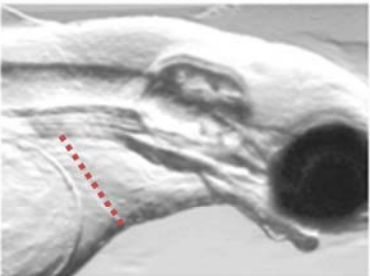
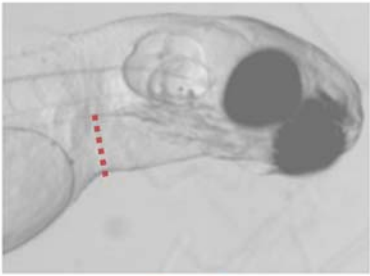
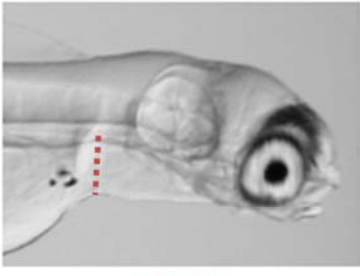
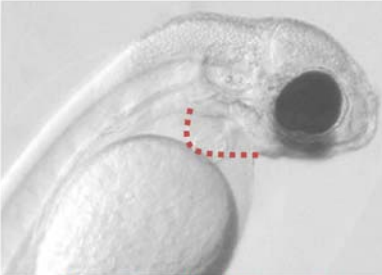
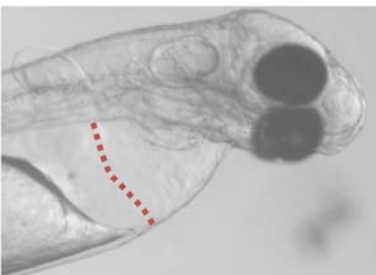
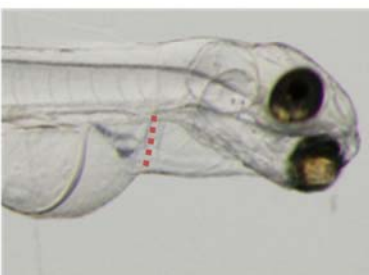


SI Figure 1. Examples of developmental staging of larvae based on Hill and Johnston, 1997 (*Photo resources from archived digital records, 2008 NRDA field investigation*). Photographs in blue box show variability in dechorionated larvae; photograph in red box shows allometric relationships in naturally hatched larvae. Dechorionation was performed at variable pre-hatch development stages during the NRDA studies, ranging from 6 to 9 days post fertilization (dpf) representing developmental stages o, p, q, r).

Control or Reference Specimens	<p>A. Field Investigation</p>  <p><i>Reference - San Rafael Bay</i></p>	<p>C. Mesocosm Study</p>  <p><i>Control</i></p>	<p>E. Environmental Stressor</p>  <p><i>Control</i></p>
	2008	2009	2010
Anomalous Specimens	<p>B. Field Investigation <i>Location: Sausalito</i></p>  <p><i>Immature Development</i></p>	<p>D. Mesocosm Study <i>Treatment: CBO 0.3 UVB</i></p>  <p><i>Pericardial/Yolk Sac Edema</i></p>	<p>F. Environmental Stressor <i>Treatment: A- TS</i></p>  <p><i>Yolk Sac Edema</i></p>

SI Figure 2. Position of transverse septum in representative control and abnormal larvae (*Note: red dotted line denotes transverse septum; the specimen in Figure 2B had been dechorionated and did not hatch normally*)

