Alamitos Generating Station

Appendix B

Field Sampling and Sample Processing Procedures

B1. Entrainment Field Sampling

B2. Entrainment Sample Sorting

B3. Entrainment Sample Identification

B4. Impingement Field Sampling

APPENDIX B1: PROCEDURE FOR COLLECTING PLANKTON SAMPLES FOR ENTRAINMENT STUDIES

1.0 PURPOSE

The purpose of this document is to define the steps and equipment necessary to accurately collect plankton samples using a wheeled bongo frame near the Alamitos Generating Station (AGS).

2.0 RESPONSIBILITIES

2.1 Task/Field leader:

- Notify the station of the proposed sampling dates.
- Schedule and coordinate sampling surveys and notifying the U.S. Coast Guard prior to sampling.
- Verify that all investigating biologists conducting the sampling have read and understand these procedures.
- Verify that procedures have been followed during sample collection and that the sampling has been conducted safely.
- Verify that information on data sheets have been reviewed and properly recorded.

2.2 Investigating biologist:

- Conduct sampling using the following procedures.

3.0 PROCEDURES

3.1 Mobilization

a. Notify plant personnel of the dates of field sampling prior to the sampling day.

b. Ensure there are enough jars, labels, and preservative (formalin) for the sample collection. Print the required number of blank field data sheets on waterproof paper.

c. Inspect the wheeled bongo frame, nets and codends for any damage. If damaged, repairs must be made before sampling begins. Ensure that the flowmeters have been calibrated within the past 90 days and that they are operational. Attach a flowmeter in approximately the center of each frame mouth.

d. Ensure that all additional equipment (Table B1-1) is in good operating condition. Make repairs if necessary.

3.2 Sample Collection

a. Samples will be collected every six hours in a 24-hr period (four cycles) according to the schedule developed by the Task Leader. A survey team consists of at least a boat driver and two investigating biologists to conduct the sampling.

b. Locate the station using the latitude/longitude coordinates. Determine the water depth with the fathometer and record the water depth on the field data sheet.

c. Ensure that the winch line and a weight (15-20 lb salmon ball) are securely attached to the center of the bongo frame. Ensure that the nets, codends and flowmeters are securely attached. The nets should be 333-µm mesh.

d. Record each flowmeter's serial number on the field data sheet (Attachment B1-1). Record the number from the flowmeter counter spins on the field data sheet prior to lowering the frame into the water. Record the start time (local time) on the field data sheet.

e. Using the measured marks on the winch cable, lower the frame and nets through the water column until the wheels on the sides of the frame are on the bottom. When the cable starts to slack, the boat is motored forward and the cable is retrieved trying to maintain a 45-degree tow angle. When the frame reaches the surface, carefully pull it into the boat. Verify that the nets have not picked up any sediment from the bottom. If there is any sediment in the nets or codends, discard both samples by detaching the codends and rinsing the nets of collected material and then reattach the codends. Repeat the sample collection at that station.

f. Check that the number of spins on each flowmeter counter to verify that the target volume of 15-20 m^3 has been collected (number of spins should be about 2,000). If the target volume has not been met with one tow, subsequent tows will be performed at the station until the target volume has been collected.

g. If the correct volume has been collected record the end number of spins from each flowmeter on the field data sheet. Subtract the initial number of spins from the end number and record the total on the field data sheet. If the integrity of either or both flowmeter readings is questionable (e.g., seaweed wrapped around the propellers), discard both samples by detaching the codends and rinsing the nets of collected material and then reattach the codends. Repeat the sample collection at that station.

h. Record the end time (local time) on the field data sheet.

i. Beginning at the top of the net, rinse the collected material down into the codend. Since the wash water is not filtered and may contain plankton, rinse the net from the outside ensuring that unfiltered water does not contaminate the sample. Inspect the net to ensure that it has been thoroughly rinsed. Samples will then be carefully transferred to prelabeled jars with preprinted internal labels. The sample from each net will be placed in separate labeled jars.

j. Detach the codend from net #1 and rinse the sample from the codend into a labeled sample jar using a squirt bottle containing seawater. Then, using a graduated cylinder or other measuring device, add enough formalin to make a 10%-formalin seawater solution. Rinse and inspect the codend of net #1 before reattaching to the net. Follow the same procedure for net #2. Sample preservation should be completed soon after collection.

k. If the collected material will fill over ½ of the sample jar, split the sample into at least two labeled jars so that there is enough ethanol for proper preservation.

l. Ensure that the sample jar contains both an inner label and an exterior label.

m. The following is an explanation of the coding for the field datasheet survey and station numbers and jar labels:

1. Each survey number on the data sheet consists of a series of 5 letters followed by 2 numbers (ABEA##). The first two letters are "AB" refers to Alamitos Bay, and the "EA" refers to entrainment abundance. The two numbers refer to the survey number with the first survey being 01. The survey number increases by one for each new 24-hour sampling effort.

2. The station designation consists of a letter-number-letter-number combination. This letter/numbering system was set up for the two Alamitos Bay generating stations (Alamitos and Haynes). The first letter refers to the station being an Outer, Shore, Harbor, or Entrainment station (see map in Attachment B1-2). The first number refers to the number of the station that links to the station letter. The numbers for each of the stations listed above are as follows:

Station letter	Station number
Outer	1-3
Shore	1-3
Harbor	1-4
Entrainment	1-3

3. Entrainment Stations E1 and E2 are located near AGS intake structures.

4. The second letter designates the replicate, either "A" or "B". The source water stations only have one sample so will always use the letter "A". There are two samples collected at the entrainment

location so the letters "A" and "B" will be used to separate these two replicates. The second number designates the net number, either "1" or "2." For example, O3A1 means that the sample was collected from Station O3, Sample A, and Net 1.

