Appendix 4A

Intake Effects Assessment Report Technical Appendices

ESA

West Basin Municipal Water District
Demonstration Desalination Facility

Appendix A

Impact Assessment Models

A1. Calculating Total Entrainment

A2. Estimating Proportional Entrainment and the Empirical Transport Model Calculations

A1. Calculating Total Entrainment

The following section describes calculations used for estimating entrainment for the proposed scwd[2] pilot desalination plant offshore intake. The entrainment estimate for a selected taxon is built on estimates of larval concentration for each survey period, which was determined by sampling approximately monthly at a location neared the proposed intake. Two plankton samples were collected at the station during two sampling cycles within a day. The within-day sampling was based on a stratified random design with two temporal cycles (night and day) and two replicates per cycle. The associated statistics based on the stratified sampling were based on Gilbert (1987). For the calculations let

i = survey period $(i = 1, \ldots, N)$;

j = day within survey period $(j = 1, \ldots, N_i)$;

k = cycle within day $(k = 1, \ldots, N_{ij})$; and

l = number of replicate tows $(l = 1, \ldots, N_{ijk})$.

The average concentration $\bar{\rho}_{ijk}$ for cycle k within day j within survey period i was calculated as follows:

$$\bar{\rho}_{ijk} = \frac{1}{n_l} \sum_{l=1}^{N_{ijk}} \rho_{ijkl} \text{ , where } n_l = 2, \text{ the number of replicate tows,} \qquad \text{(A1-1)}$$

with associated variance of

$$Var(\bar{\rho}_{ijk}) = \frac{1}{n_l (n_l - 1)} \sum_{l=1}^{N_{ijk}} (\rho_{ijkl} - \bar{\rho}_{ijk})^2 . \qquad \text{(A1-2)}$$

The average concentration $\bar{\rho}_{ij}$ for day j within survey period i was calculated as follows:

$$\bar{\rho}_{ij} = \sum_{k=1}^{N_{ij}} \frac{n_l}{n_{kl}} \bar{\rho}_{ijk} \text{ , where } n_{kl} = 4, \text{ the total samples over 2 cycles,} \qquad \text{(A1-3)}$$

with associated variance of

$$Var(\rho_{ij}) = \sum_{l=1}^{N_{ij}} \left(\frac{n_l}{n_{kl}} \right)^2 Var\left(\bar{\rho}_{ijk} \right). \qquad \text{(A1-4)}$$

where

$\bar{\rho}_{ij}$ = the average concentration of larvae on the jth day in the ith survey period.

The estimates of average daily concentration and their associated variances were used in many of the data presentations in the report, as well as in calculating estimates of daily entrainment that were used in proportional entrainment (*PE*) calculations for the empirical transport model (*ETM*). The estimate of daily entrainment, E_{ij}, was calculated as:

$$E_{ij} = \overline{\rho}_{is} V_{ij}, \tag{A1-5}$$

where

V_{ij} is the volume of water entrained by the plant on day *j* of survey period *i*.

In addition, for any period *i*, daily entrainment is estimated by using the average survey density from the one sampling day times the daily entrainment volume. Similarly, the survey period's variance of density is estimated using the variance of the survey day. The variance of daily entrainment was calculated as:

$$Var(E_{ij}) = Var(\overline{\rho}_{ij}) V_{ij}^{\ 2}. \tag{A1-6}$$

Estimates of entrainment for a survey period were calculated by using the estimate of the daily average concentration, $\overline{\rho}_{ij}$, and calculating an estimate of daily entrainment and the associated variance using Equations A1-5 and A1-6, respectively, after substituting the appropriate volume for the days in each period, V_{ij}, into the equations. The start and end dates of a survey period were defined as the midpoint between successive sampling dates. Data collected on the sampling date between the midpoints was assumed to be representative of the entire survey period. Total larval entrainment was calculated as follows:

$$E_T = \sum_{i=1}^{N} \sum_{j=1}^{N_i} E_{ij}, \tag{A1-7}$$

The associated variance was calculated as:

$$Var(E_T) = \sum_{i=1}^{N} \sum_{j=1}^{N_i} Var(E_{ij}).. \tag{A1-8}$$

Estimates of total entrainment for the yearlong study period were calculated by summing the estimates from the individual survey periods.

A2. Estimating Proportional Entrainment and the Empirical Transport Model Calculations

The empirical transport model (*ETM*) is used to estimate the probability of mortality due to power plant entrainment. The estimate is based on periodic estimates of the probability of entrainment based on daily sampling. Generally, sampling takes place over the course of a year so that larval mortality of various species is estimated, and the estimate is for the period of one year.

The daily probability of entrainment during survey period *i* can be defined as

$$PE_i = \frac{\text{abundance of entrained larvae}_i}{\text{abundance of larvae in source population}_i}$$
$$= \text{probability of entrainment in } i\text{th survey period } (i = 1, \ldots, N).$$

where the daily probability can be estimated and expressed as

$$PE_i = \frac{E_i}{R_i} \qquad \text{(A2-1)}$$

where

E_i = estimated abundance of larvae entrained in the i^{th} survey period $(i = 1, \ldots, N)$ calculated per Equation A1-5; and

R_i = estimated abundance of larvae at risk of entrainment from the source population in the i^{th} survey period $(i = 1, \ldots, N)$.

Estimating Numbers of Larvae at Risk

The daily abundance of larvae in the sampled source water (*S*) stations at risk can be estimated by

$$R_i = \sum_{l=1}^{N_l} V_{S_l} \cdot \bar{\rho}_{S_{il}}, \qquad \text{(A2-2)}$$

where V_{S_l} denotes the static volume for each of the *l* sampled source water stations , and $\bar{\rho}_{S_{il}}$ denotes the average concentration in each of the *l* sampled source water stations during survey period *i*. The variance of Expression A2-2 can be written as

$$Var\left(R_i \mid R_i\right) = \sum_{i=1}^{N_l} V_{S_l}^2 \cdot Var\left(\bar{\rho}_{S_{il}} \mid \bar{\rho}_{S_{il}}\right) \qquad \text{(A2-3)}$$

Formula A2-3 describes the temporal-spatial variance in the sampled source water population during the day of sampling. Three source water locations were sampled offshore from DCPP, in addition to samples taken at the intake location. Ideally, tow samples would be collected randomly through time and space during a sampling day over a potential source population. However, practical limitations due to sampling a large area required a directed and fixed time and location sampling scheme. Source water estimates of population and variance were made for each period using only one day, i.e. $R_i = R_{ij}$ and $Var\left(R_i\right) = Var\left(R_{ij} \mid R_{ij}\right)$.

A total sum over all survey periods used in calculating the fraction (f_i) of the source water population present during survey *i* was calculated as follows:

$$R_T = \sum_{i=1}^{N} \sum_{j=1}^{N_i} R_i, \tag{A2-4}$$

The associated variance was calculated as:

$$Var(R_T) = \sum_{i=1}^{N} \sum_{j=1}^{N_i} Var(R_i).. \tag{A2-5}$$

Period Entrainment and *ETM* Calculations

The sampled source water represents some proportion of the total source water population which was estimated in this study using current data collected during each i^{th} survey period. The ratio C_{S_i} described the fraction sampled to extrapolated source water for each survey period. C_{S_i} was used to adjust the estimate of the source water population, R_i, to allow the direct calculation of PE_i by dividing estimated period entrainment (A1-5) by the corresponding source population (A2-2) as

$$PE_i = \frac{E_i}{R_{i-Adj.}} = \frac{E_i}{R_i \bullet C_{S_i}} \tag{A2-6}$$

Variance for the Estimate of PE_i

The variance for the period estimate of PE_i can be expressed as

$$Var\left(PE_i \middle| PE_i\right) = Var\left(\frac{E_i}{R_{i-Adj}} \middle| E_i, R_{i-Adj}\right).$$

Assuming zero covariance between the entrainment and source and using the delta method (Seber 1982), the variance of an estimator formed from a quotient (like PE_i) can be effectively approximated by

$$Var\left(\frac{A}{B}\right) \approx Var(A)\left(\frac{\partial\left[\frac{A}{B}\right]}{\partial A}\right)^2 + Var(B)\left(\frac{\partial\left[\frac{A}{B}\right]}{\partial B}\right)^2 .$$

The delta method approximation of $Var(PE_i)$ is shown as

$$Var\left(PE_i\right) = Var\left(\frac{E_i}{C_{S_i} \cdot V_S \cdot \bar{\rho}_{S_i}}\right)$$

which by the Delta method can be approximated by

$$Var\left(PE_i\right) \approx Var\left(E_i\right)\left(\frac{1}{C_{S_i} \cdot V_S \cdot \bar{\rho}_{S_i}}\right)^2 + Var\left(C_{S_i} \cdot V_S \cdot \bar{\rho}_{S_i}\right)\left(\frac{-E_i}{C_{S_i} \cdot V_S \cdot \left(\bar{\rho}_{S_i}\right)^2}\right)^2 \qquad \text{(A2-7)}$$

and is equivalent to

$$= PE_i^2\left[CV\left(E_i\right)^2 + CV\left(C_{S_i} \cdot V_S \cdot \bar{\rho}_{S_i}\right)^2\right]$$

where

$$R_{i-Adj} = C_{S_i} \cdot V_S \cdot \bar{\rho}_{S_{ij}} \text{ and}$$

$$CV\left(\theta \middle| \theta\right) = \frac{Var\left(\theta \middle| \theta\right)}{\theta^2} .$$

Regardless of whether a species has a single spawning period per year or multiple overlapping spawnings the estimate of total larval entrainment mortality can be expressed by

$$P_M = 1 - \sum_{i=1}^{N} f_i\left(1 - PE_i\right)^q \qquad \text{(A2-8)}$$

where

q = number of days of larval life, and

$\hat{f}_i$ = estimated annual fraction of total larvae hatched during the *i*th survey period.

Formula A2-8 is based on the total probability law where

$$P(A) = \sum_{i=1}^{N} P(A|B_i) \cdot P(B_i).$$

In the above example, the event A is larval survival and event B is hatching with $P(B)$ estimated by f_i where

$$f_i = \frac{R_i}{R_T},$$

where R_i = static source population at risk in the *i*th survey period. Then based on the Delta method

$$\begin{aligned} Var(f_i) &= Var\left[\frac{R_i}{R_T}\right] \\ &= Var\left[\frac{R_i}{R_i + \sum_{j \neq i}^{N} R_j}\right] \\ &= f_i^{\,2}(1-f_i)^2\left[\frac{Var(R_i)}{R_i^{\,2}} + \frac{Var(R_T)}{R_T^{\,2}}\right]. \end{aligned}$$

The estimates of PE_i and f_i and their respective variance estimates can be combined in an estimate of the variance for P_M following the Delta method (Seber 1982) for variance and covariance as follows:

$$\begin{aligned} Var(P_M) &= Var\left(1 - \sum_{i=1}^{N} f_i(1-PE_i)^q\right) \\ &= Var\left(\sum_{i=1}^{N} f_i(1-PE_i)^q\right) \\ &= \sum_{i=1}^{N}\left[Var(f_i)(1-PE_i)^{2q}\right] \\ &\quad + \sum_{i=1}^{N}\left[Var(PE_i)(f_i q(1-PE_i)^{q-1})^2\right]. \end{aligned}$$

Literature Cited

Gilbert, R. O. 1987. Statistical Methods for Environmental Pollution Monitoring. Van Nostrand Reinhold Company. New York. 320 pp.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. McMillan, London. 654 p.

Tenera Environmental, Inc. 2000. Diablo Canyon Power Plant 316(b) Demonstration Report. Report No. E9-055.0. Prepared for Pacific Gas and Electric Company. March 2000. 596 p.

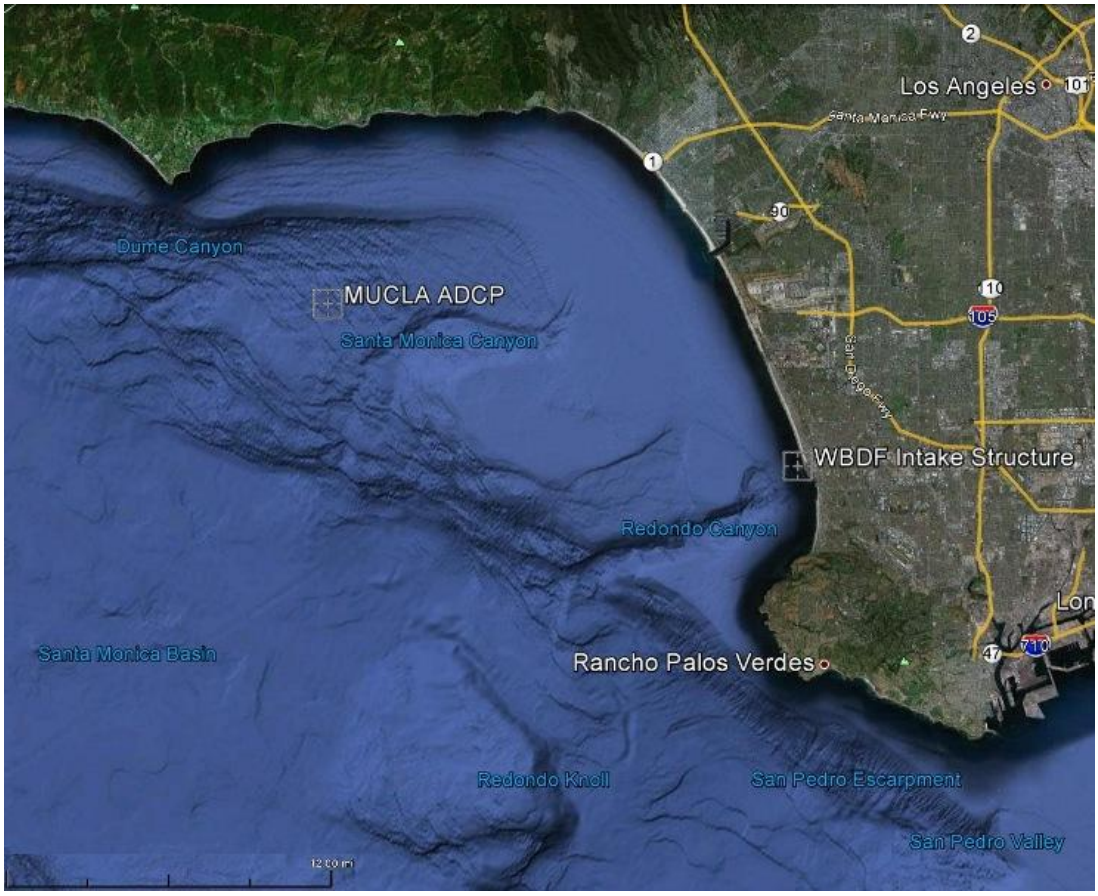
West Basin Municipal Water District
Demonstration Desalination Facility

Appendix B

Ocean Surface Currents from March 2011 Through March 2012

West Basin Desalination Facility

Back-projection of Trajectories from the Intake Structure off Redondo Beach for March 2011 Through March 2012 Source Water Samples



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Approach

Measurements of the speed and direction of the ocean waters throughout Santa Monica Bay, California were used to provide data for modeling the extent of the source water potentially entrained through the West Basin Desalination Facility (WBDF) seawater intake structure at Redondo Beach. Surface currents were measured hourly off the entirety of the coastline of southern California by a network of CODAR Ocean Sensors, Ltd. SeaSonde® high-frequency (HF) radars available through the Southern California Coastal Ocean Observing System (SCCOOS) using the network of instruments deployed by the State of California's Coastal Ocean Currents Monitoring Program (COCMP). Nearshore sub-surface currents were measured by the acoustic Doppler current profiler (ADCP) deployed on the Santa Monica Bay Observatory oceanographic mooring (MUCLA), managed by University of California, Los Angeles' Institute of the Environment & Sustainability (**Figure 1**).

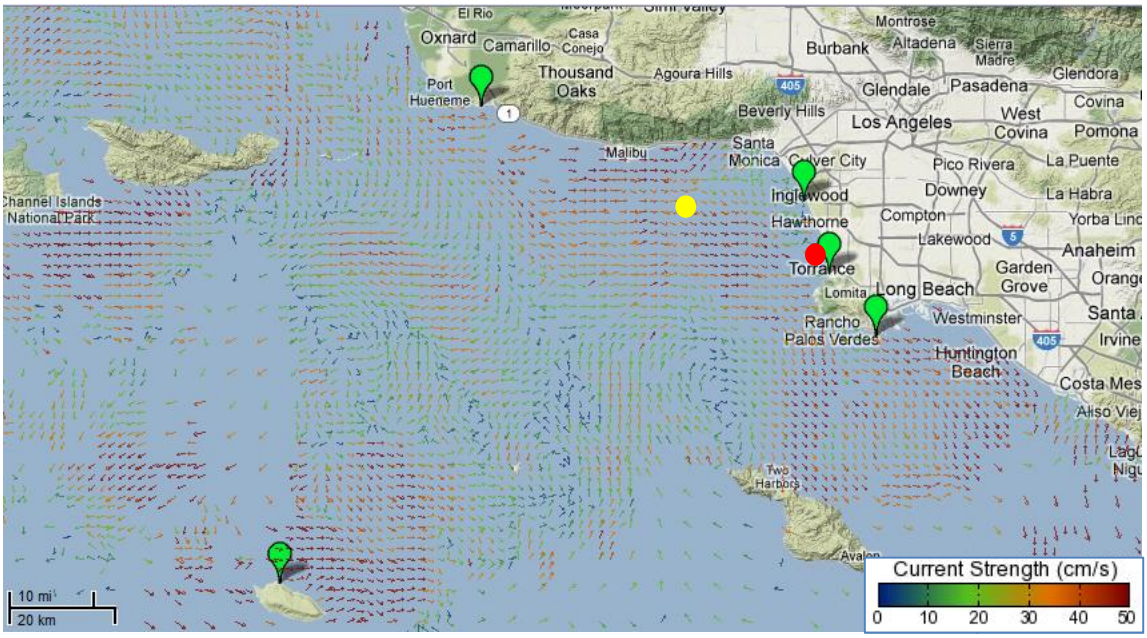

Figure 1. Ocean surface current vectors representative of data coverage throughout the study period; measured on October 1, 2011 at 0000 UTC in the Santa Monica Bay, California region by SCCOOS' CODAR SeaSonde stations (green markers). Shown are vectors of both the 6 km (3.7 mile) resolution coverage offshore and the higher 2 km (1.2 mile) resolution coverage closer to the coast, shaded according to their speed per the color-bar. The MUCLA ADCP is located at the yellow marker and the WBDF intake structure is at the red marker.

A combination of these velocity measurements was used to project the extent of water that could be transported to the WBDF intake over selected planktonic durations.

Larvae within the Water Column – Scaling Currents to these Habitats

In order to better model the extent of waters entrained by the WBDF intake, respective of larvae living sub-surface, the CODAR-derived surface currents were scaled to approximate sub-surface magnitudes. The velocity of ocean currents measured at the water's surface typically decays

with increasing depth and this relationship was seen in the velocities measured. Due to their offshore and surface origin, CODAR speeds interpolated to the location of the MUCLA mooring were about twice the magnitude of the averages measured there at mid-depths by the ADCP.

The MUCLA ADCP data were collected hourly, at the same frequency as the CODAR measurements, from May 18, 2007 to April 15, 2009 in 4 m (13 ft.) bins, with the center of the first bin located at 8 m (26 ft.) depth. Means were then calculated from the 8-20 m (26-66 ft.) ADCP bins to derive averages centered at 14 m (46 ft.); in approximation to the mid-depth of the waters at the WBDF intake location.

The proximity of the MUCLA mooring to the CODAR measurement field allowed the surface current values measured over the aforementioned time period to be linearly interpolated to the MUCLA location. The U (east-west) and V (north-south) components of the CODAR and ADCP velocities were considered separately in their relationship with depth. Further, as there are seasonal variations in the currents, each calendar month was assessed independently.

The difference between the CODAR and ADCP, as a percentage of the CODAR magnitude, was calculated. Absolute values of each component measured hourly in the same calendar month by CODAR were subtracted from the absolute values of the like 14 m (46 ft.) averaged ADCP component. The mean of these differences for the month was then divided by the mean of the absolute value of the CODAR component measured that month. This produced a percentage by which to scale-down CODAR data from the month to sub-surface magnitudes.

Application of these scaling factors to the CODAR data produced significantly tighter agreement with the mid-water-column speeds measured by ADCP, while still preserving the similarity in the directional component of velocity (**Table 1, Figure 2**). These ratios were applied to the hourly surface currents measured by CODAR during the source water sampling to produce a second data-set applicable to the sub-surface waters at the intake.

Table 1. Ratio by which components of ocean surface current velocity (CODAR) measured each calendar month were multiplied to approximate mid-water-column values at the WBDF intake. U = East-west velocity component, V= North-south velocity component.

January		**February**		**March**		**April**		**May**		**June**	
U	***V***	***U***	***V***	***U***	***V***	***U***	***V***	***U***	***V***	***U***	***V***
.843	.824	.652	.771	.729	.684	.745	.775	.807	.726	.725	.723
July		**August**		**September**		**October**		**November**		**December**	
U	***V***	***U***	***V***	***U***	***V***	***U***	***V***	***U***	***V***	***U***	***V***
.703	.723	.878	.724	.750	.755	.702	.736	.671	.737	.746	.754

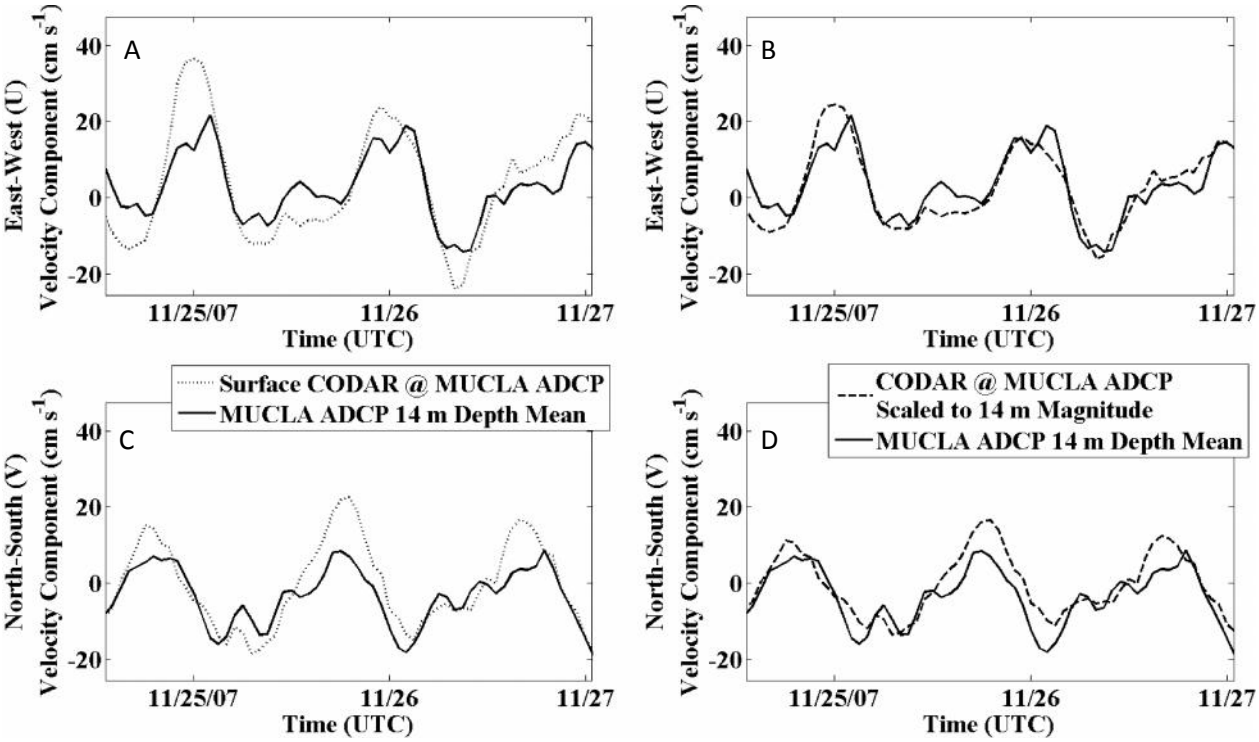

Figure 2. Shown in panels *A* and *C* on the left are the U and V components of velocity, respectively, measured over a representative period at the surface by CODAR and at depth by the MUCLA ADCP, as per the left legend. Panels *B* and *D* to the right again show the ADCP velocity components, but now with the surface CODAR scaled to 14 m (46 ft.) depth per **Table 1** as noted in the right legend.

Back-projection

A computer model was developed in MATLAB® with forcing from the combined CODAR and MUCLA ADCP measurements to reverse-track source-water flowing to the position of the WBDF intake (33.8498167° N, 118.40365° W for durations of up through 50 days. This time period allowed assessment for a range of larval or planktonic durations exhibited by the most prevalent species from larval surveys conducted in 2011 and 2012 near the intake.

The ocean current data set analyzed incorporated both the 6 km (3.7 mi) and 2 km (1.2 mi) resolution surface currents from CODAR, scaled to 14 m (46 ft.) depth. These data provided time-series from the WBDF intake site, extending from the region nearshore of Redondo Beach out through 150+ km (93+ miles) offshore and alongshore.

Trajectories were back-projected from the intake location using these hourly data sets for the 50 days leading up to 30 unique random hours within ± 2 days of each of the March 31, 2011 (WBN001), May 5, 2011 (WBN003), June 9, 2011 (WBN005), July 19, 2011 (WBN007), August 9, 2011 (WBN009), September 6, 2011 (WBN011), October 12, 2011 (WBN013), November 1, 2011 (WBN015), December 8, 2011 (WBN017), January 12, 2012 (WBN019), February 6, 2012 (WBN021), and March 5, 2012 (WBN023) Tenera survey dates.

The velocity components of the currents (U, positive to east and V, positive to north) were calculated for each of the 9,481 hours from February 7, 2011 [(March 31, 2011 -2 days) - 50 days] through March 7, 2012 [(March 5, 2011) + 2 days] and collated into files each representing an hour of measurements. For each survey date, the scaled U and V components of the ocean current velocity measured that hour were first linearly interpolated to the location of the intake. The sign of the U and V components were then reversed to calculate the location a particle (or presumably planktonic species) would have originated from the hour before and been carried by the ocean currents toward the intake. This process was repeated for each prior hour from the survey date, through 50 previous days, interpolating the U and V components of velocity at each hour to the location calculated in the prior time-step and reversing sign to back-project the location the particle would have been the hour before.

If the back-projection of a particle caused its track to cross a land boundary, the distance the particle was projected to travel was applied first to the direction in U. If land was still encountered the distance was then applied to the V direction. If both attempts to move the particle alongshore failed, it was held in position for that time-step and the process repeated the next hour, until the current moved the particle past or away from the land mass. The result at the WBDF intake of the source water analysis, for 10, 20, 30, 40, and 50 days' duration back-projections, is shown in **Figures 3 to 14** that correspond to the twelve aforementioned surveys.

Uncertainty

Error estimation for the trajectory products based on CODAR observations is an ongoing research area[1]. Given measurement error, which is on the order of 8 cm s^{-1} (0.16 kts.)[2], error in the Lagrangian path estimates will grow with time and will be larger for conditions with larger horizontal current variations. Fortunately for the present applications, species with the longest larval durations also tend to have the largest source water body volumes. Since the intake volume is fixed, the percent error caused by uncertainty in the source water body volume is relatively low.

Distance from Land

For further analyses by Tenera Environmental of source water extent alongshore, the distance of each back-projected point from the nearest point of land was calculated. Land points were defined from World Vector Shoreline data collected by the National Geospatial-Intelligence Agency and obtained from the National Geophysical Data Center at http://www.ngdc.noaa.gov/mgg/coast/.

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[1] Frolov, S., J.D. Paduan, M.S. Cook, and J. Bellingham, 2012: Improved statistical prediction of surface currents based on historic HF-radar observations. *Ocean Dynamics*, In Press.

[2] Paduan, J.D., K.C. Kim, M.S. Cook, and F.P. Chavez, 2006: Calibration and validation of direction-finding high frequency radar ocean surface current observations. *IEEE J. Oceanic Engin.*, 10.1109/JOE.2006.886195, 862-875.

Results

The calculation of source water trajectories from the ADCP-scaled CODAR ocean current velocities allowed for a more realistic estimation of source water extents of both nearshore and offshore species than would be possible using measurements from local current meters alone. Shown in **Figures 3** to **14** are plots of the 30 trajectories back-projected from within +/- 1 day of each of the 12 survey dates from the WBDF intake.

A database of these results was provided to Tenera Environmental that, for each hour, included the features given in **Table 2**.

Table 2. Values provided for each back-projected track-point.

Survey Date	Back-projection Latitude (°)
Survey Date Index (1-12)	Back-projection Longitude (°)
Number of Hours Back from Projection Start-time (1-1200; 50 days=1200 hours)	Nearest Coastline Longitude (°)
Back-projection Time (UTC)	
Back-projection Track Index (1-30)	Distance to Nearest Coastline Point (m)

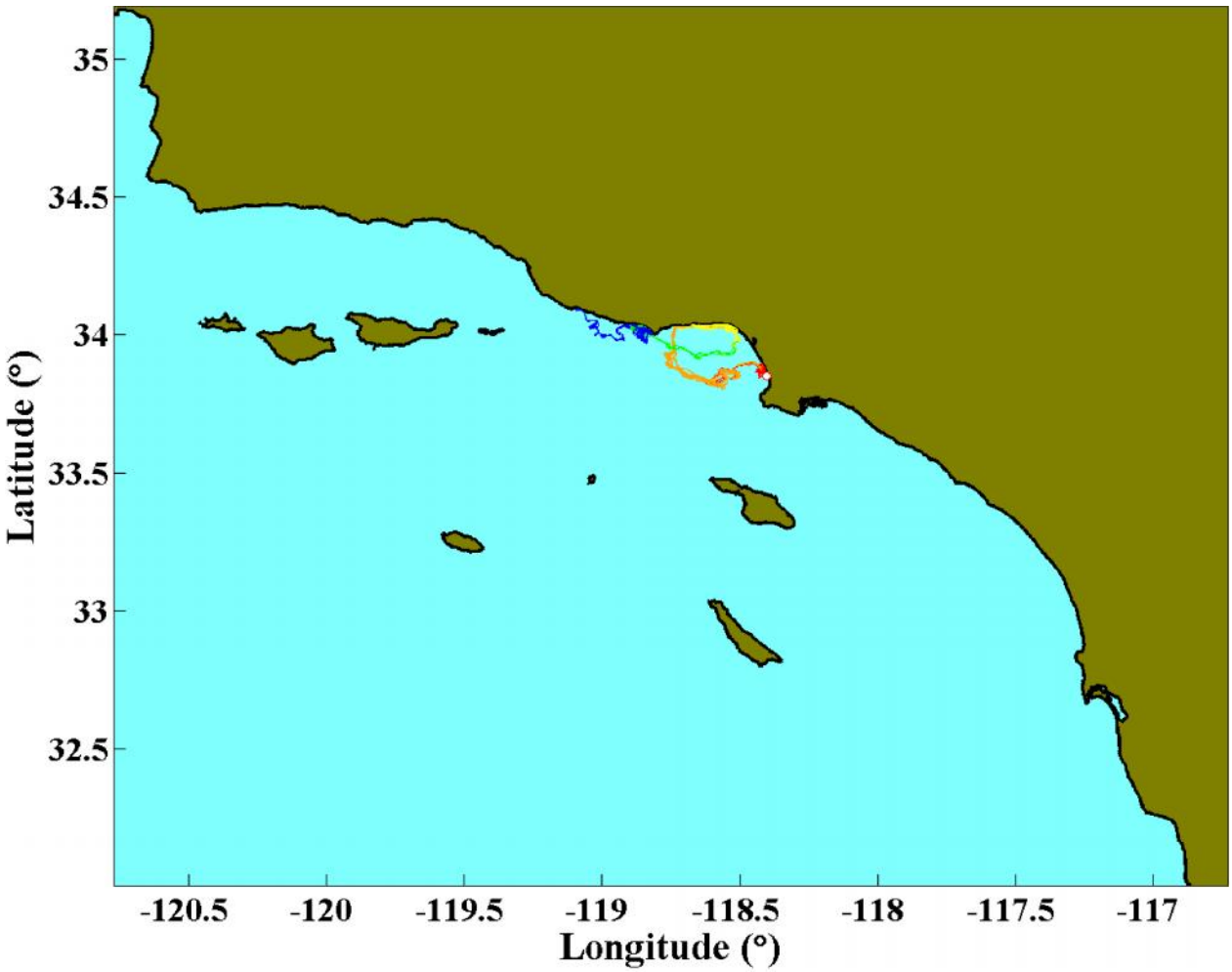


Figure 3. Thirty 50-day source water back-projections from WBDF intake with end dates centered on March 31, 2011 (WBN001). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

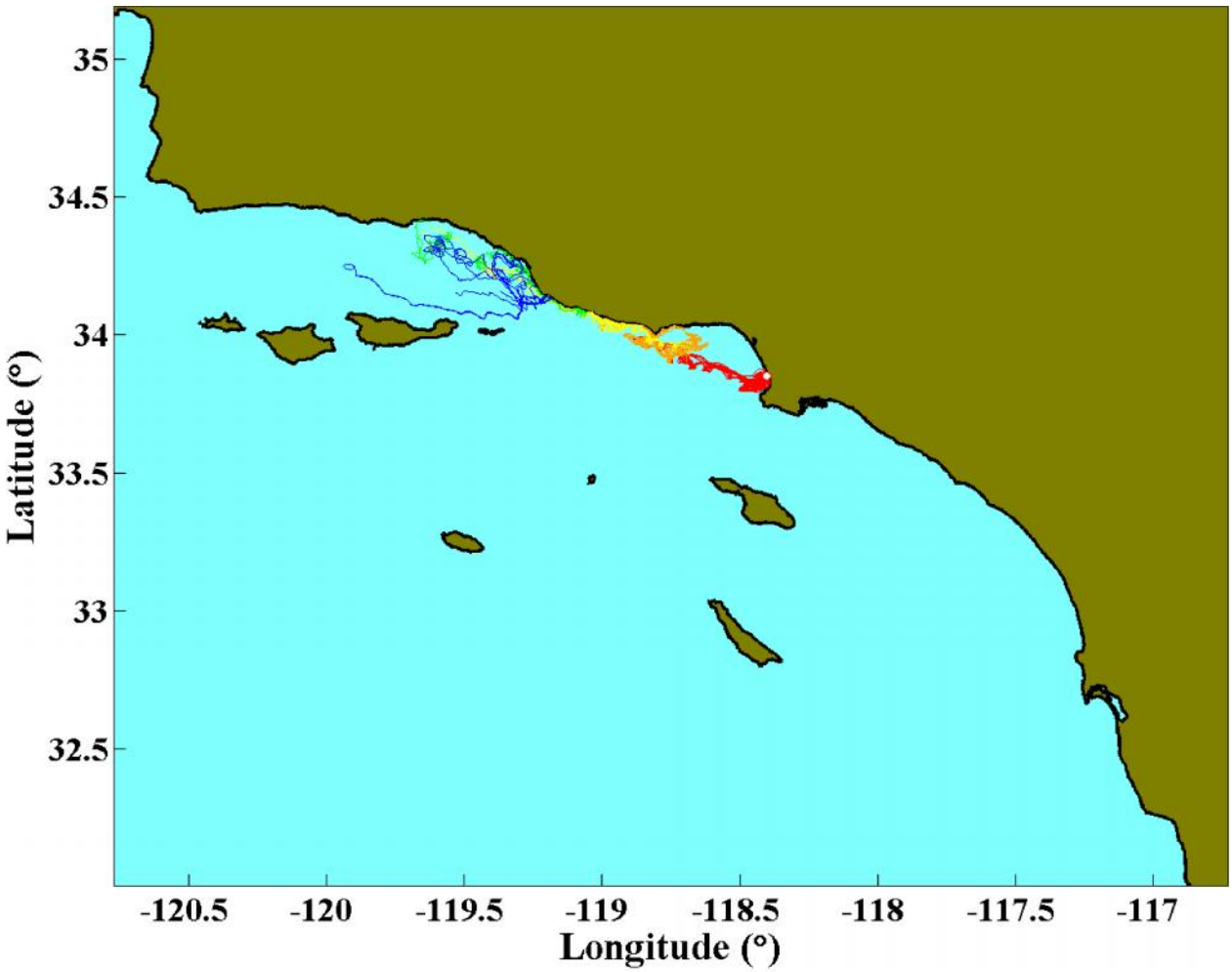


Figure 4. Thirty 50-day source water back-projections from WBDF intake with end dates centered on May 5, 2011 (WBN003). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

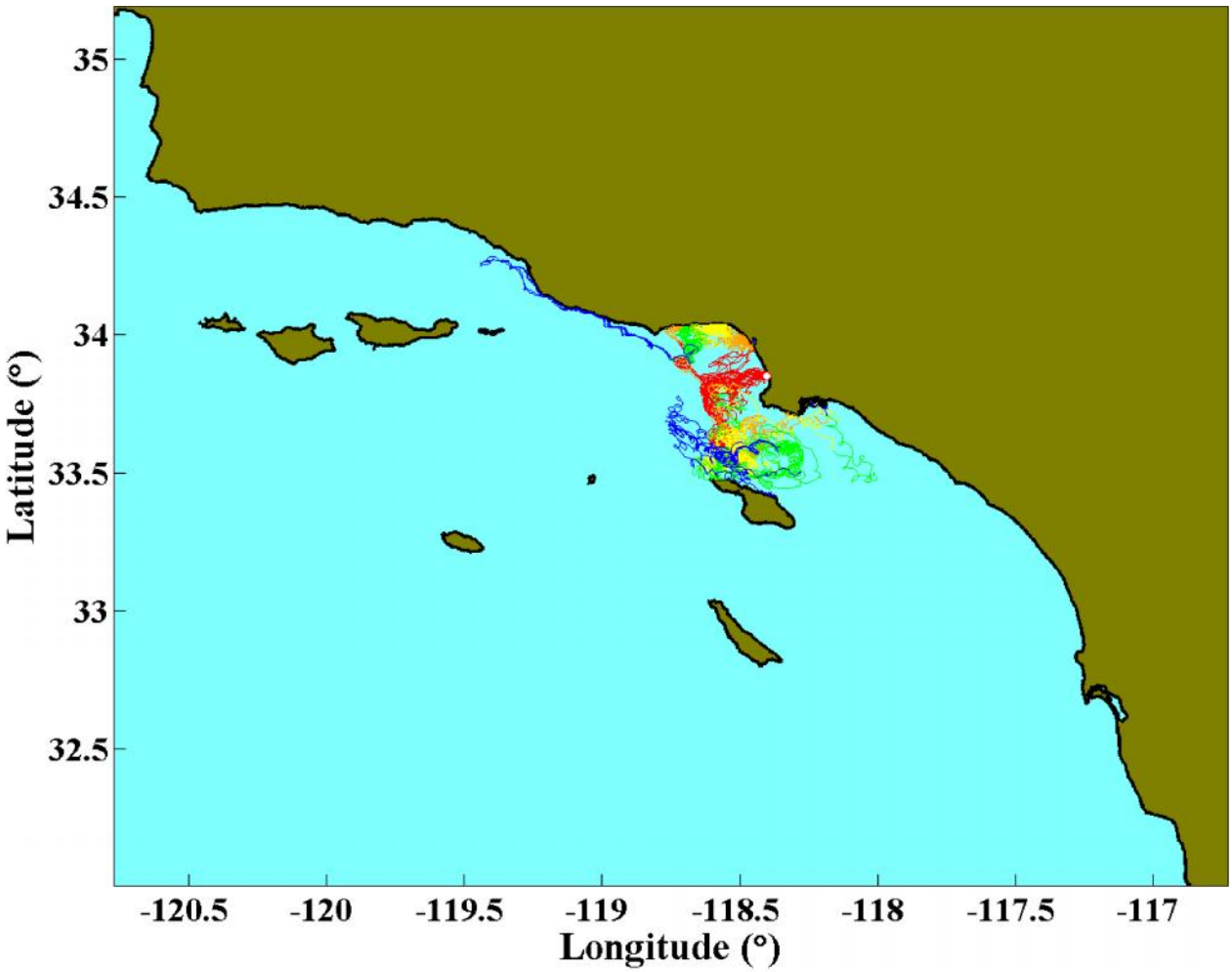


Figure 5. Thirty 50-day source water back-projections from WBDF intake with end dates centered on June 9, 2011 (WBN005). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

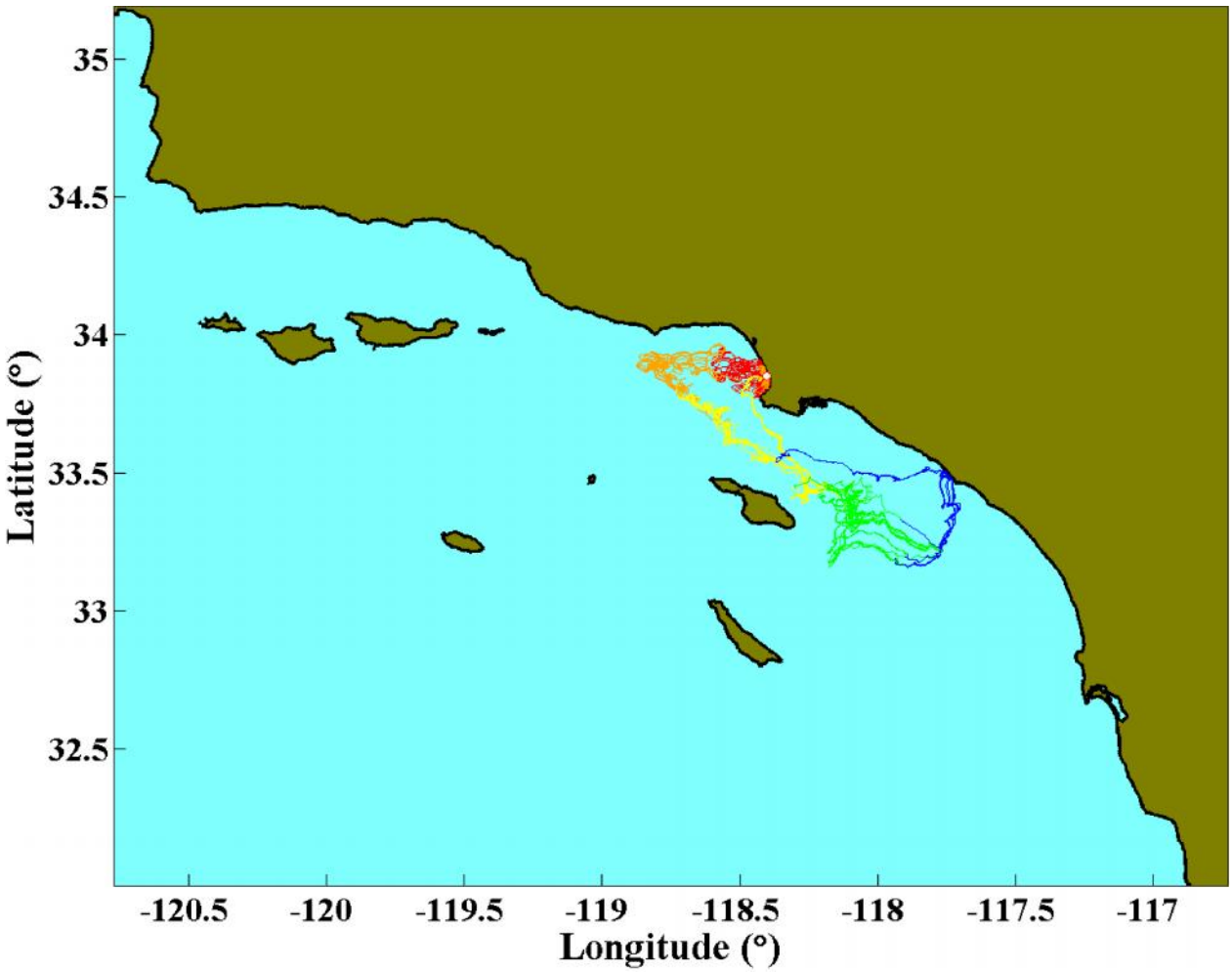


Figure 6. Thirty 50-day source water back-projections from WBDF intake with end dates centered on July 19, 2011 (WBN007). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

