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**TECHNICAL REPORT
TO THE
CALIFORNIA COASTAL COMMISSION**

E. Kelp Forest Invertebrates

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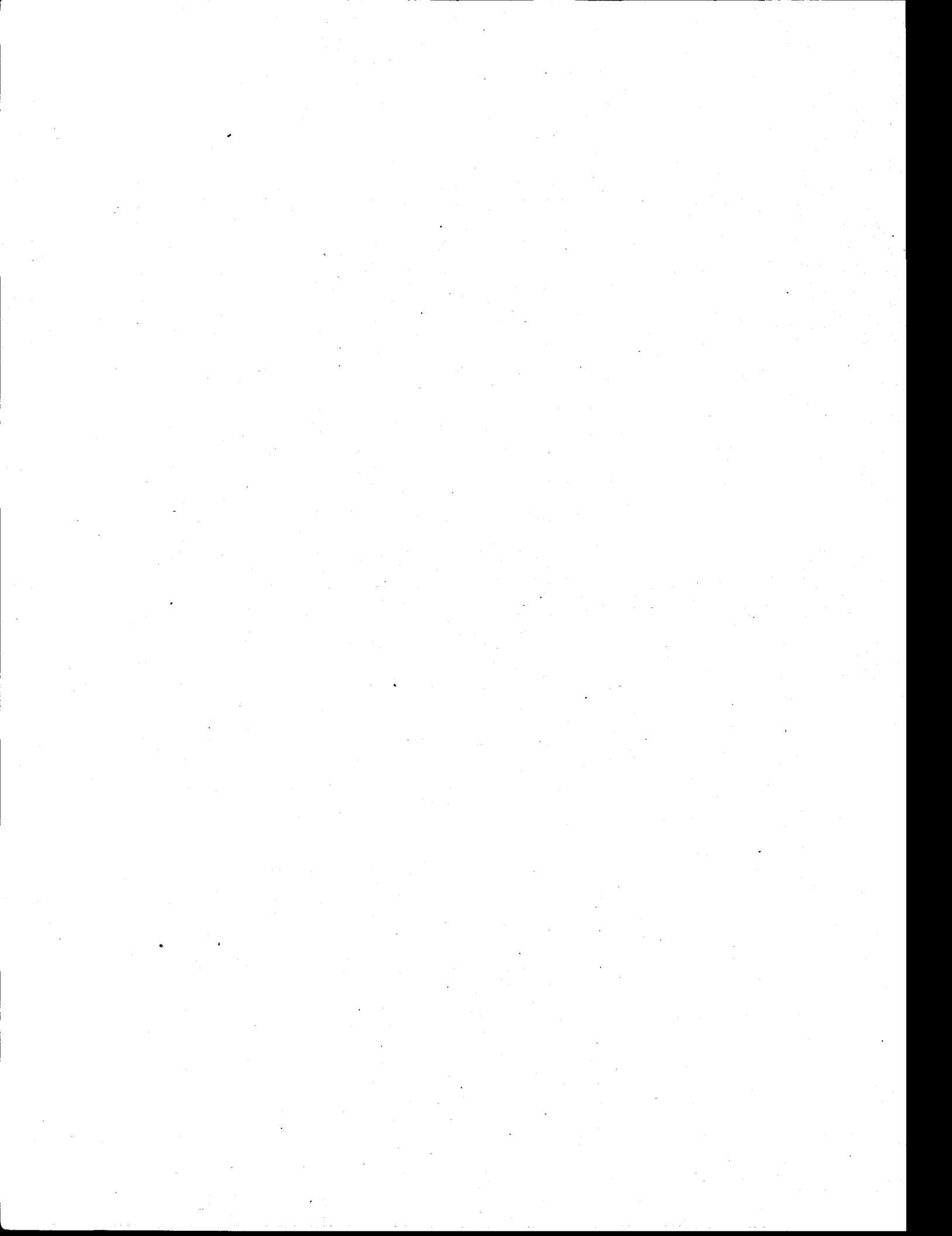


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SUMMARY

The study of large benthic invertebrates found in kelp forests began in the fall of 1980. Ten Before (preoperational) and eight After (operational) surveys were conducted at four stations. The two impact stations were approximately 500 m and 1.5 km from the diffusers, within the San Onofre kelp forest (SOK). Two stations were located far enough from the San Onofre Nuclear Generating Station (SONGS) so that impacts from the power plant were expected to be minimal. However, one of these potential control stations (Barn kelp forest = BK) was not used in our primary analyses because giant kelp declined to near zero abundance there at the beginning of the study. In addition, the substrate at our primary control (San Mateo Kelp forest = SMK) and at SOK consists of cobbles in a sand matrix, while consolidated reef predominates at BK.

