habitat are already addressed. Location of these sites will be based on land availability, but should be proximate to occupied sites.

Successful implementation of these management actions depends on the timely development of habitat enhancement techniques as described above. Management actions may also include captive rearing of butterflies for release at sites where habitat remains but butterflies have been extirpated. Detailed guidance for such efforts is not provided here, but techniques for mass rearing of endangered butterflies are available (Herms *et al.* 1996; Mattoni *et al.* 2003; Mattoni 1988; Mattoon *et al.* 1971).

The conservation and recovery of the Quino checkerspot butterfly depends on the maintenance of a reserve design that protects existing populations and the habitat patches that support them, and minimizes their isolation from other occupied sites and natural lands. We have concluded that active management is necessary to maintain existing Quino checkerspot butterfly habitats; that need will be increased if non-native invasive plants, human disturbance, and air pollution (e.g., N deposition) increase near occupied and suitable but unoccupied habitats. This monitoring and management plan must be accompanied by the application of sound conservation planning principles to construct a reserve design adequate to protect a mosaic of occupied and unoccupied Quino checkerspot butterfly habitat able to support a dynamic metapopulation. Within this framework, a sound monitoring and management plan provides guidance necessary to allocate limited resources to maximize the probability that the Quino checkerspot butterfly will recover from its endangered status.

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Appendix: Introduction to Biological Monitoring

The success of a biological monitoring program is contingent on the reliability of population estimates. If a population estimate is inaccurate, then any decision based on it may be flawed (Barnett 1991; Huff 1954; Thompson and Seber 1996). Serious problems may arise when a population estimate is accurate, but the precision of the estimate is unknown or improperly calculated. When the precision of an estimate is not properly assessed and communicated, this uncertainty is often ignored. As a result, an artificial sense of certainty is attributed to the estimate, frequently with disastrous results. If monitoring programs do not carefully evaluate and communicate the accuracy of their estimates, then the utility and credibility of monitoring programs will erode.

Monitoring biological populations is challenging because of their inherent complexity and variability. Processes that influence population dynamics can change across space, either as smooth gradients (e.g., elevation) or in heterogeneous patches (e.g., patches of wet depression in a meadow). Similarly, population densities can change through time smoothly, for instance, a gradual decreasing trend, or erratically, for example, as a sudden, precipitous population decline. The simplest populations to monitor are likely to be spatially expansive and slow changing (e.g., forest trees). In contrast, the most difficult populations to monitor are those that are spatially localized, that is, clustered or rare, or that experience erratic boom-bust population, such as insects with boom-bust population dynamics. To be effective and cost efficient, biological monitoring programs must respect the nature and scales of population dynamics across space and through time. As a result, the design of a biological monitoring program requires careful attention to match sampling to the natural history of the population of interest.

Quino checkerspot butterflies appear to exhibit the kind of spatial clustering, rarity, and erratic population changes that make monitoring extremely difficult. As a consequence, an effective monitoring program will require significant effort to document the spatial extant of populations, changes in population size, and the ecological interactions that determine population fluctuations.

The Role of Statistics Theory in Ecological Monitoring

Statistical sampling theory provides a conceptual foundation for the design of an ecological monitoring program. Sampling theory provides formal development of key concepts, such as the bias and variance of an estimate (Rao 1998). It also provides a framework for estimating the power and efficiency of different sampling schemes. In developing a monitoring program, sampling theory can be used to compare the relative merits of several alternative sampling plans by a direct comparison of their biases, efficiency, and cost (Barnett 1991; Rao 1998). In some circumstances, this can inform criteria for choosing an effective sampling plan.

Statistical sampling theory, however, does not provide clear insight into many of the fundamental challenges associated with environmental monitoring. Statistical sampling theory cannot help identify the right questions to ask (Velleman 1997). Even if a question is well posed, it will be difficult to evaluate alternative sampling plans without detailed prior knowledge of the system. Prior knowledge must take the form of preliminary population estimates, estimates of variability, and an understanding of how estimates change across space and time. Without this information, specific recommendations from sampling theory will be of limited usefulness.

Statistical sampling theory, like all branches of science, is not without controversy. Many topics remain contentious among statisticians. As a result, even a well-posed question with abundant prior knowledge may not lead to a clear sampling strategy. In ecological monitoring, many questions need to be asked simultaneously. It is likely that for most of these questions, no single design will be clearly optimal. In fact, multiple objectives may lead to contradictory solutions. Different statisticians are likely to favor different approaches depending on their own viewpoint (Huff 1954; Velleman 1997). As a result, environmental monitoring is as much an art as a science.