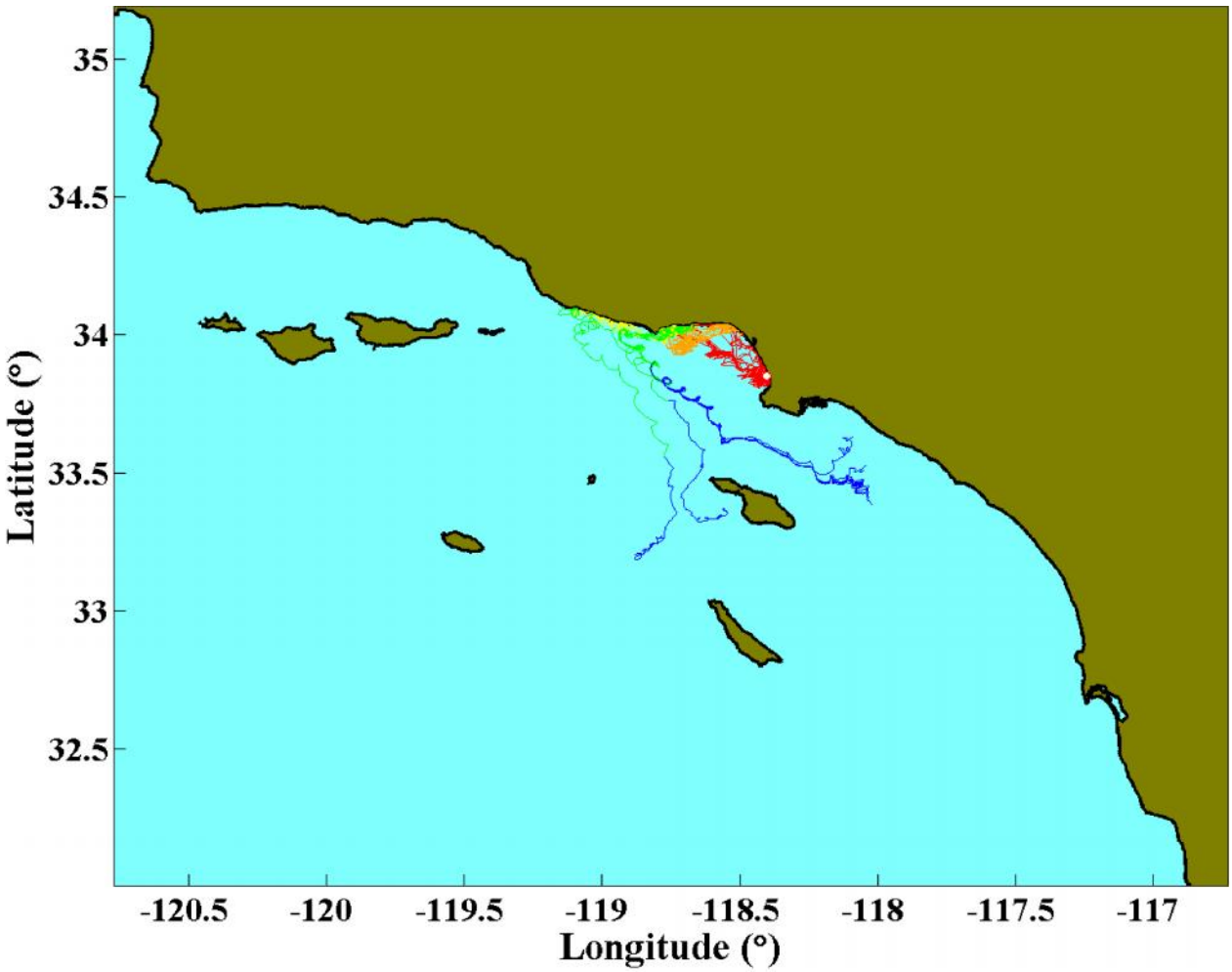


Figure 7. Thirty 50-day source water back-projections from WBDF intake with end dates centered on August 9, 2011 (WBN009). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

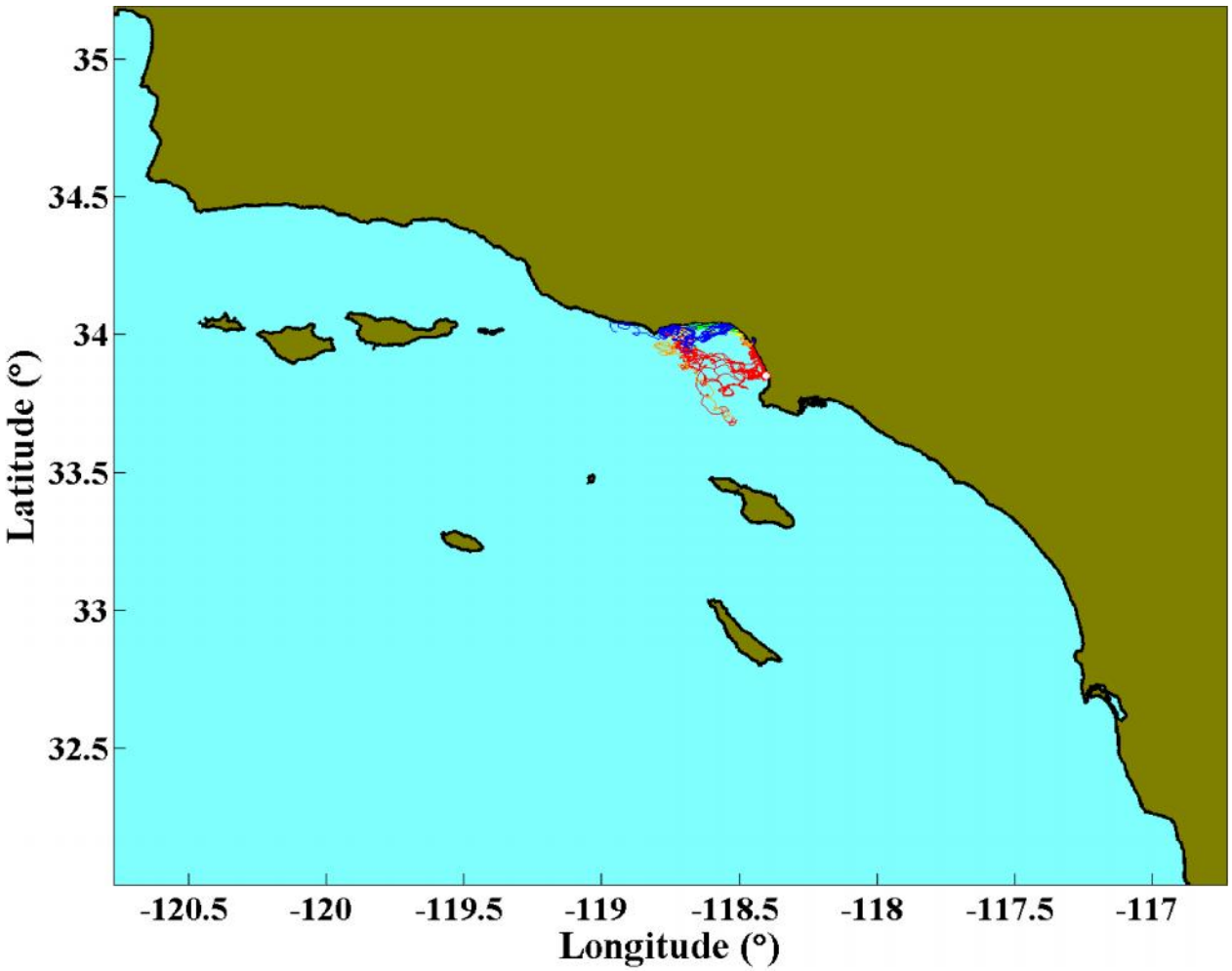


Figure 8. Thirty 50-day source water back-projections from WBDF intake with end dates centered on September 6, 2011 (WBN011). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

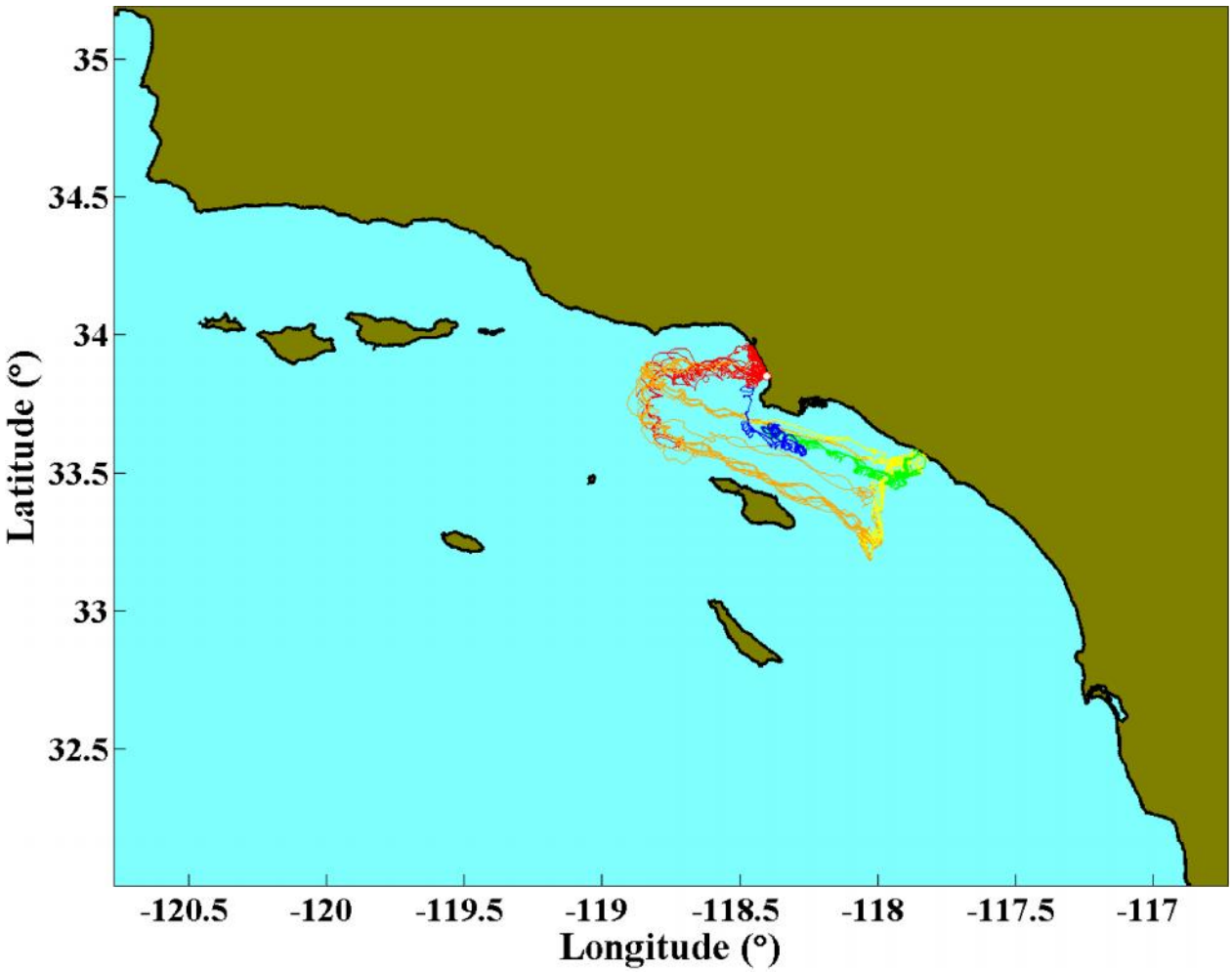


Figure 9. Thirty 50-day source water back-projections from WBDF intake with end dates centered on October 12, 2011 (WBN013). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

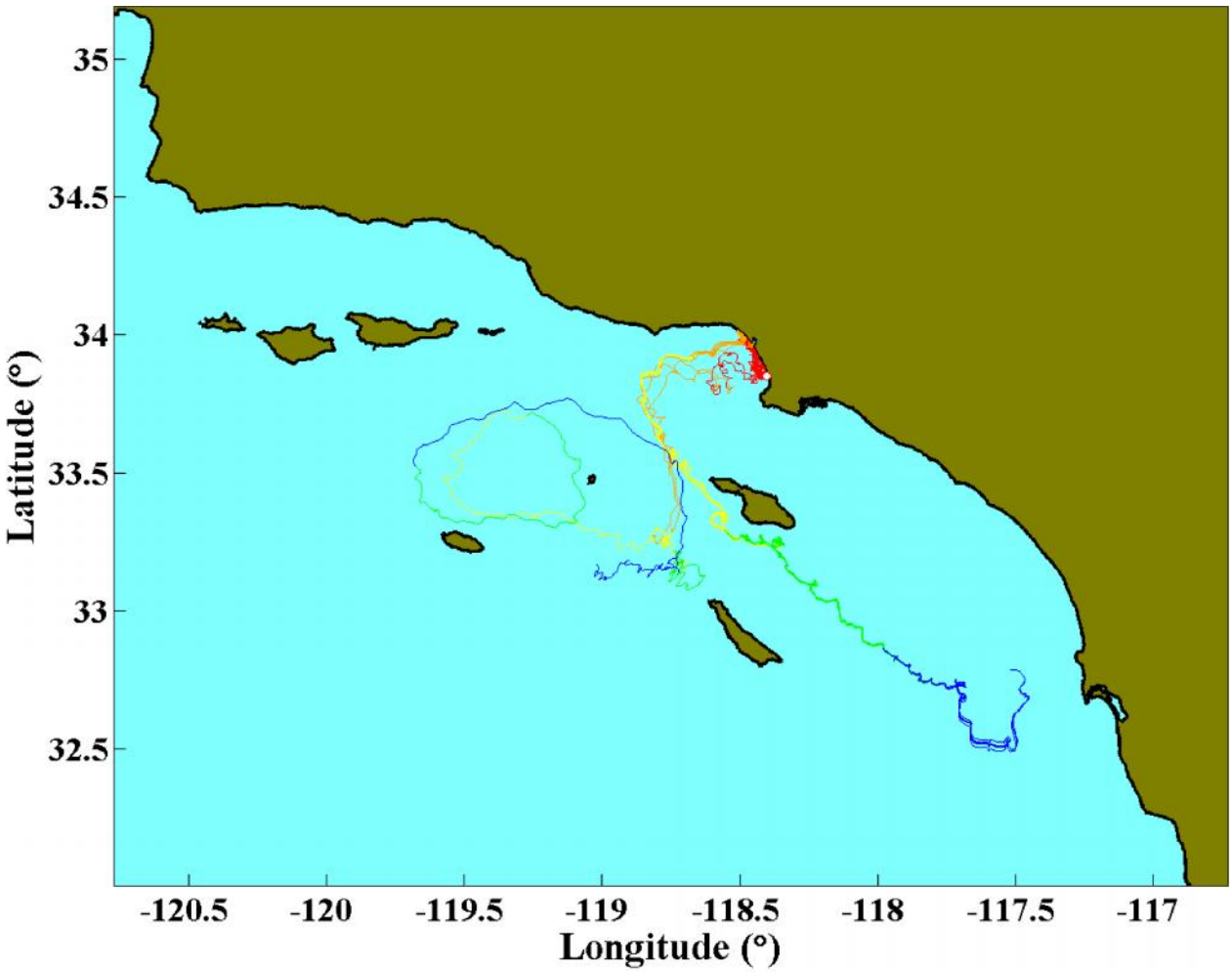


Figure 10. Thirty 50-day source water back-projections from WBDF intake with end dates centered on November 1, 2011 (WBN015). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

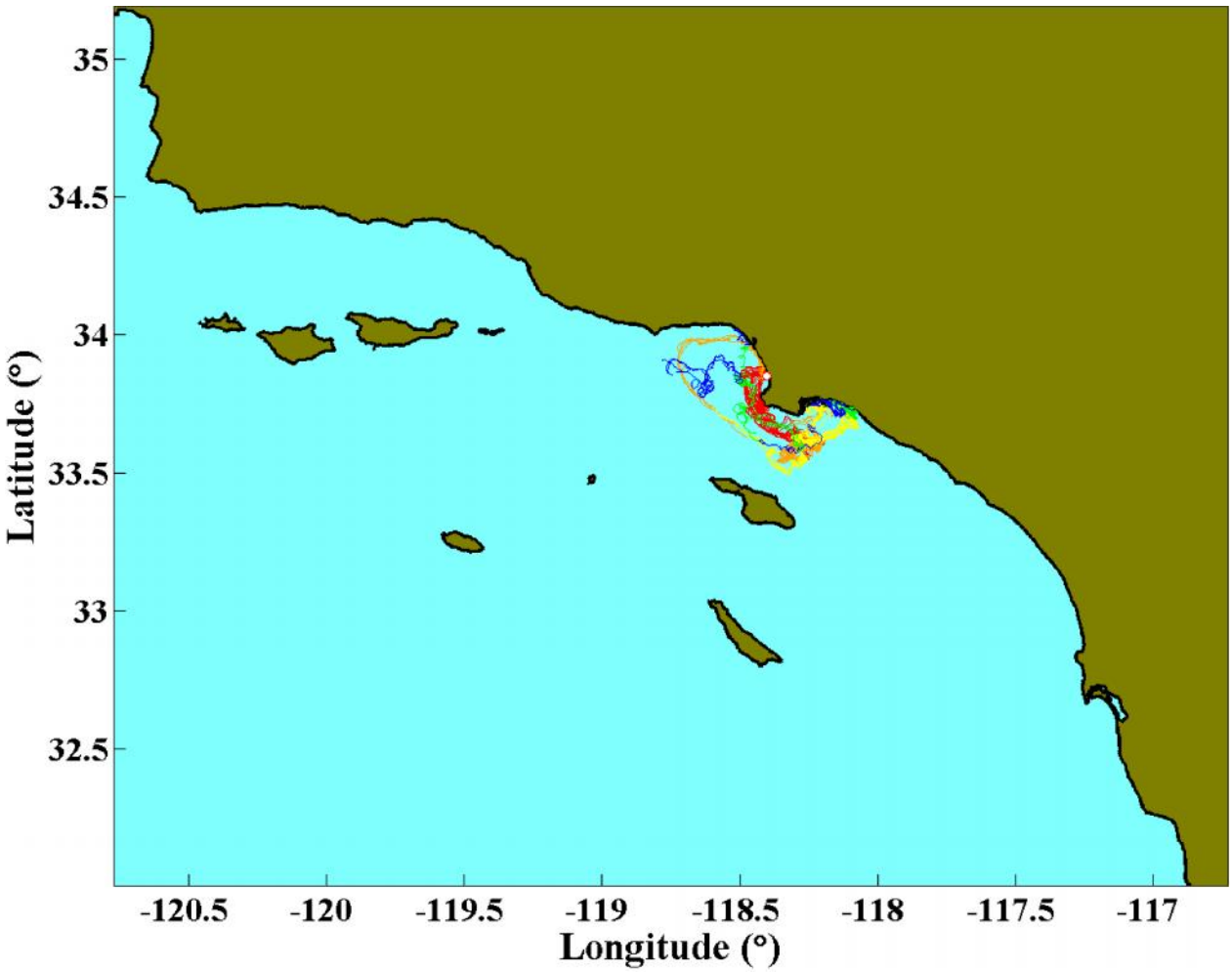


Figure 11. Thirty 50-day source water back-projections from WBDF intake with end dates centered on December 8, 2011 (WBN017). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

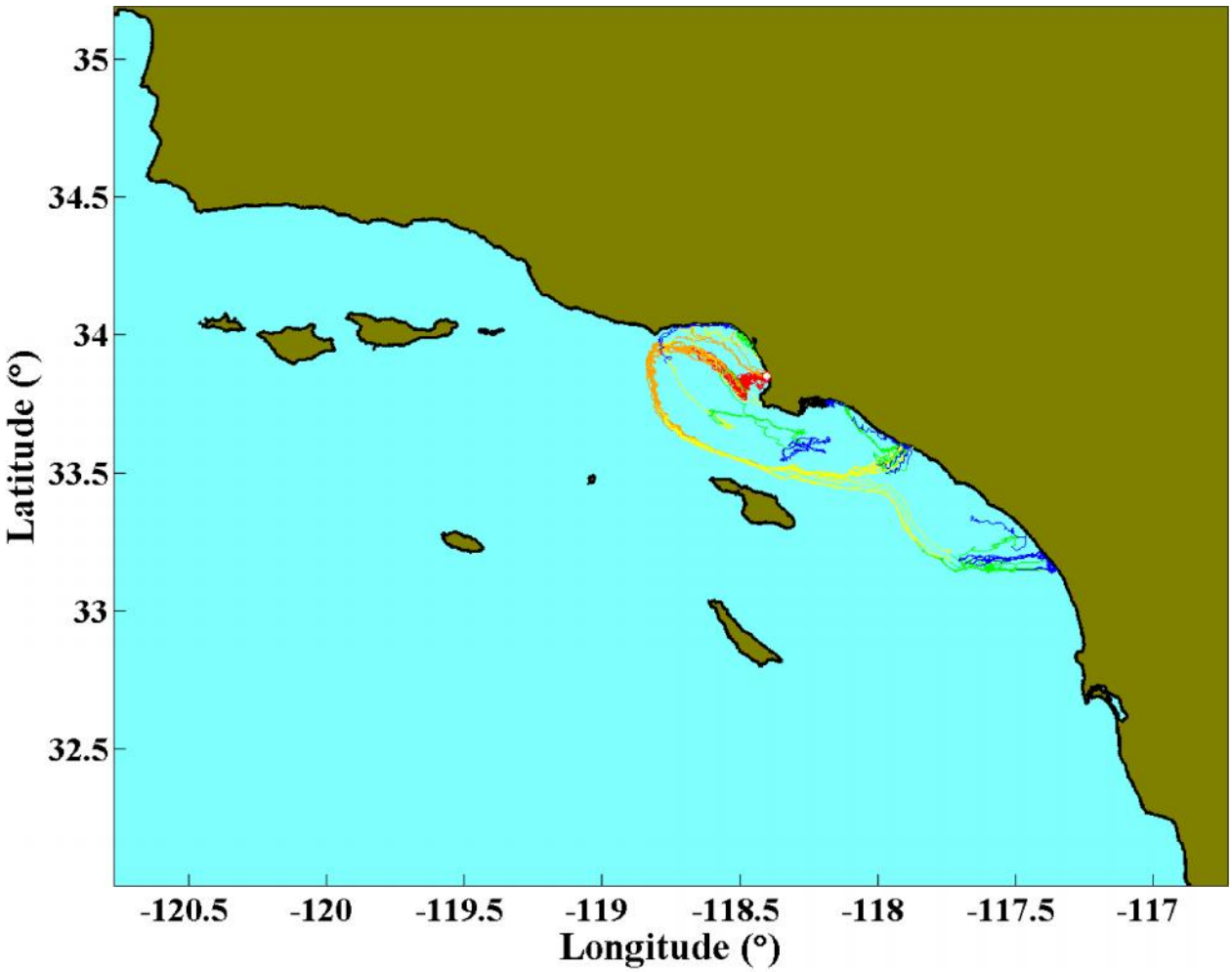


Figure 12. Thirty 50-day source water back-projections from WBDF intake with end dates centered on January 12, 2012 (WBN019). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

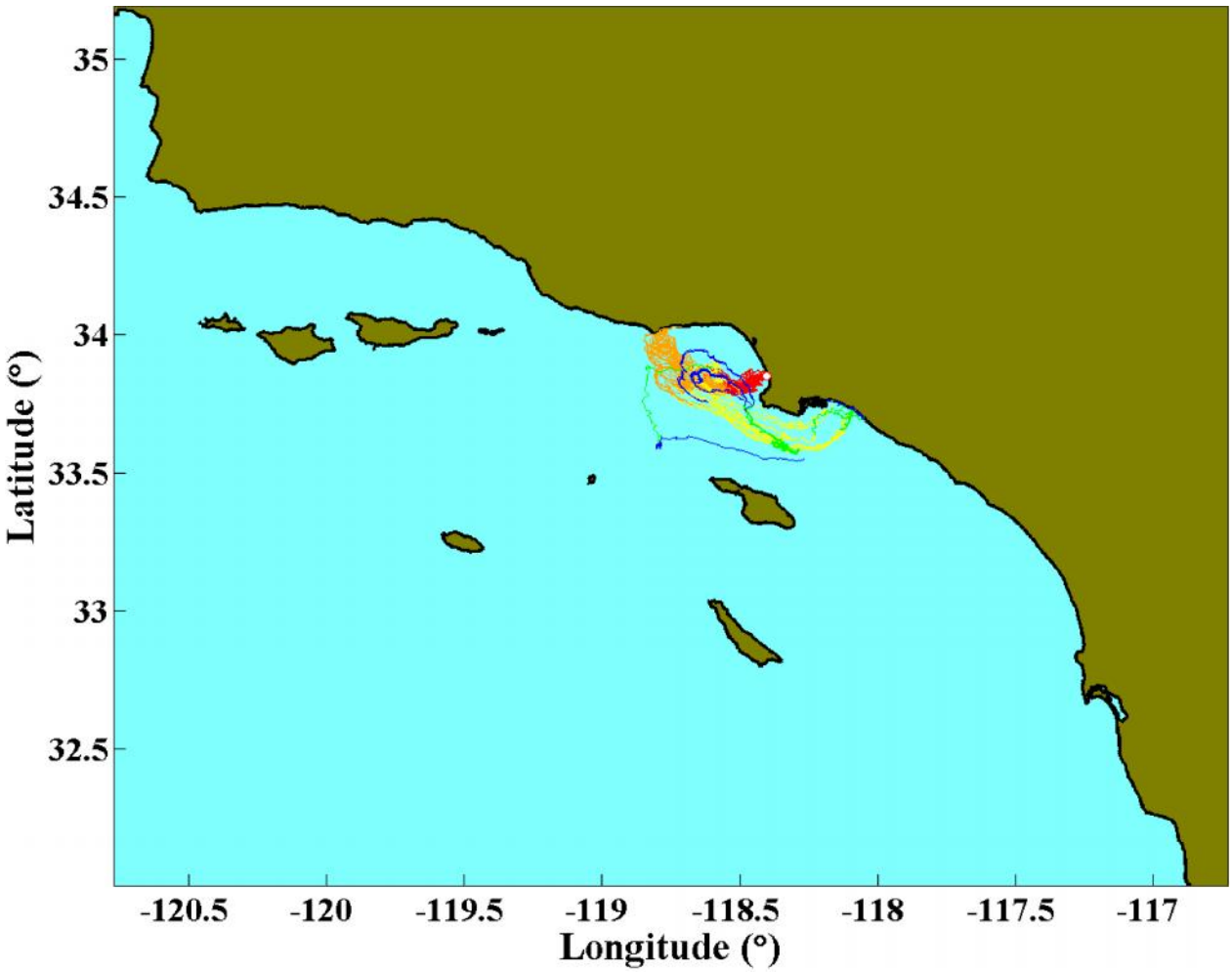


Figure 13. Thirty 50-day source water back-projections from WBDF intake with end dates centered on February 6, 2012 (WBN021). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

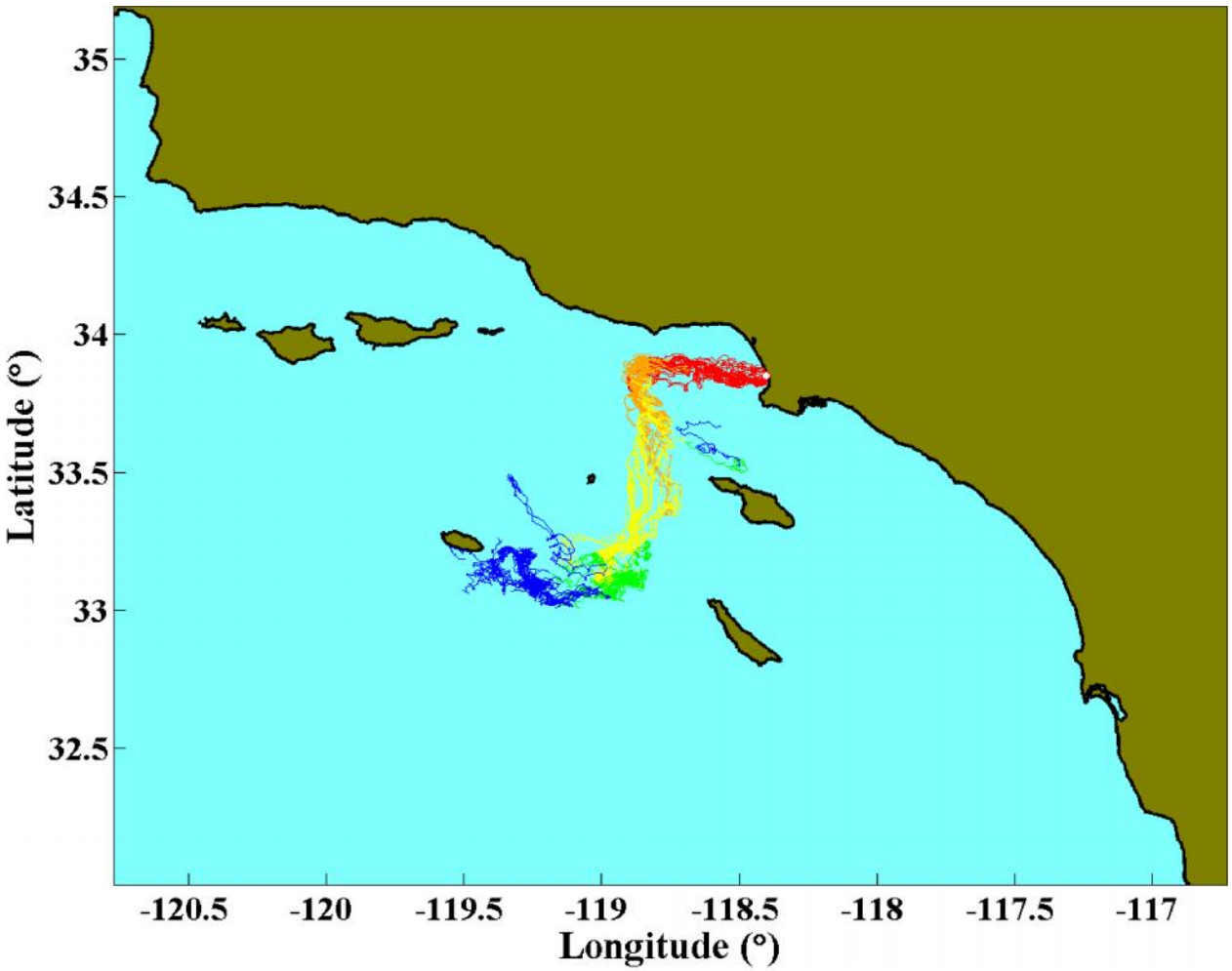


Figure 14. Thirty 50-day source water back-projections from WBDF intake with end dates centered on March 5, 2012 (WBN023). Extents of the trajectories back through 1-10 days are red, through 11-20 days are orange, through 21-30 days are yellow, through 31-40 days are green, and through 41-50 days back are blue. The site of the intake is marked by a white dot.

West Basin Municipal Water District
Demonstration Desalination Facility

Appendix C

Larval Fish Sampling Results: Net Sampling by Survey

C1. Entrainment Station Larval Concentrations

Table C1-1. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN001
Date: March 31, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Gibbonsia spp.	kelpfishes	12	15.50
Atherinopsis californiensis	silversides	4	5.17
Citharichthys stigmaeus	sanddabs	4	5.03
Genyonemus lineatus	white croaker	3	3.84
Engraulis mordax	anchovies	2	2.58
Pleuronectoidei	flatfishes	1	1.14
	Total Fish Larvae:	**26**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	8,878	10,775.08
Paralichthyidae (eggs)	sand flounder eggs	2,475	3,067.22
Pleuronichthys spp. (eggs)	turbot eggs	1,776	2,299.12
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	980	1,235.98
fish eggs (damaged)	damaged fish eggs unid.	40	45.73
Sciaenidae (eggs)	croaker eggs	20	22.99
	Total Fish Eggs:	**14,170**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	261	347.22
Metacarcinus anthonyi (megalops)	yellow crab megalops	14	18.79
Cancridae (megalops)	cancer crabs megalops	8	10.80
Cancer productus/Romaleon spp. (megalops)	rock crab megalops	2	2.70
	Total Invertebrate Larvae:	**285**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-2. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN003
Date: May 6, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Parophrys vetulus	English sole	16	19.49
larval fish - damaged	damaged larval fishes	12	14.14
Zaniolepis frenata	shortspine combfish	12	14.62
Chitonotus/Icelinus spp.	sculpins	4	4.71
Cottidae	sculpins	4	4.14
Pleuronectidae	righteye flounders	4	4.87
Sebastes spp. V	rockfishes	4	4.87
Stenobrachius leucopsarus	northern lampfish	4	4.87
	Total Fish Larvae:	**60**	
Non-Entrainable Fishes			
Sebastes miniatus	vermilion rockfish	4	4.71
	Total Non-Entrainable Fishes:	**4**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	7,526	8,497.65
Pleuronichthys spp. (eggs)	turbot eggs	398	453.90
fish eggs (damaged)	damaged fish eggs unid.	316	337.75
Pleuronectidae (eggs)	righteye flounder eggs	80	97.47
Citharichthys spp. (eggs)	sanddab eggs	40	48.73
Engraulis mordax (eggs)	northern anchovy eggs	40	41.37
fish eggs	fish eggs	38	44.78
Paralichthyidae (eggs)	sand flounder eggs	38	44.78
	Total Fish Eggs:	**8,476**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	52	59.35
Metacarcinus anthonyi (megalops)	yellow crab megalops	16	18.02
	Total Invertebrate Larvae:	**68**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-3. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN005
Date: June 9, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsypops rubicundus	garibaldi	22	57.09
larvae, yolksac	yolksac larvae	16	43.75
Gibbonsia spp.	kelpfishes	4	6.55
Hypsoblennius spp.	combtooth blennies	2	5.19
	Total Fish Larvae:	**44**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	5,200	12,712.36
Sciaenidae (eggs)	croaker eggs	380	811.42
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	240	656.22
Labridae (eggs)	wrasse eggs	180	396.11
Citharichthys spp. (eggs)	sanddab eggs	80	131.05
Engraulidae (eggs)	anchovy eggs	40	65.52
fish eggs	fish eggs	40	109.37
Paralichthyidae (eggs)	sand flounder eggs	40	65.52
	Total Fish Eggs:	**6,200**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	2	5.19
	Total Invertebrate Larvae:	**2**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-4. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN007
Date: July 19, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsypops rubicundus	garibaldi	30	61.74
Syngnathidae	pipefishes	5	10.28
Gobiesox spp.	clingfishes	3	6.06
CIQ goby complex	gobies	2	4.12
Engraulis mordax	northern anchovy	2	4.00
Pleuronichthys spp.	turbots	2	4.83
Genyonemus lineatus	white croaker	1	2.42
Gibbonsia spp.	kelpfishes	1	1.93
Heterostichus rostratus	giant kelpfish	1	2.06
larvae, yolksac	yolksac larvae	1	2.42
Scorpaena guttata	California scorpionfish	1	1.93
Syngnathus spp.	pipefishes	1	2.14
	Total Fish Larvae:	**50**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	7,882	15,925.00
Labridae (eggs)	wrasse eggs	242	486.42
Pleuronichthys spp. (eggs)	turbot eggs	141	290.83
Citharichthys spp. (eggs)	sanddab eggs	90	204.26
Sciaenidae (eggs)	croaker eggs	26	62.57
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	26	62.57
Haemulidae (eggs)	grunt eggs	13	31.29
	Total Fish Eggs:	**8,420**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	4	8.12
Metacarcinus anthonyi (megalops)	yellow crab megalops	2	4.00
	Total Invertebrate Larvae:	**6**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-5. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN009
Date: August 9, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsoblennius spp.	combtooth blennies	24	65.28
larvae, yolksac	yolksac larvae	9	20.53
Gibbonsia spp.	kelpfishes	1	1.79
Gobiesox spp.	clingfishes	1	1.79
Labrisomidae	labrisomid blennies	1	2.50
Seriphus politus	queenfish	1	1.79
Trachurus symmetricus	jack mackerel	1	1.79
	Total Fish Larvae:	**38**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	10,060	24,924.50
Citharichthys spp. (eggs)	sanddab eggs	793	1,748.06
Sciaenidae (eggs)	croaker eggs	585	1,196.80
Pleuronichthys spp. (eggs)	turbot eggs	487	1,106.59
Paralichthyidae (eggs)	sand flounder eggs	210	524.42
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	125	224.13
	Total Fish Eggs:	**12,260**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	1	2.78
	Total Invertebrate Larvae:	**1**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-6. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN011
Date: September 6, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsoblennius spp.	combtooth blennies	27	64.94
larvae, yolksac	yolksac larvae	16	43.13
Engraulis mordax	northern anchovy	12	29.57
Genyonemus lineatus	white croaker	5	13.30
Paralabrax clathratus	kelp bass	5	13.46
Gibbonsia spp.	kelpfishes	3	7.84
Menticirrhus undulatus	California corbina	3	7.24
Syngnathidae	pipefishes	3	7.27
Cheilotrema saturnum	black croaker	2	5.46
Peprilus simillimus	Pacific butterfish	2	5.01
Atractoscion nobilis	white seabass	1	3.04
Blennioidei	blennies	1	2.40
Hypsypops rubicundus	garibaldi	1	2.40
Labrisomidae	labrisomid blennies	1	3.04
Opisthonema spp.	thread herrings	1	2.40
Pleuronichthys spp.	turbots	1	2.42
Sardinops sagax	Pacific sardine	1	2.42
Seriphus politus	queenfish	1	2.58
	Total Fish Larvae:	**86**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	3,248	8,404.72
Citharichthys spp. (eggs)	sanddab eggs	326	915.92
Pleuronichthys spp. (eggs)	turbot eggs	186	490.34
Paralichthyidae (eggs)	sand flounder eggs	50	145.82
fish eggs	fish eggs	10	30.40
Haemulidae/Paralichthyidae (eggs)	fish eggs	10	30.40
Sphyraena argentea (eggs)	Pacific barracuda eggs	10	24.22
Trachurus symmetricus (eggs)	jack mackerel eggs	10	24.22
	Total Fish Eggs:	**3,850**	
Target Invertebrate Larvae			
Panulirus interruptus (phyllosome)	California spiny lobster (larval)	16	38.53
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	7	16.82
Metacarcinus anthonyi (megalops)	yellow crab megalops	1	2.40
	Total Invertebrate Larvae:	**24**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-7. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN013
Date: October 12, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Genyonemus lineatus	white croaker	48	115.54
larvae, yolksac	yolksac larvae	7	17.25
Gibbonsia spp.	kelpfishes	6	14.80
CIQ goby complex	gobies	4	9.75
Citharichthys stigmaeus	speckled sanddab	3	7.40
Pleuronichthys spp.	turbots	3	7.40
Engraulis mordax	northern anchovy	2	4.91
Blennioidei	blennies	1	2.35
Gobiesox spp.	clingfishes	1	2.46
Hypsoblennius spp.	combtooth blennies	1	2.49
Labrisomidae	labrisomid blennies	1	2.49
Syngnathidae	pipefishes	1	2.35
Xystreurys liolepis	fantail sole	1	2.35
	Total Fish Larvae:	**79**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	4,236	10,255.07
Pleuronichthys spp. (eggs)	turbot eggs	867	2,110.14
Citharichthys spp. (eggs)	sanddab eggs	240	591.12
Paralichthyidae (eggs)	sand flounder eggs	16	40.45
Sciaenidae (eggs)	croaker eggs	16	40.45
	Total Fish Eggs:	**5,375**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	2	4.91
Metacarcinus anthonyi (megalops)	yellow crab megalops	1	2.46
	Total Invertebrate Larvae:	**3**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-8. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN015
Date: November 1, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Genyonemus lineatus	white croaker	8	19.43
Engraulis mordax	northern anchovy	5	12.10
CIQ goby complex	gobies	3	7.00
Clupeiformes	herrings and anchovies	2	4.46
Gibbonsia spp.	kelpfishes	2	4.46
Leptocottus armatus	Pacific staghorn sculpin	1	2.53
Pleuronichthys verticalis	hornyhead turbot	1	2.34
Syngnathidae	pipefishes	1	2.34
	Total Fish Larvae:	**23**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	674	1,585.63
Citharichthys spp. (eggs)	sanddab eggs	150	350.10
Paralichthyidae (eggs)	sand flounder eggs	145	349.04
Pleuronichthys spp. (eggs)	turbot eggs	73	175.00
Engraulidae (eggs)	anchovy eggs	22	49.68
	Total Fish Eggs:	**1,064**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	3	7.03
Metacarcinus anthonyi (megalops)	yellow crab megalops	2	4.88
Cancridae damaged (megalops)	damaged cancer crab megalops	1	2.53
	Total Invertebrate Larvae:	**6**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-9. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN017
Date: December 8, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Atherinopsis californiensis	jacksmelt	13	34.12
Citharichthys stigmaeus	speckled sanddab	9	25.19
Citharichthys sordidus	Pacific sanddab	6	17.10
larvae, yolksac	yolksac larvae	5	13.84
Engraulis mordax	northern anchovy	4	11.68
Sebastes spp. V_	rockfishes	4	10.72
Neoclinus spp.	fringeheads	3	7.97
Gibbonsia spp.	kelpfishes	2	5.55
Leptocottus armatus	Pacific staghorn sculpin	2	5.35
larval/post-larval fish	larval fishes	1	2.67
Pleuronichthys spp.	turbots	1	2.75
Rhinogobiops nicholsi	blackeye goby	1	2.67
	Total Fish Larvae:	**51**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	2,728	7,305.22
Pleuronichthys spp. (eggs)	turbot eggs	814	2,179.09
Citharichthys spp. (eggs)	sanddab eggs	668	1,819.25
Paralichthyidae (eggs)	sand flounder eggs	60	163.69
fish eggs	fish eggs	30	83.49
Genyonemus lineatus (eggs)	white croaker eggs	20	60.03
Labridae/Paralichthyidae (eggs)	fish eggs	20	56.75
Sciaenidae (eggs)	croaker eggs	10	26.74
Sciaenidae/Paralichthyidae (eggs)	fish eggs	10	30.02
	Total Fish Eggs:	**4,360**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	25	65.48
Metacarcinus anthonyi (megalops)	yellow crab megalops	5	13.14
Doryteuthis opalescens	market squid	2	5.68
	Total Invertebrate Larvae:	**32**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-10. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN019
Date: January 12, 2012

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Atherinopsis californiensis	jacksmelt	136	345.72
Ruscarius creaseri	roughcheek sculpin	20	50.80
Engraulis mordax	northern anchovy	11	27.47
Neoclinus spp.	fringeheads	5	12.02
Engraulidae	anchovies	2	4.60
Sebastes spp. V_	rockfishes	2	4.98
Gibbonsia spp.	kelpfishes	1	2.38
Heterostichus rostratus	giant kelpfish	1	2.30
Leptocottus armatus	Pacific staghorn sculpin	1	2.30
Paralichthys californicus	California halibut	1	2.30
Pleuronichthys spp.	turbots	1	2.30
Sebastes spp. V	rockfishes	1	2.30
Seriphus politus	queenfish	1	2.82
	Total Fish Larvae:	**183**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	124	317.42
Engraulidae (eggs)	anchovy eggs	31	80.83
Citharichthys spp. (eggs)	sanddab eggs	30	79.57
Pleuronichthys spp. (eggs)	turbot eggs	14	34.63
Paralichthyidae (eggs)	sand flounder eggs	7	17.61
fish eggs	fish eggs	1	2.82
Sciaenidae (eggs)	croaker eggs	1	2.30
	Total Fish Eggs:	**208**	
Target Invertebrate Larvae			
Metacarcinus anthonyi (megalops)	yellow crab megalops	2	5.20
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	2	4.98
	Total Invertebrate Larvae:	**4**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-11. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN021
Date: February 6, 2012

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Clupeidae	herrings	55	146.51
Atherinopsis californiensis	jacksmelt	29	74.46
Genyonemus lineatus	white croaker	15	43.10
Paralichthys californicus	California halibut	14	38.21
larvae, yolksac	yolksac larvae	7	20.19
Engraulis mordax	northern anchovy	6	17.44
Pleuronichthys spp.	turbots	6	16.24
Sebastes spp. V	rockfishes	4	11.47
CIQ goby complex	gobies	2	4.77
larval fish - damaged	damaged larval fishes	1	2.76
	Total Fish Larvae:	**139**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	5,467	14,665.94
Clupeidae (eggs)	herring (eggs)	2,384	6,560.65
Citharichthys spp. (eggs)	sanddab eggs	2,251	6,182.41
Pleuronichthys spp. (eggs)	turbot eggs	1,057	2,806.84
Engraulidae (eggs)	anchovy eggs	654	1,786.75
Paralichthyidae (eggs)	sand flounder eggs	197	551.20
	Total Fish Eggs:	**12,010**	
Target Invertebrate Larvae			
Cancridae (megalops)	cancer crabs megalops	4	11.47
	Total Invertebrate Larvae:	**4**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C1-12. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN023
Date: March 5, 2012

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Ruscarius creaseri	roughcheek sculpin	28	72.14
Atherinopsis californiensis	jacksmelt	11	29.83
Oxylebius pictus	painted greenling	4	11.73
Artedius spp.	sculpins	2	5.87
CIQ goby complex	gobies	2	5.87
Syngnathidae	pipefishes	2	4.77
Gibbonsia spp.	kelpfishes	1	2.93
Orthonopias triacis	snubnose sculpin	1	2.93
Stenobrachius leucopsarus	northern lampfish	1	2.69
	Total Fish Larvae:	**52**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	1,537	4,126.13
Pleuronichthys spp. (eggs)	turbot eggs	443	1,198.56
Citharichthys spp. (eggs)	sanddab eggs	269	732.45
Sciaenidae/Paralichthyidae (eggs)	fish eggs	37	107.90
Paralichthyidae (eggs)	sand flounder eggs	34	96.86
Sciaenidae (eggs)	croaker eggs	24	69.76
fish eggs	fish eggs	10	27.10
Pleuronectidae (eggs)	righteye flounder eggs	8	18.59
Engraulidae (eggs)	anchovy eggs	4	11.29
	Total Fish Eggs:	**2,366**	
Target Invertebrate Larvae			
Doryteuthis opalescens	market squid	16	38.14
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	11	29.97
	Total Invertebrate Larvae:	**27**	

Some samples may have been split. Count and concentration are based on adjusted split count.