Most of the species for which we were able to make a determination of SONGS' effects were gastropod mollusks, and there was a general decline in the density of these species in SOK (relative to their density in SMK) after the SONGS' Units 2 and 3 began operating. This decline was largest at the station closest to SONGS' diffusers, in the upcoast portion of San Onofre kelp forest. The most likely mechanisms underlying the observed changes are changes in habitat caused by loss of kelp and understory algae, and an increase in seston flux and sedimentation. One of the striking changes during the After period was the accumulation of relatively fine sediment with apparently cohesive properties *in situ* at the upcoast station at San Onofre. This sediment was first seen in the fall of 1985. Its spatial distribution and the timing of its appearance suggest that it may be related to the operation of SONGS Units 2 and 3 (Final Technical Report B).

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1. INTRODUCTION

Here we report on the effects of the operation of Units 2 and 3 of the San Onofre Nuclear Generating Station (SONGS) on populations of large benthic invertebrates in the San Onofre kelp forest (SOK). The field work was carried out by the Kelp Forest Invertebrate Project=KIP (University of Southern California). Over the course of the project, Drs. J. Kastendiek, J. Dixon and S. Schroeter acted as principal or co-principal investigators. Like most kelp forests in southern California, San Onofre contained a diverse group of plants and animals, including hundreds of species of benthic invertebrates. Among the latter were species of sport and commercial importance (e.g., abalones, lobsters, sea urchins, and sea cucumbers), and those of functional importance in structuring the kelp bed community (e.g., sea urchins and sea stars). The species composition of the large benthic invertebrates seen at SOK did not appear especially unusual in comparison with that seen in other southern California kelp beds (Dixon *et al.* 1988).

Thirty-six species of invertebrates were counted in non-trivial numbers (i.e. at least four animals ($0.1/m^2$) one time at one of the four stations). Species chosen to be counted 1) are characteristic of kelp forests in southern California, and/or 2) have been shown to influence the structure and dynamics of kelp bed communities, and 3) can be counted easily by divers in the field under conditions of relatively poor visibility. Small sessile invertebrates, which form a turf on many hard substrates (such as bryozoans, hydroids, and sponges), are difficult to reliably identify in the field and are not included as part of this study. Several larger species of sessile invertebrates were counted, however.

Of the 36 species that sometimes exceeded $0.1/m^2$, 16 were rare (as defined in section 3.12) on most surveys. In this report we evaluate the effects of SONGS on the remaining 20 species that were relatively common at the Impact stations during the Before period, along with five pooled groups of species constituting larger taxonomic groups, and thereby including the rarer species. The pooled groups analyzed were (1) all snails, (2) non-muricid snails, (3) muricid snails, (4) sea urchins, and (5) (large) sessile invertebrates. Muricids (family Muricidae) are small predatory and scavenging snails. We were able to evaluate the effects of SONGS statistically on all but one of these species groups; we could not do so for sessile invertebrates because of violations of the statistical assumptions of our procedures as discussed below in the Methods. The natural history of the kelp forest invertebrate species are reviewed in Dixon *et al.* (1988), and relevant background is included with the Results.

Of the species of sport and commercial interest [Abalones (*Haliotis* spp.), lobsters (*Parulirus interruptus*), crabs (*Cancer* spp.), and red sea urchins (*Strongylocentrotus franciscanus*)], all are harvested locally, and were therefore excluded from the analysis. With the exception of red sea urchins, all are also normally too rare to be effectively sampled on a continuing basis.

Of crucial importance to the study was the selection of the stations at which the species were monitored. Ideally, the Impact area and the Control areas to which it would be compared would differ only in the impact. Such a situation rarely exists in nature. In the vicinity of SONGS there were only three more or less persistent giant kelp forests (Final Technical Report K). Besides San Onofre, there were the San Mateo kelp forest (SMK) and the Barn kelp forest (BK) (Figure 1). Despite some differences in bottom composition and relief, we chose these latter areas as

Control stations. The use of two widely spaced Controls was intended to estimate natural variability on the scale of several kilometers. Unfortunately, the giant kelp population at BK declined to near zero during the first year of our study, and did not reestablish itself through the end of the study in 1986. The loss of this giant kelp forest may be related to a very local increase in sedimentation by sand during the 1978-1980 period (Final Technical Report K). Consequently we do not use data from this station in our primary analyses. However, qualitatively similar conclusions are reached when this station is included in our analyses (Appendix A). In addition, the substrate characteristics at our primary control more closely matched those at the impact station, consisting of cobbles in a sand matrix rather than consolidated reef as was the case at Barn kelp forest (Table 1, for further Discussion of these kelp forests see Final Technical Report K).

