Technical Note

Review of Assessment of Injury to Pacific Herring from the COSCO BUSAN Oil Spill

WH Pearson

January 21, 2009

ABSTRACT. This technical note synthesizes outcomes of injury assessment studies by NOAA and other scientists, assesses hypotheses concerning the cause of the observed injury, and makes recommendations concerning future studies. Salient outcomes of injury studies to this point include finding that artificially spawned embryos deployed in cages in the shallow subtidal (water depth of -3 to -6 feet MLLW) showed no injury. The SFB herring spawning biomass and juvenile herring index in 2007/2008 are low, slightly greater than those from 2006/2007, and not outside the range of observed variation. Shifts in spawning patterns in 2007/2008 are not an oil spill injury but are related to ongoing changes in subtidal vegetation that began before the CBOS occurred. The observed injury was confined to the intertidal where low hatching rates and high larval abnormality rates were observed in naturally spawned embryos dechorionated before hatch and in naturally spawned embryos and larvae at hatch. The pattern of the abnormalities in the naturally spawned embryos does not fully match that expected from exposure to petrogenic PAHs. The PAH tissue burdens in the herring eggs were quite low (below 55 ng/g) and did not have a signature indicative of the ship oil. As causes of the injury in the naturally spawned embryos, direct effects from PAH exposure from CBOS, low dissolved oxygen from the sewage spill, high egg density, silt on the eggs, direct effects from the sewage exposure, and environmental temperature effects were eliminated. Hypotheses still outstanding include transport effects, legacy pollutants, some PAH or non-PAH compound in the ship oil that has not been measured, photo-toxicity of PAHs from ship oil, indirect effects from the sewage spill, and a combination of younger herring spawning in suboptimal salinities. A credible and simple alternative hypothesis for the observed injury is that the decreased hatch and higher abnormalities found in naturally spawned embryos derive from younger herring spawning at sites where the salinity regime was suboptimal for fertilization and incubation. The sum of the available and admittedly sparse data strongly suggests that the salinity mechanism warrants further study and definitely indicates that salinity needs to be a controlled factor in any experiments and a well measured factor in any further field work.

INTRODUCTION

Background

In early November 2007, the container ship COSCO BUSAN collided with the San Francisco Bay Bridge and spilled about 54,000 gallons of bunker fuel oil (IFO380 cut with 3% marine gasoil) into San Francisco Bay. The oil stranded on shorelines of the Central Bay including shorelines where Pacific herring, *Clupea pallasi,* were expected to spawn. Herring in San Francisco Bay (SFB) spawn on subtidal and intertidal vegetation from November to March with peak spawning in January and February (O'Farrell and Larson 2003, Spratt 1981, Watters 2002, CDFG 2006, 2007, 2008). Because of the known toxicity of crude and refined oils to herring eggs (Linden et al. 1978, 1980, Smith and Cameron 1979, Pearson et al. 1985, 1995, 1999, Hay et al. 1995, Carls et al. 1999, 2002) and the intertidal and shallow subtidal distribution of SFB herring eggs, herring eggs in the central SFB were considered at risk for injury derived from the COSCO BUSAN Oil Spill (CBOS). A cooperative program to assess the injury to herring was mounted and included examinations of artificially spawned and naturally spawned herring embryos at oiled and reference areas as well as chemical analyses of water, herring eggs, PolyEthylene Membrane Devices (PEMDs), and sediments.

Purpose and Scope

The purpose of this technical note is to review and synthesize the nature of the injuries observed by the NOAA and other scientists and to assess the hypotheses concerning the cause of the observed injury. Also, this technical note considers what items require attention in any field or laboratory work to be performed during 2009. This note describes the nature of the injury observed and assesses the various hypotheses for its causation. The note concludes with recommendations concerning the conduct of field and laboratory work during 2009.

MATERIALS EXAMINED

The materials examined for this technical note included the NOAA CBOS Fish Injury Assessment Report prepared by the NOAA NW Fisheries Center and the Bodega Marine Laboratory (NOAA/BML 2008) as well as presentations made by NOAA scientists in September 2008 (Incardona 2008). Additional materials specific to the CBOS included the NewFields Review of Chemistry Data (Douglas 2008), the NewFields NW analysis of digital images of herring embryos (NewFields NW 2009), and information on herring provided by California Department of Fish and Game (CDFG). The general scientific literature on pollutant toxicity to fish eggs and herring biology was also consulted.

SUMMARY OF APPROACHES IN THE INJURY STUDIES

NOAA and BML used two main approaches to assess injury to herring from the CBOS (NOAA/BML 2008, Incardona 2008). The first examined artificially spawned herring embryos. Adult herring were captured from SFB, and eggs and sperm from ripe herring were used to provide artificially fertilized eggs mounted onto screening. The artificially spawned eggs were then deployed in cages just off the bottom in -3 to -6 feet MLLW. The deployment sites and the oiling conditions given in NOAA/BML (2008) were as follows:

Site	Abbr.	Reported Oiling Condition
Point San Quentin	PSQ	Reference
San Rafael Bay	SRB	Reference
Sausalito	SA	Very light-light
Peninsula Point	PP	Light
Horseshoe Cove	HC	Moderate-light
Keil Cove	KC	Heavy-light

All the above sites had received herring spawn in recent years. Cages were deployed at five stations at each of the six sites. PEMDs were deployed with the cages. The PEMDs, and sediments near the cages were collected for analyses of PAHs and Persistent Organic Pollutants (POPs). After about 7 days of incubation and before hatching, the cages were recovered, and the eggs were subsampled to provide eggs for three efforts. First, artificially spawned herring eggs were analyzed for PAHs and POPs. Second, artificially spawned herring embryos were dechorionated for determination of heart beat, arrhythmia, and frequencies of morphological abnormalities. Third, artificially spawned eggs recovered from the cages were incubated at 12 degrees C and half-strength sea water (about 14 to 16 practical salinity units [psu]) for about 5 days until the eggs hatched. Eggs and hatched larvae were examined to measure the rate of hatching and the frequency of live normal larvae at hatch. Swimming performance of nominally normal larvae was measured.

The second collected naturally spawned eggs from eight stations at four of the cage deployment sites: SFB, SA, PP, and KC. No spawning occurred at Horseshoe Cove and no eggs were collected in the intertidal at Point San Quentin. The naturally spawned eggs were all collected on macro-algae (e.g., *Fucus*, *Gracilaria* spp) from the intertidal zone. Sediments were collected for subsequent analysis of PAHs and POPs. Egg samples returned to the laboratory were split for three analyses. The first subsamples of eggs were analyzed for PAHs and POPS. The second subsamples of eggs were dechorionated and examined for heart beat variation and morphological abnormalities as was done for the artificially spawned embryos. The third egg subsamples were incubated in the Bodega Marine Laboratory until hatch. At hatch, the eggs and larvae were examined to determine hatching rate and frequencies of live normal larvae. For two stations (SRB and KC) from which sufficient larvae hatched, swimming performance of nominally normal herring larvae was assessed.

There were two additional analyses subsequent to NOAA/BML (2008). The first performed QA assessment, fingerprint assessment, and statistical analysis of the chemistry data associated with the NOAA/BML study (Douglas 2008). The second examined of the digital imagery taken during the NOAA/BML study of artificially spawned and naturally spawned embryos dechorionated before hatch (NewFields NW 2009).

RESULTS

What Injury Was Observed?

The salient outcomes concerning injury to this point are the following:

- Artificially spawned embryos deployed in cages in the shallow subtidal (water depth of -3 to -6 feet MLLW) showed no injury.

- SFB herring spawning biomass and juvenile herring index in 2007/2008 are low, slightly above those from 2006/2007, and not outside the range of observed variation.

- Shifts in spawning patterns in 2007/2008 are not an oil spill injury but are related to ongoing changes in subtidal vegetation that began before the CBOS occurred.

- The observed injury was confined to the intertidal where low hatching rates and high larval abnormality rates were observed in naturally spawned embryos dechorionated before hatch and in naturally spawned embryos and larvae at hatch.

- The pattern of the abnormalities in the naturally spawned embryos does not match that expected from exposure to petrogenic PAHs.