C2. Source Water Stations Larval Counts and Mean Concentrations

Table C2-1. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN001
Date: March 31, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Citharichthys stigmaeus	speckled sanddab	15	15.54
Engraulis mordax	northern anchovy	8	8.09
Leuresthes tenuis	California grunion	8	5.77
Genyonemus lineatus	white croaker	5	5.38
Gibbonsia spp.	kelpfishes	4	3.60
larvae, yolksac	yolksac larvae	4	4.18
Rhinogobiops nicholsi	blackeye goby	4	4.19
Cottidae	sculpins	3	2.93
Lepidopsetta bilineata	rock sole	2	2.09
Scorpaenichthys marmoratus	cabezon	2	2.09
Atherinopsis californiensis	jacksmelt	1	0.98
Citharichthys sordidus	Pacific sanddab	1	1.11
Engraulidae	anchovies	1	1.11
Sciaenidae	croakers	1	1.09
	Total Fish Larvae:	**59**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	10,416	10,300.14
Paralichthyidae (eggs)	sand flounder eggs	2,125	2,199.02
Pleuronichthys spp. (eggs)	turbot eggs	307	289.53
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	200	213.71
Engraulidae (eggs)	anchovy eggs	123	119.72
fish eggs (damaged)	damaged fish eggs unid.	20	21.75
Sciaenidae (eggs)	croaker eggs	10	10.88
	Total Fish Eggs:	**13,200**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	138	143.35
Metacarcinus anthonyi (megalops)	yellow crab megalops	23	23.81
Cancridae (megalops)	cancer crabs megalops	2	1.95
Doryteuthis opalescens	market squid	2	2.09
	Total Target Invertebrate Larvae:	**165**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-2. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN003
Date: May 6, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Parophrys vetulus	English sole	32	20.98
Gobiesox spp.	clingfishes	12	9.10
larvae, yolksac	yolksac larvae	12	8.70
Engraulis mordax	northern anchovy	8	5.19
Hypsoblennius spp.	combtooth blennies	8	5.35
Pleuronichthys spp.	turbots	8	5.19
Zaniolepis frenata	shortspine combfish	8	5.12
Artedius spp.	sculpins	4	2.76
Chromis punctipinnis	blacksmith	4	2.59
Cottidae	sculpins	4	3.58
Gibbonsia spp.	kelpfishes	4	2.76
Liparis spp.	snailfishes	4	2.59
Myctophidae	lanternfishes	4	2.53
Pleuronectidae	righteye flounders	4	2.76
Pleuronichthys verticalis	hornyhead turbot	4	3.05
Stenobrachius leucopsarus	northern lampfish	4	2.76
	Total Fish Larvae:	**124**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	12,305	8,412.18
Pleuronichthys spp. (eggs)	turbot eggs	406	278.13
Citharichthys spp. (eggs)	sanddab eggs	338	227.79
fish eggs	fish eggs	298	201.91
Pleuronectidae (eggs)	righteye flounder eggs	191	154.93
Paralichthyidae (eggs)	sand flounder eggs	178	122.38
Labridae (eggs)	wrasse eggs	98	70.51
Engraulis mordax (eggs)	northern anchovy eggs	80	53.54
Labridae/Paralichthyidae (eggs)	fish eggs	40	27.60
	Total Fish Eggs:	**13,936**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	52	33.98
Metacarcinus anthonyi (megalops)	yellow crab megalops	8	5.19
	Total Target Invertebrate Larvae:	**60**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-3. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN005
Date: June 9, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
larvae, yolksac	yolksac larvae	8	30.62
	Total Fish Larvae:	**8**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	2,935	10,141.90
Sciaenidae (eggs)	croaker eggs	120	374.38
Pleuronichthys spp. (eggs)	turbot eggs	61	228.12
Labridae/Paralichthyidae (eggs)	fish eggs	60	178.70
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	60	229.65
Engraulis mordax (eggs)	northern anchovy eggs	40	153.10
Citharichthys spp. (eggs)	sanddab eggs	20	59.57
fish eggs	fish eggs	20	76.55
Labridae (eggs)	wrasse eggs	20	59.57
Paralichthyidae (eggs)	sand flounder eggs	20	76.55
	Total Fish Eggs:	**3,356**	
Target Invertebrate Larvae			
Metacarcinus anthonyi (megalops)	yellow crab megalops	4	14.85
	Total Target Invertebrate Larvae:	**4**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-4. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN007
Date: July 19, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsypops rubicundus	garibaldi	29	41.43
Hypsoblennius spp.	combtooth blennies	14	20.98
Gibbonsia spp.	kelpfishes	8	11.32
Pleuronichthys spp.	turbots	7	11.13
Labrisomidae	labrisomid blennies	4	5.76
CIQ goby complex	gobies	2	2.93
Genyonemus lineatus	white croaker	2	3.06
larvae, yolksac	yolksac larvae	2	3.28
Opisthonema spp.	thread herrings	2	3.43
Citharichthys stigmaeus	speckled sanddab	1	1.56
Clinidae	kelp blennies	1	1.37
Clinocottus analis	woolly sculpin	1	1.37
Girella nigricans	opaleye	1	1.72
Heterostichus rostratus	giant kelpfish	1	1.37
Neoclinus spp.	fringeheads	1	1.37
Paralichthys californicus	California halibut	1	1.50
Pleuronichthys verticalis	hornyhead turbot	1	1.50
Sardinops sagax	Pacific sardine	1	1.37
Syngnathidae	pipefishes	1	1.72
Xystreurys liolepis	fantail sole	1	1.56
Zaniolepis frenata	shortspine combfish	1	1.72
	Total Fish Larvae:	**82**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	10,799	16,316.33
Pleuronichthys spp. (eggs)	turbot eggs	1,671	2,525.32
Citharichthys spp. (eggs)	sanddab eggs	166	250.99
Labridae/Paralichthyidae (eggs)	fish eggs	98	145.88
Sciaenidae (eggs)	croaker eggs	38	58.20
	Total Fish Eggs:	**12,772**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	18	28.60
Metacarcinus anthonyi (megalops)	yellow crab megalops	4	5.47
Panulirus interruptus (phyllosome)	California spiny lobster (larval)	3	4.23
Cancer productus/Romaleon spp. (megalops)	rock crab megalops	1	1.56
	Total Target Invertebrate Larvae:	**26**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-5. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN009
Date: August 9, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsoblennius spp.	combtooth blennies	32	45.47
larvae, yolksac	yolksac larvae	23	33.07
Hypsypops rubicundus	garibaldi	13	19.26
Gibbonsia spp.	kelpfishes	3	4.86
CIQ goby complex	gobies	2	3.10
Sardinops sagax	Pacific sardine	2	2.70
Atractoscion nobilis	white seabass	1	1.17
Chromis punctipinnis	blacksmith	1	1.32
Clupeiformes	herrings and anchovies	1	1.38
Engraulis mordax	northern anchovy	1	1.48
Labrisomidae	labrisomid blennies	1	1.55
Rhinogobiops nicholsi	blackeye goby	1	1.17
Seriphus politus	queenfish	1	1.55
Umbrina roncador	yellowfin croaker	1	1.32
	Total Fish Larvae:	**83**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	19,307	27,531.55
Citharichthys spp. (eggs)	sanddab eggs	2,271	3,143.89
Pleuronichthys spp. (eggs)	turbot eggs	1,660	2,118.21
Paralichthyidae (eggs)	sand flounder eggs	559	854.29
Engraulidae (eggs)	anchovy eggs	190	293.91
Sciaenidae/Paralichthyidae/Labridae (eggs)	fish eggs	190	293.91
fish eggs	fish eggs	172	238.09
Sciaenidae (eggs)	croaker eggs	172	238.09
	Total Fish Eggs:	**24,520**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	4	4.66
Metacarcinus anthonyi (megalops)	yellow crab megalops	2	2.77
Panulirus interruptus (phyllosome)	California spiny lobster (larval)	2	2.55
	Total Target Invertebrate Larvae:	**8**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-6. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN011
Date: September 6, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Hypsoblennius spp.	combtooth blennies	41	69.40
Engraulis mordax	northern anchovy	38	59.53
larvae, yolksac	yolksac larvae	35	55.76
Opisthonema spp.	thread herrings	21	34.63
Genyonemus lineatus	white croaker	10	16.01
Paralabrax clathratus	kelp bass	8	15.20
Paralabrax spp.	sea basses	8	11.89
Sciaenidae	croakers	8	12.68
CIQ goby complex	gobies	7	11.48
Paralichthys californicus	California halibut	7	11.02
Gibbonsia spp.	kelpfishes	5	9.63
Citharichthys stigmaeus	speckled sanddab	4	5.99
Symphurus atricaudus	California tonguefish	4	6.66
Peprilus simillimus	Pacific butterfish	3	5.33
Pleuronichthys ritteri	spotted turbot	3	4.78
Syngnathidae	pipefishes	3	5.14
Triphoturus mexicanus	Mexican lampfish	3	5.02
Blennioidei	blennies	2	2.99
larval fish - damaged	damaged larval fishes	2	3.64
Cheilotrema saturnum	black croaker	1	2.06
Chilara taylori	spotted cusk-eel	1	1.49
Menticirrhus undulatus	California corbina	1	2.03
Paralabrax maculatofasciatus	spotted sand bass	1	1.50
Pleuronichthys guttulatus	diamond turbot	1	1.80
Pleuronichthys verticalis	hornyhead turbot	1	1.49
Sardinops sagax	Pacific sardine	1	1.85
Scomber japonicus	Pacific mackerel	1	1.85
Umbrina roncador	yellowfin croaker	1	1.80
	Total Fish Larvae:	**221**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	3,087	5,278.11
Citharichthys spp. (eggs)	sanddab eggs	908	1,527.77
Pleuronichthys spp. (eggs)	turbot eggs	203	372.94
Paralichthyidae (eggs)	sand flounder eggs	102	168.21
fish eggs	fish eggs	41	61.96
Sciaenidae (eggs)	croaker eggs	31	46.98
Labridae (eggs)	wrasse eggs	11	17.03
Labridae/Paralichthyidae (eggs)	fish eggs	10	14.97
Paralabrax spp. (eggs)	sand bass eggs	10	17.97
Sciaenidae/Paralichthyidae (eggs)	fish eggs	10	14.97
	Total Fish Eggs:	**4,413**	
Target Invertebrate Larvae			
Panulirus interruptus (phyllosome)	California spiny lobster (larval)	18	31.35
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	6	10.53
Metacarcinus anthonyi (megalops)	yellow crab megalops	1	1.80
	Total Target Invertebrate Larvae:	**25**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-7. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN013
Date: October 12, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Genyonemus lineatus	white croaker	78	141.08
larvae, yolksac	yolksac larvae	18	30.95
Engraulis mordax	northern anchovy	7	12.23
Pleuronichthys spp.	turbots	7	13.12
Gibbonsia spp.	kelpfishes	5	8.55
Citharichthys stigmaeus	speckled sanddab	4	7.05
Pleuronectoidei	flatfishes	4	7.50
Pleuronichthys ritteri	spotted turbot	3	4.74
Xystreurys liolepis	fantail sole	3	5.24
CIQ goby complex	gobies	2	3.25
Citharichthys sordidus	Pacific sanddab	2	3.56
Hypsoblennius spp.	combtooth blennies	2	3.50
Peprilus simillimus	Pacific butterfish	2	3.80
Paralichthys californicus	California halibut	1	1.73
Pleuronectidae	righteye flounders	1	1.63
Pleuronichthys verticalis	hornyhead turbot	1	1.49
Syngnathidae	pipefishes	1	2.07
	Total Fish Larvae:	**141**	
Fish Eggs			
fish eggs (early development stage)	fish eggs	4,622	7,606.49
Pleuronichthys spp. (eggs)	turbot eggs	778	1,194.50
Citharichthys spp. (eggs)	sanddab eggs	380	678.03
fish eggs	fish eggs	20	36.18
Sciaenidae (eggs)	croaker eggs	20	34.64
Haemulidae/Paralichthyidae (eggs)	fish eggs	10	20.68
Labridae/Paralichthyidae (eggs)	fish eggs	10	20.68
	Total Fish Eggs:	**5,840**	
Target Invertebrate Larvae			
Metacarcinus anthonyi (megalops)	yellow crab megalops	1	2.07
	Total Target Invertebrate Larvae:	**1**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-8. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN015
Date: November 1, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Engraulis mordax	northern anchovy	11	20.97
Genyonemus lineatus	white croaker	11	21.81
larvae, yolksac	yolksac larvae	8	14.48
CIQ goby complex	gobies	4	7.14
Atherinopsis californiensis	jacksmelt	2	3.57
Gibbonsia spp.	kelpfishes	2	3.54
Pleuronichthys spp.	turbots	2	3.87
Leptocottus armatus	Pacific staghorn sculpin	1	1.62
Leuresthes tenuis	California grunion	1	1.84
Paralichthys californicus	California halibut	1	2.04
	Total Fish Larvae:	**43**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	1,303	2,426.30
Citharichthys spp. (eggs)	sanddab eggs	194	346.23
Pleuronichthys spp. (eggs)	turbot eggs	136	247.96
Paralichthyidae (eggs)	sand flounder eggs	57	102.43
Labridae/Paralichthyidae (eggs)	fish eggs	20	33.07
fish eggs	fish eggs	17	32.28
Engraulidae (eggs)	anchovy eggs	2	3.67
Sciaenidae (eggs)	croaker eggs	1	1.84
Sciaenidae/Paralichthyidae (eggs)	fish eggs	1	1.84
	Total Fish Eggs:	**1,732**	
Target Invertebrate Larvae			
Metacarcinus anthonyi (megalops)	yellow crab megalops	3	5.13
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	2	3.57
	Total Target Invertebrate Larvae:	**5**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-9. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN017
Date: December 8, 2011

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Atherinopsis californiensis	jacksmelt	18	31.99
Citharichthys stigmaeus	speckled sanddab	13	25.17
larvae, yolksac	yolksac larvae	9	15.90
Citharichthys sordidus	Pacific sanddab	7	12.23
Oxylebius pictus	painted greenling	6	10.47
Engraulis mordax	northern anchovy	5	8.58
Syngnathidae	pipefishes	5	8.27
Genyonemus lineatus	white croaker	4	7.55
Leptocottus armatus	Pacific staghorn sculpin	4	7.23
Sebastes spp. V_	rockfishes	3	4.86
Stenobrachius leucopsarus	northern lampfish	3	5.48
Clinocottus analis	woolly sculpin	2	3.45
larval fish - damaged	damaged larval fishes	2	3.41
Sebastes spp. V	rockfishes	2	3.52
Alloclinus holderi	island kelpfish	1	2.07
Artedius lateralis	smoothhead sculpin	1	2.07
Chaenopsidae	tube blennies	1	1.45
CIQ goby complex	gobies	1	1.72
Hypsoblennius spp.	combtooth blennies	1	1.72
Neoclinus spp.	fringeheads	1	2.01
Opisthonema spp.	thread herrings	1	2.07
Rhinogobiops nicholsi	blackeye goby	1	2.01
Ruscarius creaseri	roughcheek sculpin	1	1.72
Synodus lucioceps	California lizardfish	1	1.89
	Total Fish Larvae:	**93**	
Non-Entrainable Fishes			
Syngnathidae	pipefishes	1	1.89
	Total Non-Entrainable Fishes:	**1**	1.89
Fish Eggs*			
fish eggs (early development stage)	fish eggs	4,682	8,101.44
Citharichthys spp. (eggs)	sanddab eggs	1,663	2,944.08
Pleuronichthys spp. (eggs)	turbot eggs	752	1,344.69
Sciaenidae/Paralichthyidae (eggs)	fish eggs	94	183.37
Paralichthyidae (eggs)	sand flounder eggs	40	80.43
Opisthonema spp. (eggs)	thread herring eggs	20	34.10
fish eggs	fish eggs	10	20.72
	Total Fish Eggs:	**7,261**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	28	49.44
Doryteuthis opalescens	market squid	5	7.26
Cancridae (megalops)	cancer crabs megalops	2	3.61
Cancridae damaged (megalops)	damaged cancer crab megalops	2	3.78
	Total Target Invertebrate Larvae:	**37**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-10. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN019
Date: January 12, 2012

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Engraulis mordax	northern anchovy	24	43.11
Atherinopsis californiensis	jacksmelt	11	18.87
Ruscarius creaseri	roughcheek sculpin	9	16.02
Gibbonsia spp.	kelpfishes	7	10.51
Sebastes spp. V	rockfishes	5	7.91
Citharichthys stigmaeus	speckled sanddab	4	7.17
larvae, yolksac	yolksac larvae	4	6.73
Genyonemus lineatus	white croaker	3	5.38
Pleuronichthys spp.	turbots	2	3.23
Stenobrachius leucopsarus	northern lampfish	2	3.00
Sciaenidae	croakers	1	1.89
Scorpaenichthys marmoratus	cabezon	1	1.50
Sebastes spp. V_	rockfishes	1	1.50
Syngnathidae	pipefishes	1	1.90
	Total Fish Larvae:	**75**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	803	1,422.26
Citharichthys spp. (eggs)	sanddab eggs	220	397.17
Engraulidae (eggs)	anchovy eggs	197	352.48
Pleuronichthys spp. (eggs)	turbot eggs	165	307.21
Sciaenidae (eggs)	croaker eggs	36	66.92
Paralichthyidae (eggs)	sand flounder eggs	8	15.43
Pleuronectidae (eggs)	righteye flounder eggs	4	7.95
Sciaenidae/Paralichthyidae (eggs)	fish eggs	4	6.16
fish eggs	fish eggs	2	3.25
	Total Fish Eggs:	**1,439**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	4	7.44
Metacarcinus anthonyi (megalops)	yellow crab megalops	1	1.75
	Total Target Invertebrate Larvae:	**5**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-11. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN021
Date: February 6, 2012

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Atherinopsis californiensis	jacksmelt	106	190.98
Clupeidae	herrings	92	180.22
Genyonemus lineatus	white croaker	18	35.88
larvae, yolksac	yolksac larvae	17	34.46
Pleuronichthys spp.	turbots	17	33.73
Engraulis mordax	northern anchovy	14	27.23
Paralichthys californicus	California halibut	12	24.26
Sardinops sagax	Pacific sardine	10	19.09
CIQ goby complex	gobies	2	3.35
Citharichthys stigmaeus	speckled sanddab	2	4.00
Pleuronichthys guttulatus	diamond turbot	2	3.32
Sebastes spp. V_	rockfishes	2	3.91
Labrisomidae	labrisomid blennies	1	2.04
Leptocottus armatus	Pacific staghorn sculpin	1	1.91
Platichthys stellatus	starry flounder	1	1.91
Ruscarius creaseri	roughcheek sculpin	1	2.04
Zaniolepis frenata	shortspine combfish	1	2.04
	Total Fish Larvae:	**299**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	7,118	13,087.12
Citharichthys spp. (eggs)	sanddab eggs	3,368	6,249.70
Clupeidae (eggs)	herring (eggs)	2,156	3,703.60
Pleuronichthys spp. (eggs)	turbot eggs	1,049	1,955.96
Engraulidae (eggs)	anchovy eggs	669	1,295.34
Paralichthyidae (eggs)	sand flounder eggs	218	360.86
Sciaenidae (eggs)	croaker eggs	98	172.71
fish eggs	fish eggs	42	82.03
	Total Fish Eggs:	**14,718**	
Target Invertebrate Larvae			
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	16	30.60
Metacarcinus anthonyi (megalops)	yellow crab megalops	4	7.90
	Total Target Invertebrate Larvae:	**20**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table C2-12. Net Sample Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBN023
Date: March 5, 2012

Taxon	Common Name	Count	Mean Concentration (#/1,000 m^3)
Fish Larvae			
Engraulis mordax	northern anchovy	64	105.20
Atherinopsis californiensis	jacksmelt	35	61.65
Citharichthys stigmaeus	speckled sanddab	12	20.97
Ruscarius creaseri	roughcheek sculpin	12	23.23
Genyonemus lineatus	white croaker	9	14.00
Oxylebius pictus	painted greenling	8	15.28
Stenobrachius leucopsarus	northern lampfish	5	7.85
Chaenopsidae/Labrisomidae	tube/labrisomid blennies	4	6.15
Parophrys vetulus	English sole	4	7.29
Pleuronectidae	righteye flounders	4	6.15
Sebastes spp. V	rockfishes	4	6.15
Pleuronichthys spp.	turbots	3	5.59
Artedius spp.	sculpins	2	3.66
Gibbonsia spp.	kelpfishes	2	3.42
Pleuronectiformes	flatfishes	1	1.72
	Total Fish Larvae:	**169**	
Fish Eggs*			
fish eggs (early development stage)	fish eggs	1,822	3,264.52
Citharichthys spp. (eggs)	sanddab eggs	481	849.81
Pleuronichthys spp. (eggs)	turbot eggs	351	643.88
Paralichthyidae (eggs)	sand flounder eggs	116	200.66
Pleuronectidae (eggs)	righteye flounder eggs	24	38.02
Sciaenidae (eggs)	croaker eggs	13	24.95
Sciaenidae/Paralichthyidae (eggs)	fish eggs	12	19.58
Engraulidae (eggs)	anchovy eggs	8	14.66
Vinciguerria lucetia (eggs)	Panama lightfish eggs	4	7.29
fish eggs	fish eggs	3	5.83
	Total Fish Eggs:	**2,835**	
Target Invertebrate Larvae			
Doryteuthis opalescens	market squid	68	124.48
Romaleon anten./Metacarcinus grac. (meg.)	cancer crabs	19	33.88
Metacarcinus anthonyi (megalops)	yellow crab megalops	8	15.07
Cancridae damaged (megalops)	damaged cancer crab megalops	4	6.15
Cancridae (megalops)	cancer crabs megalops	2	3.66
	Total Target Invertebrate Larvae:	**101**	

Some samples may have been split. Count and concentration are based on adjusted split count.

C3. Entrainment Station Larval Fish Lengths

Table C3-1. Entrainment Station Larval Fish Lengths.

Survey: WBN001
Date: March 31, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Gibbonsia spp.	kelpfishes	6	4	4.15-4.43	4.24
Citharichthys stigmaeus	speckled sanddab	3	2	0.91-1.09	1.00
Atherinopsis californiensis	jacksmelt	2	2	7.94-8.03	7.99
Genyonemus lineatus	white croaker	2	2	2.60-4.78	3.69
Engraulis mordax	northern anchovy	1	1	2.42	2.42
Pleuronectoidei	flatfishes	1	1	0.78	0.78
		Total: 15	**12**		

Table C3-2. Entrainment Station Larval Fish Lengths.

Survey: WBN003
Date: May 6, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Parophrys vetulus	English sole	4	3	2.63-3.54	3.02
larval fish - damaged	damaged larval fishes	3	0	-	-
Zaniolepis frenata	shortspine combfish	3	3	2.82-3.17	2.96
Chitonotus/Icelinus spp.	sculpins	1	1	1.92	1.92
Cottidae	sculpins	1	1	2.42	2.42
Pleuronectidae	righteye flounders	1	1	2.76	2.76
Sebastes miniatus	vermilion rockfish	1	0	-	-
Sebastes spp. V	rockfishes	1	1	2.97	2.97
Stenobrachius leucopsarus	northern lampfish	1	1	3.39	3.39
		Total: 16	**11**		

Table C3-3. Entrainment Station Larval Fish Lengths.

Survey: WBN005
Date: June 9, 2011

Taxon	Common Name	Total Count	Measured Count	Range (mm)	Length (mm)	Average Length (mm)
Hypsypops rubicundus	garibaldi	11	8	2.49-2.69		2.59
larvae, yolksac	yolksac larvae	4	0	-		-
Gibbonsia spp.	kelpfishes	1	0	-		-
Hypsoblennius spp.	combtooth blennies	1	1	2.18		2.18
		Total: 17	**9**			

Table C3-4. Entrainment Station Larval Fish Lengths.

Survey: WBN007
Date: July 19, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Hypsypops rubicundus	garibaldi	30	28	2.23-2.64	2.47
Syngnathidae	pipefishes	5	5	6.51-10.54	8.83
Gobiesox spp.	clingfishes	3	3	2.83-3.18	2.99
CIQ goby complex	gobies	2	2	2.71-2.75	2.73
Engraulis mordax	northern anchovy	2	2	8.35-8.82	8.59
Pleuronichthys spp.	turbots	2	2	1.81-2.34	2.08
Genyonemus lineatus	white croaker	1	1	1.49	1.49
Gibbonsia spp.	kelpfishes	1	1	4.80	4.80
Heterostichus rostratus	giant kelpfish	1	0	-	-
larvae, yolksac	yolksac larvae	1	0	-	-
Scorpaena guttata	California scorpionfish	1	1	1.61	1.61
Syngnathus spp.	pipefishes	1	1	9.85	9.85
		Total: 50	**46**		

Table C3-5. Entrainment Station Larval Fish Lengths.

Survey: WBN009
Date: August 9, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Hypsoblennius spp.	combtooth blennies	24	23	2.03-2.52	2.19
larvae, yolksac	yolksac larvae	9	0	-	-
Gibbonsia spp.	kelpfishes	1	1	5.11	5.11
Gobiesox spp.	clingfishes	1	1	3.27	3.27
Labrisomidae	labrisomid blennies	1	1	3.57	3.57
Seriphus politus	queenfish	1	1	1.65	1.65
Trachurus symmetricus	jack mackerel	1	1	2.35	2.35
		Total: 38	**28**		

Table C3-6. Entrainment Station Larval Fish Lengths.

Survey: WBN011
Date: September 6, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Hypsoblennius spp.	combtooth blennies	27	26	2.12-3.72	2.59
larvae, yolksac	yolksac larvae	16	0	-	-
Engraulis mordax	northern anchovy	12	8	2.32-18.79	10.00
Genyonemus lineatus	white croaker	5	5	1.40-1.95	1.59
Paralabrax clathratus	kelp bass	5	4	1.05-1.35	1.21
Gibbonsia spp.	kelpfishes	3	3	4.15-5.01	4.48
Menticirrhus undulatus	California corbina	3	3	1.56-1.89	1.73
Syngnathidae	pipefishes	3	3	8.68-14.42	11.16
Cheilotrema saturnum	black croaker	2	2	1.56-1.57	1.57
Peprilus simillimus	Pacific butterfish	2	2	1.59-1.65	1.62
Atractoscion nobilis	white seabass	1	1	1.45	1.45
Blennioidei	blennies	1	0	-	-
Hypsypops rubicundus	garibaldi	1	1	2.66	2.66
Labrisomidae	labrisomid blennies	1	1	3.79	3.79
Opisthonema spp.	thread herrings	1	0	-	-
Pleuronichthys spp.	turbots	1	1	2.43	2.43
Sardinops sagax	Pacific sardine	1	0	-	-
Seriphus politus	queenfish	1	1	1.66	1.66
	Total:	**86**	**61**		

Table C3-7. Entrainment Station Larval Fish Lengths.

Survey: WBN013
Date: October 12, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Genyonemus lineatus	white croaker	48	41	1.59-5.26	2.58
larvae, yolksac	yolksac larvae	7	0	-	-
Gibbonsia spp.	kelpfishes	6	6	4.18-5.14	4.51
CIQ goby complex	gobies	4	4	2.42-2.72	2.58
Citharichthys stigmaeus	speckled sanddab	3	3	1.15-1.29	1.23
Pleuronichthys spp.	turbots	3	3	1.47-2.01	1.82
Engraulis mordax	northern anchovy	2	2	9.95-10.55	10.25
Blennioidei	blennies	1	1	4.46	4.46
Gobiesox spp.	clingfishes	1	1	4.59	4.59
Hypsoblennius spp.	combtooth blennies	1	1	2.08	2.08
Labrisomidae	labrisomid blennies	1	1	3.54	3.54
Syngnathidae	pipefishes	1	1	9.54	9.54
Xystreurys liolepis	fantail sole	1	0	-	-
	Total:	**79**	**64**		

Table C3-8. Entrainment Station Larval Fish Lengths.

Survey: WBN015
Date: November 1, 2011

Taxon	Common Name	Total Count	Measured Count	Range Length (mm)	Average Length (mm)
Genyonemus lineatus	white croaker	8	8	2.30-3.74	2.94
Engraulis mordax	northern anchovy	5	5	7.82-21.76	13.39
CIQ goby complex	gobies	3	3	2.61-2.96	2.84
Clupeiformes	herrings and anchovies	2	1	1.47	1.47
Gibbonsia spp.	kelpfishes	2	2	4.81-5.63	5.22
Leptocottus armatus	Pacific staghorn sculpin	1	1	6.25	6.25
Pleuronichthys verticalis	hornyhead turbot	1	1	11.09	11.09
Syngnathidae	pipefishes	1	1	8.48	8.48
		Total: 23	**22**		

Table C3-9. Entrainment Station Larval Fish Lengths.

Survey: WBN017
Date: December 8, 2011

Taxon	Common Name	Total Count	Measured Count	Range Length (mm)	Average Length (mm)
Atherinopsis californiensis	jacksmelt	13	13	7.65-12.31	8.60
Citharichthys stigmaeus	speckled sanddab	9	6	2.21-3.33	3.01
Citharichthys sordidus	Pacific sanddab	6	5	2.59-4.40	3.63
larvae, yolksac	yolksac larvae	5	0	-	-
Engraulis mordax	northern anchovy	4	3	8.92-11.26	9.91
Sebastes spp. V_	rockfishes	4	4	3.01-4.13	3.52
Neoclinus spp.	fringeheads	3	3	4.94-5.32	5.08
Gibbonsia spp.	kelpfishes	2	2	5.17-8.63	6.90
Leptocottus armatus	Pacific staghorn sculpin	2	2	4.14-4.32	4.23
larval/post-larval fish	larval fishes	1	0	-	-
Pleuronichthys spp.	turbots	1	1	1.84	1.84
Rhinogobiops nicholsi	blackeye goby	1	1	2.89	2.89
		Total: 51	**40**		

Table C3-10. Entrainment Station Larval Fish Lengths.

Survey: WBN019
Date: January 12, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Atherinopsis californiensis	jacksmelt	136	132	7.20-17.64	10.31
Ruscarius creaseri	roughcheek sculpin	20	20	2.43-2.88	2.72
Engraulis mordax	northern anchovy	11	3	10.04-13.20	11.14
Neoclinus spp.	fringeheads	5	5	4.37-4.63	4.46
Engraulidae	anchovies	2	1	1.34	1.34
Sebastes spp. V_	rockfishes	2	2	4.44-4.53	4.48
Gibbonsia spp.	kelpfishes	1	1	6.79	6.79
Heterostichus rostratus	giant kelpfish	1	1	6.89	6.89
Leptocottus armatus	Pacific staghorn sculpin	1	1	4.53	4.53
Paralichthys californicus	California halibut	1	1	1.76	1.76
Pleuronichthys spp.	turbots	1	1	1.18	1.18
Sebastes spp. V	rockfishes	1	1	4.33	4.33
Seriphus politus	queenfish	1	1	1.57	1.57
		Total: 183	**170**		

Table C3-11. Entrainment Station Larval Fish Lengths.

Survey: WBN021
Date: February 6, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Clupeidae	herrings	34	16	1.62-4.09	2.61
Atherinopsis californiensis	jacksmelt	19	17	7.42-13.22	8.71
Genyonemus lineatus	white croaker	12	3	1.81-2.15	2.04
Paralichthys californicus	California halibut	7	6	1.54-2.29	1.99
Engraulis mordax	northern anchovy	4	4	2.73-3.44	2.97
larvae, yolksac	yolksac larvae	4	0	-	-
Pleuronichthys spp.	turbots	3	3	1.89-2.16	1.99
Sebastes spp. V	rockfishes	2	2	4.35-6.09	5.22
CIQ goby complex	gobies	1	0	-	-
larval fish - damaged	damaged larval fishes	1	0	-	-
		Total: 87	**51**		

Table C3-12. Entrainment Station Larval Fish Lengths.

Survey: WBN023
Date: March 5, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Ruscarius creaseri	roughcheek sculpin	19	19	2.37-2.88	2.66
Atherinopsis californiensis	jacksmelt	9	8	7.11-18.07	10.64
Oxylebius pictus	painted greenling	4	4	3.13-3.53	3.40
Artedius spp.	sculpins	2	2	2.35-2.39	2.37
CIQ goby complex	gobies	2	2	3.10-3.38	3.24
Gibbonsia spp.	kelpfishes	1	1	4.41	4.41
Orthonopias triacis	snubnose sculpin	1	1	2.85	2.85
Stenobrachius leucopsarus	northern lampfish	1	1	3.75	3.75
Syngnathidae	pipefishes	1	1	9.00	9.00
		Total: 40	**39**		

West Basin Municipal Water District
Demonstration Desalination Facility

Appendix D

Larval Fish Sampling Results: Pump Sampling by Survey

D1. Unscreened Boat Station, 1.0 mm and 2.0 mm Wedgewire Screen (WWS) Intake Onshore Pump Station: Larval Counts and Mean Concentrations

D2. Pump Station Larval Fish Lengths

D1. Unscreened Boat Station, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations

Table D1-1. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP001 **Date: March 31, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake** Count	**2.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)
Fish Larvae								
Typhlogobius californiensis	blind goby	**8**	-	-	-	-	8	64.52
larval fish - damaged	damaged larval fishes	**4**	-	-	1	8.03	3	24.08
Ruscarius creaseri	roughcheek sculpin	**4**	-	-	-	-	4	31.79
Genyonemus lineatus	white croaker	**3**	2	16.16	1	8.03	-	-
Stenobrachius leucopsarus	northern lampfish	**2**	-	-	1	8.03	1	7.90
Artedius lateralis	smoothhead sculpin	**1**	-	-	1	8.12	-	-
Cottidae	sculpins	**1**	-	-	1	8.03	-	-
Gibbonsia spp.	kelpfishes	**1**	-	-	-	-	1	8.09
Ruscarius meanyi	Puget Sound sculpin	**1**	-	-	1	7.51	-	-
	Total Fish Larvae:	**25**	**2**				**17**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**3,856**	1,430	11,561.10	1,162	9,231.57	1,264	9,986.59
Paralichthyidae (eggs)	sand flounder eggs	**205**	70	566.86	95	736.67	40	317.94
Pleuronichthys spp. (eggs)	turbot eggs	**110**	50	410.60	20	161.45	40	319.80
Labridae/Paralichthyidae (eggs)	fish eggs	**70**	13	106.47	20	160.59	37	280.51
fish eggs	fish eggs	**10**	-	-	10	80.29	-	-
Engraulidae (eggs)	anchovy eggs	**8**	-	-	8	58.86	-	-
	Total Fish Eggs:	**4,259**	**1,563**		**1,315**		**1,381**	
Target Invertebrate Larvae								
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**27**	11	89.27	2	16.23	14	111.23
Metacarcinus anthonyi (megalops)	yellow crab megalops	**26**	2	15.89	15	121.22	9	70.51
Cancridae (megalops)	cancer crabs megalops	**7**	-	-	1	8.12	6	47.83
	Total Invertebrate Larvae:	**60**	**13**		**18**		**29**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-2. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP002 **Date: April 14, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
No Fish		**0**	-	-	Not Sampled		Not Sampled
	Total Fish Larvae:	**0**					
Fish Eggs							
fish eggs (early development stage)	fish eggs	**420**	420	6,963.66			
fish eggs	fish eggs	**60**	60	993.25			
Pleuronichthys spp. (eggs)	turbot eggs	**9**	9	142.41			
	Total Fish Eggs:	**489**	**489**				
Target Invertebrate Larvae							
No Invertebrates		**0**	-	-			
	Total Invertebrate Larvae:	**0**	**35**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-3. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP003 **Date: May 5, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake** Count	**2.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)
Fish Larvae								
Cottidae	sculpins	**4**	-	-	4	21.25	-	-
larval fish - damaged	damaged larval fishes	**3**	1	6.00	-	-	2	15.64
Chromis punctipinnis	blacksmith	**1**	1	6.17	-	-	-	-
Pleuronichthys spp.	turbots	**1**	-	-	-	-	1	6.01
Sciaenidae	croakers	**1**	-	-	-	-	1	9.63
	Total Fish Larvae:	**10**	**2**		**4**		**4**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**2,012**	715	4,389.32	724	4,241.62	573	3,684.43
Pleuronichthys spp. (eggs)	turbot eggs	**666**	353	2,167.12	109	595.03	203	1,225.17
fish eggs	fish eggs	**216**	74	454.62	64	346.08	78	482.41
Engraulidae (eggs)	anchovy eggs	**33**	13	80.57	-	-	20	119.39
Citharichthys spp. (eggs)	sanddab eggs	**30**	10	61.45	20	106.80	-	-
Sciaenidae/Paralichthy./Labri. (eggs)	fish eggs	**28**	13	80.57	15	80.83	-	-
Labridae (eggs)	wrasse eggs	**23**	13	80.57	10	51.79	-	-
Paralichthyidae (eggs)	sand flounder eggs	**5**	5	30.57	-	-	-	-
	Total Fish Eggs:	**3,013**	**1,197**		**942**		**874**	
Target Invertebrate Larvae								
Metacarcinus anthonyi (megalops)	yellow crab megalops	**3**	1	6.14	2	10.68	-	-
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**3**	-	-	2	17.40	1	6.01
Cancridae (megalops)	cancer crabs megalops	**2**	1	6.14	1	8.70	-	-
	Total Invertebrate Larvae:	**8**	**2**		**5**		**1**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-4. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP004 **Date: May 25, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
Atherinopsidae	silversides	**1**	1	9.61	Not Sampled		Not Sampled
larvae, yolksac	yolksac larvae	**1**	1	9.60			
	Total Fish Larvae:	**2**	**2**				
Non-Entrainable Fishes							
Scorpaenichthys marmoratus	cabezon	**1**	1	9.60			
	Total Non-Entrainable Fishes:	**1**	**1**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**925**	925	8,918.67			
Pleuronichthys spp. (eggs)	turbot eggs	**243**	243	2,336.30			
Engraulidae (eggs)	anchovy eggs	**5**	5	48.64			
fish eggs	fish eggs	**5**	5	49.31			
Sciaenidae/Paralichthy./Labri. (eggs)	fish eggs	**1**	1	9.92			
	Total Fish Eggs:	**1,179**	**1,179**				
Target Invertebrate Larvae							
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**3**	3	29.15			
Cancridae (megalops)	cancer crabs megalops	**2**	2	19.86			
Cancer produc./Romale. spp. (meg.)	rock crab megalops	**1**	1	9.61			
	Total Invertebrate Larvae:	**6**	**6**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-5. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP005 **Date: June 9, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED Count**	**UNSCREENED Mean Concentration (#/1,000 m^3)**	**1.0 mm WWS Intake Count**	**1.0 mm WWS Intake Mean Concentration (#/1,000 m^3)**	**2.0 mm WWS Intake Count**	**2.0 mm WWS Intake Mean Concentration (#/1,000 m^3)**
Fish Larvae								
Hypsoblennius spp.	combtooth blennies	**18**	2	11.96	5	30.50	11	66.79
larval fish - damaged	damaged larval fishes	**8**	3	18.20	3	18.24	2	12.16
Oxylebius pictus/Zaniolepis frenata	paint. green./shortspine comb.	**4**	-	-	4	23.75	-	-
Ruscarius creaseri	roughcheek sculpin	**4**	-	-	-	-	4	24.28
Cottidae	sculpins	**2**	1	6.07	1	6.13	-	-
Gibbonsia spp.	kelpfishes	**1**	-	-	-	-	1	6.08
Gobiesox spp.	clingfishes	**1**	-	-	-	-	1	6.05
	Total Fish Larvae:	**38**	**6**		**13**		**19**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**6,076**	1,711	10,284.09	1,804	11,046.72	2,561	15,557.58
Pleuronichthys spp. (eggs)	turbot eggs	**389**	110	663.73	198	1,212.29	81	495.82
fish eggs	fish eggs	**150**	70	412.66	80	491.19	-	-
Engraulidae (eggs)	anchovy eggs	**118**	-	-	10	61.30	108	653.11
Labridae (eggs)	wrasse eggs	**90**	20	122.55	40	245.19	30	182.44
Sciaenidae (eggs)	croaker eggs	**30**	10	58.95	10	61.30	10	60.81
Citharichthys spp. (eggs)	sanddab eggs	**10**	10	58.95	-	-	-	-
Sciaenidae/Paralichthy./Labri. (eggs)	fish eggs	**10**	-	-	-	-	10	60.81
Vinciguerria lucetia (eggs)	Panama lightfish eggs	**10**	-	-	-	-	10	60.81
	Total Fish Eggs:	**6,883**	**1,931**		**2,142**		**2,810**	
Target Invertebrate Larvae								
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**1**	1	5.90	-	-	-	-
	Total Invertebrate Larvae:	**1**	**1**		**0**		**0**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-6. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP006 **Date: June 21, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
Hypsoblennius spp.	combtooth blennies	**7**	7	68.75	Not Sampled		Not Sampled
larval fish - damaged	damaged larval fishes	**3**	3	29.76			
Hypsypops rubicundus	garibaldi	**2**	2	19.85			
Gibbonsia spp.	kelpfishes	**1**	1	9.91			
Syngnathidae	pipefishes	**1**	1	9.91			
	Total Fish Larvae:	**14**	**14**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**399**	399	3,936.54			
Pleuronichthys spp. (eggs)	turbot eggs	**66**	66	652.37			
Labridae (eggs)	wrasse eggs	**16**	16	159.31			
fish eggs	fish eggs	**11**	11	103.42			
Citharichthys spp. (eggs)	sanddab eggs	**8**	8	78.68			
Engraulidae (eggs)	anchovy eggs	**1**	1	9.09			
	Total Fish Eggs:	**501**	**501**				
Target Invertebrate Larvae							
Metacarcinus anthonyi (megalops)	yellow crab megalops	**1**	1	9.91			
	Total Invertebrate Larvae:	**1**	**1**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-7. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP007 **Date: July 19, 2011**			**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**	
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**		
Fish Larvae								
larval fish - damaged	damaged larval fishes	**3**	2	12.27	-	-	1	6.14
Gobiesox spp.	clingfishes	**2**	-	-	-	-	2	12.28
Gibbonsia spp.	kelpfishes	**1**	-	-	-	-	1	6.14
Hypsoblennius spp.	combtooth blennies	**1**	-	-	-	-	1	6.02
	Total Fish Larvae:	**7**	**2**		**0**		**5**	
Fish Eggs								
Pleuronichthys spp. (eggs)	turbot eggs	**1,169**	321	1,971.22	424	2,469.58	424	2,613.89
fish eggs (early development stage)	fish eggs	**511**	173	1,068.04	186	1,072.82	152	923.24
fish eggs	fish eggs	**47**	26	162.62	9	51.39	12	72.48
Citharichthys spp. (eggs)	sanddab eggs	**24**	8	49.75	5	28.26	11	66.27
Sciaenidae (eggs)	croaker eggs	**20**	-	-	1	5.65	19	115.82
Paralichthyidae (eggs)	sand flounder eggs	**2**	-	-	2	11.43	-	-
Labridae (eggs)	wrasse eggs	**1**	-	-	1	5.65	-	-
	Total Fish Eggs:	**1,774**	**528**		**628**		**618**	
Target Invertebrate Larvae								
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**4**	4	24.57	-	-	-	-
Panulirus interruptus (phyllosome)	Calif. spiny lobster (larval)	**1**	-	-	1	5.88	-	-
	Total Invertebrate Larvae:	**5**	**4**		**1**		**0**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-8. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP008 **Date: July 28, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
No Fish		**0**	-	-	Not Sampled		Not Sampled
	Total Fish Larvae:	**0**					
Fish Eggs							
fish eggs (early development stage)	fish eggs	**2,698**	2,698	23,424.06			
Citharichthys spp. (eggs)	sanddab eggs	**169**	169	1,335.13			
fish eggs	fish eggs	**114**	114	965.04			
Pleuronichthys spp. (eggs)	turbot eggs	**67**	67	476.57			
Paralichthyidae (eggs)	sand flounder eggs	**29**	29	222.09			
Sciaenidae (eggs)	croaker eggs	**10**	10	90.68			
	Total Fish Eggs:	**3,087**	**3,087**				
Target Invertebrate Larvae							
No Invertebrates		**0**	-	-			
	Total Invertebrate Larvae:	**0**					

