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FINAL

1981 CON EDISON AUTOMATED
ABUNDANCE SAMPLING (AUTOSAM) AND
LABORATORY PROCESSING STANDARD
OPERATING PROCEDURES

Prepared for

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**Con
Edison**

12/28/82

TO Kevin

This is a replacement
page for EA's
draft 1981 Auto Sam
SOP.

Document can now
be considered a
final. Eileen

b) If a reworked sample contains more than 30 organisms, apply the following criteria: if any one (1) of the reworked samples exceeds 10 percent error, the entire lot size in question will be reprocessed.

c) The formula used to determine the percent error is as follows:

$$\text{Percent error} = \frac{QCE + 1}{Oe + 1} \times 100$$

where: $QCE + 1$ = the number of eggs and larvae misidentified from the sample in the quality control analysis

and: $Oe + 1$ = the total number of eggs and larvae identified from the sample in the quality control analysis.

d) Errors below the AOQL will not be added to the original data sheet.

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1. STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF ENTRAINMENT ABUNDANCE SAMPLES WITH THE EA AUTOMATED ABUNDANCE SAMPLER

1.1 INTRODUCTION

1.1.1 Objective

Entrainment abundance samples will be collected to determine the species composition and abundance of fish eggs and larvae, specifically Atlantic tomcod, striped bass, white perch, bay anchovy, and herring entrained through the Indian Point Generating Station condenser cooling system.

1.1.2 Sampling Design

An Ecological Analysts, Inc. Automated Abundance Sampler (AUTOSAM) will be used to collect ichthyoplankton samples twice a week during May, June, July, and August. AUTOSAM entrainment abundance samples will be collected at Discharge Station D2 (see Figure 1-1) from middepth, with the intake hose oriented into the flow. Each sampling effort will consist of collecting 90-minute composite samples within eight 3-hour sampling intervals extending over a 24-hour period. Each 90-minute composite sample will be obtained by intermittent operation of the AUTOSAM (e.g., 30 minutes on, 30 minutes off, 30 minutes on, 30 minutes off, etc.) throughout each 3-hour sampling interval. Samples will be analyzed at Ecological Analysts' Central Laboratory in Middletown, New York, in accordance with procedures described in Chapter 2.

1.2 GENERAL CHARACTERISTICS OF THE AUTOMATED ABUNDANCE SAMPLER (AUTOSAM)

1.2.1 Introduction

The AUTOSAM is designed to sample continuously over a given period of time and to preserve the ichthyoplankton and macrozooplankton which are collected. Control is implemented through a series of front panel thumbwheel and toggle switches (Figure 1-2) which allow operator versatility in controlling final outputs, routine maintenance, or troubleshooting. A four-digit light-emitting diode (LED) display allows the operator to read accumulated flow or time values for up to eight samples or the current cycle. The LED also displays a real time clock and the system's operational phase, number of the sample being collected, and number of the cycle within a sample.

The AUTOSAM requires a service of 50 amps at 230 volts, typically delivered via an electrical outlet encased in a waterproof box. The sampler's operation is unaffected by temperature as long as the interior of the trailer remains between 5 and 35 C.

AUTOSAM samples 700-1,300 liters per minute at suction lifts up to 20 feet (7 m). Flow and volume are measured by a Signet inline flowmeter ensuring accurate density estimates.

A sequence of a pump run, washdown/rinse, and transfer of the plankton and detritus to the codend is defined as one cycle. To reduce time between collection and preservation of the organisms and the consequent physical

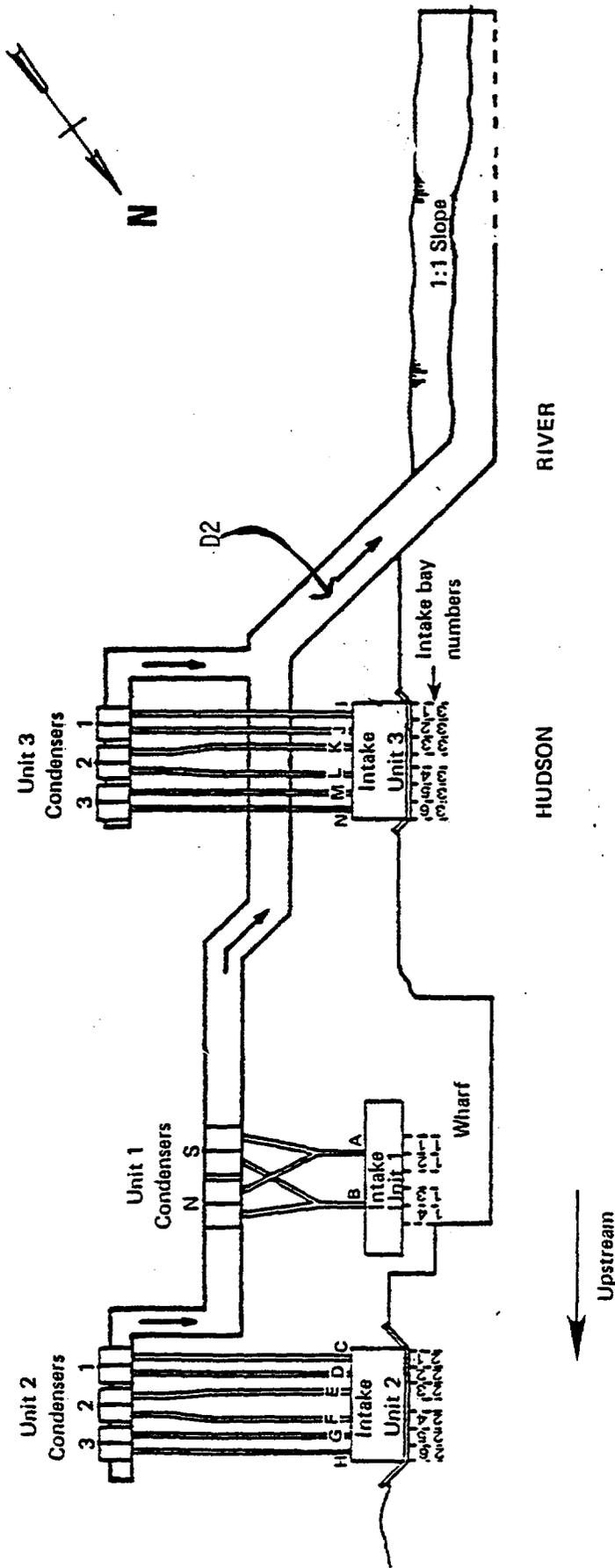


Figure 1-1. Diagram of the Indian Point Generating Station circulating water system showing location of sample stations and circulator pumps (circulator pump codes are indicated by the letters A through N at the rear of intake units).

