



**Southern California Association of
Marine Invertebrate Taxonomists**

3720 Stephen White Drive
San Pedro, California 90731

November 1989

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NEXT MEETING: Holothuroidea Workshop

GUEST SPEAKER: Dr. Mary Bergen, California State
Lands Commission

DATE: Friday, December 8, 1989, 9:30 AM

LOCATION: Cabrillo Marine Museum
3720 Stephen White Drive
San Pedro, CA 92009

MINUTES FROM MEETING ON NOVEMBER 13, 1989

Sampling Standards Meeting: This meeting was conceived, organized, and chaired by Larry Lovell, Vice-President of SCAMIT. Larry began the meeting by introducing Dr. Suzanne Lawrenz-Miller, Director of the Cabrillo Marine Museum, who welcomed the participants and presented a short summary of the Museum and its close relationship with SCAMIT.

The purpose of this meeting was fourfold: 1) presentation of sampling procedures by various wastewater treatment plants and consulting firms, 2) discussion of similarities and differences among these various procedures, 3) discussion of advantages and disadvantages of each, and 4) discussion of possible recommendations for standardization. Differences in benthic sampling procedures do affect the data and present a problem when comparing data collected from various agencies. The National Academy of Sciences and the Southern California Bight Review Committee are interested in standardizing sampling techniques. Recommendations for standardization were not solicited from SCAMIT by these two groups; however, SCAMIT took the initiative to hold this meeting for the purpose of drafting such a document.

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Eleven 20-minute talks were presented by representatives of various California agencies: 1) Dave Montagne, Los Angeles County Sanitation Districts, 2) Scott Johnson, Hyperion Treatment Plant, City of Los Angeles, 3) Doug Diener, MEC Analytical Systems, Inc., 4) Tim Rothans, Pt. Loma, City of San Diego, 5) Marilyn Smith, City and County of San Francisco, 6) Jim Laughlin, SCCWRP, 7) Skip Newton, Kinetics Labs, 8) Tim Mikel, ABC Labs, 9) Don Cadien for MBC Applied Environmental Services, 10) Larry Lovell for ECOMAR, and 11) Rick Ware for the City of Avalon (Santa Catalina Island Outfall). A summary gleaned from these seminars are presented below and tabulated (see attached pages).

Sampling is conducted on a quarterly, semiannual, or annual basis. Sampling stations are arranged in a grid array, with all agencies abandoning the formerly used rosette arrangement; the grid array is based on depth with stations located along certain isobaths. The stations are located by the use of predominantly Loran C, but to a lesser extent the Mini-ranger. In combination with the primary navigation system, most agencies utilize position buoys and/or visual line-ups. One agency occasionally even uses radar to verify station location. Buoys can be positioned at each station on a permanent basis as is the situation for ABC Labs at the Oxnard Outfall. Alternatively, marker buoys can be deployed on station to facilitate finding the exact location of the station for replicate sampling.

The most used sediment sampler is the galvanized single or double Van Veen. The Los Angeles County Sanitation Districts have a double cradle structure which supports their double Van Veen. A great deal of interest in this cradle was generated. Although there are certainly advantages to the double Van Veen, a question arose as to whether the second sample of this set-up is a true replicate. The participants agreed that it did not represent a true replicate. In any case, two samples are taken at each station: one used for biology, analyzed for infauna, and one used for chemistry and grain size. Five monitoring programs use teflon coating on the Van Veen with the only advantage appearing to be ease in cleaning and better penetration into the sediment. Kinetics Lab has a modified stainless steel Van Veen built by Battelle, Duxbury. This modified Van Veen has a superstructure built around it to ensure that the grab will work on slopes. The rigid Van Veen, with a superstructure, is the better choice for use on uneven surfaces such as slopes, but the increased mass makes it dangerous in rough seas. The free-swinging Van Veen is not as efficient on slopes, but the single Van Veen is less dangerous in rough seas. ABC Labs has replaced the chains with cable to prevent entanglement with the grab. Monitoring agencies should use the Van Veen best suited to their needs. Dr. Doug Diener, MEC, suggested checking two items: 1) an extra link or shackle in the take-up chain which causes the Van Veen to be partially, not completely (1/10 square meter) opened;

2) a large gap between axis and screened flap valves should not be present.

