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Point Loma Outfall Review

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**Point Loma Outfall Project**  
**Scripps Institution of Oceanography**  
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**I. Introduction**

Scripps Institution of Oceanography (SIO) was hired by the City of San Diego to assess the adequacy of the Point Loma Ocean Outfall (PLOO) monitoring system in providing the scientific understanding necessary to answer relevant questions about the effects on the ocean environment of the outfall and the potential issues that could arise if there were increases in the discharge volume and solids. SIO also was asked to recommend changes required to meet this need. A team of scientists conducted a review of existing monitoring capabilities in San Diego, as well as examining programs elsewhere that address similar outfalls.

The City of San Diego's ocean monitoring program has been underway since 1991, in response to regulatory requirements associated with the discharge of wastewater from the PLOO. This effort provides a very significant foundation, particularly in the benthos, for understanding environmental impacts. The City's program has been useful to regulatory agencies in assessing requirements for treatment and provides the context for future work.

The City, working with other interested stakeholders, recognized that new information is available from ongoing research, and new monitoring capabilities are being developed. As a result, the City asked for this report to help consider enhancements to its ongoing monitoring effort making it more effective in assessing the impact to human and ecosystem health and preparing for the possibility of increased output from PLOO in the future. The report and its recommendations should not be taken as a criticism of the City's program. Rather, they represent a forward-looking long-term view of the broad needs of the region. The report provides a means for the City to gain a more quantitative understanding of the role of the PLOO in the local and regional context of water motion, planktonic and benthic ecosystems, and potential human health effects. We have also addressed emerging technologies that may soon be suitable for inclusion in routine monitoring programs to help responsible agencies anticipate and prepare.

The City and SIO recognize the need for an ecological risk assessment, which will enable the City to determine priorities for implementation. The approach to this study is to look at the entire region and consider the activities not just of PLOO, but of other possible sources of contamination as well. The proposed studies should help in providing a firm scientific base for evaluating future policy decisions regarding the impacts of expanding capacity of the Point Loma outfall. The recommendations from this report, taken in the

context of an ecological risk assessment and human health risk assessment, can provide a framework for more informed decision-making in determining public priorities, scientific and technical feasibility, and cost.

The complexity of the San Diego coastal region and the multitude of potential sources for contamination led the team to take a fresh, regional look at monitoring systems that have been implemented to meet regulatory requirements. The team gave priority to a regional assessment of the monitoring needed to understand the potential impacts of contaminant and particulate materials from the Point Loma Ocean Outfall integrated with consideration of other possible sources (e.g., International Wastewater Treatment Plant, South Bay Ocean Outfall, EPA Dump Site, San Diego Bay, Mission Bay, Tijuana River, and San Diego River). Each jurisdiction may have a dedicated monitoring system that meets the specific requirements imposed by the relevant regulatory agencies. However, the discharges from the various sources ignore political boundaries and legal jurisdictions and interact with the natural environment and with each other, changing over time with seasons, weather, and other variables. As a result, a good understanding of the potential impact of any one source on the coastal marine ecosystem can best be achieved with the data from a coherent regional monitoring program that addresses all the sources and their receiving waters.

The specific goals were to

- identify for the City of San Diego the information required to assess the impact of the Point Loma Ocean Outfall on receiving waters, including the potential impact of an increased outflow;
- identify areas and issues for which the information currently available is insufficient; and
- make recommendations on required observations and sampling strategies.

The study was not an evaluation of the impact of the outfall, it was not a risk assessment to determine the levels of acceptable water quality, and it was not a scientific research project. The team did not attempt to assess the City's compliance with its regulatory obligations. Rather, the team considered the scientific questions relevant to understanding the potential impact on the ocean environment and developed recommendations for a monitoring approach that would provide the information needed to understand the potential impact, whether required through regulation or not. The study was an assessment and offers recommendations for developing a monitoring strategy. The team was not asked to provide implementation details or to assess costs.

## **II. Summary of Findings**

The primary findings are summarized below.

1. The coastal waters relevant to the Point Loma Ocean Outfall are complex and could be subject to impact from a number of potential contamination sources in the region. Presently, it is not always possible to differentiate impacts from PLOO and other possible sources. There is no interpretation of contributions from the many separate sources of contamination of the shelf off San Diego. These include tidal outflows from two large bays, two large rivers draining large watersheds loaded with potential pollutants, sewage discharge from two large municipal outfalls, and a disposal site.

### **Physical Oceanography, Plankton And Modeling**

2. The City does not adequately monitor or understand the physical circulation of the coastal waters relevant to the Point Loma Ocean Outfall in terms of spatial and temporal variability and synoptic patterns (e.g., seasonal variability or in response to episodic events), or the geographic extent of the “receiving waters.”
3. The location, movement, and dispersal of the plume from the outfall is also inadequately monitored and understood.
4. Because of the lack of knowledge of the plume’s location, its impact on the planktonic community is unclear. The spatial and temporal resolution, and the types of measurements currently made are inadequate to quantify the effects of chronic nutrient loading on the plankton relative to natural nutrient sources and other anthropogenic sources.

### **Benthic Monitoring**

5. Understanding the impact of the outfall on the benthic environment requires modification of the existing monitoring program, primarily to provide more appropriate control stations. Currently the control sites, because they are substantially different in the character of their sediments from the other monitoring sites, and because they may be contaminated from sources other than Point Loma, do not provide a basis for evaluating benthic impacts with confidence.
6. Present monitoring does not include integration of littoral transport cells. Therefore, it is possible that contaminated sediments are accumulating downslope from the shelf, and because this area is not monitored, there is presently no way to know if the effects of the PLOO or other sources of contaminants are accumulating in these areas. The Silver Strand littoral cell accretes into areas that are certain to advect into the Bay and the kelp forest.

7. There are data suggesting possible contamination phenomena; these have not yet been evaluated. Several examples are given in Section IV of this report. Further analysis may provide insights into other hazardous sources of contamination and enhanced understanding of coupling between patterns observed in the water column with those observed in the sediments and benthos (benthic-pelagic coupling). This may enhance the interpretation of spatial pattern observed over the shelf.

#### Microbial Monitoring

8. More rapid techniques than are presently being used for identifying indicator bacteria and pathogens are in late stages of development.
9. There are opportunities for San Diego to rapidly assess health risks as well as to more precisely track the source of contaminating microbes, working in partnership with other agencies.

### **III. Summary of Recommendations**

A fundamental goal for the City should be to understand where the effluent plume is and where it will go, in order ultimately to assess its potential impact. Additional work is needed in analyzing the physical oceanography relevant to the outfall. There is no clear definition of the “receiving waters” for the outfall and no comprehensive understanding of the location and movement of the plume from the outfall. This makes it impossible to distinguish impacts of the Point Loma Ocean Outfall from those of other sources. The following recommendations address general approaches, physical oceanography and modeling, benthic monitoring, and microbial monitoring. The recommendations are based on the scientific principles and our assessment of the key questions and current monitoring capabilities. Where possible we have related the recommendations to the structure of the City’s current monitoring program, which includes a core program, regional monitoring, and special studies. Within each section, recommendations are in priority order. However, the team did not prioritize between the different sections as all are interdependent and need to be addressed.

1. Because of the complexities of the Point Loma Ocean Outfall area, we recommend that the City join with other relevant jurisdictions to design and implement a regional monitoring approach. This would enable more effective deployment of monitoring resources on a shared basis and provide the information needed to enable analysts to distinguish among the various sources of problems. The data from such a regional system must be made openly available in a timely manner for all interested parties to use.

2. The City should undertake a human and ecosystem health risk assessment to use in developing implementation plans for enhanced monitoring capabilities and special studies.
3. To enhance the value of the past investment in monitoring programs, to identify and fill some gaps in current knowledge, and to enhance future studies, data collected by the City and other agencies should be made available to enable re-evaluation and further analysis using regional and long time-series perspectives as well as multivariate techniques. Examples of areas that could benefit from further use of existing data include some of the special studies recommended in this report, such as plume location, sediment maps, mass balance calculations, benthic reference conditions, and ecosystem risk assessment.

### **Physical Oceanography, Plankton And Modeling Recommendations**

4. In order to understand where the PLOO plume goes, the SIO team recommends that the core monitoring system include the capability to observe the three-dimensional circulation in the area with emphasis on the spatial and temporal variability. This includes defining the major synoptic patterns found in the region. The full report provides details of instrumentation, experimentation, and modeling recommended to understand and predict the movement and variability of the coastal waters, including moored profilers, coastal radar, a tracer experiment, and nutrient monitoring.
5. To define the geographic scope of an enhanced regional monitoring program, the City should conduct a special study to define the PLOO “receiving waters” that are estimated to go from the Mexican–U.S. border north to Point La Jolla and offshore to the 100 m isobath. We recommend validation or refinement of this definition using an integrated observing system.
6. The SIO team recommends the implementation of circulation models into the core program to support effective management decisions and adaptive monitoring. A model is needed to help synthesize the data, and to aid in identification of recurring patterns of circulation and their impact on the plume’s location and dynamics. This is important in distinguishing among possible sources of contamination and their effects on the planktonic and benthic ecosystems. Special studies are recommended to ensure a capability to provide hindcasts after a problem is detected, to establish if there are patterns that can help identify likely sources.

### **Benthic Recommendations**

7. We recommend establishment of a regional benthic monitoring program designed to evaluate the effects of discharges from the two outfalls as well as both bays, rivers, and the dredge disposal site off Point Loma. This will involve integrating the present

core monitoring programs for the Point Loma and South Bay Ocean Outfalls to enable a better definition of contaminant sources.

8. A key element of this regional approach should be the selection of suitable reference sites independent of the major contamination sources. There are no suitable reference sites between La Jolla and the Mexican border. The SIO and Oceanside nearshore zones should be considered.
9. A special studies sampling program should examine the need to extend the core benthic monitoring stations to additional areas where sediments may be accumulating.
  - a. There should be a one-time (special studies) development of a shelf sediment budget to determine the spatial distribution of sedimentation and erosion, with the goal of identifying sites where contaminated sediments accumulate.
  - b. Specific targets should be areas further offshore (slope and submarine canyons), the dispersal shadows of the toxic dump site LA5, and the Silver Strand littoral cell.
  - c. Depositional sites should be subject to more sensitive analysis of sewage exposure (e.g., linear alkyl benzenes, compound-specific stable isotope signatures) to document sewage-related sources.
  - d. These results should be used to modify the core benthic monitoring stations and possibly the sediment geochemical measurements.
10. A special studies program should identify the transport and dispersal of fine sediments in the entire region. The dispersal of these fine sediments is very poorly understood in general, but it is obviously an important transport system for pollutants as well as total carbon and nitrogen in the regional context.
11. Because hormones are important and have been shown to be present and have significant effects at other wastewater facilities, we recommend that the City, in conjunction with a regional monitoring program, participate in the growing number of collaborations presently forming to address effects of endocrine disruptors on the benthos and their consumers.
12. We recommend the establishment of a program to determine the spatial extent of source-specific contamination using outplanted organisms such as mussels (or possibly biological mimics) that assimilate contaminants.



## Microbiology Recommendations

13. Because the origin of the bacteria being identified is not always known, we recommend that microbial source tracking methods should be implemented in-house in the near future. Capability needs to be developed regionally with participation by the City so that the City can focus management actions effectively.
14. The City should participate in special studies on a regional basis to bring these new microbial source tracking techniques to fruition and implement them when they become available.
15. A public health risk assessment should be conducted to determine what pathogens pose the greatest potential risk and use that as a basis for developing a monitoring capability for the relevant bacteria and viruses.
16. Special studies should be conducted to monitor human viruses and follow their abundances on an ongoing basis in an experimental mode. Human viruses should be monitored using either molecular methods which directly test their presence or by adopting bacteriophage such as F<sup>+</sup>-specific RNA coliphage or *Bacteroides* phage as a proxy for their possible presence.
17. Since molecular methods make it possible to follow the presence of many potential pathogens relatively quickly, risks from other infectious agents whose presence does not correlate with sewage should also be considered.
18. Likewise, advances in detection strategies such as those employing DNA arrays may make it cost-effective in the future for the city to consider monitoring the abundance of pathogens which are capable of infecting marine life such as marine mammals, and whose presence may or may not correlate with sewage.

The remainder of this report provides more detail on the findings and recommendations summarized above. Together with the references provided at the end, this report provides the most current and comprehensive assessment of ocean effluent monitoring and modeling relevant to San Diego and the Point Loma Ocean Outfall.

#### **IV. Physical Oceanography, Plankton and Modeling**

The City of San Diego is considering increasing the daily discharge from the treatment plant located on Pt. Loma, from the current value of about 175 millions of gallons per day (MGD) to the full 240 MGD permitted by the State Regional Water Quality Review Board. To assess the impact of this increased discharge on the environment, and on the quality of coastal waters, there is a need to understand how the discharged waters are transported by, and mix with, the coastal waters off San Diego.

The assessment of the impact of any anthropogenic activity begins with a description of the natural environment where the activity is to take place. Once such a description is available, the next step is to define the areas that are affected and the degree of disruption that is caused. The required descriptions and the impact evaluation are complicated by the wide range of spatial and temporal scales over which physical, chemical, and biological processes occur. The weather is a good analogy for how the coastal ocean behaves. While we have a certain idea of what weather to expect at different times of the year, the actual weather conditions (wind, temperature, rainfall) vary considerably over a broad range of spatial and time scales, and our skill at predicting the weather is still limited. Similarly, in the ocean, variables such as currents, temperature, and density that determine the eventual fate of the products released at the outfall, vary over times ranging from seconds (surface waves) and minutes (internal waves) to several years and even decades (e.g., El Niño). Spatial variability exists over a comparably broad range of scales. Weather and climate are unpredictable. They are described in terms of their statistical properties, just as weather forecasts are given as statistical descriptions. The circulation in the coastal ocean is comparably unpredictable, but with sufficient observation the circulation can still be described in terms of statistical properties.

While the City has performed extensive monitoring in compliance with the National Pollution Discharge Elimination System (NDPES) permit issued by the California Water Quality Control Board, this monitoring was intended to show compliance with permit standards, not to develop the mechanistic understanding of how the plume is transported by and mixed with coastal waters. This information is required to make any assessment of the impact of increased discharge.

As stated above, the circulation in the coastal ocean off San Diego is as variable as the weather, and any description of impact must reflect the inherent variability of naturally occurring processes. In contrast with other coastal locations in the Southern California Bight, the coastal ocean off the City of San Diego, the receiving waters for the Point Loma Ocean Outfall, is relatively unknown.

## **A. Issues To Be Addressed**

From the point of view of physical circulation, before the impact of the outfall can be assessed, it is recommended that the following issues be addressed, through closely coordinated observational and modeling studies.

### *1. Definition of the physical properties of the receiving waters.*

Physical properties include the stability of the water column (because the outfall depends by design on the vertical stability to reduce the possibility that bacteria-laden waters might reach the surface), as well as horizontal circulation (that is responsible for the eventual fate of the introduced waters). This description needs to cover the relevant range of spatial and temporal time scales.

### *2. Definition of episodic events.*

Many important processes in the ocean (e.g., sediment transport) are only active during short-lived episodic events and can be completely missed by regular but infrequent sampling. In terms of this study, it will be important to determine what happens to contaminants released from all sources during extreme events such as winter storms or Santa Ana conditions, as well as during other times.

### *3. Variability of the location of the plume.*

As effluent is discharged at depth, it mixes with ambient seawater as it rises toward the surface under its own momentum and buoyancy. During this mixing, the effluent is diluted and becomes denser, losing momentum and buoyancy to the surrounding fluid. Eventually the diluted effluent will either reach the surface or stop rising at some depth below the surface where it is neutrally buoyant, forming a plume. The region of active mixing is known as the “near field” (Roberts 1999a, 1999b; Roberts et al., 1989a, 1989b). The region of plume spreading after the cessation of buoyancy-driven turbulence is known as the “far field.” The near field is established within minutes, and typically extends tens to hundreds of meters horizontally from the diffuser. The dynamics influencing the far field occur over spatial scales of hundreds of meters to tens of kilometers and time scales of tens of minutes to weeks. Knowledge of the vertical and horizontal location of the plume and the temporal variability of its structure is key to understanding the impact of the plume on the waters, plankton, benthos and beaches.

The amount of dilution of the effluent is a critical parameter influencing the probability of effluent bacteria reaching beaches. In the near field, the dilution is inversely related to the ambient density stratification and effluent discharge rate (higher stratification and discharge tend to force less dilution) and directly related to the speed of the ambient horizontal current (stronger currents force higher dilution; Roberts et al., 1989a, 1989b). The depth of the plume below the surface is directly related to the stratification and the ambient horizontal velocity (higher stratification and higher velocity give deeper plume). Thus, understanding the location of the plume at the end of the near field depends critically on knowing the vertical profile of density and the ambient horizontal currents and their vertical shear. Because the ambient currents can change on time scales of tens

of minutes (nonlinear internal waves) to hours (tidal motions) and days (remotely forced currents), it is apparent that even over a tidal cycle significant changes in plume dilution and depth would be expected even if everything else (effluent discharge rate, stratification, etc.) were constant.

Given the significant changes in horizontal velocity over a tidal cycle, and the anticipated changes in the depth and dilution of the plume, it may be more realistic to envision the discharge from the diffuser as forming “puffs” (e.g., Csanady, 1983a, 1983b) than a single coherent plume. Once these puffs are formed and have found their neutral buoyancy, they will be advected with the ambient currents at that depth. In stratified waters, these currents can change significantly in speed and direction through the water column, giving strong vertical shears. The strongest shears will be found at the strongest vertical density gradients, where vertical mixing (and dissipation of energy from the vertical shear) is suppressed. It is therefore critical to know the vertical distribution of velocities through the water column and how this changes with time. Puffs at different depths may follow completely different trajectories in space; puffs of different ages may even cross over and under each other (e.g., Petrenko et al., 1997).

