GENERAL:

Section 4.3.3 of the draft Damage Assessment and Restoration Plan for the Cosco Busan Oil Spill, "Fish and Other Aquatic Organisms" ("Draft Fish DARP"), documents, among other things, the Trustees' determination that the Cosco Busan oil spill caused injuries to Pacific herring in and around San Francisco Bay. The Draft Fish DARP also documents the Trustees' conclusions regarding the scale of this injury.

On April 14, 2011, Regal Stone Limited and Fleet Management Ltd., collectively the Responsible Party ("RP") for the oil spill submitted comments to the Trustees on the Draft Fish DARP along with two scientific reports that address injury assessment issues. The Trustees herein respond to the main concerns/issues raised by the RP for which further information is relevant; however, this document is not intended to address the comments, suggested edits, and conclusions discussed in the RP's documents point-by-point. In addition, textual edits were made to the Draft Fish DARP in response to a number of the RP's comments where the Trustees deemed it appropriate.

EVIDENCE OF EXPOSURE OF HERRING EMBRYOS TO OIL: GENERAL

Contrary to the RP's contentions, the Trustees have identified significant evidence that herring embryos were exposed to Cosco Busan oil. Several revisions have been made to the Draft Fish DARP to more fully describe the evidence of exposure, which includes the following:

- A probable match for Cosco Busan oil in water and sediment collected at Keil Cove (one herring spawning location) in November 2007
- A probable match for Cosco Busan oil in a sediment sample collected at Keil Cove at the time of the herring spawn collection in March 2008
- Observations of Cosco Busan oil in Keil Cove, including tarball events and the
 presence of oil on the shoreline, as close to the spawning dates as January
 2010, and subsequent observations documented by U.S. Geological Survey
 subsequent to spawning events
- Severe and unusual herring embryo mortality and deformities found <u>only</u> in oiled areas.

Much of this information is summarized in revised Section 4.3.3.2 of the Draft Fish DARP and is discussed in greater detail below.

EVIDENCE OF EXPOSURE: OIL FINGERPRINTING

Sediment

As stated in the revised Draft Fish DARP, Section 4.3.3.1, "Composited sediment samples collected from all sites were analyzed for PAHs at the request of the responsible party. The Trustees did not consider these samples to be a strong line of evidence, given the likely heterogeneous nature of subtidal and intertidal oiling in sediments at the time of collection and the compositing of the samples. Forensic analyses of sediment samples revealed that 1 sample collected from the intertidal zone at the Keil Cove site contained a PAH fingerprint that was interpreted as a probable match to Cosco Busan oil; remaining samples from all sites were either indeterminate (i.e. not possible to tell whether they did or did not match the source oil) or did not match. The Trustees did not pursue further chemistry and biomarker interpretive work, which could have been undertaken to attempt to draw more conclusive evidence for indeterminate sediment samples. The Trustees concluded that herring embryos were exposed to oil, or at least to trace levels of constituents of Cosco Busan oil, and used this as the basis of their claim for herring injury - thereby rendering this additional work unnecessary."

PEMDs

As stated in the revised Draft Fish DARP, Section 4.3.3.1, "[Polyethylene membrane devices or PEMDs] were analyzed to determine whether they revealed a presence of PAHs in the water at the various subtidal sites where cages were deployed, and whether the patterns of PAHs varied between the sites. PEMDs were not deployed where natural spawn was collected, and so only reflected conditions at the deeper caged sites where herring embryo effects were more subtle than at shallower natural spawn sites. As expected, analysis of the PEMDs indicated that the water contained detectable concentrations of both typically urban (pyrogenic), and oil spill derived (petrogenic) PAHs at each of the sampled sites. While the Trustees detected an elevated petrogenic to pyrogenic PAH ratio in PEMDs collected from one oiled sampling location (Keil Cove), analytical issues prevented the Trustees from drawing more conclusive information from these sampling devices."

<u>Water</u>

The Trustees did not take water samples in conjunction with the 2008 field embryo collection. However, a water sample was taken at one spawning location in November 2007 (Keil Cove), and that sample showed a probable match to Cosco Busan oil.

Embryos

The Trustees note in the Draft Fish DARP that a clear Cosco Busan fingerprint was not discernable above background PAH levels in egg tissue samples taken at the time of the 2008 field study. From this, the RP erroneously concludes that the embryos must not have been exposed to Cosco Busan oil. This, however, ignores the fact that one would not necessarily expect to see a clear fingerprint under these circumstances.