The City of Avalon uses a 1-liter core sampler and divers to collect samples. SCUBA divers ensure that the sampler penetrates into 10 cm of sediment.

There was a considerable amount of interest in box core samplers. MEC plans to run test trials on the samplers off Orange County in January. The results will be compared to the samples taken from a Van Veen. The advantage of the box core sampler is that it can be used in rough seas, e.g. 20 ft seas, which is not possible with the Van Veen; also, penetration depth may be adjusted by the addition or the subtraction of weights. This type of sampler has an added advantage over other bottom samplers because it doesn't disturb surface sediments. Unfortunately, it weighs about 100 lbs.

The minimum acceptance criteria varies greatly among the dischargers due to the type of sediments that prevail in the respective geographic areas. The more compact the sediment is the shallower the penetration depth. The shallowest acceptable penetration depth was reported by Morro Bay and Goleta at 4 cm and the deepest by Watsonville and Oceano at 14 cm. If penetration is a problem, a free fall can be employed in the last few meters of depth to ensure a deeper grab. For the majority of agencies, volume of sediment collected in each sample does not play a crucial role in the acceptance or rejection criterion. If the Van Veen has excessive leakage from cobble caught in the sampler, the sample is rejected. Instead of trying to estimate the volume of the sample when penetration is uneven, a pre-calibrated bucket can be used.

After the sample is brought on board, the sediment is usually washed into a sluice box, down to a weir, and eventually into a collecting screen. All agencies use a 1.0 mm screen or a nested 1.0 mm and 0.5 mm screen combination. When the nested combination is utilized, the organisms retained on the 1.0 mm screen are preserved in separate sample jars than those retained on the 0.5 mm screen. An in-line filter is employed to filter out contaminant organisms, e.g. plankton from wash water. A fan nozzle is attached to a garden hose in order to obtain a gentler spray so that animals aren't fragmented. The organisms retained on the screen are collected by a spatula, then finally forceps. The screen is inverted, then cleaned with a vegetable brush. The sediment used for chemistry analyses is taken from the middle of the sample, not avoiding^{FF} the sediment touching the inner surface of the Van Veen.

Kinetics Lab utilizes an elutriation device with a 1.0 mm screen; this essentially floats or percolates the sample. The elutriator can handle up to 15 liters at any one time and requires 8-10 minutes to complete. The organisms are retained on a 0.5-1.0 mm Nytex sock (screen). The advantages are obvious: the procedure is quick and clean.



Once the organisms are collected from the screen, they are placed in relaxants, fixatives, and preservatives. Six agencies use propylene phenoxytol as a relaxant. These agencies reported that the use of the relaxant does seem to make a difference in the preserved condition of the specimens, especially in the polychaetes and in some crustaceans. Objection to the use of propylene phenoxytol is its carcinogenic nature.

A 10% borax buffered formalin solution is the fixative of choice for the majority of agencies with the duration of fixation ranging from 24-168 hrs. The mode is approximately 48 hrs followed by preservation of the sample in 70% ethanol. Since the addition of 10% buffered formalin is diluted by the water retained between the sand and mud grains after decanting, Dr. Doug Diener recommended that the resultant solution of preservative in the sample should be 10-12% buffered formalin. Poison labels should mark the jars containing formalin. He also suggested buffering ethanol with marbel chips since it is usually acidic, pH 5.5. The acidity will disintegrate small mollusk shells. Some agencies, i.e. Morro Bay, Goleta, and Santa Barbara, use 70-75% isopropanol, but a few participants at the meeting expressed dissatisfaction with this preservative. Apparently, isopropanol can cause crustaceans to become quite brittle.

Each time the sample is transferred to a solution, whether relaxant, preservative, or fixative, the sample should be screened with a 0.5 mm screen and the sample jar should be inverted several times to ensure that the added solution is well mixed throughout the sample. Labelling of the sample can be on the lid, on the inside, or on the outside of the container. Two agencies, Hyperion and Serra, require all three aforementioned labels. All other agencies, except one, utilize varying combinations of two of the above. After all these steps are taken, the samples are finally archived for subsequent identification. Rose bengal, a stain, is added to the sample by a limited number of agencies to facilitate sorting. Several participants complained about the use of stains because of subsequent identification problems associated with Rose bengal.

At the Hyperion Treatment Plant, a procedural checklist ensures that all appropriate steps have been taken on the sample in order to preserve, sort, and identify the organisms. Sample custody records provide a historical record of the steps to the final destination of the sample. If the sample is lost, the chain of custody helps to trace the lost sample.