5. The date of sampling will correspond to the actual start date of each sample. At the start of a new day (midnight), use a new field data sheet.

n. Deliver the samples to the laboratory at the completion of the sampling effort.

3.3 Sample Voiding in the Field

a. Samples should be voided if any of the following occurs: 1) possible flowmeter obstruction due to kelp or other debris on the propeller, 2) obviously malfunctioning or damaged flowmeters; 3) damaged (torn) nets found after a sample is collected; 4) large quantities of sediment in the net that were collected when the wheeled bongo frame was on the bottom; 5) gear failure which prevents completion of any tows/hauls; 6) an incident or situation which may prevent reliable data collection; 7) an incident or situation which may jeopardize the safety of sampling personnel.

b. If a hole or tear is found in the net mesh, mark the damaged area and either repair or replace the net. Discard both samples and repeat the sample collection. Record this on the data sheet.

c. The number of flowmeter spins from the paired bongo nets needs to be checked in the field to confirm that the measured volumes were similar.

Table B1-1. Equipment List.

1. Wheeled bongo net frame, attached 333/335 micron mesh nets, codends, and calibrated flowmeters (include at least 1 back up net and flowmeter)
2. Winch (davits) and line for net deployment and retrieval
3. Stock solution of formalin
4. Squeeze bottles
5. Labeled jars for sample storage
6. Data sheets, pencils, permanent markers, and labels
7. Wash-down pump
8. Watch
9. Fathometer
10. GPS

Alamitos Bay Entrainment Abundance Field Data Sheet - Alamitos Generating Station (AGS) and Haynes Generating Station (HnGS)

Sheet #: ____________ **Date:** ____________ **Mesh:** **0.335** mm **Flowmeter 1:** ____________ **Conversion 1:** ____________

Survey #: A B E A ____ **Crew:** ____________ **Net Dia.:** ____________ **Flowmeter 2:** ____________ **Conversion 2:** ____________

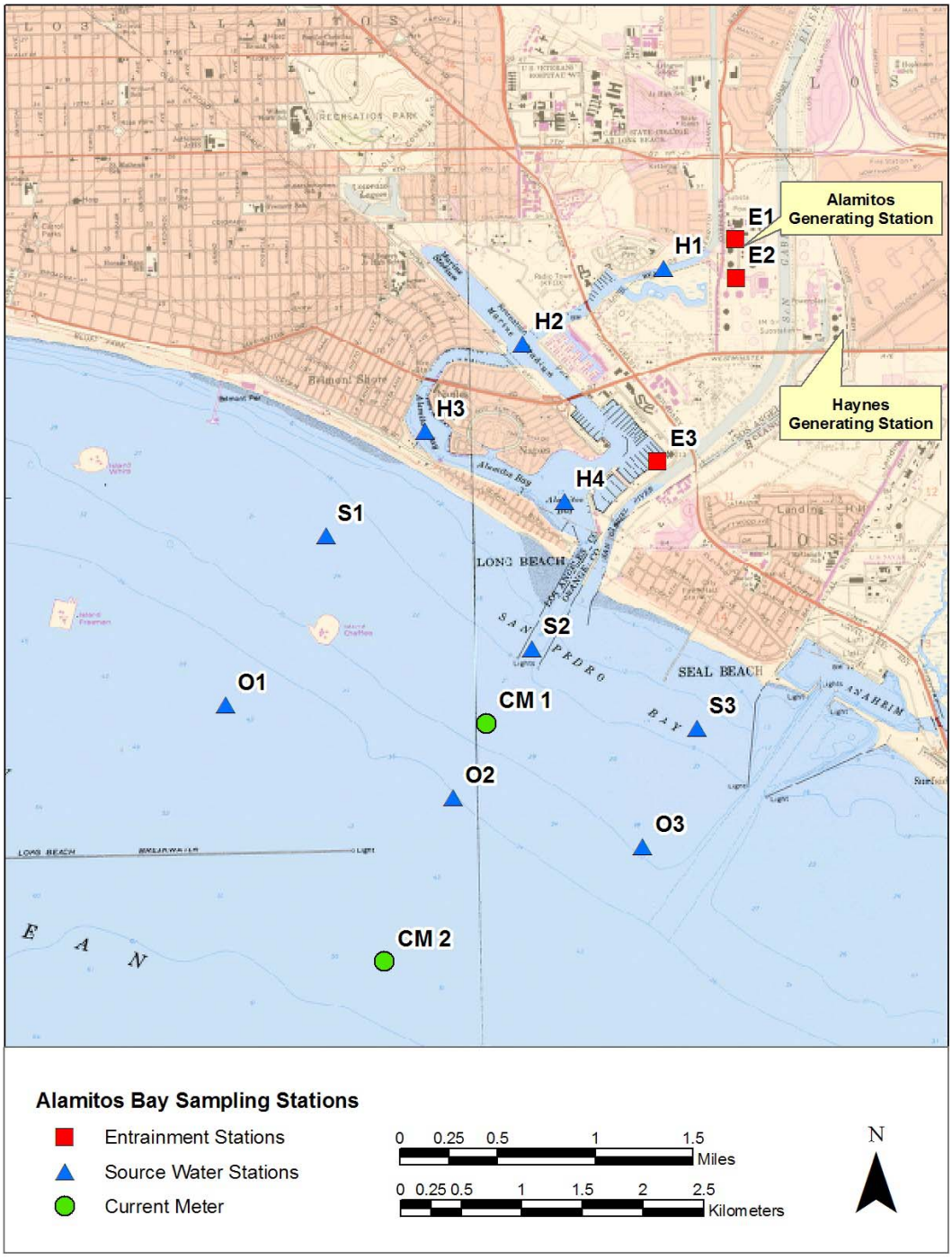
Station (A#A#)	Flowmeter Start	Flowmeter End	Total Flow	Volume (cu. m)	Sample Number	Cycle (1-4)	Temp °C	Salinity (ppt)	Tide (E, F, HS, LS)	Station Depth (ft)	Start Time (PST)	End Time (PST)	Total (min)
1													
2													
1													
2													
1													
2													
1													
2													
1													
2													
1													
2													
1													
2													
1													
2													