Artificially spawned embryos

The sum of all the results (NOAA/BML 2008, Incardona 2008, NewFields NW 2009) from the artificially spawned embryos reveals no injury to these embryos that incubated in the shallow subtidal. The hatching rates of artificially spawned herring eggs recovered from both oiled and reference areas and incubated at BML were excellent - all above 90% of total embryos - and with no significant difference between oiled and reference (NOAA/BML 2008) . Similarly, larval survival at hatch did not differ significantly among all the sites (NOAA/BML 2008). Abnormality rates for all sites were low. Frequencies of live normal larvae were at or above 80% of total embryos at all sites (NOAA/BML 2008). NOAA/BML (2008) does not provide a tabulation of frequencies of abnormality by type or an explicit statement of the abnormalities observed in the artificially spawned larvae at hatch. These are excellent results for a laboratory incubation of herring eggs for which hatching rates of 75% and abnormality rates of 15% in controls are not uncommon. Struhsaker et al. (1974) found that herring eggs spawned and incubated in SFB but brought into the laboratory just prior to hatch had rates of larval abnormalities rates of 20% to 25% at hatch.

Among the nominally normal larvae hatched from artificially spawned eggs, critical swimming speeds were lower at two oiled sites (PP and KC) compared to the rest of the oiled and reference sites (NOAA/BML 2008). Examination by cage station reveals that stations with lower swimming speeds occur at both oiled and reference sites. It is unclear why the site means for swimming speed given in Table 3-15 of NOAA/BML (2008) cannot be derived from the station means presented in Table 3-16. Further analysis of the swimming speed data is warranted to assess how the means were derived and whether significant differences occurred.

Artificially spawned herring embryos were also examined for pericardial edema and for decreased heart rate (bradycardia) and arrhythmia. These embryos were dechorionated in the laboratory after recovery from the cages deployed in the field for about 7 days. Video and still digital imagery was taken for later quantitative analysis. Pericardial edema was quantified by two separate measurements: by measuring the area of pericardium and by measuring the distance from the artioventricular junction at the heart to the ventral surface of the pericardium (AVPC distance).

Pericardial area was not significantly increased among cage stations from oiled and reference sites except for two cage stations at HC (NOAA/BML 2008). The other three cages stations at HC had significantly and substantially lower pericardial areas than the remaining oiled and reference cage stations (NOAA/BML 2008) so that site means for pericardial area in the NOAA/BML study probably do not differ significantly. Measurements of pericardial area from the NOAA/BML imagery by NewFields NW show the SA site to have significantly larger pericardial area than the other sites examined (NewFields NW 2009).

Flattening of the anterior end of the yolk sac was used by NOAA in their assessment of the presence of pericardial edema for the naturally spawned embryos but not for the artificially spawned embryos. NewFields NW used a graphic template applied to the digital imagery to identify flattened yolk sacs among artificially spawned embryos from four sites and found that the frequencies of yolk sac flattening at the oiled sites examined were not significantly greater than that at the control site (NewFields NW 2009). The sum of all the observations is that pericardial area as a measure of pericardial edema indicates no injury.

The interpretation of the AVPC distance as a measure of pericardial edema has evolved from that in NOAA/BML (2008) as further information has become available, and the AVPC distance results can now be interpreted to indicate no injury among the artificially spawned embryos. NOAA/BML (2008) reported that the site means for AVPC distances were significantly elevated at KC and SA compared to reference areas and the station means for AVPC distance were significantly elevated at KC (2 stations) SA (5 stations) and HC (2 stations). These results do not align with the oiling conditions presented in NOAA/BML (2008). More importantly, these measurements of pericardial edema in the dechorionated artificially spawned embryos did not translate into effects on hatching rate, larval survival at hatch, and frequency of larval abnormalities at hatch. The pericardial ventral surfaces in lateral views of artificially spawned embryos were predominantly concave, and not the convex or bulging shape observed after oil exposure (Linden et al. 1978, Pearson et al. 1985, Carls et al. 1999). Pericardial edema in oil exposed herring embryos can also show an “unusually large clear area” (Carls et al. 1999). NOAA/BML (2008) did not use the criteria of Carls et al. (1999), and a review of the NOAA digital imagery suggests that few, if any, of the artificially spawned embryos would pass the “convex” shape criterion of Carls et al. (1999).

The best way to view the site and stations differences in AVPC distances is as being within the inherent natural variability for that characteristic. Examination of the NOAA video imagery reveals that the position of the heart changes during the beat cycle (NewFields NW 2009). Measurements on the NOAA video imagery of the variation in the AVPC distances at different stages in the beat cycle by NewFields NW(2009) show that the inherent variability in the AVPC distance due to heart beat leads a coefficient of variation of 36%. The station means for AVPC distance in NOAA/BML (2008) range from about 60 to 124 pixels with a mid-point of 92 pixels. The inherent variability in the measurement is such that one standard deviation encompasses a range from 58 to 125, the entire range of AVPC distances from all sites and stations. NewFields NW (2009) found that more than one developmental stage was present in artificially spawned embryos examined and a reasonable hypothesis is that the AVPC distance varies with developmental stage. NewFields NW (2009) did not specifically conduct statistical analyses to determine whether pericardial edema measurements are confounded by differences among the cage stations in the portions of embryos in different developmental stages.

The overall outcomes of the analyses on artificially spawned embryos provide three main points. First, there was no injury to artificially spawned embryos. Second, there was no injury through water column exposures in the shallow subtidal. Third, more work is urgently needed to improve the quantification of abnormalities. These improvements should include standardization of the criteria and measurement procedures. The procedures must take into account variations from developmental stage on the measurement of interest.

SFB Herring Spawning Biomass and Juvenile Index

There is no evidence of adverse CBOS effects on SFB herring at the population level. The overall spawning biomass and juvenile herring index in 2007/2008 were low but not out of alignment with those in recent years (CDEQ 2008). In 2006/2007, SFB herring spawning biomass was 10,935 tons while in 2007/2008, it was 11,183 tons, a slight increase. O’Farrell and Larson (2003) found that neither spawning biomass nor number of deposited eggs predicted year class strength in SFB herring. CDFG (2007, 2008) attributed the low biomass levels in 2006/2007 and 2007/2008 to unfavorable ocean climate which influences recruitment at the subadult and early adult stage. The condition of the adult SFB herring was better in 2007/2008 than in the previous season (CDFG 2008), suggested somewhat improved feeding conditions.

The SFB juvenile herring index has been low from 2004 through 2008 (CDFG 2008). These low juvenile index values were attributed to poor conditions for growth within the Bay. There is poor correlation between the juvenile herring index and subsequent recruitment to the spawning population (CDFG 2007, 2008).

Natural Shifts in Spawning Patterns of SFB Herring

The 2007/2008 season showed a shift in SFB herring spawning patterns. In 2007/2008, herring returned to spawn along the San Francisco waterfront after not spawning along the waterfront for a number of years (CDFG 2008). About 43% of the total 2007/2008 spawning in SFB occurred along the SF waterfront and was intertidal. Also, the recent trend in a shift from predominantly subtidal spawning to more intertidal spawning continued in 2007/2008 (CDFG 2006, 2007, 2008). It is well known that herring change spawning grounds, using an area for a time, leaving that area for some years, and then returning (McQuinn 1997). It is not appropriate to interpret these shifts in spawning pattern as a response to the CBOS for several reasons.

First, close examination reveals that shifts in spawning locations and depths are related to ongoing changes in the SFB subtidal vegetation that began before the CBOS. CDFG (2007) written before the CBOS and CDFG (2008) written after the CBOS attributed shifts in spawning locations and between the subtidal to intertidal zone and waterfront piers to "dramatic" declines in subtidal vegetation observed in recent years. In 2005/2006, the two major spawning substrates, eelgrass (*Zostera marina*) and the macroalgae *Gracilaria* spp were abundant in Richardson Bay, and *Gracilaria* spp covered a wide area of the shallow subtidal in Central SFB (CDFG 2006). In 2005/2006, the spawning biomass was high (145,054 tons) and spawning was predominantly in the Richardson Bay area and predominantly subtidal in SFB (CDFG 2006). Between 2005/2006 and 2006/2007, densities of eelgrass and *Gracilaria* spp declined 43% and 99.5%, respectively (CDFG 2007). The proportions of intertidal spawning in 2006/2007 and 2007/2008 were 41% and 82%, respectively. Following the 1982/1983 El Nino, a similar shift from subtidal to intertidal herring spawning was observed in association with a dramatic decline in subtidal vegetation.