Design of Ecological Monitoring Programs

Environmental and ecological sampling situations require special methods because the problems go beyond classical sampling framework in several ways. The literature on environmental monitoring is fairly small and dispersed throughout the fields of ecology, natural resource management, and statistics. There are few coherent texts and the few available texts tend to focus on a particular problems, such as air pollution, or systems, such as temperate hardwood forests . The primary literature covering statistical issues related to ecological monitoring programs is fairly small and scattered with the notable exceptions of the two journals, *Environmental Monitoring and Assessment* (Kluwer Academic Publishers) and the *Journal of Agricultural, Biological, and Environmental Statistics* (Published by the American Statistical Association and the International Biometric Society). Much of the literature on environmental monitoring focuses on non-statistical issues, including program objectives, indicators and attributes, database design, and quality control. These are important issues, but they do not fully explore the statistical issues that underlie the design of monitoring programs.

In the last decade, there has been renewed interest and theoretical advances in environmental sampling and design of long-term monitoring programs. One major advance is the development of adaptive sampling. Adaptive sampling is based on the powerful idea that the design of the sample can and should change throughout the sampling process. In other words, the sampling design should evolve as more information becomes available. This is a very attractive idea, but one that leads to important statistical challenges. The other major advance is the development of a body of literature specifically focused on monitoring status and trend. This recent literature is focused on the additional constraints and opportunities presented when regional sampling (status) is conducted on an ongoing basis to detect changes through time (trend).

Questions that are specific to monitoring include: whether the design of the sample should be allowed to change as information grows, whether some or all sampling locations should be revisited, and how should samples taken at different times be related. The answer to these questions lies in two issues — the relative importance of description of status versus detection of trend, and the magnitude and scale of heterogeneity across space and through time.

There is a fundamental trade-off between these two components. This can be easily illustrated with a simple example (Figure 9). Imagine a landscape with nine sites that are monitored for a total of nine years. Total sampling effort is limited to nine site visits that can be allocated in any way. Sampling all nine sites in a single year provides complete information about the status of the population in year one, but no information about rate of change. At the other extreme, choosing a single site and sampling it in each of the census periods provides complete information on temporal trend at that single site, but no information about status. Between these two extremes lay a continuum of designs that value status and trend differently. A fairly balanced design might include choosing three sites and visiting them three times. This provides information on status and trend, although the information is incomplete.

A key component of any monitoring design is the allocation of effort to describing status versus trend. The optimal monitoring strategy will depend critically on the magnitude of temporal variation relative to spatial variation. If sites are heterogeneous but change slowly through time, then optimal design will include many sites visited infrequently. If sites are homogenous but fluctuate rapidly and synchronously, the optimal design will include few sites visited frequently.

Figure 9. Trade-off between effort for status and trend. Simple representation of several alternative monitoring strategies. The system consists of nine sites that are monitoring through nine surveys. The total cost of the monitoring program is limited so that only nine samples can be taken (of a possible 81). The designs are arrayed on a continuum from effort focused on describing status (left) to trend (right).

Common Designs for Monitoring Status and Trend

Common designs suggested for monitoring status and trend range from revisiting every site in each sampling period to visiting new sites each period. In practice, many monitoring protocols default to a strategy in which sites are selected at the beginning of the monitoring process and all sites are revisited in all subsequent surveys (Table 3). More careful reflection about this approach is required. Assuming that sampling effort is limited (which it always is), this approach allocates fairly large effort to site revisits, and as a direct consequence will be restricted to a very few sites (Table 3:"Repeated Visits"). This design provides more information about trend than about regional status. This design is most effective when sites are similar, but fluctuate erratically through time. As a result, multiple revisits are important to describe trends accurately.

In contrast, monitoring designs can be established in which sites are selected at random at the start of each sampling period (Table 3; "New Sites"). As a result of continual randomization, new sites are added every sampling period, but sites are sampled only once. Over the course of a several years, many sites are visited, providing reliable information about status. Because sites are not revisited, change through time can only be gauged in aggregate and the quality of information about trend will depend on sites having similar characteristics, or on the assumption that all sites are changing in (approximately) the same trajectory.

Many monitoring designs balance the relative effort allocated to estimating status and trend. One common design calls for sampling of several alternative sets of sites (Table 3; "Serial Alternating"). Typically sites are divided into a few groups and then each group is visited sequentially. In this design, all sites are revisited, but not during every sampling period. The alternation among different sets of sites allows for the monitoring of more sites than in a pure revisit design. The serial alternating design gives some information on both status and trend.

Most monitoring projects span a heterogeneous region and must satisfy the needs of a diverse group of stakeholders. As a result, there are often multiple objectives for the monitoring project. With multiple objectives, different sites or regions may warrant differential sampling effort. A mixture of these three fundamental strategies will probably best serve monitoring programs with multiple competing objectives.