Once the circulation and mixing in these receiving waters are established, and the dynamics of the effluent under current and projected operating conditions are known, the impact of an increase in effluent volume and mass loading can be evaluated given a description of the engineering parameters associated with the new loading.

Other sources of effluent and pollution will also form three-dimensional patches that vary in space and time. The characteristic spatial and temporal scales of the patches will depend on the source (e.g., point source, distributed source) and the physical dynamics and hydrographic properties of the receiving waters. Quantifying the scales of patchiness and the regions of influence of the various sources of contamination is an essential element of the proposed monitoring program. Distinguishing among the various potential sources of contamination may be fundamental to demonstrating compliance for the PLOO.

#### *4. Nearshore transport.*

The nearshore region includes the surf zone offshore to the outer edge of the coastal boundary layer—approximately 10 km. The circulation and the dynamics of the narrow strip of coastal ocean known as the surf zone can be quite different from the waters farther offshore. Relatively large currents directed alongshore can occur, driven by the incident shoaling surface gravity wave field. This surf zone circulation field interacts with the deeper waters on the continental shelf through turbulent mixing processes, including rip currents, internal waves and tides, wind-driven circulation, tidal flushing of bays and estuaries, runoff, and flow over and around topography. While the circulation that affects the Point Loma Ocean Outfall plume is probably not affected by the dynamics of the surf zone region, coastal sources of contamination, either point sources as in the case of the South Bay Ocean Outfall, the Tijuana and San Diego Rivers, San Diego and Mission Bays, or distributed sources as in the case of storm drains, certainly are. In

addition, nearshore circulation dynamics can transport effluent plumes into the surf zone circulation, with potential for powerful alongshore transport and beach contamination. To develop a true regional understanding of the quality of coastal waters in San Diego, therefore, consideration will have to be given to the circulation in the nearshore and surf zone regions and their variability. Here we briefly summarize some of the dominant physical dynamics.

*Surf zone transport:* The breaking of surface waves on a sloping beach will generate significant ( $1\text{--}2\text{ m s}^{-1}$ ) alongshore currents in the surf zone. These currents are often interrupted by rip currents that transport material offshore/onshore. The speed and direction of the surf zone currents are influenced by the amplitude and direction of the surface wave field. There is the potential for nearshore effluent plumes near the surface to become entrained into the surf zone circulation, and transported along shore far beyond the offshore location of the plume (indeed, potentially in the opposite direction of the plume's motion). These dynamics have received little investigation in the context of effluent transport.

*Internal waves:* Linear (small amplitude) and nonlinear (amplitude is a large fraction of the water depth) waves are prevalent features of the coastal circulation along the coast of the SCB (Lennert-Cody & Franks 1999; Pineda, 1994, 1999;) and have been shown to influence the transport of effluent plumes (Boehm et al., 2002; Petrenko et al., 2000). These waves form as the tide moves over submerged banks offshore, and they propagate with speeds of about  $20\text{ cm s}^{-1}$  and amplitudes of up to 15 m. With horizontal wavelengths of 100 m – 1 km, internal waves and the internal tide can generate significant vertical shear of the horizontal currents through the water column. In shallow water the waves lose energy and may break, causing vertical mixing. Internal waves need a stratified water column to propagate.

*Tidal currents:* The dominant tidal components in this region are the semidiurnal tide and the fortnightly spring-neap cycle. The tidal currents can be up to  $20\text{ cm s}^{-1}$ , and the dominant period is 12.42 hours giving significant changes in speed and direction over time scales of several hours. As the tidal flows move onshore into shallow waters, the current directions change to predominantly along shore.

*Freshwater flow:* Buoyant freshwater plumes occur infrequently in this region, but are obvious during rainy periods. They are often trapped near the coast (depending on the wind) and can propagate at speeds of tens of  $\text{cm s}^{-1}$ .

*Wind-driven flows:* Wind forcing can cause local upwelling and downwelling, as well as alongshore currents of tens of  $\text{cm s}^{-1}$ . Upwelling takes some time (at least one inertial period—about one day) to spin up, and requires a reasonably steady wind over more than one day. The daily sea breeze blows onshore in the afternoon, with initial surface water motions in the direction of the wind.

*Topographically forced flows:* The coastal topography around PLOO is complicated by the presence of Point Loma. Horizontal currents tend to follow bathymetric contours; if these contours have strong curvature, the currents may overshoot, leading to local upwelling or downwelling. Flows past headlands can also generate eddies that may persist (retaining water locally) or propagate away. Such eddies are known to form at the mouth of San Diego Bay at each tidal cycle.

*Remotely forced flows:* Winds off Baja can generate coastally trapped waves that can propagate up the coast at speed of 2–3 m s<sup>-1</sup> (Pringle & Riser, 2003). These waves can create sudden elevations of the pycnocline, with the upwelling of bottom waters inshore. Other large-scale forcings determine the speed and direction of the ambient coastal currents in the region over time scales of days to years.

## **B. Present Monitoring Program**

**Plume location:** The present monitoring program uses CTD casts to survey an area that is likely to contain much of the diluted effluent plume. Unfortunately, the temporal resolution of the sampling is inadequate to properly capture the variability of the plume location and concentration over most of the dominant time scales: hours to days. Episodic events cannot be resolved by this sampling program, and most of the dominant physical forcings are not resolved. Furthermore, CTD casts by themselves cannot resolve the actual motion of fluid parcels. This would require current meters, drifters, tracers, etc., as described below.

**Other sources:** The present monitoring program cannot resolve the location of the PLOO effluent over many of the relevant time scales. Furthermore, it cannot distinguish among the various potential sources of contamination in the region. With the present monitoring program it is impossible to determine whether a local contamination event was caused by the PLOO waters or waters from the South Bay Ocean Outfall, bay flushing, episodic river flooding, storm water runoff, or other sources. It is entirely possible that the PLOO is completely compliant with the permits, and that contamination in the region arises from other sources. Distinguishing these sources is critical to evaluation of the sewage treatment program.

## **C. What Can We Learn From Other Locations?**

The temporal and spatial variability of the processes that determine the eventual fate of the products released at the outfall must be determined before environmental impact can be assessed. Throughout the past decade, several studies have successfully documented the variability in circulation and mixing in the Southern California Bight. Three of these are briefly reviewed here, to form a context for the recommendations.

**Huntington Beach Study:** The Orange County Sanitation District (OCSD) operates a sewer outfall that discharges approximately one million cubic meters per day (250 MGD) of treated wastewater through a diffuser located 7 km offshore, in water depth of 55 m. The operating characteristics are similar to those that characterize the Point Loma Ocean Outfall, though the depth of the diffuser and its distance offshore may have important implications for the development of the near field plume. In response to a series of closures in Huntington Beach, caused by high levels of indicator bacteria, OCSD sponsored a number of oceanographic studies to determine whether the discharge contributed to the poor water quality on the beach. The program consisted of several observational and modeling studies, and results from the studies that are most relevant to the PLOO are summarized below.

Moored observations of selected physical parameters (Science Applications International Corporation, 2001) were obtained continuously from June 1999–June 2000, and a second period of moored observations was conducted in the summer and fall of 2001. Boehm et al. (2002) reported a separate analysis of these observations. Year-long observations of temperature at the outfall suggest that the temperature and density field occur in two states. During winter and early spring, the temperature difference across the 55 m deep-water column is weak, about 2°C. This difference is nearly four times larger during the rest of the year. The temperature varies on all the time scales enumerated above, and the stability of the water column changes in consequence. Currents measured at the outfall fluctuate primarily at synoptic periods (a few days to weeks). They are directed alongshore, with typical amplitude of 0.1 ms<sup>-1</sup> corresponding to displacements of about 10 km per day in either direction. There is little vertical shear during the winter. In summer, the largest currents are near the surface and the velocities decrease almost linearly with depth. Internal tides, accompanied by large cross-shore current fluctuations occur year-round between the beach and 40 m depth.

Shipborne surveys of the plume were made on 10 occasions, during the period September 1999–March 2000. The results show that the plume is transported by the alongshore current: upcoast current carries the plume upcoast, and vice-versa. The plume was found closest to the coast (1 to 1-1/2 kilometers from shore) when currents were downcoast (toward the southeast) and weak. The plume was always found more than 20 m beneath the surface. In 9 out of 10 cruises, the plume could be identified at least 7.5 km away from the outfall, and in some cases it was detected 12.5 km away. The plume width varied between 1 and 7.5 km, depending on distance from the outfall. In the vertical, the center of the plume was located about 40 m beneath the surface.

Models of the near field and far field mixing phases have been developed (Science Applications International Corporation, 2002). The near field model suggests that the plume can, on rare occasion, reach the surface during the winter. The stronger stratification during the rest of the year keeps the plume beneath 25 m. The far field model results describe the size-dependent distribution of wastewater solids over the bottom. The largest accumulation occurred over a relatively small area located inshore and to the south of the diffuser.

The Huntington Beach program represents a model for a minimal program to develop the information required to anticipate the impact of increased discharge at Point Loma. Particular strengths of the OCSB program are (a) the integration of moored and plume tracking studies to develop a mechanistic understanding of where the plume can go and (b) the further inclusion of near and far field models to synthesize the observational results. Furthermore, these physical models can be augmented with other models to predict fields such as settling of different sized particles or the impact of dissolved nutrients on the planktonic ecosystem.

**Santa Barbara Channel–Santa Maria Basin Circulation Study:** The Santa Barbara Channel–Santa Maria Basin (SBC–SMB) Circulation Study was sponsored by the Minerals Management Service with the dual goal of predicting how future oil and gas exploration and production might affect the environment, and of developing information required for emergency response. The program combined field observations for more than 10 years, with the development of numerical programs to synthesize the observations and to produce hindcast capability.

The observational program included a meteorological component described by Dorman and Winant (2000) an extensive program of moored observations (Harms & Winant, 1998; Winant et al., 2003), and drifter observations (Dever et al., 1998; Winant et al., 1999, 2003). This was the first program to describe the circulation in terms of characteristic flow patterns. Three patterns were found to describe the circulation in the area between Morro Bay and Ventura. An upwelling pattern consists of a prevailing equatorward flow at the surface and at 45 m depth, except in the area immediately adjacent to the mainland coast in the SBC where the prevailing cyclonic circulation is strong enough to reverse the equatorward tendency and the flow is toward the west. In the surface convergent pattern, north of Point Conception, the surface flow is equatorward while the flow at 45 m depth is poleward. East of Point Conception, along the mainland coast, the flow is westward at all depths and results in a convergence at the surface between Point Conception and Point Arguello, with offshore transport over a distance of the order of 100 km. Beneath the surface layer the direction of the flow is consistently poleward. The relaxation pattern is almost the reverse of the upwelling pattern, with the exception that in the SBC the cyclonic circulation is such that the flow north of the Channel Islands remains eastward, although weak. The upwelling pattern is more likely to occur in March and April, after the spring transition, when the winds first become upwelling-favorable and while the surface pressure is uniform. The surface convergent pattern tends to occur in summer, when the wind is still strong and persistently upwelling-favorable, and the alongshore variable upwelling has built up alongshore surface pressure gradients. The relaxation pattern occurs in late fall and early winter, after the end of the period of persistent upwelling-favorable winds.

The moored observations extended for more than 10 years and captured the very strong El Niño event that took place in late 1997 and early 1998. During the El Niño period the physical properties of the coastal ocean were modified in two ways that could affect



outfall operation: (a) the vertical stratification became very weak relative to other times and (b) the coastal current was anomalously strong, directed toward the north.

The large temporal and spatial variability described earlier is present in the SBC–SMB area. It would have been difficult to relate the different synoptic patterns to the forcing. A three-dimensional numerical model (Oey et al., 2001, 2004) has been developed to simulate the circulation in this region. The model assimilates near-surface temperature observations and reproduces well all major features of the observations. This study demonstrates how a real-time (nowcast) model that assimilates a few observations could be used to define the state of the circulation in the waters surrounding the Point Loma Ocean Outfall at any time.

**San Diego Coastal Ocean Observing System (SDCOOS):** Just as in Huntington Beach, high levels of indicator bacteria have forced repeated closure of Imperial Beach. SDCOOS ([www.sdcoos.ucsd.edu](http://www.sdcoos.ucsd.edu)) monitors oceanographic, weather, and water quality parameters in an area between the Mexican–U.S. border and Point Loma, extending from shore to the Coronado Islands.

The condition of any beach, in terms of fecal indicator bacteria (FIB) levels, is determined by a number of public health agencies. When shoreline water quality fails to meet the standard, warning signs are posted to notify the public. Kim and Grant (2004) point out that because of the time required to determine the FIB content of any sample, by the time the beach has been posted, the water quality may have improved to where it meets state standards. This and other factors result in a misnotification problem. The ultimate goal of the SDCOOS is to construct a statistical relationship between water quality and circulation parameters that can be determined rapidly, thus improving the reliability of the warning system.

The project, funded by the California Clean Beach Initiative, merges well-understood measuring systems with advanced current mapping radars to provide near real-time observations of relevant parameters. Hourly maps of the surface circulation in the SDCOOS area can be consulted on the web site in near real time, along with a large number of related parameters, including recent beach water quality samples and vertical profiles of current.

The area covered by SDCOOS is the southern half of the receiving waters from PLO, as defined here. While the measurement program currently in place is not exactly what is required in order to develop the required information, every attempt should be made to insure the continuation of this program.

## **D. Physical Oceanography, Plankton and Modeling Recommendations**

Given the current lack of information available to describe circulation and mixing in the coastal area affected by PLO, it is recommended that the City implement a program that combines observations and modeling as a way to fill this important knowledge gap. The different components of this plan are described in the following.

There are two specific goals. The first is to *describe the three dimensional circulation in the area, with emphasis on the spatial and temporal variability*. A useful approach to this objective would be to develop a description of the circulation in terms of a few synoptic patterns, comparable to weather patterns that forecasters use to make predictions (e.g., Santa Ana). The second specific goal is to *describe the structure of the plume and where the plume goes*, again emphasizing spatial and temporal variability. The second objective will effectively define the “receiving” waters.

A basic premise behind the recommendations presented here is that the synoptic view taken by forecast meteorologists in making short-term weather predictions is a useful starting point for describing the state of the Point Loma Ocean Outfall plume. Synoptic meteorologists have in mind, at the time they are making a forecast, a finite number of possible situations that experience has shown to capture most of the variability in the local weather. For instance a Santa Ana weather condition is often forecast during summer and fall, and weather fronts are common occurrences in the winter. In the context of Point Loma, experience suggests that the plume occupies a finite number of states, and that these can be related to oceanographic parameters that are relatively easy to monitor in real time. The basic recommendation is therefore to identify the different possible states of the plume, as well as the corresponding distributions of anthropogenic products, and relate those states, either statistically, through a stochastic model, or mechanistically, through a three-dimensional circulation model, to available oceanographic parameters.

**A straw-man definition of receiving waters:** To design an observing system, there has to be an initial definition of the size of the area of interest. The Orange County plume tracking study described earlier concludes that the OCSO plume can extend as far as 20 km up or down coast from the outfall. Based on this result, and because the discharge rates at Huntington Beach and Point Loma are of comparable magnitude, the area of interest will be taken to extend from the Mexican–U.S. border north to Point La Jolla (Fig. 1) and offshore to the 100 m isobath. This definition represents no more than a strawman, based on the best information currently available. The first task of the combined observing and modeling program will be to determine whether this definition is adequate.

### **1. Observations**

Experience shows that the most effective way to resolve the large temporal and spatial variability in the coastal ocean is to combine different sampling schemes. Some are designed to resolve temporal fluctuations at a selected location (in situ), others are intended to provide snapshots at a few times (synoptic surveys), while still others observe

properties of water parcels as they are followed (drifters). The observing component should *integrate in-situ physical, chemical, and biological observations from moorings and coastal stations (piers and beach sites) with synoptic aircraft and shipborne surveys and with releases of drifters drogued near-surface and at mid-depth*. The observations will include not only physical properties but also parameters that describe the chemical and biological content of the waters. The overarching goal of the observational program is to identify patterns of covariation in the field of observed variables, or, in other words, the synoptic patterns of variability. Because the ocean circulations change from one year to the next, as is clear for instance when an El Niño condition occurs, it is of critical importance that the period during which observations are acquired span several years, including one El Niño season.

**Moored observations:** Moored observations, supplemented by a network of land-based meteorological and environmental stations, are a core component of the recommended observing system, and are recommended as the highest priority. Moored measurements are ideally suited to determine the temporal structure of the circulation. Moreover, the relative importance of episodic events can only be determined through time series measurements.

The highest priority is to install a mooring near the Y of the diffuser, similar to the mooring deployed by OCS D at Station P. The mooring will at a minimum measure the vertical profile of current with an acoustic Doppler current profiler (ADCP) and profiles of relevant properties of the water column with sufficient vertical resolution to show the location of the plume. Observations need to be telemetered back to a central land station where they are processed and made available on the web. This has three advantages: it will allow rapid assimilation of the data into predictive models; it will allow adaptive sampling of events; and it will verify the functioning of the moored system. These observations alone will establish in real time at what depth the plume is spreading and, given the current profile, toward what direction it is spreading.

At least three additional moorings will be required on a transect between the central mooring and shore. One should be deployed halfway between the Y and shore, and the other two will be located on either side of the kelp bed. Minimum instrumentation includes a current profiler and a vertical array of temperature sensors to determine the stability of the water column. As in the case of the central mooring, relevant instrumentation will be integrated into each mooring as it becomes available. At least initially, these moorings do not need to telemeter observations back to shore.

In addition to this core program, a few more moorings deployed close to the perimeter of the receiving waters will be required to determine scales of covariation in the circulation and pertinent variables. These moorings may be moved in the course of the program, once the coherence properties have been established.