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-9. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP009 **Date: August 9, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake** Count	**2.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)
Fish Larvae								
Hypsoblennius spp.	combtooth blennies	**7**	-	-	3	18.40	4	24.56
Gibbonsia spp.	kelpfishes	**1**	-	-	-	-	1	6.17
	Total Fish Larvae:	**8**	**0**		**3**		**5**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**7,899**	4,310	26,571.37	1,620	9,869.08	1,969	12,079.93
Pleuronichthys spp. (eggs)	turbot eggs	**305**	155	949.01	103	625.41	48	294.20
Citharichthys spp. (eggs)	sanddab eggs	**200**	17	105.89	23	143.53	160	976.20
fish eggs	fish eggs	**6**	-	-	4	24.23	2	11.97
Sciaenidae (eggs)	croaker eggs	**3**	-	-	2	12.11	1	5.99
Labridae (eggs)	wrasse eggs	**2**	-	-	2	12.11	-	-
	Total Fish Eggs:	**8,415**	**4,482**		**1,754**		**2,179**	
Target Invertebrate Larvae								
Metacarcinus anthonyi (megalops)	yellow crab megalops	**1**	-	-	1	6.14	-	-
	Total Invertebrate Larvae:	**1**	**0**		**1**		**0**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-10. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP010 **Date: August 22, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** **Count**	**Mean Concentration (#/1,000 m^3)**	**1.0 mm WWS Intake** **Count**	**Mean Concentration (#/1,000 m^3)**	**2.0 mm WWS Intake**
Fish Larvae							
No Fish		**0**	-	-	Not Sampled		Not Sampled
	Total Fish Larvae:	**0**					
Fish Eggs							
fish eggs (early development stage)	fish eggs	**1,613**	1,613	10,001.09			
Citharichthys spp. (eggs)	sanddab eggs	**50**	50	309.71			
Pleuronichthys spp. (eggs)	turbot eggs	**47**	47	290.03			
fish eggs	fish eggs	**20**	20	124.00			
Labridae (eggs)	wrasse eggs	**10**	10	62.19			
Paralichthyidae (eggs)	sand flounder eggs	**10**	10	61.95			
Sciaenidae (eggs)	croaker eggs	**10**	10	62.19			
Sciaenidae/Paralichthy./Labri. (eggs)	fish eggs	**10**	10	62.19			
	Total Fish Eggs:	**1,770**	**1,770**				
Target Invertebrate Larvae							
Metacarcinus anthonyi (megalops)	yellow crab megalops	**1**	1	6.22			
	Total Invertebrate Larvae:	**1**	**1**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-11. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP011 **Date: September 6, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED Count**	**UNSCREENED Mean Concentration (#/1,000 m^3)**	**1.0 mm WWS Intake Count**	**1.0 mm WWS Intake Mean Concentration (#/1,000 m^3)**	**2.0 mm WWS Intake Count**	**2.0 mm WWS Intake Mean Concentration (#/1,000 m^3)**
Fish Larvae								
CIQ goby complex	gobies	**5**	2	12.43	1	5.78	2	12.14
Hypsoblennius spp.	combtooth blennies	**3**	-	-	1	5.79	2	11.99
larval fish - damaged	damaged larval fishes	**2**	-	-	2	11.63	-	-
Citharichthys stigmaeus	speckled sanddab	**1**	-	-	-	-	1	6.00
Engraulis mordax	northern anchovy	**1**	-	-	-	-	1	6.00
Gibbonsia spp.	kelpfishes	**1**	1	6.21	-	-	-	-
Gobiesocidae	clingfishes	**1**	1	6.21	-	-	-	-
larvae, yolksac	yolksac larvae	**1**	-	-	1	5.85	-	-
Paralichthys californicus	California halibut	**1**	-	-	1	6.04	-	-
Syngnathidae	pipefishes	**1**	-	-	-	-	1	6.00
	Total Fish Larvae:	**17**	**4**		**6**		**7**	
Fish Eggs								
Pleuronichthys spp. (eggs)	turbot eggs	**929**	320	1,986.90	229	1,333.06	380	2,295.40
fish eggs (early development stage)	fish eggs	**709**	296	1,836.44	231	1,376.79	182	1,093.87
fish eggs	fish eggs	**188**	2	12.40	108	647.42	78	469.31
Citharichthys spp. (eggs)	sanddab eggs	**113**	111	688.00	1	5.79	1	6.03
Labridae (eggs)	wrasse eggs	**17**	-	-	-	-	17	102.29
Paralabrax spp. (eggs)	sand bass eggs	**2**	1	6.21	-	-	1	6.00
Paralichthyidae (eggs)	sand flounder eggs	**2**	2	12.40	-	-	-	-
Sciaenidae (eggs)	croaker eggs	**2**	-	-	-	-	2	12.07
Scomber japonicus (eggs)	Pacific mackerel eggs	**1**	-	-	-	-	1	6.00
	Total Fish Eggs:	**1,963**	**732**		**569**		**662**	
Target Invertebrate Larvae								
Panulirus interruptus (phyllosome)	Calif. spiny lobster (larval)	**5**	-	-	2	11.69	3	18.06
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**1**	1	6.21	-	-	-	-
	Total Invertebrate Larvae:	**6**	**1**		**2**		**3**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-12. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP012 **Date: September 22, 2011**			**UNSCREENED**		**1.0 mm WWS Intake**	**2.0 mm WWS Intake**
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count** **Mean Concentration (#/1,000 m^3)**	
Fish Larvae						
Citharichthys stigmaeus	speckled sanddab	**5**	5	31.08	Not Sampled	Not Sampled
larval fish - damaged	damaged larval fishes	**3**	3	18.65		
Clupeiformes	herrings and anchovies	**1**	1	6.22		
Hypsoblennius spp.	combtooth blennies	**1**	1	6.22		
	Total Fish Larvae:	**10**	**10**			
Fish Eggs						
fish eggs (early development stage)	fish eggs	**843**	843	5,239.21		
Pleuronichthys spp. (eggs)	turbot eggs	**191**	191	1,189.45		
Citharichthys spp. (eggs)	sanddab eggs	**18**	18	111.49		
fish eggs	fish eggs	**16**	16	97.73		
	Total Fish Eggs:	**1,068**	**1,068**			
Target Invertebrate Larvae						
No Invertebrates		**0**	-	-		
	Total Invertebrate Larvae:	**0**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-13. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP013 **Date: October 12, 2011**			**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**	
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**		
Fish Larvae								
Syngnathidae	pipefishes	**3**	1	6.22	-	-	2	12.36
Genyonemus lineatus	white croaker	**2**	-	-	1	6.09	1	6.21
larval fish - damaged	damaged larval fishes	**2**	-	-	2	12.41	-	-
Gibbonsia spp.	kelpfishes	**1**	-	-	-	-	1	6.18
Hypsoblennius spp.	combtooth blennies	**1**	-	-	1	6.14	-	-
Paralichthys californicus	California halibut	**1**	1	6.22	-	-	-	-
	Total Fish Larvae:	**10**	**2**		**4**		**4**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**1,949**	356	2,212.53	636	3,906.33	957	5,929.29
Pleuronichthys spp. (eggs)	turbot eggs	**792**	295	1,836.40	328	2,016.15	169	1,049.39
Citharichthys spp. (eggs)	sanddab eggs	**255**	37	229.04	198	1,205.67	20	123.56
fish eggs	fish eggs	**84**	34	211.24	18	109.85	32	200.66
Sciaenidae (eggs)	croaker eggs	**18**	1	6.20	17	102.86	-	-
Trachurus symmetricus (eggs)	jack mackerel eggs	**17**	-	-	17	102.86	-	-
Paralichthyidae (eggs)	sand flounder eggs	**2**	2	12.39	-	-	-	-
	Total Fish Eggs:	**3,116**	**725**		**1,213**		**1,178**	
Target Invertebrate Larvae								
Metacarcinus anthonyi (megalops)	yellow crab megalops	**2**	1	6.22	1	6.16	-	-
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**2**	2	12.44	-	-	-	-
	Total Invertebrate Larvae:	**4**	**3**		**1**		**0**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-14. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP014 **Date: October 26, 2011**			**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**	
Fish Larvae							
larval fish - damaged	damaged larval fishes	**3**	3	18.67	Not Sampled		Not Sampled
larval/post-larval fish	larval fishes	**2**	2	10.92			
Syngnathidae	pipefishes	**1**	1	6.22			
	Total Fish Larvae:	**6**	**6**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**226**	226	1,372.74			
Engraulidae (eggs)	anchovy eggs	**63**	63	358.51			
fish eggs	fish eggs	**25**	25	155.54			
Citharichthys spp. (eggs)	sanddab eggs	**22**	22	127.80			
Pleuronichthys spp. (eggs)	turbot eggs	**17**	17	105.77			
Paralichthyidae (eggs)	sand flounder eggs	**5**	5	31.11			
Labridae (eggs)	wrasse eggs	**2**	2	12.44			
Labridae/Paralichthyidae (eggs)	fish eggs	**2**	2	12.44			
	Total Fish Eggs:	**363**	**363**				
Target Invertebrate Larvae							
Metacarcinus anthonyi (megalops)	yellow crab megalops	**12**	12	74.66			
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**12**	12	74.65			
Cancridae damaged (megalops)	damaged cancer crab meg.	**4**	4	24.88			
	Total Invertebrate Larvae:	**28**	**28**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-15. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP015 **Date: November 1, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED**		**1.0 mm WWS Intake***		**2.0 mm WWS Intake**
			Count	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**	
Fish Larvae							
Girella nigricans	opaleye	**1**	1	9.38	-	-	Not Lab Processed
larval fish - damaged	damaged larval fishes	**1**	1	9.38	-	-	
	Total Fish Larvae:	**2**	**2**		**0**		
Fish Eggs							
fish eggs (early development stage)	fish eggs	**349**	310	2,910.70	39	1,434.92	
Pleuronichthys spp. (eggs)	turbot eggs	**62**	61	573.45	1	36.79	
fish eggs	fish eggs	**19**	17	155.35	2	73.59	
Paralichthyidae (eggs)	sand flounder eggs	**16**	16	151.85	-		
Citharichthys spp. (eggs)	sanddab eggs	**16**	16	146.00	-		
Engraulidae (eggs)	anchovy eggs	**13**	13	123.81	-		
Scomber japonicus (eggs)	Pacific mackerel eggs	**1**	1	9.35	-		
	Total Fish Eggs:	**476**	**434**		**42**		
Target Invertebrate Larvae							
Metacarcinus anthonyi (megalops)	yellow crab megalops	**4**	4	37.53	-	-	
	Total Invertebrate Larvae:	**4**	**4**		**0**		

Some samples may have been split. Count and concentration are based on adjusted split count.

* Only one out of four samples was lab processed

Table D1-16. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP016 **Date: November 21, 2011**			**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**	
Fish Larvae							
Citharichthys sordidus	Pacific sanddab	**1**	1	6.01	Not Sampled		Not Sampled
	Total Fish Larvae:	**1**	**1**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**1,165**	1,165	7,174.40			
Citharichthys spp. (eggs)	sanddab eggs	**19**	19	116.71			
Pleuronichthys spp. (eggs)	turbot eggs	**8**	8	49.17			
fish eggs	fish eggs	**2**	2	12.02			
	Total Fish Eggs:	**1,194**	**1,194**				
Target Invertebrate Larvae							
Metacarcinus anthonyi (megalops)	yellow crab megalops	**2**	2	12.33			
	Total Invertebrate Larvae:	**2**	**2**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-17. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP017 **Date: December 8, 2011** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** **Count**	**Mean Concentration (#/1,000 m^3)**	**1.0 mm WWS Intake** **Count**	**Mean Concentration (#/1,000 m^3)**	**2.0 mm WWS Intake**
Fish Larvae							
Citharichthys stigmaeus	speckled sanddab	**2**	2	19.80	Not Lab Processed		Not Lab Processed
Citharichthys sordidus	Pacific sanddab	**1**	1	9.96			
Hypsoblennius spp.	combtooth blennies	**1**	1	9.94			
larvae, yolksac	yolksac larvae	**1**	1	9.96			
larval fish - damaged	damaged larval fishes	**1**	1	9.95			
	Total Fish Larvae:	**6**	**6**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**473**	473	4,700.78			
Pleuronichthys spp. (eggs)	turbot eggs	**188**	188	1,869.11			
Citharichthys spp. (eggs)	sanddab eggs	**138**	138	1,368.03			
Paralichthyidae (eggs)	sand flounder eggs	**78**	78	772.48			
fish eggs	fish eggs	**58**	58	571.21			
Sciaenidae/Paralichthyidae (eggs)	fish eggs	**23**	23	226.82			
Genyonemus lineatus (eggs)	white croaker eggs	**6**	6	59.77			
	Total Fish Eggs:	**963**	**963**				
Target Invertebrate Larvae							
No Invertebrates		**0**	-	-			
	Total Invertebrate Larvae:	**0**					

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-18. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP018 **Date: January 12, 2012** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** **Count**	**Mean Concentration (#/1,000 m^3)**	**1.0 mm WWS Intake** **Count**	**Mean Concentration (#/1,000 m^3)**	**2.0 mm WWS Intake**
Fish Larvae							
Gibbonsia spp.	kelpfishes	**1**	1	8.47	Not Sampled		Not Sampled
	Total Fish Larvae:	**1**	**1**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**522**	522	4,578.68			
Engraulidae (eggs)	anchovy eggs	**40**	40	357.28			
Pleuronichthys spp. (eggs)	turbot eggs	**16**	16	140.68			
Citharichthys spp. (eggs)	sanddab eggs	**10**	10	89.24			
fish eggs	fish eggs	**3**	3	26.74			
Paralichthyidae (eggs)	sand flounder eggs	**2**	2	17.28			
	Total Fish Eggs:	**594**	**594**				
Target Invertebrate Larvae							
No Invertebrates		**0**	-	-			
	Total Invertebrate Larvae:	**0**					

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-19. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP019 **Date: January 18, 2012**			**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**	
Fish Larvae							
Genyonemus lineatus	white croaker	**1**	1	6.16	Not Lab Processed		Not Lab Processed
	Total Fish Larvae:	**1**	**1**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**649**	649	4,004.93			
fish eggs	fish eggs	**26**	26	159.83			
Citharichthys spp. (eggs)	sanddab eggs	**22**	22	135.44			
Pleuronichthys spp. (eggs)	turbot eggs	**21**	21	128.89			
Sciaenidae (eggs)	croaker eggs	**6**	6	36.80			
Paralichthyidae (eggs)	sand flounder eggs	**1**	1	6.21			
Sciaenidae/Paralichthyidae (eggs)	fish eggs	**1**	1	6.17			
	Total Fish Eggs:	**726**	**726**				
Target Invertebrate Larvae							
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**1**	1	6.16			
	Total Invertebrate Larvae:	**1**	**1**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-20. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP020 **Date: January 26, 2012** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
Cottidae	sculpins	**4**	4	37.62	Not Sampled		Not Sampled
larval fish - damaged	damaged larval fishes	**4**	4	37.62			
Genyonemus lineatus	white croaker	**2**	2	18.81			
Gibbonsia spp.	kelpfishes	**2**	2	17.79			
Citharichthys stigmaeus	speckled sanddab	**1**	1	9.41			
	Total Fish Larvae:	**13**	**13**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**394**	394	3,656.62			
Pleuronichthys spp. (eggs)	turbot eggs	**122**	122	1,129.34			
Citharichthys spp. (eggs)	sanddab eggs	**11**	11	99.27			
fish eggs	fish eggs	**6**	6	56.40			
Pleuronectidae (eggs)	righteye flounder eggs	**1**	1	9.41			
	Total Fish Eggs:	**534**	**534**				
Target Invertebrate Larvae							
No Invertebrates		0	-	-			
	Total Invertebrate Larvae:	**0**					

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-21. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP021 **Date: February 6, 2012**			**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**	
Taxon	**Common Name**	**Total Count**	**Count**	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**		
Fish Larvae								
Clupeidae	herrings	**8**	-	-	Not Sampled		8	49.07
Genyonemus lineatus	white croaker	**4**	3	18.60			1	6.18
larvae, yolksac	yolksac larvae	**4**	3	18.66			1	6.19
Atherinopsidae	silversides	**1**	-	-			1	6.10
Paralichthys californicus	California halibut	**1**	1	6.17			-	-
	Total Fish Larvae:	**18**	**7**				**11**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**1,473**	655	4,068.80			818	5,024.89
Citharichthys spp. (eggs)	sanddab eggs	**283**	79	493.85			204	1,246.46
Pleuronichthys spp. (eggs)	turbot eggs	**233**	142	879.55			91	563.95
fish eggs	fish eggs	**127**	74	461.52			53	324.96
Engraulidae (eggs)	anchovy eggs	**109**	45	280.69			64	395.75
Clupeidae (eggs)	herring (eggs)	**58**	4	24.67			54	327.97
Paralichthyidae (eggs)	sand flounder eggs	**11**	9	55.48			2	12.38
Sciaenidae/Paralichthyidae (eggs)	fish eggs	**5**	5	29.53			-	-
	Total Fish Eggs:	**2,298**	**1,013**				**1,285**	
Target Invertebrate Larvae								
Cancridae, damaged (megalops)	damaged cancer crab meg.	**2**	-	-			2	12.28
	Total Invertebrate Larvae:	**2**	**0**				**2**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-22. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP022 **Date: February 22, 2012** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
larval fish - damaged	damaged larval fishes	**1**	1	8.60	Not Sampled		Not Sampled
Sciaenidae	croakers	**1**	1	8.60			
	Total Fish Larvae:	**2**	**2**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**583**	583	4,993.80			
Citharichthys spp. (eggs)	sanddab eggs	**83**	83	719.44			
fish eggs	fish eggs	**44**	44	383.37			
Pleuronichthys spp. (eggs)	turbot eggs	**39**	39	326.79			
Engraulidae (eggs)	anchovy eggs	**10**	10	86.15			
Pleuronectidae (eggs)	righteye flounder eggs	**2**	2	17.27			
	Total Fish Eggs:	**761**	**761**				
Target Invertebrate Larvae							
Cancridae (megalops)	cancer crabs megalops	**2**	2	17.20			
	Total Invertebrate Larvae:	**2**	**2**				

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-23. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP023 **Date: April 3, 2012** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED**		**1.0 mm WWS Intake**		**2.0 mm WWS Intake**	
			Count	**Mean Concentration (#/1,000 m^3)**	**Count**	**Mean Concentration (#/1,000 m^3)**		
Fish Larvae								
fish, damaged	damaged fish	**4**	4	23.58	-	-	-	-
Typhlogobius californiensis	blind goby	**2**	-	-	1	6.08	1	6.13
Atherinopsis californiensis	jacksmelt	**1**	1	6.09	-	-	-	-
CIQ goby complex	gobies	**1**	-	-	-	-	1	6.13
Genyonemus lineatus	white croaker	**1**	-	-	1	5.70	-	-
Parophrys vetulus	English sole	**1**	-	-	1	6.07	-	-
Ruscarius creaseri	roughcheek sculpin	**1**	-	-	-	-	1	6.14
Stenobrachius leucopsarus	northern lampfish	**1**	-	-	1	5.89	-	-
Syngnathidae	pipefishes	**1**	1	6.14	-	-	-	-
	Total Fish Larvae:	**13**	**6**		**4**		**3**	
Fish Eggs								
fish eggs (early development stage)	fish eggs	**2,810**	1,391	8,310.46	893	5,364.84	526	3,208.45
Citharichthys spp. (eggs)	sanddab eggs	**353**	98	585.70	183	1,050.28	72	441.65
Pleuronichthys spp. (eggs)	turbot eggs	**83**	32	188.70	15	90.82	36	217.04
fish eggs	fish eggs	**73**	23	134.97	22	136.16	27	166.80
Paralichthyidae (eggs)	sand flounder eggs	**17**	6	33.19	10	60.66	1	8.22
Sciaenidae (eggs)	croaker eggs	**8**	2	10.50	2	13.61	3	21.01
Platichthys stellatus (eggs)	stary flounder eggs	**1**	-	-	-	-	1	6.14
	Total Fish Eggs:	**3,345**	**1,552**		**1,126**		**667**	
Target Invertebrate Larvae								
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**108**	94	553.27	-	-	14	85.62
Cancridae (megalops)	cancer crabs megalops	**12**	11	63.48	-	-	1	6.13
Metacarcinus anthonyi (megalops)	yellow crab megalops	**8**	5	29.91	-	-	3	18.31
Cancridae damaged (megalops)	damaged cancer crab	**2**	1	5.89	-	-	1	6.09
	Total Invertebrate Larvae:	**130**	**111**		**0**		**19**	

Some samples may have been split. Count and concentration are based on adjusted split count.

Table D1-24. Unscreened Boat, 1.0 mm and 2.0 mm WWS Intake Onshore Pump Stations Larval Counts and Mean Concentrations (#/1,000 m^3).

Survey: WBP024 **Date: March 22, 2012** **Taxon**	**Common Name**	**Total Count**	**UNSCREENED** Count	**UNSCREENED** Mean Concentration (#/1,000 m^3)	**1.0 mm WWS Intake** Count	**1.0 mm WWS Intake** Mean Concentration (#/1,000 m^3)	**2.0 mm WWS Intake**
Fish Larvae							
Genyonemus lineatus	white croaker	**2**	2	17.91	Not Sampled		Not Sampled
Citharichthys stigmaeus	speckled sanddab	**1**	1	8.72			
	Total Fish Larvae:	**3**	**3**				
Fish Eggs							
fish eggs (early development stage)	fish eggs	**363**	363	3,146.50			
Pleuronichthys spp. (eggs)	turbot eggs	**107**	107	953.51			
Citharichthys spp. (eggs)	sanddab eggs	**24**	24	200.22			
fish eggs	fish eggs	**13**	13	106.39			
Pleuronectidae (eggs)	righteye flounder eggs	**9**	9	76.84			
Paralichthyidae (eggs)	sand flounder eggs	**8**	8	72.14			
Sciaenidae (eggs)	croaker eggs	**2**	2	15.90			
Vinciguerria lucetia (eggs)	Panama lightfish eggs	**2**	2	16.05			
Platichthys stellatus (eggs)	stary flounder eggs	**1**	1	8.10			
Sciaenidae/Paralichthyidae (eggs)	fish eggs	**1**	1	8.72			
	Total Fish Eggs:	**530**	**530**				
Target Invertebrate Larvae							
Romal. anten./Metacar. grac. (meg.)	cancer crabs	**49**	49	430.54			
Metacarcinus anthonyi (megalops)	yellow crab megalops	**2**	2	17.91			
Cancridae (megalops)	cancer crabs megalops	**1**	1	8.95			
	Total Invertebrate Larvae:	**52**	**52**				

Some samples may have been split. Count and concentration are based on adjusted split count.

D2. Pump Stations Larval Fish Lengths

Table D2-1: Pump Stations Larval Fish Lengths.

Survey: WBP001
Date: March 31, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Typhlogobius californiensis	blind goby	8	6	2.68-3.01	2.84
larval fish - damaged	damaged larval fishes	4	0	-	-
Ruscarius creaseri	roughcheek sculpin	4	4	2.17-2.80	2.57
Genyonemus lineatus	white croaker	3	1	3.98	3.98
Stenobrachius leucopsarus	northern lampfish	2	0	-	-
Artedius lateralis	smoothhead sculpin	1	1	2.29	2.29
Cottidae	sculpins	1	0	-	-
Gibbonsia spp.	kelpfishes	1	1	4.56	4.56
Ruscarius meanyi	Puget Sound sculpin	1	1	2.61	2.61
		Total: 25	**14**		

Table D2-2: Pump Stations Larval Fish Lengths.

Survey: WBP002
Date: April 14, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
No Fish		0	0	-	-
		Total: 0	**0**		

Table D2-3: Pump Stations Larval Fish Lengths.

Survey: WBP003
Date: May 5, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Cottidae	sculpins	4	4	2.34-2.50	2.40
larval fish - damaged	damaged larval fishes	3	0	-	-
Chromis punctipinnis	blacksmith	1	1	2.31	2.31
Pleuronichthys spp.	turbots	1	0	-	-
Sciaenidae	croakers	1	0	-	-
		Total: 10	**5**		

Table D2-4: Pump Stations Larval Fish Lengths.

Survey: WBP004
Date: May 25, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Atherinopsidae	silversides	1	0	-	-
larvae, yolksac	yolksac larvae	1	0	-	-
Scorpaenichthys marmoratus	cabezon	1	0	-	-
		Total: 3	**0**		

Table D2-5: Pump Stations Larval Fish Lengths.

Survey: WBP005
Date: June 9, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Hypsoblennius spp.	combtooth blennies	18	18	1.88-2.53	2.18
larval fish - damaged	damaged larval fishes	8	0	-	-
Oxylebius pictus/Zanio.frenata	painted green./shortspine comb.	4	0	-	-
Ruscarius creaseri	roughcheek sculpin	4	4	2.25-2.64	2.44
Cottidae	sculpins	2	0	-	-
Gibbonsia spp.	kelpfishes	1	1	4.42	4.42
Gobiesox spp.	clingfishes	1	1	3.11	3.11
		Total: 38	**24**		

Table D2-6: Pump Stations Larval Fish Lengths.

Survey: WBP006
Date: June 21, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Hypsoblennius spp.	combtooth blennies	7	7	1.95-2.54	2.32
larval fish - damaged	damaged larval fishes	3	0	-	-
Hypsypops rubicundus	garibaldi	2	0	-	-
Gibbonsia spp.	kelpfishes	1	0	-	-
Syngnathidae	pipefishes	1	1	9.77	9.77
		Total: 14	**8**		

Table D2-7: Pump Stations Larval Fish Lengths.

Survey: WBP007
Date: July 19, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
larval fish - damaged	damaged larval fishes	3	0	-	-
Gobiesox spp.	clingfishes	2	2	2.80-2.96	2.88
Gibbonsia spp.	kelpfishes	1	1	4.03	4.03
Hypsoblennius spp.	combtooth blennies	1	1	1.94	1.94
		Total: 7	**4**		

Table D2-8: Pump Stations Larval Fish Lengths.

Survey: WBP008
Date: July 28, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
No Fish		0	0	-	-
		Total: 0	**0**		

Table D2-9: Pump Stations Larval Fish Lengths.

Survey: WBP009
Date: August 9, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Hypsoblennius spp.	combtooth blennies	7	7	1.80-2.31	1.98
Gibbonsia spp.	kelpfishes	1	0	-	-
		Total: 8	**7**		

Table D2-10: Pump Stations Larval Fish Lengths.

Survey: WBP010
Date: August 22, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
No Fish		0	0	-	-
		Total: 0	**0**		

Table D2-11: Pump Stations Larval Fish Lengths.

Survey: WBP011
Date: September 6, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
CIQ goby complex	gobies	5	2	2.53-2.87	2.70
Hypsoblennius spp.	combtooth blennies	3	2	2.01-2.15	2.08
larval fish - damaged	damaged larval fishes	2	0	-	-
Citharichthys stigmaeus	speckled sanddab	1	0	-	-
Engraulis mordax	northern anchovy	1	0	-	-
Gibbonsia spp.	kelpfishes	1	1	6.84	6.84
Gobiesocidae	clingfishes	1	0	-	-
larvae, yolksac	yolksac larvae	1	0	-	-
Paralichthys californicus	California halibut	1	0	-	-
Syngnathidae	pipefishes	1	1	8.17	8.17
		Total: 17	**6**		

Table D2-12: Pump Stations Larval Fish Lengths.

Survey: WBP012
Date: September 22, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Citharichthys stigmaeus	speckled sanddab	5	1	1.21	1.21
larval fish - damaged	damaged larval fishes	3	0	-	-
Clupeiformes	herrings and anchovies	1	0	-	-
Hypsoblennius spp.	combtooth blennies	1	1	2.19	2.19
		Total: 10	**2**		

Table D2-13: Pump Stations Larval Fish Lengths.

Survey: WBP013
Date: October 12, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Syngnathidae	pipefishes	3	2	7.34-8.54	7.94
Genyonemus lineatus	white croaker	2	1	2.32	2.32
larval fish - damaged	damaged larval fishes	2	0	-	-
Gibbonsia spp.	kelpfishes	1	1	4.30	4.30
Hypsoblennius spp.	combtooth blennies	1	0	-	-
Paralichthys californicus	California halibut	1	1	1.92	1.92
		Total: 10	**5**		

Table D2-14: Pump Stations Larval Fish Lengths.

Survey: WBP014
Date: October 26, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
larval fish - damaged	damaged larval fishes	3	0	-	-
larval/post-larval fish	larval fishes	2	0	-	-
Syngnathidae	pipefishes	1	0	-	-
		Total: 6	**0**		

Table D2-15: Pump Stations Larval Fish Lengths.

Survey: WBP015
Date: November 1, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Girella nigricans	opaleye	1	1	1.66	1.66
larval fish - damaged	damaged larval fishes	1	0	-	-
		Total: 2	**1**		

Table D2-16: Pump Stations Larval Fish Lengths.

Survey: WBP016
Date: November 21, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Citharichthys sordidus	Pacific sanddab	1	1	2.93	2.93
		Total: 1	**1**		

Table D2-17: Pump Stations Larval Fish Lengths.

Survey: WBP017
Date: December 8, 2011

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Citharichthys stigmaeus	speckled sanddab	2	0	-	-
Citharichthys sordidus	Pacific sanddab	1	0	-	-
Hypsoblennius spp.	combtooth blennies	1	1	2.14	2.14
larvae, yolksac	yolksac larvae	1	0	-	-
larval fish - damaged	damaged larval fishes	1	0	-	-
		Total: 6	**1**		

Table D2-18: Pump Stations Larval Fish Lengths.

Survey: WBP018
Date: January 12, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Gibbonsia spp.	kelpfishes	1	0	-	-
		Total: 1	**0**		

Table D2-19: Pump Stations Larval Fish Lengths.

Survey: WBP019
Date: January 18, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Genyonemus lineatus	white croaker	1	0	-	-
		Total: 1	**0**		

Table D2-20: Pump Stations Larval Fish Lengths.

Survey: WBP020
Date: January 26, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Cottidae	sculpins	4	0	-	-
larval fish - damaged	damaged larval fishes	4	0	-	-
Genyonemus lineatus	white croaker	2	0	-	-
Gibbonsia spp.	kelpfishes	2	1	5.53	5.53
Citharichthys stigmaeus	speckled sanddab	1	0	-	-
		Total: 13	**1**		

Table D2-21: Pump Stations Larval Fish Lengths.

Survey: WBP021
Date: February 6, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Clupeidae	herrings	8	3	1.21-1.41	1.32
Genyonemus lineatus	white croaker	4	3	2.38-3.01	2.73
larvae, yolksac	yolksac larvae	4	0	-	-
Atherinopsidae	silversides	1	0	-	-
Paralichthys californicus	California halibut	1	1	2.39	2.39
		Total: 18	**7**		

Table D2-22: Pump Stations Larval Fish Lengths.

Survey: WBP022
Date: February 22, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
larval fish - damaged	damaged larval fishes	1	0	-	-
Sciaenidae	croakers	1	1	6.15	6.15
		Total: 2	**1**		

Table D2-23: Pump Stations Larval Fish Lengths.

Survey: WBP023
Date: April 3, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
fish, damaged	damaged fish	4	0	-	-
Typhlogobius californiensis	blind goby	2	2	2.59-2.65	2.62
Atherinopsis californiensis	jacksmelt	1	1	8.76	8.76
CIQ goby complex	gobies	1	0	-	-
Genyonemus lineatus	white croaker	1	1	2.39	2.39
Parophrys vetulus	English sole	1	0	-	-
Ruscarius creaseri	roughcheek sculpin	1	1	2.21	2.21
Stenobrachius leucopsarus	northern lampfish	1	1	2.70	2.70
Syngnathidae	pipefishes	1	1	11.11	11.11
		Total: 13	**7**		

Table D2-24: Pump Stations Larval Fish Lengths.

Survey: WBP024
Date: March 22, 2012

Taxon	Common Name	Total Count	Measured Count	Length Range (mm)	Average Length (mm)
Genyonemus lineatus	white croaker	2	0	-	-
Citharichthys stigmaeus	speckled sanddab	1	1	2.37	2.37
		Total: 3	**1**		

West Basin Municipal Water District
Demonstration Desalination Facility

Appendix E

Regression Plots from Intake Screening Technology Support Studies: Morphology of Larval Fish Head Capsules. Tenera. 2011

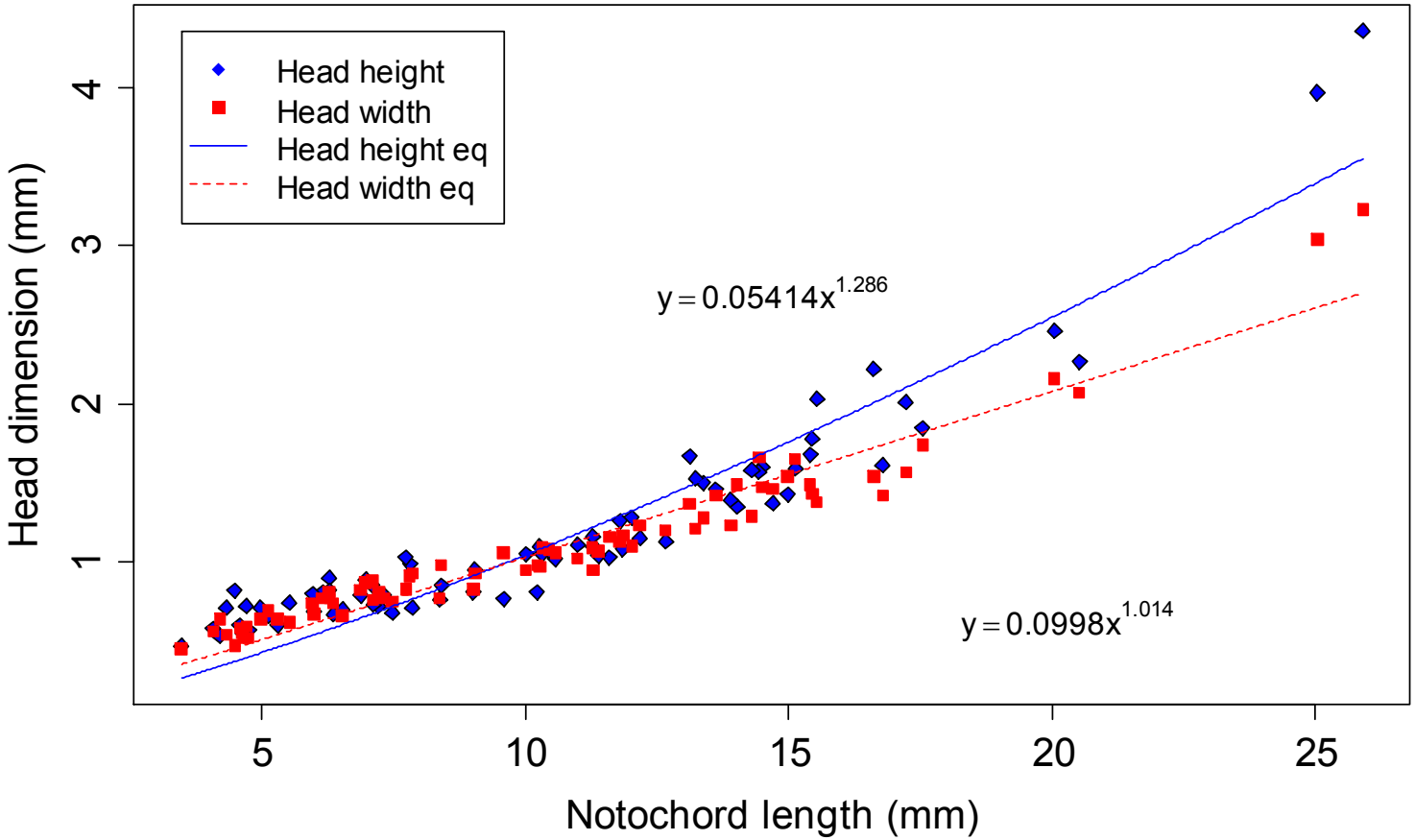


Figure 1. Kelpfishes (*Gibbonsia* spp.) allometric regression plots.

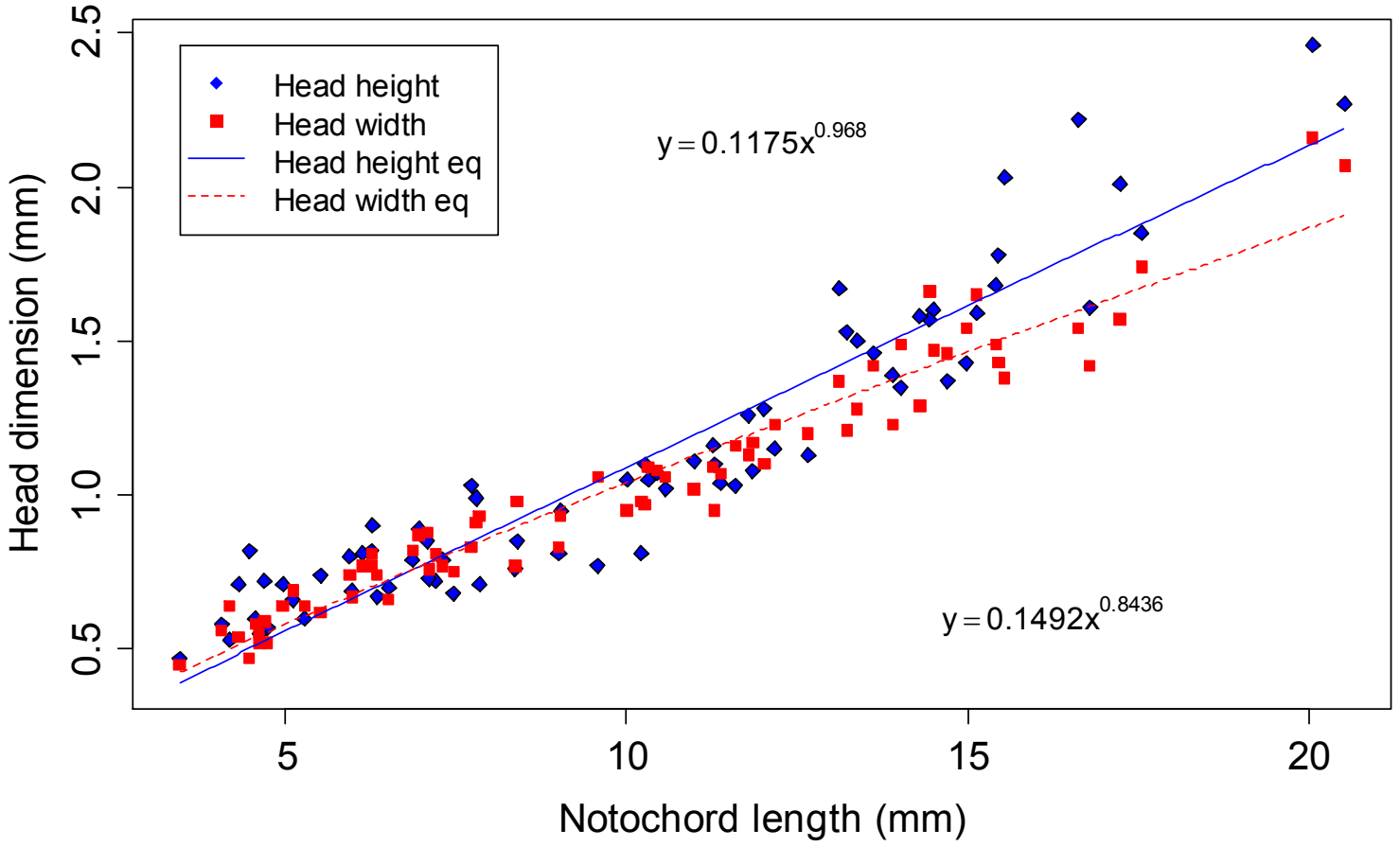


Figure 2. Kelpfishes (*Gibbonsia* spp.) allometric regression plots for fish smaller than 21 mm notochord length.

