

Cosco *Busan* Oil Spill Natural Resource Damage Assessment

Data Report of Laboratory and Field Herring Injury Studies Performed 2008-2009



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I. INTRODUCTION

Contemporary research on oil toxicity, largely in response to the *Exxon Valdez* spill, has focused on crude oil, and in particular petrogenic polycyclic aromatic hydrocarbons (PAHs). The hallmark of “canonical” crude oil toxicity in fish embryos is cardiogenic edema, attributable to the tricyclic PAH fraction of unrefined petroleum such as Alaska North Slope crude oil (ANSKO). It was anticipated that if any lingering oil toxicity followed the *Cosco Busan* spill, it would be observed as a small increase in the detection of pericardial edema in herring embryos incubated near oiled shoreline. The 2007-2008 Fish Injury studies were designed to detect such differences. While there were statistically significant increases in measures of sublethal pericardial edema in caged embryos incubated in the subtidal zone of oiled shorelines, embryos that incubated in the intertidal zones of oiled shoreline apparently succumbed to a dramatically different type of *lethal* toxicity. The complete absence of this lethality at the non-oiled site, plus the inability to associate lethality with other chemical or abiotic stressors, strongly suggests a link to exposure to *Cosco Busan* oil. While canonical petrogenic PAH toxicity is sublethal, previous laboratory studies with ANSKO and herring larvae showed that oil can produce acutely lethal toxicity when combined with exposure to ambient sunlight or UV wavelength light. At the same time, modern residual fuel oils such as that carried on board the *Cosco Busan* have distinct chemical differences from unrefined crude oil that could result in different types of toxicity. On this basis, the novel lethal effect observed in 2007-2008 natural spawn samples and the differences in effects observed in subtidal vs. intertidal incubation leads to these specific aims: **(1) Does the inherent toxicity of Cosco Busan bunker oil differ significantly from unrefined Alaska North Slope crude oil? (2) Did sunlight exposure of beached Cosco Busan bunker oil produce novel toxic compounds through photo-oxidation? (3) Was the observed necrosis in natural spawn samples due to phototoxicity of PAHs or other bunker oil constituents?**

In order to test these specific aims, a laboratory study was designed by investigators at NOAA's Northwest Fisheries Science Center and the UC-Davis Bodega Marine Laboratory, and implemented at the Bodega Marine Laboratory December 2008 through March 2009. Oiled gravel columns were used to generate water contaminated with dissolved-phase oil constituents in a way that mimics intertidal conditions following an oil spill. The basic principle was to expose herring embryos to oil during weathering by initiating weathering of the columns in January with continuously flowing seawater, and incubating herring embryos in the column effluents at different points between January and whenever the availability of gametes ceased (potentially April). A replicate design tested effluents from columns containing clean gravel, gravel from a non-oiled urbanized beach in San Francisco Bay, gravel coated with three concentrations of ANSKO as a positive control, and gravel coated with three concentrations of *Cosco Busan* oil. Both the columns and the incubation reservoirs for embryos were exposed outdoors to either full

sunlight or sunlight with reduced UV wavelengths with the use of covers constructed from UV transmitting (UVT) or UV blocking (UVB) plastic.

Due to the constraints of obtaining sufficient masses of herring gametes and the time to analyze the embryos from a single experiment, only Aim 3 was rigorously tested. The study as it was executed could not rule in or out a contribution of photo-oxidation to toxicity. The toxicity of the two oils was not directly compared in the laboratory without the additional stressor(s) of outdoor exposure. Embryos were incubated in the column effluents at four points between late January and late March 2009. After incubation to 8 days post-fertilization (just before hatch), embryos were examined for signs of necrosis. In order to verify the oiled gravel dose response relationships, PAHs were measured in water samples at the start and end of embryo incubation, and in embryos tissues at the end of incubation. However, PAH concentrations were not intended to be used as the sole determinant in the interpretation of toxic effects, as there may be other unmeasured compounds contributing to toxicity of a given oil.

A second goal of 2008-09 studies, described in Part 2 below, was to assess the status of naturally spawned embryos in and near the Cosco Busan spill zone in the second spawning season following the spill. The sampling design was identical to that used in the 2007-08 season: eight replicate samples of natural spawn on intertidal substrate collected along an isobathic transect. The plan was to obtain repeat samples at the same spill zone and reference sites as last year and identify new reference sites. Reconnaissance revealed no intertidal spawning at any of the reference or oiled sites sampled in 2008. Intertidal spawning was observed at a new non-oiled site along the Marin shoreline at Paradise Cove, but this spawning occurred at a higher level and on different substrate relative to 2008 samples.

Finally, an additional lab study addressed whether incubation at higher than optimal salinity could account for some of the abnormalities observed in embryos from oiled sites in 2008. This study is described in Appendix 1.

II. METHODS

Field Collection of Spawned Eggs

Field collection of herring spawn was carried out as described in the general CBOS Fish Injury Study SOP manual from 2007-2008. The workplan is described in two documents, "*Cosco Busan Herring Studies Work Plan 2008-2009 Draft*" and "*2008field_workplan*". Files are found in the folder "Work plans and SOPs":

- CBOS_SOP.pdf
- Cosco Busan Herring Studies Work Plan 2008-2009 Draft.pdf
- 2008field_workplan.pdf.

Laboratory Exposure and Phototoxicity Study

Study design, methods and SOPs are included in the following documents, found in the folder "Work plans and SOPs":

- Cosco Busan Herring Studies Work Plan 2008-2009 Draft.pdf
- SOP Illustrated spray method.doc
- 2008 Dec oiled gravel prep.doc
- SOP for collecting water from exposure reservoirs.doc
- CBOS_SOP.pdf

Salinity Study

Study design, methods and SOPs are included in the following documents, found in the folder "Work plans and SOPs":

- experimental design salinity age 29jan09.doc
- CBOS Salinity Experiments.doc

III. RESULTS

Part 1. Herring Egg Laboratory Exposure and Phototoxicity Study

Raw data files:

Folder "Data files and lab notes"

- CBOS09 fem wts.xls (weights of females supplying gametes)
- column temp log 031809.xls (continuous temperature data in control columns for Trial 4)
- column temp log Jan-Mar09.xls (continuous temperature data, Trials 1-3)
- ColumnWQJan14toFeb23.pdf (ad hoc water quality measures Trials 1 and 2)
- fertilization_tests.pdf (lab notes on individual female fertilization tests)
- Folder "Trial 1", image files
- Folder "Trial 2", image files
- Folder "Trial 3", image files and data files, Trial 3 key to egg chemistry.doc, Trial 3 tissue PAH.xls, Trial 3 water PAH.xls
- Folder "Trial 4", image files and data files, Trial 4 key to egg chemistry.doc, Trial 4 tissue PAH.xls, Trial 4 water PAH.xls
- WaterQuality022609.xls (Trial 3 ad hoc water quality measurements)
- WaterQuality031809.xls (Trial 4 ad hoc water quality measurements)

Experimental Conditions and Findings

Production of embryos

Capture and handling of adult fish and generation of embryos followed the same SOP and published methods for 2007-08 studies. Briefly, small numbers of adults (<100) were captured and immediately placed on ice for transport to the lab within 2-3 hours. Gonads were dissected on arrival and stored moist at 4°C. Mass fertilization utilized the polyvinyl alcohol method to prevent clumping of eggs prior to fertilization (Griffin et al., 1998 *Biol. Bull.* 194: 25). To generate embryos for the oiled gravel column studies, ripe prespawn adults were captured by gillnet or trawl in the Central Bay/Richardson Bay area. After obtaining adult length and weight data, ovaries and testes were dissected. Eggs from individual females were tested for fertilization success with milt pooled from 3-4 of the largest testes, and those showing rates of 90% or better were selected. All ovaries were then stripped of eggs, which were pooled and well mixed for mass fertilization with the pooled milt on microscope slides for observation and nitex sheets for PAH analysis. For each trial, mass fertilization rates were about 90%. Embryos for Trial 1 were generated from fish captured in Richardson Bay 01/22/09 with an average female weight of 104 g (n = 5); Trial 2 embryos were sourced from fish capture off Point Chauncey 02/11/09 with average female weight of 60 g (n = 26); Trial 3 embryos were sourced from Point Chauncey fish captured 02/23 and 02/25/09 with average female weight of 61 g (n = 33); Trial 4 embryos were sourced from Richardson bay fish captured 03/16 and 03/17/09 with average female weight of 63 g (n = 13). Embryos for the salinity study were obtained from Richardson Bay fish captured 03/05/09 with average female weight of 91 g (n = 4).

Trial 1, January 22-30 2009

This trial was considered a “dry run” in which there would not be a full work-up of samples for chemical analysis. The primary purpose was to check that the oiled gravel doses were correctly targeted, and that the positive controls were producing the expected sublethal toxicity. Embryos on randomly selected replicate slides were examined daily from 5 dpf through 8 dpf. Representative images were collected from most but not all dose and light combinations.

Results at 8 dpf are shown in Figure 1. In general, the positive and negative controls produced expected results. Normal development was observed in clean and urban gravel effluents. Embryos exposed to ANS under UVB plastic showed dose-dependent pericardial edema (not shown), as did embryos exposed to ANS under UVT plastic (arrows, Fig. 1, second column, top). In addition, at the highest dose of ANS under UVT plastic, there was a reduction in the size of the embryos, and a slight dorsal curvature. This novel effect of oil + UV exposure was also observed in the CB 0.3 g/kg UVT treatment (fourth column, middle). Dose-dependent pericardial edema was also observed in embryos exposed to CB oil under UVB plastic (arrows, Fig. 1, third column). While embryos exposed to CB oil under UVT plastic appeared viable at 6 dpf (Figure 2), by 8 dpf embryos exposed to 1 g/kg CB oil and UV were completely necrotic (Fig. 1, third column), making them impossible to dechorionate.