Many factors govern the design of the moored array. The temporal scales to be resolved run from several minutes to several years. Consequently, measurements (or at least a

subset thereof) need to span several years and be sampled at sufficiently high frequency to resolve internal waves and changes in the surface wave field, as they determine the circulation within the surf zone. Given currently available technology, resolving the high-frequency end of the spectrum poses no significant challenge. The need for long-term measurements is directly (and nearly linearly) related to the cost of the program.

Deciding in which areas moored observations should be made, and determining how densely sampled that area is, are challenging tasks. There is currently no accepted definition of the receiving water for the PLOO operation. Given the regional outlook that is promoted in this report, the area of interest extends southward at least to the Mexican border, northward to Point La Jolla, and offshore to the 100 m isobath.

While some measurements of physical parameters (temperature and currents) are relatively simple to make, the moorings can and should be used as platforms to measure biological (e.g., fluorescence) and chemical parameters as autonomous instrumentation becomes available to do so. Some analysis will be required to determine what the most effective instrumentation is to determine where in the vertical the plume is spreading. The plume is known to have high values of beam attenuation (Beam C). As sensors are developed to measure other variables, they can be tested and installed on this mooring. As in the OCSD, mooring measurements of this site will be telemetered to shore. The system will have to be flexible enough to include a wide variety of instruments, as they become available.

Profiling moorings are being developed that allow an instrument package to continuously profile through a portion of the water column. McLean Research Laboratories (East Falmouth, MA) manufactures the MMP, a moored profiler that uses a motor to move the profiling package along a vertical wire. These systems have the advantage that they will resolve thin features that might be missed by discrete instruments on a wire.

In general, two kinds of questions can be addressed using such observations: questions about the amplitude and time dependence of the measured parameters and, to a more limited extent, questions related to the spatial structure of the flow. Additionally, these observations serve two other important purposes: (a) provide rough estimates of different terms in the various balance equations that govern the distribution of relevant parameters (mass, momentum, and concentration), highlighting the most important dynamical relationships, and (b) provide measurements that can be assimilated into numerical models of the circulation.

**Plume tracking:** At this point we don't know where the water goes, or where the plume goes. Therefore, the highest priority special study is to determine the plume's location on a repeated basis, as done by the OCSD study described above (MEC and AOS, 2001). In San Diego County, the definition of the plume is complicated by the existence of two major point sources (PLOO and SBOO). Nonetheless, the techniques used to identify the plume in the Orange County study will be able to describe the location of both plumes,

identify conditions when they merge, and should be able to describe incidences of transport of effluent from the San Antonio de los Buenos treatment plant, in Mexico.

The plume needs to be mapped at different times during the year, and related to synoptic patterns of circulation. This means a minimum of twelve realizations, generating twelve plume maps. It is also necessary to resolve interannual variability of the plume by, for instance, conducting plume surveys during El Niño and non-El Niño years. At the end of the year-long sampling period, the plume(s) will have been fully described on at least 12 occasions, and the remaining task will be to identify, first by simply sorting the maps, then through more sophisticated methods of pattern analysis, what the main characteristic plume configurations are. The final step will be to relate each characteristic configuration to indicators of regional forcing, such as wind and alongshore pressure differences.

The basic methodology consists in conducting surveys with a towed underway vehicle (fish) from a research vessel, as described in MEC (2001). The towed fish is instrumented with a conductivity–temperature–depth (CTD) that measures temperature, salinity, fluorescence, attenuation, and depth. At the same time, water is pumped to the ship's laboratory to sample nitrate, coliform bacteria, and other relevant properties. Because of the variability of the circulation, it is of the utmost importance that the surveys be conducted at least on a monthly basis over a period of one year. The basic procedure for tracking the plume, as described by Wu et al. (1994) identifies plume water through a combination of properties, including transmissivity, salinity, and fluorescence.

A tracer for diluted effluent that could be analyzed in real time would be a significant improvement to plume-mapping studies. Petrenko et al. (1997) showed that fluorescence emitted at 340 nm by excitation at 228 nm was strongly correlated with the presence of the plume. The fluorescence was thought to be caused by aromatic amino acids such as tryptophan and was not correlated with chlorophyll fluorescence. Wet Labs (Philomath, OR) manufactures a submersible instrument called SAFire that measures fluorescence at six excitation and emission wavelengths.

Currently, the monitoring program does not measure nutrients. Several instruments now exist that allow real-time, continuous measurement of dissolved nutrients in situ. The ISUS (Satlantic; Dartmouth, Nova Scotia, Canada) uses an optical method to measure nitrate and can be used on a CTD profiling package. EnviroTech (Chesapeake, VA) sells the NAS in situ nutrient analyzer that uses wet chemistry to quantify nutrient concentrations. Both these instruments can be put on moorings for extended periods of time to obtain continuous measurements at a location.

**Surface and mid-water drifters:** Drifters provide the best way to identify the path of fluid parcels released at some point in the ocean. Therefore a second-priority special study is to release drifters in groups at locations near the plume outfalls and at several depths. Such a program could operate with a stable of 100 drifters, half of which are drogued near the surface, and the other half drogued at depth.

A typical drifter consists of a drag element (drogue) located at some distance beneath the surface, tied to an electronic package at the surface that measures position by the Global Position System (GPS), and transmits the information in real time to shore through one of a variety of communication systems (packet radio, wireless networks, paging systems). Once the drifter leaves the area of interest (receiving waters), they are recovered by a small boat and can be redeployed.

The usefulness of drifter observations depends on developing a statistical picture of many trajectories, and useful descriptions require large (order 1,000) trajectories to be observed. As a consequence, it is important to keep the cost of each drifter to a minimum. With current technology, and the advent of relatively cheap GPS units, drifters that can be reused many times should be available for about \$500 each. By their very nature, drifters are relatively easy to lose. A program of drifters released at regular (monthly) intervals at two depths (surface and mid-water) would be of tremendous benefit in identifying where products contained in the plume end up. The value of such a program would be even greater if the drifter releases were conducted at the same time as the plume tracking surveys.

**Surface current maps:** Radar technology makes it possible to describe the surface velocity field continuously over distances of 20 km. The second-priority core monitoring program enhancement is to extend the San Diego Coastal Ocean Observing System, which is already monitoring the portion of San Diego County south of Point Loma, north to Del Mar. This should be a relatively inexpensive task because much of the infrastructure needed to produce data, and to disseminate over the web, is already in place.

**Aerial and satellite imagery:** Aerial photographs and, to a limited extent, satellite images have been shown to identify the location of the plume, when it surfaces. Because of its high-resolution and large spatial coverage, the incorporation of remote sensing data is a high regional priority. The aerial photographs created by Ocean Imaging Corporation (San Diego, CA) are composite images based on measurements from a digital multispectral camera sensor flown at relatively low altitudes. In contrast to satellite imagery, aircraft can almost always be flown beneath the clouds that frequently cover the ocean off San Diego County and limit the utility of many sensors carried on satellites. Under some circumstances, the aerial photographs show very clear plume boundaries, and suggest that the plume can have a relatively narrow surface expression. Continuing to support the acquisition of imagery by Ocean Imaging, as currently sponsored by the State Water Resources Control Board and other operators, is an obvious priority. The value of these observations would be considerably enhanced if they were acquired immediately before and during plume tracking and drifter deployments.

**Ancillary observations:** The coastal ocean is forced by a combination of local winds and distant forcing that acts locally through pressure gradients. The synthesis of the observational products described above, and the operational models described below, will require definition of these forcing functions. Meteorological observations are made on a

routine basis by the National Oceanic and Atmospheric Administration (NOAA), both at coastal airports and a small number of buoys maintained by the National Data Buoy Center (NDBC). Sea-level observations are made continuously at several sites along the coast, and these data, along with the weather observations need to be acquired and integrated into the observational stream. There is clear evidence (Pringle & Riser, 2003) that the variability in the coastal circulation near San Diego depends on winds and sea level in the coastal ocean to the south, off Mexico. The Científicos del Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) maintains a network of meteorological and pressure sensors along the coast of Baja California that would be of great help to the program outlined here. Every effort should be made to support the continuation of that observational program.

## **2. Modeling**

It is customary to divide models of the plume dynamics into near-field and far-field components (as described above). Near-field models of the PLOO were developed as part of the engineering study for the construction of the extension, and are not addressed further here. However, models should be developed as an enhancement to the core program with a priority equal to the deployment of the central mooring at the diffuser. Models, combined with data, can give realistic predictions of the concentration of products carried by the effluent in the coastal ocean. An observational description of the circulation, as produced by the recommended program described earlier, will necessarily be limited. The variability in time and space of the coastal ocean is so large that there can never be enough observational resources to fully resolve the entire flow. The studies described above have dealt with this difficulty by developing sophisticated three-dimensional numerical models that have the required resolution in time and space to describe the evolution and structure of the flow.

When combined with a well-designed observational program, models are powerful tools for understanding the observed dynamics. The power of any observational program is limited by the temporal and spatial distance among samples: If the samples are taken too far apart in space or time they will be uncorrelated, and it will be difficult or impossible to understand the dynamics. The distance in time and space over which observations become uncorrelated is the “decorrelation scale.” The decorrelation scales are determined by the physical (chemical, biological, etc.) dynamics, and will vary with time and location. Spatial decorrelation scales in the PLOO region are likely to be larger along bathymetric contours than across them, and shorter in regions of sudden changes in coastline shape (e.g., Point Loma). Temporal decorrelation scales get longer with increasing time scales of the flow: Turbulence has very short decorrelation scales, while coastal trapped waves have decorrelation scales of hundreds of kilometers and several days.

For the purposes of the PLO, models can be used in two main ways: (a) to assimilate data and give a detailed picture of the spatial and temporal patterns of the flow during the observational program or (b) to give a statistical picture of flow patterns and probability of water parcel location under different forcing regimes. Interesting (and cautionary)

examples of both uses of these models are given in the Mamala Bay Sand Island Ocean Outfall Chlorination Study (SICS; Mamala Bay Study Commission, 1996; Noda & Associates, 1999). In the first instance, a three-dimensional model of Mamala Bay (and the waters surrounding Oahu, Hawaii) was run, forced by tides and wind (Mamala Bay Study Commission, 1996). The model did not, however, assimilate data gathered in Mamala Bay at the time (some current meter data and temperature–salinity data near the diffuser). The model was used to simulate water motions around the Sand Island outfall. Because the model did not assimilate the field data, it was found that the model currents were sometimes in the opposite direction of those measured, weakening any statistical confidence in the model results (Noda & Associates, 1999). Subsequently, Roberts (1999a, 1999b) used the field data to derive statistical probabilities for the presence of diluted plume waters in the near and far field around the diffuser. However, the statistics and trajectories of the flow were based on a limited number of current meter moorings, spaced several kilometers apart. To derive the trajectory statistics, it had to be assumed that the flow was uniform in the area between current measurements and identical to the flow at the nearest current meter. In other words, it was assumed that the current meters were separated by less than one decorrelation length of the flow field. All the conclusions concerning the plume trajectory statistics then rest on the validity of this assumption, which was not supported with analysis. A carefully developed three-dimensional circulation model—appropriately forced—and assimilating the data would give statistically robust estimates of the flow variability, without having to make such unsupported assumptions.

Given the relatively short time and space scales over which the effluent plume would be expected to vary, it will be impossible to model the plume without continuous input of appropriate field data. These data must define the boundary conditions for the model: (a) the currents and water properties that control material entering and leaving the domain and (b) the boundary forcings in the domain (heat flux, wind stress, etc.). Within the model domain, the model will respond to these forcings and give predictions of the motions of the water and plume. The skill of such predictions falls off rapidly in time, because the autocorrelation time scales of the dominant physical forcings is short (~1–2 d).

**Simple stochastic models—Regressions:** Stochastic models and statistical regression models can be used to estimate probabilities of events, given certain forcings (for example, flow patterns, wind conditions, etc.). For instance, whether under certain oceanographic circumstances water quality on the beach tends to be worse than under other conditions), a stochastic or regression model can be constructed to determine whether such a relationship exists. If so, then what are the associated confidence levels? The advantage of such a model is that, given the observations of cause (oceanographic conditions) and effect (water quality at the beach), it is reasonably straightforward to implement. The major disadvantage is that this kind of model does not identify the processes responsible for the effect, and thus makes it difficult to determine how the system might be modified to improve the outcome. This is particularly true when there are multiple sources of contaminants. Even if there were real predictive skill associated



with predicting water quality, given, say, the occurrence of onshore flow, a regression model would not identify whether the ultimate source for the contaminant is the Point Loma Ocean Outfall, the South Bay Ocean Outfall, or the numerous storm drain outfalls distributed along the coast.

**Three-dimensional numerical models:** A number of three-dimensional numerical models have been designed to simulate the circulation in the coastal ocean. For example, Oey et al. (2001; Oey et al., 2004) describe the application of the Princeton Oceanographic Model (POM) to the region of the California Continental shelf between Ventura and Morro Bay. That study provides an excellent example for what a three-dimensional model could do in the San Diego area. It is important to note that there currently exist many different numerical models (e.g., POM, Regional Ocean Modeling System [ROMS], and Finite-Volume Coastal Ocean Model [FVCOM]), and any of these could be used in the projected application. Typical models of coastal circulation use a horizontal grid of order one kilometer, and resolve between 25 and 50 positions in the vertical so that in 100 m depth, the vertical resolution can be as fine as 2 meters.

Experience has shown that with sufficiently good boundary conditions, and suitable choice of domain, models produce circulation fields that are consistent with observations. Because the modeled fields have high resolution in space, and continuous coverage in time, they can be used to identify where the products released at the outfall go and the concentration of these products. Because it is possible to run the model with different discharge rate, it becomes straightforward to determine whether an increased discharge will result in a negative environmental impact or in worse water quality at the beach.

In addition to using models to describe and predict the movement of waters, models can be used to assess the impact of biological activity on human health.

**Biological model:** Hundreds of models of the planktonic ecosystem exist, however, it is not clear that one is required for the purposes of the PLOO. Two factors of concern are as follows: (a) Does effluent containing harmful bacteria reach levels of danger to human health? (b) Does the effluent plume deleteriously affect the planktonic ecosystem?

**Bacteria:** The human health effects of effluent bacteria depend on the number of bacteria in the water and the location of the water containing the bacteria. The bacterial concentration will be related to the initial concentration in the effluent, the dilution of the effluent as it enters the ambient seawater, and the growth/mortality rates of the bacteria in the plume. The bacterial concentrations are thus controlled by physical factors (mixing and transport) and biological factors (growth and death). The physical factors can be well understood in the context of the models discussed earlier. Numerous studies have attempted to quantify the mortality rates of human pathogens in effluent plumes: The rates tend to be higher in sunlight and high temperatures. The bacteria appear not to grow once introduced to seawater. Thus, a conservative assumption would be that the bacteria have no mortality once introduced into the plume, and that concentrations are influenced solely by physical factors. If no bacterial exceedences are predicted to occur with no

mortality, then a reasonable conclusion would be that dilution of the effluent is strong enough, and the flow is favorable enough that bacterial mortality would only decrease the likelihood of local contamination. It would be necessary to find out whether a statistically meaningful number of exceedences occurred under the no-mortality assumption and the conditions under which these events took place. This process will identify the primary set of physical dynamics that generates excessive bacterial concentrations in a given region. This information can then be used to modify the bacterial sampling program to be more responsive to the relevant forcings (e.g., wind stress, heat flux, and phase of moon) and to motivate improvements in the bacterial model to include appropriate sources of mortality. This will be particularly important in the regional context we advocate here: while the PLOO may not be responsible for beach contamination, such contamination still occurs. It is important to try to identify the sources and dynamics of such contamination events—whether from other outfalls, tidal flushing of the bays, or episodic flushing of watersheds. Models combined with data are excellent tools for this type of problem.

**Plankton:** Many of the fundamental properties of the plankton can be measured quickly and continuously with extant instruments, such as those that are used in the present PLOO monitoring program. These properties include chlorophyll concentration, oxygen concentration, and light transmittance. From the present monitoring program, these indicators suggest a minimal impact of the plume on the bulk plankton and light quality. Unfortunately, as discussed above, the spatial and temporal resolution of the present monitoring program makes it difficult to determine how completely the plume has been sampled. In particular, it is difficult to estimate the impact of the effluent nutrient loading on the planktonic ecosystem in the absence of well-resolved dissolved nutrient measurements. Furthermore, without continuous measurements of the natural variability of the ambient flow, it is impossible to put the nutrient loading of the PLOO into a natural context to quantitatively assess its contribution to the planktonic production, and how that contribution varies with the flow conditions.

One aspect of the plankton that is not addressed by the present monitoring program is the influence of nutrient ratios on phytoplankton species composition. Effluent high in nitrate but low in silicate, for example, would more likely stimulate the growth of flagellates, which form the “red tides” in this region, than diatoms that have silica shells. It would be relatively easy to measure the nutrient composition of the effluent and the plume, and has probably been done in earlier studies, to address this issue. At a more basic level, it is important (and relatively easy) to determine when and where the local phytoplankton are nutrient limited. Without nutrient limitation, the loading from the outfall will have a negligible effect.