Survey: ABEA## (Alamitos Bay Entrainment Abundance)
Date: MM/DD/YY
Station: A#A#
A#: (Station Designation - Outer 1-3, Harbor 1-4, Shore 1-3 or Entrainment 1-3)
A (Replicate A or B - Only Entrainment samples have two replicates)
#: (Net 1 or 2)
Tide: Ebb, Flood, High Slack (HS), or Low Slack (LS)

NOTES:

Reviewed By / Date: ____________ Entered By / Date: ____________ Copied By / Date: ____________

Attachment B1-1. Example field datasheet for Alamitos Bay station sampling.



Attachment B1-2. Map of AGS and HnGS entrainment and source water sampling stations. E = Entrainment, H = Harbor, S = Shore, O = Offshore, and CM = Current Meter.

APPENDIX B2: PROCEDURE FOR SORTING PLANKTON SAMPLES IN THE LABORATORY

1.0 PURPOSE

The purpose of this procedure is to define the steps for sorting target organisms from plankton samples collected at Alamitos Generating Station, and to describe the Quality Control Program (QC) used to monitor the sorting accuracy of individual sorters.

2.0 RESPONSIBILITIES

- Laboratory Supervisor is responsible for assuring that plankton sample sorting is in accordance with written procedures.
- The Quality Control Supervisor is responsible for implementing the Quality Control Program, which monitors sorting accuracy in accordance with written procedures.
- Investigating biologists are responsible for sorting samples in accordance with written procedures.

3.0 INSTRUCTIONS

3.1 Sorting Procedures

3.1.1 Sample Processing

a. Ensure that the proper equipment necessary for sample processing is available (Table B2-1).

b. Samples that were originally fixed in formaldehyde after collection must be transferred to 70% ethanol before laboratory processing. This is done outside to lessen the exposure to formaldehyde fumes. Only qualified personnel who have read and signed the information about the hazards of working with formaldehyde may transfer samples.

   1. A funnel with the appropriate mesh size attached to its bottom opening is placed into a jar or can. The mesh must not be larger than that used during sample collection. Place the jar and funnel in a tray so the sample can be retrieved if spillage occurs.

   2. Pour the sample carefully into the canning funnel. The sample jar and jar lid are rinsed with water, directing the water and organisms into the funnel. Rinse the sample with water to flush the formaldehyde from the sample.

   3. Rinse the sample into a labeled jar with 70% ethanol from a squeeze bottle. Make certain that the jar has both an inner label and a jar top label. Additional ethanol is added to the sample jar to cover the sample.

   4. The waste formaldehyde and rinse water is then discarded into the appropriate hazardous waste container.

c. Consult the sorting schedule posted in the processing laboratory to determine sorting priorities.

d. Sign out the sample in the Laboratory Tracking MS Excel file and the Laboratory Sample Tracking Sheet (Attachment B2-1) by writing your initials under the ‘sorter’ column. Transcribe information from the sample label into the Sorter’s Log Book (Attachment B2-2) and into the sorter's notebook (each sorter has separate log sheets and a notebook for this purpose).

e. Take two clean canning funnels with attached mesh netting, one labeled ‘sorted’ and the other labeled ‘unsorted’. The mesh size should be no larger than that used to collect the samples.

f. Place the 'unsorted' canning funnel over the 'used alcohol' bottle and funnel, which is located in a dish so samples can be retrieved if a spill occurs. Pour the sample into the canning funnel. The canning funnel will contain the material to be sorted, while the ethanol will drain into the 'used alcohol' bottle.

g. Using 70% ethanol or 70% used alcohol in a squeeze bottle, rinse any remaining sample from the sample jar, the jar lid and inner sample label into the canning funnel containing the unsorted sample.

h. Place the 'unsorted' funnel containing the sample and the empty 'sorted' funnel into individual glass bowls in a tray. Make sure the sample is covered with water so it will not dehydrate during processing.

i. Using forceps, transfer a small amount of the sample from the 'unsorted' funnel to the sorting tray. Add enough water to cover the sample. Distribute the sample in the sorting tray.

j. Place the sorting tray on the base of the dissecting microscope. Adjust the magnification so that the field of view is slightly larger than the width of an individual marked grid.

k. Arrange the light source to provide adequate illumination.

l. Carefully scan the entire sorting tray using the grids for orientation. Remove the target organism with forceps and place them either into a shell vial containing 70% ethanol or into a small dish containing water. Count the organisms as they are removed. A list of what target organisms and when to pull them is posted in the lab.

m. Log the number of organisms removed from the sample in the sorter notebook.