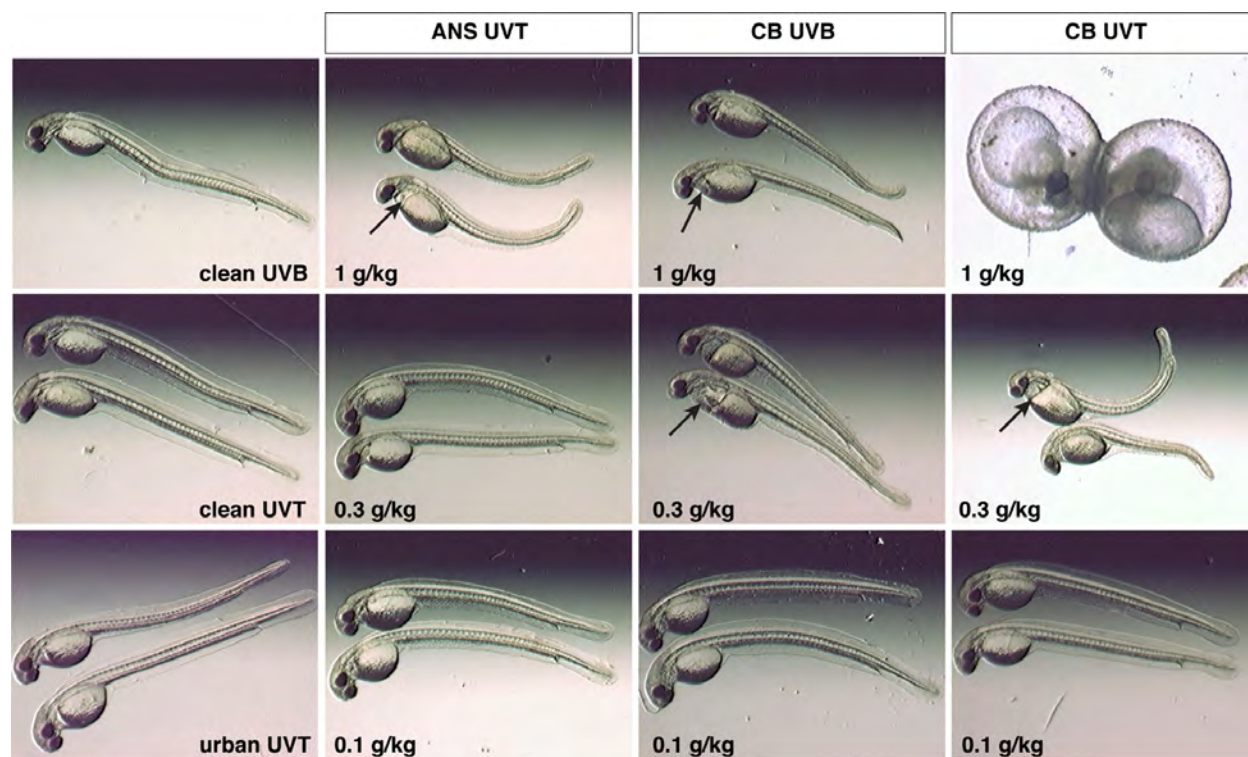


Figure 1: Morphology of 8 dpf embryos, Trial 1. Arrows indicate pericardial edema

During this trial, continuous temperature loggers were placed in one control effluent aquarium on each table. Temperature in the exposure aquaria fluctuated around the desired 12°C

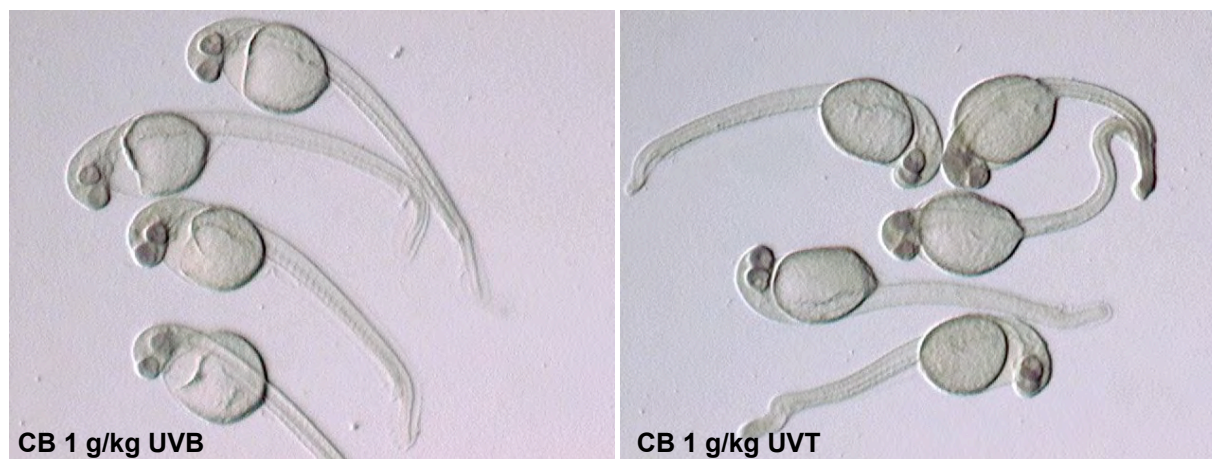


Figure 2: Morphology of high dose CB-exposed embryos at 6 dpf, Trial 1

incubation temperature on a diurnal basis, peaking around 18°C between 12:00 and 14:00 (Fig. 3). However, all tables showed very similar temperature profiles. Spot checking temperatures randomly in other column effluents showed that the logger data were broadly representative of all columns in a table. Data was not collected from Table 4 due to failure of the logger.

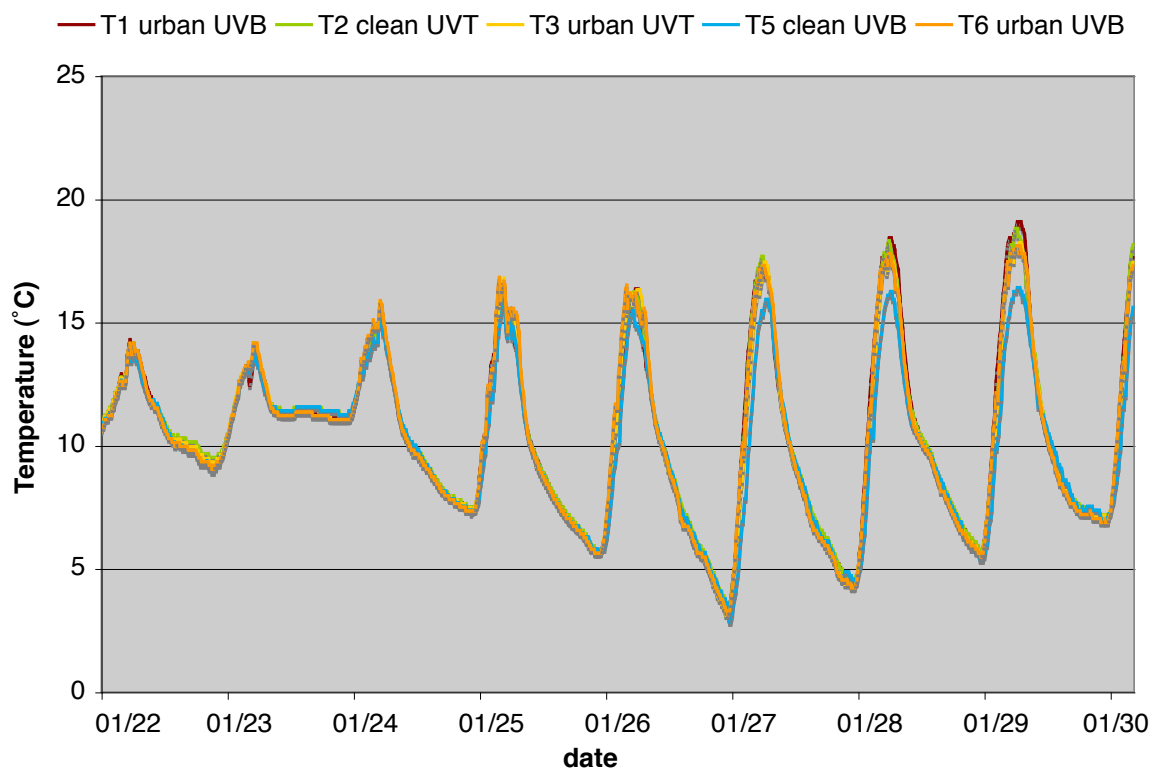


Figure 3: Trial 1 temperature logger data from control column effluents, recorded every 10 min.

Trial 2, February 13-22 2009

During this trial, it was noted at 5 dpf that there were high rates of abnormal early embryos in lab incubator controls. These embryos had gastrulation defects. Because of this, it was decided to abort this trial due to the time it would take to distinguish oil-associated lethality from

background. However, a small-scale analysis was completed. So that the columns could be prepared for a new batch of embryos, one or two replicates of each treatment were removed from the column effluents at 7 dpf and placed in 20-gal aquaria with 22 ppt seawater, immersed in a 4-ft tank for temp control, and incubated an additional 2 days under the appropriate UVT or UVB plastic. Thus oil exposure stopped at 7 dpf, while sunlight exposure continued to 9 dpf when embryos were analyzed.

Results are shown in Figure 4. Embryos were scored as viable eyed embryos. Similar to what was observed in Trial 1, high rates of cytolized eyed embryos were observed in the CB 1 g/kg UVT treatment. Only a few embryos from the CB 1 g/kg UVT treatment were sufficiently intact to dechorionate (Figure 5).

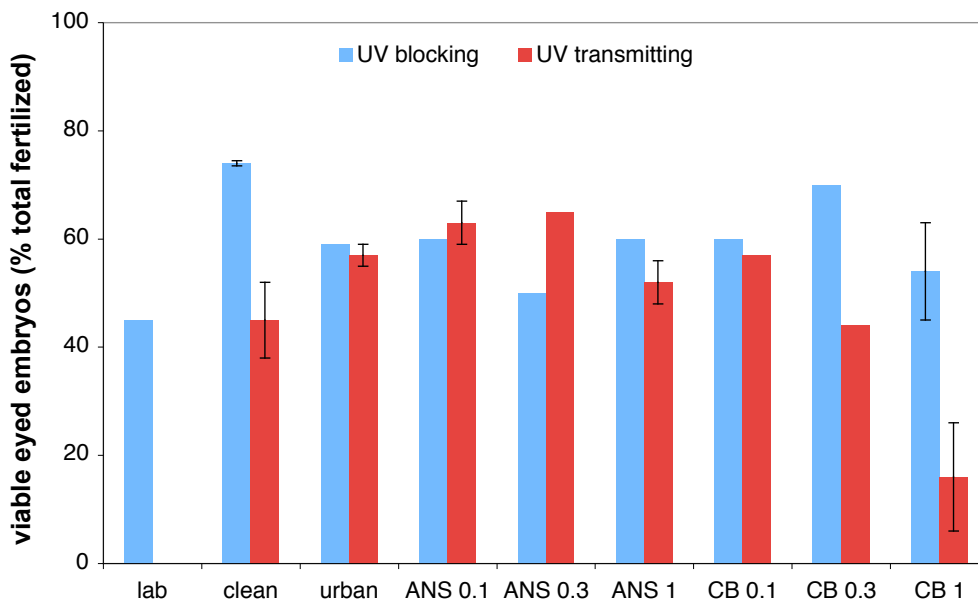


Figure 4: Viable eyed embryos, Trial 2. Error bars present for samples with n = 2.