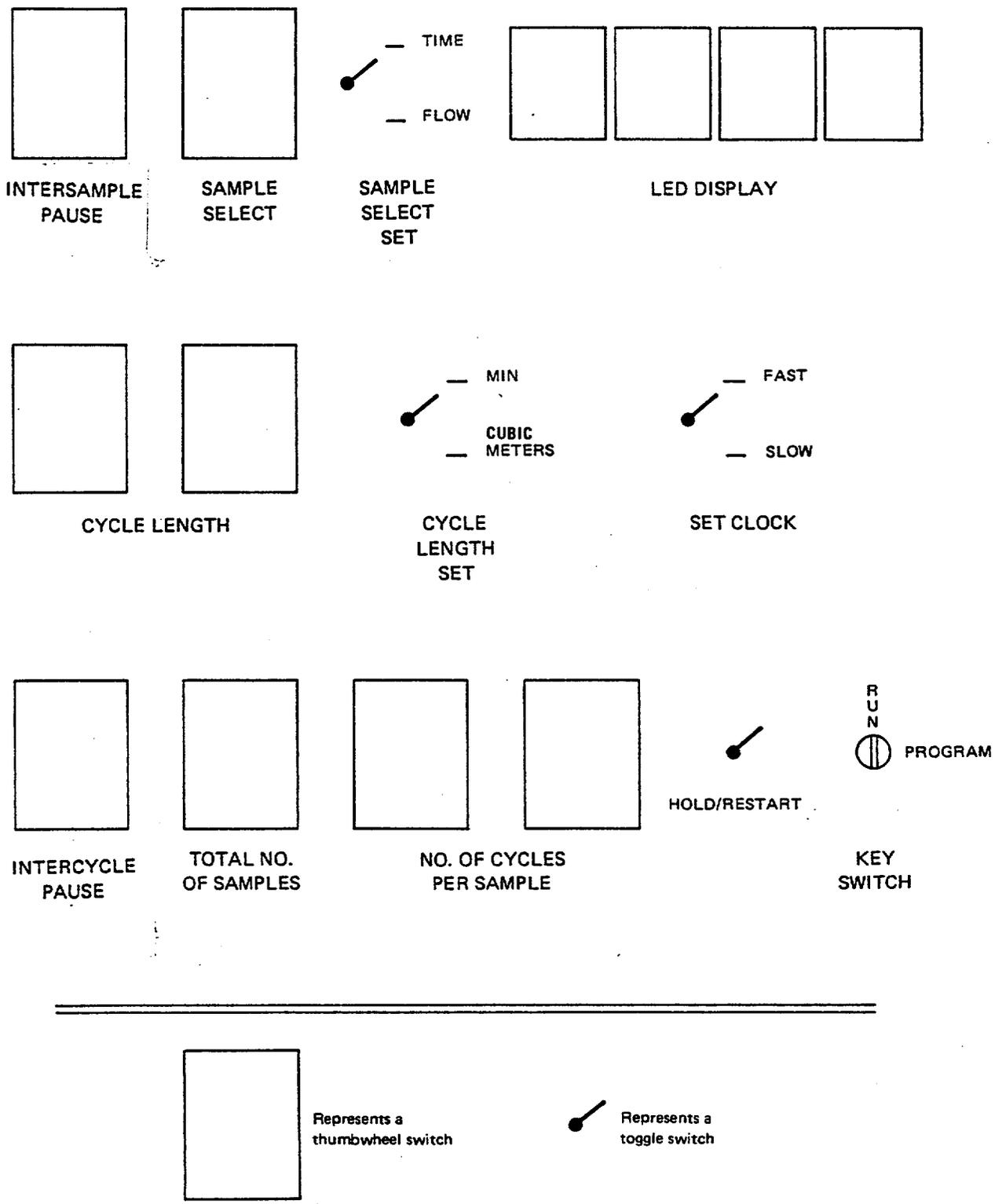


Figure 1-2. Front panel of Auto Sam, Indian Point Generating Station, 1981.

damage to sensitive species, 1-24 cycles of 5-99 minutes may be composited into one sample, with eight sample spaces provided for on the turntable. The length of a cycle may also be determined by volume pumped (adjustable from 1-99 cubic meters).

1.2.2 Microcomputer Inputs

Operation of the AUTOSAM is controlled through the use of microcomputer inputs to select the cycle length, the number of cycles per samples, and the number of samples over a specific time period to best meet the experimental design. The front panel switches which control the operation of the AUTOSAM are described below. Refer to Figure 1-2 for placement of switches.

1. INTERSAMPLE PAUSE
Thumbwheel - Between every sample a pause (adjustable from 0 to 27 hours) can be inserted. Intersample pause length = 3 hours x whatever is entered via the switch.
2. CYCLE LENGTHS
Thumbwheels - Two thumbwheel switches determine the length of a cycle (adjustable from 5 to 99 minutes or cubic meters); cycle length in minutes or volume pumped in cubic meters is determined by the associated CYCLE LENGTH SET toggle switch.
3. CYCLE LENGTH SET
Toggle
4. INTERCYCLE PAUSE
Thumbwheel - Between every cycle a pause can be inserted, adjustable from 0 to 2-1/4 hours. Intercycle pause length - 1/4 hour x specific number entered via the switch.
5. TOTAL NO. OF SAMPLES - Determines total number of samples (adjustable from 1 to 8) and thus provides a stopping point.
6. NO. OF CYCLES PER SAMPLE - These two thumbwheel switches determine the number of cycles composited in each individual sample (adjustable from 1 to 24).
7. SET CLOCK - This two-position (up for fast forward, down for slow forward) momentary toggle switch sets the real time clock. (The real time clock appears on the LED display when the SAMPLE SELECT switch is set at "9" and the SAMPLE SELECT SET toggle at "time").

8. HOLD/RESTART

- This momentary toggle switch is used in conjunction with the KEY SWITCH (in run position) to start and stop (hold) the machine. With the KEY SWITCH in program position, a restart pulse from the toggle switch will reprogram the machine to start sampling on whatever sample is entered via the SAMPLE SELECT.

1.2.3 Microcomputer Outputs

There are three types of outputs from the AUTOSAM microcomputer: an operator-controlled LED display, a printer for important data sheet information (optional), and seven AC lines to control the sampling process.

The SAMPLE SELECT thumbwheel is used in conjunction with the SAMPLE SELECT SET toggle to provide output information through the LED display. If the SAMPLE SELECT SET toggle is turned to the up ("time") position, and the SAMPLE SELECT thumbwheel is set on any number 1 through 8, the LED will display the accumulated time of any of the first through the eighth samples.