n. Scan the tray a second time. If target organisms are found on the second pass, repeat a third time. Continue this process until a scan does not produce any additional target organisms.

o. Once sorted, pour the sorted sample into the 'sorted' funnel and rinse with a small amount of water. Take a second aliquot from the 'unsorted' funnel as described above. Repeat the above steps until the entire sample has been sorted.

p. If the sorter thinks there will be more than 500 fish eggs in a sample then the sample may be "sub sampled" for eggs. When "sub sampling" the sample should be processed first for fish larvae and selected invertebrate larvae. When ready to "sub sample" put the sorted sample back in the original sample jar and fill the jar with 70% ethanol up to the lip of the jar. Jar size varies, but they will typically be 500 ml (if sizes varies there will be a posting in the lab). A 'sub sample' should be 10%of the sample volume so the sorter will use the aliquot transfer pipette with the 10 ml attachment and take 5 aliquots. The sample should be stirred up in order to get a fair amount of sample in the aliquot. Once the aliquot is processed for fish eggs it may be returned to the original sample jar with the rest of the sorted sample. Make sure it is noted in the logbook and record the total volume of the sample and the volume of the sub sample. There will be an extra data sheet in the laboratory tracking sheets and a column in the MS Excel tracking sheets to record the sub sample information. On top of the sample jar put a white dot with survey number, sample number, sorters initials, sub sample date, and "SS".

q. When the sorting has been completed, the sorted organisms should be placed into a shell vial containing 70% ethanol. Fill the shell vial completely with clean 70% ethanol then place cotton into the top end of the vial to keep the organisms inside. Place the vial into a labeled snap cap containing 70% ethanol. Make sure the shell vial and cotton are completely covered with 70% ethanol.

r. Label each jar lid with the appropriate colored dot label. Prepare a waterproof inner label for the jar containing the shell vial. Both labels should contain the following information:

   1. Survey number
   2. Collection date
   3. Station, cycle and sample number

4. Collection start time
5. Jar number (if more than one jar)
6. Sorter's initials
7. Number of organisms in shell vial

s. The total number of sorted organisms and the total time required to process the sample is recorded in the sorter's notebook.

t. Put the sorted sample back into the original sample jar. Used alcohol may be used to fill sample jar to at least ¾ full. Rinse any remaining sample from the funnel into the jar using a squirt bottle containing ethanol. Make sure the inner waterproof label is in the sample jar. Thoroughly clean the funnels of all remaining sample.

u. If a sample must be stored before completion:

1. Put the sorted portion of the sample back into the original sample jar. Rinse any remaining material from the funnel into the jar using a squirt bottle containing ethanol. Make sure that the sample is adequately covered with ethanol.
2. Put the unsorted sample into a second jar. Rinse any sample from the 'unsorted' funnel into the jar using a squirt bottle containing ethanol. Using a dot label, label the jar lid with the sample identification information, sorter's initials, and the word "unsorted". Make an additional inner label with the sample identification information and marked 'unsorted'. Place the label inside the jar with the 'unsorted' sample. Make certain that the 'unsorted' sample is adequately covered with ethanol.
3. The sorted and unsorted portion of the sample should be stored until sorting can continue.

3.1.2 Once the sample is completed, place an appropriately colored dot label on the jar top with the sorter's initials and date of sorting. Return the jar to the box from which it was originally removed.

a. Transcribe the information recorded in the sorter's notebook to the computer on the Laboratory Tracking Sheets and the Quality Control log and on the Laboratory Sample Tracking Sheet (Attachment B2-1), and to the Sorter's Log (Attachment B3-2).

3.2 Sorting Quality Control Program

3.2.1 QC Sorting Criteria

a. The first ten samples that are sorted by an individual are completely resorted by a designated QC sorter. A sorter is allowed to miss one target organism when the original sorted count is 1–19. For original counts above 20 a sorter must maintain a sorting accuracy of 90%.

b. After the sorter has passed 10 consecutive sorts, the program is switched to a '1 sample in 10' QC program for that sorter. After the sorter has completed another 10 samples, one sample is randomly selected by the designated QC sorter for a QC resort.

c. If the sorter maintains the 90% accuracy sorting rate for this sample, then the sorter continues in the '1 sample in 10' QC mode.

d. If a sample does not meet the 90% accuracy rate their subsequent samples will be resorted until 10 consecutive samples meet the criteria.

3.2.2 QC Resorting

a. Sorting procedures used during the QC resort are the same as the sorting procedures described in Section 3.1.

b. All fish and selected invertebrate larvae that were missed by the sorter are removed during the QC resort.

c. For the QC process, a larval fish is defined as having a head plus at least 50% of the body. Any parts without a head and/or less than 50% of the body will be considered fragments and will not be counted against the original sorter as a missed fish. However, it is important for each sorter to remove all fish and fragments from each sample that is sorted and correctly record them as # fish / # fragments in the sorter's notebook and on the tracking sheet.

d. Any vials of fish larvae or selected invertebrate larvae generated from the resort are labeled with an orange dot label, and labeled as described in the sorting procedures with the addition of "QC" added to the label.

e. An orange dot label should also be placed on the top of the jar of the sample that was resorted and labeled with the QC person's initials, survey number, sample number, and date the resort was completed.

f. The vials are stored in the appropriate location.

3.3 Waste Disposal

3.3.1 No formaldehyde or water contaminated with formaldehyde should be disposed of into the sewage system. Dispose of any water contaminated with this chemical in the designated waste water container to be disposed of at a local hazardous materials waste depository.