Two stations were established in the San Onofre kelp forest. The cooling waters from Units 2 and 3 are discharged from a series of ports upcoast from the kelp bed. The two stations were placed under the kelp canopy, one station as close to these diffuser ports as possible, and the second as far away as possible. This design was intended to provide a rough estimate of the distance over which the cooling water discharge had an effect.

The sampling design approximates the Before-After/Control-Impact-Pairs (BACIP) design discussed in Interim Technical Report 2 and by Stewart-Oaten *et al* (1986), but differs in that we had two Impact stations. Replicate samples were taken through time both Before and After Units 2 and 3 began operating. For each species or species group, the difference in density between the Control station and each of the two Impact stations was calculated for each survey. If the power plant had no effect, one would expect that the average difference between Impact and

Control stations would be the same after Units 2 and 3 began operating as before. In this report we use the test of this hypothesis to evaluate SONGS' impact on the abundance of kelp forest invertebrates.

2. FIELD METHODS

2.1 Station Locations and Configuration

The studies reported here were done in kelp forests in northern San Diego County, California (Fig. 1). Four monitoring stations were established in Fall 1980. The station nearest the Units 2 and 3 diffusers was chosen to be within the area predicted to be most affected by the operation of Units 2 and 3 (Murdoch *et al.* 1980, see Fig. 2, SOKU). A second "Impact" station was located further downcoast in the San Onofre kelp bed (SOKD). Distant Control stations were located in the San Mateo kelp forest (Figure 3, SMK), and in the Barn kelp forest (BK). As stated previously we do not use data from BK in our primary analyses.

The stations were placed in about the same depth of water (approximately 14 meters) in locations where giant kelp had been present prior to the beginning of the study. SMK was thought to be far enough away from SONGS in the upcoast direction so as not to be affected by the discharged cooling water. All stations were located on hard substrates at least 10 meters from the nearest sand plain.

Each station consisted of a surface buoy anchored by a metal plate which marked the origin of four 40-m transects (oriented at 35°, 125°, 215°, and 305°). The transects were marked with 1/4-inch steel reinforcing bar stakes. Ten permanent 1-m² quadrats were positioned every 4 meters along each transect.

2.2 Sampling Schedule

By May 1983, Units 2 and 3 were pumping water at rates near the levels they are expected to operate at over the long-term (but see Discussion). For the analyses

presented here, the period from October 1980 through the end of April 1983 is considered to be the "Before" period. Eleven Before surveys were done during this period at two to four month intervals. Since the species that were monitored tend to be long-lived and to recruit sporadically, we thought there should be a time lag between the start of normal plant operation and the beginning of the "After" (operational) period. We therefore designated the period from the beginning of May 1983 to the beginning of October 1984 as the "Interim" period during which sampling frequency was reduced, and only two surveys were done. The period from the beginning of October 1984 to the end of December 1986 is considered the After period, during which eight quarterly surveys were done. SONGS' operating characteristics during each of these periods are presented in Table 2.

The first survey of the study was not used in our analyses because methods were still being developed and field technicians were still being trained. Data collected during the two surveys in the Interim period are also not used in the analyses presented here.

2.3 Sampling Protocol

In addition to the target species whose individuals were counted throughout the study, individuals of twelve new species of snails were counted beginning on the next to last survey during the Before period. This was possible because of the increased experience of the field crew by that time.

In order to avoid disturbing the sessile species, the quadrats were sampled non-destructively; i.e., only those animals on the surface of the substrate were counted. Animals which could escape out of the quadrat were counted first; those which crawled into the quadrat after the census began were not counted. Although

it is possible (even likely) that the proportion of the invertebrates that was exposed varied seasonally, or through time for other reasons, we have no reason to expect that this proportion would change differently from the Before to After periods at the different stations. Thus, such temporal changes in behavior might decrease our power of detecting effects, but would be unlikely to produce biases.