Secondly, herring do not consistently avoid oiled areas. The evidence for herring shifting spawning in response to shoreline oiling was equivocal following the NESTUCCA oil spill (Hay et al. 1995). Herring spawned in both oiled and non-oiled areas at the time of the EXXON VALDEZ oil spill and in the years following (Pearson et al. 1995, 1999).

Thirdly, the observed shift in spawning pattern from subtidal to intertidal is contrary to expectation if herring are presumed to avoid oiled areas. If the shoreline is oiled, than the intertidal would be expected to have higher levels of oiling in the subtidal. A shift in spawning as an avoidance response would be expected to be from intertidal to subtidal rather than that observed from subtidal to intertidal.

The sum of information in this section is that the shifts in spawning patterns of SFB herring are being driven by natural ongoing changes in subtidal vegetation unrelated to the CBOS.

Naturally spawned embryos and larvae

The examination of the naturally spawned embryos dechorionated before hatch and the larvae hatched from naturally spawned embryos revealed lowered hatching rate and higher abnormality rates at the oiled sites compared to the reference site (NOAA/BML 2008, Incardona et al. 2008). SRB had a hatching rate of 84% (+/- 11%) of the total embryos while hatching rates at SA, PP, and KC were 44%, 24%, and 42%, respectively. At hatch, the rates of normal larvae were 74%, 0.1%, 0%, and 14%, at SRB, SA, PP, and KC, respectively. Essentially 100% of the larvae that hatched at SA and PP were found to be abnormal. Frequency of abnormal larvae at hatch varied from 10% of total embryos at SRB to 44% at SA (Table 1). Among the oiled sites, only KC had any normal larvae at hatch. NOAA/BML (2008) does not provide any tabulation of abnormalities observed by type for the naturally spawned larvae at hatch. There was no significant difference in swimming speed between KC and RFB, the two sites from which sufficient numbers of normal larvae could be tested (NOAA/BML 2008).

Naturally spawned embryos were examined after dechorionation about 5 days before hatch and showed higher rates of abnormalities at SA, PP, and KC compared to SRB (Table 1) (NOAA/BML 2008). The abnormalities observed in the dechorionated naturally spawned embryos included body axis defects, tissue opacity, pericardial edema, and cardiac arrhythmia (NOAA 2008). The body axis defects dominated.

Table 1. Comparison of Results for Various Parameters of Hatching Success in Herring. NOAA and BML data from NOAA/BML (2008) and NewFields data from NewFields NW (2009).

		% of Total Embryos Failed to Hatch	% of Total Embryos with Hatched Abnormal Larvae	% of Total Embryos with Body Axis Defects in Dechorionated Embryos Before Hatch		Edema - % of Total Embryos Examined with Flattened Yolk Sacs		Edema - Pericardial Area (Sq Pixels)		Edema - AVPC Length (Pixels)		% of Total Embryos with Cardiac Arrhythmia		Heart Beat (Beats per 20 Sec)	
Site	Site ID	BML	BML	NOAA	Newfields	NOAA	Newfields	NOAA	Newfields	NOAA	Newfields	NOAA	Newfields	NOAA	Newfields
SRB	MRU01	16	10	0	12	1	37	NM	31325	NM	12.2	0	11	NR	37
SA	MRQ10/P01	56	44	60	78	33	36	NM	22677	NM	13.7	48	11	NR	39
PP	MRQ01	76	24	98	99	11	NM	NM	NM	NM	NM	91	0	NR	22
KC	MRR20	57	32	90	94	11	56	NM	30665	NM	21.3	70	33	NR	41.8

The examination of the digital imagery by NewFields NW provided more information on the nature of these abnormalities and their frequencies (Table 1). For the reference site, SRB, NOAA reported 0% of the dechorionated embryos had body axis defects while NewFields NW reported 19% of the dechorionated embryos had such abnormalities and BML reported 10% abnormal larvae at hatch. As their criterion for edema, NOAA used flattened anterior ends of the yolk sac (Incardona 2008) and reported the frequency of flattened yolk sacs to be 1% at SRB and 11 to 33% among the oiled sites (NOAA 2008, Table 1 here). In contrast, NewFields NW found that frequency of dechorionated embryos with flattened yolk sacs was 37%, 36% and 56% at SRB, SA, and KC, respectively (NewFields NW 2009, Table 1 here). NewFields NW (2009) found the imagery of dechorionated embryos from PP was of insufficient quality to support analysis of edema. NewFields NW used two other measures of edema in the naturally spawned embryos: pericardial area and AVPC length. For all three measures of edema by NewFields NW, there was no significant difference among the sites examined (NewFields NW 2009). NewFields NW reports substantially lower frequencies of arrhythmia than does NOAA at all sites examined (NOAA 2008, NewFields NW 2009, Table 1 here).

Tissue opacity was reported in substantial frequencies in naturally spawned dechorionated embryos from the oiled sites in NOAA (2008). In contrast, the examination by NewFields NW found significant regressions relating tissue opacity to the level of background illumination in the digital imagery for these larvae. This outcome indicates that opacity may be an artifact of laboratory procedure. Regardless, consistency of background illumination will need to be tightly controlled in future work of this type. Smith and Cameron (1979) observed that embryos that died in the egg case during development under oil exposure were liberated from the egg cases during the hatching of adjacent viable embryos and that these nonviable embryos were “highly contorted” and “lacked the transparency of living larvae.”

Explanation for the differences between NOAA and NewFields NW include the following:

- The imagery for naturally spawned embryos is poor quality and poor orientation.
- The sets of specific images examined by NOAA and NewFields may not have completely overlapped.
- Definitions and procedures for the measurements differed when examining the same images.
- NewFields used more types of measurements than did NOAA.

The differences in the results leave open the question of whether edema occurred in the naturally spawned embryos and, if so, to what extent. This is an unfortunate outcome because pericardial edema is an abnormality often considered to indicate exposure to PAHs (NOAA 2008). The differences also point out the urgent need for explicit, detailed definitions for scoring the abnormalities and for workable procedures to support such scoring.

While the results from BML, NOAA, and NewFields NW differ from one another in several important aspects, the overall outcome still indicates injury – lowered hatch and higher larval abnormalities – in the oiled sites (SA, PP, and KC) and perhaps slight injury – elevated abnormalities but normal hatching rate at the reference site SRB. Further examination and quantification of the nature of the abnormalities has substantial merit, especially to assess whether edema occurs in the naturally spawned embryos and, if so, at higher levels in the oiled areas. To this end, examination of the raw data from BML’s scoring of larvae for abnormalities at hatch is urgently needed.

Does the Pattern of Abnormalities Match That from Exposure to Petrogenic PAHs?

Exposure of Pacific herring embryos to the WSF of oils has been found to induce several morphological abnormalities: body axis defects (scoliosis, lordosis, and kyphosis), pericardial edema, jaw deformities, yolk sac edema or other yolk sac abnormalities, fin ray malformations, and retarded growth evidenced by shorter length and larger yolk sac volumes (Linden et al. 1978, 1980, Smith and Cameron 1979, Pearson et al. 1985, 1995, Middaugh et al. 1998, Carls et al. 1999, Incardona et al. 2009). Hatching rate may or may not be decreased under oil exposure. Lowered heart beat rate (bradycardia) was reported by Linden et al. (1978) for herring embryos exposed to a fuel oil and two crude oils. Bradycardia and cardiac arrhythmia are physiological abnormalities observed to precede pericardial edema and mortality in Pacific herring exposed to effluents from gravel coated with weathered Alaska North Slope crude oil (ANSCO) (Incardona et al. 2009). In addition, cardiac arrhythmia has been observed in Pacific herring embryos under exposure to oil and creosote (Vines et al. 2000, Incardona et al. 2009). Moreover, pericardial edema is taken as an indicator of injury from oil exposure (NOAA/BML 2008) in part because the other abnormalities can be induced by natural and man-made stressors other than oil (Westernhagen 1988). In his presentation, Incardona (2009) posited that the pattern of abnormalities seen in the naturally spawned embryos does not match that expected from exposure to petrogenic PAHs.