4.0 RECORDS

4.1 All data sheets are later reviewed by the Lab Manager or designated staff.

4.2 Original data sheets are permanently stored.

Table B2-1. Equipment List

1. Tray or dish
2. Bowls
3. Sample jars
4. Two canning funnels with attached plankton mesh netting, labeled with mesh size, and labeled ‘sorted’ and ‘unsorted’
5. Squeeze bottle containing 70% ethanol (denatured)
6. Squeeze bottle containing fresh water
7. Sorting tray or petri dish marked with a sorting grid
8. Dissecting microscope with light source
9. Glass shell vials and cotton
10. Jar/vials with lids
11. Forceps
12. Waterproof labels
13. Dot labels
14. Sorter's notebook
15. Plankton splitter

ALAMITOS BAY (AGS) AND (HnGS) – Lab Tracking Sheet

Survey: ______________________________

Sample Information							Sort Information							QC Information			
Project #	Sample Date	Station	Cycle	Sample	Start Time	# Jars	Sorter	Date Sorted	Time (hrs)	# Fish	Fish Eggs	# Megs	# Lobster/ Squid	QC'd By	QC Date	QC Fish	QC Other *

*specify which (fish eggs(E), megs(M), lobster(L), or squid(S))

Attachment B2-1. Lab Tracking Sheet

Alamitos Bay Power Plants-Sorter's Log

(ABEA)

Name: _____

Sample Information					Sort Information							Quality Control					
						Sort Time											
Survey Number	Station	Cycle	Sample	Collection Time	Date Sorted	AGS	HnGS	# of Fish	# of Fish Eggs	# of Megs	Lobster / Squid	QC'ed By	QC'ed Date	QC Fish	Fish P / F	QC Other *	Other P / F

*specify which (fish eggs(E), megs(M), lobster(L), or squid(S))

Attachment B2-2 Sorter's Log Sheet

APPENDIX B3: PROCEDURES FOR THE IDENTIFICATION OF LARVAL FISHES and TARGET INVERTEBRATES

1.0 PURPOSE

The purpose of these procedures is to define the steps for identifying planktonic organisms, and to describe the Quality Control (QC) Program used to monitor the accuracy of each individual's identification performance.

2.0 RESPONSIBILITIES

- The Lead Taxonomist is responsible for assuring that plankton identifications are performed in accordance with written procedures and for implementing the Quality Control Program.
- Investigating biologists are responsible for plankton identifications and for monitoring accuracy in accordance with written procedures.

3.0 INSTRUCTIONS

3.1 Identification procedures for larval fishes, *Cancer* spp. crab and *Panulirus* lobsters.

a. Ensure that the proper equipment necessary for the identification of target organisms is available (Table B3-1).

b. The fish and target invertebrates from each sample are kept in separate containers and processed following this procedure in essentially the same manner.

c. The container of target organisms to be identified is carefully emptied into a dish. The dish is placed on the microscope stage and the lighting adjusted to provide adequate illumination.

d. Each target organism is identified to the lowest taxonomic classification possible. The total number of each taxon is recorded on the Entrainment /Source Water Plankton Tow Lab Data Sheet (Attachment B3-1).

e. All individuals of each identified taxon of larvae from a sample should be put into a shell vial containing 100% ethanol. Each vial should contain a label with the taxon name and sample number. Cotton should be pushed into the upper end of the vial to keep the label and organisms enclosed.

f. Mutilated larvae (partial organisms that are missing body parts and are unable to be identified) are placed in a separate labeled vial. Whole larvae that are unidentified, are placed in a separate labeled vial.

g. All vials containing target organisms from an individual sample should be put into a labeled jar containing enough ethanol to cover the vials. The jar should contain both an inside label and a label attached to the outside of the lid denoting the sample number, date and time collected, and identifier's initials. Tighten the jar lid to prevent evaporation of the preservative. Samples with many different fish taxa may require more than one labeled jar.

h. On the Laboratory Sample Tracking Sheet, record the identifier's initials and date sample was logged in. The identifier's log will contain the total number of larvae identified and the date identified. If more than one day was needed to complete the identification, record the date the sample identification was completed.

i. Place the jar into the appropriate box containing identified samples.

j. Dispose of any liquids containing ethanol into the appropriate waste container.

3.2 Identification Quality Control (QC) Program

3.1.2 Fishes

a. The first ten samples of larval fishes that are identified by an individual identifying biologist will be completely re-identified by a designated identification QC biologist. A total of at least 50 individuals from at least 5 taxa (50/5 criteria) must be present in these first ten samples. If the first 10 consecutive samples do not pass the 50/5 criteria, additional samples must be re-identified until this criteria is met.

b. The identifying biologist must maintain a 95% identification accuracy level in these first 10 samples. For all samples, if a sample contains between 1–19 larvae, one larvae can be misidentified and the sample will not fail the QC check.

c. If the identifying biologist identifies a larval fish to a certain family or genus and subsequently the identification QC biologist is able to refine the identification to a lower taxonomic level, this will not be considered a misidentification pertaining to the 95% identification accuracy level. A misidentification will be one in which the identifying biologist identifies the fish as belonging to a certain family, genus or species, and then the identification QC biologist determines that the initial identification was incorrect and changes the identification to a different family, genus or species or changes it to a higher taxonomic group.

d. After the identifying biologist has passed 10 consecutive samples, the program is switched to a "1 sample in 10" QC program. After the identifying biologist has completed another 10 samples, one sample is randomly selected by the designated identification QC biologist for a QC review.

e. If this sample maintains the 95% accuracy level as determined by the identification QC biologist, then the identifying biologist continues in the "1 sample in 10" QC mode. If a sample does not meet the 95% accuracy level, their subsequent samples will be re-identified until 10 consecutive samples meet this level of accuracy.

f. Any misidentified fish found by the identification QC biologist, will be placed into the appropriate labeled vial for that sample. This information will be recorded on the Fish Identification Data Sheet.