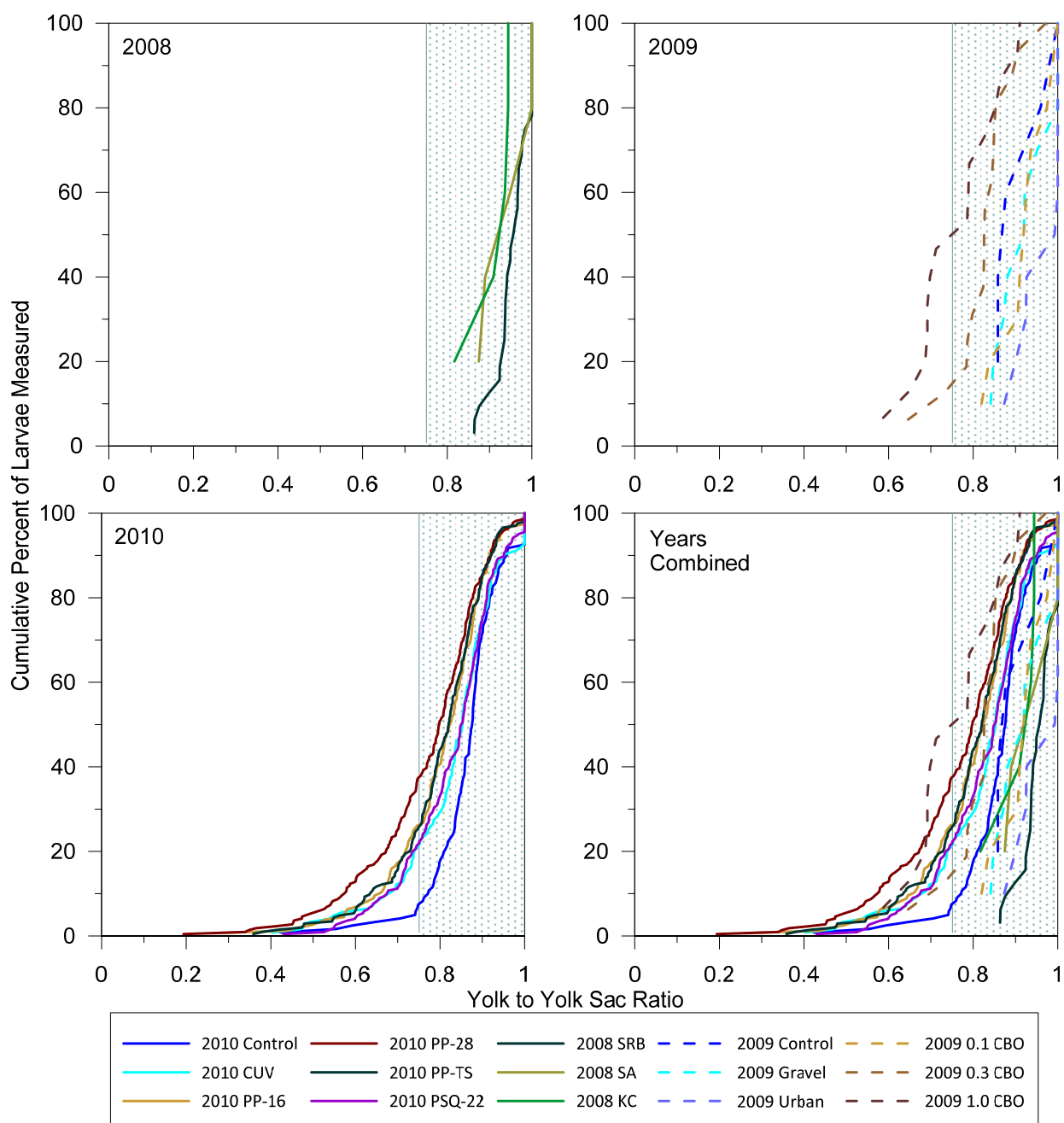
Once our methodology for precise determination of the pericardium was established as reliable and repeatable using the large dataset from the 2010 environmental stressor study (729 individuals), we re-evaluated the occurrences of pericardial and yolk sac edema from archived digital records from the NRDA investigations (42 records from 2008 and 56 records from 2009 that were of comparable developmental stage); results are illustrated in Figures 4 and 5. The precision and repeatability of results in making comparisons of multiple datasets was based on a five-step process (shown below)

- 1) Standardization of datasets for consistency in developmental stage (pre- and post-hatch);

The developmental stage of the herring embryo and larvae has a bearing on physiological development and associated allometric parameters. Consequently, the developmental stage of each larva was determined using criteria developed by Hill and Johnston [33]; varying developmental stages are illustrated in Figure 3. The use of standard length and head-to-trunk angle (HTA) measurements were used to confirm development status.