Discussion

Formalin disposal was the subject of a short discussion. Several agencies, e.g. Hyperion Treatment Plant, MEC, and Los Angeles County Sanitation Districts pour the formalin down the drain with the

addition of copious amounts of water. The solution is already at 10%, then is further diluted by the addition of water. The City of San Francisco and Pt. Loma, City of San Diego, puts the used formalin in polyethylene containers for disposal by the hazardous waste group. Tom Parker, Los Angeles County Sanitation Districts, mentioned a paper that describes the breakdown of formaldehyde to CO₂ and several harmless compounds by the addition of ferric chloride and hydrogen peroxide.

Several similarities in sampling programs were mentioned by Dave Montagne. All agencies are sampling at the depths of the effluent discharge and on a grid basis. Most agencies use a single or double Van Veen with replicate samples taken. The sediment is put through a 1.0 mm screen with the material retained on the screen put in a fixative (10% formalin) and later preserved in 70% ethanol with a label identifying station, date, and other pertinent collection data.

Several topics were discussed:

- 1) Many agencies send their least experienced people out in the field. Unfortunately, field mistakes can't be rectified; information lost in the field can't be retrieved for that specific collection. It was suggested that the most experienced personnel should be sent in the field, not the least.
- 2) A fan nozzle should be used in washing the sediment through the screen to decrease water pressure to prevent damage to soft-bodied organisms, e.g. polychaetes.
- 3) In cases of body fragments, some agencies identify heads or significant posterior portions of organisms if no head exists. This procedure prevents the double counting of a single specimen. Some agencies count only heads, no other fragments. Either rule may be used, but consistency within a monitoring program is the most important rule.
- 4) A set of recommendations may be difficult to follow for agencies that have specific permit requirements and that are set in their ways. The participants of the meeting were reminded of a point of which they were all very much aware: "The permit determines the monitoring program; the monitoring program does not dictate the permit". SCAMIT can not unilaterally decide to standardize the sampling.
- 5) Dr. Bruce Thompson, SCCWRP, suggested that various agencies must first get the Regional Water Quality Control Board members to agree on the feasibility and possible acceptance of sampling standardization. Then, perhaps, a set of recommendations can be written and set forth. It must be emphasized that these recommendations are pertinent only to wastewater monitoring programs



and not to other scientific benthic sampling. SCAMIT certainly does not want to undermine such scientific investigations as the MMS/Santa Maria Basin Project which has a totally different objective than the wastewater monitoring groups. Dr. Thompson also warned SCAMIT not to venture too far from the original purpose of the association which is taxonomy. Many members agreed with this suggestion. Perhaps this suggestion as well as others presented here can be further discussed at an executive meeting of the SCAMIT officers.

6) The last suggestion was a laboratory analysis QA/QC meeting of SCAMIT.

Holothuroidea Workshop: On 8 December of this year, Mary Bergen will host a SCAMIT workshop on the southern California Holothuroidea at the Cabrillo Marine Museum. Be sure to have slides of holothuroid ossicles and tube feet already prepared before the start of the workshop.

SCAMIT Christmas Party: This year's Christmas party will be held at the Cabrillo Marine Museum on 9 December from 5:00 - 10:00 P.M. The main dish and drinks will be provided by SCAMIT, but it's potluck on the side dishes and desserts. There will also be a surprise visit from the man in red, Big John Claus, for the kiddies and for us adults who never grew up. We need volunteers to help set up and clean up. RSVP to Mas Dojiri, also indicate side dishes or desserts and whether or not you can help with the party.

Biological Illustrator Available for Free Lance Work: Therese Trebaol is available for free lance biological illustrations. Her education includes a Master's degree in medial illustration from University of California, San Francisco. She has worked extensively with pen and ink, stippling, carbon dust, acrylics, watercolors, and airbrush for publication in books, biomedical journals, and television. Pricing depends on the complexity of the illustration. Telephone: (213) 545-5176.

