APPENDIX C — ATTACHMENT 1

Benthic Monitoring Program Description

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Program Design and Approval

An initial benthic monitoring program design and sampling plan was submitted to EPA Region IX by CCH on June 25, 1986, in its response of August 6, 1986. EPA Region IX requested specific alterations to the sampling program. The requested changes included the movement of one proposed near-field station to a position 500 meters (m) to the southwest of the ZID, and the addition of a ZID boundary station on the southeast boundary. The letter requested placement of the reference stations at distances of 3.5 kilometers (km) east and west of the outfall. All changes requested by EPA Region IX were incorporated into the final sampling plan submitted to EPA Region IX on September 8, 1986. Initial benthic sampling was carried out on September 15, 1986.

Station Positioning

The exact positioning of each station is determined using a Motorola Mini-Ranger navigation system. The locations of stations in relation to latitude, longitude and bathymetric contours are shown on Figure C-3-1. Ranges are determined from the navigation system for each replicate grab sample at each station on each sampling date and are given in the appendices to the annual monitoring reports. Depths for all stations are also recorded for each of the five replicate grab samples and reported in the annual reports. Original station positions within and at the boundaries of the ZID were located precisely during the original sampling using the submarine "Makali'i," its tender ship "Kila," and the CCH vessel "Noi' I Kai" (Nelson et al., 1987b).