While a careful estimate of the nutrient budget of the effluent plume is difficult (and requires measurements of the effluent dilution and plume location in relation to hydrography and a thorough understanding of the near and far field mixing dynamics), a quick calculation helps to put the discharge into a broader context. Ocean waters near the diffuser have a dissolved nitrogen (nitrate) concentration of about 25  $\mu\text{M/l}$ . Waters leaving the diffuser have a total nitrogen concentration (ammonium plus nitrate) of about

25 mg/l, or about 1.8 mM/l. Assuming a 100:1 dilution with deep waters, the effluent plume would have a concentration of about 43  $\mu$ M/l—about twice the deep nitrate concentration. Assuming a flow of 180 MGD ( $680 \times 10^6$  l/d) from the PLOO, and a 100:1 dilution, this would inject about  $68 \times 10^9$  l/d of water into the receiving waters, with an additional 18  $\mu$ M/l of dissolved inorganic nitrogen in the form of nitrate and ammonium. If this were in a plume 10 m thick by 1 km wide, each day's output would stretch about 7 km along shore—approximately the daily alongshore displacement by the ambient coastal current (10 cm/s). While these calculations should not be considered better than order-of-magnitude guesses, they suggest that the nutrient loading by the diluted effluent could be a significant local nitrogen source in the nearshore region—probably greater than that due to the episodic upwelling that occurs along this coast (a flux that has not been quantified due to lack of appropriate data). However, much of the effluent plume will remain below the pycnocline, and often below the euphotic zone (approximately 30–40 m locally), and have little effect on phytoplankton production during much of the year. Therefore, to understand the effects of the plume on the planktonic ecosystem it will be necessary to ensure that the plume is adequately sampled (as discussed above), and to quantify the conditions under which the plume reaches the euphotic zone, and the magnitude of natural nutrient sources. The physical sampling/modeling program described above, augmented with instruments for biological properties, will significantly increase our understanding of the role of the PLOO in the local planktonic ecosystem.

Given that the inorganic nutrients in the diluted plume are about equal to the deep nitrate concentration of the ambient waters, we might expect about a doubling of the dissolved nutrient concentrations at the surface during deep mixing events (for example, during winter weak stratification and wind-driven mixing). This would lead to approximately a doubling of the phytoplankton concentration. However, this doubling of the phytoplankton biomass would likely occur disproportionately in the larger size classes of phytoplankton – the diatoms and dinoflagellates. While the smallest phytoplankton are efficient at taking up nutrients, their biomass tends to be held in check by their efficient, fast-growing grazers. The larger phytoplankton tend to show the greatest increases in response to nutrient inputs. Thus the diluted effluent plume may have local effects on the planktonic ecosystem; however, their importance, impact, and ramifications are difficult to address. One might suspect a link with local “red tides”—dense blooms of phytoplankton (usually dinoflagellates). However, red tides have been recorded in the Southern California Bight for over a century, and there is no evidence of changes in their frequency, intensity, or geographic coverage in the recent past. While a link of red tide occurrence with urbanization is tempting, we still do not understand the dynamics that allow red tide formation, and cannot predict their occurrence. Until we can, we cannot quantify the impact of outfalls such as the PLOO on such phenomena.

Presently no models exist that would allow us to predict the occurrence of local red tides. A simple nutrient-phytoplankton-zooplankton-detritus model coupled to the physical model would aid in quantifying the nutrient budget of the plume, and its effect on phytoplankton biomass (though not community structure). In addition, modification of the planktonic sampling program in light of the physical model results and physical field

studies (e.g., rapid response to unusual events or plume tracking) will build confidence that the effect of the effluent is generally minor, and the conditions under which it is significant.

### **E. Physical Oceanography, Plankton and Modeling Conclusions**

We don't know where the water goes or where the plume goes. The City of San Diego is considering an increase in the daily discharge from the treatment plant located on Point Loma from the current value of about 175 MGD to the full 240 MGD permitted by the State Regional Water Quality Review Board. To assess the impact of this increased discharge on the environment, and on the quality of coastal waters, understanding how the discharged waters are transported by and mixed with the coastal waters off San Diego is necessary.

A review of the existing monitoring program has shown that while there is evidence that products associated with the plume remain in the vicinity of the outfall, there is no available description of the location of the plume comparable, for instance, with the extensive information available for the Huntington Beach facility operated by OCSD. Nor does there exist a description of the variability in the circulation in the receiving waters, which was determined for Orange County. A major conclusion of this review is that *there is currently insufficient information to determine how the projected increase in the discharge at Point Loma would affect water quality* because the information that is available for other sites has not been generated. The second major conclusion is that an *observation plan as outlined previously would provide the information on which to base a confident assessment of the impact of the increased discharge.*

We recommend the simultaneous development of observational and modeling capabilities to identify the location of the plume within the highly variable coastal ocean. The highest priority is to enhance the core program with the simultaneous deployment of a mooring at the diffuser site, and the development of a data-assimilating circulation model. Other high priorities include special studies to locate the plume, to extend the surface-mapping radar and remote sensing, and to track the plume with drifters.

## **V. Benthic Monitoring**

In this section, we evaluate the present benthic monitoring program, and make recommendations for modifying the program to better gauge the effects of the PLOO against a background of several other sources of pollution on the shelf offshore from San Diego. This represents a more regional approach to understanding the nature and distribution of affected sediments and benthos (plants and animals living in, on, or near the ocean bottom) from multiple sources of pollution. This approach has been the long-term objective of the Southern California Coastal Water Resources Project at the scale of the Southern California Bight (e.g., the Bight 2003 project; SCCWRP, 2003), and we recommend a similar approach at the scale of the shelf off San Diego County but with greater spatial intensity focusing on the potentially most affected areas, with the objective of discrimination among the sources.

### **A. Background**

The ocean shelf offshore from San Diego County is complex, mostly composed of soft sediments, and extends to the 125–150 m contours (Fig. 1). The slope offshore from the shelf is steepest near La Jolla where the La Jolla Submarine Canyon is located. The shelf is much broader south of Pt. Loma and extends to Punta Descanso ~32 km south of the Mexican border. The “9-Mile Bank” is an extension of the shelf but separated from it by the Loma Sea Valley to the northeast and the Coronado Submarine Canyon to the south. There are also areas of rocky bottom, mainly limited to waters off La Jolla and Point Loma where two of the largest giant kelp forests in the SCB are located. Additionally, there are smaller kelp forests and an associated rocky bottom located in northern San Diego County and near the Tijuana River Estuary located near the Mexican border.

The shelf contains two littoral cells, the Oceanside Cell and the Silver Strand Cell, and one subcell, the Mission Bay Subcell, between Point Loma and La Jolla. Littoral cells are coastal segments in which a complete cycle of sediment supply, transport, and loss occurs (Inman, 1976). There is little or no mixing of sediments or small benthos among littoral cells, and sediments with pollutants discharged into one cell are likely limited to the receiving cell.

Contamination of the shelf began well over 170 years ago when the population of San Diego began to grow significantly. Major impacts began in the 1850s when the San Diego River was redirected from San Diego Bay to Mission Bay to prevent the siltation of San Diego Bay. During this time, raw sewage ran directly into both bays. The combined effect devastated the prime estuarine habitat in both of these bays. Sewage treatment did not commence in San Diego until 1943. Until this time, raw sewage ran into coastal waters and bays through more than 20 outfalls that discharged sewage right at the shoreline. It was not until 1963 that a comprehensive sewage system with a proper offshore subsurface discharge was established in Point Loma. By this time, San Diego Bay was in a constant quarantine. Also during this period, San Diego Bay was subject to

repeated episodes of dredging for the creation of a deep-water channel while the fill was used to reclaim land for development or dumped offshore onto the shelf. Major development of Mission Bay began in the 1940s with the establishment of a channel, and major dredging and fill activities persisted into the early 1960s. The re-engineering and pollution of San Diego Bay and Mission Bay vastly altered the physiography, estuarine dynamics, and ecosystems of these biologically important wetlands, and contributed to the contamination of the shelf off San Diego. Clearly any regional monitoring program of sewerage discharges offshore must include these two sources of contamination.

Presently, the main sources of contaminants to the shelf off San Diego include the Point Loma Ocean Outfall, the South Bay Ocean Outfall, the San Diego and Tijuana Rivers, as well as the aforementioned outflows from Mission Bay and San Diego Bay. Both bays have been sites of landfills sometimes composed of toxic materials, and serve as receiving waters for urban runoff from large watersheds. San Diego Bay has also been subjected to industrial and sewage discharges, is the largest home base for the U.S. Navy on the West Coast, and is the site of a major shipyard. Industrial discharges and leaching anti-fouling paints associated with port activities have led to some of the highest concentrations of heavy metals and polychlorinated biphenyls (PCBs) in the United States (Fairey et al. 1998; McCain et al. 1992; San Diego Bay Interagency Water Quality Control Board, 1994). Sediments dredged from San Diego Bay are routinely dumped ~10 km SW of Point Loma at the LA-5 disposal site, less than one kilometer distant from one of the present PLOO benthic reference stations (E3). The transport of contaminated sediments from this dumpsite via resuspension or bedload likely contaminates a much larger area than the site itself, and is therefore another source of contamination for the shelf that must be addressed in any regional monitoring program.

The Point Loma Ocean Outfall presently discharges enhanced primary-treated urban sewage (~85% removal of suspended solids) approximately 7.25 km from shore and 95 m deep through two diffuser pipes configured as a Y. The PLOO was lengthened and deepened to its present configuration in 1993. Prior to 1993, effluent was discharged ~3.4 km from shore and 65 m deep. Sampling of sediment and benthos was conducted before the initiation of discharge through the present outfall, and has continued to the present at the same stations. This has enabled comparisons of sediment conditions and benthic populations before and after discharge to detect possible impacts attributable to the onset of sewage discharge.

## **B. What Benthic Monitoring Is Now Being Conducted?**

The components of the present benthic monitoring program include the following.

**Sediments:** The texture and chemical composition of sediments is determined at 22 stations (Fig. 2). The sampling is conducted using a 0.1m<sup>2</sup> grab on a semi-annual basis. The stations are stratified into three depth groups – 88 m, 95 m (diffuser depth), and 116 m. Six stations to the north are intended as distant controls (cf, reference sites), and the

remaining stations are located with respect to gradients of depth and distance from the diffuser. Sediments are analyzed for texture, indicators of organic enrichment, trace metals, pesticides, polyaromatic hydrocarbons (PAHs), and PCBs. Spatial and temporal patterns of the contaminants are inspected, and pre-discharge values are compared to post-discharge values to detect contamination. A subset of these constituents is then compared to values from the entire Southern California Bight to determine relative contamination on this larger spatial scale.

**Benthic Infauna** (animals that live in the sediment): Two replicate samples are collected quarterly, also using a 0.1m<sup>2</sup> grab, from the same sites where sampling for sediment texture and chemistry are conducted. Samples are sieved through a 1,000-micron sieve, identified to taxonomic groups and species, and enumerated. The following parameters are calculated: number of species per grab, number of species per station, abundance per grab, wet-weight biomass per grab, Shannon Diversity Index per grab, Pielou's Index of Evenness per grab, Swartz Dominance Index per grab, and Infaunal Trophic Index per grab. Spatiotemporal patterns are examined using multivariate ordination and clustering techniques. Additionally, a before/after control/impact (BACI) analysis is performed to determine if the discharge from the PLOO is affecting the benthic infauna.

**Megabenthos:** Until earlier this year, demersal (bottom and near bottom) fish and megabenthic (large enough to see unaided and live on or near the bottom) invertebrates were sampled quarterly at eight stations using an otter trawl. Sampling stations were stratified by depth (Fig. 3). These included three inshore stations 60 m deep and eight offshore stations 100 m deep. Currently, the sampling program consists of sampling at 6 of the 100 m stations on a semiannual basis. Catches are identified, enumerated, and inspected for parasites and external anomalies. Fish are measured and weighed (individually) and invertebrates are weighed wet by species. Spatial patterns are inspected within each depth along distance gradients from the diffuser to detect impacts. Statistical parameters generated for each station include number of species, abundance, Shannon Diversity Index, and biomass (wet). Spatio-temporal patterns are examined for benthic infauna, as indicated above, using total abundance for each species. Time series of species number and abundance data are visually inspected.

**Fish bioaccumulation:** Annual otter trawl samples are collected from some of the offshore trawling stations (see above) and two rig-fishing stations (Fig. 4) to detect contaminants in fish liver and muscle. The relationship between contaminant levels and distance from the diffuser is investigated within species.

**Kelp forest habitat:** The Point Loma kelp forest, one of the largest kelp forests in California, is also one of the most urban kelp forests in the world as it is located between the entrances to two large bays, each of which includes runoff from river systems fed by large watersheds, is traversed by the outfall pipe, is washed by the water in the immediate coastal zone that has been exposed to all the storm drains in the area, and on occasion is probably exposed to effluent from the South Bay Outfall. The oceanographic processes off Imperial Beach often deposit sediments near the opening of San Diego Bay, and much

of this material can be advected to the kelp ecosystem. Within the forest there is intense sport and commercial fishing for sea urchins, spiny lobsters, and fin fishes, and the kelp itself is harvested for the production of alginates. This multi-use resource is also important to San Diego's large diving community. Thus, the health of this ecosystem is of concern to all aspects of society.

**Sediment Mapping Study:** As part of a recently mandated special study (NPDES Permit No. CA0107409, Order No. R9-2002-0025, Addendum No. 1), the City of San Diego is conducting a sediment mapping program in the areas of the Point Loma Ocean Outfall and the South Bay Ocean Outfall to create maps of sediment composition, condition and infaunal benthos that are "scientifically defensible" (City of San Diego Metropolitan Wastewater Department and Southern California Coastal Water Research Project, 2004). The sediment mapping study consists of two phases. The first phase is intended to develop an understanding of spatial variability of sediment composition, and the second phase is the development and execution of a sediment sampling program based on information from the first phase. More than two hundred sites will be sampled as part of this program.

### **C. What Does the Current Monitoring System Show?**

Wastewater discharge through the PLOO does not appear to significantly affect sediment quality except at the site nearest the diffuser. At all sites, most measured parameters do not exceed levels of natural variability within the Southern California Bight. The effects that have been observed occur at the station nearest the diffuser (E14). These have included higher levels of sulfides and biochemical oxygen demand (BOD). Sediments at the northern B sites (the intended control sites for the before/after analysis) are different from those at the E sites closer to the outfall. This lack of an appropriate control has rendered the interpretation of most benthic data difficult.

Throughout the monitoring area, infaunal benthos are dominated by an assemblage of ophiuroids and polychaetes typical of the Southern California Bight. Results of the before/after analyses indicate that changes to the infauna have been apparent because of the onset of sewage discharge at the site nearest the diffuser (E14). The overall pattern at this site (well within the zone of initial dilution [ZID]) has been a shift in biomass by phyla, with echinoderms decreasing (mainly *Amphiodia* sp.) and polychaetes increasing. This is a pattern consistent with organic enrichment. Control sites used for the before/after comparison with the impact site were E26 and B9. Other sites intended as controls included the other B sites, but these were deemed dissimilar from the impact site because of their different sediment structure (Fig. 5) and benthic assemblages that were apparent prior to the onset of discharge. Infaunal assemblages are, to a large degree, structured by the grain-size distribution of the sediments (see, e.g., Rhoads & Young 1970). Therefore, different infaunal assemblages would be expected between the B and E sites. Results of the Infaunal Trophic Index analysis, an index that is widely applied for determining the presence of disturbed benthic assemblages in southern California (Word, 1980), indicates that none of the sites are affected. Values of the ITI at E14 (site nearest



the diffuser) have typically indicated that it is the most disturbed of all the sites but have still been within the range of no impact. However, the greater variability of species richness and abundance observed at E14 over the course of the monitoring program is consistent with enrichment. Contrary to expectations of a disturbance, the general pattern at all of the monitoring stations has been an increase in species richness and abundances since discharge began. In summary, the results of the infaunal monitoring program indicate that the site nearest the diffuser (E14) appears affected by discharge, whereas there is no evidence that these effects extend to the other monitoring stations where natural processes appear to dominate.

The results of the megabenthic component of the benthic monitoring program indicate no specific effects of discharge through the diffuser. The megabenthic invertebrate complex at the monitoring sites is dominated by the white urchin, *Lytechinus pictus*. Other species that are common include the sea pen, *Acanthoptilum* spp., and the asteroids *Astropecten verrilli* and *Luida foliolata*, as well as the warty sea cucumber *Parasthicipus californicus*. Variability in species richness and abundances has been high, and the variability of offshore stations has been much greater than inshore stations, mostly because of the variability of the white urchin. For fish, the dominant species has been the Pacific sanddab (*Citharichthys sordidus*), while other species such as the yellowchin sculpin (*Icelinus quadriseriatus*), the longfin sanddab (*Citharichthys xanthostigma*), the longspine combfish (*Zaniolepis latipinnis*), the plainfin midshipman (*Porichthys notatus*), the Dover sole (*Microstomus pacificus*), the California tonguefish (*Symphurus atricauda*), the pink seaperch (*Zalembius rosaceus*), the stripetail rockfish (*Sebastes saxicola*), the California scorpionfish (*Scorpaena guttata*), and the bigmouth sole (*Hippoglossina stomata*) occur frequently in the samples. Species richness has been stable throughout the monitoring program, including the period prior to discharge, while abundance has been quite variable. The variability of fish abundance appears related to El Niño events. El Niños are characterized as periods when the thermocline is deeper and the shelf is bathed by warm nutrient-poor waters. During these periods, the Pacific sanddab, a cool-water species, is the dominant species on the shelf (range: Bering Sea to southern Baja California most commonly observed 50 to 150 m deep), declines in abundance at the monitoring sites, thereby significantly affecting total fish abundances. This most likely reflects general ocean warming and a depressed thermocline. Throughout the monitoring period, there has generally been a lack of fin rot, tumors, lesions, and physical abnormalities, indicating that demersal fish are not subjected to a combination of contaminant concentrations and exposure periods necessary for these to occur.

There does not appear to be evidence of bioaccumulation in fish muscle and liver tissues that can be associated with the discharge of sewage from the diffuser. This is evidenced by a general lack of spatial pattern in the data and by a lack of pristine control areas with which to make species-specific comparisons for these data.

