

**Sanitation Districts of Los Angeles County
Joint Water Pollution Control Plant
Proposed Special Study
2016**

**ASSESSMENT OF ICHTHYOPLANKTON META-BARCODING
FOR ROUTINE MONITORING (JWSS-16-003)**

Goals and Objectives:

Discharge of treated wastewater through deep ocean outfalls results in the creation of a rising diluted effluent plume that interacts with the pelagic ecosystem, yet little to no NPDES monitoring is conducted to assess potential impacts to this ecosystem. Recognizing this limitation, ichthyoplankton monitoring is a specific objective within Pelagic Ecosystem component of the Comprehensive Monitoring Program (CMP) for Santa Monica Bay. Further, support for implementing the SMBRC CMP is a requirement in the NPDES permits for the Hyperion Treatment Plant (HTP) and Joint Water Pollution Control Plant (JWPCP). Historically, the number of samples combined with the time and specialized taxonomic skills needed to identify fish larvae have made implementation of meaningful ichthyoplankton monitoring prohibitive. However, the use of genetic meta-barcoding may make analysis of samples collected at the intensive spatial and temporal scales required for such monitoring cost-effective.

The overall goal of this research is to develop a framework for cost-effective and informative ichthyoplankton monitoring within the coastal waters of Santa Monica Bay (SMB) and beyond. The main objectives for this Special Study are:

1. Build capacity among local NPDES monitoring groups to collect standardized ichthyoplankton samples for meta-barcoding, consistent with California Cooperative Oceanic Fisheries Investigations (CalCOFI) procedures, in the coastal ocean.
2. Participate along with other large POTWs and other organizations to collect ichthyoplankton samples during Spring 2016
3. Obtain a snapshot of the ichthyoplankton population within SMB coastal waters
4. Test if meta-barcoding is a viable method to identify ichthyoplankton compared to traditional methodology used by CalCOFI.
5. Determine whether meta-barcoding significantly reduces time, cost, and specialized taxonomic expertise needed for ichthyoplankton monitoring.
6. Assess whether meta-barcoding based ichthyoplankton community analysis would allow a meaningful status and trends monitoring program to be implemented within SMB and or/ the Southern California Bight (SCB).

Benefits:

This study will provide a long overdue snapshot of the current ichthyoplankton community within SMB and allow comparison with other nearshore areas of the SCB using traditional morphological taxonomic identifications. If meta-barcoding is deemed an accurate and cost-effective substitute for traditional taxonomic analysis, existing National Pollutant Discharge Elimination System (NPDES) monitoring programs within SMB may implement an ichthyoplankton status and trends monitoring program consistent with the SMBRC CMP.

Further, similar monitoring may be incorporated within future Bight Regional Monitoring Program administered by the Southern California Coastal Water Research Project (SCCWRP) to assess the entire SCB and allow comparisons with conditions in SMB.

Approach:

Samples of ichthyoplankton, including fish eggs, will be collected from sites associated with our NPDES demersal fish and epibenthic invertebrate trawl program (**Figure 1**). Similar samples will be collected on the same day by the City of Los Angeles from their HTP trawl monitoring stations. Samples will be collected via vertical tows with a 150-um mesh paironet (25-cm diameter mouth) and placed in 95% ethanol. Samples will then be transported to SCCWRP where the ethanol will be changed over a series of days. Sample contents will be sorted and morphologically identified to the lowest possible taxonomic level by National Oceanic and Atmospheric Association (NOAA) Southwest Fisheries staff in La Jolla, CA.

After all of the individuals in a sample have been sorted and morphologically identified, the ichthyoplankton species will be reconstituted as a single sample and a random aliquot of the sample will be removed and homogenized for meta-barcoding analysis, while the remaining material will be archived. Meta-barcoding is the use of universal genetic markers to identify multiple species within a single sample, simultaneously. The work involves extraction, amplification, and sequencing of targeted pieces of DNA from a homogenized sample. A yet to be determined contract laboratory will be selected by NOAA Southwest Fisheries to perform the meta-barcoding on these samples. The sequence data will be cleaned, filtered, and then queried against a reference library for adult fish from the Southern Californian region. The reference library is being constructed at SCCWRP by mining the Barcode of Life and GenBank databases for sequences from species known to be present in CalCOFi and Scripps Institution of Oceanography pier sampling.

SCCWRP staff will compare ichthyoplankton sample composition, characterized by meta-barcoding, to that produced by traditional morphological methods to evaluate if the genetic-based methods can produce similar results. Similarity between the methods will be evaluated from ecological (e.g., species composition, functional composition, etc), environmental assessment (e.g., species indicative of water quality issues), and fisheries (e.g., anchovies, Kelp Bass, sardines) perspectives.

Project Duration:

Initial sample collection will occur in the late spring/early summer of 2016. Samples will be processed for morphological and meta-barcoding identification by winter of 2017. Analyses and manuscripts will be completed by summer 2017.