3.1.3 *Cancer* spp. and *Panulirus* spp.

a. The first ten samples identified by an individual identifying biologist will be completely re-identified by a designated identification QC biologist.

b. The identifying biologist must maintain a 95% accuracy level in these first 10 samples. For all samples, if a sample contains between 1-19 larvae, one larvae can be misidentified and the sample will not fail the QC check.

c. After the identifying biologist has passed 10 consecutive samples, the program is switched to a "1 sample in 10" QC program. After the identifying biologist has completed another 10 samples, one sample is randomly selected by the designated identification QC biologist for a QC review.

d. If this sample maintains the 95% accuracy level as determined by the identification QC biologist, then the identifying biologist continues in the "1 sample in 10" QC mode.

e. If an identifier's sample does not meet the 95% accuracy level, their subsequent samples will be re-identified until 10 consecutive samples meet this level.

f. Any misidentified larva found by the identification QC biologist, will be placed into the appropriate labeled vial for that sample and recorded on the appropriate laboratory identification data sheet.

3.2 Larval Fish Measuring

3.2.1. Larval Fish Measuring Procedure

a. Turn on the computer, camera, and light source at the measuring station.

b. Consult the lab schedule near the measuring station to determine measuring priorities and retrieve the binder containing the appropriate data sheets.

c. Locate the box containing the fish to be measured and place it in a easily accessible area close to the measuring station.

d. Open the Optimas Image Analysis or ImageJ software by clicking with the mouse on the appropriate software icon.

e. Open the Larval Fish Measuring macro in Optimas, or the FishMeasure2 macro in ImageJ and follow the macros' directions.

f. Select the jar of fish to be measured and consult the jar label. Compare data on the jar label with the inner label and the data sheet for this sample. Consult an identifier regarding discrepancies between labels.

g. Enter the data queried for by the macro including the last five digits of the serial number, the measurer's initials, the data sheet sequence number and the species code.

h. Open the jar and remove the vials for the target taxa to be measured as per the posted list. Place the vials in a rack designed to allow the vials to maintain an upright posture so as to reduce spillage.

i. Select the first vial to be measured. Remove the cotton and the label. Compare the label with the data sheet for confirmation.

j. Empty the vial into a shallow dish. Remove any fish that have adhered to the vial, cotton, the label, or any tools used in the transferring process and place the fish in the dish. Add alcohol to the dish if necessary to prevent desiccation.

k. If the number of larval fish in the vial exceeds what can be reasonably measured on a single image capture, transfer some of the fish to another glass dish and immerse them in alcohol.

l. Place the dish on the stage of the microscope. Arrange the fish so that all fish appear on the screen. Adjust the zoom, focus, and lighting for the best possible image. If this is the first group of larval fish being measured, or if the magnification has been changed, it is necessary to re-calibrate. Place the micrometer on the stage of the microscope and re-calibrate by drawing a line from one of the micrometers millimeter marks to another, noting the distance between the two marks, and entering that value when queried. Replace the dish containing the larval fish to be measured.

m. Measure larval fish by drawing a line from the pre-maxillary to the end of the notochord, being careful to follow the contours of the fish. If the fish is too damaged to find either the pre-maxillary or to estimate the path taken by the notochord, do not measure, and proceed to the next larval fish. If the line does not adequately approximate the larval fish's length it must be re-measured.

n. Note the program's display of the measurement, check that it seems reasonable. If it does not seem reasonable, it may be necessary to re-calibrate and re-measure. If the problem persists, contact an identifier. Make note of any problems in measuring and post near the measuring station.

o. The macro will store the measurement in separate data files along with the necessary sample information.

p. Repeat the above steps for all fish in the dish.

q. When all larval fish in the dish have been measured, fill the vial that originally contained the fish with alcohol and transfer the measured fish to the vial.

r. If the larval fish from this vial have been segregated into two or more groups, place another group into the dish, being careful to submerse them in alcohol, and measure as above. Do not measure more than fifty larval fish of any one taxon from each survey.

4.0 RECORDS

4.1 All data sheets are later reviewed by the Lab Manager or designated staff.

4.2 Original data sheets are permanently stored.

Table B3-1. Equipment List

1. Dissecting microscope, with camera attachment connected to computer equipped with Optimas 6.2 or ImageJ if measuring larvae
2. Light source
3. Micrometer
4. Sorting tray or petri dish
5. Squeeze bottle containing 70% ethanol (denatured)
6. Glass shell vials
7. Holder for shell vials
8. Jar containing target organisms to be identified
9. Cotton
10. Forceps
11. Waterproof labels
12. Dot labels
13. Data sheets
14. Identifier's log sheet
15. Taxonomic references

HAYNES GENERATING STATION (HnGS) - Lab Data Sheet **Sheet:** ______

Survey: ________ **Date:** ________ **Start Time:** ______ **Cycle:** ______ **Station:** ________

Species Code	Taxon	Count	QC Resort Additional Count	Total Count	Stage	Entrain-able	Comments

NOTES: **Total:** ______

	ID'ed By / Date	ID QC By / Date	QC Resort By / Date	Entered By / Date
Fish				
Eggs				
Inverts				

Attachment B3-1. Entrainment /Source Water Plankton Tow Lab Data Sheet

APPENDIX B4: IMPINGEMENT FIELD SAMPLING PROCEDURES

1.0 PURPOSE

The purpose of this document is to identify the procedures and equipment necessary to accurately collect and process impingement samples at the Alamitos Generating Station (AGS).