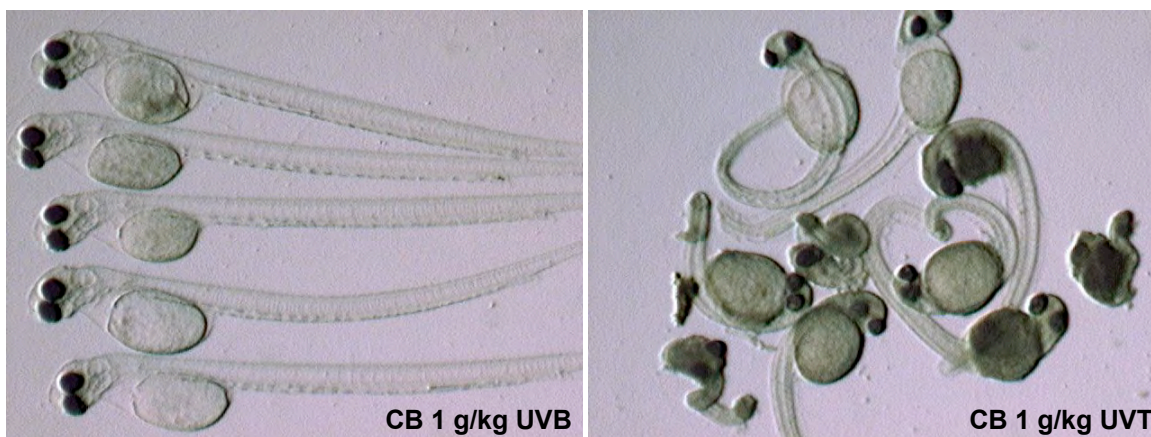


Figure 5: Morphology of non-cytolyzed CB 1 UVT embryos compared to CB 1 UVB

Some changes were made for this trial to improve the temperature control. A chiller was added to cool the water leaving the head tank providing source water for the columns. The water baths

were switched from freshwater to seawater to dampen the night time chilling effect. Incubation temperatures still fluctuated several degrees around the desired 12°C, with higher temperature peaks (20°C) coming with warmer sunny days. Consistent with Trial 1, all tables showed similar temperature trends (Fig. 6). The last two days of incubation for this trial were carried out in 20-gal aquaria submerged in 4-foot tanks held at 12°C. It was noted during this run that diatom

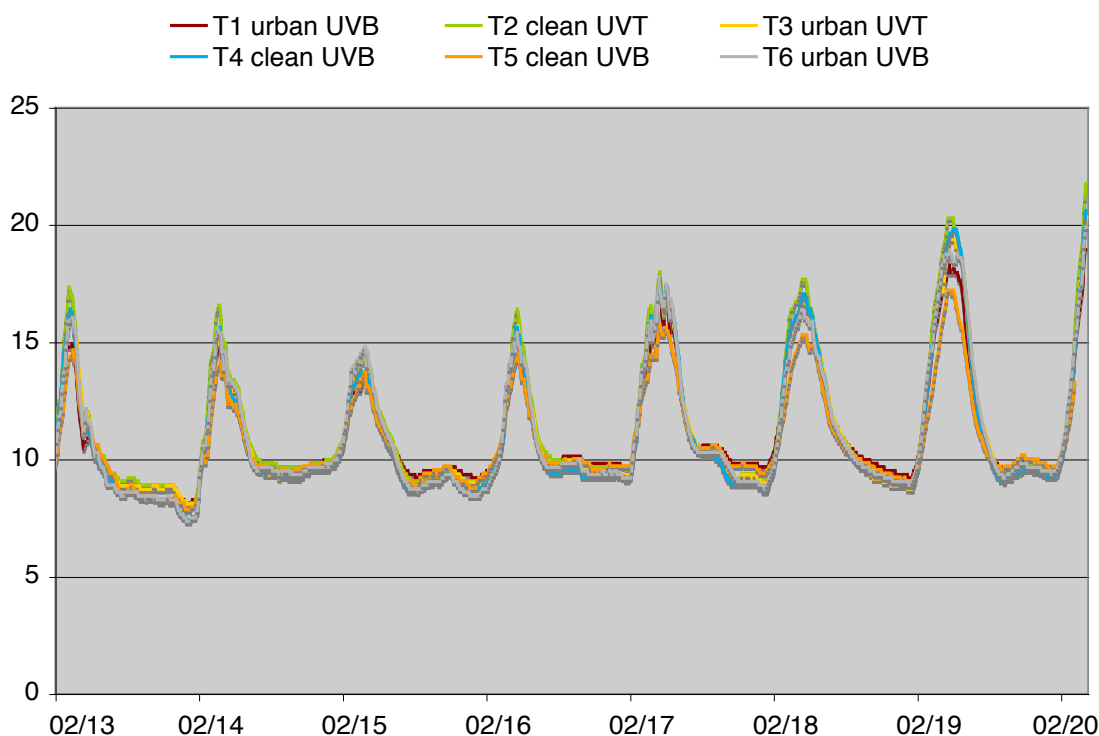


Figure 6: Trial 2 temperature logger data from control column effluents, recorded every 10 min.

growth was becoming visible in the incubator tanks and tubing feeding the columns. Tanks were scrubbed prior to placement of embryo slides.

Trial 3, February 26 - March 7 2009

Laboratory controls were satisfactory for this trial (about 10% background embryos with abnormal early development), and a full experiment was completed with samples taken for water and tissue PAH analysis. Embryos were incubated to 9 dpf and analyzed. Due to higher daytime temperatures than the previous trials, development was accelerated, and some hatching had occurred by 9 dpf. Therefore, embryos were scored as unfertilized eggs, dead during segmentation or earlier (no eye pigment), viable with eye pigment, necrotic with eye pigment, or hatched (empty chorion). During this trial, heavy growth of singular and filamentous diatoms had occurred between days 6 and 9 of incubation, coating the outer chorions. Embryos on slides were gently scraped with forceps to allow visual scoring through the chorion.

This was the first trial for which there was a complete set of samples taken for PAH analysis in both water and tissue. Two duplicate sets of column effluent samples (200-ml) were collected and analyzed for PAHs. One was analyzed by the NOAA NWFSC without filtering. The second set was analyzed by the Alpha Analytical Woods Hole Division (Mansfield, MA), after filtration ostensibly to remove particulate oil or oil droplets. The Alpha Analytical data are reported here. Aqueous PAH levels correlated well with oil doses loaded on gravel (Fig. 7). PAH levels were

trace, with the highest CB oil dose producing total PAHs (TPAH) less than 1 ppb ($\mu\text{g/l}$). The lowest oil doses (0.1 g/kg) produced aqueous TPAH that were nominally higher than but difficult to distinguish from background levels of about 100 ppb.

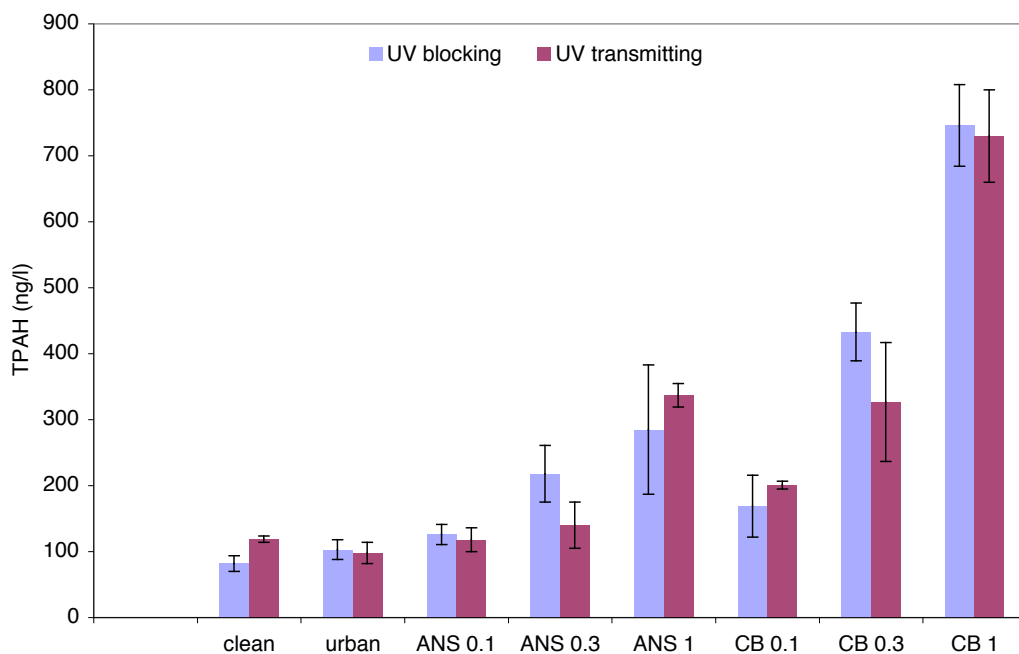


Figure 7: Total aqueous PAHs in column effluents (38 analytes). Values are mean \pm SE for three replicate columns from samples taken at the start of incubation.

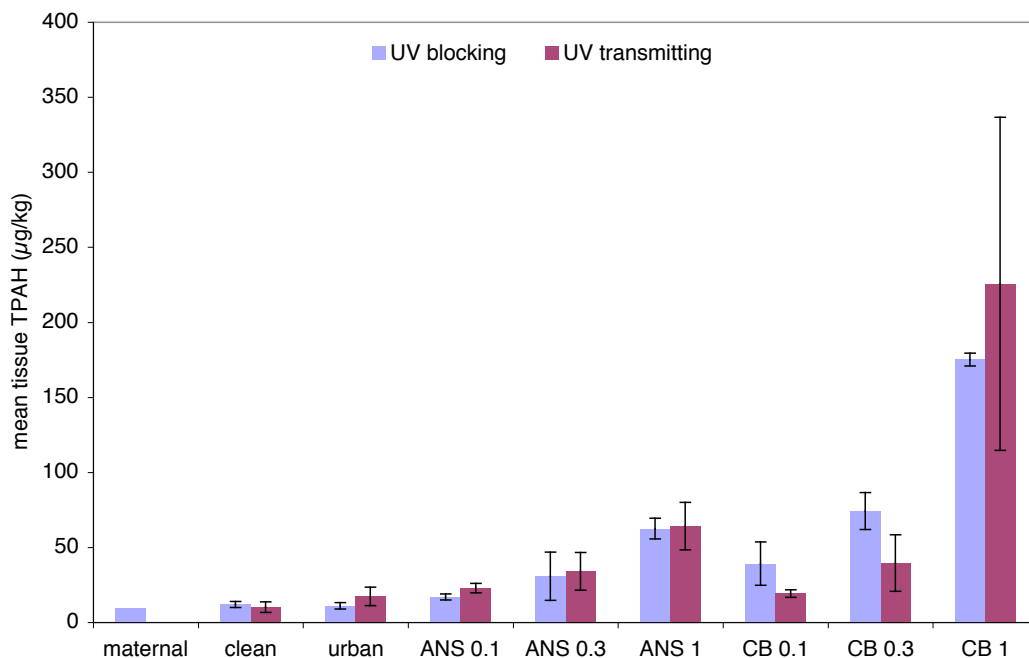


Figure 8: Total PAHs in embryos (38 analytes). Values are wet weight mean \pm SE for three replicates.

TPAH measured in embryo tissues correlated with aqueous TPAH and oil loading dose (Fig. 8). The highest TPAH values were found in embryos exposed to the highest (1 g/kg) dose of CB oil, where both UVB and UVT treatments accumulated about 200 ppb TPAH. For reference, the doses of ANSCO producing sublethal effects on heart rate in herring resulted in TPAH at 480 ppb at the lowest tested dose (0.4 g/kg) at an earlier phase of weathering (Incardona et al., 2009 *Environ Sci Technol* 43:201). The lower doses of both CB oil and ANS produced TPAH below 75 ppb.