BENTHIC FIELD METHODS

DISCHARGER:	CITY OF S.F.	SANTA CRUZ	WATSONVILLE	OCEANO	MORRO BAY	GOLETA	SANTA BARE
FLOW RATE (MGD)	17	10-12	8	20	1.5	6	10-15
NAVIGATION							
Mini-ranger		X	X			X	
Diff. Loran C							
Loran C	X			X	X		X
Buoys	X						X
Depth					X		
Visuals				X	X		
Radar							
SAMPLER							
Single VanVeen		X					
Double VanVeen					X	X	
Smith-MacIntyre	X						
#3 Coffee Can Core			X	X			X
1-Litre Core							
Galvanized					X	X	
Stainless	X						
Teflon		X					
MINIMUM ACCEPT. RITERIA							
Penetration (cm)	7	9	14	14	4-10	4-10	14 +/-
Volume (l)			3	3			3
SCREEN SIZE							
1.0 mm	X	X	X	X	X	X	X
0.5	X						
LABELLING							
Top	X						
Inside	X	X	X	X	X	X	X
Outside		X	X	X	X	X	X
RELAXANTS							
MgCl ₂							
MgSo ₄							
Propylene phenoxytol		X	X	X			
Duration (min)		10	10	10			
FIXATIVE							
Formalin (%)	10	10	10	10	10	10	10
Borax buff	X	X	X	X			
Seawater buff					X	X	
Duration (hrs)	48	24-72	24-72	24-72	48-72	48-72	
PRESERVATIVE							
Ethanol (%)	70	70	70	70			
Isopropanol (%)					70	70	75

BENTHIC FIELD METHODS

DISCHARGER:	OXNARD	HYPERION	AVALON	LA CO.	SCCWRP	OR CO.	ALISO
FLOW RATE (MGD)	20	400	.6-.9	375		250	4.2
NAVIGATION							
Mini-ranger							
Diff. Loran C						X	
Loran C		X		X	X		X
Buoys	X	X				X	
Depth		X		X		X	X
Visuals		X	X	X			
Radar		X					
SAMPLER							
Single VanVeen	X	X			X		X
Double VanVeen				X		X	
Smith-MacIntyre							
#3 Coffee Can Core							
1-Litre Core			X				
Galvanized	X	X		X	X		X
Stainless							
Teflon						X	
MINIMUM ACCEPT. CRITERIA							
Penetration (cm)		5	10	10	8-10		10
Volume (l)	3		1			4	3
SCREEN SIZE							
1.0 mm	X	X	X	X	X	X	X
0.5 mm					X		
LABELLING							
Top	X	X		X	X	X	X
Inside		X	X		X	X	X
Outside	X	X	X		X		X
RELAXANTS							
MgCl ₂							
MgSo ₄						X	
Propylene phenoxytol		X					
Duration (min)		30				30	
FIXATIVE							
Formalin (%)	10	10	5	10	10	10	10
Borax buff		X	X	X	X	X	X
Seawater buff		X	X			X	
Duration (hrs)	24-48	48-96	48-120	48-168	24-48	72-120	48
PRESERVATIVE							
Ethanol (%)	70	70	70	70	70	70	70
Isopropanol (%)							

BENTHIC FIELD METHODS

DISCHARGER:	SERRA	OCEANSIDE	ENCINA	SAN ELIJO	SAN DIEGO
FLOW RATE (MGD)	19	16.5	20	22	190
NAVIGATION					
Mini-ranger		X	X	X	
Diff. Loran C					
Loran C	X	X		X	X
Buoys					
Depth	X		X		X
Visuals	X				
Radar					
SAMPLER					
Single VanVeen	X	X		X	X
Double VanVeen			X		
Smith-MacIntyre					
#3 Coffee Can Core					
1-Litre Core					
Galvanized	X				X
Stainless					
Teflon		X	X	X	
MINIMUM ACCEPT. CRITERIA					
Penetration (cm)	10	9		9	4-10
Volume (l)			4		
SCREEN SIZE					
1.0 mm	X	X	X	X	X
0.5					
LABELLING					
Top	X		X		
Inside	X	X	X	X	X
Outside	X	X		X	X
RELAXANTS					
MgCl ₂					
MgSo ₄			X		
Propylene phenoxytol		X		X	
Duration (min)		10	30	10	
FIXATIVE					
Formalin (%)	10	10	10	10	15
Borax buff	X	X	X	X	X
Seawater buff			X		X
Duration (hrs)	48	24-72	72-120	24-72	48-168
PRESERVATIVE					
Ethanol (%)	70	70	70	70	70
Isopropanol (%)					