If the SAMPLE SELECT thumbwheel is on "9" and the SAMPLE SELECT SET is on "time," the LED displays a real time clock. If the SAMPLE SELECT thumbwheel is on "9" and the SAMPLE SELECT SET is on "flow," the LED will display operational phase in the first digit, sample number in the second digit, and cycle number in the last two digits. The five cycle phases are: (1) pumping, (2) rinsing, (3) turntable, (4) pause, and (5) error.

The output signals to the water pumps, valves, and the sampling mechanism are routed through an Opto 22 Input/Output (I/O) card. At present, seven AC output modules are used; these are assigned and linked to the LED phase display as follows:

<u>AC Output Module Number</u>	<u>Device to Which Module is Assigned</u>	<u>Phase</u>
0	Sampling Pump	1
1	Rinse Pump No. 1	2
2	Rinse Pump No. 2	2
3	Ball Valve open	2
4	Ball Valve closed	2
5	Turntable Advance	3
6	Formaldehyde Pump	4
7	(spare)	

If the SAMPLE SELECT thumbwheel is set on "0," a "time" setting of the SAMPLE SELECT SET toggle will cause the LED to display the elapsed time of the present cycle. Knowing the number of cycles in a sample, the length of a cycle, and the cycle number, the amount of time left in a sample can be determined. If the SAMPLE SELECT thumbwheel is on "0," a "flow" setting of the SAMPLE SELECT SET toggle will cause the LED to display the amount of flow accumulated in the present cycle.

1.3 FIELD COLLECTION PROCEDURES

The AUTOSAM operator will be responsible for setting up the AUTOSAM for sampling, safe operation of the AUTOSAM, the collection of samples according to Standard Operating Procedures, and the accurate entry of information on the Fisheries and Entrainment Abundance data sheets.

1.3.1 Equipment

The following equipment will be available from the AUTOSAM trailer or the accompanying vehicle. Care should be taken to ensure that all equipment listed is available before and during all sampling operations.

- Ecological Analysts' Automated Abundance Sampler (AUTOSAM)
- Intake and discharge flexible hoses (4-inch)
- Extension cord (220-volt)
- Chocks for the trailer wheels
- Nine codend collection cups (includes one backup)
- Five gallons of 100 percent buffered formalin
- Squeeze-type wash bottle filled with 10 percent formalin
- Ball jars
- In-jar labels
- Jar-top labels
- Ball jar sieve (500 μm)
- Wristwatch
- Martek chemistry unit and datalogger
- Field notebook
- Fisheries and Entrainment Abundance data sheets (Figure 1-3)
- No. 2 grade pencils
- Waterproof marking pens
- Flowmeter calibration check unit
- Sample concentration funnel

1.3.2 Presampling Procedures¹

1. Set the trailer level by adjusting the tongue jack and stabilizers.
2. Chock the wheels.
3. Connect the 4-inch intake hose and the 4-inch discharge hose to the trailer discharge.
4. Attach the AUTOSAM trailer to the power source with the extension cord provided.
5. Turn on the main circuit breaker.
6. Prime the sampling pump (requires 2 gallons of water).
7. Check secondary concentrator screen for tears or net separation from the framing.
8. Check the main tank net and rinse system.

1. The intake line should slant gently upwards to the 4-inch sampling pump in the trailer such that there are no sags in the line where the water can remain.

SITE: INDIANPT HH SERIAL NO. BBAA0039

GEAR: ENTA50 COLLECTORS: JS

START DATE: Day 25 Month MAY Year 81 TIME Hr. 12 Min. 00 REMARKS: _____

END DATE: Day 25 Month MAY Year 81 TIME Hr. 15 Min. 00

STATION: IPD1

Sample void: sample jar dropped, spilled or lost; contents improperly preserved; collection tubing, nets, etc., discovered to be faulty at the conclusion of the sample; proper number of cycles not obtained (generally, for every six scheduled cycles, five must be obtained). Cycles void: unit stops; coarand, net or tubing defective during cycle.

TIDE: 1 1 = Flood 4 = Low slack 2 = Ebb 9 = Not taken 3 = High slack
DEPTH: 2 1 = Bottom 4 = Oblique 2 = Mid-depth 9 = Not taken 3 = Surface

WATER QUALITY DATA READ BY JS INSTRUMENT NO.: M-14 PROBE NO.: M14

	DEPTH	TEMP. (C)	D.O.	COND. (µmho)	pH	BATTERY VOLT.	LAB. TEMP. (C)	LAB. COND. (µmho)
B:		24.6	10.2	625	7.8	11.9	2	
E:								

READ BY: JS/ND

CIRCULATING PUMPS OPERATING: -C-D-E-F-G-H

TIDAL HEIGHT: +0.6 THROTTLED: Unit 1 Unit 2 N Unit 3 Unit 4 Unit 5 Y = Throttled N = Not throttled

SAMPLE DURATION: 81 MINUTES CYCLE DURATION: 27 MINUTES NO. OF CYCLES: 3

METER READING (m³) VOLUME FINAL: 324.5 INITIAL: 0.0 NET: 324.5 COMPOSITE LENGTH: 9 1 = 3 hours 2 = 4 hours 3 = 6 hours 4 = 10 hours 5 = 24 hours 9 = unique

CORRECTION: _____ UNIQUE COMPOSITE LENGTH: Hr. 1 Min. 30 CORRECTED NET: 324.5

SAMPLE DURATION (Minutes) FINAL: _____ INITIAL: _____ NET: _____ FLOWMETER NUMBER _____ NO. OF CYCLES FINAL: _____ INITIAL: _____ NET: _____



Figure 1-3. Auto Sam data sheet.

9. Turn all circuit breakers on to provide power to the AUTOSAM.
10. Place codends on turntable.
11. Fill carboy with 100 percent formalin.
12. Set up the ambient water collection system designed to collect ambient water for water quality measurements from within AUTOSAM.
13. Set up the Martek water quality unit and datalogger. The probe should be lying in the bottom of the ambient water collection tank.

1.3.3 Sampling Procedures

This section details the actual sampling procedures to be used at the Indian Point plant for the collection of ichthyoplankton for abundance studies. As each point is defined, it may be necessary to record information on the Fisheries and Entrainment Abundance data sheet (Figure 1-3). The required information and the appropriate location for recording it on the data sheet are described. All entries on the data sheet must be filled in to ensure that pertinent information is available for analysis (see Section 1.3.5 for Fisheries and Entrainment Abundance data sheet format).