Unlike other materials contaminated with oil (such as water, sediment, rock, or bird feathers), organisms like fish embryos metabolize oil compounds. The embryos are, therefore, not necessarily contaminated with the whole oil or with the suite of compounds that are used to fingerprint the oil; rather, they take up select compounds (such as PAHs) that are dissolved from whole oil through the weathering process. Moreover, because fish tissues metabolize PAH compounds, and because different PAH compounds are dissolved from whole oil at different rates, the PAHs are taken up by tissues at different rates. In short, the compounds taken up by tissues do not necessarily chemically resemble the whole oil from which they came. Therefore, since fingerprinting of oil depends on identifying a pattern of PAHs that closely matches the pattern of the source oil, it is predictable that PAH patterns in whole oil would not match the patterns found in fish tissues. Further complicating any fingerprinting exercise is the fact that the field collected embryos were incubated in San Francisco Bay, where they were exposed to multiple sources of PAHs, including boat fuel and exhaust, highway runoff, air pollution, etc. This mixed PAH signature was, in fact, seen in the field collected embryos. However, the background PAHs cannot explain the observed mortality, since embryos from the non-oiled reference sites were similarly exposed to the general, low-level urban background that contaminates San Francisco Bay on a daily basis.

In counterpoint, the RP erroneously cites the Trustees' 2009 laboratory studies for the proposition that exposure must necessarily lead to an identifiable chemical signature in embryonic tissues. In those studies, herring embryos were exposed only to Cosco Busan oil or a reference crude oil from Alaska (rather than a mixture of sources). Chemical analysis of the tissues in these studies showed a sufficient "signature" to differentiate between the chemically complex Cosco Busan bunker oil and the crude oil. However, to imply that this is equivalent to a "fingerprint" is a significant overstatement. While the "signature" may have been sufficient to differentiate between two very different types of oils under controlled circumstances, it would likely have been insufficient to differentiate between Cosco Busan oil and, for example, another bunker oil, particularly in the natural environment outside of a lab.

It is also not surprising that the embryos in the lab studies would show a more distinct signal than embryos in the field that were exposed to the same oil. As noted above, the lab embryos

were exposed to only one type of oil rather than a mixture, and with no potentially masking urban background. In addition, the concentrations observed in the lab studies were up to ten times higher than what was observed in the field, and the oil in the lab studies had been weathered for two months rather than the four months of weathering that occurred in the field collected embryos. In short, there is no reason to believe that conditions in the field and in the lab would have yielded a similar signature in herring embryo tissues.

EVIDENCE OF EXPOSURE: EMBRYONIC EFFECTS

The RP ignores what is perhaps the most significant indicator of exposure: the presence of embryonic abnormalities throughout the oiled zone for which there is no other evident explanation. As indicated in the Draft Fish DARP, herring embryos throughout the oiled zone uniformly suffered from gross mortality and deformities that were not found at the reference sites. In disputing this evidence of exposure, the RP contends that these effects may have been caused by some combination of other localized environmental factors; however, the RP is unable to identify a plausible deformity-causing factor or factors that would <u>only</u> affect embryos in the oiled zone.

Also noteworthy is the fact that the deformities observed in the field only occurred during the 2008 spawning season, shortly after the spill. There were no similar mortalities or deformities assessed in subsequent spawning seasons.

ALTERNATE THEORIES FOR EMBRYONIC EFFECTS

There is no dispute that Cosco Busan oil is capable of producing the effects discussed above, as evidenced by the consistent results of the Trustees' laboratory studies. The RP, however, goes to great lengths to demonstrate that there exists in nature the possibility of similar effects in the absence of Cosco Busan oil, suggesting alternative theories ranging from salinity and temperature extremes to poor maternal health. The Trustees do not dispute this point. In fact, similar effects occurred independent of Cosco Busan oil during one of the laboratory experiments conducted by the Trustees (potentially a result of algal blooms). However, in the laboratory studies the effects occurred in all embryos (both oiled and control), thereby suggesting a cause other than Cosco Busan oil. This was not the case in the field, where only embryos in the oiled zone exhibited such effects. None of the RP's theories provides a plausible

¹ It is noteworthy that, even in the study impacted by algal blooms, the occurrence of effects was consistently highest in embryos exposed to Cosco Busan oil and sunlight.

explanation for mass mortality and deformities in herring embryos exclusively within the spill zone.²

Nevertheless, the Trustees considered several proposed non-oil alternate theories, independent of the spatial distribution issues discussed above, and found them to be unconvincing.

<u>Density-Dependent Hypoxia</u> – Hypoxia has been shown to occur when eggs are deposited in layers greater than eight eggs in thickness. Data from the field in 2008 showed that eggs were deposited in layers of four eggs or less at all sites, so the Trustees discounted this as a potential cause.