Training dives were made before all but one survey. Training was done in SMK, which was considered the most difficult station due to the high diversity and abundance of invertebrates. Several quadrats were randomly chosen from the sampling array, and were sampled by each diver. The results were used immediately to standardize methods used by each diver during each survey. After the training dive, counts were compared to those of the field leader, sampling techniques discussed, and another set of quadrats was sampled. This was repeated two or three times, in order to ensure that all divers were sampling in the same manner.

In October 1985, patches of fine anomalous sediments were first seen. Thereafter their presence was periodically mapped in the field, and samples were collected for laboratory analysis (Final Technical Report B). The presence/absence of this new substrate type was recorded for each kelp forest invertebrate sample quadrat beginning in October 1985, the first survey on which it was noted. These sediments were distinguished from other sediments through a set of field tests described in Final Technical Report B. These field tests identified this sediment by its apparently cohesive nature (for example it could hold a divers hand print while other sediments within the kelp forest could not). The sediments identified as anomalous in the field differ from other sediments in the area. The organic content of these deposits is higher than surrounding sediments, and they are generally finer than the sands at the same depth in and around SOK (Table 3, see Final Technical

Report B). In grain size and organic content they were similar to the sediments found in 30 m depths, about 1 km offshore of the kelp forests (Table 3). The timing of their appearance (after SONGS began operating) and the location of these deposits (found primarily at the station closest to the diffusers), it seems possible that these fine deposits are related to the operations of SONGS Units 2 and 3 (Final Technical Report B).

3. ANALYSES

All programs used to do analyses, and to prepare tables and figures, are listed in flow charts in Appendix B. The programs implemented before January 1, 1988 were saved on the "report" disk of the Kelp Forest Invertebrate Project following procedures outlined in the MRC's Data Standards Document (Titan 1988). New programs implemented for this revised version of the report have been saved on a read only disk space on the University of California at Santa Barbara's mainframe computer system, and copies of all these programs will be saved on tape.

We test for an impact of SONGS' Units 2 and 3, and estimate the size of its effects, using a modification of the BACIP procedure outlined by Stewart-Oaten (1986). Basically, our approach is the same here as in the other MRC studies (Interim Technical Report 2). We calculate the "delta" (the mean difference between the density (usually log transformed) at Impact and Control stations) during periods both Before and After Units 2 and 3 became operational. We test whether, on average, the size of the deltas was different between the After period and Before periods, and we use the size of the Before to After change to estimate the percentage increase or decrease in abundance that could be attributed to the operations of SONGS Units 2 and 3.

Our implementation of the BACIP design needs to take into account some special attributes of the Kelp Forest Invertebrate Studies design. First, we have two potential Control stations, located in Barn and San Mateo kelp forests. During the design phase of this study both kelp forests were persistent, but nearly all kelp was lost from Barn kelp forest prior to the first sample date in November 1980. We therefore excluded the station in Barn kelp forest from our primary analyses, and

used only the San Mateo kelp forest station as a Control. Analyses in Appendix A show that our general conclusions do not depend upon the exclusion of the Barn kelp forest data. In addition, the substrate at San Mateo kelp forest, like the substrate at San Onofre kelp forest consists of cobbles in a sand matrix, while the substrate at Barn kelp forest consists of much more consolidated reef (Table 1, see Final Technical Report K).

Just prior to the next to last survey during the After period a large moving aggregation (termed a front) of red sea urchins (*Strongylocentrotus franciscanus*) invaded a portion of SMK. Fronts of sea urchins are known to have tremendous effects on invertebrate communities (Ebert 1978), and the front in SMK probably affected the abundance of some invertebrates. We therefore exclude those impacted quadrats from our calculation of the mean density at each station during the final two surveys. Quadrats that were impinged by the sea urchin front were determined from field notes and maps made by the Kelp Forest Invertebrate Project.