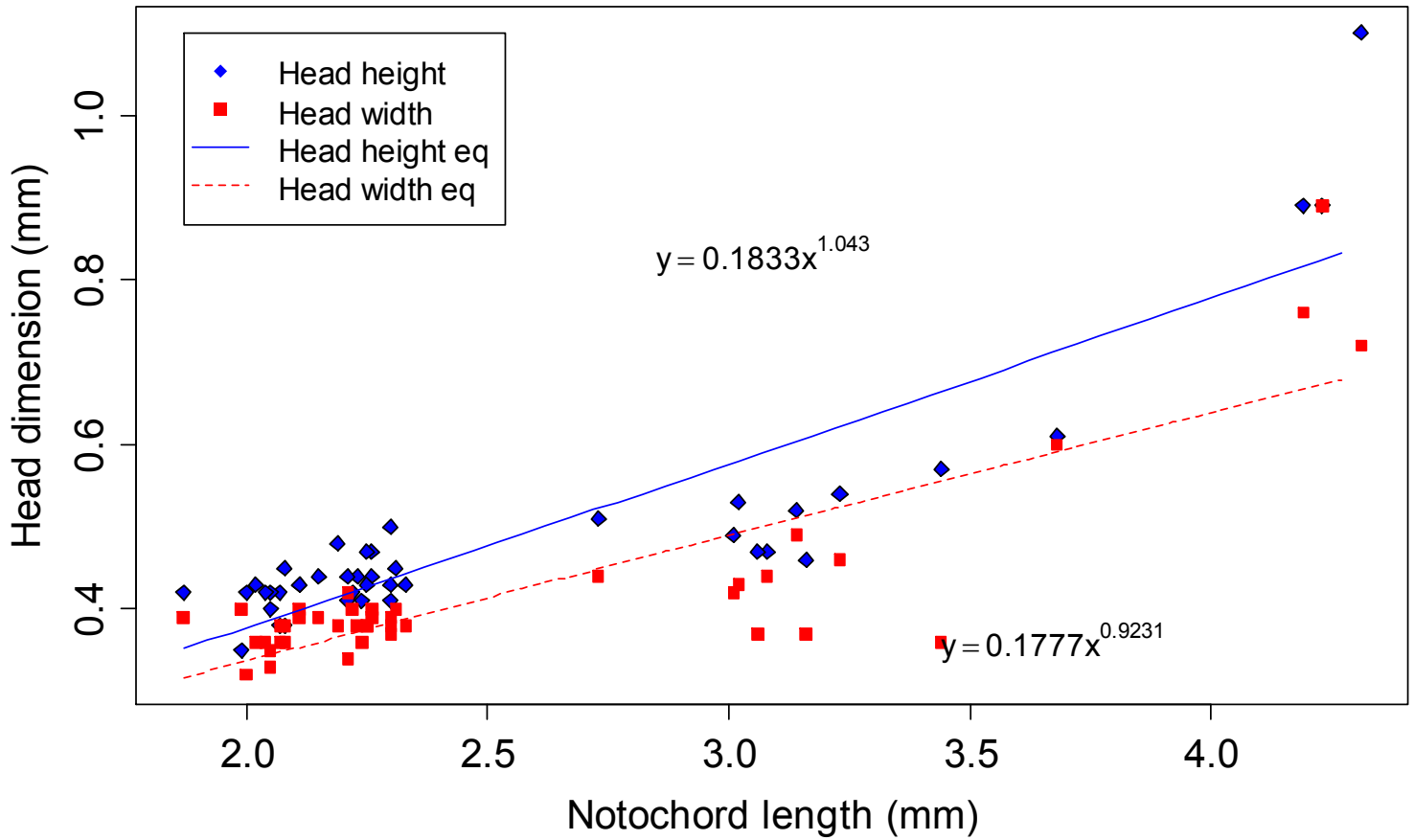


Figure 6. Combtooth blennies (*Hypsoblennius* spp.) allometric regression plots.

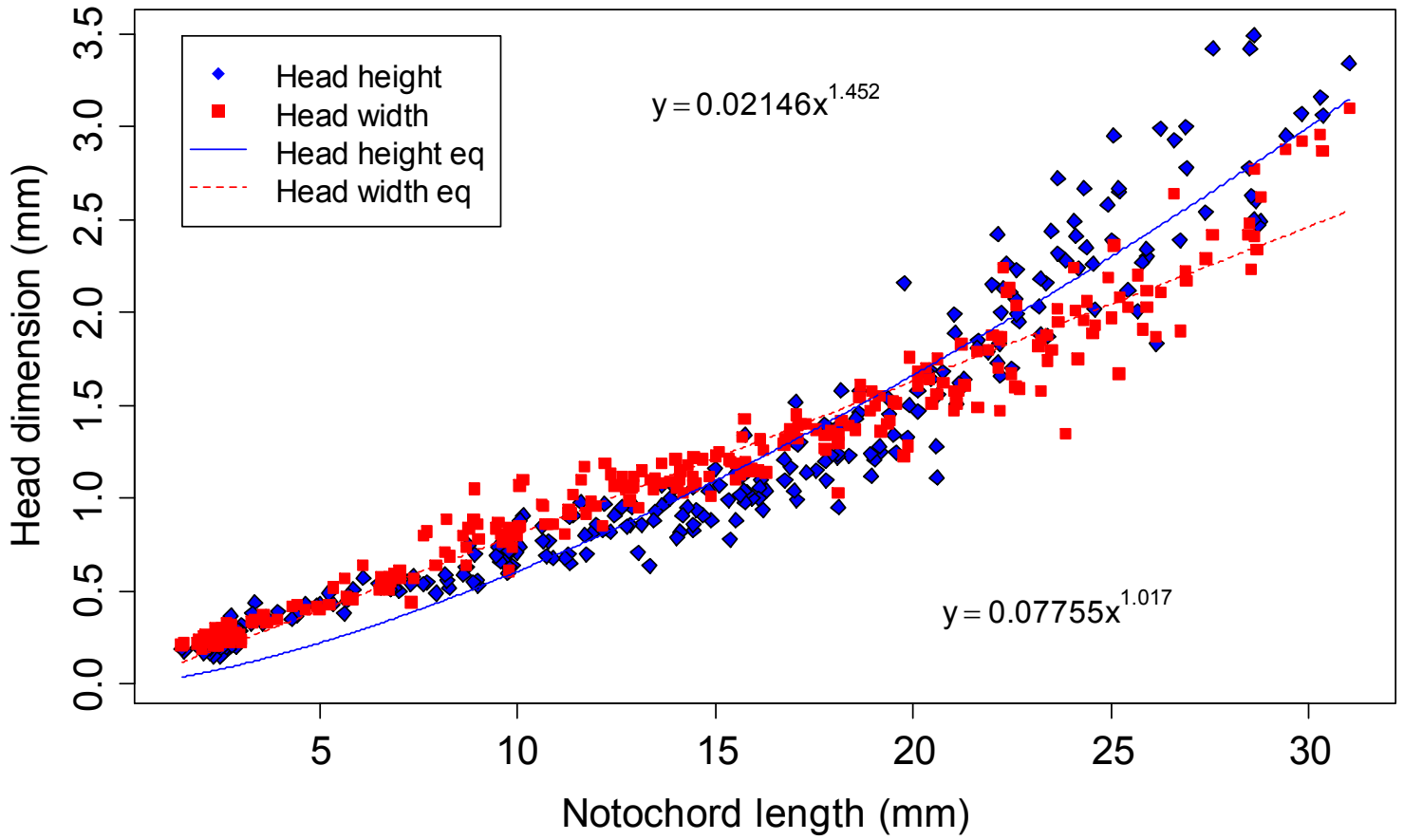


Figure 8. Anchovies (Engraulidae and *Engraulis mordax*) allometric regression plots.

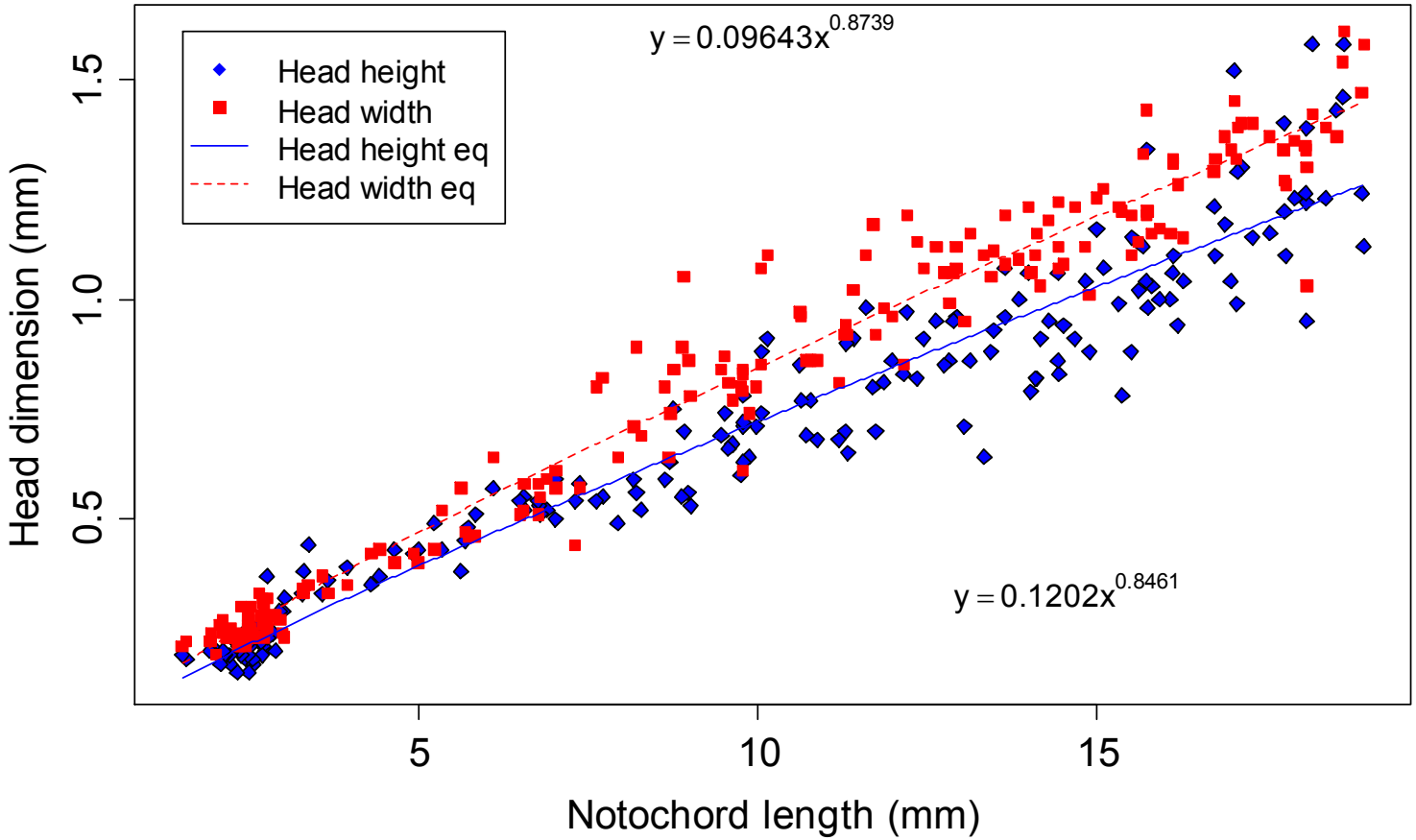


Figure 9. Anchovies (Engraulidae and *Engraulis mordax*) allometric regression plots for fish less than or equal to 19 mm notochord length.

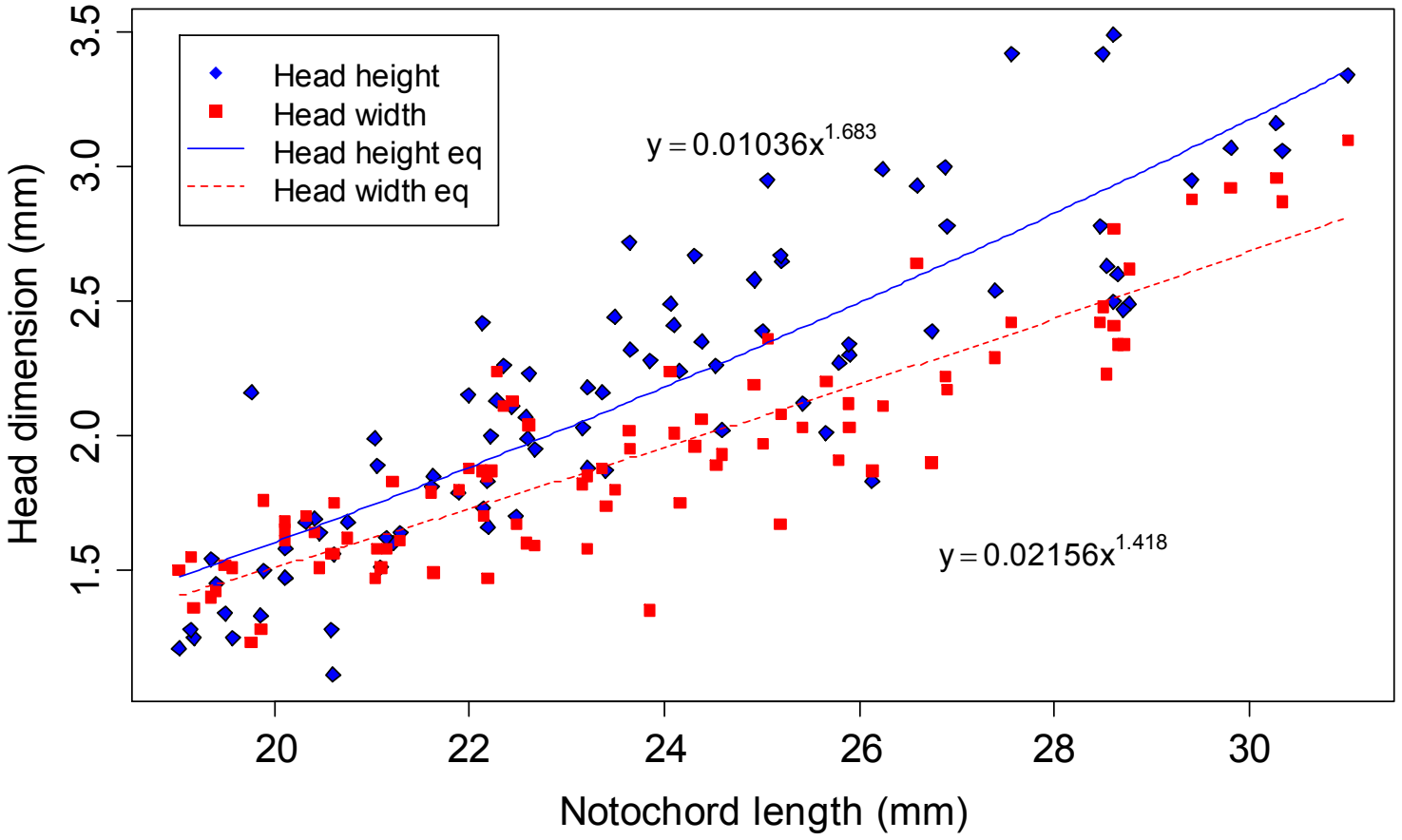


Figure 10. Anchovies (Engraulidae and *Engraulis mordax*) allometric regression plots for fish equal to or larger than 19 mm notochord length.

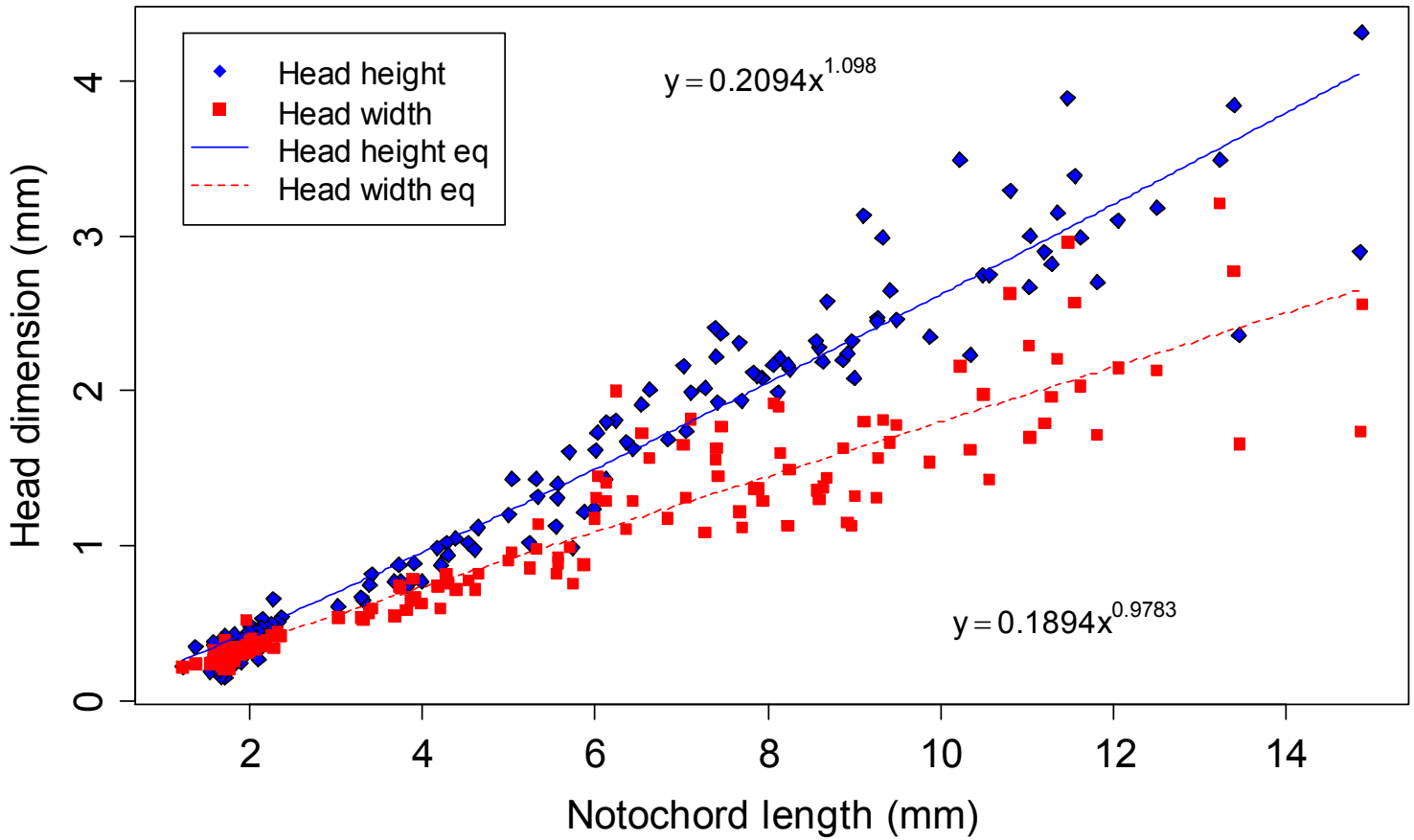


Figure 11. Croakers (*Seriphus politus* and *Genyonemus lineatus*) allometric regression plots.

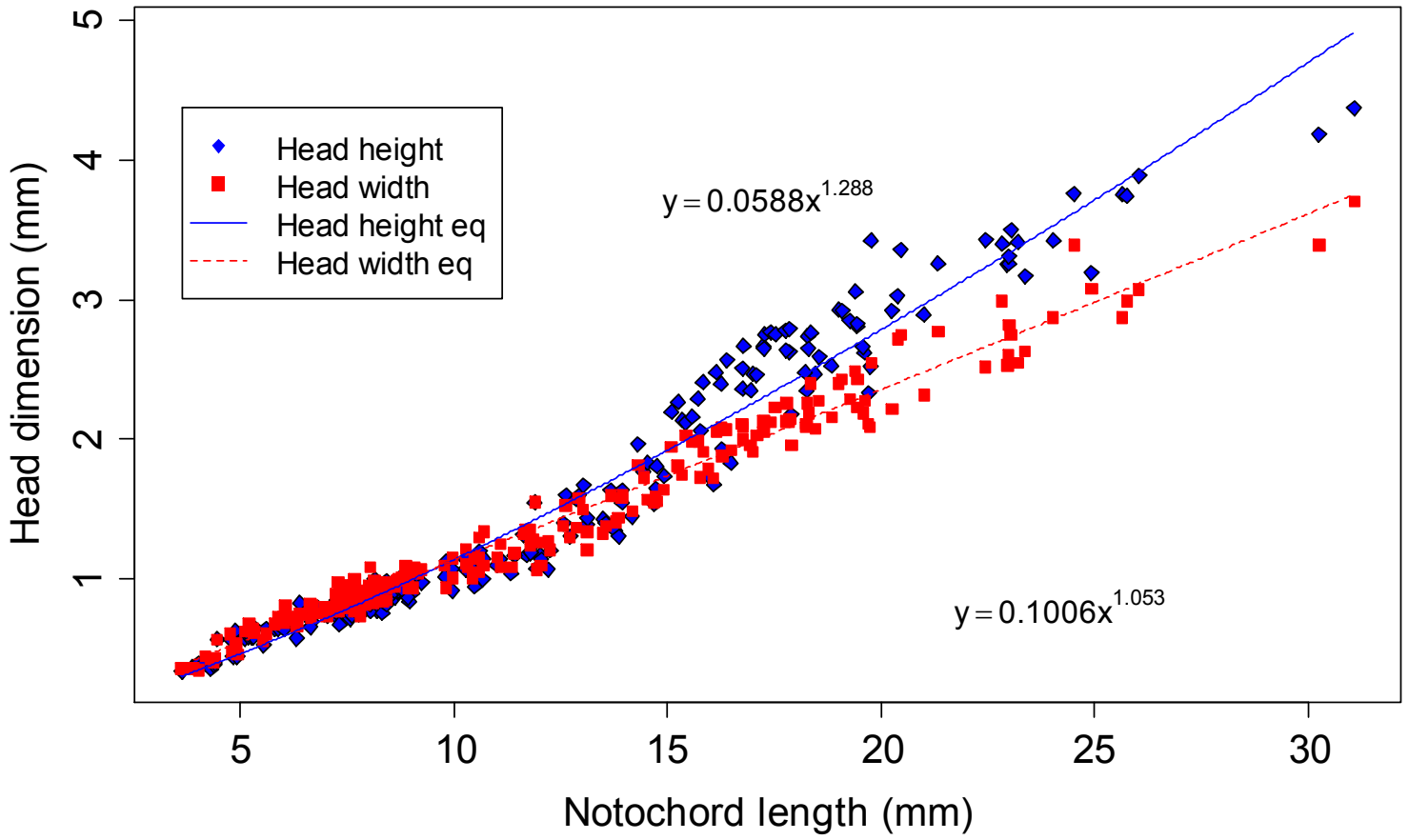


Figure 13. Silversides (Atherinopsidae, *Atherinopsis californiensis, Atherinops affinis, and Leuresthes tenuis*) allometric regression plots.

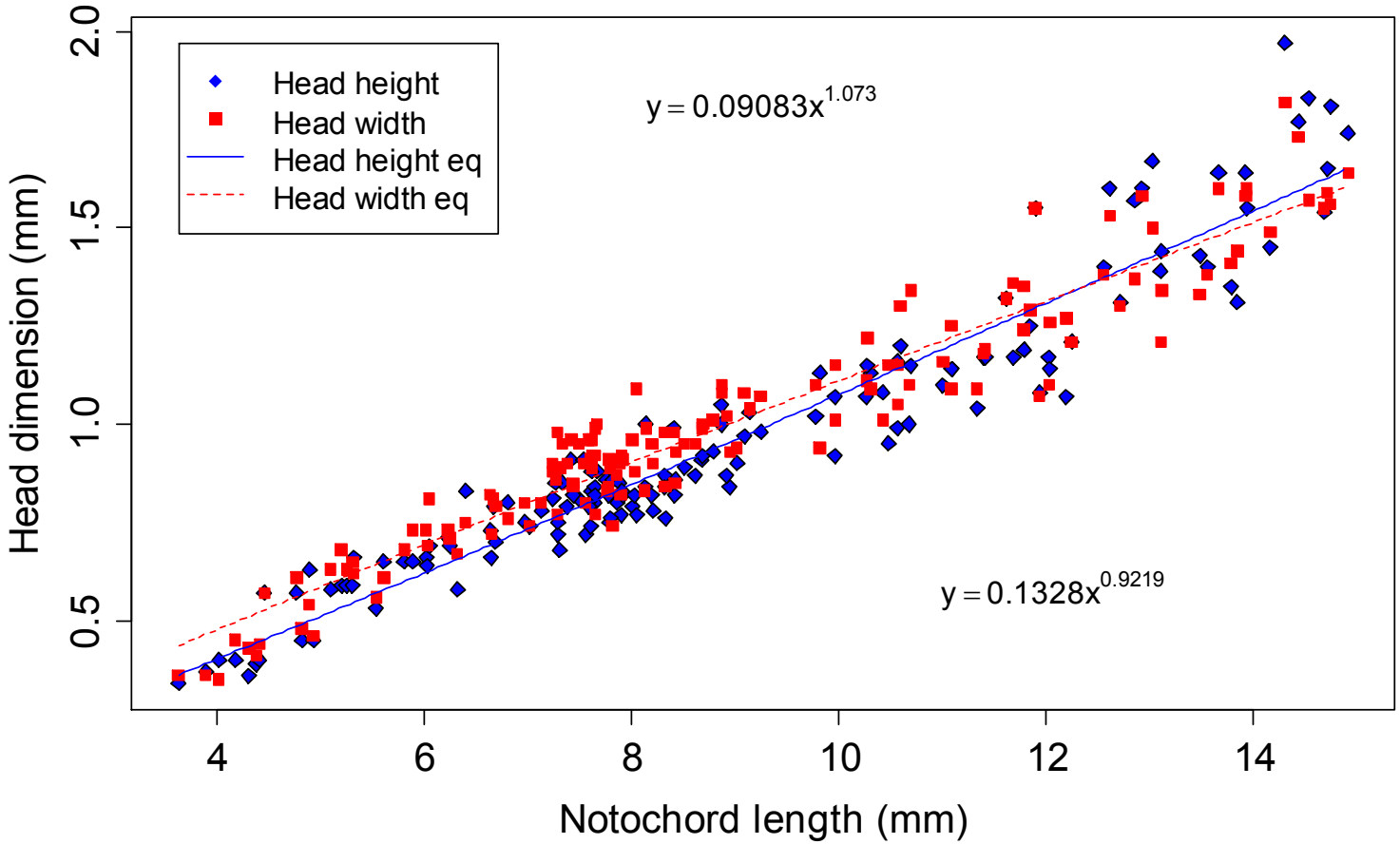


Figure 14. Silversides (Atherinopsidae, *Atherinopsis californiensis, Atherinops affinis, and Leuresthes tenuis*) allometric regression plots for fish smaller than 15 mm notochord length.

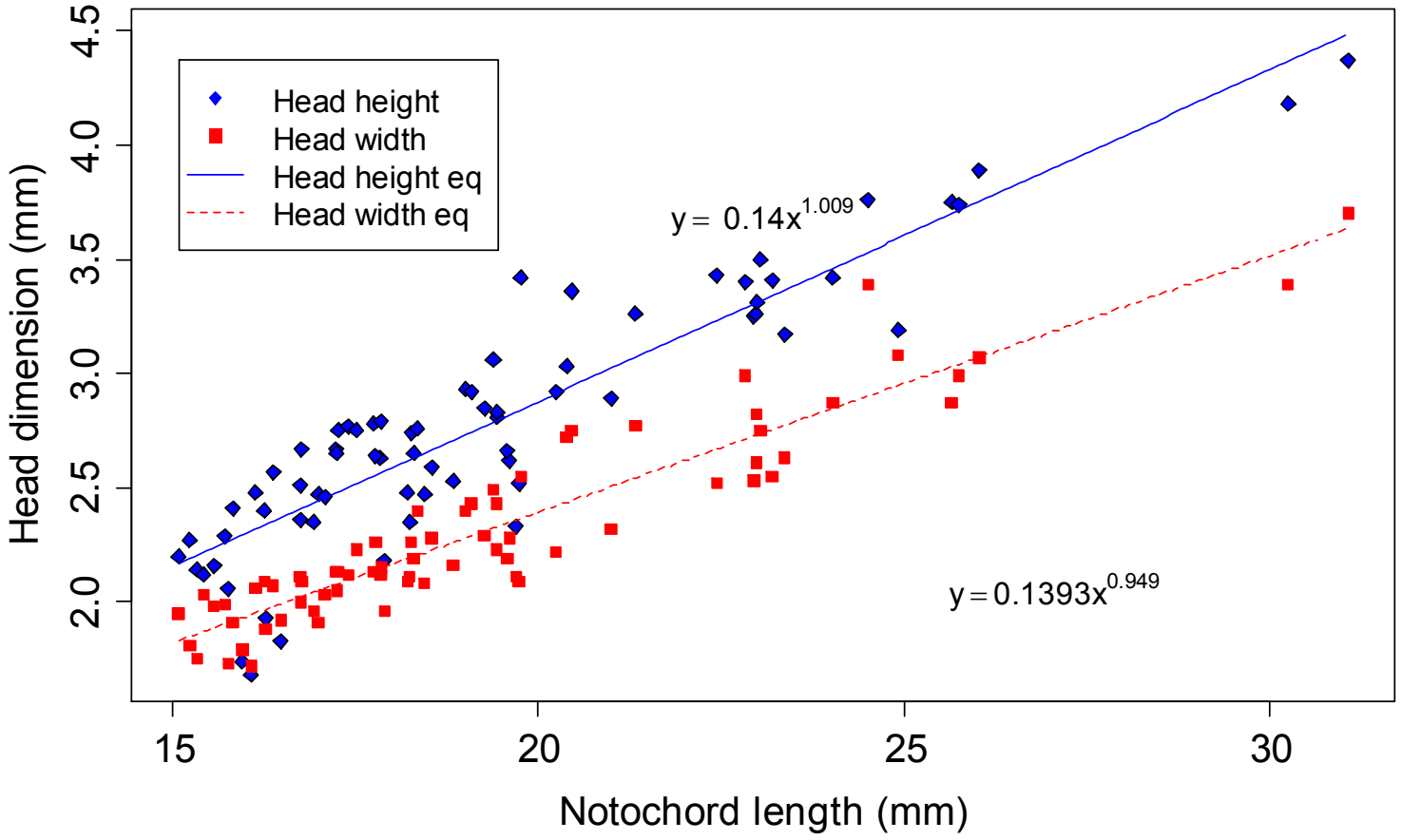


Figure 15. Silversides (Atherinopsidae, *Atherinopsis californiensis, Atherinops affinis, and Leuresthes tenuis*) allometric regression plots for fish larger than 15 mm notochord length.

West Basin Municipal Water District
Demonstration Desalination Facility

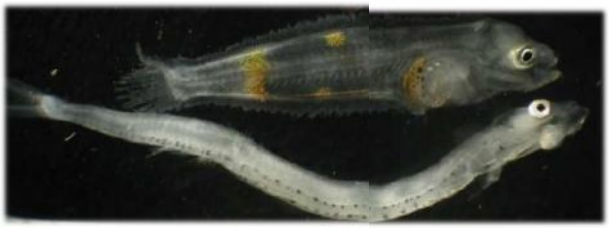
Appendix F

Intake Effects Assessment Study Plan

F1. Field Plankton Sampling Procedures

F2. Tenera Laboratory Procedures

Standard Operating Procedures for Zooplankton Collection



TENERA Environmental

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Attachments

INTRODUCTION

Zooplankton encompass an array of macro and microscopic animals and includes haloplankton, meroplankton, and ichthyoplankton. Zooplankton species play a vital role in the marine food chain. The herbivorous zooplankton feed on phytoplankton and in turn constitute an important food item to animals in higher trophic levels, including fish. The occurrence and abundance of ichthyoplankton (fish eggs and fish larvae) facilitate the location of probable spawning and nursery ground of fishes. Zooplankton are ubiquitous. The success of zooplankton estimation and productivity largely depends upon the use of correct methodology which involves collection of samples, fixation, sorting, identification, length analysis, and preservation. The detailed procedures on field sample collection and fixation are given in this manual.

RESPONSIBILITIES

The Project Manager is responsible for:

- verifying that all staff working on the project have read and understand these procedures;
- ensuring adherence to all vessel requirements (safety, etc.) during surveys;
- designing and implementing the Quality Assurance/Quality Control Program in accordance with written procedures;
- maintaining these procedures and ensuring that any approved changes made to the procedures are understood and implemented by field personnel and recorded in Section 16.0 of this procedure.

The Field Biologist/Supervisor is responsible for:

- ensuring that the California Department of Fish and Game (CDFG) Scientific Collecting Permits are valid and that CDFG has been notified regarding the sampling;
- ensuring that plant personnel are contacted prior to sampling according to this procedure;
- ensuring that plankton samples are collected in accordance with written procedures;
- verify that procedures have been followed during sample collection and that sampling has been conducted safely;
- ensuring that all the necessary supplies and equipment are available and sampling gear is in proper working order.

Field Biologists are responsible for:

- reading and understanding these procedures;
- ensuring all on-board and pump station sampling, sample handling, labeling, and sample fixation/preservation activities related to zooplankton tows and pump collection are in accordance with these procedures;
- Understanding and implementing all QA/QC requirements described in this procedure.

STANDARD OPERATING PROCEDURE FOR ZOOPLANKTON COLLECTION

1.0 SCOPE AND APPLICATION

These standard operating procedures describe the field sampling and fixation/preservation of marine zooplankton samples collected from locations off California.

2.0 SUMMARY OF METHODS

Zooplankton collection is generally accomplished using a small-meshed net to filter planktonic organisms from seawater. As water passes through the net planktonic organisms are filtered from the water and concentrated in a receptacle (codend) at the end of the net. Once a target volume of seawater has been filtered the net is lifted from the water, washed down, and the cod end removed. The contents of the codend are then emptied into sample jars and preserved for later processing in a laboratory. Sampling success involves the selection of suitable gear that is selected based on the objectives of the investigation. Gear considerations include net frame type, net material mesh size, time of collection, water depth of the study area and depth strata to be sampled, and sampling strategy.

2.1 Towed Net Sampling

A common method of zooplankton collection is by towing a plankton net through the water column from a boat. Plankton nets used are of various sizes and types. In addition to the mesh size, the frame type, length and mouth area of the net, towing speed, time of collection, and type of tow (surface, oblique, etc.) will determine the quality and quantity of zooplankton collected.

The bongo frame used for zooplankton sampling is a double net frame on to which two plankton nets are mounted in a side by side configuration. Calibrated flowmeters in the net frame opening are used to record the volume of ocean water filtered by each net. Once the desired volume of seawater has been filtered the nets are lifted from the water and thoroughly rinsed down from the outside so that any planktonic organisms adhering to the net are pushed into the codend. The contents from each codend may be preserved as separate replicates or combined into a single sample. Debris or extraneous material is removed from the codend and the zooplankton sample is transferred into clean, dry sample containers. Prior to the next tow the nets are sprayed to prevent contamination of samples with collections from the previous tows. Washing the nets will also prevent clogging, especially when there is a bloom or if a finer mesh is used for obtaining the samples. Samples are preserved in 10% buffered formalin or 95% ethanol in the field and delivered to the Tenera San Luis Obispo Plankton Lab for processing.

2.2 Pump Sampling

Pump gear can be used on board a vessel or pump sampling can be carried out from a pier or shore station. In this method, an inlet pipe is lowered into the source water and the outlet pipe is positioned in the mouth of a net of suitable mesh size. The net is submerged in a tank of seawater to prevent damage to the planktonic organisms that are filtered through the net. A flowmeter on the inlet pipe records the water volume filtered. This method is used for quantitative estimation and to study the small scale distribution of plankton.

After lifting the nets from the tank they are thoroughly washed so that any planktonic material adhering to the plankton net is pushed down into the codend. Debris or extraneous material is removed from the

codend and the zooplankton sample is transferred into clean, dry sample containers. Prior to the next tow the nets are sprayed to prevent contamination of samples with collections from the previous tows. Washing the nets will also prevent clogging, especially when there is a bloom or if a finer mesh is used for obtaining the samples. Samples are preserved in 10% buffered formalin or 95% ethanol in the field and delivered to the Tenera San Luis Obispo Plankton Lab for processing.

3.0 SAFETY AND WASTE HANDLING

Refer to the *Tenera Injury and Illness Prevention Plan (*IIPP) (January 2009, or as amended) and individual procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) chemical and waste handling procedures; and 3) accident prevention. Never compromise your personal safety or that of a field partner to collect a sample. Always wear appropriate safety gear.

It is the responsibility of the user of these procedures to comply with all applicable safety and waste handling rules. Additionally, all relevant chemical disposal and waste regulations are to be followed. All containers storing reagents and wastes used in sample collection must be properly identified through appropriate labeling and hazard definition.

Every chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Please refer to the IIPP for more detailed descriptions of the potential risks associated with any chemicals used in these procedures.

4.0 EQUIPMENT AND SUPPLY LIST

(see Appendix A)

5.0 CHEMICALS

- 70% denatured alcohol *(Use Fisher Scientific 95% denatured alcohol). To make 1 L of 70% denatured alcohol, dilute 740 ml of 95% alcohol with 260 ml of distilled water.*
- 95% non-denatured ethanol *(Use Fisher [Acros Organics]) 190 proof reagent stock).*
- 10% Formalin (3.7% formaldehyde) *Prepare 8 L of 10% buffered formalin solution by mixing 1 part 37% formaldehyde (800 ml) with 9 parts clean sea water (7200 ml) in a calibrated carboy. Add borax for additional buffering capacity.*

6.0 CHAIN OF CUSTODY

The primary objective of the chain of custody procedure is to create a written record that can be used to trace the possession of the sample. The chain of custody form (Tenera COC form; Attachment A) is filled out at sample collection and follows the sample through every person involved in the chain of possession until it reaches the laboratory. All samples, whether received in person or via commercial carrier, must be accompanied by a chain of custody form. Sample custody procedures are important to ensure the integrity of the samples whether for legal or other purposes.

6.1 Chain of Custody Procedures

a. The chain of custody form should be completed by the sampler using a waterproof pen at the time of sample collection and the form should be packaged with the samples for transport to the laboratory.

b. Samples may be delivered to the laboratory by Tenera field crews or arrive by US Mail, Federal Express, or other commercial carriers. When samples arrive at the Lab, the completed chain of custody form must be signed by Tenera personnel. If there is an address slip on the package for the shipment (e.g., a Fed- Ex slip) that log is saved and marked with the Project Identification and Survey Number.

c. Every time the sample changes possession, the person relinquishing the sample and the person receiving it must complete the chain of custody form to include the date, time, their printed name, and signature. Minimally, there must be two signatures on the chain of custody.

d. The Lab representative receiving the samples must review the chain of custody data to ensure all samples are accounted for. Any discrepancies on the form must be documented.

e. A copy of the signed chain of custody form shall be returned to the party of origin. The completed, signed, chain of custody forms shall be filed and stored in the Chain of Custody binder in the laboratory (A2 Lab).

7.0 SAMPLE FIXATION

The necessity of proper fixation and preservation of zooplankton needs no emphasis. After sampling, the fixation of samples should occur as early as possible (at least within five minutes) after the collection to avoid damage to animal tissue by bacterial action and autolysis.

The most common fixing reagent is 4 to 5% formaldehyde (formalin). Analytical grade formalin should be used for fixation as the commercial formalin is often contaminated with iron compounds which produce a precipitate of iron hydroxide that may render zooplankton identification difficult. The concentrated formalin should be diluted with filtered seawater (preferably with water from the sampling area) to avoid undesirable osmotic effects. The dilution is in the ratio of 1 part formalin and 9 parts seawater. The pH of the fixative should be around 8.0. Commonly used buffers are borax (sodium tetraborate) or hexamethyene teteramine. The buffers are added in an amount of approximately 200 g to one liter of concentrated formalin. Dilutions of formalin shall be prepared in a well-ventilated area outside while using safety goggles, gloves, and protective clothing.

7.1 Formalin Mixing Procedures

- Splash goggles and chemical proof gloves must be worn when handling formalin
- Pour ½ gallon (1.85 liters) of 37% formalin into a 20 liter formalin carboy
- Fill carboy to the 20 liter mark with filtered seawater
- Add Borax buffer (approximately 370 grams) to form a super saturated solution
- Stir thoroughly until most of the Borax is dissolved

For samples involving DNA and/or otolith analysis, 95% non-denatured ethyl alcohol is used. Immediately after collection, drain the samples of excess seawater and wash the sample into a glass jar using 95% non-denatured ethyl alcohol. Add additional 95% ethyl alcohol to fill the jar; there must be three to four times more ethanol than plankton volume.

8.0 FORMALIN

Formaldehyde is a colorless, strong-smelling highly reactive gas that is composed of hydrogen, carbon, and oxygen. Formalin is usually used in a liquid solution with water or methanol. The occupational

health hazards of formalin are primarily due to its toxic effects after inhalation, after direct contact with the skin or eyes in liquid or vapor form, and after ingestion. When present in the air at levels above 0.1 ppm (parts per million) it can cause watery eyes, burning sensations in the eyes, nose, and throat, nausea, coughing, chest tightness, wheezing, skin rashes, and allergic reactions. Ingestion of as little as 30 ml of a 37% solution of formalin can result in death. Long term exposure to formaldehyde has been shown to be associated with an increased risk of cancer.

8.1 Employee's Responsibilities

a. Read this standard operating procedure and also refer to the *Tenera Injury and Illness Prevention Plan* (IIPP) (January 2009, or as amended). Discuss any questions with the Field Supervisor or Lab Director.

b. Read the Formaldehyde Material Safety Data Sheet (MSDS) before beginning field collection procedures. The MSDS is available in the plankton laboratory, UA2 Lab, and the Lab Director's office.

c. Participate in the Formaldehyde Awareness Training.

d. Report immediately to the Field Supervisor any symptoms or reactions that might be related to formalin exposure.

e. Properly use protective clothing and equipment.

f. Immediately flush with water any skin area that comes into contact with formalin.

8.2 Protective Clothing and Equipment

- Safety splash goggles/glasses
- Chemical resistant gloves
- Portable eyewash
- Formalin spill cleanup kits
- Formalin monitoring badges

8.3 Monitoring

a. Representative monitoring for airborne formalin shall be conducted at the inception of a new procedure involving handling of formalin or at the resumption of an established procedure after a long period of time has elapsed.

b. The exposure of each employee involved in sample processing will be determined, using appropriate short-term exposure or long-term exposure monitoring badges.

c. Complete records of the results of airborne formalin monitoring will be kept for 20 years.

8.4 Clean Up

a. If a formalin spill occurs on the vessel, encircle the spill at its perimeter by pouring on FSC-1 solidifier located in the Formaldehyde Spill Kit. Once the spill is covered completely, wait until the residue is a semi-solid mass (15 minutes or more).

b. Transfer the absorbed material using the pick-up scoop and scraper to the appropriate sized bag.

c. Rinse the area thoroughly with copious amounts of water. Scrub the area with a mild detergent and water.

d. If formaldehyde or formalin has contaminated your clothing, change into clean clothing immediately.

e. Do not take contaminated work clothes home. An authorized individual should launder contaminated work clothes.

f. If personal exposure to formaldehyde or formalin occurs, wash all body areas IMMEDIATELY and THOROUGHLY with copious amounts of water.