1. Check the pumps, codends, nets, chiller, and preservation system for proper condition.
2. Fill out as much of the data sheet in advance as possible, including site, gear, station, date, serial number, etc. (see Section 1.3.5).
3. When the power is turned on, the microcomputer will not be in a particular regime, as will be indicated by the LED display flashing on and off (this also occurs whenever the control process is halted or finished).
4. Set the SAMPLE SELECT thumbwheel to "9."
5. Set the SAMPLE SELECT SET toggle in the up ("time") position.
6. Correct the real time clock using the "fast" and "slow" positions of the SET CLOCK toggle.
7. Set sample start times using intercycle pause, sample number, and cycles/sample thumbwheel switches. (Refer to Figure 1-3)
 - a. Set INTERCYCLE PAUSE switch to "1"
 - b. Set TOTAL NO. OF SAMPLES to "2" (for 1200 start)
 - c. Set 1st cycles/sample switch to "0"
 - d. Set 2nd cycles/sample switch to day you wish sample to begin. Today is day "0", tomorrow, day "1", etc.
8. Turn the KEY SWITCH to "program", and press the restart toggle. Turn the key switch back to "run". Set the seven AC output toggle switches (not shown in Figure 1-2) to automatic.
9. Refer to Figure 1-2 as necessary and set the following thumbwheel switches for the desired experimental design. Set the switches in the following order:

- a. Set INTERSAMPLE PAUSE thumbwheel to "0."
 - b. Set CYCLE LENGTH thumbwheels to "27" minutes, CYCLE LENGTH SET toggle must be in the "min" position.
 - c. Set INTERCYCLE PAUSE thumbwheel to "2."
 - d. Set NO. OF CYCLES PER SAMPLES thumbwheel to "03."
 - e. Set TOTAL NO. OF SAMPLES thumbwheel to "8."
10. Start the AUTOSAM process control by turning the HOLD/RESTART toggle to the RESTART position (the LED display should stop flashing).
 11. Check the process control: set the SAMPLE SELECT thumbwheel to "9" with the SAMPLE SELECT SET toggle in the down ("flow") position. The LED display should now show 1 1 01. The information presented on the LED represents (from left to right) phase, sample number, and cycle number (the last two digits). The five different phases are: (1) pumping, (2) rinsing, (3) turntable, (4) pause, and (5) error. Similar checks can be made on the status of each sample throughout the sampling effort. Set the SAMPLE SELECT thumbwheel to "0" and the corresponding toggle to time. The LED should show "1200", the hour the sampler will start on the day selected.

1.3.4 Sample Termination

It is not necessary for the AUTOSAM operator to be at the site exactly when AUTOSAM stops sampling, but this is a good time to check the sampling system as a maintenance routine. At the end of the eighth sample, the eight samples will be removed from AUTOSAM and essential information stored in the microcomputer memory will be recorded on the Fisheries and Entrainment Abundance data sheets.

First, transfer accumulated flow and time data from the LED display onto the appropriate data sheets according to the following sequence:

1. Set SAMPLE SELECT thumbwheel to "1" for information pertaining to the first of the eight samples.
2. Switch SAMPLE SELECT SET toggle to "flow" position and record the accumulated flow (m^3) in the NET and CORRECTED NET blocks, Section 4 of the data sheet.
3. Switch SAMPLE SELECT SET toggle to "time" position and record the elapsed time in minutes in the SAMPLE DURATION block, Section 3 of the data sheet. This will require converting the number of whole hours to minutes. The time values will be the same for all samples.
4. Repeat steps 1-3 for the remaining samples (1-8 on the SAMPLE SELECT thumbwheel corresponds to a possible eight samples).
5. Remove data tape containing hourly temperature, dissolved oxygen, pH, and conductivity readings from datalogger. Record water quality information in Section 2 on the Abundance Data Sheet for all samples collected.

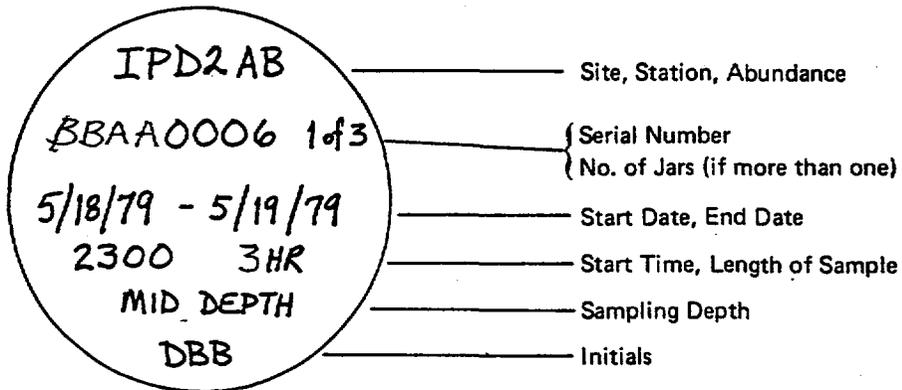
After Steps 1-6 have been completed, preserve each sample as follows:

1. Remove the codend collection cup from the turntable and pour the contents through the sample concentration funnel. Rinse the codend collection cup and pour the residue through the concentration funnel. Repeat until all of the detritus in the codend has been removed. Remove the concentration funnel tip, where the sample has been concentrated. Place the mouth of a Ball jar over the top of the tip. Carefully invert this configuration. Rinse the sample gently from the concentration tip into the Ball jar with 10 percent formalin. CAUTION: If formalin fumes are detectable in the trailer, a respirator mask should be worn.
2. Place a completed in-jar label in the sample jar so that the label can be seen through the glass. This label will contain the following information (Figure 1-4):
 - a. Type of collection, "IPAB" for Indian Point abundance
 - b. Length of sample
 - c. Serial number
 - d. Site and station IPD2
 - e. Collector's initials.
3. Place the top on the sample jar and complete a jar-top label (Figure 1-4). This label will contain the following information:
 - a. Site: IP
 - b. Station: D2
 - c. Type of collection: AB
 - d. Serial number
 - e. Number of jars (if more than one)
 - f. Start date and time
 - g. End date
 - h. Length of sample
 - i. Sampling depth
 - j. Collector's initials.
4. Properly store the sample jars.

1.3.5 Fisheries and Entrainment Abundance Data Sheet Completion

1. Section 1 of the data sheet:

SITE	- Indian Point
GEAR	- Start at extreme left, enter ENTA50
START DATE	- Day: Enter appropriate number Month: Enter first three letters of month Year: Enter last two digits of year
START TIME	- Record in military time (0001-2400)



JAR-TOP LABEL

TYPE OF COLLECTION	AB	3 HR
SERIAL#	BBAA 0006	1 of 3
STATION	IPD2	INITIALS DBB

Abundance, Length of Sample
 Serial Number, No. of Jars (if more than one)
 Site (Indian Point) & Station, Initials

IN-JAR LABEL

Figure 14. Label format for Auto Sam abundance samples.