Embryos were examined beginning at 5 dpf. As was observed in the first two trials, embryos progressed through development to the eye pigmentation stage, with high numbers of deteriorating, cytolyzed embryos in the CB 1 g/kg UVT treatment near appearing close to hatching. Results for necrotic eyed embryos are shown in Figure 9. For the CB 1.0 g/kg dose under UV transmitting conditions, a mean of 91% was found to be cytolyzed by the late eyed stage, consistent with the effect observed in Trial 1. The middle dose of CB oil (0.3 g/kg) under UV transmitting conditions produced a statistically significant increase in necrotic lethality at 16% compared to 3% under UV blocking conditions. The high dose of ANSCO also produced a statistically significant increase in necrotic lethality at 16% compared to 6% under UV blocking conditions. The percentage of necrotic embryos did not correlate with tissue PAH levels (Fig. 8

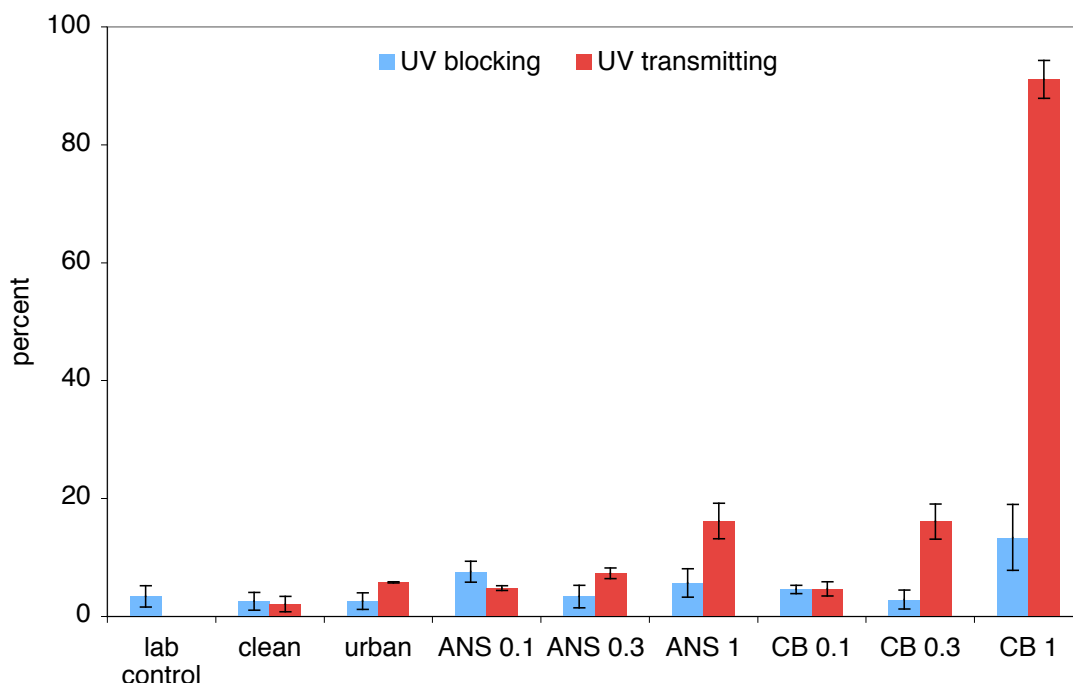


Figure 9: Necrotic eyed embryos, Trial 3. Mean and SE for $n = 3$, except CB 0.3 g/kg UVT treatment, $n = 2$. Denominator for percentage is total eyed eggs per slide (necrotic eyed + viable eyed + hatched)

vs. Fig. 9): While the CB 0.3 g/kg UVT treatment and the ANS 0.3 g/kg UVT treatment had indistinguishable PAH levels (40 ± 19 ng/g vs. 34 ± 13 ng/g, respectively), only the CB 0.3 g/kg UVT treatment produced a significantly higher percentage of necrotic embryos ($16 \pm 3\%$ vs. $4.8 \pm 0.4\%$).

With increasing daytime temperatures and day length, incubation temperatures continued to have diurnal variation and diatom growth became much heavier than the previous trial. Temperature peaked on two days at $22.5 - 24^\circ\text{C}$ (Fig. 10). All tables showed similar temperature trends. Algae growing in the columns and incubation aquaria was examined microscopically and found to consist of green filamentous (stacking) and brown singular diatoms, with some flagellated forms. Chorions were coated with clustered singular diatoms. The presence of algae

resulted in a diurnal pattern of high dissolved oxygen levels coupled with elevated pH, coincident with peak daytime photosynthesis. Daily dissolved oxygen levels, measured at mid-day, are shown for clean gravel effluents and CB 1 g/kg doses in Fig. 11. Daily pH values, measured at mid-day, are shown for clean gravel effluents and CB 1 g/kg doses in Fig. 12.

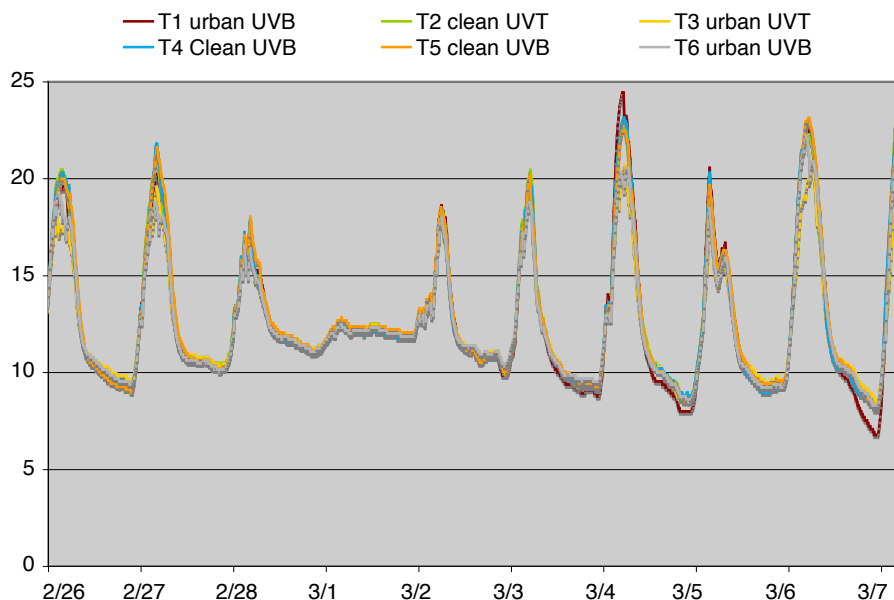


Figure 10: Trial 3 temperature logger data from control column effluents, recorded every 10 min.

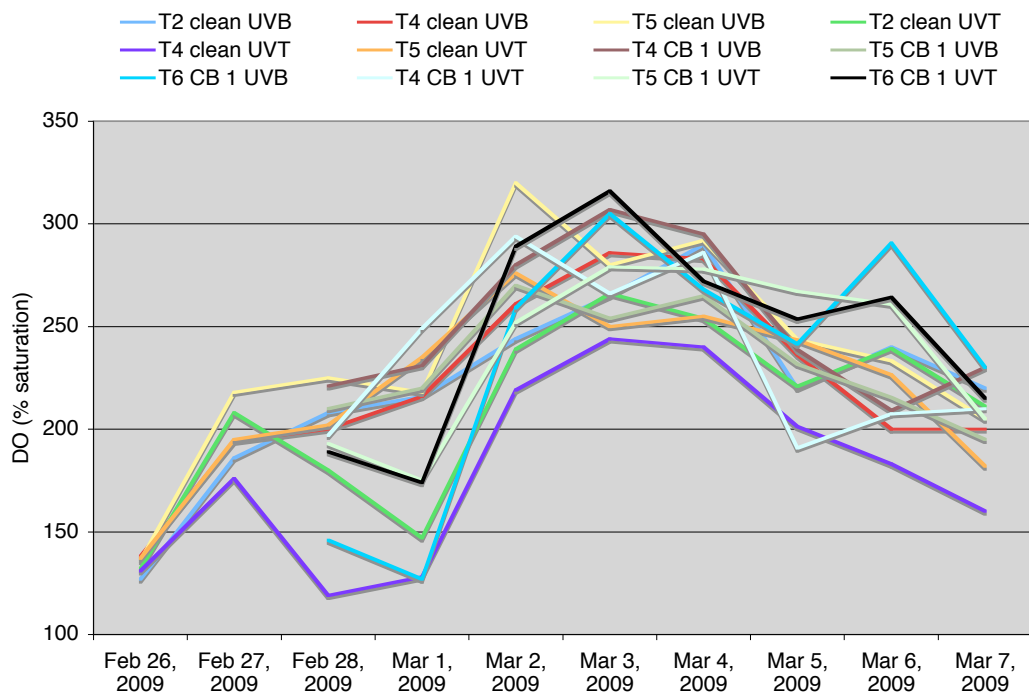


Figure 11: Trial 3 daily dissolved oxygen levels, clean gravel vs. CB 1 g/kg, UVB and UVT

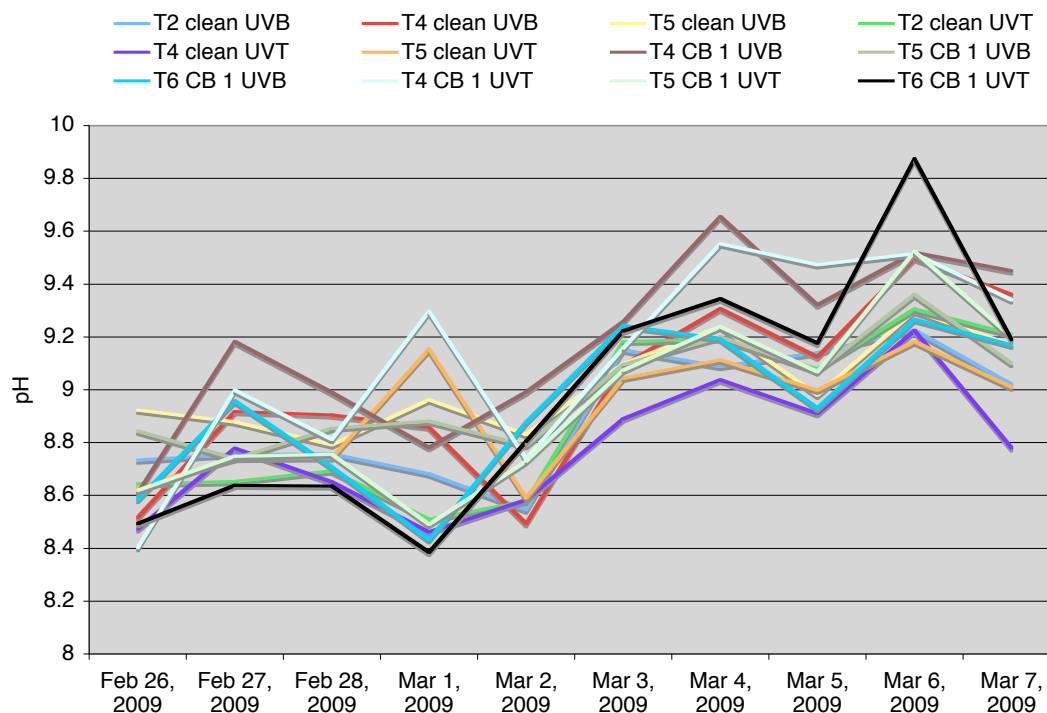


Figure 12: Trial 3 daily pH, clean gravel vs. CB 1 g/kg, UVB and UVT

Algal growth was assessed and described in a semi-quantitative manner for each column/incubation aquarium pair and noted. These data are shown in Table 1. Throughout this trial ammonia was undetected or at background levels.