NOAA/BML (2008) had also reported tissue opacity as a newly observed abnormality in the naturally spawned embryos, but whether this tissue opacity is a new abnormality is unclear. Smith and Cameron (1979) reported that some non-viable larvae observed near the end of crude oil exposure were “contorted” and lacked movement, heartbeat, and the “transparency of living larvae.” Smith and Cameron concluded that these larvae died in the egg and subsequently released. In their examination of the NOAA digital imagery of naturally spawned embryos, NewFields NW (2009) found tissue opacity to be significantly related to background illumination. If the naturally spawned embryos were moribund at the time of dechorionation and mounting, then the digital imagery may have been capturing the tissues turning opaque as the embryo died. It is well known that herring embryos become opaque when they die. Therefore, present information does not support tissue opacity as a newly observed abnormality to be added to the suite of other abnormalities already observed in the naturally spawned embryos.

Present information and analyses indicate that some but not all of abnormalities that would be expected from exposure to petrogenic PAHs or creosote are present in the naturally spawned embryos. Certainly, body axis defects and arrhythmia were observed in the naturally spawned embryos while jaw deformities, yolk sac edema, and fin ray malformations were not reported in NOAA/BML (2008). NewFields NW (2009) did not observe jaw deformities or yolk sac edema (except for potentially one instance) and was unable to score for fin abnormalities because of the orientation of the embryos in the digital images. Jaw deformities are best observed in larvae after hatch (Westernahgen 1988) so that the lack of jaw deformities in the dechorionated embryos examined about 5 days before hatch may missed the presence of jaw deformities. The raw records for the BML examination of larvae at hatch would be useful to resolve this issue. Jaw deformities, yolk sac edema, and fin abnormalities occur at higher PAH exposure levels than pericardial edema (Carls et al. 1999). Retarded growth may have occurred but may have been masked by the poor quality of the imagery from naturally spawned embryos. Specific measurements on larval length and yolk sac volume would be needed to confirm or deny the presence of retarded growth. Yolk sac abnormalities, included vacuolization, have been reported for Pacific herring exposed to oil and creosote (Pearson et al. 1985, Vines et al. 2000). NOAA/BML (2008) and NewFields NW (2009) noted granulation of the yolk sac amongst the naturally spawned embryos but this abnormality does not appear to be of the same character as the vacuolization of yolk sac reported by Vines et al. (2000) for exposure to creosote.

The key issues are whether pericardial edema exists in the naturally spawned embryos, whether the incidence of pericardial edema is elevated at oiled sites compared to controls, and whether the detailed characteristics of the edema resemble those from exposure to PAHs or other pollutants. If pericardial edema is not present or is not present at higher levels in the oiled areas, then the pattern of abnormalities would not match that expected from PAHs associated with petroleum or creosote and could match the patterns seen for other stressors.

Present analysis outcomes provide some evidence for pericardial edema in naturally spawned embryos but not of the severity or character seen previously for exposure to fuel oil or crude oils by Linden et al. (1978), Carls et al. (1999), or Incardona et al. (2009) . Two measures of edema, AVPC distance and pericardial area, measured by NewFields NW (2009) provide no indication of increased pericardial edema at the oiled sites. Both NOAA/BML (2008) and NewFields NW (2009) report edema based on flattened yolk sacs in the naturally spawned embryos. NOAA/BML (2008) report edema in only 1% of embryos from the control site and significantly increased frequencies at oiled sites (Table 1). In contrast, NewFields NW (2009) reports 37% in controls and levels at the oiled sites that are not significantly higher (Table 1). NewFields NW (2009) applied a graphical template to the imagery to detect even slight departures of the yolk sac from a curved shape so that their scores may be higher than those of NOAA/BML (2008) based on a finer-resolution in their analysis. The degree of flattening of the anterior ends of the yolk sacs observed in the imagery presented by both NOAA/BML (2008) and NewFields NW (2009) does not approach that presented by Incardona et al. (2009) for Pacific herring embryos exposed to weathered ANSCO. Carls et al. (1999) stated that the pericardial edema was indicated when the "pericardium was convex ventrally or had an unusually large clear area." NewFields NW (2009) and NOAA/BML (2008) did not systematically score or measure convex pericardiums or clear areas in the pericardium. The imagery in both NewFields NW (2009) and NOAA/BML (2008) does not show naturally spawned embryos with convex pericardiums or clear areas of the same severity as shown in the imagery of herring embryos exposed to weathered ANSCO in Incardona et al. (2009). For the analyses of pericardial edema, the imagery for the naturally spawned dechorionated embryos is suitable in only about one-third of the specimens (NewFields NW 2009). Examination of the larvae at hatch during which the scorer could change the orientation of the specimen under the dissecting scope could have enabled more of the specimens to be scored. Unfortunately, we currently lack a detailed breakdown of the abnormalities by type from BML's examination of the larvae at hatch or a clear confirmation that the BML scorers observed edema at hatch.

The outcome of the above discussion is that some sort of pericardial edema may be present in the naturally spawned dechorionated embryos but analysis of the existing digital imagery will not support credible quantification of its extent. The pericardial edema observed in the naturally spawned embryos does not appear to be as severe as that observed elsewhere under exposure to fuel and crude oils. Examination of the raw data from the BML scoring of abnormalities may be critical to resolving the pericardial edema issue. Certainly, procedures for quantifying pericardial edema in larvae with other abnormalities needs to be refined and applied in any future work.

What was the exposure of herring eggs to CBOS oil?

The major outcomes of the analysis of the chemistry data by NewFields concern the measured levels of PAHs and the signature in the PAH profile. First, the PAH levels in the herring eggs and PEMDs are quite low. In both artificially and naturally spawned embryos, tissue burdens of PAHs are below 55 ng/g. Second, the PAH patterns do not contain a CBOS signature (Douglas et al. 2008). The observed signature is that for PAHs derived from urban sources – either runoff or atmospheric deposition of combustion products.

The low PAH levels measured in the herring eggs do not approach those known to induce pericardial edema or cardiac abnormalities in Pacific herring in the recent experiments of Incardona et al. (2009). The PAH tissue burden in the naturally spawned embryos in 2008 ranged from less than 20 ng/g wet weight to about 55 ng/g. The PAH tissue burdens in the controls in Incardona et al. (2009) were 32 to 58 ng/g wet weight. The PAH tissue burden in the lowest ANSCO exposure was 480 to 620 ng/g. Whereas there are differences in the composition of ANSCO and CBOS oil (IFO380), there is still substantial overlap in PAH profile. Even if the PAH tissue burdens in the herring eggs derived entirely from CBOS oil, the levels were too low to induce the observed abnormalities. Also, the range of PAH tissue burdens in the artificially and naturally spawned embryos was essentially the same yet those burdens in the artificially spawned embryos did not lead to lowered hatch and increased abnormalities.

What caused the observed injury?

Nature of the injury

Hypotheses concerning the cause of the injury observed in the naturally spawned embryos need to explain several aspects of the nature of the injury. First, whatever caused the injuries had to be widespread through and adjacent to Richardson Bay (i.e., covering sites SA, PP, and KC) but not in San Rafael Bay (SRB). Second, the exposure mechanism had to operate in the intertidal zone but not in the subtidal zone below -3 feet MLLW where the caged embryos were deployed and showed no injury. Third, the stressor had to induce a pattern of abnormalities that included substantially lower hatching rates (high mortality in the egg), body axis defects, and perhaps pericardial edema. The pattern would not include jaw deformities, fin ray malformations, yolk sac edema, or other abnormalities. Much of the scientific literature examined larvae at hatch so that bradycardia and arrhythmia in embryos as outcomes of stressor exposure have not been consistently tested. Therefore, we cannot rely on the literature to identify bradycardia or arrhythmia as an outcome of exposure to a number of pollutant types besides oil. In reviewing the hypotheses, it is useful to keep in mind that abnormalities such as body axis defects, head and eye deformities, and retarded growth have been observed as general responses of fish embryos to stress from a wide variety of pollutants (oil, pesticides, metals) as well as from natural environmental extremes (Westernhagen 1988).

Review of Hypotheses

At issue is the cause of injury and its linkage, if any, to the CBOS. The state of information at present is not adequate to definitively assign a particular cause to the observed injury. Some hypotheses can be eliminated from further consideration.