- END DATE - Enter as per instructions for START DATE and START TIME
- STATION - Enter IPD2
- TIDE - Enter as appropriate, following the code on the data sheet
- DEPTH - Enter "2" middepth
- HH - Leave blank
- SERIAL NUMBER - Enter "BCAA" in the first four boxes, sequential numbers are used
- COLLECTORS - Enter initials
- REMARKS - Record information pertinent to the sample; e.g., problems, reason for a void sample, backup water quality equipment used.

2. Section 2 of data sheet (WATER QUALITY DATA)

- READ BY - Enter initials of person reading instrument, and individual recording readings (if different)
- INSTRUMENT NUMBER - Fill in appropriate code. For Marteks, use M- in the first two boxes followed by instrument number
- PROBE NUMBER - Enter M followed by the instrument number
- DEPTH - Leave blank. Enter chem readings on the top line
- TEMP - Enter water temperature in degrees centigrade
- DO - Enter dissolved oxygen in mg/l
- COND - Enter conductivity in μmho
- pH - Enter in pH units
- BATTERY VOLT - Must be recorded once at end of instrument use if datalogger is not used.

NOTE: Indicate in REMARKS section of the data sheet if a backup instrument was used for any particular parameter. The AUTOSAM operator is responsible for ensuring that readings obtained are within acceptable ranges.

3. Section 3 of the data sheet

- READ BY - Enter initials of individual who obtained the information in this section
- CIRCULATING PUMPS OPERATING - List letters of circulating pumps operating during the sample collection that potentially supply cooling water to the sampling station. The following codes should be used (see Figure 1-1). Each letter including the first must be preceded by a dash.
- Unit 1 - From North to South, B, A
Unit 2 - From North to South, H, G, F, E, D, C
Unit 3 - From North to South, N, M, L, K, J, I
- TIDAL HEIGHT - Enter - 999.
- THROTTLE - Indicate if the circulating pumps for a particular unit are throttled or not by using the code listed on the data sheet.
- SAMPLE DURATION - Enter duration as determined from procedures in Section 1.3.4, Item 3. Value in minutes should be 81.
- CYCLE DURATION - Enter "27"
- NUMBER OF CYCLES - Enter "3"

4. Section 4 of the data sheet (METER READINGS)

- FINAL INITIAL - Leave blank
- NET - Net sample volume is obtained from procedures in Section 1.3.4, Item 2
- CORRECTION - Leave blank
- CORRECTED NET - Enter NET value
- COMPOSITE LENGTH - Enter "9"
- UNIQUE COMPOSITE LENGTH - Enter 1 HR. and 30 MIN. in appropriate spaces

5. Section 5 of the data sheet

SAMPLE DURATION - Leave blank
FLOWMETER NUMBER - Leave blank
NUMBER OF CYCLES - Leave blank

1.3.6 Manual and Special Control Sequences

1.3.6.1 Manual Rinse Sequence

It may be desirable to utilize a manual rinse at certain intervals if exceptionally high detrital loads are present or if assurance of clean screens is important.

1. Switch the HOLD/RESTART toggle to the "hold" position to stop the machine.
2. Close ball valve.
3. Use first rinse to wash down net in the barrel.
4. Open ball valve to flush detritus into codend.
5. Use second rinse to washdown secondary concentrator.

1.3.6.2 Reprogramming

In addition to starting and stopping the sampling process, the HOLD/RESTART toggle is used to enter a change in the numerical designation of the sample being collected. This may be necessary if one or more of the samples collected are void for any reason. The following sequence should be followed:

1. Set the HOLD/RESTART toggle to the "hold" position.
2. Rotate the KEY SWITCH to the "program" position to allow the computer to accept a different sample designation.
3. Select the new sample number that is to be collected with the SAMPLE SELECT thumbwheel.
4. Set the HOLD/RESTART toggle to the "restart" position. This will clear the time and flow accumulations of previously collected samples except those represented by lower sample numbers than the new sample number.
5. Rotate the KEY SWITCH back to the "run" position. This will initiate the sampling process of the designated sample number.

1.3.6.3 Delayed Start

The AUTOSAM may be programmed to delay the initiation of sampling up to 9 days from the time of programming. This enables sampling to commence without the operator being present. The start time for the sampling activity should be

set by using the four digit thumbwheels located in the lower left-hand panel area (normally used for intersample pause, total number of samples, and the two thumbwheels used to designate the number of cycles per sample) in the following manner:

1. Set the desired start time from left to right according to the format of HR (x 10)HR (x 1) MIN (x 10). Set the desired start day in the 4th position. the present day is day "0", the next day (tomorrow) is "1", etc.
2. Set the SAMPLE SELECT thumbwheel to position "9" and the SAMPLE SELECT SET toggle to "time." At this point it is suggested that the real time clock which is now being shown on the LED display be corrected if necessary by means of the SET CLOCK toggle.
3. Rotate the KEY SWITCH to the "program" position.
4. Set the HOLD/RESTART toggle to "restart."
5. Rotate the KEY/SWITCH to the "run" position.
6. Set the SAMPLE SELECT thumbwheel to position "0." The start time should appear on the LED display.
7. Reset the four digit thumbwheels located in the lower left-hand panel area to the appropriate values of "intersample pause," "total number of samples," and "number of cycles per sample."
8. Set the INTERCYCLE PAUSE AND CYCLE LENGTH thumbwheels to the appropriate values.
9. Set the SAMPLE SELECT thumbwheel to "1" (or another number if you want to start with a different sample). If left at "0" the unit will commence with sample "1".
10. Set the HOLD/RESTART toggle to "restart" to initiate the sampling sequence. This should cause the LED display to stop blinking and the beginning of the accumulation of time towards the delayed sample start time.

NOTE: A delayed start setting of 00HR, 0MIN, and 0 Days, will cause the initiation of sampling immediately when Step 10 is completed.

1.4 MOVING THE AUTOSAM TRAILER

1. Unplug and coil the 220-volt extension cord.
2. Disconnect and stow the intake and discharge lines.
3. Disconnect the ground wire.
4. Make sure the circuit panel, pipes, codends, and other equipment are secure.
5. Secure the doors of the trailer.