The kelp forest ecosystem has been monitored over three decades through a contract with Scripps Institution of Oceanography. While the kelp forests and associated species are highly dynamic with occasional El Niño or storm induced collapses of the giant kelp (Dayton et al., 1992; 1999), there has been no indication of ecological impacts of PLOO since the present program began in 1970. There are indications however, that the southerly end of the kelp forest experiences considerable sedimentation, and the professional urchin divers report that this is especially apparent during periods when the bay is dredged (Pete Halmay and Dave Rudie, pers. comm.). Figure 6 shows the flushing bay water entrained in a northerly coastal current into the kelp forest, while the temperature signal of the broken outfall pipe reverses direction immediately offshore of the kelp forest. This figure exemplifies the extreme local nature of the shore processes.

#### **D. How Well Is the Benthos Monitored?**

Overall, we found the current benthic monitoring program to be comprehensive, particularly the surveys of the benthic community possibly impacted by PLOO. The major findings listed previously indicate that any benthic effects of sewage discharge through the diffuser at Point Loma are highly localized because all anomalous data were limited to the station nearest the diffuser. For sediments, these results provide evidence that contaminants are not accumulating in any specific spatial pattern beyond the zone of initial dilution.

The biological data are generally consistent with this pattern as well. This means that either (1) a combination of processes, both biological and physical, is effectively modifying and/or redistributing contaminants such that possible effects of discharge are subsumed by natural variability beyond the zone of initial dilution or (2) there are no effects. However, one can never assert that there are no effects because new technology or approaches may find impacts. That is, we cannot prove a negative. Thus, we need to seek a balance between the resources and technology and the risk of erroneously assuming no effect. With this in mind, we limit our critique of the monitoring program to the data and analyses performed by the City of San Diego as mandated by the Environmental Protection Agency for discharge of sewage through the diffuser at Point Loma.

We believe that there are four main problems with the present benthic monitoring program. First and foremost, there is a *lack of adequate control stations* with which to compare sediment chemistries and benthic assemblages. The sediments of the northern B stations, chosen as the original control sites during planning for the pre-discharge study, are composed of different grain sizes than the sites nearer the diffuser. Two of the sites have much coarser sediments (B13 and B12) while the remaining sites have sediments much finer (B8 and B11). Only two of the six B sites have sediment properties similar to the sediments near the outfall. A multibeam sonar survey of major portions of the shelf (Gardner et al., 1998) indicates large-scale patterns of variability in backscatter over the shelf (Fig. 7). The intensity of backscatter is indicative of seabed and subsurface roughness, sediment composition, and bulk properties, and therefore is a useful proxy for

determining the spatial pattern of bottom composition. Inspection of Figure 7 reveals large-scale patterns of backscatter that appear consistent with the median grain size values obtained at the study site (Fig. 5). The B sites (control sites) are located on or near a relatively large feature that has greater backscatter than the sites closer to the PLO, which have finer sediments. While it is too late for the present outfall, future infaunal monitoring projects utilizing a BACI approach should account for bottom composition by conducting pilot studies so that the most appropriate control stations can be located. For example, the requirements were such that the present control sites were maintained even when it was known they were different with the exception of site B13 where monitoring was discontinued in 2004. This offers an opportunity to use old monitoring sites to establish some long-term baseline stations. Such time-series offer a gold mine of long-term data that will be extremely important for understanding regional patterns and changes. At the same time, inappropriate reference sites can be discontinued. One approach to picking the optimal reference sites is offered below.

To test the utility of the backscatter data for choosing sites by sediment texture, we examined the relationship between backscatter and actual grain size values observed in the monitoring program. A random sample of 25 backscatter values was selected from the immediate vicinity (within 100 m) of each sediment sampling site of the monitoring program for correlating mean backscatter with median phi values for the stations. This resulted in a correlation coefficient of  $\sim 0.72$ , indicating that the availability of the backscatter data when this project began would have been of great value, and that these data should be utilized when future control sites are considered.

The differences in sediment composition among sites cause *a priori* expectations that some components of the sediment chemistries and benthic infaunal assemblages will be different. For example, concentrations of biochemical oxygen demand, nitrogen, and sulfides are negatively correlated with grain size. For infauna, species richness is generally positively correlated with increasing grain size. Aside from a few outliers, these are the general patterns observed in the monitoring program. The differences among sites for sediment composition are shown in Figure 8. Parameters of sediment composition were analyzed using multivariate, multidimensional scaling (MDS) analysis, which reduces data from multidimensional space to two variables that when plotted against one another reflect similarities among samples (stations). Increasing distance in the MDS plot reflects increasing dissimilarity among the stations. Inspection of Figure 8 reveals that stations B8, B13, and B11 are quite different from the core E stations nearer the outfall. Therefore, many of the control stations are too different from the impact stations to be of value. For example, the relationship of total organic carbon for all the stations associated with both the PLOO and the South Bay Ocean Outfall (SBO) for 2001 are plotted against grain size in Figure 9. Grain size accounts for more than 64% of the variation in total organic carbon among the sites. Similar significant relationships exist for many other sediment constituents such as sulfides, nitrogen, and so forth.

A second area of concern for the present monitoring program is the *lack of any integration of the littoral transport cells*. In the Silver Strand Cell, sediments accumulate

in an area ~4 km south of Point Loma (Inman, 1976). Considering the concentrations of very serious pollutants from SBOO and the episodic flushing of the Tijuana River, it is important that the accumulation of these sediments just south of the mouth of San Diego Bay be monitored. Not only are these sediments of concern, but the material flushed from San Diego Bay may also be entrained in this littoral cell. The most relevant sediment transport system for PLOO and Mission Bay is the Mission Bay subcell in which sediments move north and offshore into the Loma Sea Valley (Inman, pers. comm.). Presently, there are no stations along the slopes, valleys, and canyons. It is well known that material accumulates at the canyon heads and moves down the canyons (e.g., see Vetter & Dayton, 1999). Molecular markers of sewage have been detected in slope and canyon sediments of the San Pedro Shelf (Phillips et al. 1997). Coastal sediments are transported via resuspension and bedload as a result of wave motions and currents, and the average direction of sediment transport, is downward off the shelf onto the slopes, valleys, and submarine canyons. If contaminants are accumulating on the bottom, they will be transported along these pathways such that contaminated sediments may accumulate downslope from the shelf. Because this area is not monitored, there is presently no way to know if the effects of the PLOO or other contaminants, especially from LA5 are accumulating in these areas. In summary, it is important to monitor both the depositional area of the Silver Strand Littoral Cell and the canyon heads.

Our third area of concern is the *lack of interpretation of contributions from the many separate sources of contamination of the shelf off San Diego*. As previously mentioned, these include tidal outflows from two large bays, two large rivers draining large watersheds loaded with potential pollutants, sewage discharge from two large municipal outfalls, and a disposal site. Given the spatial coverage of benthic monitoring programs associated with discharges from Point Loma and South Bay (see Fig. 10), such an interpretation is possible. Many of the northern SBOO stations and the southern PLOO stations are likely affected by San Diego Bay. The southern PLOO stations, especially E3, are possibly affected by the LA-5 dredge disposal site. Finally, the northern PLOO stations are likely affected by outflows from Mission Bay. An MDS analysis of sediment organics (Fig. 11) shows that the station at the diffuser (E14) and E3 cluster separately from the other benthic sites but are very different from each other. These results support the contention that sediments at E14 and E3 are affected by the different sources. Work by Phillips et al (1997) demonstrates that it is possible to use a combination of markers in sediments to distinguish contaminants from river vs outfall discharge.

A fourth area of concern and perhaps one of the most important problems relating to sediment transport is the deposition of the fine sediments (silts and clays; < 63  $\mu$ m). The fine particulate sediments are transported and dispersed very differently than are the heavier sediments that are better understood. Figure 9 shows the close relationship of TOC with the fine sediments and it is generally understood that pollutants are also associated with the fine sediments. The dispersal of these fine sediments is very poorly understood (R. Guza, personal communication) in general. Figure 12 gives an indication of the sediment transport dynamics of the heavier sediments. Considering the sources of pollutants associated with SBOO, the Tijuana river when it floods and the normal tidal

flushing from its estuary, the watersheds flowing into San Diego Bay, possibly material even eroding from LA5 and the short dumps to be entrained into the Imperial Beach circulation cell as well as possible impacts from PLOO, one can understand the importance of better understanding the dispersion of the fine sediments.

Other critiques are more specific and are listed below.

There are unexplained observations in the data that we believe warrant further study: (a) Elevated concentrations of arsenic, chromium, and iron, relative to the other sites, have been observed at B13; (b) elevated concentrations of PAHs and aluminum have been observed at B10 and B8, respectively; (c) elevated concentrations of Al near B8 may reflect the proximity of a B-36 (the largest bomber ever built) that came apart in the air and crashed near that area in 1952; and (d) elevated concentrations of copper at the sites near the LA-5 dredge disposal area (Fig. 13)

Further analysis of the data may yield a more refined interpretation of the effects of the PLOO and discriminate these from other possible sources of contamination. These would include further examination of the relationship among variables using bivariate and multivariate analyses. One example is illustrated in the MDS analysis presented in Figure 11. Another example is the relationship between total nitrogen and total organic carbon (Fig. 14) at the Point Loma and South Bay stations. These constituents are highly correlated ( $r = .935, p < .001$ ) among stations, with two significant outliers that include E3, the site that is within a few hundred meters of the LA-5 dredge disposal site, and B13, which also has elevated levels of As, Cr, and Fe. These findings strongly indicate other sources of contamination. Multivariate analyses that link environmental variables, such as grain size, and sediment chemistries with benthic infaunal data would also be useful; one such analysis is the BIO-ENV procedure (Clarke & Ainsworth, 1993).

There is no attempt to couple patterns observed in the water column with those observed in the sediments and benthos (benthic–pelagic coupling). This may enhance the interpretation of spatial pattern observed over the shelf. For example, elevated levels of the photosynthetic pigment *chl a* are evident from water-column data at many of the northern B stations. *Chl a* is a useful proxy of the abundance of phytoplankton in the water. Higher concentrations of *chl a* in this northern area may be due to the outflow from Mission Bay or the presence of cooler more productive waters in the southern La Jolla and Pacific Beach areas (Parnell et al., in prep.). Concentrations of total organic carbon and total nitrogen in sediments at some of the B stations are elevated relative to the other sites. Part of this enrichment may be due to the occurrence of cooler, more productive waters in this area.

In summary, while the general findings of benthic monitoring indicate that the effects of the plume appear mainly limited to the station nearest the diffuser, further analyses and the investigation of anomalous spatial findings would be helpful in determining the environmental hazards present on the shelf that may be negatively affecting the benthos.

In the following section, we make recommendations for enhancing the present benthic monitoring program while building on our evaluation.

### **E. Benthic Monitoring Recommendations**

Our main recommendation is that the *monitoring program be redesigned as a regional approach* for determining areas on the shelf that are contaminated and identifying the sources of these contaminants if possible. An accurate assessment of the effects of the PLOO is only possible when the effects of all the important sources of contamination are examined so that the effects of the PLOO are not masked or confounded. As such, a regional program would include studying the effects all contaminant sources and would encompass the entire shelf off San Diego.

Our major recommendations are:

1. We recommend establishment of a regional benthic monitoring program designed to evaluate the effects of discharges from the two outfalls as well as both bays, rivers, and the dredge disposal site off Point Loma. This will involve integrating the present core monitoring programs for the Point Loma and South Bay Ocean Outfalls to enable a better definition of contaminant sources.
2. A key element of this regional approach should be the selection of suitable reference sites independent of the major contamination sources. There are no suitable reference sites between La Jolla and the Mexican border due to numerous sources of contamination. The shelf between the La Jolla and Scripps Submarine Canyons just offshore from SIO and/or the Oceanside Littoral Cell should be considered.
3. A special studies sampling program should examine the need to extend the core benthic monitoring stations to additional areas where sediments may be accumulating.
  - a. There should be a one-time (special studies) development of a shelf sediment budget to determine the spatial distribution of sedimentation and erosion, with the goal of identifying sites where contaminated sediments accumulate. This program should focus on deposition of fine particles.
  - b. Specific targets should be areas further offshore (slope and submarine canyons), the dispersal shadows of the toxic dump site LA5, and the Silver Strand littoral cell.
  - c. Depositional sites should be subject to more sensitive analysis of sewage exposure (e.g., linear alkyl benzenes, compound-specific stable isotope

signatures, and possibly additional markers such as trialkylamines and whiteners) to document sewage-related sources.

- d. These results should be used to modify the core benthic monitoring stations and possibly the sediment geochemical measurements.
4. A special studies program should identify the transport and dispersal of fine sediments in the entire region. The dispersal of these fine sediments is very poorly understood (R. Guza, personal communication) in general, but it is obviously very important transport system for pollutants as well as total carbon and nitrogen in the regional context.
5. Because hormones are important and have been shown to be present and have significant effects at other wastewater facilities, we recommend that the City, in conjunction with a regional monitoring program, participate in the growing number of collaborations presently forming to address effects of endocrine disruptors on the benthos and their consumers.
6. We recommend the establishment of a program to determine the spatial extent of source-specific contamination using outplanted organisms such as mussels (or possibly biological mimics) that assimilate contaminants.
7. Re-occupy some of the old stations discontinued when the pipe was extended in order to recapture the time-series. This gives powerful insight into long-term regional habitat changes.
8. We recommend continuation of the present fish bioaccumulation program and that it utilize more sensitive source-discriminating biomarkers or bioindicators. The present program should continue even though there appears to be no specific spatial patterns of contamination near the PLOO because discharge through the outfall is projected to increase substantially in the near future. As part of this enhanced program, we recommend a new set of sampling sites that are regional covering shelf and slope areas between La Jolla and the Coronado Islands.

## **DISCUSSION**

### **Contaminant Sources**

The possible sources of shelf contamination include San Diego Bay, Mission Bay, the Tijuana River, the LA-5 dredge disposal site, and the ocean outfalls off Point Loma (PLOO) and Imperial Beach (SBOO). The relative significance of the effects of municipal wastewater discharge and river discharge throughout the Southern California Bight has changed over time. Impacts from wastewater discharges in Southern California have declined (Schiff et al., 2000) because of increasing levels of treatment and source

control. The area of severely affected benthos near the wastewater discharger off Palos Verdes decreased from distances as great as 15 km in 1970 to 5 km by 1990 (Bergen et al., 1999; Stull, 1995). As of 1994, the area of the shelf off Southern California where the benthos was considered at least marginally affected by anthropogenic disturbance (not including fishing disturbances) was approximately 9% (Schiff et al., 2000).

Approximately 40% of the areas near river discharges were affected, while ~10% of the areas near municipal wastewater discharges were affected. We begin with a brief description of the possible sources of contamination to the shelf off San Diego.

The watershed of San Diego Bay covers ~1,074 km<sup>2</sup> and includes three major hydrographic units. The bay is shaped like a crescent and is ~25 km long and varies from 1-3km wide. The watershed includes Otay and Sweetwater rivers as well as Telegraph Canyon, Chollas, Switzer, and Paradise creeks. The mean tidal range is ~1.7m and the largest range is ~3m. A rough estimate of the tidal flushing is over 100 million cubic meters per day. The bay is on the state's list of impaired water bodies, with sediments having high concentrations of PCBs and PAHs (Mearns et al., 1991). A study of sediment contamination in the bay revealed that copper, zinc, mercury, PAHs, PCBs, and chlordane were highly elevated (Fairey et al., 1998). A hydrographic survey of the outer Bay and adjacent coastal waters revealed enrichments of Mn, Ni, Cu, Zn, and Cd (Esser & Volpe, 2002). The relative flows of San Diego Bay: PLOO is roughly 130:1.

Mission Bay receives runoff from approximately 145 km<sup>2</sup> of watershed. This includes the San Diego River system draining a very large watershed and contributing large floods. The current physiography of Mission Bay is the product of decades of dredging tidal salt marshes and mudflats. Approximately six square kilometers of the bay have been identified by the Regional Water Quality Control Board as water-quality limited because of elevated concentrations of coliforms. Other parts of the bay are also listed as a result of elevated concentrations of lead. A rough estimate of the flushing rate is 11 million cubic meters per day, or roughly 20 times the flow of PLOO.

The watershed that drains into the Tijuana River and Estuary is ~4,483 km<sup>2</sup> in area; nearly three quarters of this watershed is in Mexico. Historically, the City of Tijuana has had limited sewage treatment facilities, the result is that overflows have drained into the River and Estuary. In fact, an average of 13–20 million gallons d<sup>-1</sup> of raw sewage consistently flowed into the river during the 1980s (Desmond et al., 1999). Studies have shown that metals such as Pb, Zn, Cu, and Cr (SCCWRP, 1990) in the river are elevated, and that it contained the greatest concentrations of Cd, Cu, Pb, Ni, Zn, PCBs and suspended solids in Southern California (SCCWRP, 1992). Concentrations of metals (Cd, Cu, Ni, Pb, and Zn) in the sediments of the Tijuana Estuary increased significantly from 1989 (Gersberg et al., 1989) to 1997 (Meyer & Gersberg, 1997). Meyer and Gersberg note that the timing of this increase coincided with the introduction and expansion of the maquiladora program in Mexico.

The LA-5 dredge disposal site was designed as a predominantly nondispersive marine disposal site. These are designed so that waste material is intended to remain stationary.



