**Executive Summary for Laboratory Demonstration of Environmental Factors and Their Effects on Early Stage Development of Clupea pallasi (October 2010)**

Adverse biological responses can result from many different types of stressors. Abnormal development has been observed in response to naturally occurring changes in water quality (e.g., salinity, temperature, dissolved oxygen, and pH), exposure to ultraviolet light and to air during tidal extremes, as well as to anthropogenic stressors such as oil and chemicals in urban runoff; in addition, reduced viability of fertilized embryos has been related to the condition of gametes at spawn. The experimental studies conducted on the development of Pacific herring embryos in 2008, approximately three to four months after the *Cosco Busan* Oil (CBO) Spill did not show a CBO chemical exposure. In 2009, CBO experimental exposure arrays resulted in a diagnostic chemical signature in the developing embryos at all exposure concentrations ranging from high to low that was not observed in the embryos collected in 2008. Thus the observation of adverse effects made in 2008 for the natural spawning assessments occurred in the absence of CBO exposure; the artificially spawned larvae exhibited no adverse effects. The 2009 laboratory studies were however, also unable to demonstrate a relationship between other potential stressors and the adverse effects because of the presence of laboratory and measurement artifacts. In 2010, an experiment was developed to evaluate the potential impact of 2008 environmental stressors on developing embryos of Pacific herring. This study was designed to provide a controlled laboratory assessment where the environment mimicked the 2008 conditions. The environmental stressors evaluated were 1) donor fish condition and health of gametes, 2) fluctuating temperature and salinity defined by data collected by NOAA/BML to match two locations within the range of spawning activities, 3) the presence of UV light, and 4) the influence of aerial exposure that occurs during periods of intertidal stranding. In the 2010 laboratory studies there was no oil exposure. These conditions were effectively mimicked except the intertidal stranding which probably underrepresented the actual conditions during the 2008 development of herring eggs.

The 2010 experiments demonstrated that all of the abnormalities observed in the 2008 assessment were also found in 2010 under exposure to environmental variables but not to oil. We developed a repeatable method of distinguishing and measuring pericardial and yolk sac edema which also matched our qualitative assessments. These measurements and assessments of edema were developed on the 2010 data and then applied on the 2009 and 2008 images provided by NOAA/BML. All measurements were conducted on randomized sample designations so that assessment and measurement staff were blind to any experimental treatment information. Note that all larvae assessed hatched naturally (were not dechorionated) and therefore these descriptions refer to apparent development at hatch.

This quantitative assessment of types of edema demonstrated that pericardial edema, while present to a large degree in the 2009 experimental oiling experiments was only incidentally present in the 2008 and the 2010 assessments. Pericardial edema was only observed in larvae that had gross body axis defects where separation of pericardial and yolk sac edema was not feasible or appropriate.

Adverse effects were identified in association with each of the potential contributing factors that were examined. Significant yolk sac edema, body axis defects, and disruption of early development occurred in the absence of petroleum contamination and in the presence of nonchemical based stressors. We also determined that there are significant problems with the assessment of dechorionation of developing embryos, especially on organisms that have undergone natural spawning and that represent very different developmental ages.

The combination of observations from the 2008, 2009, and 2010 studies provide a petroleum based chemical signature that demonstrates exposure as well as a biological signature of the type of stressor that appears to be involved with the creation of the defect in development. These conclusions are summarized in the following table.

| Chemical Signature | Stressor Type | Biological Response |
|---|---|---|
| Without Exposure to Oil (CBO) | Population | Low percentage of body axis defects (~2% major and <5% less obvious); combination of pericardial and yolk sac edema only in major body axis defect individuals |
| | Gamete Condition | High early mortality (<48h); those that survive have a high percentage of normal hatch |
| | UV | Higher incidence of body axis defects (BAD) |
| | Increased Temperature | Increased incidence and intensity of yolk sac edema |
| | Intertidal/estuarine fluctuating stressors (salinity/air exposure) | Increased incidence and intensity of yolk sac edema; no pericardial edema except in individuals with major body axis defects |
| With Exposure to Oil (CBO). Relative contribution of chemical components of complex mixtures create tissue signature of exposure | Chemical Stressors (petroleum, TBT, Cu, algal) | Pericardial edema often combined with yolk sac edema and other adverse responses as exposure increases |

The examination of observations from the 2008, 2009, and 2010 studies led to the conclusion that combination of fluctuating temperature and salinity, with UV exposure and some degree of aerial exposure of the developing embryos and the developmental condition of the adult spawning fish and their gametes were the cause of abnormalities observed in 2008. The adverse biological effects, particularly a higher incidence of yolk sac edema and a low incidence of pericardial edema for naturally spawned larvae in 2008 were the same as the response by larvae exposed

to multiple environmental stressors in the absence of petroleum contamination during the 2010 environmental stressor laboratory study.

A companion report "Framework for Assessment of Causal Relationships between Early Life Stage Developmental Anomalies of *Clupea pallasi* and Cosco Busan Oil" provides an assessment framework for evaluating the body work for conducted relative to the CB Oil Spill (NewFields 2010c). This report uses an inference framework to compare all data collected relative to CBO and larval development with effects-based literature summaries to make conclusions.