The hypotheses for agents judged unlikely to induce the observed injury include the following:

- Direct effects from PAHs from CBOS oil are simply not likely.
    - The lack of a CBOS oil signature in the herring egg tissues indicates that the exposure of the intertidal herring embryos was to a stressor other than CBOS oil.
    - The levels of abnormalities did not fully align with the oiling conditions.
    - The pattern of abnormalities does not fully match that expected from petrogenic PAHs.
    - The PAH tissue burdens in naturally spawned embryos are not high enough to induce abnormalities observed.
    - Essentially the same range of PAH tissue burdens in the artificially spawned embryos did not produce lower hatching rates or greater abnormality levels but rather excellent hatching success with no difference between oiled and reference areas.

- Low Dissolved Oxygen (DO) following from the January 30, 2008 spill of raw sewage is not likely to have affected herring egg incubation.
    - Low DO does lead to embryonic mortality and abnormalities in herring (Alderdice and Hourston 1985).
    - Pattern of observed abnormalities overlaps but does not completely match that expected from low DO (Braum 1985).
        - Braum (1985) reports mortality, reduced hatch, retarded growth, and sciolosis (severe spiraling of the body) in herring larvae from eggs exposed to low DO.

        - Braum (1985) reports no pericardial edema.
    - Herring require oxygen saturation above 20% to hatch successfully (Alderdice and Hourston 1985).
    - Values of oxygen saturation at depths less than 3 m at all stations near Richardson Bay (USGS 2009) were above 88% saturation in mid-February 2008.
    - Any low DO from raw sewage spill on January 30, 2008 would not be expected to be sustained for three weeks until herring spawning in the Richardson Bay area on February 17 to 19.

- High egg density is unlikely to have induced the observed injury.
    - High egg density is known to induce mortality and abnormalities in herring eggs (Galkina 1971, Taylor 1971, Alderdice and Hourston 1985).
    - The egg densities do not align with lower hatching rates and higher abnormalities.
        - Medium density spawn at SRB (up to 4 egg layers) had the highest density reported in NOAA/BML (2008) but also had the highest hatching rate and lowest abnormality rate.
        - Spawn at SA, PP, and KC was very light to light and predominantly in single layers.
    - The observed egg density was not high enough to induce the high level of effects observed.
        - Hatching rates of light and medium spawn is expected to approach 90 to 100% while heavy spawn (above 8 egg layers) is expected to have hatching rates below 60% (Alderdice and Hourston 1985).
        - Spawning above 4 egg layers was not reported by NOAA/BML (2008).

- Suspended sediment was unlikely to have induced the observed injury.
    - Heavy deposition of suspended sediment was not observed on the naturally spawned eggs (NOAA/BML 2008).
    - Suspended sediments coating the artificially spawned eggs deployed in the subtidal was observed but did not induce an injury (NOAA/BML 2008).
    - The laboratory experiments of Kiorboe et al. (1981) did not find any effects on development in herring embryos exposed from 5 to 300 mg/L of silt during incubation.

- High temperatures during the intertidal incubation of naturally spawned eggs are unlikely to have induced the observed injury.
    - High solar isolation and high air temperatures at mid-day low tides during incubation have been observed to induce mortality and high levels of abnormalities in larvae hatched from intertidal eggs (Purcell et al. 1990).
    - The abnormalities observed in situ by Purcell et al. (1990) overlap but do not completely match those observed in NOAA/BML (2008) or NewFields NW (2009).
        - The abnormalities observed in situ were body axis defects, jaw deformities, and fin malformations (Purcell et al. 1990).
        - The abnormalities reported by NOAA/BML (2008) and NewFields NW (2009) do not include jaw and fin abnormalities and may include pericardial edema not reported by Purcell et al. (1990).
    - The weather and tides in San Francisco during in situ 2008 incubation were not as extreme as those reported by Purcell et al. (1990).
        - Mid-day low tides exposed herring eggs to extremely high (17 to 18 degrees C) air temperatures (Purcell et al. (1990).
        - The intertidal naturally spawned embryos in all sites in SFB would have been exposed to the air to some extent during incubation.

        - Review of the weather records for downtown San Francisco show typical winter weather for the month of February with air temperatures below average for most of in situ incubation period (National Climate Data Center 2008).
        - During February 2008, SFB tides were below 0.0 ft MLLW (i.e. "minus tides") in the late afternoon and early morning rather than at mid-day when solar isolation and air temperatures would have been at maximum.
        - Maximum air temperatures in San Francisco ranged from 11.6 to 16.1 degrees C during the period of in situ incubation and were 14.4 to 21.1 degrees C during collection of the naturally spawned eggs.
        - The natural air temperature regime and aerial exposure at low tides during incubation does not appear severe enough to have induced the observed lower hatch and higher abnormality rates. (See below for question of high temperatures during collection and transport.)

Hypotheses postulated by Incardona (2008) and proposed by NOAA for future study include the following:

- A toxic component of CBOS Oil not measured by the PAH and POP chemical analyses is postulated to be a potential cause of the injury.
    - Although PAHs are often considered to be the primary agent causing toxicity to fish embryos from oil exposure, recent experimental work with fresh and weathered fuel oil from the *Prestige* oil spill indicates that components of heavy fuel oil and exposure pathways other that water-borne PAH exposure are involved in oil toxicity (Gonzalez-Doncel 2008).
    - The possibility that an oil component other than those measured was an active agent cannot be completely ruled out.

- Photo-oxidation of CBOS oil compounds that leads to increased toxicity is also postulated as potential cause.

- Photo-activation of PAHs or other compounds derived from CBOS oil is postulated as a potential cause but appears unlikely based on recent experimental work.
    - UV radiation alone or in combination with contaminants has been observed to decrease hatching rate and increase abnormalities in fish embryos (Bunn et al. 2000).
    - Recent laboratory experiments indicate that photo activation of PAHs in herring eggs does not occur.
        - Laboratory experiments by Barron et al. (2002) investigated hatching rate hatch timing and a suite of larval abnormalities in Pacific herring eggs exposed to the WAF of ANSCO and sunlight treatments.
        - Although yolk sac edema at significantly increased levels occurred at a tissue burden of about 2000 ng/g wet weight in exposed herring eggs, decreased hatching rate, changes in hatch timing, and increased frequencies of other larval abnormalities at hatch did not occur at the highest level tested (about 2000 ng/g).
        - Several sunlight (UVA) treatments applied to the oil exposed eggs did not significantly decrease hatching rate or increase larval abnormalities.
        - In herring larvae, photo toxicity leading to increased mortality only occurred when a PAH tissue burden have been accumulated before UVA exposure.
        - NOAA/BML (2008) report PAH tissue burdens in naturally spawned herring eggs to range from 13 to 53 ng/g wet weight, levels are well below those

inducing yolk sac edema in herring eggs exposed in the Barron et al. (2002) experiments.

- Although laboratory experiments show photo toxicity occurs in the laboratory, the potential for such an effect in the field is considered uncertain because of the natural factors that ameliorate such photo toxicity. (MacDonald and Chapman 2002).
- Based on the tissue burdens observed in SFB herring eggs and the experimental results of Barron et al. (2002), photo activation of PAHs observed in SFB herring eggs is unlikely.

Credible hypotheses not being actively examined by NOAA include the following:

- The question of transport effects from high air temperatures during collection of naturally spawned eggs at the oiled sites in 2008 remains unanswered.
  - During collection of naturally spawned eggs air temperatures were from 14.4 to 21.1 degrees C.
  - This observation raises the question of whether high temperature and low DO occurred during transport of the eggs.
  - Data on the temperatures and DO observed during transport or upon receipt in the laboratory would resolve the questions about transport effects but may not have been taken.

- A legacy pollutant other than CBOS oil may have induced the observed injury.
  - A pollutant other than CBOS oil present in Richardson Bay from past human activity may be the active agent.
    - Creosote induces a suite of abnormalities in Pacific herring similar to that induced by oil exposure (Vines et al. 2000) and may be wide-spread in Richard Bay.
    - While the yolk sac vacuolization observed by Vines et al. (2000) in herring exposed to creosote was not observed by NewFields NW (2009) in the naturally spawned embryos, the body axis defects was.
  - Contrary to some statements made during meetings, POPs such as pesticides can induce patterns of abnormalities similar to that of PAHs, i.e., retarded growth, body axis defects, head, eye, and jaw deformities, and pericardial edema in fish embryos (Westernhagen 1988).
  - Results from analysis of egg tissue and sediments for POPs are needed to assess this hypothesis.