In addition to multiple control stations, our design also differed from the standard BACIP by having two, rather than one, impact stations. These impact stations were approximately 500 and 1500 meters from the diffusers, and thus relatively stationary invertebrates would not necessarily experience the same magnitude of impact at the two stations. This enables us to examine not only whether the density of kelp forest invertebrates was affected by SONGS Units 2 and 3, but also whether these effects were larger at the near than at the far Impact station. One way of approaching this analysis would be to do three sets of BACIP tests. We could first compare the near Impact with the Control, then the far Impact with the Control, and finally we could use the far Impact as a Control for the near Impact. The first two comparisons would test for SONGS' impacts at each station

individually. A significant result in the third comparison would indicate a difference in the size of the effect at the two impact stations. Instead of this set of three separate tests, we test for SONGS effects using a unified model. We calculate the "deltas" for each of the Impact stations using SMK as the Control. This delta vector (of length two) was then subjected to "repeated measures ANOVA" (e.g. Winer 1971) with station being a repeated (or within subject) main effect, and period (Before or After) being the other main effect. Our "subjects" here are the surveys. A main effect of period indicates that on average (across the two Impact stations) there has been a net effect of SONGS. A significant interaction between period and station indicates that the two Impact stations were affected to a different extent or in different directions. We do not report the results of tests for station effects. A main effect of station is of less interest in the context of this report, since it merely indicates that there are differences in average density at the two impact stations over the entire period of the study (and not that these differences changed after the power plant began operating).

In addition to repeated measures BACI analyses we used the BACIP t-test to compare two Control Stations (SMK and Barn) in ancillary analyses reported in Appendix A.

3.1 Details and Model Specification

3.1.1 Repeated Measures Model

Our repeated measures model can be expressed as:
$$\delta_{ijk} = P_i + S_j + T_k + PS(ij) + e_{ijk}$$
 where P is the period effect, S is the station effect, T is the time effect associated with a particular survey, PS(i,j) is the effect associated with the particular combination of period i and station j (i.e. an interaction), and e is

the error term. There is some disagreement in the literature on whether it is better to analyze such "repeated measures" data using univariate or multivariate tests, however with a vector of deltas of only length two (i.e. with two Impact stations) the two methods are identical (LaTour and Miniard 1983). Thus our analysis is equivalent to a standard univariate split-plot analysis of variance, where Periods are the whole plots and surveys are the split plots (e.g. Milliken and Johnson 1984).

3.1.2 Choosing a Transformation

The validity of our repeated measures analyses (and our ancillary BACIP analyses) depends upon several assumptions, and we test for violations of the most crucial of these. Our primary assumption tests are for additivity, lack of serial correlations in the deltas, and lack of temporal trends in the deltas in the absence of an impact of SONGS. The details of these assumption tests are described in Interim Technical Report 2. Because we use data from two Impact stations in a single analysis we need a single transformation for three stations simultaneously. To implement the repeated measures test we required additivity of, and no trends in the deltas. These assumption tests were done for one Control-Impact pair of stations at a time, as described in Interim Technical Report 2. The station pairs for which we ran our assumption tests were: near Impact-Control, far Impact-Control and near Impact-Far Impact.

We started with an *a priori* expectation of a multiplicative model, and attempted to induce additivity (perhaps the most basic assumption of the analysis) by using log transformations. Because we sometimes encountered zero values and the log of zero is undefined, we needed to add a constant. We chose a constant that results in an additive model. Our first choice of a constant was 0.025, the smallest possible nonzero station mean. If this constant failed the assumption tests, we then

considered sequentially larger constants, scaled by percentiles of "unimpacted abundances" (pooling Control and Impact in the Before period with the After data at Control). We considered the 1, 5, 10, 25, 50, 75, 95, and 99th percentiles, as well as the mean of the unimpacted abundances as possible constants. Because more than one constant might induce additivity and we select among these by the *a priori*, but somewhat arbitrary rule of picking the smallest satisfactory constant (i.e. one for which none of the assumption tests produced significant ($p < 0.05$) results). In some cases there was no constant that passed all three assumption tests. In these cases we used the smallest constant that satisfied the additivity and no trends assumptions, even if it failed on the serial correlation test. In cases where no constant satisfied both the additivity and/or trends assumptions, we used a constant of 0.025. In this case, we present results graphically and calculate percentage changes, but do not report the results of the repeated measures test. All these assumption tests are done only on the Before data because effects of the power plant can masquerade as violations of assumptions during the After period. We note with our results cases where there was serial correlation. In cases where data are available from only two surveys in the Before period no assumption tests were done (these would have involved regressions through two data points), and the data were $\log(x+0.25)$ transformed.

We present analyses for species that were chosen as follows. First, we selected those species which were present and counted at least once at one of the two Impact and at one of the two potential Control stations during at least two Before (preoperational) surveys. We also required that the species have a mean abundance, at one of the stations in the San Onofre kelp forest, of at least $0.1/\text{m}^2$ on at least one survey during the study. This is equivalent to a total of four animals at a station. The species meeting these abundance criteria are listed in Table 4.