Table 3. Common designs for monitoring status and trend. Representation of several monitoring designs. These ideas are presented as icons and described in more detail. In all three examples, total effort is equivalent (18 sites visited over a six year period). The designs differ radically in their allocation of effort to describing status and trend.

<table>
<tr><th>Sampling Method</th><th>Repeated Visits</th><th>New Sites</th><th>Serial Alternating</th></tr>
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<tr><td>Intuition</td>
<td>Sites are revisited every year. Allows for the estimation of change for each site, every year.</td>
<td>New sites are visited in each year of the study..</td>
<td>Sites are grouped into panels. One panel is sampled each year on a fixed rotation schedule.</td></tr>
<tr><td>Best When</td>
<td>〈 Information on trend through time is of paramount importance
〈 Relatively little spatial heterogeneity
〈 Short-term trends at each site are of interest</td>
<td>〈 Information on regional status is of paramount importance
〈 Adding new sites is more important than consistent re-sampling of existing sites</td>
<td>〈 Both regional status and temporal trends are important
〈 Balances spatial and temporal coverage
〈 Interest in long-term trends through time (longer than rotation period)</td></tr>
<tr><td>Pros/Cons</td>
<td>+ Simple
+ Allows for estimation of short-term temporal change
+ Change can be detected as early as the second sampling period
- Limited spatial sampling</td>
<td>+ Maximizes the sampling of sites within the region
- Comparison of trends through time is confounded with sites
- Estimate of temporal trends can only be detected after many sampling periods</td>
<td>+ Provides estimates of long-term trends without sacrificing spatial coverage
- Fine-scale temporal change is difficult to detect
- Temporal trends can only be estimated after 2 complete rotations</td></tr>
</table>

Table 4. Mixed designs for monitoring status and trend. Representation of several mixed monitoring designs. These ideas are presented as icons and described in more detail. In all three examples, total effort is equivalent (30 sites visited over a six year period). The designs differ in their relative mixture of revisit, serial alternating, and new site design elements.

<table>
<tr><th>Sampling Method</th><th>Repeated Visits
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+ New Sites</th><th>Repeated Visits
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<tr><td>Intuition</td><td>Some sites are revisited every year. Some sites are on a two-year rotation.</td><td>A groups of sites are visited on a rotation but new sites are visited in each year of the study..</td><td>Sites are grouped into panels. One panel is sampled each year on a fixed rotation schedule.</td></tr>
<tr><td>Best When</td><td>〈 Information on trend through time is of paramount importance
〈 Relatively little spatial heterogeneity
〈 Short-term trends at each site are of interest</td><td>〈 Information on regional status is of paramount importance
〈 Adding new sites is more important than consistent re-sampling of existing sites
〈 Some revisits are necessary</td><td>〈 Both regional status and temporal trends are important
〈 Balances spatial and temporal coverage
〈 Some sites are visited every year (sentinel sites), but new sites are continuously evaluated,</td></tr>
<tr><td>Pros/Cons</td><td>+ Allows for estimation of short-term temporal change
+ Change can be detected as early as the second sampling period
- Limited spatial sampling</td><td>+ Maximizes the sampling of sites within the region
+ Comparison of trends through time is not completely confounded with sites
- Limited temporal information</td><td>+ Balanced, provides estimates of long-term trends without sacrificing spatial coverage
- More complex, three types of sites
- May be too conservative</td></tr>
</table>

The appropriate mixed strategy will depend largely on the nature of spatial and temporal variability of the system and the type of questions that must be addressed (Table 4). For surveys focusing on trend, a mixed design incorporating some sites that are revisited each survey and other sites that will be part of a serial panel, provide tremendous precision for the estimation of trend but are not as limited (spatially) as pure revisit designs. In addition, the designation of two but are not as limited (spatially) as pure revisit designs. In addition, the designation of two classes of sites, namely those that will be revisited each survey and those that will be revisited less frequently as part of the panel can be based on the objectives of the monitoring program.

Sites that are revisited in each survey receive more effort; this only makes sense if they are of greater importance to addressing the objectives of the monitoring program. These sites might be located in critical habitat, areas that experience high rates of change, or areas of key importance for some reason. As a result, these sites can be thought of as “sentinel” sites. Sentinel is derived from the Latin word “sentire” which means to watch or to perceive. In this context, these sites stand watch for the monitoring program. These sentinel sites provide information on trend above and beyond the contribution that they make to the overall survey.

Alternatively, if status is paramount importance, mixing features of a serial alternative design with a new sites design can provide tremendous power to document status, but also allow for the estimation of trend. In a pure “new site” design, there is no way to evaluate that assumption. In a mixed design with many new sites, but some revisits, these assumptions can be evaluated explicitly.