- While direct effects from the sewage spill are unlikely, an indirect effect from the sewage spill cannot be ruled out.
  - Effects from direct exposure to sewage are unlikely.
    - The untreated sewage spill was three weeks before spawning in Richardson Bay.
    - Exposure of herring to raw sewage would not produce the observed effect (Incardona 2008).
    - Exposure of herring eggs to sewage sludge decreases hatching rate but there is no information on abnormalities.
      - Costello and Gamble (1992) exposed herring eggs to sewage sludge and observed precocious (early hatch) at all exposure levels (0.01 to 1.0% sludge dilutions) and decreased viable hatch at 0.1, 0.2 and 1.0% exposure levels.
      - Costello and Gamble (1992) did not score for abnormalities in larvae at hatch.

        - Costello and Gamble (1992) concluded that the direct effects from sewage dumping would be unlikely beyond the immediate vicinity of the dump site but called for more study of potential effects via the microlayer.
- Indirect effects of a microlayer contaminated by the sewage spill are plausible and the observed effects align with an exposure pathway through the microlayer.
    - Laboratory experiments have demonstrated that about 35% of the materials in sewage is floatable and will rise to contaminate the surface microlayer (Word et al. 1984) and contaminated microlayers have been observed near sewage outfalls (Wurl and Obbard 2004).
    - Concentrations of pollutants such as hydrocarbons, pesticides, and metals can be enriched in the microlayer up to 500 times the concentrations in the water column (Hardy 1982, Wurl and Obbard 2004).
    - Exposure of floating fish eggs and herring eggs to contaminated microlayers reduced hatching and increased frequency of abnormalities (Hardy et al 1987a, 1987a, Kocan et al. 1987, Westernhagen 1987).
        - The frequencies of live normal hatch decreased significantly as the concentrations of PAHs, PCBs, and heavy metals increased (Hardy et al 1987a, 1987b).
        - Abnormalities observed in herring from contaminated microlayers include reduced hatch and increased frequencies of body axis defects (sciolosis, lordosis), jaw deformities, eye defects, and yolk sac abnormalities (Kocan et al. 1987, Westernhagen et al. 1987).
        - Toxic effects on herring eggs were the greatest for microlayers contaminated with heavy metals (Westernahgen et al. 1987).
    - The mechanism by which an indirect effect from a sewage spill could occur is as follows:
        - The sewage spill contaminates the microlayer of Richardson Bay and adjacent areas (sites SA, PP, KC) but because of the wind and current patterns, the contaminates microlayer does not extend into San Rafael Bay (SRB).
        - During falling tides, the contaminated microlayer coats the intertidal vegetation at SA, PP, and KC but not SRB.
            - Tidal action deposits contaminated microlayer as “bath-tub ring” at SA, PP, and KC.
            - The microlayer coats either the eggs or the vegetation upon which the eggs are deposited.
            - Because of the currents in SFB, less deposition of contaminated microlayer released into Richardson Bay would be expected at KC compared to SA or PP.
            - The contaminated microlayer does not contact the subtidal areas where the artificially spawned embryos were deployed.
            - Further, contact between herring eggs and a contaminated microlayer deposited in the intertidal would be greater for spawn with single egg layers as reported for SA, PP, and KC than for multiple egg layers as reported for SRB by NOAA/BML (2008).
            - The observed injuries among naturally spawned embryos were heavier on the very light and light spawn density than on the medium spawn density (NOAA/BML 2008).

        - Although the above mechanism aligns with the aspects of the observed injury cited above and has support from other scientific studies, there are no direct measurements or observations that address the mechanism at the specific sites of interest here.

- A credible and simple hypothesis for the observed injury is that the decreased hatch and higher abnormalities found in naturally spawned embryos derive from a combination of poorer egg quality among young spawning herring and from salinity effects during fertilization and incubation.
    - The spawning population of SFB herring is now comprised of younger herring, which have smaller eggs with lower survival potential.
        - Beginning in the 1990s, the SFB herring stock has progressively lost the older fish (age 6 and older) from the spawning population (CDFG 2008).
            - Age 4 fish decreased from 47% of the number of spawning SFB herring in 2006/2007 to 27% in 2007/2008.
            - Age 1 and age 2 fish increased abruptly to 36% by number in the SFB spawning population in 2007/2008 from 10% in 2006/2007.
        - Egg weights in recruit herring spawning for the first time are lower than those in older, repeat spawning herring (Hay 1985).
        - Larger egg size is considered to be of advantage for survival of herring eggs and larvae (Blaxter and Hunter 1982).
            - This lower potential is probably related to the amount of yolk available to sustain embryonic and larval development.
            - Lipid content of the SFB herring ovaries used for artificial spawning in 2007/2008 was 0.4% - about 7 times less than the lipid content in currently stable Puget Sound herring stocks (NOAA/BML 2008).
        - Younger herring may also produce embryos with higher rates of abnormalities.
            - Hershberger et al. (2005) found increased levels of abnormalities in naturally spawned Pacific herring in the Cherry Point Washington stock.
            - Naturally spawned embryos collected just before hatching and hatched in the laboratory showed lower dry weight, shorter lengths, higher incidence of yolk deficiencies and skeletal abnormalities (55% in 2000 and about 40% in 2001).
            - Tissue burdens of PAHs and pesticides in the Cherry Point herring were no higher than those in other Puget Sound herring stocks.
            - Transplant experiments showed that the abnormalities in the Cherry Point stock were from some inherent factor and not environmental factors present at the spawning locations.
            - Hershberger et al. (2005) attributed the higher levels of abnormalities in the Cherry Point stock to changes in the age structure of the stock.
                - The Cherry Point stock has progressively lost the older age class among the spawning population.
                - The spawning population at Cherry Point is now about 49% age 2 herring (Hershberger et al. 2005).
            - In addition to the potential effects from younger spawning herring, are potential effects from spawning in areas with suboptimal salinity regimes.
    - Optimal salinity conditions for fertilization and incubation in SFB herring is narrower (9 to 24 psu) than that in other herring stocks (Griffin et al. 1998) so that suboptimal salinity

during field fertilization and incubation of naturally spawned eggs in SFB may have also reduced hatching success.

- Spawning in waves would have concentrated the younger fish at SA, PP, and KC rather than SRB.
  - In SFB and other regions, herring spawn in waves, with the older fish spawning fish (Spratt 1981, Hay 1985).
  - Spawning at SRB was substantial and occurred February 17, 18, and 19, 2008 (Incardona 2008).
  - Spawning at SA, PP, and KC was less intense and occurred later, February 19, 20, and 21, 2008.
  - Based on the observations that younger fish spawn later, the herring spawning at SA, PP, and KC would be expected to be younger than those spawning at SRB.
  - Younger spawning fish at SA, PP, and KC would be expected to produce poorer quality eggs at SA, PP, and KC compared to SRB.
- The salinity regimes at SA, PP, and KC appear to have had suboptimal salinities compared to SRB.
  - Based on the experimental results of Griffin et al. (1998), the salinities of about 16 psu are optimal for egg fertilization and incubation in SFB herring.
    - Fertilization rates at 16 psu are expected to be about 86% (Griffin et al. 1998).
    - Hatching rates at 16 psu constant salinity would be expected to be about 50% (Griffin et al. 1998).
  - The salinities above 25 psu are for fertilization and incubation.
    - Fertilization rates at 28 psu in SFB herring are expected to be about 30% (Griffin et al. 1998).
    - Hatching rates at 28 psu would be expected to be about 15% (Griffin et al. 1998).
  - The reference site SRB appears to have had a lower salinity regime than those at SA, PP, and KC.
    - Mean salinity at PP recorded during deployment of the artificially spawned embryos was 30 psu while PSQ north of SRB had a mean salinity of 20 psu.
    - The salinity logger at SRB failed so there is no salinity data for SRB from the cage deployment, but SRB was north of PSQ and would have had lower salinity.
    - The salinity data at depths less than 3 m taken by USGS (2009) for February 2008 is sparse but does show a definite and abrupt decrease in salinity from 25 psu at Station 18 near Angel Island to 17 psu at Station 17 west of the Tiburon Peninsula and then to 13 to 14 psu at Station 15 closest to the SRB site.
  - In comparing the naturally spawned embryos to the artificially spawned embryos, it is important to remember that the artificially spawned embryos were fertilized and held briefly in the laboratory at about 16 psu so that these embryos benefited from early conditions that were optimal before deployment to sites with less optimal salinities and furthermore also benefited from no exposure to the air during low tides.
  - The pattern of abnormalities reported by Griffin et al. (1998) is similar to that observed in the naturally spawned larvae at hatch.