6. Hook trailer to pick-up or appropriate vehicle with 2 5/16-inch ball and electric brake controller (small box under dashboard near the steering wheel).
7. Crank tongue jack all the way up!
8. Hook up light and brake cord and check operation.
9. Hook up safety chains!
10. Remove chocks from under the wheels and check for any possible transit problems.

2. CENTRAL LABORATORY ENTRAINMENT ABUNDANCE SAMPLE PROCESSING STANDARD OPERATING PROCEDURES

2.1 INTRODUCTION

Final processing of ichthyoplankton collected in the experiments described in Chapter 1 will be conducted at Ecological Analysts' (EA) Central Lab in Middletown, New York. Ball jars containing samples will be transported to Middletown by EA personnel. The Central Lab processing has three phases: (1) sorting, (2) identification, and (3) quality control. During sorting of ichthyoplankton samples, eggs and larvae are removed, the sample correctly preserved, and the vials accurately labeled. Organisms removed from the detritus are identified, and information regarding life stage and species is recorded. Quality control procedures also assure the accuracy of identification.

The Consolidated Edison studies scope of work requirements which will be met by the Central Lab are summarized in Table 2-1. When all the specimens are analyzed according to the requirements, the Entrainment Abundance data sheets are ready to be sent to data processing.

2.2 ICHTHYOPLANKTON SORTING PROCEDURES

2.2.1 Sorting Equipment

The following equipment will be available for each individual sorting a sample. Ensure that the equipment is available prior to and during all sorting efforts.

- One Pyrex sorting tray measuring 34.3 cm x 21.6 cm (13.5 in x 8.5 in), which has blackened sides and bottom
- One pair blunt-point microdissection forceps
- Three pipettes
- Three fine-point probes
- Ample supply of 4-dram vials with polyvinyl snap-top lids
- Waterproof paper for inside-vial labels
- Additional No. 2 grade pencils
- Ample supply of 3/4-in. diameter vial labels
- One waterproof marking pen
- One squeeze bottle filled with 5 percent buffered formalin solution
- 375- μ m-mesh sieve or standard 500- μ m-mesh sieve
- An illuminating magnifier
- Tally counter

2.2.2 Presorting Procedures

This section describes the procedures to be used for rinsing the formalin preservative from the field collection.

1. Remove the field label from the sample.
2. Decant the sample jar contents through a sieve made of one layer of 375- μ m mesh (or standard sieve). Collect and save formalin in a quart jar.

TABLE 2-1 SAMPLE PROCESSING--1981 CON EDISON ABUNDANCE STUDIES

Experiment	Indian Point Site Processing			Central Lab Processing		
	Approximate No. of Samples	Initial Sample Processing	Onsite Lab Processing	Level of Identification Required	Length Frequency	Quality Control
<u>Automated Abundance Sampler</u>						
Abundance Samples (Chapter 1)	272	None	None	Lowest possible taxon	Atlantic tomcod, striped bass, white perch, <u>Alosa</u> spp. and bay anchovy	Sort: CSP-1a) ID: PDA ^{b)}

- a) Continuous Sampling Plan
- b) Proportion defective analysis.

3. Rinse the inside of the sample jar and lid into the sieve.
4. Wash the sample with clean water to remove formalin.
5. Rinse the contents of the sieve into a ball jar.
6. Check the sieve to ensure that all residual material has been rinsed into the ball jar.

2.2.3 Sorting Procedures

This section describes the sorting procedures to be used for removing the fish eggs and larvae from the sample.

1. Copy the information listed on the field label on a vial label.
2. Place the vial label on the top of the vial.
3. A separate vial must be maintained for eggs and larvae.
4. Sort approximately 10 ml aliquots from ball jar of detritus and remove eggs and larvae.
5. Sort individual aliquots for fish eggs and larvae, using forceps, probes, and pipettes.
6. Place the ichthyoplankton into separately marked vials (i.e., eggs or larvae).
7. Maintain a continuous tally of each life stage using a tally counter.
8. After all the organisms are removed from the sample, record the total count for each life stage on the vial label.
9. If several vials exist for one sample, keep the vials together with a rubber band.
10. Record the total number of eggs and larvae collected in the sample on the data summary sheet.
11. If no ichthyoplankters are encountered in the sample, write "No Specimens Collected" on the laboratory work sheet.
12. If yearling or older fish are collected, record the species and number caught on the data sheet and the laboratory work sheet and place the specimens into a separate jar.

2.2.4 Quality Assurance Checking Procedures

Certain individuals have been assigned as laboratory control personnel. Quality assurance of the laboratory effort will be conducted in the following manner:

1. Sort through the sample tray to ensure that all eggs and larvae have been removed.
2. If any one fish egg or larva is found to remain in the tray, show the specimen to the original sorter. If two specimens are found to remain in the tray, have the original sorter resort the entire tray.
3. Repeat Step 2 until all eggs and larvae are removed.
4. If an original sorter does not remove all the specimens within three attempts, continue with Steps 2 and 3 until all eggs and larvae are removed, and notify the supervisor of the problem.
5. If no eggs or larvae are encountered, preserve the contents of the tray and begin sorting another 10 ml aliquot.
6. Continue to check each tray, as per Steps 1 through 5, until the entire sample is sorted.
7. Once the entire sample has been sorted, check the vial labeling and data summary recording for accuracy and legibility.
8. Once the sorting and documentation review is complete, have the original sorter initial the collection jar (white label) and the laboratory work sheet in the proper spaces.
9. The assigned laboratory control personnel will also initial the collection jar (blue label) and laboratory work sheet in the appropriate spaces.

2.2.5 Postsorting Procedures

The following procedures will be followed to assure that all sorted samples are retained for the quality control effort and documentation.

1. Decant the contents of the Pyrex sorting tray through a 375 μ m sieve.
2. Rinse the contents of the sieve into the sample jar.
3. Check the sieve to ensure that all residual material has been rinsed into the sample jar.
4. Return the field label to the sample jar.
5. Fill the sample jar with the original 10 percent formalin.
6. Place the sample jar in the appropriate designated location.

2.3 ICHTHYOPLANKTON IDENTIFICATION PROCEDURES

2.3.1 Introduction

The combination of physical and temporal data at the collection sites must first be considered in all fish egg and larval identifications. This allows

certain species to be dismissed based on the time of year, water temperature, salinity, location in the estuary, location in the water column, and habitat sampled. This precursory investigation of data also allows certain families, and occasionally species, to be singled out for identification based on temporal or physical peculiarities particular to that group.

Additional data from concurrent studies should also be incorporated to find which species are spawning in the same area and at the same time as the ichthyoplankton collections. This gives insight towards which family, or which species within a family, certain individual specimens may belong and conversely, allow for certain species to be dismissed based on collections at other times, locations, etc.