8.5 Hazardous Waste Disposal

Any absorbent materials used to clean up a spill should be placed into a heavy duty plastic garbage bag, sealed, labeled, and disposed of according to local ordinances.

8.6 Transport of Samples from a Vessel to the Laboratory

Formalin should always be carried in containers that are approved for this purpose. The containers should be of a material that is impervious to the formalin solution or vapor. All containers must be checked prior to transport. If there is any apparent leakage of liquid or vapor from the container, or there appears to be a potential for leakage, then the container is not suitable for the transport of formaldehyde solution. Ideally, concentrated formalin should be carried as 10 liter aliquots with no more than 25 liters being carried in a single container. At sea 37% formaldehyde solution is stored on deck in a chemical storage container (carboy). Plankton samples containing 10% formalin are stored on deck in labeled cartons or crates.

Formaldehyde solution must not be transported to or from a vessel in a situation where fumes generated from a spillage can come into contact with the driver or passengers. All quantities of 37% and 10% formaldehyde must be carried in an approved chemical container and securely stored in a vehicle that separates the occupants of the vehicle from the formaldehyde. Containers of formaldehyde solution must be clearly labeled with Formalin Biohazard Labels. An approved formaldehyde spill kit and formaldehyde MSDS must always be carried when transporting all concentrations of formaldehyde solution in case of accidental spillage. Spillages of formaldehyde in enclosed vehicles should, when possible, be irrigated with water. If this is not possible, the formaldehyde must be allowed to evaporate and all fumes should be dispersed before the vehicle is used again.

9.0 FIELD SAMPLING PREPARATION

a. Coordinate with the Lab Director ahead of time to ensure there are enough jars, labels, and preservative for the sample collection effort.

b. Prepare all sample jars and transport containers.

c. Prepare internal and external sample jar labels: use either waterproof paper and pen or pre-printed labels on waterproof paper. Pre-labeling will include the project code, survey number, date of collection (unless conducting a 24-hour survey), sampling station, and preservative type (if using only one type of preservative). Following successful collection of the sample, the cycle number, sample number, and time of collection are added in permanent marker.

d. Affix the appropriate chemical hazard label (i.e., formalin, alcohol) to the sample jar lid.

e. Checkout the required number of Field Data Sheets (Attachment C) from the Data Coordinator. Standard uniform field datasheets will be maintained. Field data sheets will include the following information for each sampling event:

- Sample sequence number
- Collection date
- Sampling personnel
- Sampling event start and end times
- Status of plant makeup water pump operation
- Cycle of start, end, and total time
- Cycle start and end flowmeter readings
- Total cubic meters (m^3) sampled in cycle
- Total cubic meters (m^3) collected in sampling event
- Number of jars of sample collected per cycle and total jars per sampling event
- Pump serial number ID
- Flowmeter serial number ID

f. Inspect the frame, nets, and codends for any damage. If damaged, repairs must be made before sampling begins. Ensure the flowmeters are within the necessary calibration period and they are operational.

g. Check all equipment and supplies against the Equipment and Supply List (Appendix A) and ensure that all equipment is in good operating condition. Make repairs if necessary.

h. A calibration log will be created for each instrument. The logs shall include at least the following information: name of instrument, serial number and/or identification number of instrument, date of calibration, and calibration results. Theses logs will be provided to the Project Manager and maintained in a master calibration file as part of the QA/QC.

i. All field staff should be briefed by the Field Supervisor on the sampling goals and objectives prior to arriving at the site.

10.0 COMMUNICATION REQUIREMENTS

Contact plant/site personnel or appropriate person(s) prior to sampling according to the project-specific Study Plan.

The state requires that at least one of the individuals conducting the field sampling have a Scientific Collecting Permit. Notify the local California Department of Fish and Game office of the sampling event and location of the activity prior to collecting. Notification must be made during normal business hours at least 24 hours prior to collecting and can be made using the Notification of Intent to Collect for Scientific Purposes form (Attachment B). Also, CDFG requires that the list of species collected during the sampling be submitted at the end of the study.

Cell phones will be carried by personnel in the field. All vessels will have VHF radios on board capable of contacting the United States Coast Guard and the local Harbor Master in the event of an emergency.

11.0 TOWED NET SAMPLE COLLECTION

The Study Plan will contain a detailed description of the project-specific sampling procedures based on the Study Plan's rationale and protocols. The Study Plan's appendices will also contain additional information on standard operating procedures, equipment operation and maintenance, and quality assurance programs for the field collecting efforts. The following are general procedures for towed net sampling:

a Samples will be collected during specific sampling cycles according to the schedule detailed in the project-specific Study Plan. A sampling team consists of, at minimum, a boat captain and two investigating biologists.

b. Attach the two plankton nets to the bongo frame. Assure that the plankton nets are the correct length and mesh size for the particular study. Mount a flowmeter to the mouth of each net.

c. Attach the winch line to the bongo net frame and ensure that a codend is attached to each net. The winch line is marked at regular intervals so that the sampling personnel know how much line has been deployed.

d. Navigate to the first sampling station. Locate the station using the latitude/longitude coordinates.

e. Determine the water depth with the fathometer or other depth measuring device. Record the water depth on the field data sheet.

f. Record each flowmeter's serial number and number of spins from the unit's totalizer on the field data sheet. Make sure that the propeller does not spin until the bongo frame and nets are in the water.

g. Start the hydraulic pump and capstan, attach a weight (15 to 20 lb salmon ball) securely to the center of the bongo frame and lower the nets until the frame is at the water's surface and the nets are underwater.

h. Record the start time (Pacific Standard Time) on the field data sheet.

i. Using the measured marks on the winch line lower the nets from the surface and pay out line so that the net assembly sinks mouth first to the desired depth (within 1 meter from the bottom). Shift the vessel motor into forward gear and proceed forward slowly (1 to 2 knots) for a specified period of time (usually four to six minutes). When the nets have been towed for the desired length of time activate the winch and raise the nets. The tow time may need to be adjusted so that each net on the frame has filtered the appropriate amount of water (usually at least 40 m^3 of water). When the frame reaches the surface check the number of spins on each flowmeter counter to verify that the target volume of has been collected (number of spins should be about 4,000 for 40 m^3). If the target volume has not been achieved with one tow, subsequent tows will be performed at the station until the target volume has been collected.

j. Raise the nets from the water and stabilize the flowmeters to prevent spinning. If flowmeter readings indicate that the target volume has been filtered through each net, record the end number of spins from each flowmeter on the field data sheet. Record the end time (Pacific Standard Time) and total time of the collection 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. Record the reason(s) for voiding the tow on the data sheet. Repeat the sample collection at that station.

k. Use the winch to raise the net assembly to a height where the weight can be removed. Securely stow the weight then raise the nets to their maximum height for rinsing. 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. Reattach the codends and repeat the sample collection at that station. If the nets become clogged with material during the hauls, they must be rinsed prior to the total volume being filtered. Rinse the material in the nets into the codends. Attach a closing device above the codend of each net to ensure the collected material will not be lost during the subsequent hauls. Continue the net hauls until the correct volume has been filtered.

l. Rinse the sample, using the washdown sprayer, into the codend of each net beginning at the top of the net. 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.

m. Place pre-printed labels (containing the information specified in Section 9. c. above) into the corresponding pre-labeled sample jar. Add preservative (10% formalin or 95% ethanol); allow a ratio of at least two to three times the volume of preservative to volume of the plankton sample. Use additional jars if necessary.

n. Detach the codend from net #1 and rinse the sample from the codend into the pre-labeled sample jar using a wash bottle containing sea water. 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. Depending on the specific project, samples from each net may be combined and placed in a labeled jar. Add additional preservative from the onboard carboy if necessary.

o. Ensure that all sample jars contain a corresponding inner label and jar-top label and that the Chain of Custody form is filled out correctly and completely. Pack the sample jars for transport in accordance with safety and/or Department of Transportation standards. Deliver the samples to the Tenera Plankton Laboratory at the completion of the sampling effort.

p. The following is an explanation of the coding for the field datasheet and jar labels:

- Each survey number on the data sheet consists of a series of letters followed by 2 or 3 numbers (e.g., DCPPEA##). The first letters refers to the name of the project (e.g., “Diablo Canyon Power Plant”) and the “EA” refers to “Entrainment Abundance”. The numbers refer to the survey number with the first survey being 01. The survey number increases by one for each subsequent sampling event.
- The station designation (numbers and/or letters) is project specific and is detailed in the project’s Study Plan.
- Sample labeling also includes a cycle designation. Each 24-hour sampling period is divided into four six-hour cycles or a “day” and “night” cycle, depending on the specific project. The first sampled time block of each 24-hour sampling period is Cycle 1.
- The date of sampling will correspond to the actual start date of each sample.

12.0 PUMP SAMPLE COLLECTION

The Study Plan will contain a detailed description of the project-specific sampling procedures based on the Study Plan's rationale and protocols. The Study Plan's appendices will also contain additional information on standard operating procedures, equipment operation and maintenance, and quality assurance programs for the field collecting efforts. The following are general procedures for pump sampling:

a. Samples will be collected during specific sampling cycles, usually day and night, according to the schedule detailed in the project-specific Study Plan.

b. Place pre-printed labels (containing the information specified in Section 9. c. above) into the corresponding pre-labeled sample jar. Add preservative (10% formalin or 95% ethanol); allow a ratio of at least two to three times the volume of preservative to volume of the plankton sample. Use additional jars if necessary.

c. Rig sampling pipe assemblies and flowmeters to the flange/bib fittings installed on the water supply systems from the wedgewire screen intakes (specific to the Study Plan).

d. Attach net frames to each of the plankton nets. Assure that the plankton nets are the correct length and mesh size for the particular study. Ensure that a codend is attached to each net.

e. Set up the sampling station by filling the tank(s), suspending the plankton nets so approximately three fourths of their length is submerged in the tank, and positioning the discharge pipe from the sampling pipe assemblies over the net mouth.

f. At the desired time, turn off the water flow into the tank and zero the totalizer on the flowmeter. Start the flow of water from the sampling pipe assemblies and record the start time and totalizer values on the data sheet. Collect the sample for the appropriate period of time. To lessen the impact of abrasion and predation, water flow should be stopped after about 15 minutes and the net removed. A clean net is put in the tank and the water is started again. The net that was used for the collection is then rinsed and the material in the codend is put into a labeled sample jar.

g. When the flow meter totalizer indicates that the target volume has been filtered, turn off the water, remove the net from the tank, and record on the data sheet the stop time and the total number of gallons filtered during the sample collection period.

h. Rinse the sample down into the codend beginning at the top of the net. Since the wash water is not filtered and may contain plankton, rinse the net from the outside to ensure that unfiltered water does not contaminate the sample. Inspect the net to ensure that it has been thoroughly rinsed.

i. Detach the codend from the net and rinse the sample from the codend into a pre-labeled sample jar using a wash bottle containing sea water. Rinse and inspect the codend of the net to make certain that all of the collected material is rinsed from the codend before it is reused. Follow the same procedure for the nets at the other sampling stations. Add additional preservative from the carboy at the station if necessary.

j. Ensure that all sample jars contain a corresponding inner label and jar-top label and that the Chain of Custody form is filled out correctly and completely. Pack the sample jars for transport in accordance with safety and/or Department of Transportation standards. Deliver the samples to the Tenera Plankton Laboratory at the completion of the sampling effort.

13.0 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) gear failure which prevents completion of any tows/hauls; 5) an incident or situation which may prevent reliable data collection; 6) 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 after the net has been repaired or replaced. Record the circumstance on the field 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.

14.0 RECORDS

All data sheets will be reviewed and signed off by a Field Supervisor or their designate and submitted to the Data Coordinator for logging, computer entry, and storage. Original data sheets will be stored in fireproof locations. If desired, copies of all completed data sheets will be transferred to client staff at least quarterly.

15.0 FIELD SAMPLING QUALITY ASSURANCE /QUALITY CONTROL

The appropriate Quality Assurance/Quality Control methods will be selected for each project on the basis of circumstances, objectives, and requirements. The provisions of this SOP will be adapted to these project specific requirements in the project QA plan. Current Field Sampling QA/QC consists of the following:

15.1 Plankton Net Flowmeter Calibration

a. Disconnect the flow meter from the net. Record the serial number of the flow meter on the Zooplankton Net Flowmeter Calibration Log (Attachment D). Connect the flow meter to a rod. Measure and mark a distance of at least 6 to 9 m (20 to 30 ft) on the dock and record the distance on the Calibration Log.

b. Record the initial number of spins from the readout on the flowmeter totalizer on the Calibration Log. Lower the flowmeter into the water slowly so that the propeller does not spin. Walk along the dock towing the flowmeter at a speed of between 30 to 46 cm (1 to 1.5 ft) per second for the marked distance, checking to make sure that the propeller is spinning. When the flowmeter has been towed over the measured distance, carefully raise it out of the water. Record the end number of spins from the flowmeter totalizer on the Calibration Log.

c. Repeat this procedure at least 10 times for each flow meter. Subtract the initial reading from the end reading and record the total number of spins per trial on the Calibration Log. The total spins for each of the ten trials are summed and divided by the number of trials. The resulting mean is the calculated calibration value for the measured tow length. Record all information on the Zooplankton Net Flowmeter Calibration Log and enter into the computer database.

d. Calibrate each flow meter at least once every three months. If the average of these readings differs by more than 10% from the original calibration readings, and the differences cannot be explained by sampling conditions (e.g., rough seas or boat is drifting), then the meter needs to be serviced and re-calibrated by taking 10 additional readings.

15.2 Sample Collecting Process

a. Sample labels must be properly completed, including project code, survey number, sample number, date and time of collection, sampling station, cycle number, and preservative type and placed into the sample container. The outside of the container should be labeled with the same information and the appropriate Hazardous Materials Warning label.

b. Chain of custody forms must be completed during sampling and include the same information as the sample container labels.

c. After each sample collection, all nets, codends, buckets, etc. that have come into contact with the sample should be thoroughly rinsed, examined, and picked free of organisms or debris. Any additional organisms found should be placed into the sample container.

15.3 Chain of Custody

Chain of custody procedures will follow those outlined in Section 6.0. They will be initiated when the first sample is collected and will be followed until all samples are relinquished to the plankton laboratory. Chain of custody forms will provide an unbroken trail of accountability that ensures the physical security of samples, data, and records. At the end of each sampling event all sample containers are checked against the chain of custody form(s). It is important to verify the survey and station identification numbers, sample number, collection date, collection time, and preservation as part of the QA/QC procedures.

16.0 SOP REVISIONS

In order to ensure that Tenera, client staff, and contractor(s), are using the current document, control copies of this manual are maintained. These control copies are maintained in binders in the Plankton Lab and are labeled as control copies. Electronic copies are also maintained. Revisions to the SOPs in this document are made as follows:

1) Obtain a copy of the current SOP from a control copy of the manual,

2) Obtain the electronic copy from the Quality Assurance Manager,

3) Revise the electronic copy of the SOP in Word using redline and strikeout (this will provide a record of the revisions) and prepare a clean copy of the revised SOP,

4) Update the revision number on the cover page and in the footer,

5) Provide the two versions of the revised SOP (redline/strikeout version and the clean edited version) to the appropriate scientist(s) for review and approval.

Upon approval, the final revised SOP is given to the Quality Assurance Manager for inclusion in the control hard-copy and electronic copy of the manual. All procedure revisions (summary of changes) are to be recorded in this section with the revision date and number, changes noted by page and section, and name of reviser.

History of SOP Revisions

Revision Date	Revision Number	Page/Section	Summary of Changes	Reviser

APPENDIX A
EQUIPMENT AND SUPPLY LIST

GENERAL EQUIPMENT	QUANTITY
Bongo net frame with micron mesh nets	2
Codends	4
Calibrated flowmeters	4
Metal or wooden rod for calibration	1
Winch line	2
Winch for net deployment and retrieval	1
Boat hook	2
Wash-down pump	1
Filter with attached micron mesh	2
Extra gas (for pump)	3 gal.
Salmon weights (10 to 20 lb)	2
Fathometer or other water depth measuring device	1
GPS for nearshore sampling	1
Field watch	2
Flashlight (for night sampling)	2

SUPPLIES	
Sample containers: size and type are project specific	Project specific
Inner jar labels	Project specific
Field datasheets (on Rite-in-Rain paper)	Project specific
Wash/squeeze bottles	5
Small funnel	2
37% Formalin	Project specific
5-gallon carboy with spigot	2
Carboy stand	1
Sodium borate buffer/ Borax	1 box
Formalin stirring stick	1
Container to provide secondary containment during sample transfer	2
Non-denatured 95% ethanol (for DNA/otolth studies)	Project specific
Hazardous materials labels	1 box

Hazardous waste container	1
Liquinox soap	1 bottle
Sample container rack	2 racks
Indelible ink pens (extra fine and regular sharpies)	1 box
Pencils	1 box
Scissors	1 pair
Ziploc bags	1 box
Garbage bags	1 box
Cable ties	1 box
Rubber bands	1 bag
Duct tape	1 roll
Field notebook	1
Tool box	1
Extra sample containers (to account for heavy samples, breakage, contamination)	
Extra labels for sample containers	
Extra nets	2

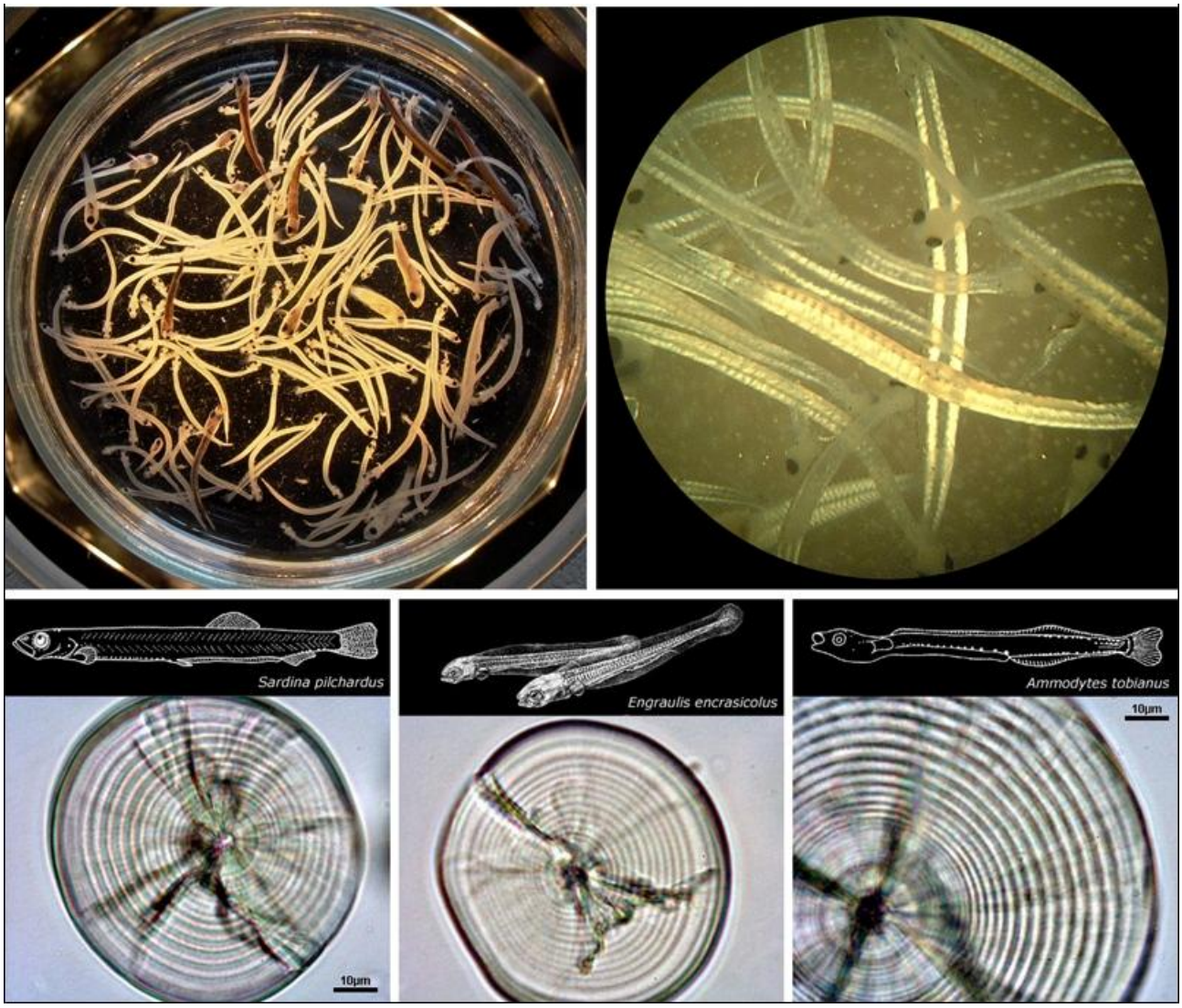
SAFETY EQUIPMENT

Life-vests	1 for each person aboard
Safety goggles/glasses	3
Eye-wash bottle	1
Formaldehyde monitoring badges – TWA	Project specific
First aid kit	1
Disposable nitrile gloves	1 box
Leather work gloves	Project specific
Rain gear	
Material Safety Data Sheets (MSDS)	Project specific

REGULATORY

Scientific Collection Permits

Standard Operating Procedures For Zooplankton Laboratory



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INTRODUCTION

Zooplankton encompass an array of macro and microscopic animals and includes haloplankton, meroplankton, and ichthyoplankton. Zooplankton species play a vital role in the marine food chain. The herbivorous zooplankton feed on phytoplankton and in turn constitute an important food item to animals in higher trophic levels, including fish. The occurrence and abundance of ichthyoplankton (fish eggs and fish larvae) facilitate the location of probable spawning and nursery ground of fishes. Zooplankton are ubiquitous. The most characteristic feature is their variability over space and time in any aquatic ecosystem. The success of zooplankton estimation and productivity largely depends upon the use of correct methodology which involves collection of samples, fixation, sorting, identification, length analysis, and preservation. The detailed procedures on all these aspects are given in this manual.

RESPONSIBILITIES

- The Laboratory Director is responsible for ensuring that plankton sample processing is being conducted in accordance with these written procedures. The Lab Director is responsible for ensuring that all samples are properly logged into the laboratory, tracked, and processed (transferred, split, sorted, identified, measured) on schedule; data are properly recorded; and samples are properly handled after analysis (stored, discarded, etc.).
- The Laboratory Director and Project Manager are responsible for designing and implementing the Quality Control Program that monitors accuracy in accordance with written procedures.
- Scientists and Research Assistants are responsible for processing samples in accordance with written procedures.

STANDARD OPERATING PROCEDURE FOR ZOOPLANKTON LABORATORY

1.0 SCOPE AND APPLICATION

These standard operating procedures describe the methods used in the laboratory processing of marine zooplankton samples collected from locations off California and Hawaii. The methods are appropriate for all sample types of collection (bongo nets used in surface, oblique, and depth-specified tows as well as pump sampling). Laboratory analysis of zooplankton samples provide data to be used in calculating percent concentrations and percent relative abundances of individual taxon.

2.0 SUMMARY OF METHOD

This procedure includes chain of custody and sample log-in guidelines and explains how to 1) prepare samples for processing, 2) separate target organisms from collected samples, 3) prepare, record, and maintain specimens for taxonomic identification and storage, 4) obtain larval fish length measurements, 5) extract and read otoliths, and 6) perform the required Quality Assurance/Quality Control procedures.

3.0 SAFETY AND WASTE HANDLING

Refer to the *Tenera Injury and Illness Prevention Plan (*IIPP) (January 2009, or as amended) and individual procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) chemical and waste handling procedures; and 3) accident prevention.

It is the responsibility of the user of these procedures to comply with all applicable safety and waste handling rules. Additionally, all relevant chemical disposal and waste regulations are to be followed. All containers storing reagents and wastes used in the laboratory must be properly identified through appropriate labeling and hazard definition.

Every chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Please refer to the IIPP for more detailed descriptions of the potential risks associated with any chemicals used in these procedures. It is good laboratory practice to wear a lab coat, safety glasses/goggles, and gloves when handling chemicals.

4.0 EQUIPMENT LISTS

(see Appendix A)

5.0 CHEMICALS

- 70% denatured alcohol *(Use Fisher Scientific 95% denatured alcohol). To make 1 L of 70% denatured alcohol, dilute 740 ml of 95% alcohol with 260 ml of distilled water.*
- 95% non-denatured ethanol *(Use Fisher [Acros Organics]) 190 proof reagent stock).*
- 10% Formalin (3.7% formaldehyde) *Prepare 8 L of 10% buffered formalin solution by mixing 1 part 37% formaldehyde (800 ml) with 9 parts clean sea water (7200 ml) in a calibrated carboy. Add borax for additional buffering capacity.*

6.0 CHAIN OF CUSTODY

The primary objective of the chain of custody procedure is to create a written record that can be used to trace the possession of the sample. The chain of custody form (Tenera COC form; Attachment A) is filled out at sample collection and follows the sample through every person involved in the chain of possession until it reaches the laboratory. All samples, whether received in person or via commercial carrier, must be accompanied by a chain of custody form. Sample custody procedures are important to ensure the integrity of the samples whether for legal or other purposes.

6.1 Chain of Custody Procedures

a. The chain of custody form should be completed by the sampler at the time of sample collection and the form should be packaged with the samples for transport to the laboratory.

b. Samples may be delivered to the laboratory by Tenera field crews or arrive by US Mail, Federal Express, or other commercial carriers. When samples arrive at the Lab, the completed chain of custody form must be signed by Tenera laboratory personnel. If there is an address slip on the package for the shipment (e.g., a Fed- Ex slip) that log is saved and marked with the Project Identification and Survey Number.

c. Every time the sample changes possession, the person relinquishing the sample and the person receiving it must complete the chain of custody form to include the date, time, their printed name, and signature. Minimally, there must be two signatures on the change of custody.

d. The Lab representative receiving the samples must review the chain of custody data to ensure all samples are accounted for. Document any discrepancies on the form.

e. A copy of the signed chain of custody form shall be returned to the party of origin. File and store completed, signed, and returned chain of custody forms in the Chain of Custody binder in the laboratory (A2 Lab).

7.0 SAMPLE LOG-IN AT LABORATORY

All samples must be recorded on the Sample Log-In Sheet (Attachment B) upon delivery to laboratory personnel. The Sample Log-In Sheet is posted in the A2 Storage Area. Samples are logged into the laboratory to verify sample arrival and sample condition. The integrity of the samples is checked (correct preservation used, lids and jars intact, fixation times fall within the requirements, and so on) and missing or damaged samples must be reported to the Laboratory Director immediately. During log in, samples are assigned a laboratory storage location (within the A2 Storage Area).

7.1 Sample Log-In Procedures

a. Place samples preserved in ethanol on the Prep Table against the north wall of the A2 Storage Area. Place samples preserved in formalin on the Transfer Shelf under the fume hood.

b. Confirm receipt of the samples by completing the Sample Log-In Sheet.

c. Check sample fluid levels and, if needed, add additional preservative or fixative. Check the integrity of the sample jars and lids. Document any discrepancies or problems.

d. Verify the field data sheets (Project Manager) and enter data into the Access database (Senior Data Analyst).

e. Create the electronic LAB_SORT ID TRACKING spreadsheet for the samples/survey.

f. Update the SAMPLE_PLANKTON ARCHIVING RECORD database to include new samples.

g. Generate pre-printed labels for individual sample jars and sample boxes, including hard copy tracking sheets and ID logs for all samples (Senior Data Analyst). Label samples by placing the pre-printed labels on the jar lid; label sample boxes with pre-printed labels. Label data must include the survey number, collection date, collection time, station, cycle, sample number, and if appropriate, split code.

h. Place 95% ethanol identification stickers on the appropriate ID data sheets. Place tracking sheets and ID data sheets into the appropriate Lab and Project binders. Place correct alcohol label (70% or 95%) on the side of the sample jar.

i. Place samples in appropriate labeled and color-coded project boxes. Place sample boxes in the A2 Storage Area designated for the project.

8.0 SAMPLE TRANSFERRING

Plankton samples that are originally fixed in formalin must be transferred to 70% alcohol before laboratory processing. Allow 72 hours as the minimum fixation period. If ethanol is used to initially preserve samples (e.g., for otolith or DNA analysis) the samples should be transferred into fresh ethanol within 24 hours because water in the tissues of gelatinous plankton or fish flesh quickly dilutes the preservative.

The chemical formaldehyde (CH_2O) plays an indispensable role in fixing the proteins in a fresh specimen in order to prepare it for wet preservation. Until recently, various solutions of formaldehyde have also been used as a preservative for long-term storage of specimens. The most common preservative used is formalin, a 10% solution of formaldehyde mixed in water (i.e., 9 parts water and 1 part formaldehyde). However, it is now recognized that formalin, because of its acidity, makes a very poor long-term preservative for many specimens, resulting in the decalcification of bones, distortion of tissues, and acidic decomposition of specimens. Today, the recommended long-term preservative for maintaining a variety of vertebrate and invertebrate specimens is a 70%-75% solution of ethanol.

Only qualified personnel who have the approval of the Lab Director may transfer samples. Personnel who work with formaldehyde must have signed documentation that he/she understands the hazards and has been trained in how to work with formaldehyde safely. Transferring must be conducted in a fume hood or in a well ventilated area to lessen the exposure to formaldehyde fumes. Proper Personal Protection Equipment (PPE) should be worn while preparing and handling samples for transfer.

8.1 Sample Transferring Procedure (Formalin to Alcohol)

a. After a sample has been collected in the field, the appropriate information as to the identity of the sample shall be written on the sample lid. Verify that the sample information is correct by comparing the data on the sample jar lid to the pre-printed sample labels.

b. Samples should be processed one at a time. Place a mesh funnel with the appropriate mesh size into a Ball® jar. The mesh size must not be larger than that used during sample collection. Place the Ball® jar and mesh funnel in a Pyrex® tray so the sample can be retrieved if a spill occurs.

c. Working under the fume hood, transfer the sample from the collection jar into the mesh funnel. While transferring, care should be taken so that no part of the sample is lost. Using a

wash bottle containing fresh water, thoroughly rinse the zooplankton to flush the formalin from the sample. Thoroughly rinse the sample jar and lid into the mesh funnel to remove any residual organisms adhering to the walls of the sample collection jar.

d. Using a wash bottle containing 70% alcohol, rinse the sample into the appropriately labeled container. Use only new or clean containers. Ensure that the container has both an inner label and a lid top label and that the information matches and is correct. Carefully decant 70% alcohol into the container; allow a ratio of at least two to three times the volume of alcohol to volume of the plankton sample. Label the jar with a "70% alcohol" label.

e. The waste formalin and rinse water shall be discarded into the appropriate labeled waste containers.

f. After transferring, all field sample collection jars are to be thoroughly cleaned and allowed to air dry.

g. Fill in the required information on the LAB SORT_ID TRACKING datasheet and the Transfer Log on the computer.

8.2 Sample Transferring Procedure (Ethanol to Ethanol)

a. After a sample has been collected in the field, the appropriate information as to the identity of the sample shall be written on the sample lid. Verify that the sample information is correct by comparing the data on the sample jar lid to the pre-printed sample labels.

b. Samples should be processed one at a time. Place a mesh funnel with the appropriate mesh size into a Ball® jar. The mesh size must not be larger than that used during sample collection. Place the Ball® jar and mesh funnel in a Pyrex® tray so the sample can be retrieved if a spill occurs.

c. Transfer the sample from the collection jar into the mesh funnel. While transferring, care should be taken so that no part of the sample is lost. Using a wash bottle containing 95% non-denatured ethanol, thoroughly rinse the sample jar and lid into the mesh funnel to remove any residual organisms adhering to the walls of the sample collection jar.

d. Using a wash bottle containing 95% non-denatured ethanol, rinse the sample into the appropriately labeled container. Use only new or clean containers. Ensure that the container has both an inner label and a lid top label and that the information matches and is correct. Carefully decant 95% non-denatured ethanol into the container; allow a ratio of at least two times the volume of ethanol to volume of the plankton sample. Label the jar with a blue "95% ethanol" label.

e. The original ethanol preservative should be placed into an approved safety container labeled "To Be Recycled" for recycling through the Alcohol Recycling System (ARS).

f. After transferring, all field sample collection jars are to be thoroughly cleaned and allowed to air dry.

g. Fill in the required information on the LAB SORT_ID TRACKING datasheet and the Transfer Log on the computer.

9.0 SAMPLE SPLITTING

When samples are particularly dense, a Folsom Plankton Splitter may be used to divide samples into smaller, more manageable subsamples. Aliquot portions of approximately ½, ¼, ⅛, etc. of the original sample are obtained and the process is continued until the sample is small enough for sorting. Plankton concentrations of split samples are calculated based upon the number of splits and the volume of water filtered. Multiplying the organism count in the m^{th} fraction by 2^m gives an estimate of the number in the original sample.

Formalin-fixed samples that have not yet been transferred into ethanol should not be split. Since the plankton splitter is not alcohol-resistant, ethanol samples must be transferred into and split in water using the following procedure.

Folsom Plankton Splitter

9.1 Sample Splitting Procedure

a. Set up the Folsom Plankton Splitter in the designated lab area (Prep Table). Level the splitter using the adjustable legs until the air bubble is centered within the circle. Make sure the drum and trays are clean. Wash with mild soap and water, rinse with distilled water before drying.

b. Because the field volume may not fully represent how much material is in the sample, each sample jar should be individually inspected to determine how many splits, if any, may be necessary. The number of splits determines how many jars will be needed:

Number of Splits	No Split	Split in half (½)	Split in fourths (¼)	Split in eighths (⅛)	Split in sixteenths ($^1/_{16}$)	Split in thirty-seconds ($^1/_{32}$)
Number of Jars	1	2	3	4	5	6

c. Prepare the needed number of sample jars, re-print the appropriate pre-printed jar lid labels, and re-label each new jar, initially leaving the "split code" designation blank. (See Section 9.2 Labeling and Recordkeeping for Split Samples.)

d. Place a mesh-sieve funnel into a regular funnel over the alcohol bottle used for plankton splitting. Place the alcohol bottle into a Pyrex® tray so the sample can be retrieved if a spill occurs. Pour the sample and ethanol into the mesh funnel. The funnel will contain the material to be split while the ethanol will drain into the alcohol bottle.

e. Using specimen forceps, place the sample material back into its original sample jar. Rinse any remaining sample from the funnel into the jar using a wash bottle containing water. Make sure the entire sample is returned to the original sample jar. Fill the jar with water until the sample is completely covered.

f. Carefully pour the sample into the drum of the plankton splitter. Use a wash bottle filled with water to rinse any remaining sample from the jar into the rotating drum and to get any

sample off the sides of drum and the drum axel. Carefully, but thoroughly, mix the sample with a glass stirring rod.

g. Place the trays, labeled "A" and "B", underneath the drum. Place one hand over the drum opening and rotate the drum 120° to divide the stirred sample with the separating blade. Continue rotating the drum 120° approximately six (6) times or until the sample looks evenly distributed between the two compartments of the drum. Water may be added as needed. Once the sample is evenly distributed, rotate the drum carefully so the sample and water pour into the trays. Use a water wash bottle to flush any residual sample from the drum into the trays.

h. For samples involving just one split (i.e., split in half) pour the contents of Tray A into a mesh-sieve funnel that is placed over a Ball® jar; the sample will remain in the funnel, while the water will drain into the empty jar. The water in the jar may be disposed of down the drain.

i. Transfer the sample material from the funnel into a newly labeled jar. Using a wash bottle containing the appropriate ethanol (i.e., the same type as the original sample) rinse any remaining sample into the jar. Ensure that the container has both an inner label and a lid top label and that the information matches and is correct (see labeling below). Carefully decant the ethanol from the original sample (i.e., from the plankton splitting alcohol bottle) into the sample jar; allow a ratio of two to three times the volume of ethanol to volume of the plankton sample. Repeat the process for Tray B. Additional ethanol may be added to the sample jar if needed.

j. When additional sub-sampling is needed, the contents of one tray are placed into a new sample jar while the other tray is returned to the drum for further splitting. Use a random 1 & 2 number chart (1=A, 2=B) to select the tray (Tray A or Tray B) that is to be further subdivided. Pour the contents of the selected tray into the drum and completely rinse out the tray using a water wash bottle. Replace the clean trays under the drum and repeat step 9.1.g. above. Continue the process until the sample is small enough for sorting.

k. The jars should be of sufficient size so that when filled the volume of ethanol is at least three times greater than the volume of the sample (i.e., the sample volume should be no more than 25% of the volume of the storage jar).

l. Use mild soap and fresh water to clean the plankton splitting drum. Do not use chemicals such as acetone or ketone for cleaning.

9.2 Labeling and Recordkeeping for Split Samples

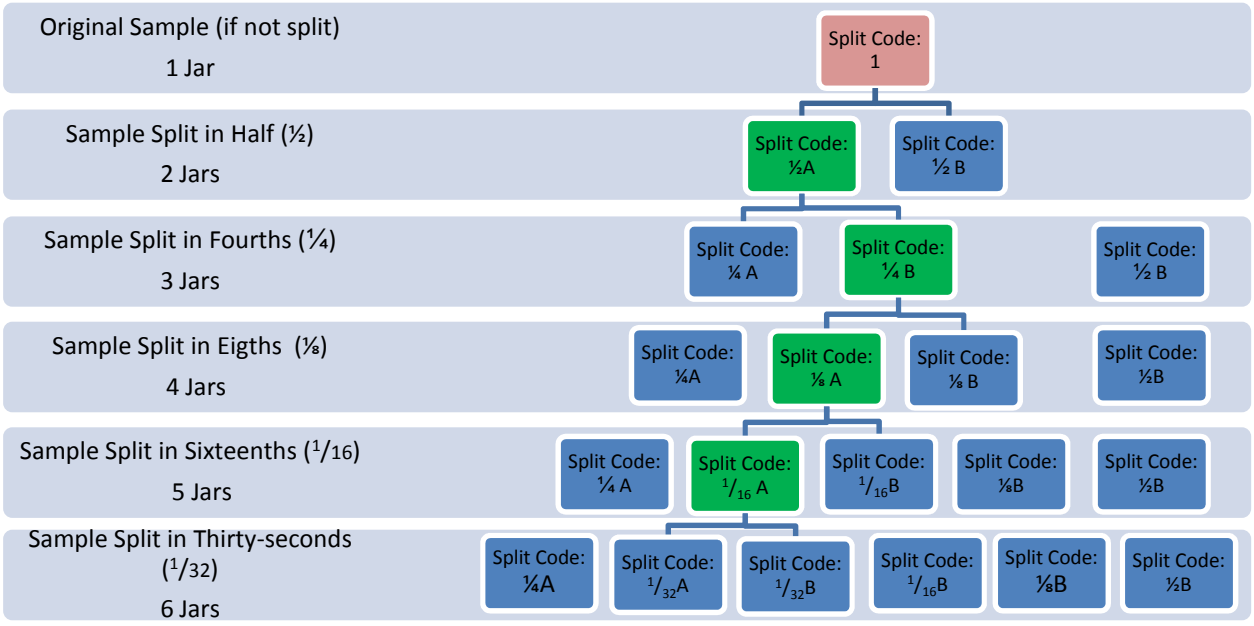
Sample labels and tracking/ID sheets must reflect when a sample has been divided into sub-samples. Split code information is essential to making zooplankton density calculations from the samples.

The split codes are in the form of a fraction (½, ¼, ⅛, etc.) and the letters A or B. The fraction indicates what proportion of the original sample is contained in the sample jar. The A and B correspond to the individual plankton splitting trays. For example, the split code ½A indicates that the original sample has been split into two sub-samples and the jar contains the plankton material from Tray A and is ½ the original sample.

Likewise a sample containing the split code $^{1}/_{32}$ on the sample label indicates the jar contains one thirty-second of the sample. However, the entire sample has not been split into thirty-two sub-samples. Only one sub-sample or tray (designated A or B) from each split is chosen (by random number) for subsequent splitting. Therefore a sample split into thirty-seconds will have six jars: one

labeled ½ (A or B), one ¼ (A or B), one ⅛ (A or B), one $^{1}/_{16}$ (A or B), one $^{1}/_{32}$ A and one $^{1}/_{32}$ B. See Figure 1 for clarification.

Figure 1. Split Code Designations for Samples Divided into Subsamples.
Note that only one tray or "subsample" (shown in green) is selected for subsequent splitting. The other tray/subsample (shown in blue) is not split but remains intact.



9.2.1 Labeling and Recordkeeping for Sample Split In Half (½)

a. Sample labels should be made from waterproof paper and be placed inside and on the outside of each jar. Use a heavy weight cotton fiber paper (Rite in the Rain) and a No. 2 pencil or Pigma Micron pen if labeling by hand.

b. After the sample has been split in half, fill in the split code on the new jar lid labels (pre-printed) with ½A for the split from tray 'A' and ½B for the split from tray 'B'. The ½ indicates that the jar contains ½ of the sample, and the A or B corresponds to the tray.

c. Use a random number chart (1=A, 2=B) to select the sample "split" (subsample) to be sorted. A '1' on the random number chart means that ½A will be sorted and ½B will be stored. A '2' on the random number chart means that ½B will be sorted and ½A will be stored.

d. Take the inside label from the original sample jar, write in the proper split code, and put the label into the sub-sample (or "split") that was selected to be sorted. Copy the original sample information onto a new inside label (on waterproof paper) for the sample not to be sorted. Place the sample to be sorted in the box that is designated "To be Sorted" and place the sample that was not chosen into the box designated "Not to be Sorted". Proper labeling for sample (split in half with subsample "1" selected for sorting):

DCPPEA 05 9/15/08
E1A1 Cycle 1 10:08
Sample #3 Split: ½ A

e. On the hardcopy tracking sheets in the appropriate Project binder, enter "2" under the column title '# of subsamples'. In the 'Split Code' column enter either ½A or ½B, depending on which subsample was selected for sorting.

f. Enter the number of subsamples and split code data onto the LAB SORT_ID TRACKING computer tracking sheets:

Survey Number	Sample #	Station	Cycle	# of Sub-Samples	Split Code
DCPPEA05	03	E1A1	1	**2**	**½ A**

g. Fill out the Split Log (density, field and sort volumes, number of jars, etc.) located in the Project's folder in the LAB SORT_ID TRACKING database. Record initials, date, and time data under the Split Information on the LAB SORT_ID TRACKING computer tracking sheet for the Project.

h. Record the appropriate Split Multiplier data (i.e., number of subsamples) for the sorted sample on the corresponding Lab ID Data Sheet.