        - Griffin et al. (1998) found lowered hatching rate, immobile hatched larvae, body axis defects, and partially hatched larvae but not pericardial edema.
        - If the abnormalities observed in SFB do not include pericardial edema and tissue opacity is not confirmed as an abnormality, then the pattern of abnormalities observed in 2008 for the naturally spawned embryos is similar.
        - Once again, there is an urgent need to examine the raw data from the BML's scoring of herring larvae at hatch.
    - Although the data is sparse, the sum of the available data strongly suggests that the salinity mechanism warrants further study and definitely indicates that salinity needs to be a controlled factor in any experiments and a well measured factor in further field work.

Summary on Hypotheses

As causes of the injury in the naturally spawned embryos, several hypotheses have been eliminated. These eliminated hypotheses include the following:

- Direct effects from PAH exposure from CBOS
- Low dissolved oxygen
- High egg density
- Silt on the eggs
- Direct effects from the sewage spill in late January
- Temperature effects

Hypotheses still outstanding include the following:

- Potential high temperatures during transport
- Legacy pollutants
- Some PAH or non-PAH compound in the fuel oil that has not been measured
- Photo-toxicity of PAHs from CBOS oil
- Indirect effects from the sewage spill
- Combination of younger herring spawning in suboptimal salinities.

A credible and simple hypothesis for the observed injury is that the decreased hatching rates and higher abnormality levels found in naturally spawned embryos derive from a combination of younger herring spawning at sites where the salinity regime was suboptimal for fertilization and incubation. Although the available data is sparse, the sum of the available data strongly suggests that the salinity mechanism warrants further study and definitely indicates that salinity needs to be a controlled factor in any experiments and a well measured factor in any further field work.

RECOMMENDATIONS FOR FURTHER STUDIES

NOAA has proposed studies for winter 2009 to address the issue of whether COSCO BUSAN oil can be tied to the observed injury through some mechanism not yet studied but still derived from the oil spill, such as, photo toxicity or some other ship oil compounds not measured previously. Recommendations concerning the 2009 studies derived from the assessment above include the following:

1. Before commencing any studies, examination of the raw data from BML’s scoring of larvae for abnormalities at hatch is urgently needed to assess the presence of pericardial edema and jaw deformities and to aid the development of other methods of scoring.

2. Before the 2009 work begins, detailed attention is urgently needed to develop and apply effective procedures for characterizing and scoring pericardial edema in herring embryos with other abnormalities.

    a. These improvements should include standardization of the criteria and measurement procedures.

    b. Also needed are specific explicit, detailed definitions for scoring pericardial edema and workable procedures to support such scoring.

    c. Procedures should take into account variations from developmental stage on the measurement of interest.

    d. Procedures to ensure consistent background illumination will be needed.

    e. Development and application of procedures needs to be observed during site visits.

3. The results of scoring embryos and larvae at hatch for abnormalities need to be tabulated by abnormality type.

4. Sample sizes for embryo and larval examination should be increased from 20 per unit to 40 per unit.

5. Oiled gravel columns will be used in the 2009 work and require continuously flowing seawater.

    a. Seawater flow should be maintained continuously even during periods when embryos are not under exposure.

    b. If the columns lose seawater flow, they should be emptied and refilled rather than simply restarted.

6. Ideally, the field works will sample the primary sites about the same time as last year.

7. The in situ shading experiment was a serious shortcoming.

    a. During a field deployment, we can expect the shades to foul with debris and an algal turf. An algal turf sufficient to impair UV transmission might only take a day or so to develop.
    b. Cleaning the in situ shades each day appears impractical and unlikely to be effective.
    c. A viable alternative approach for the in situ shading should be developed or the task dropped.

8. The study design must address other credible hypotheses besides those centered on CBOS oil, and the potential effects of younger fish spawning in suboptimal salinities warrants experiment study at the same level of effort given to exposures to CBOS oil.

    a. Although experimental control of temperature, salinity, and age class is a must, we urge that salinity and age class be addressed as specific experimental variables in the overall experimental study.

b. With appropriate replication in the experimental design, age class should be addressed by using two age classes, Age 2 and Age 5, as gamete donors. The eggs and larvae would be tracked by age class through the experiments.

c. With appropriate replication in the experimental design, salinity should be addressed by using two salinities, 16 psu, the known optimal salinity, and 28 psu, a suboptimal salinity for which other data is available and which is close to the salinities observed in 2008 in and near Richardson Bay.

9. The 2009 experiments and studies should focus potential effects related to the CBOS. The comparison of CBOS oil effects with those of ANSCO is not necessary to discern CBOS effects and the effort and resources planned for ANSCO can be re-allocated to tasks more directly relevant to CBOS.

10. Given Barron et al. (2002), the hypothesis concerning photo-toxicity does not appear plausible enough to warrant study and may be dropped in favor of more urgent efforts.

11. Any field work to resample the previous study sites needs to provide for more measurements of temperature and salinity and for the age structure of the herring spawning at each of the sites under study.

12. Exposure regimes well characterized by high quality chemistry are the hall mark of studies such as those proposed so that the resources devoted to chemistry should be more than minimal.

    a. Eggs should be analyzed for tissue burdens of PAHs and POPs.

    b. Water samples of effluent from the oiled gravel and controls need to be filtered, and both the filtrand (on the filter) and the filtrate (water passing the filter) should be analyzed for PAHs and perhaps other selected compounds.

    c. A written analysis plan should be developed beforehand.

13. At a minimum, site visits should occur during set up, field collection, laboratory incubation, and larval examination.

14. REFERENCES

Alderdice, DF, and AS Hourston. 1985. Factors influencing development and survival of Pacific herring (*Clupea pallasi*) eggs and larvae to beginning of exogenous feeding. Canadian Journal of Fisheries and Aquatic Sciences 42: 56-68.

Alderdice, DF, and FPJ Velson. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). Journal of the Fisheries Research Board of Canada 28: 1545-1562.

Barron, MG, MG Carls, JW Short, SD Rice. 2002. Photoenhanced Toxicity of Aqueous Phase and Chemically-Dispersed Weathered Alaska North Slope Crude Oil on Pacific Herring Eggs and Larvae. Final Report to Prince William Sound Regional Citizens' Advisory Council for Contract Number 602.01.2.

Blaxter, JHS, and JR Hunter. 1982. The biology of clupeid fishes. Advances in Marine Biology 20: 1-223.

Braum, E. 1985. Experiments on the respiration and in situ development of Baltic herring (Clupea harengus L.) eggs. Int. Rev. ges Hydrobiol. 70: 87-100.

Bunn, NA, CJ Fox, and T Webb. 2000. A Literature Review of Studies on Fish Egg Mortality: Implications for the Estimation of Brood Stock Biomass by the Annual Egg Production Method. Science Series Technical Report Number 111. Centre for Environment, Fisheries, and Aquaculture Science, Lowestoft.

California Department of Fish and Game. 2006. Pacific Herring Commercial Fishing Regulations. Final Supplemental Environmental Document. SCH Number 98052052.

California Department of Fish and Game. 2007. Pacific Herring Commercial Fishing Regulations. Final Supplemental Environmental Document. SCH Number 98052052.

California Department of Fish and Game. 2008. Pacific Herring Commercial Fishing Regulations. Final Supplemental Environmental Document. SCH Number 98052052.

Carls MG, GD Marty, JE Hose. 2002. Synthesis of the toxicological impacts of the *Exxon Valdez* oil spill on Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, USA. Canadian Journal of Fisheries and Aquatic Sciences 59(1): 153–172.

Carls MG, SD Rice SD, JE Hose. 1999. Sensitivity of fish embryos to weathered crude oil: Part I: Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). Environmental Toxicology and Chemistry 18(3):491–493.

Costello, MJ, and JC Gamble. 1992. Effects of sewage sludge on marine fish embryos and larvae. Marine Environmental Research 33: 49-74.