Upon comprehensive assimilation of these data, all positive identification is completed by visual observations and measurements of characteristics. This has historically been the means of species separation and a variety of literature exist to describe individual species identification and development. This technique does leave potential for human error, and could prevent various species from being separated due to their similarities in early life stages.

2.3.2 Identification

Positive identification begins at the family level. Each individual of a particular collection is separated into a representative group based upon the characteristics. A general description of the various characteristics which can be used to separate and identify fish eggs and larvae are listed in Tables 2-2 and 2-3.

After individual specimens are categorized into groups and into respective families, identification procedures are initiated to segregate the individuals into species. Two criteria exist for completion of species identification. These are meristics (i.e., counts) and morphometrics (i.e., form and structure). Although pigmentation characteristics are a morphometric comparison, the use of pigment (or lack thereof) is generally deemphasized due to variability within species and changes occurring after preservation.

By definition, meristics and morphometrics in egg and larval identification are separate entities. In actual practice, the meristics of a particular larva cannot be separated from the morphology of the structure in question. It is necessary, however, to recognize that the literature available for identification of species is generally from a variety of geographic regions and relatively little published information exists for the egg and larval development of Hudson River stocks. Development rates also change from region to region, and, therefore, these characteristics are frequently variable. However, the combination of both are used to complete the identification process.

At this point, two factors must be assessed to determine how extensive an identification process will continue. The first is determined by any specific requirement listed in Consolidated Edison's scope of work which states that all fish will be identified to the lowest taxonomic level practicable. The second factor is the relative merit of identifying unusual specimens for inclusion in the data base being developed. In general, the specific requirements of the

TABLE 2-2 GENERAL CHARACTERISTICS USEFUL FOR EGG IDENTIFICATION

<u>Meristic</u>	<u>Morphometric</u>
1. Oil globule(s) - number	1. Embryonic cap
2. Longitudinal and lateral measurements of - chorion - yolk - oil globule	2. Germinal disc
3. Proportionalities with egg capsule of - perivitelline space - yolk diameter	3. Embryo shape, either attenuated or truncate
4. Proportionalities with yolk of - oil globule(s)	4. Position of oil globule in yolk to embryonic cap
5. Some have distinct coloration; however, not very useful on preserved samples.	5. Position of oil globule to embryonic cap
	6. Single or clustered egg
	7. Chorion - granular or smooth - attachment disc - filaments attached - turgid or pliable - adhesive or nonadhesive
	8. Yolk - granular or smooth - type of granulation
	9. Fertilized or unfertilized
	10. Live or dead

TABLE 2-3 GENERAL CHARACTERISTICS USEFUL FOR LARVAL IDENTIFICATION

Meristic

1. Myomere numbers
 - total
 - preanal
 - postanal
 - cleithrum to vent
 - posterior yolk to vent
 - dorsal anlage to vent
2. Vertebrae number
3. Teeth
4. Fin rays
5. Brachioistegal rays
6. Gill rakers
 - including serrations on gill rakers
7. Dorsal anlage (n)
8. Proportionalities with total length of
 - yolk
 - eye
 - caudal peduncle
 - head
 - snout to vent
 - depth
9. Proportionalities with depth of
 - caudal peduncle
10. Proportionalities with head of
 - eye
 - opercle
 - mouth
11. Proportionalities with fins of
 - dorsal fin to vent
 - between fin
12. Proportionalities with eye of
 - mouth extension
 - auditory vessicle
13. Proportionalities with yolk of
 - oil globule(s)
14. Heavy or light
15. Chomotophore size and shape

16. Location
 - dorsally
 - ventrally
 - laterally
 - in eye
 - in viscera
 - in myocepta
 - in yolk
 - in vent
17. Pattern
 - clustered
 - linear
 - distinct

Morphometric

1. Size at hatch
2. Yolk
 - presence or absence
 - position or oil globule(s)
 - type of oil globule(s)
3. Eye
 - presence or absence of pigment
4. Fins
 - multiple or single dorsal
 - spinous or soft
5. Teeth
 - presence or absence
6. Intestine
 - convoluted or linear
 - striated
7. General shapes
 - head
 - fin
 - mouth
 - body
8. Finfold junction
9. Attached or separate multiple dorsal fins

scope of work require species identification in families exhibiting a high economic and social importance. Compliance with the scope of work must be met, since this is a technical document upon which the contract is based.

Scope of work requirements for entrainment abundance experiments demand separation of species within common genera. The requirements come from differing economic, social, or political importance of species in the same genus (e.g., Morone americana vs. M. saxatilis; Alosa sapidissima vs. A. aestivalis and/or A. pseudoharengus). This frequently necessitates species identification for specimens difficult to separate because of overlapping of morphological characteristics. Because the scope of work requires this degree of critical separation, a systematic analysis must be employed to aid the identification process. A combination of progressive and regressive development is used to identify those larvae in the critical areas of overlapping morphological characteristics. Known larvae which are of an earlier and later stage of development than those in the critical stages will be examined for distinct characteristics. These distinct characteristics are then developmentally traced both progressively and regressively through the critical stages to separate the species.

Whenever such difficult species separation is required by a scope of work, the following will be made available to all laboratory personnel:

1. Pertinent literature and selected references
2. Specimen review and consultation with EA employees experienced in the species identification.
3. Initiate contact with known experts in the scientific community and follow-up viable contacts, as available.
4. Attempt rearing known stock as scope requirements, manpower, budgeting, and equipment are available.

2.3.3 Equipment

The following equipment must be available for every individual involved in the ichthyoplankton identification process. Ensure that all equipment is available before and during the identification process.

One dissecting microscope with 0.75X-30X magnification including a 2X lens and 10X oculars
One illuminator adaptable to the appropriate microscope
One ocular micrometer with divisions of 0.1 mm at 10X magnification
One four-quadrant Pyrex culture dish or glass petri dish
One pair fine-point microdissection forceps
One pair blunt-point microdissection forceps
Three dropping pipettes
Three microdissection teasing needles
Ample supply of 4-dram vials with polyvinyl snap-top lids
Waterproof paper for inside labels
Extra No. 2 grade pencils
One waterproof marking pen

- One squeeze bottle filled with 5 percent buffered formalin
- One squeeze bottle filled with water
- Lens paper
- One ruler with divisions in 1.0 mm increments
- 3/4-in. vial labels.
- White labels are used for all abundance vials.