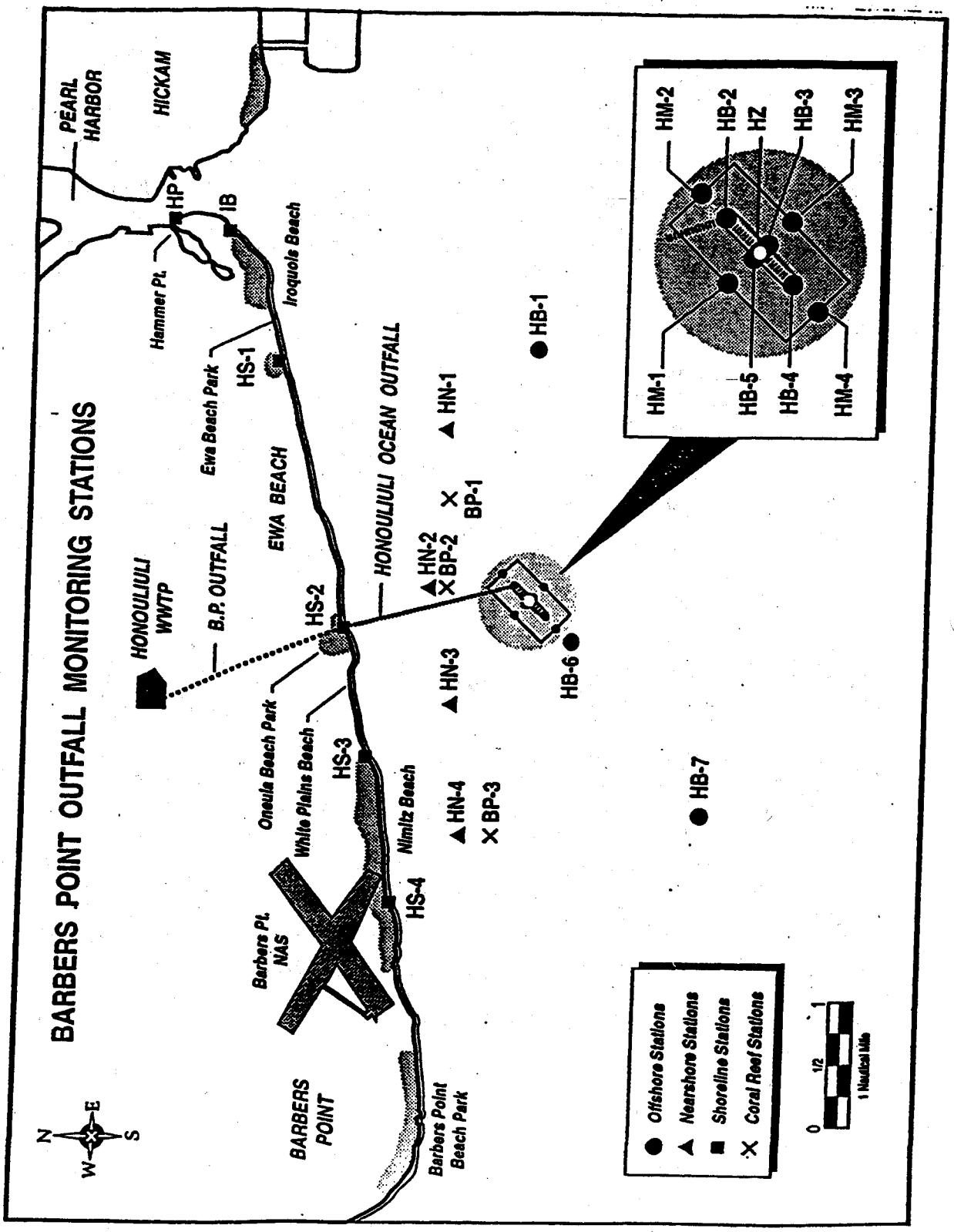
Sampling Methods

The sampling methodology used in the monitoring program has been approved by EPA and embodied in the NPDES permit. The methods generally follow the recommendations of Swartz (1978) and EPA guidelines (1987c), herein referred to as EPA procedures.

The sediment sampling is done using a modified 0.1 square meter (m^2) Van Veen grab sampler. The sampler is normally deployed from a stern-mounted A-frame of the city's research vessel Noi' I Kai. Penetration of the sampler is recorded and summarized in each annual report and generally ranges from 6 and 10 centimeters (cm). Replicated Van Veen grab samples are taken at each station. Sampling effort for the initial 1986 survey was six grabs per station, with an additional four samples being taken at Stations HB1 and HB4 at the request of EPA. In the 1990 survey, replication was 10 grabs per station, again at the request of U.S. EPA. Evaluation of the 1990 survey showed little differences in conclusions based on the higher sampling effort (per a power analysis); and with consultation with EPA, sampling effort was set at five replicates per station in the subsequent surveys. (Note: Adequacy of the benthic sampling effort was reviewed by Tetra Tech (1987) and found to be satisfactory.)

FIGURE C-3-1

HWWTP OCEAN MONITORING STATIONS



To obtain both biological and physical-chemical samples, a subsample of each replicate grab is taken using a coring tube (7.6 cm diameter by 5 cm deep) (methods established by National Pollutant Discharge Elimination System permit no. HI0020877). Such subsampling is necessary because the small size and abundance of epifauna and infauna in the area (Nelson 1986 and Russo et al., 1988), and processing and analysis of the entire grab sample would be prohibitively expensive and time-consuming. Use of replicated grab samples at each station, rather than replicated subsamples from one grab sample, is done to provide data for determining intrastation variability, which is required for statistical analysis. All biological cores subsampled from grabs are processed on a 0.5-mm mesh screen for sediment removal. This screen size was selected after testing a 0.25-mm mesh screen for comparison in 1986. Four samples from Stations HB1 and HB4, and two samples from all other stations were compared and found to be similar to the 0.5-mm fraction; thus, this additional processing was not carried out in subsequent surveys.

Geochemical samples for analysis (total organic carbon [TOC], oxidation-reduction potential [ORP], grain size) are also obtained from the grabs from which the biological sub-cores are taken. Each replicate Van Veen grab contains more than enough sediment for both biological and geochemical sample types. A minimum of three subsamples, 7.6 cm diameter and 5 cm deep, (one from each of three different grab samples) are generally taken (oftentimes more are taken for replication). The top 2 cm of sediment of each subsample is used for geochemical analysis. Samples for total volatile solids are put in screw-cap jars which are placed on ice and taken to the laboratory for analysis. Sediment ORP is measured on board the monitoring vessel immediately after a sample is obtained. Methods for analyses of sediment grain size and sediment ORP followed methods described in EPA procedures specified in the NPDES permit.

Measurements of the organic content of the sediments have changed over the course of the monitoring program. Initially, organic content was estimated by measurement of the percentage total volatile solids (TVS) in the sediments (Nelson, 1987b). More precise methods to give direct measurements of organic content of sediments were begun in 1990 with the measurement of total organic carbon (TOC). Considerable variability occurs in the sampling results from year to year because of inter-laboratory variability. This is a result of the changes in the bidding for the analytical support contracts over the years. In 1990, TOC was measured by Radian Corporation as a subcontractor to Science Applications International Corporation. In 1991 and 1992, analysis of total organic carbon was carried out by Geochemical and Environmental Research Group under subcontract to National Environmental Testing, Pacific Division, using the coulometric titration method. In 1993 and 1994 the TOC analysis was done by Coast-to-Coast Analytical Services, Inc. using the measurement of chemical oxygen demand (COD) by the closed reflux, colorimetric method. Values for COD were then converted to TOC multiplying the result by 0.25.

Sample Processing

Handling and processing of biological samples have followed EPA procedures specified in the permit and in guidance publications cited above. Non-mollusk samples are fixed with buffered 10 percent formalin for a minimum of 24 hours. Following fixation, all samples are placed in alcohol until they are processed. Mollusk samples are places in labeled jars in the field, then placed on ice and transported to the lab and refrigerated prior to being washed in fresh water (to minimize loss of fine sediments), fixed in 75 percent isopropyl alcohol for 24 hours, and then air

dried. A subsample in a 10-cm^3 aliquot was removed from each mollusk sample for sorting and subsequent identification.