2.0 RESPONSIBILITIES

2.1 Task/Field leader:

- Contact plant to obtain clearance for personnel that will be conducting the sampling.
- Verify that all investigating biologists conducting the sampling have read and understand these procedures.
- Verify that procedures have been followed during sample collection and that the sampling has been conducted safely.

2.2 Investigating biologist:

- Conduct sampling using the following procedures.

3.0 PROCEDURES

Impingement sampling will only be undertaken when there is water passing through the traveling screens of Units 1-6. If only one pump is in operation, proceed with sample collection using the following procedures. The bar rack area does not require daily cleaning, thus no collections will be conducted from this area. Each normal operation impingement survey consists of an initial cleaning period followed by four 6-hr sampling cycles. Table B4-1 presents the proposed schedule for each survey.

3.1 Mobilization

a. Notify plant personnel of the dates, times, and names of the biologists that will be onsite during each survey. All personnel will require photo identification (driver's license, passport, etc.) to obtain access to the plant site.

b. The equipment listed in Table B4-2 is required for sampling and should be checked before leaving for the plant. Verify that any scales used for the sampling have been calibrated within the previous three months.

3.2 Traveling Screen Sample Collection

Impingement sampling procedures will generally be the same at all units. All units are equipped with traveling screens (TS), and when sampling occurs they will be rinsed and cleaned every 6 hours during the 24-hr period (see Table B4-1). At Units 1 through 4, impinged material is transferred from traveling screens to a collection bin via conveyor belt. At Units 5&6, impinged material is washed from traveling screens into a metal collection tray at ground level.

a. Make sure that the collection basket has been emptied prior to the survey. If there is any debris in it, request to have the basket switched out.

b. The initial screenwash is for cleaning purposes only; do not process any materials from the bin/tray after the cleaning rinse. The time at the end of the initial TS rinse is the beginning of Cycle 1. Record this on the appropriate datasheet.

c. Secure a mesh net/vexar basket (with mesh of the same size or smaller than the TS mesh) inside of the collection basket so that impinged materials can easily be removed

d. Have plant personnel activate the TS wash system thirty minutes prior to the end of each cycle so that all impinged material is rinsed from the screens into the collection basket.

e. Remove all impinged fish and invertebrates from the impinged debris.

f. Replace the mesh net/vexar basket in the rectangular bin before initiation of the screenwash for the next cycle.

g. All collected impinged material will be processed using the procedures in following section.

3.3 Sample Processing

a. Remove all fishes and invertebrates from the impinged debris. Record the volume of the debris (gallons) on the datasheet. Also record the composition and percentage of the debris.

b. All fishes, crabs, shrimps and prawns, and cephalopod mollusks are identified, counted, measured (see measurement criteria below), and weighed. This information is recorded on the appropriate datasheet. All other invertebrates are identified and recorded as present by entering a "P" in the count box.

Organism Group	Length Measuring Criteria
Fishes	Total body length for sharks, disc width for skates and rays and standard lengths for bony fishes
Crabs	Maximum carapace width
Spiny lobster and Shrimps	Carapace length, measured from the anterior margin of carapace between the eyes to the posterior margin of the carapace
Octopus	Maximum "arm" spread, measured from the tip of one tentacle to the tip of the opposite tentacle
Squid	Dorsal mantle length, measured from the edge of the mantle to the posterior end of the body

c. Record all organism names on the appropriate datasheet, using their scientific names whenever possible. The taxa codes are recorded after the datasheets are returned to the office.

d. Make certain of all identifications before recording the name on the datasheets. If an organism cannot be positively identified it should be saved. The voucher specimen should be placed in a plastic bag with a waterproof label indicating the location, cycle, date, time, and the initials of the collector. If personnel on the next cycle can positively identify the organism record the organism's name on the datasheet. If positive identification cannot be made the organism is returned to the laboratory for identification.

e. If a large number (more than 30) of any individual countable species is collected during a cycle, individually measure and weigh 30 randomly selected individuals of this species and then count and measure the remaining individuals and record this information on a separate line on the datasheet. For example: if 198 deepbody anchovies were collected, randomly select 30 individuals and record on the first row *Anchoa compressa*, count = 1, Length = standard length (the distance from the tip of the snout to the posterior vertical margin of the hypural plate) to the nearest mm, weight = weight to nearest gram, sex = "-" (if the sex cannot be determined without dissection, record a "-"), and condition = alive (A), dead (D), or mutilated (M). Continue this procedure for the other 29 randomly chosen deepbody anchovies. Then batch weigh the remaining individual. If all of the individuals are alive, put an "A" in the Cond. box. If some are dead and some mutilated, use additional rows to fill in the appropriate information in the corresponding row(s).

f. Determine the sex of the countable organisms to the extent possible without dissection. Assign the letter M to refer to males, F for females, J for juveniles, G for gravid. Put a "-" if the sex cannot be determined without dissection.