2.3.3.1 Procedures for Measurement of Ichthyoplankton

All the Consolidated Edison entrainment abundance samples analyzed in the Central Lab require larval length measurements for striped bass, white perch, Alosa sp., Atlantic tomcod and bay anchovy. These measurements will be conducted in the following manner:

1. All specimens will be measured in total length (TL) to the nearest 0.1 millimeter (i.e., from the tip of the snout to the end of the caudal fin or caudal finfold). NOTE: if the specimen is damaged and the caudal finfold is destroyed or frayed, consult your supervisor before continuing. If more than 50 percent of the specimen is present, it is not measured, but is counted as an organism.
2. For specimens under 10.0 mm TL, measure with an ocular micrometer.
3. For specimens over 10.0 mm TL, measure with a ruler.
4. Do not attempt to match equal halves of larvae and measure the result.
5. Randomly select up to 30 individuals of each species and life stage for measurement.
6. If particular species and life stages occur at a rate of less than 30 individuals per sample, measure all of them.

2.3.3.2 Guidelines for Life Stage Separation

Actual separation of life stages will be conducted on developmental characteristics of the individual specimens. The following descriptions will be used to separate ichthyoplankton life stages and are adapted and modified from Balon (1975).

1. Egg: Described as the interval between fertilization and hatching.
2. Yolk-sac: Described as the interval between hatching and the transition of the embryonic median finfold.
3. Post-larvae: Described as the interval from the end of the yolk-sac stage until juvenile transformation is completed.

2.3.3.3 Vial Labeling

Vial labeling, in an accurate manner, is necessary to ensure that the various specimens are available for reference and QC. The following information should be recorded on every vial label:

1. Collection information from abundance collections (Figure 1-4).
2. The identifier will record the species, life stage, and number of organisms.

2.3.4 Data Recording--Entrainment Abundance Samples

This section describes how to complete the back of the Entrainment Abundance Data Sheet (Figure 2-1). This data sheet will be used for samples collected with the Automated Abundance Sampler.

The identifier will:

1. Ensure that the sample vial(s) has a serial number identical to that appearing on the front of the data sheet.
2. Record the serial number, his/her initials or (printed) last name and date that the sample was identified in the appropriate locations on the data sheet.
3. As each new species and life stage is identified, record the species name, life stage, and species code in the appropriate column.
4. Record the total numbers of specimens (species and life stage) identified in the space provided for plus (+) count.
5. Record measurements (to nearest 0.1 mm) in the space provided for each species and life stage, up to 30 measurements.

2.4 QUALITY CONTROL

This section describes the error analysis tests and procedures used by Central Lab for abundance samples in the Central Lab.

2.4.1 Continuous Sampling Plan (CSP)

Continuous sampling plans (CSP) are an extremely effective quality control system when applied to a situation of a common laboratory and analysis group. CSP will be used for sorting on a sample-by-sample basis. CSP-1 as described by Duncan (1974) is very effective for ichthyoplankton sample processing.

This system involves reprocessing a continuous number of samples and an associated percentage of the next whole group. Duncan (1974) provides a chart for developing any particular grouping for a specific average outgoing quality limit (AOQL). EA uses a 10 and 10 method to provide an AOQL of not to exceed 10 percent. In this format, ten consecutive samples are reprocessed; if all are acceptable, then one of the next 10 samples are selected at random and reprocessed. This is the selection of the 10 and 10 method (i.e., 10 in a row and 10 percent from the next same group [10]). If a sample is encountered that is unacceptable, the process repeats itself by processing samples until 10 consecutive samples are acceptable. It must be noted that all the samples in a row must be acceptable, or repeated reprocessing occurs until the consecutive target number of all samples are acceptable.

The formula used to determine the percent error for a sample is as follows:

$$\text{Percent error} = \frac{QCe + 1}{(Oe + 1) + (QCe + 1)} \times 100$$

where

$QCe + 1$ = the number of eggs and larvae from the sample in the quality control analysis,

and

$(Oe + 1) + (QCe + 1)$ = the sum of the original eggs and larvae from the sample, and the number of eggs and larvae from the sample in the quality control analysis.

2.4.2 Proportion Defective Analysis (PDA)

1. Introduction

PDA has been selected as the QC system to be used for abundance sample identification. It is an extremely strict plan, since the system is designed to allow no bad samples (exceeding the average outgoing quality limit, AOQL, of 10 percent) to pass. This design causes the probability of rejecting acceptable lots, ≤ 10 percent AOQL to be high. An advantage to this system is that any lot that passes is guaranteed to be good, ≤ 10 percent AOQL.

2. Selection Process

Samples will be selected for rework based upon completion of a lot (i.e., a given number of consecutive samples completed). A lot size of 50 will usually be QC'd and seven samples will be reworked. If smaller lot sizes are chosen less samples will be reworked (See below). Samples within each lot size will be selected at random, using a random number table.

The number of samples to be reworked for a particular lot size are as follows:

<u>Lot Size</u>	<u>Number to be Reworked</u>
5	4
6 - 10	5
11 - 20	6
21 - 50	7

If any lot consists of less than 5 samples, all must be reworked.

3. Determination of Defective Analysis:

- a) If a reworked sample contains 30 or less organisms, apply the following criteria: if any one (1) of the reworked samples exceeds 3 incorrectly identified organisms, the entire lot size in question will be reprocessed.

b) If a reworked sample contains more than 30 organisms, apply the following criteria: if any one (1) of the reworked samples exceeds 10 percent error, the entire lot size in question will be reprocessed.

c) The formula used to determine the percent error is as follows:

$$\text{Percent error} = \frac{QCE + 1}{Oe + 1} \times 100$$

where: QCE + 1 = the number of eggs and larvae misidentified from the sample in the quality control analysis

and: Oe + 1 = the total number of eggs and larvae identified from the sample in the quality control analysis.

d) Errors below the AQL will not be added to the original data sheet.

b) If a reworked sample contains more than 30 organisms, apply the following criteria: if any one (1) of the reworked samples exceeds 10 percent error, the entire lot size in question will be reprocessed.

c) The formula used to determine the percent error is as follows:

$$\text{Percent error} = \frac{QCe + 1}{(Oe+1) + (QCe+1)} \times 100$$

where: $QCe + 1$ = the number of eggs and larvae sorted from the sample in the quality control analysis,

and: $(Oe+1) + (QCe+1)$ = the sum of the original number of eggs and larvae sorted from the sample, and the number of eggs and larvae sorted from the sample in the quality control analysis.

d) Errors below the AOQL will not be added to the original data sheet.

REFERENCES

- Balon, E.K. 1975. Terminology of intervals in fish development. J. Fish Res. Board Can. 39(9): 1663-1670.
- Duncan, A.J. 1974. Quality Control and Industrial Statistics, 4th Edition. Richard D. Irwin, Inc. Homewood, Illinois.