Table 1: Characterization of algal growth in columns and incubator aquaria, Trial 3

Table	dose	plastic	column	aquarium	color
Table 1	ANS 0.1	UVB	slight	heavy	green
Table 3	ANS 0.1	UVB	heavy	slight/med	brown
Table 5	ANS 0.1	UVB	slight	medium	green
Table 1	ANS 0.1	UVT	slight	slight/med	green
Table 3	ANS 0.1	UVT	medium	heavy	green
Table 5	ANS 0.1	UVT	slight	slight	green
Table 1	ANS 0.3	UVB	slight	slight/med	green
Table 2	ANS 0.3	UVB	slight	heavy	green
Table 6	ANS 0.3	UVB	heavy	heavy	mixed
Table 1	ANS 0.3	UVT	slight	medium	green
Table 2	ANS 0.3	UVT	slight	slight	green
Table 6	ANS 0.3	UVT	slight	heavy	green
Table 3	ANS 1	UVB	slight	heavy	brown
Table 4	ANS 1	UVB	slight	slight	green
Table 5	ANS 1	UVB	slight	slight	green
Table 3	ANS 1	UVT	slight	slight	green
Table 4	ANS 1	UVT	slight	slight	green
Table 5	ANS 1	UVT	slight	slight	green
Table 2	CB 0.1	UVB	heavy	heavy	green
Table 4	CB 0.1	UVB	heavy	heavy	green
Table 6	CB 0.1	UVB	heavy	heavy	mixed
Table 2	CB 0.1	UVT	heavy	heavy	green
Table 4	CB 0.1	UVT	slight	slight/med	green
Table 6	CB 0.1	UVT	heavy	heavy	mixed
Table 1	CB 0.3	UVB	slight	medium	green
Table 2	CB 0.3	UVB	heavy	heavy	green
Table 3	CB 0.3	UVB	heavy	heavy	green
Table 1	CB 0.3	UVT	slight	slight/med	green
Table 2	CB 0.3	UVT	heavy	heavy	green
Table 3	CB 0.3	UVT	slight	slight	brown
Table 4	CB 1	UVB	slight	medium	green
Table 5	CB 1	UVB	slight	slight/med	green
Table 6	CB 1	UVB	slight	medium	green
Table 4	CB 1	UVT	slight	medium	green
Table 5	CB 1	UVT	heavy	medium	brown
Table 6	CB 1	UVT	medium	heavy	mixed
Table 2	clean	UVB	heavy	heavy	green
Table 4	clean	UVB	heavy	medium	green
Table 5	clean	UVB	heavy	heavy	green
Table 2	clean	UVT	slight	heavy	green
Table 4	clean	UVT	slight	slight	green
Table 5	clean	UVT	slight	medium	green
Table 1	Urban	UVB	slight	slight/med	green
Table 3	Urban	UVB	slight/med	medium	green
Table 6	Urban	UVB	heavy	heavy	mixed
Table 1	Urban	UVT	slight	medium	brown
Table 3	Urban	UVT	medium	slight	green
Table 6	Urban	UVT	medium	slight/med	green

Trial 4, March 18-26, 2009

Due to the coating of eggs by diatoms and higher temperatures during Trial 3, some modifications were made for a fourth trial. Between the trials, all components were cleaned of algae. The gravel was rinsed with cold freshwater, and the lines, aquaria, and water baths were bleached and treated with thiosulfate. Flow of 25 ppt seawater was initiated 48 hr prior to embryo incubation. A major goal was to increase the water flow rate weathering the columns to help stabilize the temperatures. The size of the head tank limited the total volume at a higher rate, so some treatments had to be eliminated. The ANS columns and urban gravel negative control were eliminated, cutting the number of columns from 48 to 24. Flow rate was increased from 12 ml/min to 30 ml/min. In addition, to reduce the exponential growth of algae typically observed in the last few days of incubation, a 90% shade cloth was used to cover each bank of columns starting on day 5 of incubation. The shade cloth was removed for the last 24 hours of exposure.

The aqueous TPAH levels in this trial were similar to Trial 3, with the highest gravel loading producing TPAH in the 0.7-0.9 ppb range (Fig. 13). Tissue TPAH levels were also similar to Trial 3, with the CB 1 g/kg treatments producing roughly 150-200 ppb by 8 dpf (Fig. 14).

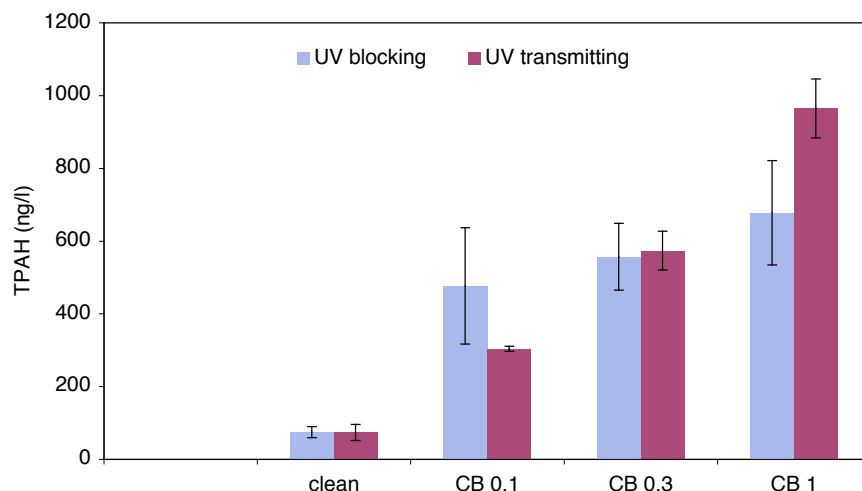


Figure 13: Total aqueous PAHs in column effluents (38 analytes). Values are mean \pm SE for three replicate columns from samples taken at the start of incubation.

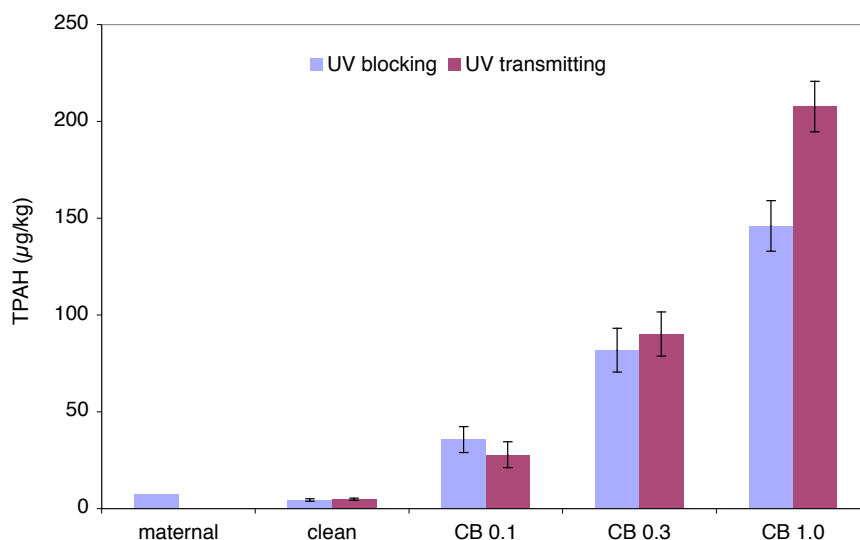


Figure 14: Total PAHs in embryos (38 analytes). Values are wet weight mean \pm SE for three replicates.

As in previous trials, embryos were examined daily from 5 dpf and were found to progress into the eye pigmentation stage. By 8 dpf, large numbers of deteriorating eyed embryos were observed in the CB 1 g/kg UVT treatment. Accumulation of diatoms on the chorions was still heavy enough to require removal by careful scraping with forceps. To streamline the scoring process, in this trial the counts included total eggs on each slide, unfertilized eggs, and viable (i.e. non-necrotic) eyed embryos. There was not a count for embryos that died at early stages for each treatment, but random checks showed a background rate of about 10% in laboratory

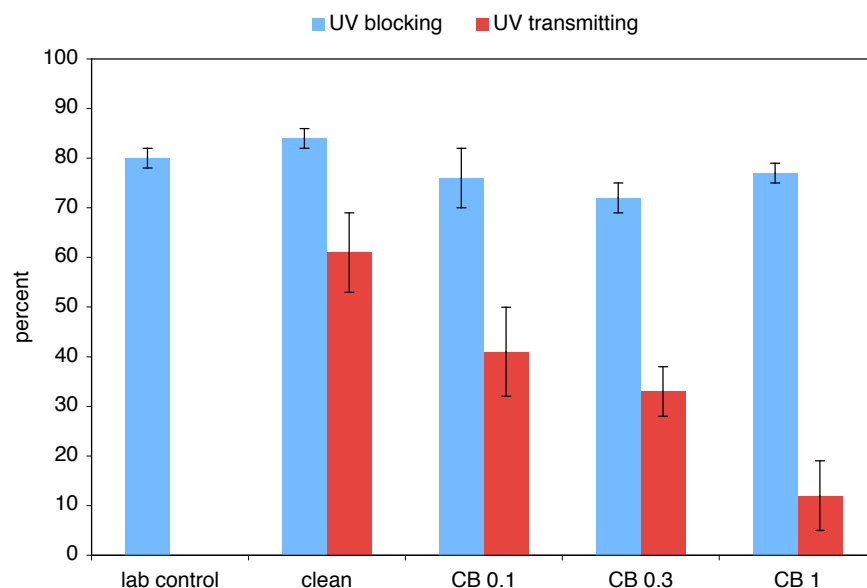


Figure 15: Viable eyed embryos, Trial 4. Mean and SE for $n = 3$. Denominator for percentage is total fertilized eggs per slide.

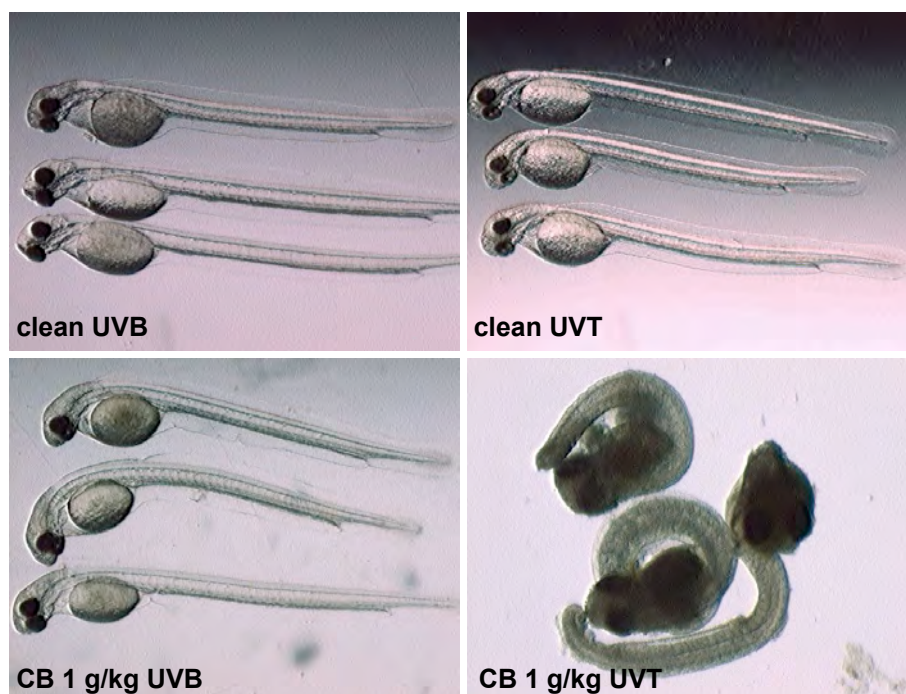


Figure 16: Lethal effects of CB 1 UVT treatment, Trial 4. Examples of viable dechorionated embryos shown for clean UVB and UVT treatments, and CB 1 g/kg UVB treatment, necrotic embryos from CB 1 g/kg UVT treatment.

controls and column specimens. The data for viable eyed embryos are shown in Fig. 15. Examples of viable and necrotic embryos are shown in Fig. 16.