g. Record the condition of each countable organisms: A for alive; D for dead; M for mutilated. If an individual is mutilated, do not measure the length. If there are more than 30 non-mutilated individuals, the mutilated individual(s) can be weighed with the batch weight of the additional individuals. If there are less than 30 non-mutilated individuals, record the weight of the mutilated individual(s) but not their lengths.

h. Record any anomalies or other notes (encountered in each cycle) in the notes section on the datasheet.

i. At the end of each cycle verify that: a) the sampling procedures have been followed correctly, b) the data has been recorded correctly and legibly, and then c) sign and date the "Reviewed by/Date" section at the bottom of the datasheet.

j. Put all dead animals and discarded debris in trash dumpsters. Make sure to double bag the material as collection of the trash may not occur for several days.

k. Quality control (QC) checks will be preformed on at least a quarterly basis to verify all organisms are being removed from the debris and that the correct identification, enumeration, length and weight measurements of the organisms are being recorded on the datasheet. The QC team will randomly choose the actual impingement cycles that will be checked and will resort the debris for any missed organisms. All organisms will then be identified, re-measured and re-weighed by the QC team to ensure that the data is being recorded correctly. If a sampling team fails a QC check, they will be retrained on fish identification and sample collection. QC checks will be performed on the sampling team until they pass the QC requirements. The QC checks will be fully documented and reported to the Project Manager.

3.4 General

a. All information recorded on the datasheets must be written legibly with a pencil.

b. Keep information separate for each cycle

c. The survey number will be determined based on the week corresponding to that survey (e.g., week 1 = survey 1). Make sure the correct survey number is recorded.

d. Make certain that the unit #s and cycle numbers are correct on the datasheet you are using. Record the date and time for the start and end of each screen wash (generally 15 to 20 minutes) and cycle duration times (generally 6 hours). Each 24-hr survey is divided into 4 six-hour cycles.

e. Record the names of all personnel present during each cycle.

f. Use military time (0000 – 2400) to record every cycle collected. Record all times as local time (Pacific Standard Time or Pacific Daylight Time).

g. During each screen wash, verify that the screens are operating properly (the screens should be moving and the water should be spraying). Check with the operator to find out how many circulating water pumps are operating.

h. If a survey cannot be completed or is cancelled, make a note on the appropriate data sheet explaining the reason for the cancellation. Write the survey number that corresponds to that week, date and sign the datasheet.

i. At the end of each screen wash, record the relevant meteorological data.

j. If the traveling screens trip before the 6-hour cycle is over, collect all material and process it as part of the upcoming cycle. If possible, have the screen wash system run at the scheduled times.

3.5 Heat Treatments

During heat treatments follow the same procedures as during normal impingement sampling. Use a separate data sheet for each species collected. A single data sheet can be used for several species if low numbers of these species are collected.

If an extremely large amount of material is collected in the collection basket, sub-sampling of the most abundant fishes/shellfishes should occur to minimize the time taken to process the sample. Sub-sampling procedures are as follows:

a. Remove the less abundant fishes/shellfishes from the impinged material; and record as individuals on the datasheets.

b. Collect a sub-sample (for example, two 3-gallon sub-samples) from the pile of impinged material. Make sure to randomly sample the pile by collecting organisms from different areas of the pile. Discard the remaining material and record the volume discarded on the data sheets.

c. The number and weight of the organisms collected from each sub-sample should not be recorded with the other data. Record the sub-sample data on a separate datasheet. Make certain that record of the organisms from the sub-samples can be linked back to the quantity of material discarded and not sampled.

Table B4-1. Target schedule for 24-hour impingement sampling effort (schedule assumes that at least one circulating water pump is in operation). Based on 30 minutes for complete rotation of travel screens at each unit.

Time	Units 1/2	Units 3/4	Units 5/6
8:00	Rinse and Clean		
8:30	Start Cycle 1		
9:00		Rinse and Clean	
9:30		Start Cycle 1	
10:00			Rinse and Clean
10:30			Start Cycle 1
11:00			
11:30			
12:00			
12:30			
13:00			
13:30			
14:00			
14:30			
15:00		Rinse Cycle 1	
15:30		Start Cycle 2	
16:00		Process Cycle 1	Rinse Cycle 1
16:30			Start Cycle 2
17:00			Process Cycle 1
17:30			
18:00			
18:30			
19:00			
19:30			
20:00			
20:30			
21:00		Rinse Cycle 2	
21:30		Start Cycle 3	
22:00		Process Cycle 2	Rinse Cycle 2
22:30			Start Cycle 3
23:00			Process Cycle 2
23:30			
0:00			
0:30			
1:00			
1:30			
2:00			
2:30			
3:00		Rinse Cycle 3	
3:30		Start Cycle 4	
4:00		Process Cycle 3	Rinse Cycle 3
4:30			Start Cycle 4
5:00			Process Cycle 3
5:30			
6:00			
6:30			
7:00			
7:30			
8:00	Rinse Cycle 1		
8:30	Process Cycle 1		
9:00		Rinse Cycle 4	
9:30		Process Cycle 4	
10:00			Rinse Cycle 4
10:30			Process Cycle 4
11:00			

Note: Schedule is separated into 30-minute increments to show activities associated with each cleaning and collection cycle at the two sets of traveling screens.

Table B4-2. Equipment List.

1. Datasheets printed on waterproof paper
2. Pencils
3. Scales (Electronic and spring)
4. Measuring boards
5. Fish and invertebrate identification keys
6. Buckets and plastic totes
7. Floodlights and extension cords
8. Calipers
9. Calculator
10. Hardhats
11. Safety Glasses
12. Rubber/latex gloves
13. Clipboard