Species were excluded from the repeated measures analysis which were absent during the entire After period at both stations of any of three possible station pairs in the primary analysis (i.e. SOKU-SMK, SOKD-SMK, SOKU-SOKD). In these cases it was still sometimes possible to estimate percent change in abundance at SOKU and/or SOKD if abundances were sometimes not zero at both SMK and the appropriate SOK station. Species for which we were able to estimate percent change for at least one impact station are indicated in Table 4. Any of the species satisfying the criteria for calculation of percent change in abundance at one or more of the SOK stations were then subjected to tests of the assumptions underlying the BACIP analysis.

In the above discussion, and in the Results we use "species" to indicate the lowest taxonomic level at which individuals were identified. In some cases several members of the same genus are included in such a category, when they were not consistently distinguished in the field.

3.1.3 Perturbations Unrelated to SONGS

During the course of our study two events occurred which were unrelated to the operation of SONGS but which could cause period by station interactions in the abundance of some echinoderm species. In 1981, and especially in 1983 and 1984, a series of epizootics similar to the episode observed in Baja California (Dungan *et al* 1982), virtually eliminated shallow water sea star populations in the southern California Bight (Tegner and Dayton 1987). Since densities of sea stars were initially more than two fold higher in SOKU than in SMK, the decline to low densities at all stations (Table 5) would lead to an estimated relative reduction at the SOKU station. A second perturbation unrelated to SONGS was the commercial harvesting of red sea urchins. Red urchins were initially more abundant at the

Control station in the San Mateo kelp forest than in the San Onofre kelp forest (Table 6). Harvesting was heaviest during the Interim period. By late 1984, fishermen could no longer find sufficiently dense populations in SOK to make harvesting economically feasible (See discussion in Final Technical Report K). However, dense populations were still found at SMK, where harvesting continued. We have not included sea stars or red urchins in our analysis because of these severe perturbations. All other species were analyzed using the repeated measures approach if they met the abundance and statistical criteria described above.

3.1.4 Statistical Power

Statistical power is the probability of detecting a significant effect when the null hypothesis of no effect is false, and a particular (specified) alternative is true. Power depends upon variability among the samples, the number of samples taken, and the particular alternative hypothesis that is specified. Power is of particular concern for the kelp forest invertebrates because many species were only sampled a few times during the Before period. We chose to evaluate the power for detecting a 50% decline in density at the near Impact station, as detected by the Period x Station interaction in the repeated measures analysis. This is equivalent to the power of detecting a 50% reduction in density at the near Impact station relative to the far Impact station using the BACIP procedure.

3.1.5 Evidence of an Effect of the Power Plant

Our repeated measures test evaluates whether the differences between the abundance of organisms at the Control and the average of the two Impact stations changed after Units 2 and 3 began operating (the period effect), and whether the Impact - Control changes differed in size between the two Impact stations (the

period x station effect). We take a statistically significant result for either of these effects as evidence that SONGS has affected the density of the organism in question. Our inference of a SONGS effect is greatly strengthened in cases where there are plausible mechanisms by which SONGS could produce the observed changes, and weakened by the existence of probable causes unrelated to SONGS.

3.2 Potential effects of anomalous sediments

During the After period there was a marked increase in anomalous sediments (see Section 2.3) at the near Impact station and surrounding areas (Table 7, see Final Technical Report B). Because changes in substrate could reasonably be expected to affect the abundance of benthic animals, the origin of the sediment and the cause of its deposition in the kelp forest immediately became topics of inquiry. It seems possible that this substrate is related to the operation of SONGS Units 2 and 3, and this a topic still under investigation as this report is being prepared (Final Technical Report B).

In the context of the kelp forest invertebrate studies there is no way to completely isolate the effects of sediments from possible effects of SONGS which are not directly related to sediments. However we were able to explore the effects of the sediments at the near Impact station by comparing "impacted" and "unimpacted" quadrats Before and After the sedimentation event using the BACIP procedure. This test should control for most potential SONGS effects unrelated to the sediments, because the quadrats are close to one another (all within an 80 m x 80 m area), and thus should experience roughly the same exposure to SONGS' effluents. We defined "impacted" quadrats as those which had any anomalous sediments on any survey. From October 1985 until the end of the study, anomalous