The increased flow rate did lead to better temperature control, but there were still diurnal fluctuations with peaks around 18°C at 12:00-14:00 on three days (Fig. 17).

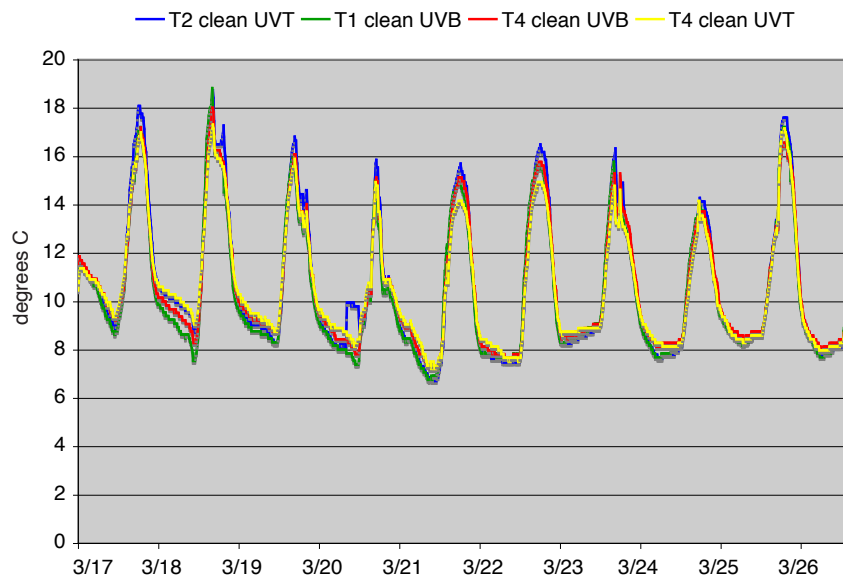


Figure 17: Trial 4 temperature logger data from control column effluents, recorded every 10 min.

Although growth of diatoms was reduced in this trial relative to Trial 3, there was still a gradual increase of algal growth throughout the incubation period, which resulted in a gradual increase in dissolved oxygen levels (Fig. 18) and pH (Fig. 19). However, there was little variation among

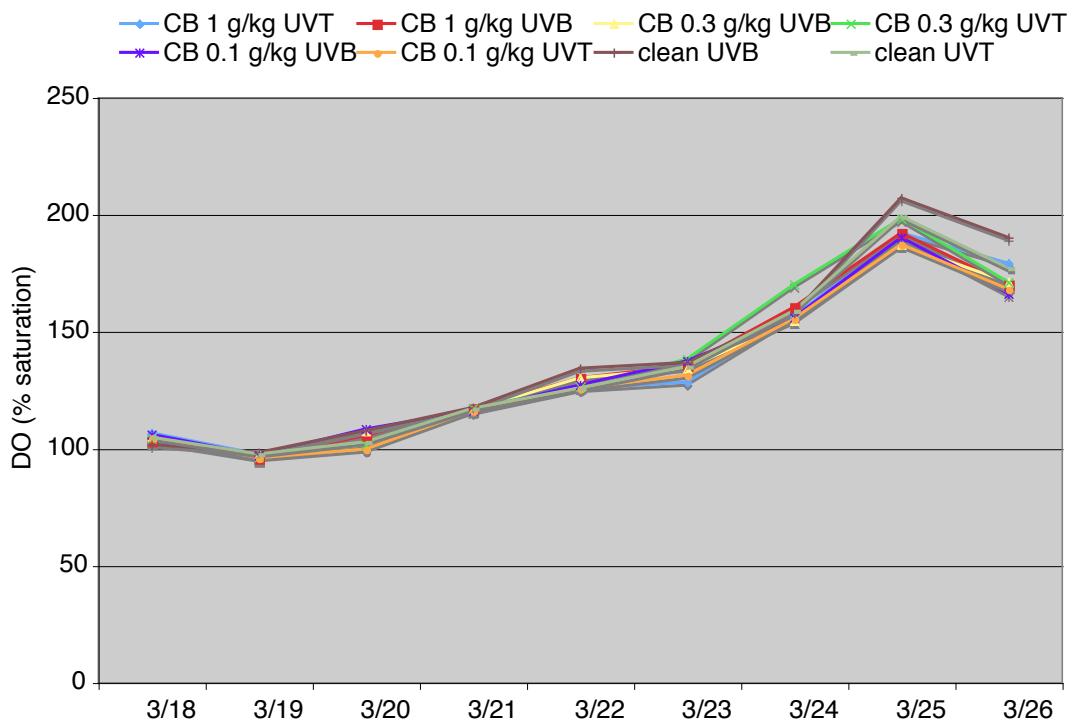


Figure 18: Trial 4 daily dissolved oxygen levels in one replicate of each treatment

treatments with respect to temperature, dissolved oxygen, and pH. Throughout this trial ammonia was undetected or at background levels.

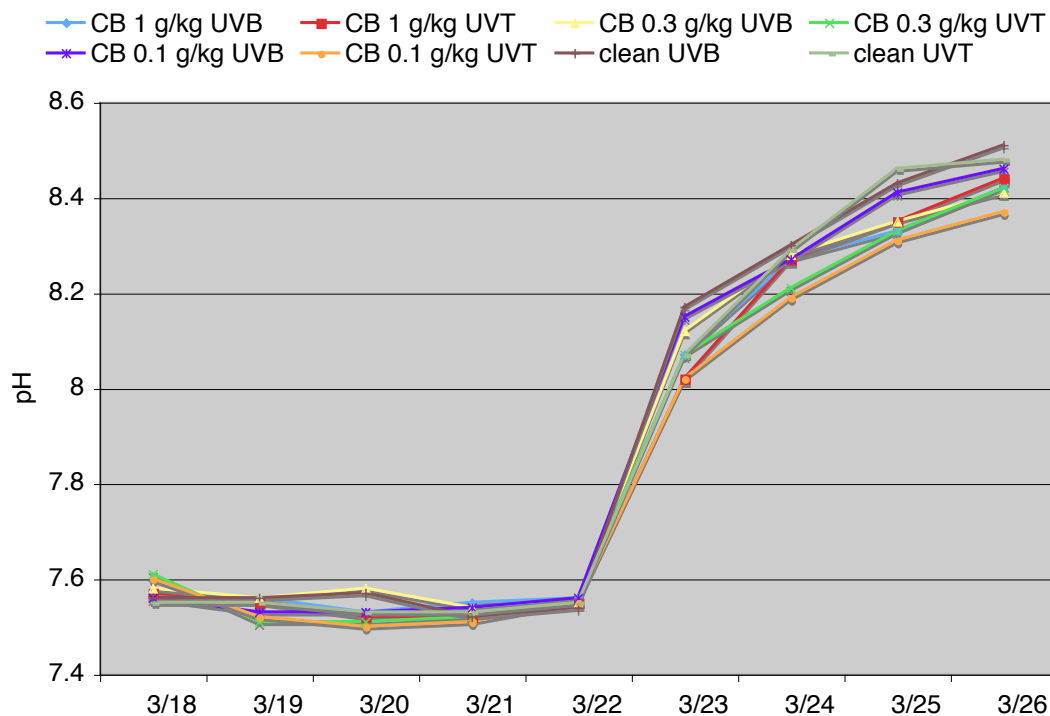


Figure 19: Trial 4 daily pH in one replicate of each treatment

Trials 1-4, Assessment of hatching and larval morphology in the Laboratory Exposure and Phototoxicity Study

These data do not directly address the Specific Aims of the Laboratory Exposure and Phototoxicity Study as described in the Introduction. The primary focus of this study was to determine whether Cosco Busan bunker oil could produce lethal embryonic necrosis. Data on larval hatching rates and morphology are provided as supplemental information in Appendix 2 of this report.

Part 2. San Francisco Bay Field Collection of Spawned Herring Eggs

A. Previously Oiled or Reference Marin County Shoreline Areas

No spawning was observed in the intertidal zones of previously oiled sites.

B. Paradise Cove: Paradise Cove was considered an additional reference site which did not have visible oiling in 2007. Samples of natural spawn were collected 28 January 2009. The same approach was used as in 2007-08, with collection of 8 samples along a transect, and analysis of at least 25 embryos per sample. Collection of these samples deviated from the SOP established for spawn sampling during 2007-08. The spawning habitat at Paradise Cove was unlike sites sampled last year, with little spawning on intertidal algae. Most of the spawn was higher in the intertidal on rocks and seawall. The samples were collected from locations several feet higher than directed in the SOP (see image below).



Location of Paradise Cove spawn samples on seawall.

Embryo results: Many of the samples had high numbers of embryos that died early (segmentation stages), and some were discolored with a rust-colored pigment. A mortality rate for each of the 8 samples was determined by counting 100 random eggs. Dechorionated embryos were imaged at low magnification, and any abnormalities documented at high magnification. Any abnormalities were documented on a phenotypic checklist. Dechorionated embryos were fixed in 4% paraformaldehyde and placed in storage. Results are shown in Table 1. Only the types of defects observed are listed in the table, i.e. unaffected structures on the phenotypic checklist are not listed in Table 2-1.

Table 2-1: Abnormalities in embryos

sample	% early mort	embryo N	% edema	% yolk opacity	% body axis curved
N1	68	55	7	5	5
N2	23	51	12	0	0
N3	51	48	4	4	0
N4	18	56	4	4	0
N5	12	59	0	5	8
N6	21	26	0	0	0
N7	5	59	0	7	0
N8	24	51	2	4	0

Hatching Results: A subset of approximately 100 embryos from each grab sample (N1-N8) were removed from substrates (rocks, algae) and placed into 250 ml finger bowls containing 150 ml $\frac{1}{2}$ FSW and incubated at 12°C with daily water changes. At commencement of hatching, cultures were counted on a daily basis until all viable embryos had hatched. Counts consisted of unhatched eyed embryos, non-viable embryos (unfertilized and dead), partial hatched embryos, hatched larvae live, hatched larvae normal, and hatched larvae abnormal. Morphology was evaluated for the following: scoliosis, edema, opaque yolk or head, kinked tails, bent heads, jaw abnormalities, and motor abnormalities (twitching or sluggish behavior). Percent total hatch was calculated as hatched live a percentage of the total number embryos/larvae (the sum of eyed unhatched, non-viable, partial hatched, hatched larvae dead, and hatched larvae live). Percent normal hatch was calculated as hatched larvae normal as a percentage of total embryos/larvae (the sum of eyed unhatched, non-viable, partial hatched, hatched larvae dead, hatched larvae live).