As such, the material is dumped deep enough so that resuspension that is due to wave motion is limited. The depth of the site ranges from 100–125 m. The source of the material dumped at LA-5 is primarily sediments dredged from San Diego Bay. To our knowledge, the composition of sediments and the condition of the benthos at this site have not been studied. However, because the material at LA-5 is from San Diego Bay, which is known to be highly contaminated, then it is likely that sediments at this site are also contaminated. The results of a recent multibeam sonar survey (Gardner et al., 1998) indicate that waste material is not all located within the designated disposal area. A total of 252 mounds were observed outside the disposal site (Fig. 15), and many of these were elliptical, indicating that material was dumped while vessels were underway. Within LA-5, 10 mounds were observed covering ~54% of the area. The fact that material was dumped inshore of the disposal site represents a significant risk that these sediments may not be stationary because they are located at shallower depths. The LA-5 site is located immediately offshore of a ~50m scarp, therefore, mounds dumped just inshore of the site are much shallower than intended. Resuspension from the shallower mounds must be recognized as another source of contamination that should be monitored. It should be noted that the location of disposal mounds outside LA-5 compromises Stations E3, E2, and E1 (where Cu has been elevated; Fig. 13) as stations intended to monitor for the effects of the Point Loma Ocean Outfall. Effects of these illegal dumps are easily confused with PLOO effects in the sampling south of the outfall.

### **Sediment Budgets and Transport**

The development of a sediment budget, with a focus on fine particles, should be pursued using two methods. The first addresses transport of sediment by wave motions in shallow shelf waters (< 50 m). The transport of sediments in these areas is typically dominated by resuspension that is due to wave interactions with the bottom (see, e.g., Inman, 1976; Lyne et al., 1987). As part of a regional coastal zone planning and shore protection project in California, Inman developed a model of sediment accretion and erosion in the Silver Strand Cell (Fig. 12). The model shows two principal areas of sediment accumulation and an area near the mouth of San Diego Bay where the sediment climate is erosional. Coupling these patterns of sediment transport with the Largier et al. (in prep) observation of a dominant northward (near shore) gyre circulation in this area strongly suggests that contaminants discharged through the South Bay Ocean Outfall may be accumulating in these areas where Inman's model suggests. These results are extremely helpful in choosing locations for monitoring sediments and benthos. A similar sediment model is not available for the transition area between the Silver Strand Cell and the Mission Bay Subcell. Therefore, we recommend the *development of such a model that would be useful for determining where sediments and benthos of these cells are being affected by the various sources of contaminants along the shelf*. The recent Nearshore Canyon Experiment (NCEX), part of which was conducted in Northern La Jolla, exemplifies the type of program that would be ideal for the Silver Strand and Mission Bay Subcells.

The second method utilizes sediment profiles of radioisotopes and patterns of bioturbation (sediment mixing by animals) to determine sedimentation rates in shelf, slope, and canyon environments off San Diego. Rates of sedimentation over the last 50–100 years should be evaluated in these areas using  $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ , and  $^{234}\text{Th}$  geochronologies. Pb-210 chronology has been employed extensively to evaluate sedimentation patterns in lacustrine, estuarine, shelf and deep-sea environments (e.g., Alexander et al., 1991; Alexander & Venherm, 2003; Nittrouer et al., 1979). Because of the high particle affinity of lead (especially to fine sediments),  $^{210}\text{Pb}$  supplied to the water column by runoff, atmospheric precipitation and the decay of dissolved  $^{226}\text{Ra}$  is rapidly scavenged by sinking particles (Nittrouer et al., 1979). The rapid adsorption of  $^{210}\text{Pb}$  causes particles reaching the sediment to typically have substantial “excess” activities of  $^{210}\text{Pb}$ ; that is, activities above levels supported by decay of its precursor  $^{226}\text{Ra}$  within the sediment. Because  $^{210}\text{Pb}$  has a half-life of 22.3 years, this excess  $^{210}\text{Pb}$  signal can be used to track particle dynamics for time scales up to 100 years after particles have reached the seafloor.

A typical profile of  $^{210}\text{Pb}$  activity in sediments sustaining essentially continuous deposition exhibits three regions: a surface mixed layer, a region of radioactive decay, and a zone of background  $^{210}\text{Pb}$  levels (i.e., a zone of no  $^{210}\text{Pb}$  excess). The slope of the excess activity profile for  $^{210}\text{Pb}$  (or similarly behaved radionuclides) within this zone frequently allows estimation of the intensity of sediment mixing that is due to bioturbation (e.g., Nittrouer et al., 1983; Smith et al., 1992).

The region of radioactive decay occurs immediately below the surface mixed layer. Usually little or no bioturbation occurs in this region and  $^{210}\text{Pb}$  activity (and excess activity) declines because of increasing age of sediment with depth. Fitting curves to the activity data within this zone allows estimation of sediment accumulation rates. Major discontinuities in the  $^{210}\text{Pb}$  profile within this region (with or without concomitant changes in grain size) can indicate pulsed sediment input, resulting, for example, from storm events or turbidity flows (e.g., DeMaster et al., 1991). Below the region of radioactive decay,  $^{210}\text{Pb}$  activities should equal background levels supported by  $^{226}\text{Ra}$  decay within the sediment.

Radioisotope profiles collected along a distance gradient from the diffuser would also enable the examination of patterns of sediment accumulation and bioturbation to help determine the community effects of toxicant and organic matter loading. Because  $^{210}\text{Pb}$  integrates over long time scales (up to 100 years), it would also be useful for studying patterns of sedimentation prior to the existence of any deepwater outfalls and human modifications of the shorelines, bays, and estuaries of San Diego.

Cs-137 was produced from above-ground nuclear testing in the 1950s and 1960s. Therefore, in the absence of bioturbation, it should exist in sediment cores to depths where sediments are no older than ~50 years. If  $^{137}\text{Cs}$  penetrates deeper, based on estimates of sedimentation using  $^{210}\text{Pb}$ , then there is deep mixing of the sediments because of bioturbation. If there is deep mixing, then sedimentation rates based on  $^{210}\text{Pb}$

represent maximum estimates of sedimentation.  $^{234}\text{Th}$  can be used to determine rates of bioturbation because of its short half-life of 24 days.  $^{234}\text{Th}$  is present in the sediment only as deeply as it is mixed downward by recent (last 100 da) animal activities. It has been used as a proxy of bioturbation intensity at sites sampled along a distance gradient from the ocean outfall located off Palos Verde (Los Angeles County; Wheatcroft & Martin, 1996). Due to its short half-life  $^{234}\text{Th}$  is also useful to study seasonal effects, special events such as El Niños, and localized changes.

## Source Discrimination and Sewage Exposure

**Biomarkers:** There have been major advancements in the use of bioindicators and biomarkers and molecular geochemical markers to detect sewage exposure and effect in recent years (e.g., see Eganhouse, 1997; de Besten, 1998; Huggett et al., 1992; Lam & Grey, 2003). Bioindicators typically refer to measurements at the organismal, population, or community level, whereas biomarkers measure cellular, biochemical, molecular, or physiological changes. Biomarkers provide a sensitive indication of exposure to effluent via food supply or environment, thereby helping to address whether population changes at specific locations are correlated to differential contaminant exposure. They also provide a means of determining whether the causes of altered benthic populations at a site (such as E14 off Point Loma) are due to sewage exposure or other factors such as the effect of the diffuser on bottom circulation or differing sediment composition. (N.B., we emphasize that exposure does not necessarily reflect a negative influence on fitness; additional biomarkers linked to stress response may provide further indication of negative exposure effects.). Molecular markers, measured in sediments or water, provide a direct tracer of sewage effluent and associated particulates.

Responses to sewage exposure may be detected and have effects at multiple levels of organization: molecular, biochemical pathway, organelle, cell, tissue, organ, individual, population, community and ecosystem (Huggett et al. 1992). **Biomarkers** may provide either (a) indication of contaminant effects (early warning signals) or (b) quantitative measure of changes in a biological system (e.g., in response to sewage). Typically such biomarkers measure cellular, biochemical, molecular, or physiological changes. They may include markers for geneotoxicity, mutagenicity, and endocrine disruption. While biomarkers act at the suborganismic level, the term **bioindicator** is used to refer to measurements at the organismic, populations or community level. In many cases biomarkers will provide earlier warning of change or response than will bioindicators.

Effective biomarkers must have a small Type I error (false positive) and Type II error (false negative) must be kept to a minimum. In monitoring for sewage effects Type II error is potentially most important and a highly sensitive marker may not be desirable. One cause of false negatives may be found in changes that are repaired by the biological system. These could appear as lack of exposure when that is not the case. Level of exposure and timing of measurement affect whether a biomarker response is observable.

One of the most difficult aspects of biomarker application is the need to distinguish observed 'change' in the biological system (indicating exposure) from a significant 'biological consequence' (ie decrease in Darwinian fitness). Biomarkers indicating effects on feeding, growth, reproduction, or survival are likely to be most useful. However, translating from sub organismal level effects to population dynamics is one of the most difficult challenges in biomarker application.

Application of biomarkers in conjunction with chemical measurements may be valuable in predicting threshold effects. Also, biomarkers are good at indicating general stress level when multiple stressors (contaminants) are involved, as could be the case with sewage exposure. When chemical measurements are expensive, bioassays indicating exposure could be less costly.

**Biomarker Uses in Monitoring Sewage:** Biomarkers may have the following uses in a sewage monitoring program. They can be used to screen for exposure or for toxic effects. Isotopic, fatty acid and sterol biomarkers may indicate ingestion of sewage-derived organic matter. These markers have varying degree of specificity for specific contaminants. These markers may also be incorporated into assays to test potential for ingestion of environmental water or sediment samples. Biomarkers may be used to diagnose sewage-related effects, signaling the cause for observed changes effects. For example, declining populations could be tested for exposure to sewage with biomarkers, to determine whether sewage could be a cause. Application in this mode requires that affected vs reference populations be compared and that relationships among homeostasis, response and fitness are known. Biomarkers may be applied to trend monitoring, documenting changes over time (i.e., in response to changes in effluent volume) or to assess improvement after remediation. Typical applications also involve monitoring spatial variation, to assess site-specific responses or gradients (ie, with distance from an outfall). For this approach, biomarkers must be applicable in common species and reference values and modifying factors (e.g., effluent dispersion patterns) must be known. Site-specific risk assessment can be achieved to evaluate hotspots or signal effects at higher levels of organization. Finally, biomarkers may facilitate risk assessment at the level of ecosystem structure and function. To do this effectively biomarkers must (1) be applied to a range of species (2) estimate the % of potentially affected species (3) be applied to key organisms and (4) be amenable to sensitivity comparisons among species.

**Molecular Markers and Biomarkers of Sewage Exposure:** A number of markers have been applied to the detection of sewage and sewage exposure in seawater, sediments and living tissues. Examples are summarized in Appendix 1A. Numerous molecular markers, including trialkylamines (TAMs), linear alkylbenzene (LABs), linear alkylbenzene sulfonates (LASs) and fluorescent whitening agents (FWAs) act as tracers of sewage-derived material. Many are hydrophobic and adsorb to particulates (Takada et al. 1997). The TAMs, LABs and FWAs have their origins with detergents and fabric softeners and do not seem to be degraded by sewage treatment (Reemtsma et al. 1997; Stoll et al. 1997, Bayona et al. 1997). They have different transport potentials, due to their association with different particulate size fractions. For example, LABs are more typically

associated with the colloidal fraction, and thus travel farther from effluent sources, and often further offshore than TAMs, which are clay associated (Bayona et al. 1997). Both have been found to accumulate in deep-water basins in southern California, which have a propensity to collect fine particles and to hypoxia-related reductions in biodegradation (Bayona et al. 1997). Various coprostanols (sterols), selected fatty acids, C and N stable isotopic signatures of bulk sediments and specific compounds provide additional biochemical markers of sewage presence or exposure. The coprostanols are associated with (mostly human) feces and often are strongly correlated with TAM distribution (Bayona et al. 1997). Sometimes these markers are used as ratios to PCBs or PAHs to track specific sources such as sludge (Takada et al. 1997).

Isotopic signatures and to a lesser extent the fatty acids have been analyzed in animals living in or on sewage-affected sediments. Animal tissues reflect the isotopic and fatty acid composition of the organic matter in their food (with little or known changes) so that tissue analyses may reflect exposure to and incorporation of sewage-derived organic matter. These markers may provide good indication of exposure but do not necessarily yield information about deleterious consequences that might affect fitness or ecosystem function. However, strong indication that sewage-derived organic matter is fueling the food web, will necessarily indicate that the more normal food sources are not in use, and a problem could exist.

We recommend *characterization of dissolved and particulate constituents* from the sources mentioned above to aid the discrimination of sources that lead to the contamination of the shelf and benthos. Priorities would include heavy metals, PAHs, chlorinated hydrocarbons, and fecal bacteria. In addition, we recommend analysis of stable isotopic ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) compositions of dissolved inorganic material, suspended particulates, sediments, and sewage-related compounds (linear alkyl benzenes, specific fatty acids) as well as fecal sterols in suspended particulates and sediments. Time series collections should be established to capture major flood events at river/bay mouths and storm resuspension on the shelf. Suspended particulates should be sampled using pumps and sediment traps, and samples for dissolved constituents should be collected using pumps. We recommend that multivariate approaches (PCA, SIMCA, MDS) be adopted to analyze molecular and biomarkers in sediments, as a means to identify sewage-specific influence and exposure from different contaminant sources (*sensu* Phillips et al. 1997).

## **Effects and Responses**

**Endocrine disruptors:** While the study of the effects of endocrine disruptors on the benthos is still in its infancy, it is advisable that the City of San Diego participate in the growing number of collaborations presently forming to address this issue. The field of endocrine disruptors and their effects on the benthos is beyond the expertise of our panel. However, there is a growing literature that indicates significant influence of sewage-associated hormones and other endocrine disrupting chemicals (EDCs) on the reproductive physiology of fishes (e.g., Purdom et al. 1994, Lye et al. 1998, Rodgers-

Gray et al. 2001). Male feminization is a particularly prevalent effect. Endocrine disruption may be an alternative explanation for decreased abundances of the indicator brittlestar *Amphiodia urtica* near the PLOO. Decreased abundance of *A. urtica* is common near many outfalls on the west coast. The most common hypothesis has been that these decreased abundances were due to increased fish predation as many species of fish are more abundant near outfalls than surrounding featureless sandy areas due to an artificial-reef effect.

The results of a manipulative experiment by Thompson et al. (1993) suggested that the effects of sewage on *Amphiodia urtica* are age specific with increased levels of mortality observed for early juveniles. This effect may be due to endocrine disruption, since it has been shown that larval and juvenile stages of some invertebrates are highly susceptible to endocrine disruption (Oberdörster & Cheek, 2000). As a caveat, indicator amphipods in the genera *Rhepoxynius* and *Ampelisca*, do not appear to be affected by the PLOO since their abundances near the outfall are not different from other sites. Endocrine disruption has been observed for juvenile development and molting in some species of crustaceans (McKenney et al., 1998).

There has not been a great deal of study of the effects of endocrine disruptors on invertebrates. However, there is evidence that vertebrate sex steroid hormones affect echinoderm reproduction, that some echinoderms metabolize progesterone, and that many xenobiotics (e.g., Cd and PCBs) can affect steroid metabolism (reviewed in Oberdörster & Cheek (2000).

**Protein Biomarkers:** Molecular and biochemical alterations are usually the first detectable response to the chemical environment, serving as markers of both exposure and effect. These markers are usually more sensitive than indicators at higher levels of organization, and may underlie these more complex changes. Use of protein biomarkers offers the following benefits (1) potential to relate magnitude of change in biomarker to magnitude of exposure (2) most sensitive response and earliest effect detectable (3) response indicates biochemically significant exposure (4) ideal for evaluation of chemical risk - providing a first screening, and (5) could be used in a tiered approach - with varying degrees of specificity and ability to detect chemical combinations. Key protein systems known to respond to pollutants in the environment include cytochrome P450 monooxygenases, metallothioneins, stress/heat shock proteins, and Phase II enzymes. Oxidant-mediated responses (radicals) and heme/porphyrin synthesis offer alternative processes that may be sensitive to exposure. **More details about these are given in Appendix IB.**

**Criteria for an effective 'effect' biomarker** include the following:

1. Baseline data for concentration or activity should be known to distinguish between natural variability and contaminant-induced stress
2. Basic biology and physiology of the test organisms must be known so that sources of uncontrolled variation can be minimized
3. All intrinsic and extrinsic factors that affect the biomarker should be known

4. Changes in biomarker concentration must be attributable to physiological acclimation or genetic adaptation
5. Increased levels should be correlated with a change in health or fitness of the test organism

For few of the protein biomarkers discussed in Appendix IB are all these criteria met rigorously. Thus, most of the markers remain experimental and in a research phase.

**Community Indicators.** Spatial or temporal changes in community composition, detectable through ordination and other multivariate approaches, may reflect change at the assemblage level due to exposure to sewage (Appendix IA). These techniques may be very sensitive to changes in community structure, and can be valuable in providing indication of change that is not detectable with summary or parametric statistics. However, many confounding factors, including other forms of environmental forcing may be responsible. In the case of the Point Loma Outfall, inputs from other contaminant sources may be involved. Accurate interpretation of the role of sewage and the significance of the community change may require assays linked to organism fitness or ecosystem function.

**Recommendations:** The suitability of bioindicators and biomarkers as indicators of PLOO influence should be explored at sites shown to be depositional hotspots (new and existing monitoring stations). We suggest a special study in which sediments and organisms are collected from the present benthic monitoring sites to screen for sewage exposure (e.g.,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , fatty acid, coprostanols, linear alkyl benzene sulphonates). We also suggest analysis of the organisms identified as (a) most representative of the region (e.g., *Lanassa* sp. D, *Amphiodia urtica*, *Spiophanes duplex*, Pacific sandab, longfin sanddab, stripetail rockfish, yellowchin sculpin and plainfin midshipman, *Lytechinus pictus*, *Sicyonia ingentis*, *Astropecten verrilli*, *Acanthoptilum* sp., and *Parastichopus californicus*); (b) taxa most sensitive to change (*Amphiodia* spp, *Euphilomedes carcharodonta*, *Capitella 'capitata'*, ampeliscid, and phoxocephalid amphipods); and (c) assay taxa that can be compared nationally (e.g., *Mytilus*, *Emerita*). These organisms represent a range of lifestyles and feeding modes and thus exposure mechanisms (e.g., via sediment ingestion, porewater exposure, suspended particulate ingestion, overlying water exposure). In addition, algae should be assayed if they occur within the sampling regions (e.g., kelp) because they are known to be sensitive indicators of sewage exposure.