9.2.2 Labeling for Sample Requiring More than One Split (¼, ⅛, $^{1}/_{16}$, or $^{1}/_{32}$)

a. Sample labels should be made from waterproof paper and be placed inside and on the outside of each jar. Use a heavy weight cotton fiber paper (Rite in the Rain) and a No. 2 pencil or Pigma Micron pen if labeling by hand.

b. After the second split (split into fourths [¼]) there are three subsamples: one ½ subsample and two ¼ subsamples. If the sample is not to be divided into any additional splits, put both ¼ splits into jars, copy the original sample information onto waterproof paper for the inside labels, attach the new pre-printed lid labels, and write ¼A and ¼B for the split code on both inside and lid top labels.

c. Use the random number chart to pick which ¼ sample will be sorted. Place the sample to be sorted in the box that is designated "To be Sorted" and place the other two samples (½ and ¼ subsamples) into the box designated "Not to be Sorted".

d. On the hardcopy tracking sheets in the appropriate Project binder, enter "4" under the column title '# of subsamples'. In the 'Split Code' column enter either ¼A or ¼B, depending on which subsample was selected for sorting.

e. Enter the number of subsamples and appropriate split code data onto the LAB SORT_ID TRACKING computer tracking sheets:

Survey Number	Sample #	Station	Cycle	# of Sub-Samples	Split Code
DCPPEA05	03	E1A1	1	**4**	**¼ A**

f. Record the appropriate Split Multiplier data (i.e., number of sub-samples) for the sorted sample on the corresponding Lab ID Data Sheet.

g. If additional splits are necessary, repeat the procedure, indicating the proper number of sub-samples and the split code on the jar labels (inside and top). Enter the information on the hardcopy tracking sheets, on the LAB SORT_ID TRACKING computer tracking sheets, and the corresponding Lab ID Data sheets.

10.0 SAMPLE SORTING PROCEDURES

10.1 Sample Sorting Procedure for 70% Alcohol Samples

a. Upon arrival check the bulletin board and white board for messages, updates, meetings, etc.

b. Ensure that the necessary equipment for sample processing is available (Appendix A-1).

c. Consult the Plankton Lab sorting schedule posted in the laboratory to determine sorting priorities. A list of target organisms to pull for each Project is posted in the lab.

d. Obtain the sample to be sorted from the Sample Storage Area and check it out by initialing under the "sorted by" column on the Laboratory Sample Tracking Sheet (hardcopy, Attachment C) and the LAB SORT_ID TRACKING electronic spreadsheet (Attachment D). Transcribe all information from the sample label into the Sorter's Log Book (Attachment E) and into the Sorter's Notebook (each sorter has separate log sheets and a notebook for this purpose). See Attachment F for an example of the appropriate notebook entry.

e. Rinse two mesh-sieve funnels (one labeled "S" [sorted] and the other labeled "U" [unsorted]) with fresh water prior to use. The mesh size should be no larger than that used to collect samples. The mesh size is written on the sieve funnel.

f. At the sample preparation station near the Lab sink, place the 'unsorted' mesh funnel into the mouth of a clean Ball® jar. Place the Ball® jar and mesh funnel into a Pyrex® tray so the sample can be retrieved if a spill occurs. Pour the sample and alcohol into the mesh funnel. The funnel will contain the material to be sorted while the alcohol will drain into the Ball® jar.

g. Using 70% alcohol in a wash bottle, rinse any remaining sample from the sample jar, the jar lid, and inner sample label (hold the label with forceps) into the mesh funnel containing the unsorted sample.

h. Place the 'unsorted' mesh funnel containing the sample material over the funnel situated in the container labeled "Alcohol Water Waste". Using fresh water in a wash bottle, carefully rinse the entire sample with fresh water.

i. Place the 'unsorted' mesh funnel containing the sample and the empty 'sorted' funnel into individual glass bowls and place both bowls in a Pyrex® tray. Place enough water into the bowl to cover the sample. Continue adding water as needed. Do not let the unsorted or sorted sample desiccate during processing.

j. Pour the 70% alcohol that was filtered through the mesh funnel back into the original sample jar. Keep the original alcohol-filled sample jar with the sample.

k. Place the "sorting tray" (a petri dish marked with a sorting grid) 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 on the sorting tray.

l. Arrange the light source to provide satisfactory illumination.

m. Using specimen forceps carefully transfer a small amount of the sample from the 'unsorted' funnel to the sorting tray. Using a wash bottle, slowly add enough water to cover the sample. Carefully distribute the sample evenly in the sorting tray using insect handling forceps.

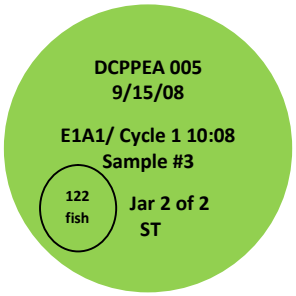
n. Look thoroughly through the entire sorting tray using the grid for orientation. Remove the target organisms (fish, crab, etc.) with insect handling forceps and place into a culture dish ("fish dish") containing water. Count and record the type of organism removed from the sample and keep a running tally of each organism in the Sorter's Notebook.

o. Carefully re-distribute the sample in the sorting tray and inspect 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.

p. Once the tray is sorted, pour the sorted sample into the 'sorted' funnel and rinse the sorting tray 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.

q. When sorting is completed, fish and eggs should be re-counted (to verify the count recorded in the Sorter's Notebook) and transferred from the fish dish into ½ dram shell vials containing 70% alcohol. Use separate vials and snap caps for fish and eggs. Wet one end of a small piece of cotton with alcohol and place the cotton into the top end of the vial to keep the organisms inside. Place the shell vial into a snap cap vial and add enough 70% alcohol to completely cover the shell vials. For fish samples, label the snap cap lid with a green colored dot label. For egg samples, label the snap cap lid with a white colored dot label. Prepare waterproof inner labels for the snap cap vial containing the shell vial. Both the inner label and snap cap label should contain the following information:

- Survey serial number
- Date the sample was collected
- Station, cycle, and collection start time
- Sample number
- Jar number (if more than one jar)
- Sorter's initials
- Number of organisms in shell vial



Green snap cap fish lid label (70% alcohol)

r. Recount and transfer invertebrates from the fish dish into a 2 dram screw-top vial and fill with alcohol. Prepare a white rectangular outside label and a waterproof inner label for the screw-top vial containing the same information as shown in step q above. Place the white label on the side of the vial and the appropriate small color coded dot containing the survey and sample number on the screw-top lid. Refer to the invertebrate color code chart posted in the Lab.

s. Record the total number of sorted organisms and the total time required to process the sample in the Sorter's Notebook. In addition, input the following information into the LAB SORT_ID TRACKING spreadsheet:

- Sample start date
- Sample completion date
- Sort time
- Number of organisms (fish, eggs, invertebrates)
- Number of fish fragments

t. Return the sorted sample back into the original sample jar containing 70% alcohol. Rinse any remaining sample from the funnel into the jar using a wash bottle containing 70% alcohol. If needed, use recycled 70% alcohol to fill the sample jar to at least ¾ full to allow for any evaporation. Ensure the correct inner waterproof label is in the sample jar.

u. Using fresh water, thoroughly clean the mesh sorting funnels, sorting tray, bowls, Pyrex® dish, fish dishes, and forceps.

v. If a sample must be stored before completion, 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 wash bottle containing 70% alcohol. Make sure that the sample is adequately covered with 70% alcohol. Put the unsorted sample into a second jar. Rinse any sample from the 'unsorted' funnel into the jar using a wash bottle containing 70% alcohol. 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. Ensure that the 'unsorted' sample is adequately covered with alcohol. The sorted and unsorted portion of the sample should be stored together in a container until sorting can continue. Record the number of organisms found and the time spent in the Sorter's Notebook.

w. Once the sample is completed, place a green colored dot label on the sample jar top with the survey number, sample number, sorter's initial, and date sorting was finished. Return the jar, in the proper order, to the box from which it was originally removed. The vials containing organisms should be stored in the appropriate labeled box in the lab.

x. Fill out the required information in the LAB SORT_ID TRACKING datasheet and Sorter's QC log on the computer. Transcribe the information recorded in the Sorter's Notebook to the Laboratory Sample Tracking Sheet and to the Sorter's Log.

10.2. Sample Sorting Procedures for 95% Ethanol (non-denatured) Samples

a. Rinse two mesh-sieve funnels (one labeled 'sorted' and the other labeled 'unsorted') with fresh water prior to use. The mesh size should be no larger than that used to collect samples. The mesh size is written on the sieve funnel.

b. At the sample preparation station near the Lab sink, place the 'unsorted' mesh funnel into the mouth of a clean Ball® jar. Place the Ball® jar and mesh funnel into a Pyrex® tray so the sample can be retrieved if a spill occurs. Pour the sample and ethanol into the mesh funnel. The funnel will contain the material to be sorted, while the ethanol will drain into the jar.

c. Using a 95% ethanol wash bottle, rinse any remaining sample from the sample jar, the jar lid, and inner sample label (holding the label with specimen forceps) into the mesh funnel containing the unsorted sample.

d. Place a funnel into the mouth of an empty ethanol wash bottle and pour the ethanol from the Ball® jar through the funnel into the empty bottle. Check that the ethanol wash bottle is labeled "95% non-denatured ethanol". This ethanol will be used in the sorting process instead of water. If needed, add more ethanol to the wash bottle from the stock 95% non-denatured ethanol container.

e. Place the 'unsorted' mesh funnel into a glass bowl in a dish and cover the sample with 95% ethanol. Do not let the sample desiccate during processing. Ethanol evaporates quickly; continually check the condition of the sample, adding ethanol as needed.

f. Using specimen forceps transfer a small amount of sample from the 'unsorted' mesh funnel into the sorting tray. Add enough 95% ethanol to cover the sample. Carefully distribute the sample evenly in the sorting tray using insect handling tweezers.

g. 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 on the sorting tray.

h. Arrange the light source to provide adequate illumination.

i. Carefully inspect the entire sorting tray using the grid for orientation. Remove the target organisms (fish, crab, squid, etc.) with insect handling tweezers. Place fish into a small fish dish containing 95% ethanol. Invertebrates found in 95% ethanol samples shall be placed in an invertebrate vial with 70% ethanol. Count and record the type of organism removed from the sample and keep a running tally of each organism in the Sorter's Notebook.

j. Carefully re-distribute the sample in the sorting tray and inspect the sample 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.

k. Once the tray is sorted, pour the sorted sample into the 'sorted' mesh funnel and rinse the sorting tray into the mesh funnel with a small amount of 95% ethanol. Take a second aliquot from the 'unsorted' funnel as described above. Repeat the above steps until the entire sample has been sorted.

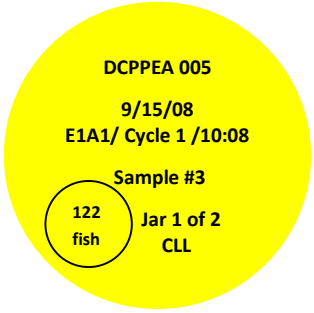
l. Approximately every hour recount the fish and place them into a shell vial containing 95% ethanol. Place a small piece of cotton into the top of the shell vial and place the shell vial into a snap cap container filled with 95% ethanol. Keep the snap cap container closed.

m. If there is not enough ethanol in the ethanol wash bottle to sort the entire sample, place the 'sorted' sample into the original sample jar. Use either the sorter's ethanol wash bottle or the clean 95% ethanol wash bottle to rinse the sample from the mesh funnel into the sample jar. Cover the sample with enough ethanol to prevent desiccation. At the sink, use a funnel to refill the sorter's ethanol wash bottle with the ethanol from the 'sorted' glass bowl or refill from the stock 95% non-denatured ethanol container.

n. If a sample will take more than approximately two hours to sort, keep the unsorted sample in a jar with ethanol instead of the mesh funnel. This will prevent desiccation. To get the last bit of sample out of the jar it may be necessary to put the sample into the mesh funnel.

o. When the sample is complete make sure all fish and invertebrates are counted.

p. Add enough 95% ethanol to completely cover the shell vials and label each snap cap lid with a yellow dot to denote the snap cap vial contains 95% ethanol. Prepare a waterproof inner label for the snap cap vial containing the shell vial. Both the inner label and snap cap label should contain the following information:
   - Survey serial number
   - Date the sample was collected
   - Station, cycle, and collection start time
   - Sample number
   - Jar number (if more than one jar)
   - Sorter's initials
   - Number of organisms in shell vial



Yellow snap cap fish lid label (95% ethanol)

q. Place a yellow '95% ethanol' sticker on the side of the snap cap container.

r. When the sample is completed, place a green dot on the sample jar lid labeled with the survey number, sample number, sorter's initial, and date sorting was finished:

s. Return the jar, in the proper numerical order, to the box from which it was originally removed. The snap cap vials containing fish and eggs and the invertebrate vials should be stored in the appropriate location in the lab.

t. Fill out the required information in the Laboratory Tracking Sheets binder, the Sorter's Logbook binder, and on the LAB SORT_ID TRACKING datasheet and Sorter's QC log on the computer.

10.3. Sample Sorting Procedures for Sub-Sampling Fish Eggs

a. In samples to be processed for fish eggs, if the sorter thinks there will be more than 500 eggs in a sample then the sample should be "subsampled" for eggs. In some instances, all samples may be subsampled for eggs. When subsampling, the sample should be processed first for fish larvae and selected invertebrate larvae, ignoring any eggs. When ready to subsample put the sorted sample back in the original sample jar and fill the jar with 70% alcohol up to the lip of the jar. Jar size varies, but will typically be 500 ml (if sizes varies there will be a posting in the lab). A 'sub sample' should be 10 percent of the sample volume.

b. After thoroughly mixing the sample, take the subsample as quickly as possible to avoid biasing the subsample as organisms begin to sink. For a 500 ml sample use a Hensen-Stempel pipette with a 10-ml plunger and take 5 aliquots (totaling 50 ml). There should be no air bubbles inside the Hensen-Stempel pipette. If there are, replace the subsample into the jar, completely dry the pipette, and begin again. When the subsample is bubble-free, dry the outside of the pipette and dispense the subsample into the mesh funnel. Mix the sample again and repeat the procedure to obtain 10 percent of the sample volume. Rinse the pipette into the mesh with water, washing the ethanol out of the sample through the mesh.

c. Once the aliquot is processed for fish eggs return it to the original sample jar with the rest of the sorted sample. Record in the Sorter's Notebook that the sample was subsampled and record the total volume of the sample and the volume of the subsample. Additionally, fill in the subsample information on the laboratory tracking sheets and on the computer tracking sheets. Place a white dot sticker labeled with the survey number, sample number, sorters initials, subsample date, and the abbreviation "SS" on top of the sample jar.

11.0 IDENTIFICATION OF LARVAL FISHES AND MACROINVERTEBRATES

11.1 Target Organism Identification Procedures

a. Ensure that the proper equipment necessary for processing is available (Appendix A-2).

b. Consult the Plankton Lab schedule posted in the laboratory to determine ID priorities.

c. Obtain the sample to be identified from the ID Station and check it out by initialing under the "identified by" on the LAB SORT_ID TRACKING electronic spreadsheet (Attachment D). Ensure that the correct sample is signed out. Transcribe all information from the sample label into the Taxonomist's Log (Attachment G) and into the identifier's individual Notebook (each taxonomist has separate log sheets and a notebook for this purpose).

d. The fish and target invertebrates from each sample are to be kept in separate containers. Keep fish and eggs in shell vials and target invertebrates in screw-top vials.

e. Carefully empty the container of target organisms to be identified into a small watch glass. Place the dish on the microscope stage and adjust the lighting to provide adequate illumination.

f. Unless otherwise specified, identify each target organism to the lowest taxonomic classification possible.

g. A fish is considered to be "whole" when 1) the head and 50% or more of the body is present or 2) a headless fish with at least 80% of the body present. Fish are considered to be "fragments" when less than 50% of the body is present (with or with a head). Place mutilated larvae (partial organisms that are unable to be identified) and fragments in a separate vial labeled "Unidentified fragments". Place whole larvae that are unidentified in a separate vial labeled "Unidentified larvae". Uncertain identifications should be checked with other Tenera identifiers and the reference collection. The conservative guidance for IDs is not to push rare organisms beyond a verifiable identity.

h. Record initial identifications and counts in the individual identifier's Notebook, which should also include notes and comments on the organisms in each sample.

i. Record the species identification, including life stage(s), and total number of each taxon on the Laboratory Identification Data Sheet (Attachment H); sign and date the sheet. In addition, record the total number of organisms in the sample, the ID time, date, and identifiers initials on the electronic LAB SORT_ ID TRACKING database for the project and in the identifiers personal QC log. If more than one day is needed to complete the identification, the date the sample identification was completed is to be recorded on the tracking sheets.

j. All individuals of each identified taxon of fish larvae from a sample should be put into a shell vial containing either 70% alcohol or 95% non-denatured ethanol, as appropriate. A yellow "95% ethanol" sticker on the snap cap container indicates the specimens are stored in 95% non-denatured alcohol. Each vial should contain an inside label with the taxon name, survey number, and sample number. Use a heavy weight cotton fiber paper [Rite in the Rain] and a No. 2 pencil or Pigma Micron pen to make the label. To avoid specimen damage, the label shall be placed in the vial before the specimens. If this is not practical, care must be taken that larvae are not trapped between the label and vial, either on the walls or floor of the vial. Cotton should be pushed into the upper end of the vial to keep the label and organisms enclosed.

DCPPEA 05 Sample #9

Genyonemus lineatus

Inside label with the taxon name, survey number, and sample number.

k. If greater than 100 fish of any stage of larval development are present in a sample, then each stage shall be grouped and placed in individual labeled shell vials. The exception is fish within the family Engraulidae; each developmental stage, regardless of the total number in a sample, shall be grouped and placed in individual labeled shell vials.

l. Place all vials containing larval fish from an individual sample into a whirlpak bag containing enough 70% alcohol, or 95% non-denatured ethanol, as appropriate, to cover the vials. Place the whirlpak into a Ball® jar that contains both an inside label and a label attached to the outside of the lid denoting the survey number, 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 jar, vial, or whirlpak bag.

m. All individuals of each identified taxon of macroinvertebrate larvae from a sample should be put into a 2 dram screw-top vial containing 70% alcohol. Each vial should contain an inside label with the taxon name, survey number, and sample number. Use a heavy weight cotton fiber paper [Rite in the Rain] and a No. 2 pencil or Pigma Micron pen to make the label. To avoid specimen damage, the label shall be placed in the vial before the specimens. If this is not practical, care must be taken that larvae are not trapped between the label and vial, either on the walls or floor of the vial. Cap the vial tightly with a screw-top lid.

n. Fill out the required information in the Identification Tracking Sheets binder, the Taxonomist's Logbook binder, on the LAB SORT_ID TRACKING datasheet, and the identifier's ID QC log on the computer.

o. At the end of work, all in-progress identification sheets shall be placed in the fire proof box located in the Lab. The datasheets are to be placed into the individual identifier's folder under the appropriate Project file.

p. The Ball® jar containing fish larvae shall be placed into the appropriately labeled box containing identified samples. Marcroinvertebrate vials shall be placed in labeled racks.

q. If needed, archive representative identified specimens in the reference/voucher collection.

r. Place any liquids containing ethanol into the appropriate waste container.

11.2 Identification of Sort QC Organisms

a. Identification procedures used to identify those organisms resulting from a Sort QC are the same as the identification procedures described above (Section 11.1 Target Organism Identification).

b. Once identified, record the species identification and the total number of each taxon on the Sort QC Laboratory Identification Data Sheet (color-coded to indicate a Sort QC). Sign and date the datasheet and file it behind the original Lab ID Data Sheet in the appropriate Project Binder.

c. Transcribe all taxonomic Sort QC data onto the original Lab ID Data Sheet and update taxa counts.

d. Remove the orange QC sticker from the snap cap containing the QC organism(s) and place the sticker onto the original Lab ID Data Sheet and sign and date the datasheet.

e. Place the vials containing fish larva from the SORT QC sample into a whirlpak bag containing enough 70% ethanol, or 95% non-denatured ethanol, as appropriate, to cover the vials. Place the whirlpak into a Ball® jar that contains both an inside label and a label attached to the

outside of the lid denoting survey number, sample number, date and time collected, identifier's initials and the words "SORT QC".

f. Place macroinvertebrates from the SORT QC sample back into the screw-top vial and fill with 70% alcohol. Place both an inside label and a label attached to the outside of the vial denoting survey number, sample number, date and time collected, identifier's initials and the words "SORT QC". Place the vials on the invertebrate shelf in the Lab.

12.0 LABORATORY QUALITY ASSURANCE /QUALITY CONTROL

12.1 Quality Assurance

The appropriate Quality Assurance methods will be selected for each project on the basis of circumstances, objectives, and requirements. The provisions of this SOP will be adapted to these project specific requirements in the project QA plan. Current Laboratory QA consists of the following:

QA-1. All samples are to be logged into the Laboratory and sample information (through the entire process) will be recorded on the LAB SORT_ID TRACKING datasheet and SAMPLE_ARCHIVING PLANKTON RECORD to track the progress of each sample within the Project/Survey. Tracking of each sample will be updated as each step is completed (i.e., transferring, splitting, subsampling, sorting, taxonomy, measuring, QC checks, storage, maintenance, and disposal).

QA-2. The target control limit for sorting efficiency is 90%. Precautionary measures are taken when sorting efficiency falls below 95%. Corrective action is taken when sorting efficiency falls below 90%.

QA-3. After laboratory processing is complete for a given sample, all mesh-sieves, forceps, bowls, trays, etc. that have come into contact with the sample are to be carefully examined, thoroughly rinsed, and picked free of organisms or debris; organisms found will be added to the sample residue.

QA-4. All procedures outlined in this SOP will be followed. Any deviations known ahead of time must be approved by the Project Manager and/or Lab Director. Any deviations made during the project must be recorded and also approved by the Project Manager and/or Lab Director as soon as practicable.

QA-5. Procedures are established to evaluate the quality of the taxonomy. At least 10% of the samples are identified again by a second taxonomist. The taxonomy passes the QC check if a prescribed percentage (PTD of 10%) of the identifications is in agreement between the original taxonomist and the second taxonomist.

QA-6. Voucher Collection/Identifications: A voucher collection of all samples is to be maintained. These samples should be properly labeled, preserved, and stored in the laboratory for future reference. A taxonomist not responsible for the original identifications will check samples corresponding to the identifications on the Voucher Collection ID sheets. Labels with specific taxa names are added to the vials of specimens by the taxonomist. Individual specimens may be extracted from the sample to be included in a reference collection or to be verified by a second taxonomist. Identifications are initialed by the identifying taxonomist. The word "validated" and the first initial and last name of the person validating the identification should be added to Voucher Collection ID sheets. Specimens sent out for taxonomy validations should be recorded in the "Taxonomy Validation Notebook" showing the label information and the date sent out. Upon return of the specimens, the date received and the finding should also be recorded in the notebook along with the name of the person who performed the validation.

QA-7. A library of basic taxonomic literature is essential in aiding identification of specimens and will be maintained (and updated as needed) in the taxonomic laboratory. Taxonomists will participate in periodic training of specific taxonomic groups to ensure accurate identifications.

12.2 Quality Control

12.2.1 Quality Control Program for Laboratory Sample Sorting

The basic processing of zooplankton samples involves the time-consuming removal of organisms from large amounts of debris. Inevitably, processing errors occur during this sorting phase regardless of the diligence of the technician and those errors must be estimated. Therefore, the first QA/QC component of sample processing is the requirement to assess the sorting efficiency (i.e., the proportion of total organisms extracted from the sample upon sorting). High sorting efficiencies will ensure that endpoint calculations are reasonably reliable and without bias between samples.

The recommendation for assessing sorting efficiency is that at least 10 percent of all samples are to be resorted and any organisms found on the second sort be enumerated. The criterion for an acceptable sort is that > 90 percent of the total number of organisms are recovered during the initial sort. Sorted samples (and unsorted samples/subsamples) are retained until taxonomy and sorting efficiency (QC) are confirmed and the data are reviewed.

All laboratory personnel receive basic instruction and evaluation in the sample processing procedure by experienced laboratory staff. Experienced technicians are used to remove target organisms from the sample. A Quality Control (QC) technician must be present when samples are processed by an inexperienced individual (i.e., sorter in training).

12.2.2 Sorting Efficiency Requirements

a. A QC technician or trainer will check all sorted trays from the first ten samples processed by a sorter in training to ensure that each sample meets the >90 percent sorting efficiency. Qualification as an experienced technician may only occur when a sorter achieves >90 percent sorting efficiency for ten consecutive samples, however additional samples may be checked until the trainer decides that training is complete.

b. After the sorter has passed 10 consecutive samples with an efficiency rate of at least 90 percent, the program is switched to a '1 sample in 10' QC program for that sorter. Using a random number chart, one of the next ten samples is selected for a QC check.

c. A QC technician re-sorts the selected sample, retrieving and counting any organisms found. If the sorter maintains the 90% efficiency sorting rate for this sample, then the sorter continues in the '1 sample in 10' QC mode. If a sample does not meet the 90% efficiency rate the sorter's subsequent samples will be resorted until 10 consecutive samples meet the criterion.

d. A sorter is allowed to miss one target organism when the original sort count is 1 through 19. For original sorter counts above 20 organisms a sorter must maintain a sorting efficiency of 90% based on the total combined count of individuals found by both the original sorter and the QC re-sorter. The sorting efficiencies obtained on each sample are calculated as below and recorded on the SORTER's QC LOG datasheet.

Determination of Sorting Efficiency

$$\textit{Sorting efficiency (\%)} = [\textit{Total organism} - \textit{QC organisms}] \div \textit{Total organisms} \times 100$$

Where:
Total organisms = total number of organisms found in the sample plus the QC organisms

QC organisms = total number of organisms found in the QC check

Example:

- 18 organisms are found in the QC re-sort
- 193 organisms were recovered in the original sort process

Total organisms = 211
QC organisms = 18

% Sorting Efficiency = [211 – 18] ÷ 211 x 100 = 91.5%

e. After all organisms sorted from the original sample have been counted, the total number of organisms found in the sample and the total number found in the QC check are entered on the SORTER's QC LOG and LAB SORT_ID TRACKING datasheet.

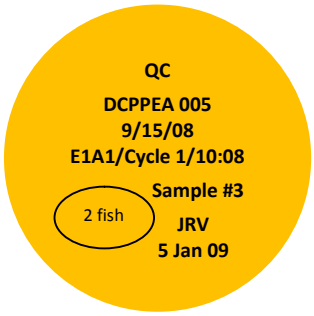
12.2.3 Sorting Procedure for QC Resort

a. Sorting procedures used during the QC resort are the same as the sorting procedures described above (Section 10.0 Sample Sorting Procedures). Any fish and selected invertebrate larvae that were missed by the sorter are removed during the QC resort.

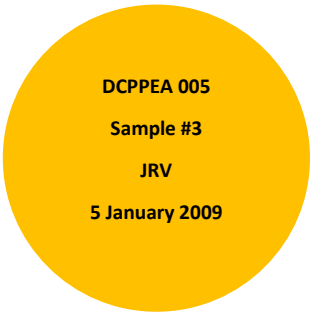
b. For the QC process, a larval fish is defined as having a head plus at least 50% of the body. Any parts without a head and/or less than 50% of the body will be considered a fragment and will not be counted against the original sorter as a missed fish (even if subsequently identified by a taxonomist). However, it is important for each sorter to remove all fish and fragments from each sample and correctly record them as # fish/# fragments in the Sorter's Notebook and on the tracking sheets.

c. Any fish larvae generated from the QC are put into shell vials topped with cotton and then into snap cap vials. Both the inner waterproof label and snap cap should be labeled as described in the sorting procedures (10.0); however use an orange dot label for the snap cap lid with "QC" added to the label. Any invertebrates generated from the QC are put into screw-top vials with a QC label placed on the side of the vial.

d. 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 technicians' initials, survey number, sample number, and date the QC resort was completed.



Orange QC label for snap cap lid



Orange QC label for sample jar lid

e. Store the QC snap cap vials and screw-top vials on the "QC Shelf" in the A2 Lab.

f. Fill out the required QC information on the LAB SORT_ID TRACKING datasheet and in the Sorter's Notebook.

12.2.4 Taxonomic Quality Control

Tenera maintains a reference compilation with a voucher collection containing over 300 specimens. The identification of most of these specimens has been verified by outside professional taxonomists. All new taxonomists are required to identify each of these specimens. The identification of any new taxon for our laboratory is currently verified by outside taxonomists and compared to known distribution records.

A taxonomic reference list of fish species is established for each project to assist in achieving consistently correct identifications. Ichthyoplankton from sorted samples are identified to the lowest taxonomic level possible (or practical). Macroinvertebrate taxa are usually identified to genus.

Since agreements are, in part, contingent on the targeted level of identification (i.e., species, genus, family, or higher) the list will identify the lowest level of identifiable taxon for each representative specimen. For example, if genus is the target, and one taxonomist provides a name for a specimen at the species level, whereas the other leaves the name at genus level, it would be scored as an agreement. However, if one identification is at the genus level and the re-identification is at family, it would not be counted as an agreement (one identification met the target, the other did not). If disagreements affect a large number of specimens in samples throughout the entire data set, then those samples can be isolated and evaluated further for corrective re- identifications.

For projects involving new geographic areas/locations (and for new taxonomists) the first ten samples of fish identified by a taxonomist will be completely re-identified by a designated QC taxonomist. At least 50 individual fish larvae from at least five taxa must be present in these first ten samples; if not, additional samples will be re-identified until this criterion is met. Taxonomic identifications are subject to a 10 percent re-check by another in-house taxonomist (i.e., one out of ten consecutive samples is selected to be re-identified by a second senior taxonomist).

Taxonomic precision is calculated as Percent Taxonomic Disagreement and will serve to quantify the rates of error (percent disagreement) in assignment of nomenclature to individual specimens in the sample. The error rate will be quantified as the proportion of individual specimens in the sample identified differently (percent taxonomic disagreement [PTD]) by the two taxonomists calculated as:

$$PTD = [1-(\underline{com}_{\underline{pos}})] \times 100$$
$$N$$

where: $comp_{pos}$ is the number of agreements and N is the total number of organisms in the larger of the two counts. A PTD goal of less than or equal to 10% is targeted. Taxonomists are required to maintain a maximum 10% PTD accuracy level in these first ten samples. The lower the PTD value, the greater the overall taxonomic precision indicating relative consistency in sample treatment. If the PTD goal of 10% is attained, then the taxonomist will continue to have one of ten samples checked by a QA/QC taxonomist.

taxonomist interaction is used to determine problem areas, identify consistent disagreements, and define corrective actions. Questionable specimens are immediately shared with all other taxonomists and compared to voucher specimens. If consensus cannot be reached among Tenera taxonomists, the specimen is shown to an outside expert.

Taxonomists are required to maintain a maximum 10% PTD accuracy level in these first ten samples. After the taxonomist has identified ten consecutive samples with a 10% PTD or less, the taxonomist will have one of their next ten samples checked by a QC taxonomist. If the taxonomist maintains , then they

will continue tohave one of ten samples checked by a QA/QC taxonomist. If they fall below this level, then the next ten consecutive samples they have identified will be checked for accuracy. Samples will bere-identified until ten consecutive samples meet the 95% criterion. Identifications will be verified with taxonomic voucher collections maintained by Tenera.

12.2.5 Verification of Taxonomic Identification

a. After the taxonomist has identified ten samples, one of the ten samples is selected under the direction of the Lab Director from the taxonomist's ID QC LOG for verification by a second (QC) taxonomist (Consult Lab Schedule).

b. Identifying labels are removed from each shell vial of the QC sample. The labels and the original Fish Identification Data Sheet are retained by the Lab Director. Fish are kept in individual shell vials as originally grouped and identified by the first taxonomist.

c. The second (QC) taxonomist re-identifies the sample and records the species identification, including life stage(s), and total number of individuals identified for each taxon on the QC Fish Identification Data Sheet (color-coded to represent an ID QC); the sheet is signed and dated.

d. The original sample identification data is compared with the data obtained from the QC check and the PTD calculated on the Percent Taxonomic Disagreement Form (Attachment I). If there is a disagreement with an identification (not to include life stages) the original taxonomist is consulted until a consensus is reached, utilizing additional experts as warranted.

e. All taxonomic discrepancies and comments associated with the QC check are recorded on the original Fish Identification Data Sheet.

f. The cumulative taxonomic proficiency data for each taxonomist is calculate and retained.

13.0 PRESERVATION AND MAINTENANCE OF LARVAL FISH

The majority of fish larvae are stored in 70% alcohol; however those designated for DNA or otolith analysis are stored in 95% non-denatured ethanol. When possible, concentrations should be measured with an alcohol hydrometer. "Treated water" (either de-ionized or distilled) is used to dilute the ethanol in order to reduce the risk of acidity caused by chloride and fluoride ions present in most urban water supplies. The ethanol is allowed to "settle" before use and is decanted using a carboy tap to keep particles, fuzz, and other debris away from specimens.

Ethanol is used as a preservative rather than formalin because of problems in maintaining pH in formalin solutions. Unbuffered formalin becomes acidic which destroys bone. Many buffers (including the widely-used borate buffer) damage larvae or allow the pH to rise too high, thereby bleaching larvae. Calcium carbonate can crystallize out on larvae, in effect "fossilizing" them. In addition, formalin presents health problems to those who work on the samples. Formalin is therefore best avoided as a storage solution for larvae. Alcohol, however, evaporates quickly so special measures must be taken to avoid this problem (see below).

Some larvae will be fixed initially in alcohol instead of formalin (e.g., for DNA and/or otolith studies). If so, this should be noted, as some investigations (such as clearing and staining) are not possible on ethanol-fixed material. Conversely, other investigations are not possible on formalin-fixed specimens (e.g., DNA, otolith). If fixed in formalin, the buffer should also be recorded (even if only seawater), as this, too, can affect what studies are possible with the material.

a. Fish larvae are stored in ½ dram (1.8 ml) glass shell vials capped with cotton tops. Labels for larvae are placed in the vials. Vials from each sample are stored together in whirlpaks which are filled with 70% alcohol or 95% ethanol. Whirlpaks are subsequently placed in pint glass Ball® jars. A yellow "95% ethanol" sticker indicates the specimens are stored in 95% non-denatured alcohol. Storing vials in whirlpaks containing ethanol accomplishes two things: inhibits or eliminates evaporation from vials, and checking of alcohol levels involves inspecting only one "container" rather than a large number of individual vials.

b. Larval fish must be kept in the dark. Archived larvae should be stored in covered boxes. Those in the work/lab area should be stored in cabinets or behind black curtains hanging in front of the shelves; this prevents the fading of specimens caused by exposure to light. This point cannot be overstressed. Larval pigment is particularly susceptible to fading under exposure to light - even artificial light. Do not leave specimens out on a bench or desk exposed to direct sunlight. Faded specimens are usually unidentifiable.

c. Vials, whirlpaks, and jars should be securely sealed, the lids applied firmly.

d. Checking and topping off the larval fish samples with alcohol should ideally take place at least once a year. For 70% alcohol samples where the alcohol level is found to be low (i.e., well below the neck) top-off with 80% alcohol. Eighty percent is used instead of 70% because the alcohol/water mix does not form a true azeotrope (mixture) and some separation occurs. The alcohol concentration at the top of the jar is higher than that at the bottom, and thus more alcohol than water evaporates with the result that the alcohol concentration drops below 70%. If over one third of the alcohol has evaporated, dispose of the old alcohol, refill the vial and/or whirlpak with clean 70% alcohol and replace the defective cotton, whirlpak, or lid.

e. After completing the alcohol level check and topping off any samples, fill out the electronic SAMPLE_ARCHIVING PLANKTON RECORD datasheet, place a sticker on the sample jar indicating the date and initials of the person responsible, and fill out the card attached to the survey storage carton, if appropriate.

14.0 LARVAL FISH MEASURING

Length measurements are taken on a representative sample of the target larval fish taxa. Approximately 100 fish from each taxon are measured using a video capture system and ImagePro© image analysis software. The fish from each taxon will be selected from entrainment stations based on the percentage frequency of occurrence of a taxon in each survey. For example, if 20 percent of the California halibut larvae for the entire study were collected during the June survey then 20 fish will be measured from that survey. Larval fish lengths are measured to the nearest 0.1 mm using an ocular micrometer.

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

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

c. Obtain the sample containing the fish to be measured from the Fish Sample Collection and place it in an easily accessible area close to the measuring station.

d. Enter the starting date and initial under "measured by" on the LAB SORT_ID TRACKING electronic spreadsheet.

e. Open the appropriate software on the computer (See ImagePro© Procedure 14.1.) by clicking with the mouse on the ImagePro© icon. Open the Larval Fish Measuring macro in ImagePro©, and follow the macro's directions.

f. Select the jar or vial of fish to be measured and compare the data on the jar label with the inner label and the data sheet for the sample. Consult an identification biologist if any discrepancies are found.

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.

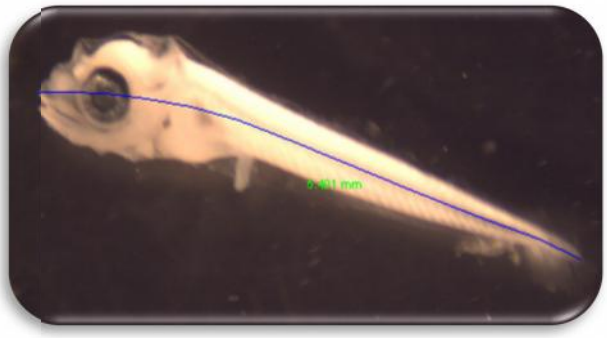
h. Open the sample jar and remove the vials for the target taxa to be measured. Place the vials in a rack designed to allow the vials to maintain an upright posture so as to reduce the risk of a spill.

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

j. Empty the vial into a shallow petri 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 the appropriate alcohol to the dish if necessary to prevent desiccation. A yellow "95% ethanol" sticker on the snap cap container indicates the specimens are stored in 95% non-denatured alcohol.

k. Place the petri dish on the stage of the microscope. With fine-tipped forceps carefully arrange the fish on the computer screen so that they can be measured. 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 the micrometer. 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.

l. 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.



m. If the fish is too damaged to find either the pre-maxillary or to estimate the path taken by the notochord, do not measure the fish, but 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 fish measurement and 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 a larval identification biologist. Make note of any problems in measuring, record in log, and post near the measuring station.

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

p. Repeat the above steps for all fish in the sample that need to be measured.

q. When all selected larval fish have been measured, put the fish back into their original labeled, ethanol filled vial.

r. If the larval fish from this vial have been segregated into two or more groups for ease of handling, place another group into the dish, being careful to submerse them in ethanol, and measure as above.

s. At the end of work, all in-progress identification sheets shall be placed in the fire proof box located in the Lab. The datasheets are to be placed into the individual measurer's folder under the appropriate Project file.

t. When all selected fish in a survey has been measured, fill in the required information on the LAB SORT_ID TRACKING datasheet.

u. The Ball® jar containing fish larvae shall be placed back into the appropriately labeled box containing identified samples within the Larval Fish Collection.

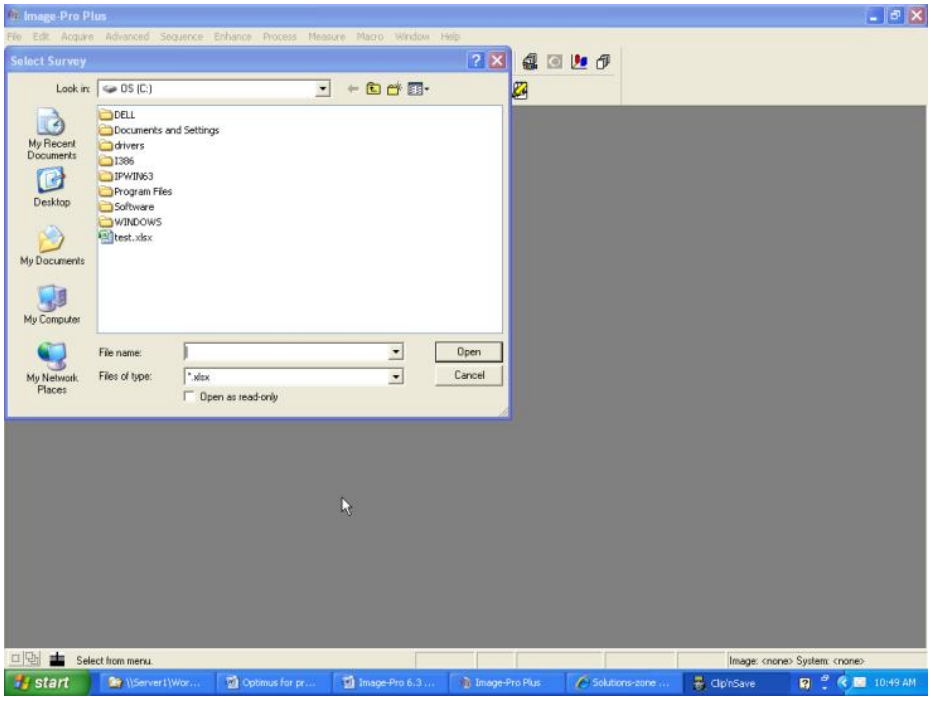
14.1 ImagePro© 6.3 Procedures for Measuring Larval Fish Length

a. Open ImagePro© by double-clicking its icon.

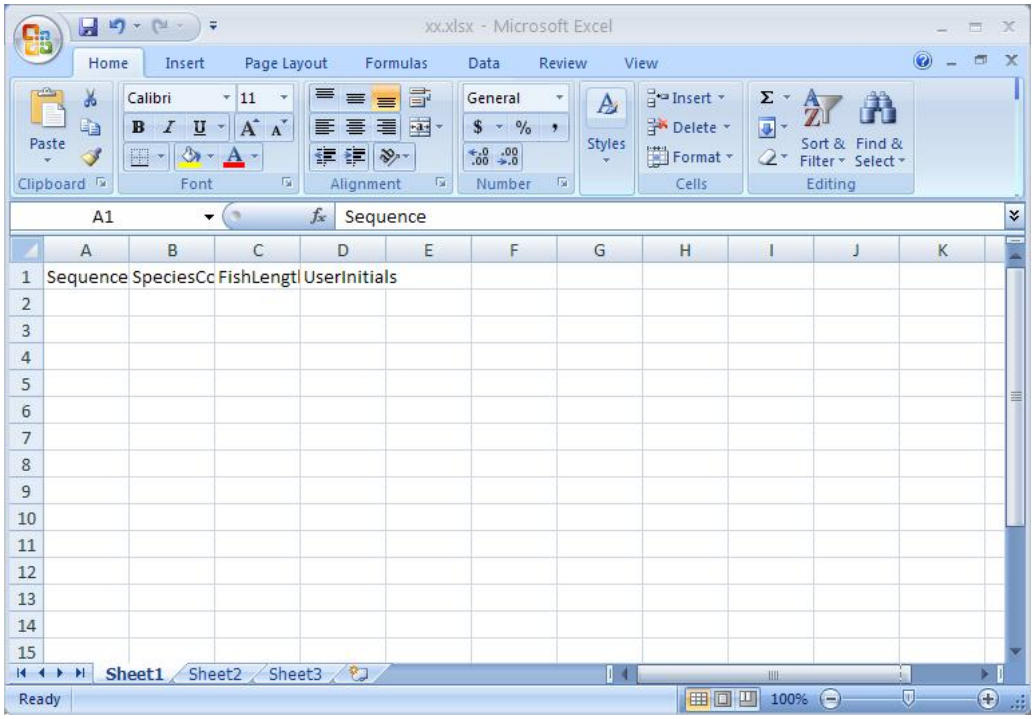
b. Turn on the camera.

c. Select the **Macro>MeasureFishLengthOnly** menu option to start the macro.