Douglas, G. 2008. Presentation on Review of Chemistry Data. Presentations concerning the COSCO BUSAN Oil Spill Fish Injury Assessment, September 2008, Fairmont, California.

Galkina, LA. 1971. Survival of spawn of the Pacific herring (*Clupea harengus pallasi* Val.) related to abundance of the spawning stock. Rapports et Proces-Verbaux des Reunions Conceil International pour L'Exploration de la Mer 160: 30-33

Gonzalez-Doncel, M, L Gonzalez, C Fernandez-Torija, JM Navas, and JV Tarazona. 2008. Toxic effects of an oils spill on fish early life stages may not be exclusively associated to PAHs: Studies with Prestige oil and medaka (*Oryias latipes*). Aquatic Toxicology 87: 280-288.

Griffin, FJ, MC Pillai, CA Vines, J Kaaria, T Hibbard-Robbins, R Yanagimachi, and GN Cherr. 1998. Effects of salinity on sperm motility, fertilization, and development in Pacific herring, *Clupea pallasi*. Biological Bulletin 194: 25-35.

Hardy, JT. 1982. The sea surface microlayer: Biology, chemistry, and anthropogenic enrichment. Progress in Oceanography 11: 307-328.

Hardy, J, S Kiesser, L Antrim, A Stubin, R Kocan and J Strand. 1987a. The sea-surface microlayer of Puget Sound: Part I. Toxic effects on fish eggs and larvae. Marine Environmental Research 23: 227-249.

Hardy, J, EA Crecelius, LD Antrim, VL Broadhurst, CW Apts, JM Gurtisen, and TJ Fortman. 1987b. The sea-surface microlayer of Puget Sound: Part II. Concentrations of contaminants and relation to toxicity. Marine Environmental Research 23: 251-271.

Hay, DE. 1985. Reproductive biology of Pacific herring (*Clupea pallasi*). Candain Journal of Fisheries and Aquatic Sciences 42: 111-126.

Hay, DE, PB McCarter, and D Grant. 1995. Nestucca oil spill and impacts of herring eggs. Candain Technical Report of Fisheries and Aquatic Sciences Number 2060. Pacific Biological Station, Nanaimo, British Columbia.

Hershberger, PK, NE Elder, J Whittouck, K Stick, and RM Kocan. 2005. Abnormalities from the once-largest herring population in Washington state result primarily from factors independent of spawning location. Transactions of the American Fisheries Society 134: 326-337.

Incardona, JP. 2008. Presentations concerning the COSCO BUSAN Oil Spill Fish Injury Assessment, September 2008, Fairmont, California.

Incardona, JP, MG Carls, HL Day, CA Sloan, JL Bolton, TK Collier, and NL Scholz. 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. Environmental Science and Technology. Online preprint

Kocan, R, H von Westernhagen, ML Landolt, and G Furstenberg. 1987. Toxicity of sea-surface microlayer: Effects of hexane extract on Baltic Herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) embryos. Marine Environmental Research 23: 291-305.

Kiorbe, T, E Frantsen, C Jensen, and G. Sorensen. 1981. Effects of suspended sediment on the development and hatching of herring (*Clupea harengus*) eggs. Estuarine, Coastal and Shelf Science 13: 107-111.

Linden, O. 1975. Acute effects of oil and dispersant mixtures on larvae of Baltic herring. Ambio 4: 130-133.

Linden, O. 1978. Biological effects of oil on early development of Baltic herring *Clupea harengus membras.* Marine Biology 45: 273-283.

MacDonald BG, and PM Chapman. 2002. PAH phototoxoicity – an ecologically irrelevant phenomenon? Marine Pollution Bulletin 44: 1321-1326.

McQuinn, IH. 1997. Metapopulations and the Atlantic herring. Reviews in Fish Biology and Fisheries 7: 297–329.

Middaugh, DP, ME Shelton, CL McKennedy, G. Cherr, PJ Chapman, and LA Courtney. 1998. Preliminary observations on responses of embryonic and larval Pacific herring, Clupea pallasi, to neutral fraction biodegradation products of weathered Alaska north slope crude oil. Archives of Environmental Contamination and Toxicology 34: 188.196.

National Climate Data Center. 2008. Local Climatological Data, San Francisco C/O., CA, February, 2008. http://www.ncdc.noaa.gov retrieved January 9, 2009.

NewFields NW. 2009. Evaluation of Measurement Endpoints for the Assessment of Potential Petroleum Related Responses by Pacific Herring: Early Development. Report prepared by NewFields NW, Port Gamble, Washington for Polaris Applied Sciences, Kirkland, Washington.

NOAA/BML. 2008. The 2007 *Cosco Busan* oil Spill: Assessing toxic injury to Pacific herring embryos and larvae in the San Francisco estuary. Report prepared by NOAA Fisheries, Seattle, Washington and Bodega Marine Laboratory, Bodega Bay, California.

O'Farrell, MR, and RJ Larson. 2003. Year-class formation in Pacific herring (*Clupea pallasi*) estimated from spawning-date distributions of juveniles in San Francisco Bay, California. Fisheries Bulletin 103: 130-141.

Pearson, WH, DL Woodruff, SL Kiesser, GW Fellingham and RA Elston. 1985. *Oil Effects on Spawning Behavior and Reproduction in Pacific Herring (Clupea harengus pallasi).* API Publication Number 4412. American Petroleum Institute, Washington, D.C.

Pearson WH, RA Elston, RW Bienert. 1999. Why did the Prince William Sound, Alaska Pacific herring (*Clupea pallasi*) fisheries collapse in 1993 and 1994? Review of hypotheses. Canadian Journal of Fisheries and Aquatic Sciences 56: 711–737.

Pearson WH, E Moksness, and JR Skalski. 1995. A field and laboratory assessment of oil spill effects on survival and reproduction of Pacific herring following the Exxon Valdez spill. In: Wells PG, Butler JN, Hughes JS (eds) *Exxon Valdez* oil spill: fate and effects in Alaskan waters. STP 1219, Publication 04-012190-16, American Society for Testing and Materials, Philadelphia, pp 626–661

Purcell, JE, D Grosse, and JJ Grover. 1990. Mass abundances of abnormal Pacific herring larvae qt a spawning ground in British Columbia. Transactions of the American Fisheries Society 119: 463-469.

Smith, RL and JA Cameron. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. Transactions of the American Fisheries Society 108: 70-75.

Spratt, JD. 1981. Status of the Pacific Herring, Clupea harengus pallasi, Resource in California 1972 to 1980. California Department of Fish and Game, Fisheries Bulletin Number 171. 107p.

Struhsahker, JW, MB Eldridge, and T Escheverria. 1974. Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. In: FJ Vernberg and MB Vernberg. Pollution and Physiology of Marine Organisms, Academic Press, New York, NY. p253-284.

USGS. 2009. Water Quality of San Francisco Bay. http://sfbay.wr.usgs.gov/cgi-bin/sfbay. Retrieved January 9, 2009.

Vines, CA, T Robbins, FJ Griffin, and GN Cherr. 2000. The effects of diffusible creosote-derived compounds on the development in Pacific herring (*Clupea pallasi*). Aquatic Toxicology 51: 225-239.

Watters, DL, and KT Oda. 2002. Pacific Herring, Clupea pallasi, Spawning Population Assessment and Fishery Management for San Francisco Bay, 1993-94. California Department of Fish and Game, Marine Region Administrative Report 02-1. 34p.

von Westernhagen, H, M Landolt, R Kocan, G Furstenberg, D Janssen, and K Kremling. 1987. Toxicity of the sea-surface microlayer: Effects on herring and turbot embryos. Marine Environmental Research 23: 273-290.

von Westernhagen, H. 1988. Sublethal effects of pollutants on fish eggs and larvae. Fish Physiology, Volume XIa: 253-346.

Word, JQ, CD Boatman, CC Ebbesmeyer, RE Finger, S Fischnaller, and QJ Stober. 1984. Vertical transport of effluent material to the surface of marine waters. Fifth International Oceanographic Disposal Symposium, Corvallis, Oregon, pp.134-149.

Wurl, O and JP Obbard. 2004. A review of pollutants in the sea-surface microlayer (SML): A unique habitat for marine organisms. Marine Pollution Bulletin 48: 1016-1030.