For those taxa whose tissues reflect significant or possible exposure at some stations, we suggest analysis of (a) endocrine disruption and (b) biomarkers to diagnose stress response to sewage exposure. We encourage to City to become involved in EDC evaluation through special studies programs linked to regional efforts. Studies of vitellogenin and spawning history may be suitable components.

More exploratory measures for fish stress response, examined with special studies, could include Cytochrome P450 for detection of biochemically significant induction by polyaromatic and polychlorinated hydrocarbons, and metallothioneins—metal-binding proteins that sequester and metabolize heavy metals. Detection of P450 can be by

catalytic enzyme activity in liver, antibodies, or DNA probes. In addition, they are induced by both essential and nonessential toxic heavy metals in fish and invertebrates. This approach may only reveal significant stress. For example, Brown et al. (1987) found that liver metal concentrations in fish around an outfall were low, despite high metal concentrations in sediments and water borne particulates. It is important that these analyses be carried out on both exposed and 'clean' specimens for appropriate interpretation. Other biochemical markers are available (e.g., see Kloepper-Sams & Stegeman, 1992), but an understanding of their significance may be insufficient to recommend their use.

### **Modification of the Present Sampling Locations**

The choice of benthic monitoring stations should be determined as being part of a shelf-wide, source-discriminating, regional monitoring program. In this sense, the choices should be based on information of plume dispersion, shelf circulation, and patterns of sedimentation as described earlier. The following criteria should also be considered when choosing benthic sampling stations.

Control sites should be chosen so that they represent similar habitat but in relatively pristine areas. This is not possible for the shelf off San Diego (Silver Strand and Mission Bay Subcells) because there are so many possible sources of contamination, some of which have been contaminating the shelf for decades. However, the Oceanside Littoral Cell, located north of the La Jolla Submarine Canyon, has not been subject to the same urban contamination and represents the best available control area.

Stations chosen to evaluate benthic, suspended particulate, and dissolved samples should be located immediately adjacent to the sources of contamination described earlier to identify useful source fingerprints. That is, it is important to attempt to chemically "fingerprint" LA-5, the two bays, the two outfalls, and the Tijuana River inputs.

### **Outplanted Biological Indicators**

The spatial extent of source-specific contamination can be studied using a bioindicator (or biological mimic) outplant approach. We recommend modification of existing Mussel Watch protocols. Mussels feed on suspended particles and adsorb dissolved compounds through their gills, thus incorporating contaminants in their tissues and serving as recorders of the local particle contamination climate. The State Mussel Watch Program in California, initiated in 1977 by the California State Water Resources Control Board, utilizes mussel assays of known contaminants as a proxy for water-body impairment. The interpretation of impairment results from a ranking of contaminant concentration in the mussels among water bodies.

This approach can be used to determine the spatial extent of bay and river plumes along the shoreline and on the shelf. Because mussels integrate the particle contamination signal over time, their tissues effectively record the relative times that they are exposed to



contaminated particles. Furthermore, the unique combination of contaminants in tissue and shell can be useful for source discrimination. Researchers at Scripps Institution of Oceanography (Parnell, Dayton, Becker and Levin) are presently investigating the utility of this method in Point Loma in collaboration with the City of San Diego Wastewater Monitoring Program.

As part of a pilot project to determine the utility of this method, Parnell put out juvenile mussels (25–50 cm in shell length) in cages near the surface and near the bottom at four sites in the vicinity of Point Loma and at a reference site near the Scripps Pier in La Jolla (Fig. 16) for two months. Unfortunately, the mooring inside the mouth San Diego Bay was lost. Chemical analyses were conducted by the City of San Diego Wastewater Chemistry Laboratory in accordance with California State Mussel Watch protocols (State Water Resources Control Board, 1996). An MDS analysis was performed on concentrations of the organics, heavy metals, and PCBs that were detected in the mussel tissue. The results (see Fig. 17) indicate that these tissue constituents clustered into three main groups: Scripps Pier, the near-bottom site closest to the tip of Point Loma (red circle in Fig. 16), and all remaining samples. The sample from Scripps Pier had low concentrations of most constituents, while the bottom sample from near the mouth of the Bay had highly elevated concentrations of aluminum, zinc, iron, lead, and some PCBs, as well as moderately elevated concentrations of nickel, manganese, and other PCBs. These results indicate that particulates in the nepheloid layer near the mouth of the Bay are contaminated and, because of its proximity to the bay, it is likely that the contaminants are coming from the bay. Similar multivariate analyses have been performed on elemental concentrations in shells of newly recruited mussels, revealing strong Bay signals and variation along the open coast (Becker et al. in press). These sort of projects need to be done over a larger scale in order to fingerprint the potential sources of pollutants, and should be done using more sensitive source tracers possibly including biomarkers and bioindicators.

A similar method of determining the spatial distribution of contaminants, but in the water column, utilizes the chemical-fingerprint recording capacity of shelled invertebrate larvae and otoliths (earbones) of fish larvae (e.g., DiBacco & Chadwick, 2001, and current work by Levin and Becker). Many species of invertebrates and fish drift as plankton during their early life stages, and can be used as tracers of estuarine and bay plumes (Parnell, 2001). Chadwick and Largier (1999) successfully used ultraviolet fluorescence as a tracer of water ebbing from San Diego Bay.

## **Mining and Synthesizing Old Data with New Analyses**

In the course of our evaluation, we have come to appreciate the sheer volume of data that have been collected by the City of San Diego Wastewater Monitoring Program as well as by several other agencies. For the most part, these data appear to be used for the production of periodic reports that document the effects of various sources of pollution in the San Diego area. To our knowledge, few of these data are used again beyond some selective use in time series. We urge that these data be merged into a single database so that they can be re-evaluated and reanalyzed with multivariate techniques using regional and long-time series perspectives.

Present multivariate approaches may be expanded to include techniques that allow statistical comparisons of community composition (e.g., multivariate, multidimensional scaling, analysis of similarity, and simple persistence). The goal would be to (a) determine to what extent communities associated with the PLOO can be distinguished from those subject to other sources and (b) whether the degree of differentiation varies seasonally or interannually (i.e., are PLOO effects on communities identifiable in some seasons but not others).

### **Additional regional issues of concern**

There are a variety of problems and issues that may be indirectly linked to the PLOO, but are considered by the report authors to be of sufficiently large scale that they have been considered outside the purview of this review and the scope our analyses. Notably among these are higher organisms with extremely large ambits, such as birds, turtles and marine mammals. They may be affected directly by contaminants in any of the described sources (outfalls, bay flushing, dumpsite or non-point source runoff) in southern California. Of particular concern are endocrine disruptors and pathogens. While some of these may be sewage associated, many may not be, and it is not considered feasible at this time for the City to address these issues. The City monitoring effort assesses the lower ends of the food chain (through fishes). Should key problems be demonstrated in contaminant levels or exposure responses in benthos, megafauna or fishes through the studies suggested in this report, then it would be wise to enlist regional agencies to examine indirect effects (through bioaccumulation of contaminants or interspecies transfer) on higher trophic levels. Similarly, it may be desirable to perform additional tests on pathogens and sediments that are clearly regional in nature. One example includes the distribution of non-sewage related pathogens that pose a possible human or marine mammal health risk. Another is sediment toxicity testing and toxicity identification evaluation procedures. Such assessments would need to be implemented by umbrella organizations such as SCCWRP. Another area where regional approaches can play a key role is in the selection of more suitable reference sites and regional reference conditions for benthic studies. Bight wide monitoring by SCCWRP again can contribute valuable insight. This list can go on, but highlights the considerable need for a regional approach to many environmental issues.

## **VI. Microbial Monitoring**

The objectives of this section were to review bacteriological water quality monitoring being conducted by the City of San Diego, with particular attention to that associated with the Point Loma Ocean Outfall. Current developments in the field of monitoring water-quality microbiology were evaluated, and recommendations concerning future monitoring practices are presented. Particular attention has been paid to issues of rapid detection and microbial source tracking.

### **A. Background**

Bacterial indicator organisms are used throughout the world to measure the infection risk present in aquatic environments such as along public beaches. Different indicators and indicator threshold levels are used in different states, countries, and regions. Indicator microbes are not necessarily pathogenic, but are monitored because they are representative of the presence of human wastes where pathogenic bacteria or viruses could exist. The most commonly measured indicators are total coliforms (TC), fecal coliforms (FC), and enterococci (EC). TC is a group of bacteria present in many different environments, including soil, plants, and animals (including humans). More specifically, they are aerobic and facultatively aerobic gram-negative, nonspore forming rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. FC is a group of coliforms that are passed through the fecal excrement of humans, livestock, and wildlife. The most well-known member is *Escherichia coli*. These organisms may be separated from the total coliform group by their ability to grow at elevated temperatures. EC are gram-positive, homofermentative chain-forming cocci that are typically of fecal origin.

Threshold levels for indicator bacteria have been derived from epidemiological studies carried out by the U.S. Public Health Service (cited in Noble, Moore, et al., 2003). Swimming-associated health effects were found in waters containing more than 2,300 colony-forming units (cfu) per 100 ml of TC and by extrapolation 200 cfu per 100 ml for FC. Later studies revealed that EC provided an even better correlation to bather illness than TC or FC (Cabelli, 1983a, 1983b; Cabelli et al., 1982; Dufour, 1984). The levels of EC in ocean waters have also been shown to correlate with the incidence of illness in swimmers from Santa Monica Bay, California (Haile et al., 1999). The utility of EC as an indicator organism is most likely a reflection of its closer association with human feces than scat from other animals and because it survives longer in aquatic environments (Griffin, 2001, Griffin et al., 2001). Accepted threshold levels for EC are more likely to fail than those of TC or FC (Noble, Moore, et al., 2003), particularly during dry weather when the numbers of sewage-associated bacteria are typically lower. To its credit, the City of San Diego is currently participating in a epidemiological study of Mission Bay, comparing indicator bacteria and virus levels with public health data <http://www.sccwrp.org/about/rspln2004-2005.html#c3>.

In 1986, the U.S. Environmental Protection Agency recommended that EC be used as the sole indicator for ocean water monitoring. As of 2001, only seven states and three

territories had adopted EC as a marine water quality indicator microbe (Griffin et al., 2001), but an increasing number of states have or are planning on incorporating EC detection into their monitoring programs (Noble, Moore, et al., 2003). Hawaii is the only state that also utilizes the anaerobic spore-forming fecal bacterium *Clostridium perfringens* (CF) as a bacterial indicator. Hawaii's bacterial limits for marine waters are also the most demanding, requiring no more than 7 EC or 5 CF per 100 ml of water sample (Griffin et al., 2001).

## **B. California State Regulations**

During the late 1990s, a number of California state assembly bills (AB) were passed that influence water-quality testing in the state. AB411 set new monitoring standards weighted toward weekly sampling. It also required the local health officer to post conspicuous warning signs and establish a telephone hotline to inform the public about a beach that fails to meet standards developed by the Department of Health Services.

AB538 directed the State Water Resources Control Board to develop protocols for identifying sources of contamination where bacteriological standards have been repeatedly exceeded at heavily visited beaches. And, AB1946 required local health officers to submit a monthly survey to the State Water Resources Control Board (SWRCB) detailing information on beach postings and closures that were due to failure to meet bacteriological standards. It also required SWRCB to establish a specific format for the surveys, make the information available to the public on a monthly basis, and publish an annual statewide report. More recently, AB639 was passed in 2001, which directs the Water Resources Control Board to work with the Department of Health Services to develop rapid diagnostic tests to measure levels of bacterial contamination in waters adjacent to public beaches.

In 1999, as a result of AB411, California changed its procedures for testing ocean microbial water quality from TC testing to quantifying the levels of three types of indicator organisms: TC, FC and EC. The standards are described in the California Code of Regulations, Title 17, State Ocean Water Quality Standards (#7958). They are as follows:

1. Based on a single sample, the density of bacteria in water from each sampling station at a public beach or public water contact sports area shall not exceed:
  - a. 1,000 TC per 100 ml, if the ratio of FC/TC exceeds 0.1,
  - b. 10,000 TC per 100 ml,
  - c. 400 FC per 100 ml, or
  - d. 104 EC per 100 ml.
  
2. Based on the mean of the logarithms of the results of at least five weekly samples during any 30-day sampling period, the density of bacteria in water from any sampling station at a public beach or public water contact sports area, shall not exceed:

- a. 1,000 TC per 100 ml,
- b. 200 FC per 100 ml, or
- c. 35 EC per 100 ml.

### **C. San Diego Bacteriological Water-Quality Monitoring**

The typical methods used to enumerate indicator bacteria are membrane filtration, or multiple tube fermentation (MTF; Madigan et al., 2003; Noble, Weisberg, et al., 2003). In membrane filtration, 100 ml or more of a water sample is passed through a sterile membrane filter that removes bacteria and then the filters are incubated on bacteriological growth medium, and the resulting bacterial colonies are counted. In MTF, samples are inoculated into a liquid medium and incubated, and evidence of microbial growth such as cell turbidity or production of a chromogenic compound is used to deduce cell numbers. The City of San Diego in conjunction with the County of San Diego Department of Environmental Health uses both membrane filtration and MPN. Membrane filtration is used to determine the numbers of TC and FC according to methods described in Protocols 9222B and 9222D listed in the 20th edition of standard methods for the examination of water and wastewater (Eaton et al., 1998). The IDEXX Laboratories Enterolert™ MPN system (ASTM Method #D6503-99) is used for EC quantitation via MTF.

The City of San Diego and other cities in the county routinely monitor water samples collected from about 100 shoreline locations on a weekly basis during the summer (and fewer locations during the remainder of the year). Specifically, the City of San Diego presently monitors 19 sites from Mission Bay to the Mexican border on a weekly basis throughout the year. A useful website to access the results of this monitoring and that of other California coastal areas is <http://www.healthebay.org/>. When monitoring indicates that water quality does not meet state bacteriological standards, a water contact advisory is issued and warning signs are posted 50 yards on either side of the sampling location in question.

The Annual Receiving Waters Monitoring Reports for the Point Loma Ocean Outfall for the last three years can be found at <http://www.sannet.gov/mwwd/environment/reports.shtml>. These documents along with the quality assurance manuals provide excellent details on how and where the measurements have been performed along with overviews of the results. The data improved in 2002 with the addition of EC counts along with those of TC and FC.

Clearly there are spatial and temporal trends in the distributions of these fecal indicator bacteria (FIB). The average concentrations of FIB measured at some of the coastal stations are much higher during our rainy months (February–March), presumably because of terrestrial runoff. This is especially apparent at the southernmost stations (previous sites D1–D3), which are influenced by discharge from the Tijuana River. Station D8 located near Ocean Beach periodically (less than 20% of the time) has bacterial

concentrations exceeding compliance standards. High bacteria numbers associated with PLOO are generally located at depths greater than 60 m and are limited to an area within about 2 km of the outfall, with occasional transport to some of the northern and southern offshore sampling locations. This is reflected in the mean indicator bacterial densities for quarterly sampling at the PLOO offshore stations versus the kelp stations closer to shore. The latter typically have counts less than 1/100 of that of the former.

#### **D. Pros and Cons of Methods Being Used**

Griffin et al. have listed a number of positive and negative aspects of current indicators being used (Griffin et al., 2001). Some of the advantages of the currently used indicators and their methods of detection are that their culture-based detection is simple and inexpensive, their presence correlates in many cases with human pathogen presence, and in some cases their presence has been correlated to public health risks. Some of the disadvantages of the methods and indicators in use are that detection takes a long time (12–18 hours), the methods are subject to false positives and false negatives, culturing does not detect the so-called “viable but nonculturable” forms of microbes (Roszak & Colwell, 1987), and all of the indicators can be shed by nonhuman animal sources, in which case the health risk to people is less clear.

The need for rapid detection is great. Changes in indicator bacteria levels in beach waters occur more rapidly than the time required to obtain assay results (Boehm et al., 2002). The result is that contaminated beaches are not posted soon enough and posted beaches are not reopened soon enough. It is because of this problem that the above-mentioned AB639 was introduced. The State Water Resources Board has requested that the Southern California Coastal Water Research Project Authority promote the development of rapid diagnostic tests. A brief description of SCCWRP subcontracts in this area can be found at [http://www.sccwrp.org/whatsnew/rapid\\_indicator/index.html](http://www.sccwrp.org/whatsnew/rapid_indicator/index.html).

A related issue to that of rapid detection is microbial source tracking (MST). This is another area of concern at State Water Resources Control Board (<http://www.sccwrp.org/about/rspln2004-2005.html#c1>). It is often desirable to determine whether the microbial assay results reflect human sewage contamination or the introduction of microbes from other sources such as cattle, dogs, or gulls. Griffith et al. have recently reviewed this issue (Griffith et al., 2003). They have referred to four basic categories of MST. The first is genotypic characterization of isolated, cultured bacteria. The second is phenotypic characterization of isolated, cultured bacteria. The third is genotypic characterization of selected genes obtained from environmental DNA samples, and the last category is direct measurement of bacterial or human viruses. In a recent study, it was concluded that the third method (involving host-specific polymerase chain reaction methods) was highly sensitive and yet also yielded very few false-positive results (Griffith et al., 2003).