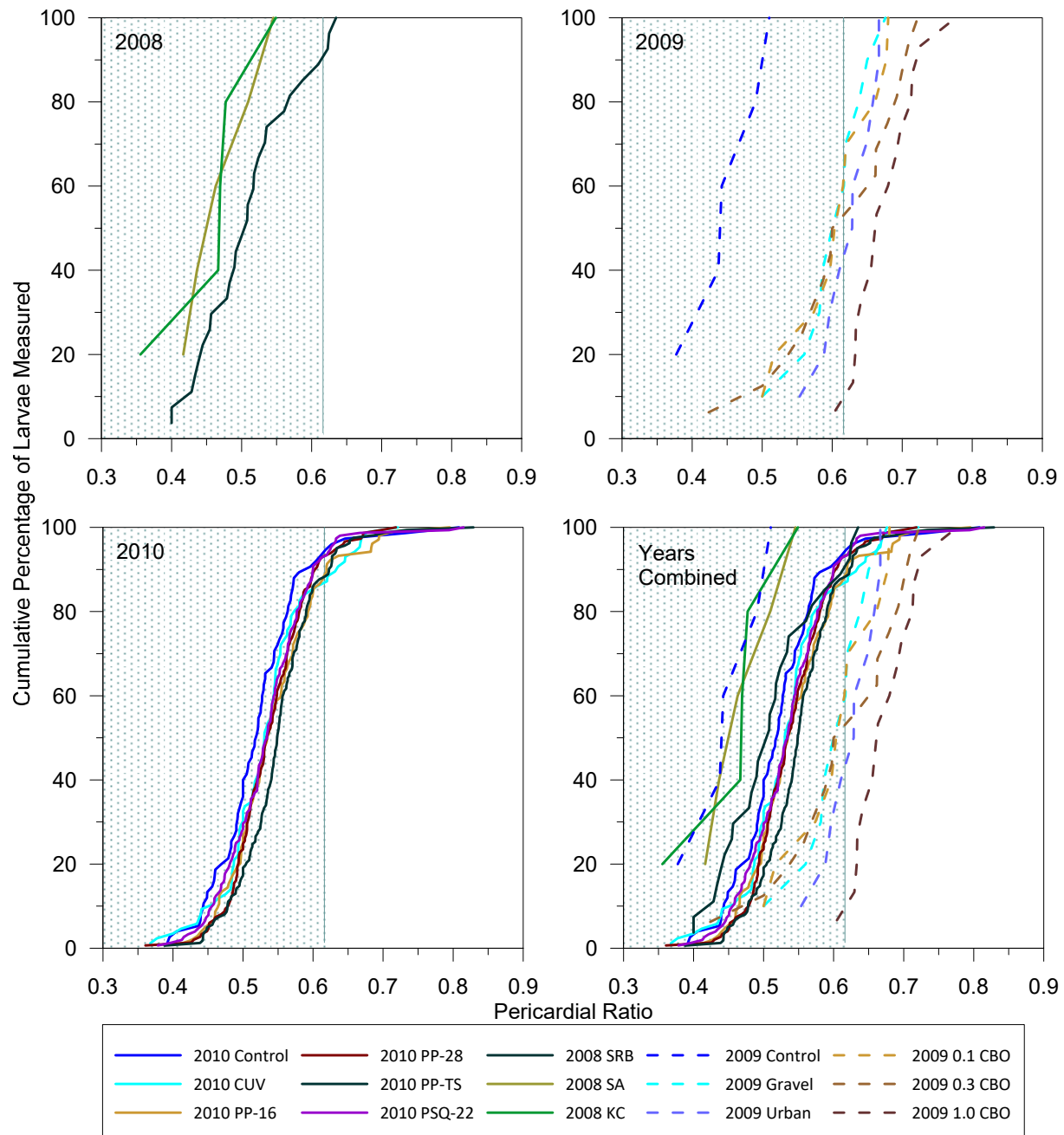
- 2) Segregation of the pericardial and yolk sac areas by identifying the location of the transverse septum;
- 3) Lineal measurement of pericardial depth (PD) and body depth (BD), and of the yolk length (YL) and yolk depth (YD) using ImageJ analysis software;
- 4) Summarization of ratio data, PD: BD and YD: YL; and
- 5) Creation of edema-specific frequency response curves for evaluation of inter-treatment variation, shown in the following figures

SI Figure 3. *Re-evaluation of previous data sets using procedures outlined for pericardial and yolk sac edema assessments*



Note: 2010 Control 97.5% 1-tailed confidence limits (shaded area) are shown on all graphs for comparison purposes.

SI Figure 4. Quantitative measurement of yolk to total yolk sac ratio for 2008, 2009, 2010, and all years combined



Note: 2010 Control 97.5% 1-tailed confidence limits (shaded area) are shown on all graphs for comparison purposes.

SI Figure 5. Quantitative measurement of pericardial depth to body depth ratio for 2008, 2009, 2010, and all years combined