Elutriation of the non-mollusk samples was done using the technique of Sanders et al. (1965). This method has been found to very efficient at successfully removing from the sediment all organisms that are not heavily calcified (Nelson et al., 1987b). Samples are then washed several times, and the water from each rinse is poured through a 0.5-mm mesh sieve so the remaining sediment can be retained for micromollusk sorting. Subsamples (10 cubic centimeters [cm]) are then sorted for micromollusks following the methods developed for Hawaii by Kay (1980) and Kay and Kawamoto (1983). Since 1992, for samples which contained rubble pieces, the rubble was acid-dissolved to find endolithic and cryptic species. This acid bath fraction could have contributed to the somewhat higher species richness and abundance recorded in the post-1992 samples as compared to previous years.

Caution must be used in the interpretation of data because the biological subcores have to be processed in two different procedures, one for micromollusks and the other for all other organisms. The two components of the fauna are not directly comparable and must be analyzed separately (Nelson et al., 1987b). Because the micromollusks are not separated into living versus dead shell fractions, these samples represent time-averaged samples but are appropriate for interpreting changes for Hawaiian waters as demonstrated by the extensive analyses done by Kay (1975, 1978, 1979b, 1982), Kay and Kawamoto (1980, 1983), Nelson (1986), and Russo et al. (1988).

Dr. E. Alison Kay, the world authority on the micromollusks of Hawaii, has described the methods used for micromollusk examination (Kay and Kawamoto, 1983) to be interpreted as follows:

> The samples analyzed are time-averaged collections which represent the last year (or perhaps even several years) accumulation of sediments. The validity of the results is based on two assumptions: (1) that transportation is minimal and (2) that total populations are accurate indicators of benthic communities. There is now a considerable body of evidence indicating that transportation does not play a major role in mixing shelly benthic assemblages (Ekdale, 1978; Warme et al., 1976) and there is increasing evidence that total microfaunal assemblages are more accurate indicators of general environmental conditions than living assemblages (Scott and Medioli 1980). (Kay and Kawamoto, 1983).

For the benthic biological samples, all specimens were identified to the lowest taxonomic level possible, generally species. Sources of taxonomic citations for the identification of Hawaiian marine benthic species was provided in Nelson et al. (1987), which is updated in an annual bibliography as appropriate. Voucher specimens have been submitted to taxonomic specialists for verification when necessary, and new species or key changes found for taxa are described in the annual reports. All specimens are archived for maintenance for six years following collection by the City & County of Honolulu laboratory.

Data Analysis

As described (Nelson et al., 1994b), all data are tested for assumptions of normality by the Kolmogorov-Smirnov test, and heterogeneity of variances by F test or Levene Median Test prior to statistical analysis in accordance to classical statistical methods (Sokal and Rohlf (1981). Comparisons of mean values among stations were made with one-way analysis of variance (ANOVA) followed by the *a posteriori* Student-Newman-Keuls (SNK) test which is used to determine which differences among stations are significant. Statistical computer packages have changed over the years, with the most recent analysis done using Sigma Stat for Windows software (Nelson et al., 1994b). In cases where heterogeneous variances were found which could not be corrected by application of standard transformations, the Games and Howell test of equality of means has been applied (Sokal and Rohlf, 1981).

Cluster analysis is used to provide an overall comparison of species composition among stations for each sample date (Pielou 1984). The Bray-Curtis similarity index (Bloom 1981) on untransformed data was calculated with clustering being carried out using the flexible sorting strategy (Nelson et al., 1994b). To make the analysis more manageable, only those non-mollusk species which contributed at least 0.05 to 0.09 percent (exact criterion determined for each year) of the total abundance were included in the cluster analyses (Nelson, 1993). For example, in 1994, this criterion resulted in the inclusion of species represented by a total of more than three individuals in the analysis (Nelson et al., 1994b). As a result, the data set for analysis was reduced from 159 to 97 species which is typical (a one-third reduction in the data set for analysis). The Cluster program developed by the EPA Corvallis Environmental Research Laboratory (Mathews, 1981) was used to compute the dissimilarity matrix (Nelson et al., 1994b).

Two derived variables were calculated, as required by the NPDES permit. The Quattro Pro spreadsheet software by Dr. Walter G. Nelson, the principal author of the annual reports prepared under contract to the University of Hawaii Water Resources Research Center, is used to calculate the Shannon-Wiener diversity index (H') ($\log_{10}$) and evenness index (J) for all stations (all replicates pooled).