An excellent example of the issue of MST is the Children’s Pool in La Jolla, which is home to a colony of harbor seals. This beach area has previously been ranked by Heal the Bay as the worst polluted beach in California (*San Diego Union Tribune*, 10/19/03). MST was used to determine the source of the high-indicator bacterial counts. In 1997, collaborative studies were performed between the County of San Diego Department of Environmental Health and the laboratory of George M. Simmons at Virginia Institute of Technology in Blacksburg, Virginia. Genotypic characterization of *Escherichia coli* isolates from water and seal scat samples was performed. The resulting DNA fingerprinting of Children’s Pool water sample isolates indicated that they were all of nonhuman origin and most appeared to be of seal origin. As a result of these efforts, the primary source of the contamination in the Children’s Pool has been established.

### **E. Additional Methods and Indicators To Consider**

The technologies now exist to determine the presence of particular pathogens of interest directly. This calls into question the need for using indicator microbes as proxies for their presence. It is also possible to detect pathogens whose presence is not necessarily associated with sewage and to rapidly detect various indicator organisms. Some of these are listed in Table 1 and are described in more detail below.

Table 1. Additional public health related microbes or detection methods to consider.

Indicator Organism	Detection Method
F+-specific RNA coliphage	Plaque forming units
<i>Bacteroides fragilis</i> bacteriophage	Host cell lysis
Adenovirus	PCR
Enterovirus	PCR
<i>Escherichia coli</i>	PCR
Shiga toxin <i>Escherichia coli</i>	PCR
<i>Bacteroides-Prevotella</i> group	PCR
<i>Vibrio parahaemolyticus</i>	PCR
<i>Vibrio vulnificus</i>	PCR
<i>Vibrio cholerae</i>	PCR

**Virus detection:** Viral pathogens found in the coastal environment include hepatitis A virus, rotavirus, enterovirus, adenovirus, and Norwalk virus. An excellent review of the types of viruses present in coastal waters and their detection has been written by Griffin et al. (2003).

Two types of bacteriophage have been promoted as useful indicators: F+-specific RNA coliphage (Hsu et al., 1995) and *Bacteroides fragilis* bacteriophage (Lucena et al., 1996). The presence of F+-specific RNA coliphages correlate with the presence of *Escherichia coli*, and oligonucleotide probing can be used to determine whether the phage are derived

from human or nonhuman sources. *Bacteriodes fragilis* is an anaerobic bacterium that makes up a large fraction of the intestinal flora of humans and other mammals. Its bacteriophage can exist at concentrations of thousands of plaque forming units per 100 ml of contaminated water. They also have similar half-lives in the environment to those of a number of human viruses (cited in Griffin et al., 2001).

Monitoring for the presence of human viruses in water samples has also been performed. The major drawbacks to this approach to water-quality monitoring is that weeks are required to determine if mammalian tissue culture lines are infected, and some viruses, such as the Norwalk or Norwalk-like viruses that are responsible for large outbreaks of shellfish poisoning, cannot be cultured in cell lines (cited in Griffin et al., 2001). A breakthrough in virus monitoring has been the application of the polymerase chain reaction (PCR). PCR methods have been used for both adenovirus and enterovirus monitoring in ocean samples in the last few years (Jiang et al., 2001; Noble & Fuhrman, 2001). These procedures involve ultrafiltration of water samples by one or more of a number of methods, followed by nucleic acid extraction, reverse transcription (in the case of RNA viruses), PCR with appropriate oligonucleotide primers, and PCR product detection. MPN analyses of PCR product detection from various DNA template concentrations can be used to infer virus concentration. Curiously, adenovirus and enterovirus PCR detection has not correlated with the concentration of standard microbial indicators or bacteriophage levels (Boehm et al., 2002; Jiang et al., 2001; Noble & Fuhrman, 2001). Adenovirus numbers have been reported to correlate with those of F+-specific coliphage (Jiang et al., 2001).

**Genotypic library methods:** Isolated microbes can be characterized at the nucleic acid level by a number of techniques for microbial source tracking purposes. This general methodology was performed to identify the source of the *Escherichia coli* in the waters of the Children's Pool in La Jolla. Genotypic library methods include pulse-field electrophoresis, ribotyping, and box-PCR (described in Griffith et al., 2003). While such methods can be highly discriminating, they still require the time and expense of microbe cultivation and isolation along with the facilities and expertise to perform the molecular diagnostic analyses.

**Genotypic nonlibrary methods:** In contrast to the genotypic library methods mentioned above, as well as the standard protocols for indicator bacteria monitoring, the genotypic nonlibrary methods are far more rapid. Thus, they hold great promise for speeding up the detection process. In addition, some of these methods have the additional bonus of being useful for microbial source tracking as well. PCR success depends on controls, controls, controls. As this technology is applied, it will be important to always use protocols that incorporate negative controls (i.e., experiments performed in the absence of added DNA or primer) and positive controls (i.e., water samples seeded with known amounts of target organisms).



Many PCR methods have already been developed for detecting *Escherichia coli* (Fricker & Fricker, 1994; Yavzori et al., 1998). While these have not yet been used for ocean monitoring, their potential for this application would seem to be very high.

One pioneer in the development and use of molecular tools for water-quality monitoring is Katherine Field at Oregon State University. She has focused on the *Bacteriodes–Prevotella* group as indicators of fecal pollution. Although these microbes are strict anaerobes, which are relatively difficult to grow, molecular methods obviate the need for any laborious culturing methods and so negate this concern. And because this group of microbes is highly abundant in feces, they represent a more plentiful fecal target group than other microbes such as *Escherichia coli*. Dr. Fields has identified host-specific small subunit ribosomal RNA gene markers for *Bacteriodes–Prevotella* group members present in humans and cows (Bernhard & Field, 2000a). Further cloning and sequencing experiments have provided enough data for the design of PCR primers, which allow the selective amplification of either human or ruminant sources of this group (Bernhard & Field, 2000b). Primers for the detection and discrimination of a greater variety of host-associated *Bacteriodes–Prevotella* groups are under development. It has already been documented that this approach is more sensitive than culture-based fecal coliform indicator assays, is comparable in complexity to standard food safety and public health diagnostic tests, and it is expected to be scaleable to high-throughput automated formats (Field et al., 2003).

PCR analyses of nucleic acids extracted from coastal waters can be used to look for a variety of organisms and genes of interest. For example, Miyagi et al. (2001) used PCR to look for shiga toxin producing *Escherichia coli* from marine waters in Japan (Miyagi et al., 2001). Not only were they successful in many cases, but the sensitivity was greater than that of culture-based detection methods.

In the future, it should be possible to further refine PCR detection methods. Multiplex PCR, in which several sets of species-specific primers are present in a single reaction, could be used for the simultaneous detection of multiple pathogens in a single PCR reaction (Brasher et al., 1998). Primers will have to be carefully designed and evaluated to ensure that they are sufficiently specific to the pathogen of interest. In addition, these methods are only semiquantitative, and additional experiments must be performed in order to verify the PCR product, either by sequencing or by Southern blot, which increases the assay time.

To resolve these problems, quantitative PCR such as that performed using the TaqMan (Perkin Elmer, Applied Biosystems; Foster City, CA) method may be useful. It has already been shown to be a sensitive, quantitative method to detect microbial pathogens in seawater (Lyon, 2001). This method detects the amplification of PCR products using a fluorescent probe that hybridizes specifically to a sequence between the PCR primers. As the number of PCR products increases, the amount of fluorescence increases, which can be detected using a quantitative PCR instrument. The PCR products do not have to be independently verified because of the specificity of the fluorescent probe, and it is

possible to detect multiple pathogens at once using different primers and different probes with different fluorescent colors (Lyon, 2001). However, to decrease the possibility of PCR product interference, it is probably easiest to set up individual reactions for each pathogen or indicator microbe to be detected. In addition, cells that are in a viable but nonculturable physiological state can be detected using this PCR method (Lyon, 2001).

These types of PCR detection methods are not necessarily a panacea. One issue is that because one is looking for the presence of particular genetic material rather than a growing microorganism, dead, dying, or viable but nonculturable forms of microbes may give a positive result. *Because of this, it will be important in the future to determine whether these methods can be correlated to culture-based standard indicator bacteria levels. Correlations of public health risk with results from promising molecular detection methods will also be necessary in the future to validate their usefulness.* For more information on assessing public risk see Haile et al (1999) and references cited in Noble et al. (2003a). Ideally, a range of illnesses would be monitored.

**Additional nonlibrary methods to consider:** For detecting multiple bacteria, viruses, and perhaps protists, another system to consider is the Luminex™ system. This employs microspheres of unique fluorochrome composition (spectral addresses), each of which can be combined to a distinct species-specific oligonucleotide capture probe. DNA samples obtained from the environment are biotinylated and subsequently provided with another fluorescent tag. Hybridization between environmental DNA and the microspheres is then performed, followed by spectral interrogation of the microspheres in a rapidly flowing fluid stream that is passed by two separate lasers. The result is that the extent of DNA hybridization to 100 different microsphere types can be quantified in a single reaction vessel in a matter of seconds. This method has been used to detect phytoplankton species with a sensitivity limit of 10 cells (Ellison & Burton, unpublished results). It has also been used to monitor the presence of several pathogenic bacteria associated with foodborne illnesses (Dunbar et al., 2003). Sensitivity can be further increased by PCR amplification of target sequences of interest. This method has the advantage over other molecular approaches for our application here in that while it enables rapid identification of multiple species, which is more difficult with real-time or quantitative PCR, it is easily modified and is cost-effective (as compared with genechips or microarrays (Yang et al., 2001). However, extensive testing would be necessary to evaluate specificity and sensitivity as described (Dunbar et al., 2003).

Additional rapid detection methods are in development. SCWRRP subcontracts in this area include methods that incorporate immunomagnetic separation of bacteria of interest coupled with fluorescent or luminescent detection methods and a dual-wavelength fluorescence detection system for monitoring species-specific exoenzyme activity ([http://www.sccwrp.org/whatsnew/rapid\\_indicator/index.html](http://www.sccwrp.org/whatsnew/rapid_indicator/index.html)). The reliability of such approaches and their comparability to existing detection methods is currently unknown. Dr. Chris Scholin at the Monterey Bay Aquarium Research Institute is also working to develop instruments that collect water samples autonomously, concentrates the microbes, performs DNA probe array assays and transmits the results real-time to a shore based

location (<http://www.mbari.org/staff/scholin>). However, the availability of such devices for routine water quality monitoring is still likely to be years in the future.

**Indigenous marine microbes and viruses to consider:** While the focus of this report deals specifically with the PLOO, it is important to document that developments in microbe detection will also make possible the detection of microbes which may be of public health concern, but whose presence is not specific to sewage. Bacteria of the genus *Vibrio* are natural residents of marine environments around the world. Their levels do not correlate with those of fecal indicator bacteria (Griffin et al., 2001). The abundance of particular *Vibrio* species can be  $10^4$  cells  $\text{ml}^{-1}$  in seawater or per individual zooplankton, and can be concentrated 100-fold by filter-feeding shellfish. *Vibrio* species can be the dominant microbial species present off the Scripps Institution of Oceanography Pier (Rehnstam et al., 1993). Many *Vibrio* species are important human pathogens whose numbers are highest during the summer months when seawater temperature is highest and recreational use of our beaches is greatest. Their route of entry is typically from wounds or ingestion of contaminated water or food. According to year 2000 data from the Centers for Disease Control Vibrio Surveillance System, 26% of all U.S. non-cholera *Vibrio* infections that year were reported in California. The principle *Vibrio* threats to human health in California waters are posed by *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* (Morris, 2003). *V. parahaemolyticus* is a major enteropathogen responsible for a striking increase in infections since the mid-1990s. *V. vulnificus* causes severe wound infections, septicemia, and gastroenteritis and is the most frequent cause of serious vibrio infections in the United States. *V. vulnificus*-related disease prompted California to implement regulatory educational initiatives in 1991 (Mouzin et al., 1997). A variety of vibrios including *V. parahaemolyticus* and *V. vulnificus* can also cause ocular infections (Penland et al., 2000). Most *V. cholerae* strains in California lack the genes necessary to cause the disease cholera, but may still cause diarrhea and in some cases septicemia. *V. cholerae* acquired in San Diego has also been reported to cause necrotizing fasciitis and septic shock (Wagner et al., 1995). Symptoms of both *V. cholerae* and *V. parahaemolyticus* infections include diarrhea (sometime bloody), abdominal cramps, nausea, vomiting, and fever.

All of these vibrios have been routinely isolated off our West Coast (Jiang, 2001; Jiang, et al., 2003; Jiang & Fu, 2001; Kaufman et al., 2002; Portillo-López & Lizárraga-Partida, 1997; Strom & Paranjpye, 2000). Explosive growth of *V. cholerae* has been observed in seawater obtained off the Scripps Pier during a phytoplankton bloom (Mourino-Perez et al., 2003).

PCR primer sets have already been designed for detecting vibrios. In the case of *V. cholerae*, a primer and probe set has been designed to amplify a segment of the *hlyA* gene that is present in most strains tested so far, including toxigenic and nontoxigenic strains (Lyon, 2001). This assay was able to detect 10 colony-forming units  $\text{mL}^{-1}$  in sterile artificial seawater. TaqMan methods have also been used to detect *V. vulnificus*, by using primers specific to the hemolysin/cytolysin gene *vhA* (Campbell & Wright, 2003).

However, the lower limit of this assay was less than 250 CFU per mL. Real-time PCR methods have also been developed to detect pathogenic *V. parahaemolyticus* by looking at strains possessing the thermostable direct hemolysin gene, *tdh* (Blackstone et al., 2003).

An example of a indigenous virus group to consider monitoring are the caliciviruses, RNA viruses that have genome sizes of 7–8 kbp. These are among the most common causes of viral-induced diarrhea and vomiting in people. Ocean caliciviruses (genus *Vesivirus*) can cause disease in terrestrial species such as a disease of pigs known by the acronym VES. Indeed, in 1932 in San Diego pigs developed VES apparently after eating raw garbage from a restaurant that contained seafood waste. Many fish, seals, sea lions, dolphins, and whales are now known to be susceptible to calicivirus infections (with fish and fish products being the usual mode of transmission to land). Gray whales may excrete  $10^{13}$  caliciviruses per whale daily, so large marine mammals could be an important source of these viruses. Serotype analyses of *Vesivirus* infections in mink, reptiles, primates (including man), cattle, skunk, fish, and nematodes suggest that most of these viruses originate in the ocean. DNA hybridization experiments also indicate that shellfish can sometimes contain caliciviruses, although it is not known if these viruses can infect them. No other virus has been shown to have its primary origin in the sea and yet emerge to cause disease in people (Smith et al., 1998).

Of course, the central issue in considering the detection of any of indigenous microbes or viruses is whether the risk to the public of infection warrants their surveillance.

**Pathogen effects on aquatic life.** It is also possible that bacteria, viruses, fungi or protozoa may be present in sewage and are capable of infecting marine life such as marine mammals. Advances in detection strategies may make it cost-effective in the future for the city to consider monitoring the abundance of these pathogens as well. Thus, it will be important to keep abreast of developments in these fields and relevant research being conducted at the regional and national level.

## **F. Concluding Remarks and Recommendations on Microbial Monitoring**

The City of San Diego currently has an active program of monitoring bacterial indicators of fecal contamination from a variety of coastal environments, including the vicinity of the PLO. TC, FC and EC are monitored and evaluated using well-accepted standard operating procedures. San Diego, along with every other coastal municipality in the country is now faced with new opportunities for more rapidly assessing health risk as well as to more precisely track the source of contaminating microbes. The City should continue to partner with other agencies to identify promising methods that warrant comparison with the monitoring practices currently in place. Future studies are also needed which correlate public health risks to results from these monitoring methods. All of the recommendations below should be considered in partnership with regional and national organizations such as SCWRRP, the Water Research Foundation (WERF) and the EPA (i.e., Beaches Environmental Assessment and Coastal Health Act grants). Some of the more specific issues to consider are as follows:

1. *Should human virus monitoring be based on molecular methods that directly test their presence or should bacteriophage such as F<sup>+</sup>-specific RNA coliphage or Bacteroides phage be used as a proxy for their possible presence?*
2. *What rapid detection practices can be implemented in the near future?* The DNA-based nonlibrary methods seem to hold great promise, but should be compared with other methods being proposed such as those involving immunomagnetic separation of bacteria of interest coupled with fluorescent or luminescent detection. PCR-based methods will be important for a variety of water-quality risk assessments in the future, so investing in this technology now will be prudent, even if primers, protocols, and target organisms are still being debated.
3. *What microbial source tracking methods can be implemented in-house in the near future?* Again, PCR-based methods hold great promise, but library-based methods (as used in the case of the Children's Pool in La Jolla) should also be considered.
4. *Should infection risks that are not sewage-related also be routinely monitored?* Because molecular methods make it possible to follow the presence of many potential pathogens relatively quickly, risks from other infectious agents whose presence does not correlate with fecal indicator bacteria should also be considered.

## **VII. Summary and Conclusions**

As described in detail earlier, the question of how to assess the impact of the Point Loma Ocean Outfall is a complex question. The City of San Diego's current monitoring program has provided important pieces of information, but it is not adequate when taking into account the regional perspective and the need to distinguish between multiple potential sources of contamination. The physical circulation of coastal waters and the movement of the effluent within those waters are not well understood, and the City does not appear to have the modeling tools needed to predict the behavior of the plume under a variety of conditions. We have described steps the City can take, based on examples in other comparable locations, to develop this capability. The impact of the effluent on the benthic environment is more extensively monitored and better understood. Nonetheless, better placement of control stations and additional analytical techniques could help clarify the benthic situation. Finally, new science and technology provide the potential to identify and respond to human health threats from ocean pathogens. The City must undertake careful studies to assess and prioritize health risks and work with other groups to determine the most appropriate monitoring and intervention strategies to address this important area.

Scripps Institution of Oceanography is confident that improved regional understanding can be achieved by following the recommendations of this report.

## Figures