Deliverables:

The results, analyses, and conclusions of this Special Study will be documented in quarterly progress reports and a final comprehensive report will be submitted to the Los Angeles Regional Water Quality Control Board by August 15, 2017. Moreover, and published journal articles resulting from this work will be provided to the LARWQCB when available.

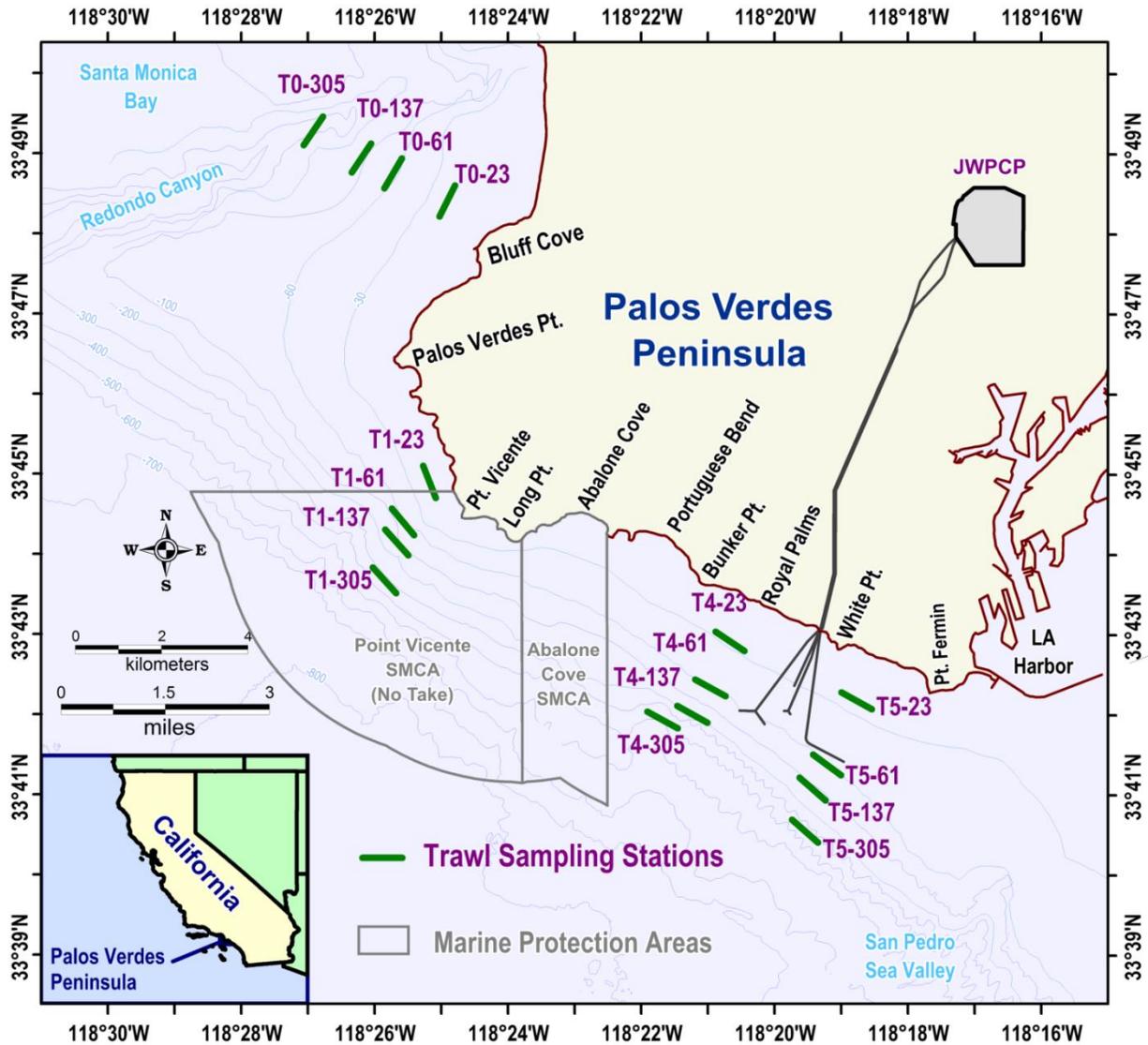


Figure 1 Map of the Sanitation Districts demersal fish and epibenthic invertebrate trawl stations. Ichthyoplankton samples will be collected from each of these sites in the late spring/early summer of 2016 in coordination with simultaneous sampling by City of Los Angeles, HTP staff at their trawl sites.

Collaborators:

The samples collected for this Special Study will be part of a larger effort by staff from SCCWRP, NOAA Southwest Fisheries, CalCOFI, UC Santa Barbara, Scripps Institute of Oceanography, and SCB POTWs, including the Sanitation Districts of Los Angeles County and the City of Los Angeles Environmental Monitoring Division.