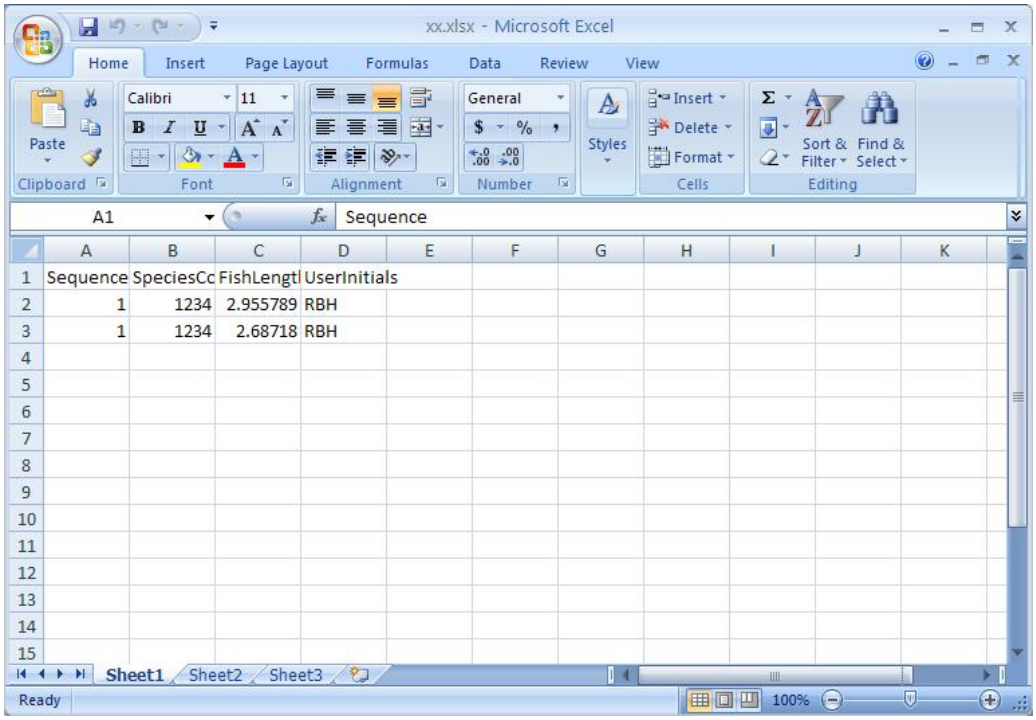
d. The macro starts by trying to open a previous survey file or starting a new one.



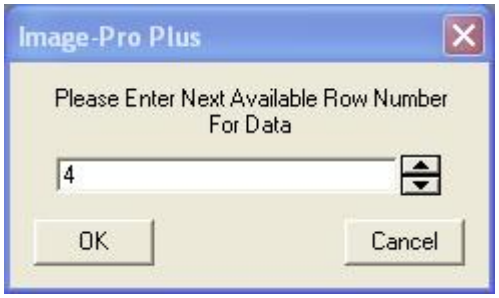
e. If the file does not exist then enter a new file name in the appropriate folder. Excel will open a new spreadsheet with column headers in the first row.



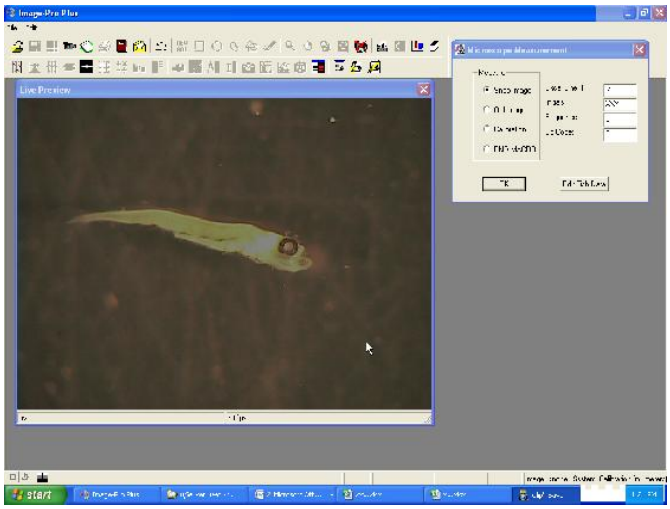
f. If the file does exist then select it from the list. Excel will open it with previously entered data.



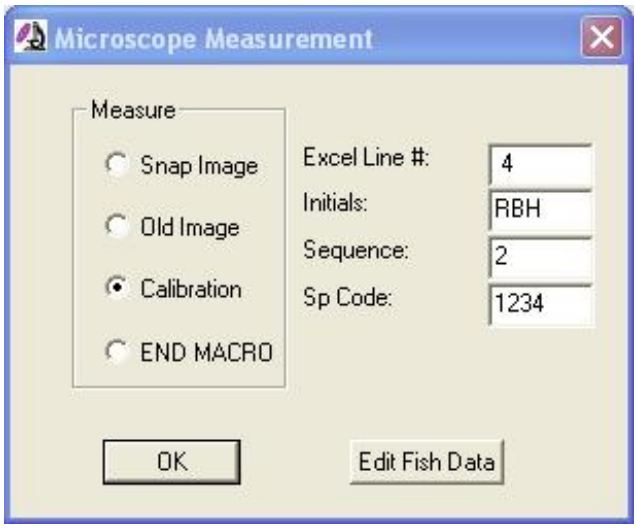
g. In this case, clicking on the rightmost ImagePro© task in the Windows taskbar should open the **Please Enter Next Available Row Number For Data** prompt. Enter this value and then press the **OK** button to continue.



h. The **Live Preview** window and **Microscope Measurement** dialogue will open.



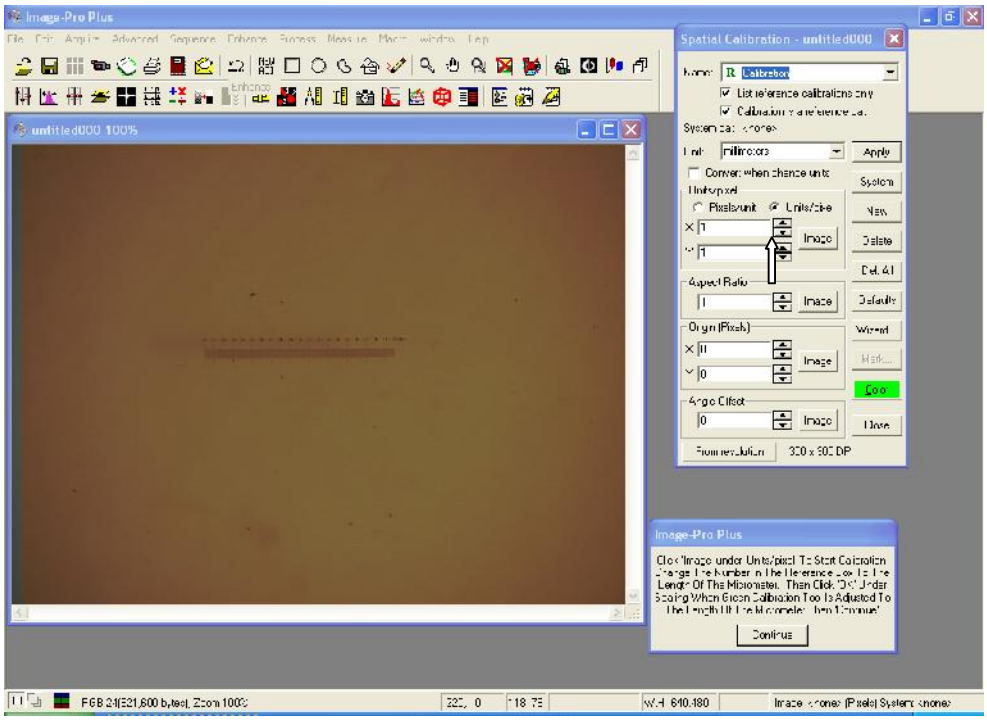
i. Select the **Calibration** option and press **OK**.



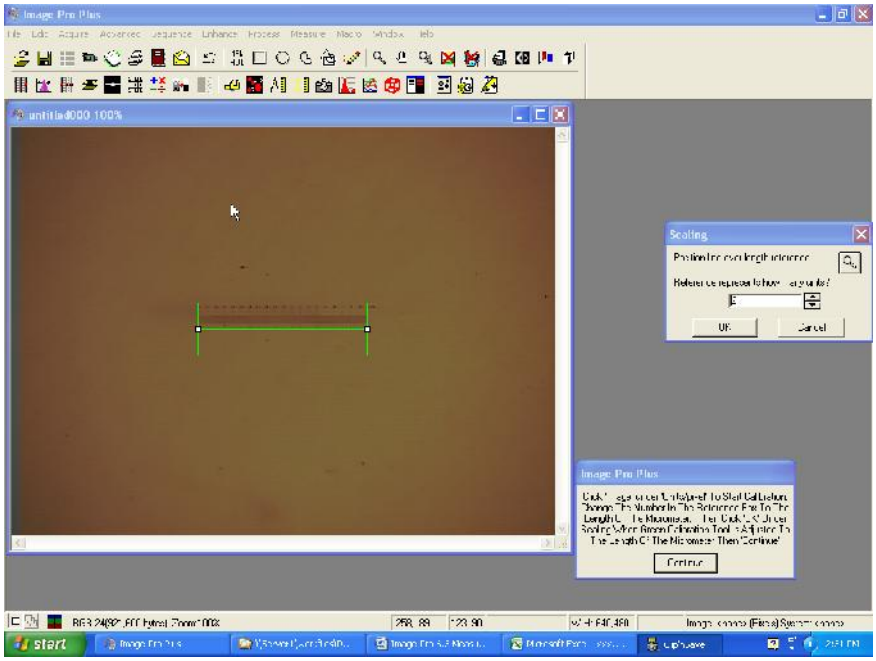
j. Place a fish under the microscope and adjust the magnification so the whole fish fits in the field of view. It is important not to adjust the magnification from this point on until the measurer wishes to recalibrate. Replace the fish with the micrometer under the microscope

and adjust the focus. Only the focus and position of the micrometer may be adjusted at this time. Press **OK** to start calibration.

k. Calibrate the measurements to the magnification. Click the **Image** button in the **Units/pixel** box to start this process.



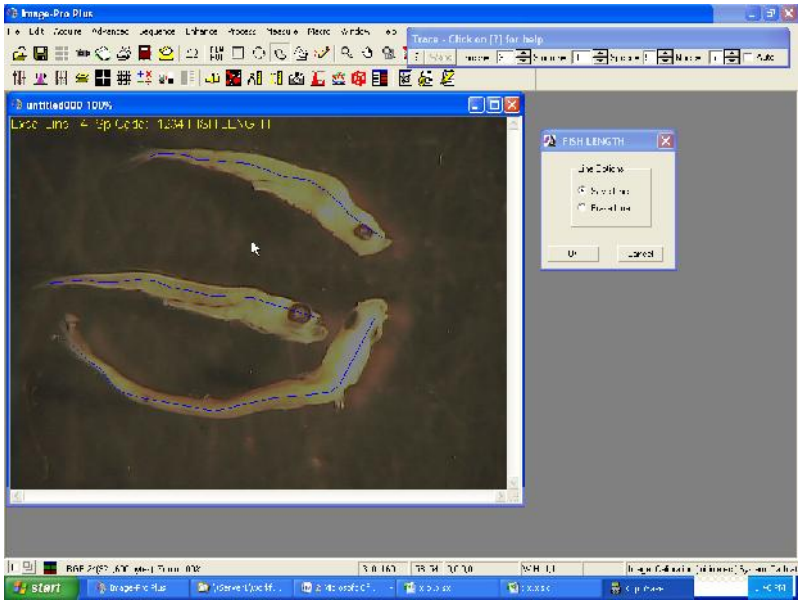
l. Position the green calibration tool over the length of the micrometer. Change how many units the green reference line represents to “2” in the Scaling box. Press the OK button and then the Continue button to go on.



m. Enter measurer's initials, the sequence number (data sheet number), and species (Sp) code in the appropriate text boxes.

n. Place fish of the same species from the same sequence under the microscope and click **OK** to start measuring.

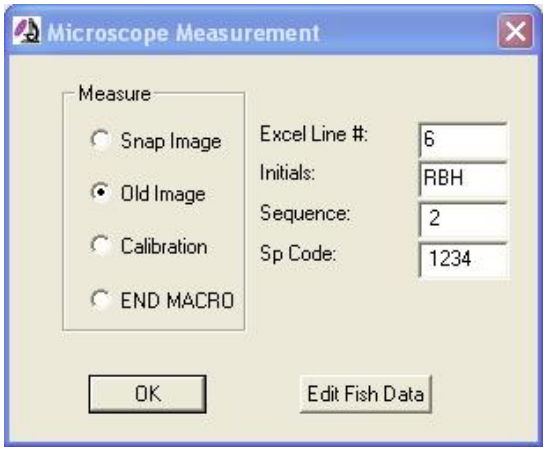
o. Measure by left-clicking at points along the length of the fish to make poly-line segments and then right-clicking after the last segment has been made. If the measurement made is not satisfactory select the **Erase Line** option and click **OK** to erase it. Once measuring is completed select the **Save Line** option and click **OK** to write the measurements to the Excel file.



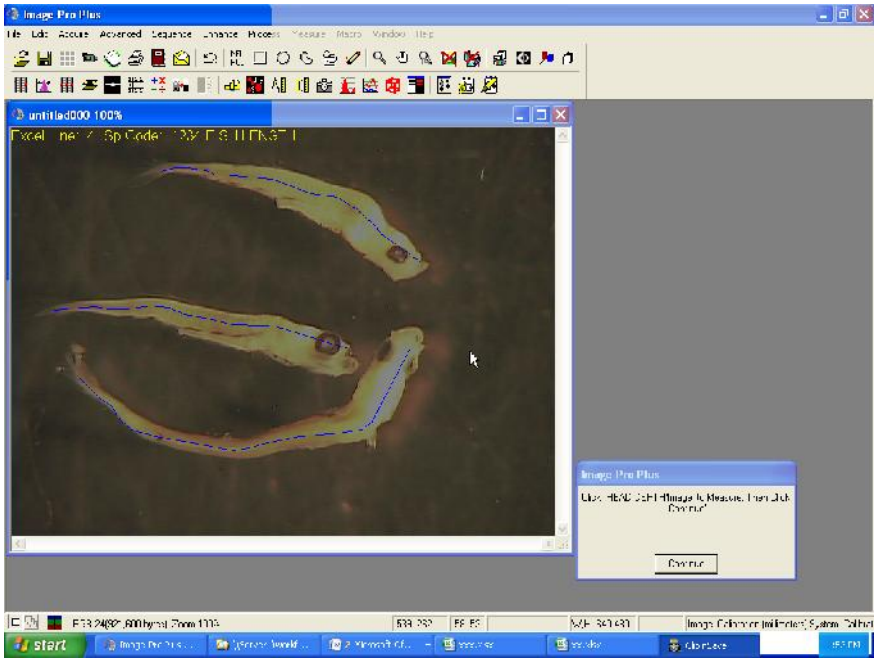
p. Continue measuring while changing the information in the text boxes as applicable (i.e., new sequence or different species code are needed) and calibrating when the magnification needs to be changed.

14.1.2 Method to Correct Measurement Error

a. If an error has been made with a measurement it is possible to re-measure from a previously captured image. Make sure the text boxes are filled in with the proper information of the measurement to be changed, especially taking care to have the proper Excel line number entered in the Excel **Line #** text box. Select the **Old Image** option and press **OK** to continue.



b. Select the image to be re-measured and click Continue.

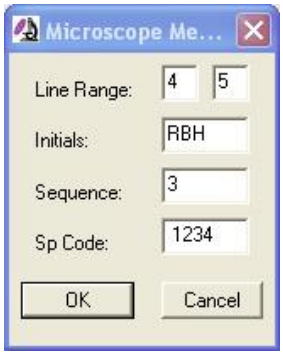


c. Follow steps m. through o. as normal to re-measure fish.

14.1.3 Method to Correct Data Entry Error

a. If the wrong fish information has been entered for a particular fish, these data may be changed by clicking the **Edit Fish Data** button.

b. Enter the correct information in all the pertinent text boxes, especially taking care to have the proper range of Excel line numbers entered in the **Line Range** text boxes.



c. When finished, select **END MACRO** and click on **OK.**

d. Be sure to save in Excel often by selecting the Excel spreadsheet and clicking the save button in the upper left corner.

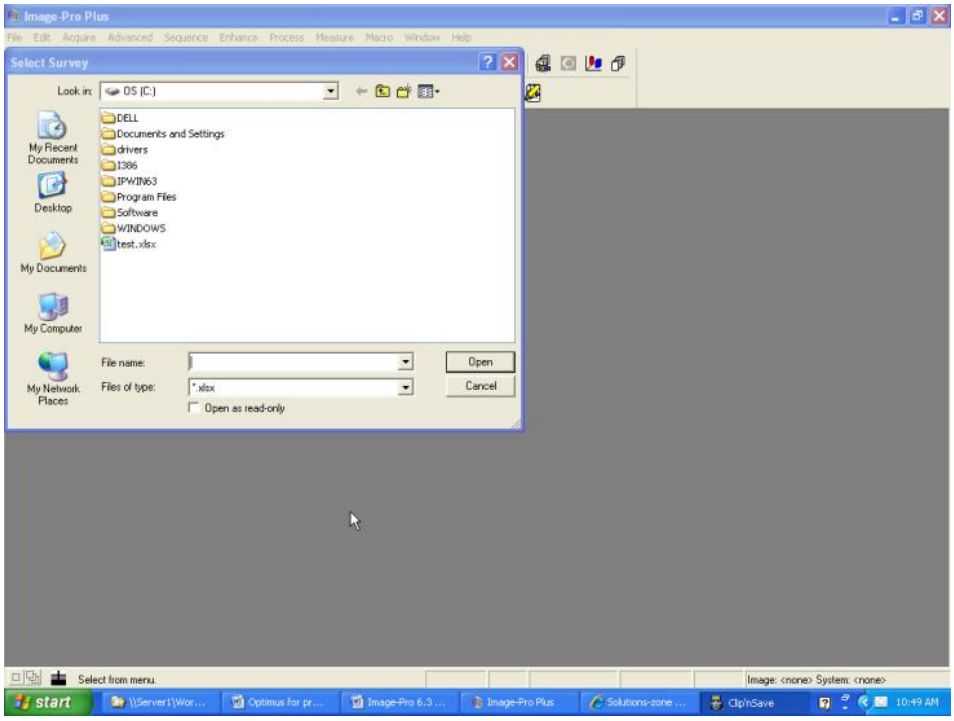
14.2 ImagePro© 6.3 Procedures for Measuring Larval Fish Length and Head Capsule

a. Open ImagePro© by double-clicking its icon.

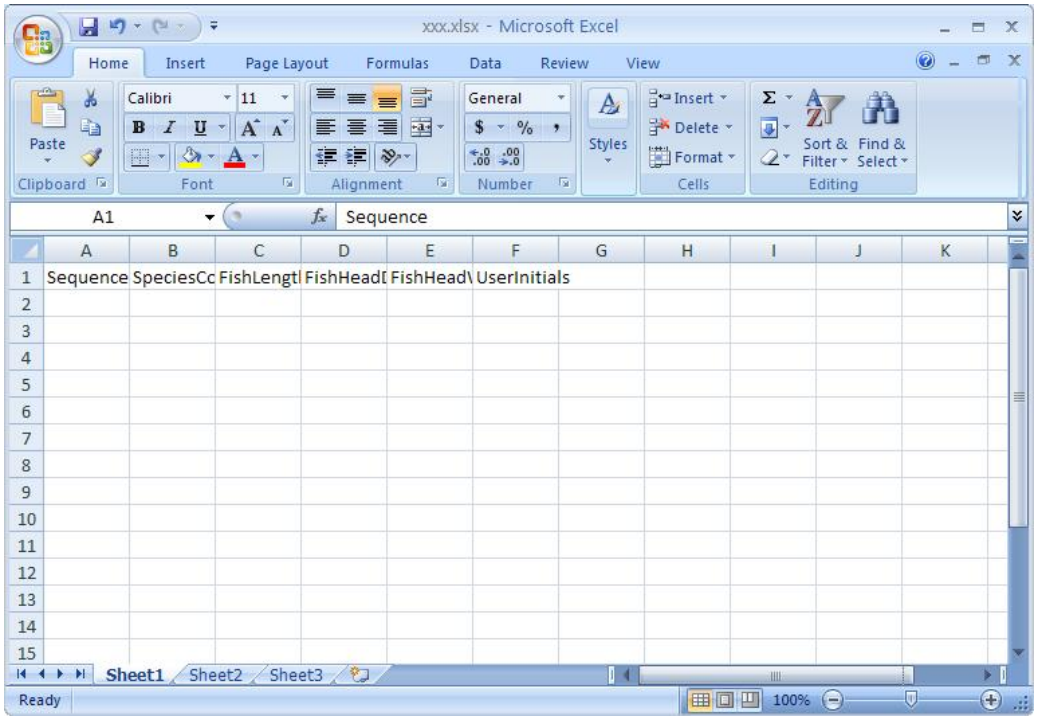
b. Turn on the camera.

c. Select the **Macro>MeasureFishLengthWidthDepth** menu option to start the macro.

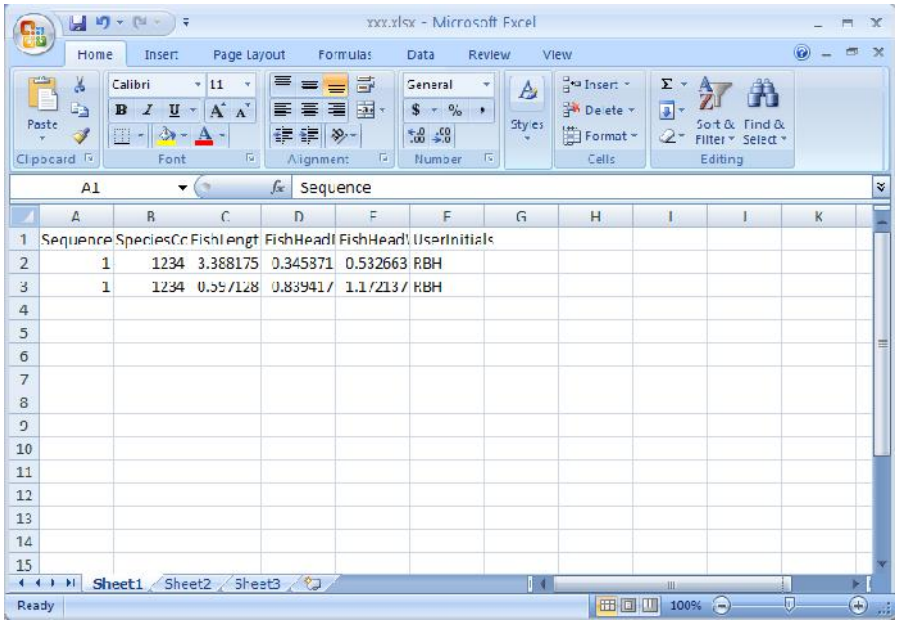
d. The macro starts by trying to open a previous survey file or starting a new one.



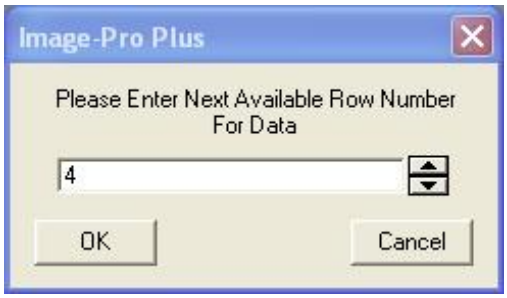
e. If the file does not exist then enter a new file name in the appropriate folder. Excel will open a new spreadsheet with column headers in the first row.



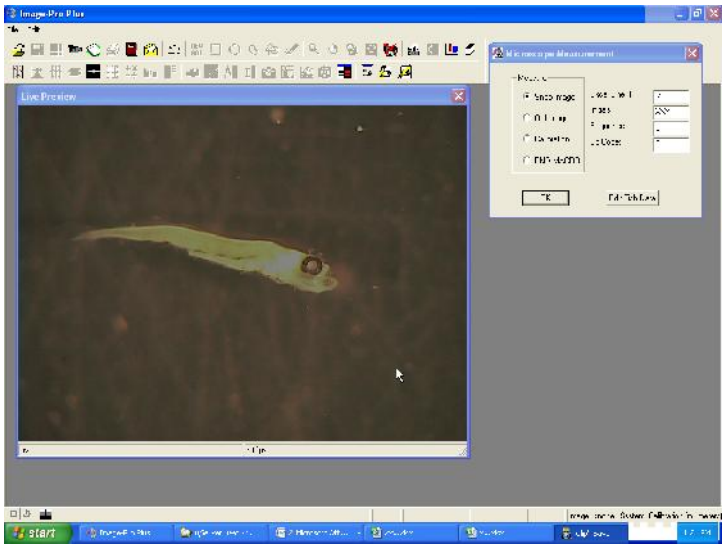
f. If the file does exist then select it from the list. Excel will open it with previously entered data.



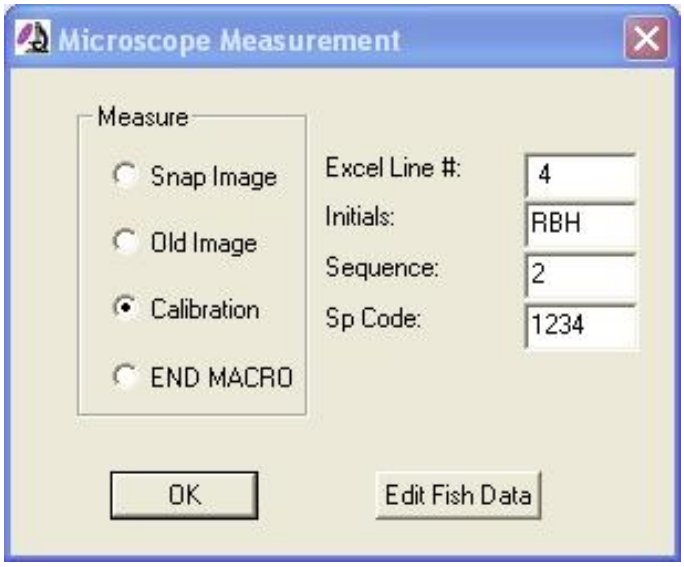
g. In this case, clicking on the rightmost ImagePro© task in the Windows taskbar should open the Please Enter Next Available Row Number For Data prompt. Enter this value and then press the OK button to continue.



h. The **Live Preview** window and **Microscope Measurement** dialogue will open.

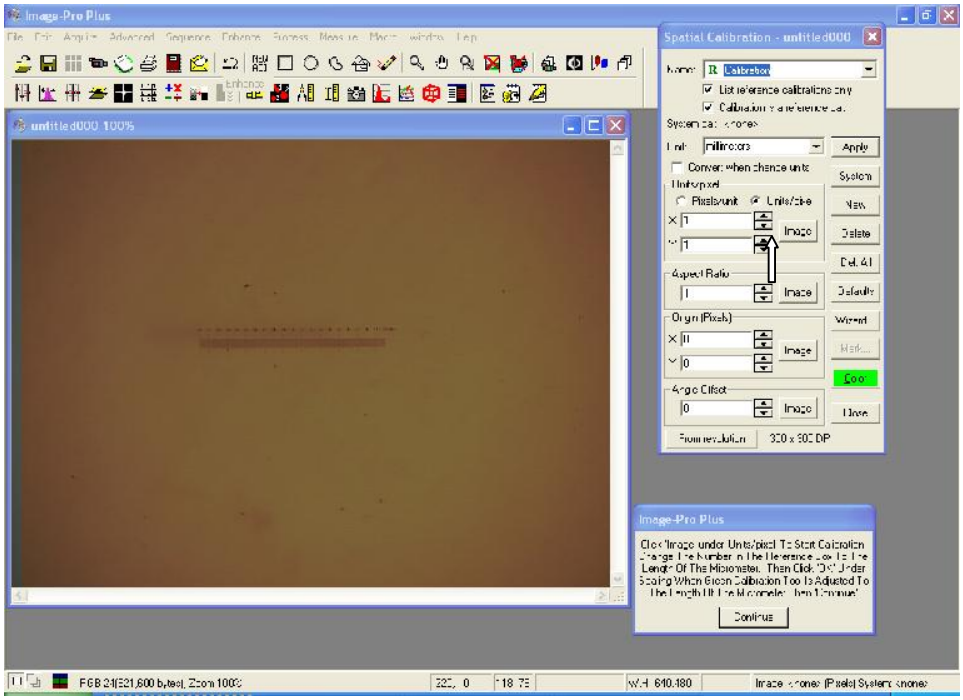


i. Select the **Calibration** option and press **OK**.

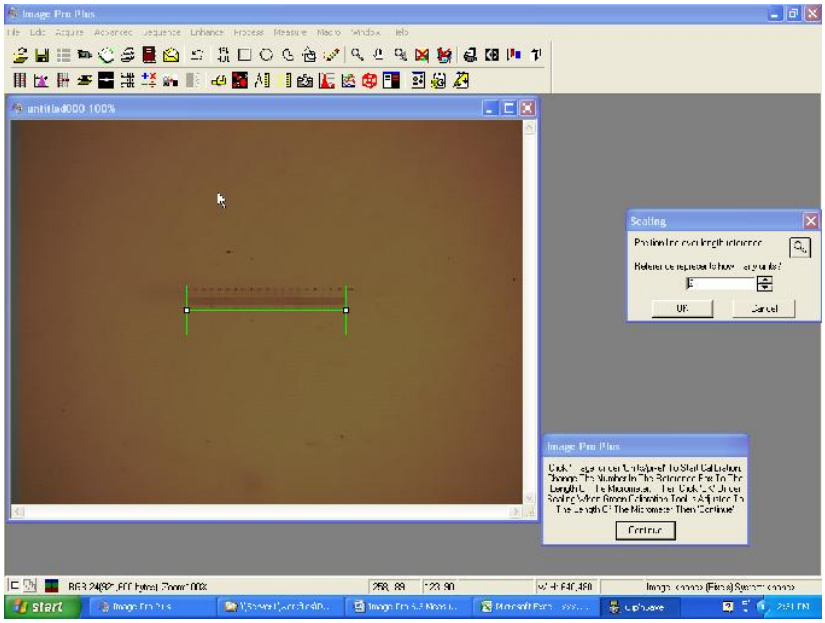


j. Place a fish under the microscope and adjust the magnification so the whole fish fits in the field of view. It is important not to adjust the magnification from this point on until the measurer wishes to recalibrate. Replace the fish with the micrometer under the microscope and adjust the focus. Only the focus and position of the micrometer may be adjusted at this time. Press **OK** to start calibration.

k. Calibrate the measurements to the magnification. Click the **Image** button in the **Units/pixel** box to start this process.



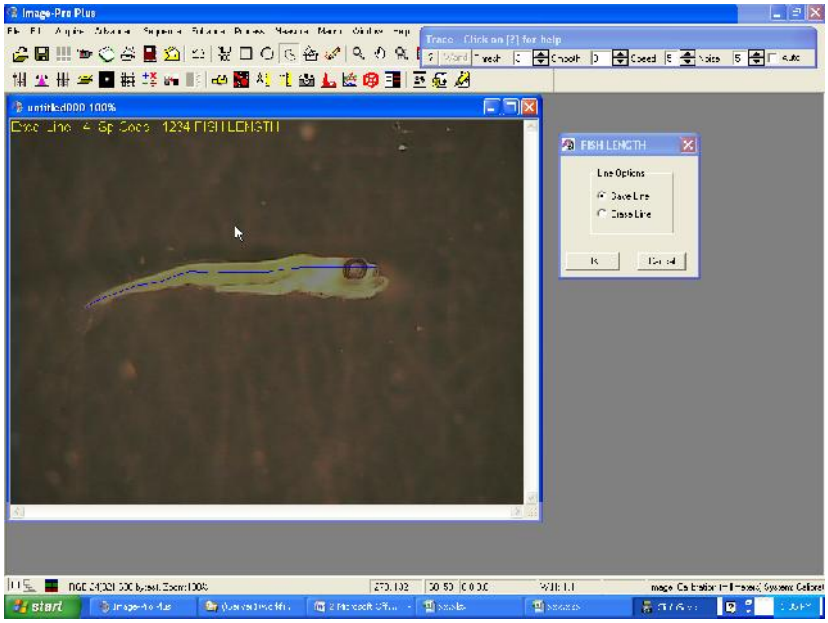
l. Position the green calibration tool over the length of the micrometer. Change how many units the green reference line represents to "**2**" in the **Scaling** box. Press the **OK** button and then the **Continue** button to go on.



m. Enter measurer's initials, the sequence number (data sheet number), and species (Sp) code in the appropriate text boxes.

n. Replace fish under microscope and select an option (**Length, Head Depth, Head Width**) under **Measurement** then click **OK** to start measuring.

o. Measure by left-clicking at points along the length of the fish to make poly-line segments and then right-clicking after the last segment has been made. If the measurement made is not satisfactory select the **Erase Line** option and click **OK** to erase it and start again. Once measuring is completed select the **Save Line** option and click **OK** to write the measurements to the Excel file.

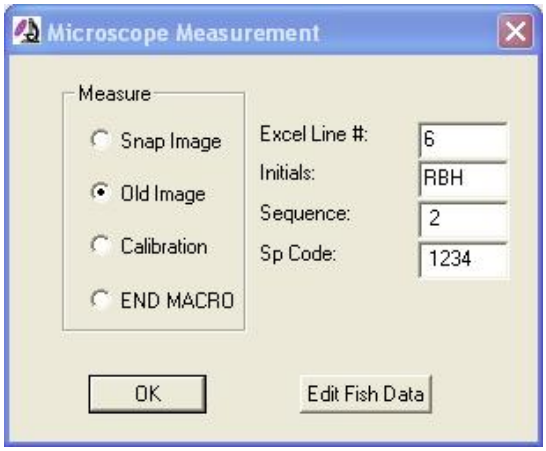


p. Repeat steps l. and m. until a measurement has been written for all three **Measurement** options while making sure to adjust the angle of the fish when the **Live Preview** window is open to acquire the proper view of the dimension to be measured. If a previously captured image can be used for multiple measurements select the **Old Image** option in the **Source** box. The macro will automatically advance to the next Excel line when all three measurements have been made. However, if the measurer is unable to make a measurement due to unsuitability of the fish (e.g., unable to measure head depth due to the mouth being open) the measurer may advance to the next line in Excel manually by entering it in the **Excel Line #** text box in the **Microscope Measurement** dialogue.

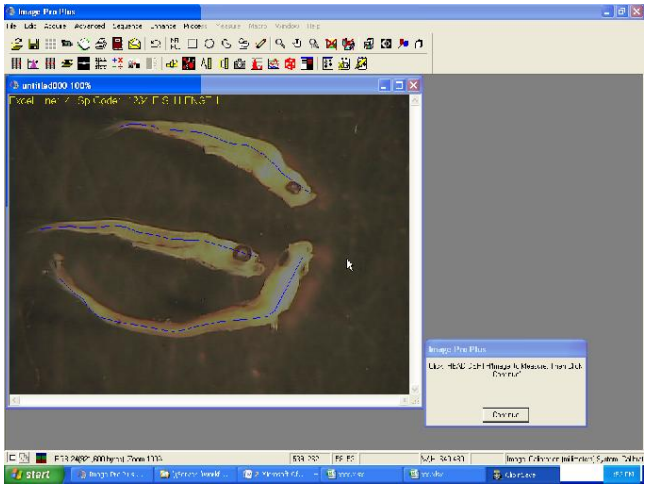
q. Continue measuring while changing the information in the text boxes as applicable (i.e., new sequence or different species code are needed) and calibrating when the magnification needs to be changed.

14.2.1 Method to Correct Measurement Error

a. If an error has been made with a measurement it is possible to re-measure from a previously captured image. Make sure the text boxes are filled in with the proper information of the measurement to be changed, especially taking care to have the proper Excel line number entered in the Excel **Line #** text box. Select the **Old Image** option and press **OK** to continue.



b. Select the image to be re-measured and click Continue.

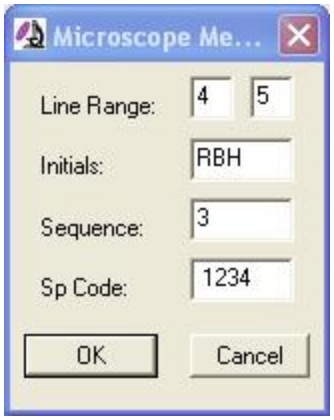


c. Follow steps m. through o. as normal to re-measure fish.

14.2.2 Method to Data Entry Error

a. If the wrong fish information has been entered for a particular fish, these data may be changed by clicking the **Edit Fish Data** button.

b. Enter the correct information in all the pertinent text boxes, especially taking care to have the proper range of Excel line numbers entered in the **Line Range** text boxes.



c. When finished, select **END MACRO** and click on **OK.**

d. Be sure to save in Excel often by selecting the Excel spreadsheet and clicking the save button in the upper left corner. 

15.0 OTOLITH EXTRACTION AND READING PROCEDURES

15.1 Otolith Extraction and Reading

a Remove otoliths from larvae that were preserved in 95% non-denatured ethanol only. Preservation in formaldehyde, which is slightly acidic, can possibly dissolve the otolith which can decrease the overall size of the otolith. All larvae for analysis will be removed from the samples and placed in individually labeled vials.

b Three microscopes are used during this procedure. A Wild dissecting microscope (referred to as Scope #1) is equipped with a camera port which is used for photographing the larvae. Another Wild dissecting microscope (referred to as Scope #2) with bottom lighting is equipped with two pieces of polarizing film to aid in finding the otoliths in the larvae. This scope is used for the otolith extraction. The Olympus compound microscope is used for photographing the otolith after extraction.

c Via USB connection, plug the Infinity 2 microscope camera into the dissecting scope camera port and turn on the computer. Open the Infinity Analyze program. A new window will open automatically to preview the field of the microscope.

d Put a small dish with a little 95% ethanol under Scope #1. Place the larvae from which you want to remove the otoliths into the dish and then into the scope's field of view. At this point calibration is not necessary, so simply click the capture button on the monitor (the button with the picture of a camera on it) once the fish is in focus, has proper lighting, and is magnified large enough to see properly.

e Save the image according to the larvae's taxonomic family. The first letter of the family name is used followed by the number assigned to the individual fish (e.g. Pomacentridae number 45 would be saved as "P45"). The vial the fish came from should have a label with this name on it.

f Remove the camera from the dissecting microscope and attach it the Olympus compound microscope. Whenever either scope that is used for photographs is not in use replace, the dust cap on the camera tube.

g Label a clean microscope slide with the same name that was used to save the initial image of the fish (e.g . "P45"). Remove the fish from the dish and place it on the slide with a few drops of 95% ethanol to ensure it does not dry up.

h Place the microscope slide under the Scope #2. Adjust the light source so that all light is directed off of the mirror underneath the microscope. This scope has a piece of polarizing film on the mirror and another within the microscope head. This causes the light to be cross polarized and otoliths to appear as a bright spot when viewed under through microscope. Adjust the lighting and the angle of the mirror while looking in the microscope until you can view the otoliths clearly.

i Otoliths are found at the anterior portion of the head and appear as a small, circular, opaque disc. Using very thin, needle like probes begin to extract the otoliths. Gently peel back the operculum of the fish and scrape any tissue away that is surrounding the otoliths.

j There are three pairs of otoliths called the lapilli, asterisci and the sagittae. Attempt to extract all otoliths that can be found. The largest, easiest to find, and most important are the sagittae. Without these a comparative measurements of the otoliths cannot be taken. Often only one or two of the pairs are found. Be sure to add 95% ethanol as needed to ensure that the fish does not go dry. Get the otoliths as clean as possible.

k Place the remains of the fish back into the vial it came from, entirely filling the vial with 95% ethanol, and save for possible DNA testing.

l Dab any excess ethanol away without touching the otolith and let the slide dry. It is important to know where the otoliths are on the slide and not to touch the otoliths when they are dry, as they are easy to lose.

m Place a small drop of immersion oil on top of the otoliths once they are dry and move the slide to the compound microscope.

n Open a new window for video preview in the infinity analyze program.

o Find the magnification power that allows the sagittal otoliths to be seen at their largest while still fitting the entire otolith into the field of view of the camera.

p Calibration for the camera has been preset. In the upper left corner of the program there is a drop down box that has choices for the various magnifications of the camera. Choose the magnification that you are using, adjust the camera focus and lighting and take a picture (if taking pictures on 100x magnification use the 10x calibrations setting and adjust any later measurements by moving the decimal place). This scope cannot be calibrated for 100x measurements.

q The naming of the otolith photo goes as follows- the name of the fish, a hyphen, the number of the otolith (based on the order which they are viewed), and a letter telling if it is one of many possible photos taken of the same otoliths. For example the first otolith picture taken

from fish P45 would be saved under the name “P45-1A”, and if a second photo were taken of the same otolith under a different focus it would be titled “P45-1B”. When the next otolith from the same fish is photographed its first photo will be titled “P45-2A”, and so on.

r Now there should be a captured image on the screen. Go to the Measure tab at the top of the program and chose the “point to point” command. The mouse will now appear as a small crosshair. Click once to begin a measurement and once to end. Take a few diameter measurements making sure you get the largest and smallest diameter lengths on the otolith. Record the largest and smallest measurement on the datasheet for that individual larvae.

s Once measurements have been taken, repeat the process taking more photographs of the otolith under different magnifications and focuses (it is not important to have the entire otolith in these photos). Get as many photos as is necessary to be comfortable that the number of growth increment on the otolith can be determined. These photos will be the only record of the otoliths so more pictures is always better than fewer. It will probably be necessary to increase magnification, if there are no 100x pictures taken yet, as well as capture images under many focus positions, making sure that all areas of the otolith on the saved images are readable.

t Repeat the above steps for all otoliths found in the extraction process. Only one of the sagittal otoliths needs to have its diameter measured.

u When finished capturing images remove the slide and place it aside. It is best not to clean the slide until a final determination has been made on the number of growth increment of the larvae.

v Print all photographs taken of the otolith on a color printer. The images are easier to read when printed on a color printer.

w Using both the printed images and those saved on the computer to determine the number of increments found on each otolith. Using a permanent marker put a dot on each of the observed increments on the printed images, and label each picture with its larval identification number and the number of increment observed. Take more pictures of the otolith at this point if necessary.

x Photographs taken with different magnifications and focuses may lead to an opposing final conclusion about the number of rings present. Make a final decision based on the clearest photos available. If none are clear enough state in the data sheet that the otoliths were unreadable.

y Record the number of rings as well as the maximum and minimum diameter lengths of the otolith on the given data sheet.

z When confident you won’t need any more pictures wash the slide with soap and water and use a tiny bit of ethanol to get the permanent marker label off the slide.

aa Close the infinity analyze program making sure all photos are properly saved and shut down the computer. All hard copies of the photographs are to be placed in the proper section of a binder labeled “Otolith Pictures”.

NOTE: These procedures are written for the extraction of otoliths from one fish at a time. When working with many fish at once it may be helpful to photograph all fish first, then pull otoliths a few at a time and take the photographs a few at a time. This will help save time by not moving the camera twice for every fish that is being analyzed.

18.0 SOP REVISIONS

In order to ensure that Tenera staff are using the current document, control copies of this manual are maintained. These control copies are maintained in binders and are labeled as control copies. Electronic copies are also maintained. Revisions to the SOPs in this document are made as follows:

1) Obtain a copy of the current SOP from a control copy of the manual,

2) Obtain the electronic copy from the Quality Assurance Manager,

3) Revise the electronic copy of the SOP in Word using redline and strikeout (this will provide a record of the revisions) and prepare a clean copy of the revised SOP,

4) Update the revision number on the cover page and in the footer,

5) Provide the two versions of the revised SOP (redline/strikeout version and the clean edited version) to the appropriate scientist(s) for review and approval.

Upon approval, the final revised SOP is given to the Quality Assurance Manager for inclusion in the control hard-copy and electronic copy of the manual. All procedure revisions are to be recorded in this section with the revision date and number, changes noted by page and section, and initials of reviser.

History of SOP Revisions

Revision Date	Revision Number	Page/Section	Summary of Changes	Reviser