Total hatching success (percent of live embryos hatched relative to total number of embryos) ranged from 64% (N2) to 98% (N4) with a mean of 83 +/- 7% (Fig. 2-1). Percent normal hatch (percent of normal hatched embryos relative to total number of embryos) ranged from 60% (N7) to 95% (N4) with a mean of 77 +/- 7%. Scoliosis was the predominant morphological abnormality observed (6 +/- 2%) (Table 2-2), while other abnormalities (edema, opaque/vacuolated yolks, opaque heads) were observed in less than 2.1% of embryos (Fig. 2-2).

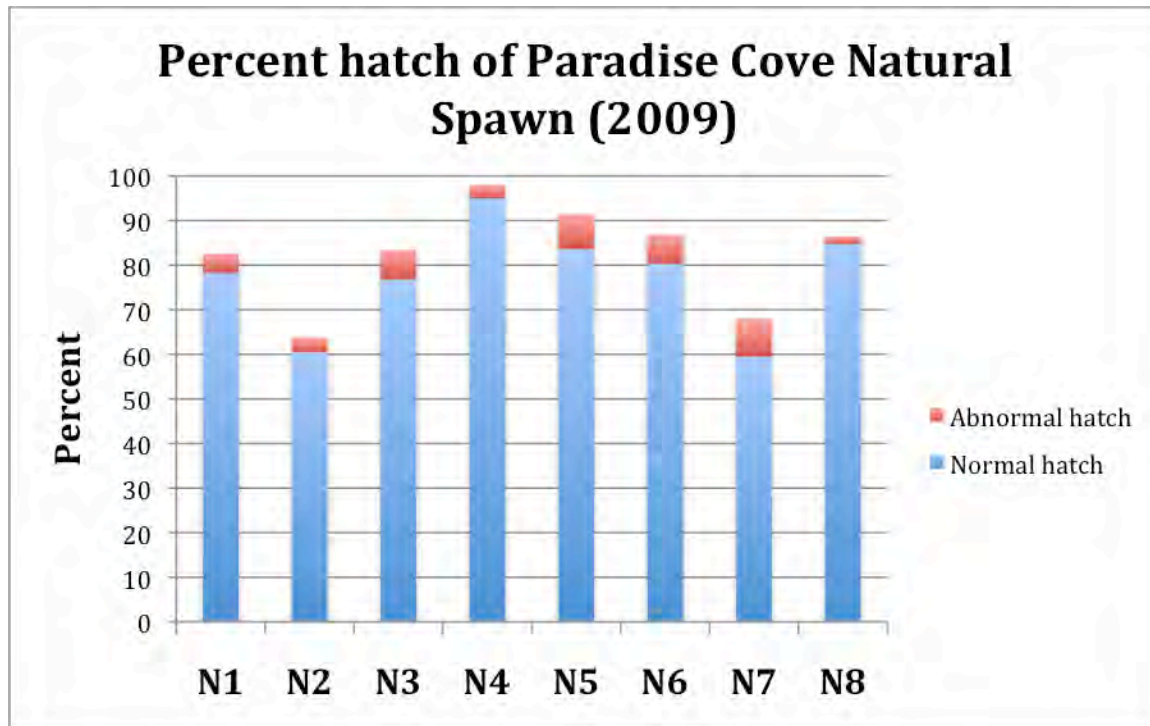


Figure 2-1: Percent hatch of Paradise Cove naturally spawned embryos. Total hatch consists of live normal (blue bars) and abnormal larvae (red bars) as a percent of total numbers of embryos.

Table 2-2: Morphological abnormalities observed in herring larvae from individual grab samples collected at Paradise Cove (percent of live hatch).

Grab #	Edema	Opaque yolk	Opaque head	Scoliosis	Jaw
N1	1.3	1.3	0	2.6	1.3
N2	1.4	5.6	1.4	12.5	0
N3	0	0	0	6.1	0
N4	0	1.0	0	2.0	0
N5	0	0.9	0	7.7	0
N6	0	1.9	0.9	6.6	0
N7	0	1.3	0	7.8	1.3
N8	0	0	0	5	0

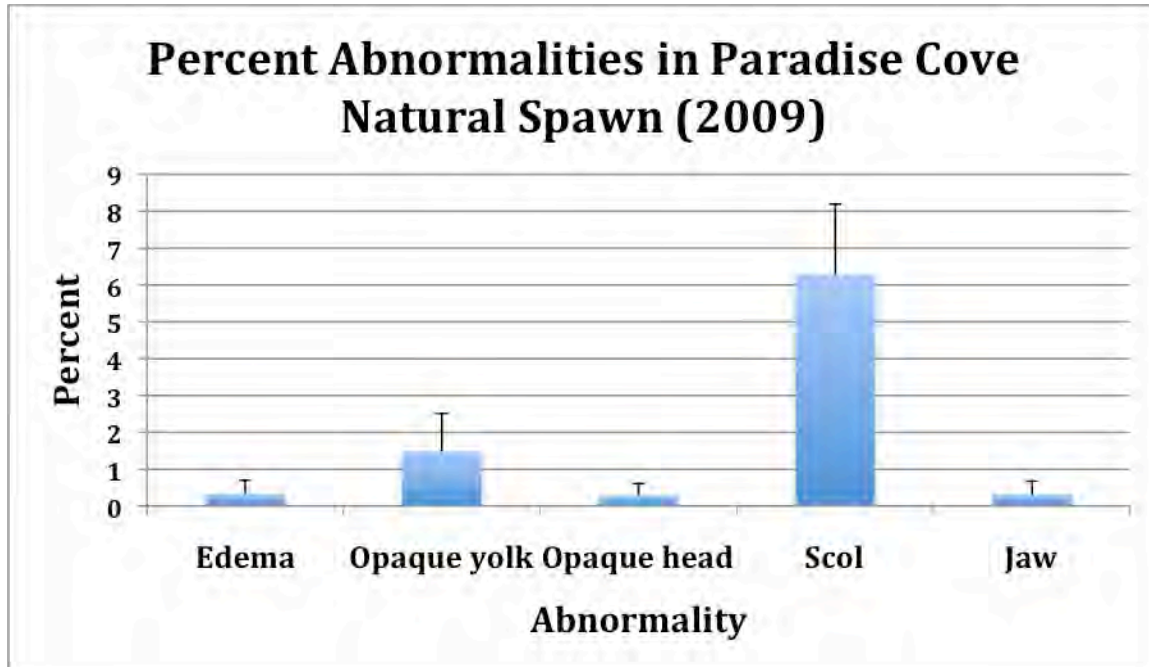


Figure 2-2: Average morphological abnormalities in herring larvae from Paradise Cove natural spawn (N1-N8). Scoliosis was observed in 6.3% of larvae, while other abnormalities observed occurred at lower levels (mean percent \pm SE).

Data files:

- CBOS external drive: Hatch data:2009:MRS-003b-FEM2 natural spawn 09.xlsx
- New MRS-003b-FEM2 natural spawn 09_v2.xls
- CBOS external drive:CBOS:CBOS images:Paradise natural spawn 1-29-09

C. Deployment of Temperature and Salinity Data Loggers

Continuous temperature and salinity recorders were deployed at five sites in order to assess these conditions during any potential incubation of natural spawn during the 2008-09 spawning season. The sites included three oiled sites from the 2007-08 study, Sausalito, Peninusal Point, and Keil Cove; and two reference sites, San Rafael Bay and Paradise Cove. The logger at Paradise Cove broke from its mooring and was found 9 Feb 2009, and brought ashore. Excel workbooks containing the data for each logger are found in the folders "Field Sampling 2009" > "Field temp and salinity".

Appendix 1: Laboratory Salinity Study

This study tested alternative hypotheses whether (1) high salinity could produce embryonic lethality such as that observed at intertidal zones of oiled sites in the 2007-08 spawning season, and (2) whether embryos fertilized and incubated at high salinity would show pericardial edema when dechorionated at low salinity (i.e. 16 ppt), as a potential explanation for signs of edema in subtidal caged embryos at oiled sites in 2007-08.

Fertilizations were carried out at the Bodega Marine Lab on March 5, 2009 according to the study plan. Duplicate slides were received at NWFSC from BML on 3/13/09 (8 dpf), along with BML 16 ppt seawater for processing. All slides were examined upon receipt and showed similar numbers of viable eyed embryos. Due to time constraints, the most relevant treatments were selected for dechorionation, i.e. optimal laboratory salinity regimen (fertilized at 16 ppt, incubated at 16 ppt) and high salinity regimen (fertilized at 30 ppt, incubated at 30 ppt). For dechorionation, slides were transferred to 16 ppt seawater and held at 12°C on a cooling stage. At least 20 embryos were dechorionated at 16 ppt from a single slide for each treatment, and held at 16 ppt for imaging.

At BML after 7 days of incubation, the remaining slides were transferred to 16 ppt salinity and incubated at 12°C with daily water changes of 16 ppt seawater. At commencement of hatching, the remaining embryos and larvae were evaluated as described previously.

Embryo Results:

A. Fertilized at 16 ppt, incubated at 16 ppt, dechorionated and imaged at 16 ppt
23/23 embryos between Hill and Johnston (1997) stages *o* and *p*. Edema present in 0/23 embryos. Body axis defects present in 0/23 embryos. Pericardial space larger than embryos from high salinity regimen (see below), but this is due to more advanced head rotation. Representative samples shown in Fig. 19, top.

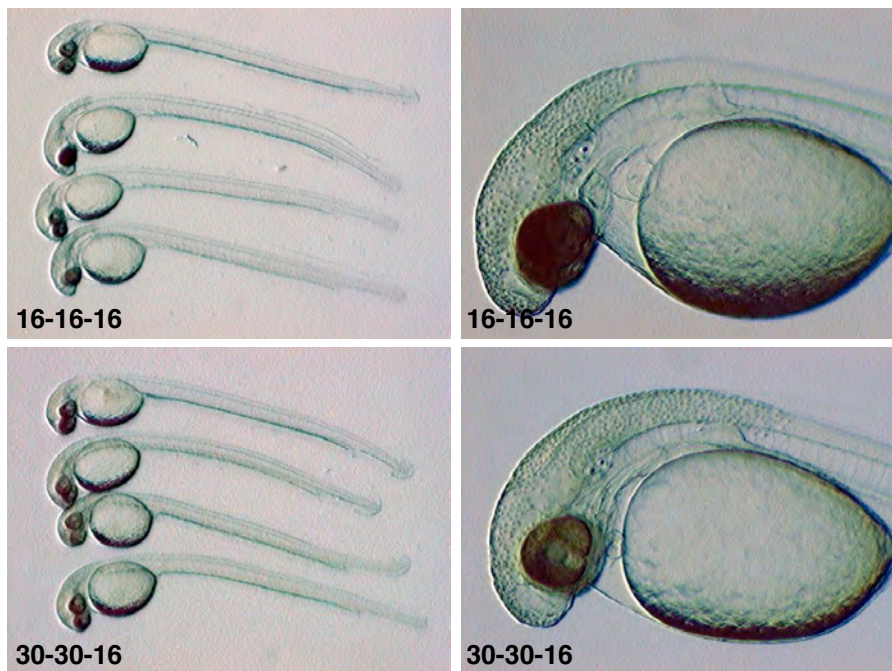


Figure 19: Morphology of embryos exposed to optimal salinity (16 ppt, top) or high salinity (32 ppt, bottom).