<u>Sub-Optimal Salinity</u> – Euryhaline Pacific herring embryos develop normally at salinities of 8-28 ppt, and even sub-optimal salinities (i.e., ~30 ppt at the Peninsula Point study area over a 4-5 day period in mid-February 2008) would not be expected to result in acute mortality in late development, as was seen in the field. Therefore, the Trustees discounted this as a potential cause.

<u>Extreme Temperatures</u> – There is no data from the 2008 field collection to suggest that the embryos were subjected to abnormally high temperatures. Even if the temperatures had been abnormally high, the field-collected embryos showed no signs of the accelerated development that one would expect to see in embryos exposed to abnormally high temperatures. Examining tidal and temperature records from San Francisco Bay during the February 2008 natural spawn period, the Trustees found no evidence that the eggs collected for analysis (i.e., taken at depths that ranged from just below the tide level to as much as two feet below the tideline) would have been subjected to extremes of temperature, sunlight, and air exposure postulated by the RP. Therefore, the Trustees discounted this as a potential cause.

<u>Sediment Coating</u> – A coating of fine sediment on herring embryos has the potential to hinder their development; however, it would not be expected to cause the late developmental mortality observed in the field-collected embryos. Therefore, the Trustees discounted this as a potential cause.

5

² Nor do studies of alternative potential causes offered by the RP convincingly demonstrate results that are truly "similar" to effects found in the field. The RP laboratory studies, which are available in the Trustees' administrative record, showed that environmental factors may produce some effects found in the field (body axis defects). However, these effects occurred in the RP's studies only a fraction as often as in field-collected embryos during the 2008 spawning season. In addition, none of the RP experiments could reproduce the types of acute tissue necrosis found in the presence of Cosco Busan oil.

<u>Sewage Spills</u> – Two sewage spills occurred during the 2008 spawning season in the general vicinity of the field-collected embryos. The available evidence indicates that sewage is not acutely lethal to herring embryos; therefore, the Trustees discounted this as a potential cause.

Immature Maternal Condition - Immature maternal condition has the potential to result in low quality eggs. However, during the 2008 spawning season, natural spawn deposition occurred as a continuous "wave" that started at the reference site at San Rafael Bay and continued west over the next several days. It is highly unlikely that there was a distinct subpopulation of females in poor condition that spawned only at the oiled sites and not at the reference site. More importantly, there is not a plausible biological mechanism that links poor maternal condition to the type of acute, necrotic mortality observed at oiled sites. These embryos appeared to have developed normally up to the hatching stage, and then succumbed to an acute insult. Accordingly, the Trustees discounted this as a potential cause.

As discussed above, none of these theories provided a plausible alternate explanation for the injuries observed in the oiled zone during the 2008 spawning season. Nor does it even appear that the RP attributes the observed injuries to any one of the factors above. Rather, the RP seems to speculate that a perfect confluence of non-oil stressors (including the size and health of donor fish, fluctuating temperatures, fluctuating salinity, ultraviolet exposure, and air exposure) occurred only within the oiled zone, thereby resulting in injuries similar to those that the Trustees' 2009 laboratory studies demonstrated are caused by exposure to Cosco Busan oil and sunlight. The Trustees find this implausibly tenuous. In addition, as discussed in the Section 5 of Appendix D to the Draft DARP, this theory is flawed because it is inconsistent with the environmental conditions at the time of the 2008 herring embryo field collection. The Trustees ultimately concluded that an injury of a type shown to be caused by Cosco Busan oil and found only in the oiled zone was most likely to have been caused by the Cosco Busan oil.

EFFECT OF CLEANUP ACTIVITIES

The Trustees concur that the cleanup activities undertaken in December 2007, prior to spawning events, which began in February 2008, were relevant to the herring injury assessment. Had the cleanup not occurred when it did, it is likely that the injuries to herring would have been far worse than those ultimately identified by the Trustees. Nevertheless, even the most effective cleanup activities are unable to remove all of the oil from a given shoreline, nor do cleanup activities generally address oil entrained in the water column or sediments. In this case, oil remained in the environment long after cleanup activities were complete. In recognition of these facts, the Trustees have added the following to Section 4.3.3.2:

"Although the cleanup of several such areas in December 2007 is likely to have significantly reduced the exposure of herring embryos to oil, there was documented oil, including multiple tarball stranding events, along the shoreline in at least one spawning location (Keil Cove) as late as January 2010. Continued shoreline surveys by the U.S. Geological Survey subsequent to herring spawning documented continued presence of stranded tar in Keil Cove (R. Rosenbauer, personal communication, May 24, 2011). The Trustees also were concerned that cleanup activities would not likely address oil that was entrained in the nearshore water column and sediments due to churning wave action in shoreline areas (as suggested by the November 30 water sample discussed above under "Herring Spawning Site Water Sampling" and the samples discussed above under "PEMD and Sediment Study")."