B. Fertilized at 30 ppt, incubated at 30 ppt, dechorionated and imaged at 16 ppt. 26/26 embryos between stages o and p, but slightly delayed relative to optimal regimen. This was evident in the degree of head rotation, eye pigmentation, and length of tail bud. Edema present in 0/26 embryos. Body axis defects present in 0/26 embryos. Representative samples shown in Fig. 19, bottom.

Hatching Results:

Cumulative daily hatch rates were collected for all tested salinity regimens: Fertilized at 16 ppt and incubated at 16 ppt (16-16), fertilized at 16 ppt and incubated at 22 ppt (16-22), fertilized at 22 ppt and incubated at 22 ppt (22-22), fertilized at 16 ppt and incubated at 30 ppt (16-30), and fertilized at 30 ppt and incubated at 30 ppt (30-30). Data (Fig. 20) are reported as percent normal hatch, that is the number of hatched larvae with normal morphology divided by the total number of embryos/larvae (unhatched embryos, dead hatched and live hatched). Total and normal hatching rates were observed to have the following trend: total hatching rates for embryos fertilized and incubated in 16 ppt > embryos fertilized in 16 ppt and incubated in 22 ppt > embryos fertilized in 22 ppt and incubated in 22 ppt > embryos fertilized in 30 ppt and incubated in 30 ppt > embryos fertilized in 16 ppt and incubated in 30 ppt (Fig. 20). The same trend was observed for normal hatching, except that the hatching rate for embryos fertilized in 16 ppt and incubated in 30 ppt was greater than that for embryos fertilized in 30 ppt and incubated in 30 ppt.

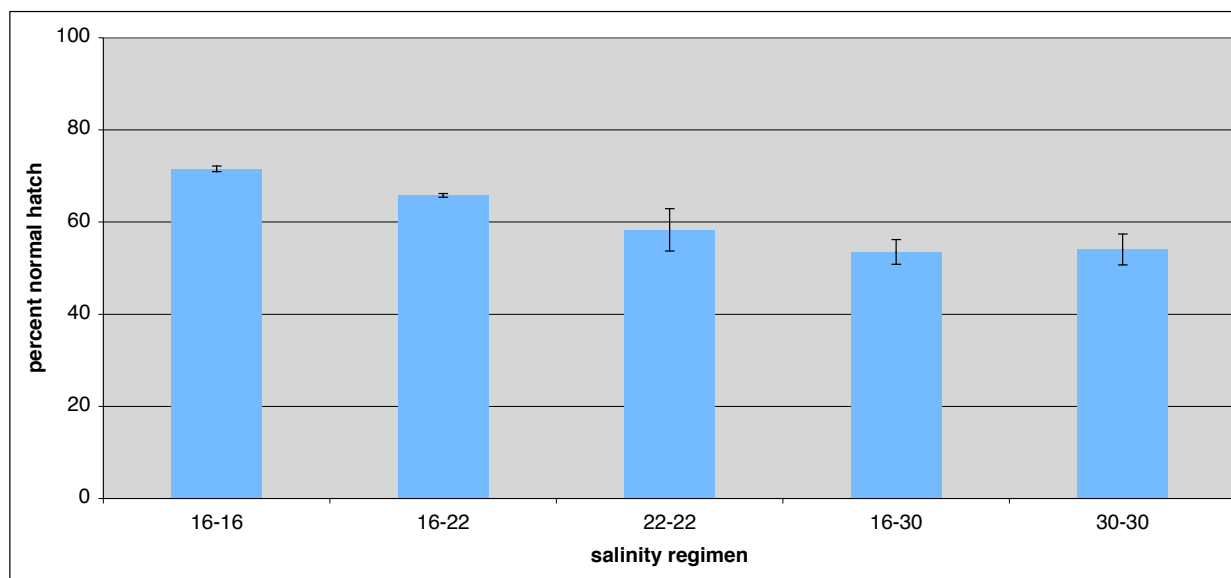


Figure 20: Normal hatch rates after fertilization and incubation under various salinity regimens. Data are mean ± SE for two replicates.

Morphological abnormalities were observed in all treatments (Fig. 21). The incidence of edema was highest for embryos fertilized and incubated in 16 ppt seawater (3.08 ± 2.57), and lowest for embryos fertilized and incubated in 22 ppt (0.97 ± 0.31). In general, the incidence of other morphological abnormalities observed (opaque yolk sac, bent heads, scoliosis, and jaw abnormalities) was similar for all treatments except for embryos fertilized and incubated in 30 ppt. Yolk opacity, bent heads, and scoliosis were higher in this treatment. Jaw abnormalities were highest in the embryos fertilized in 16 ppt and incubated at 22 ppt, and were lowest in the 16-30 and 30-30 treatment groups.

Morphological Abnormalities and Salinity

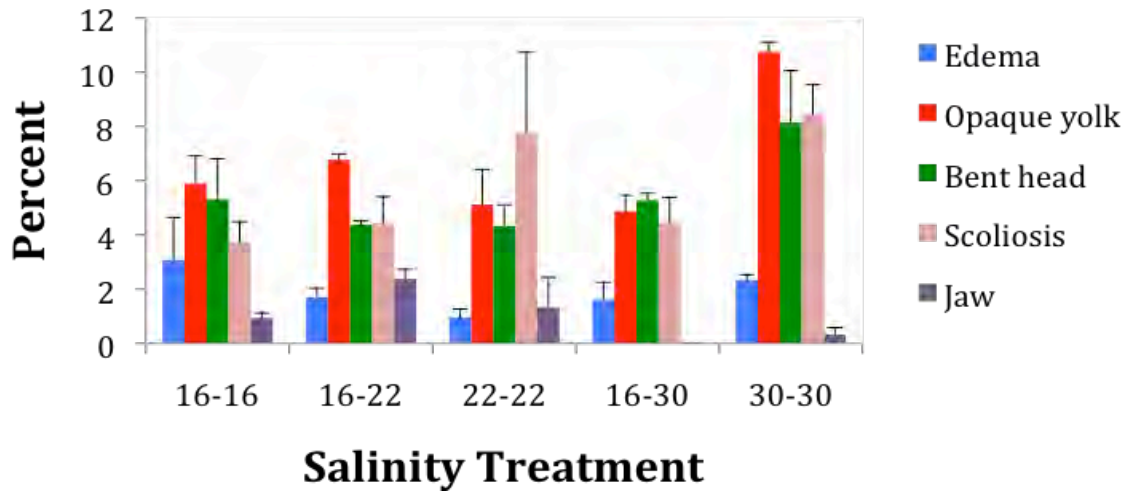


Figure 21: Morphological abnormalities in herring larvae fertilized at 16, 22, or 30 ppt and incubated at 16, 22 or 30 ppt. (Means +/- SE)

Summary of Data and Samples Collected

Trial 1 (Preliminary)

Sample Code Key: N/A

Egg Source Information: CBOS09 fem wts.xls, fertilization_tests.pdf (in folder “Data files and lab notes”)

Experimental Conditions: column temp log Jan-Mar09.xls, ColumnWQJan14toFeb23.pdf (in folder “Data files and lab notes”)

Embryo Images: in folders “Data files and lab notes”>”Trial 1”> “5 dpf”, “6 dpf”, and “8 dpf”

Trial 2 (Aborted)

Sample Code Key: N/A

Egg Source Information: CBOS09 fem wts.xls, fertilization_tests.pdf (in folder “Data files and lab notes”)

Experimental Conditions: column temp log Jan-Mar09.xls, ColumnWQJan14toFeb23.pdf (in folder “Data files and lab notes”)

Embryo Images and scoring: in folders “Data files and lab notes”>”Trial 2”; “Data files and lab notes”> Embryo Scores Final.xls (Trial 2 tab)

Trial 3

Sample Code Key: “Data files and lab notes”>”Trial 3”>Trial 3 key to egg chemistry.doc

Egg Source Information: CBOS09 fem wts.xls, fertilization_tests.pdf (in folder “Data files and lab notes”)

Experimental Conditions: column temp log Jan-Mar09.xls, WaterQuality022609.xls (in folder “Data files and lab notes”)

Embryo Images and Scoring Results: in folders “Data files and lab notes”>”Trial 3”; “Data files and lab notes”> Embryo Scores Final.xls (Trial 3 tab)

Water Sample PAH Analysis: in folders “Data files and lab notes”>”Trial 3”>Trial 3 water PAH.xls

Eggs Sample PAH Analysis: in folders “Data files and lab notes”>”Trial 3”>Trial 3 tissue PAH.xls

Trial 4

Sample Code Key: “Data files and lab notes”>”Trial 4”>Trial 4 key to egg chemistry.doc

Egg Source Information: CBOS09 fem wts.xls, fertilization_tests.pdf (in folder “Data files and lab notes”)

Experimental Conditions: column temp log 031809.xls, WaterQuality031809.xls (in folder “Data files and lab notes”)

Embryo Images and Scoring Results: in folders “Data files and lab notes”>”Trial 4”; “Data files and lab notes”> Embryo Scores Final.xls (Trial 4 tab)

Water Sample PAH Analysis: in folders “Data files and lab notes”>”Trial 4”>Trial 4 water PAH.xls

Eggs Sample PAH Analysis: in folders “Data files and lab notes”>”Trial 4”>Trial 4 tissue PAH.xls

Salinity Study

Egg Source Information: CBOS09 fem wts.xls, fertilization_tests.pdf (in folder “Data files and lab notes”)

Experimental Conditions: in folder “Work plans and SOPs”>CBOS Salinity Experiments.doc

Embryo Images and Scoring Results: in folders “2008-09 Salinity study”>”16-16-16 ppt” and “30-30-16 ppt”, data files CBOS Salinity Study embryo results.doc and CBOS salinity hatch data 09.xls

Appendix 2: Assessment of hatching and larval morphology in the Laboratory Exposure and Phototoxicity Study

Data files:

- CBOS external drive:Hatch data:2009:1-23-09 larval hatch.xlsx and 1-23-09 larval hatchv_2.xlsx
- CBOS external drive:Hatch data:2009:2-26-09 larval hatch.xlsx and 2-26-09 larval hatchv_2.xlsx
- CBOS external drive:Hatch data:2009:2-13-09 CBOS larval hatch.xlsx
- CBOS external drive:Hatch data:2009:3-18-09 larval hatch.xlsx and 3-18-09 larval hatchv_2.xlsx
- CBOS external drive:CBOS:CBOS images:Columns:1-23-09 exp
- CBOS external drive:CBOS:CBOS images:Columns:2-13-09 exp
- CBOS external drive:CBOS:CBOS images:Columns:2-26-09 exp
- CBOS external drive:CBOS:CBOS images:Columns:3-18-09 exp
- CBOS external drive:CBOS:CBOS images:Columns:Image key.xlsx

Results

Trial 1, January 22-31

This trial was designed to investigate the effects of Cosco Busan bunker oil in oiled gravel columns on development of herring embryos and compared to embryos exposed to Alaska North Slope Crude Oil, clean gravel, and gravel obtained from an urban site. The 1st trial was a dry run to test the gravel column system to insure that appropriate responses were observed in herring embryos, and to determine what, if any, adjustments were necessary to adequately assess embryonic development. Initially, hatched larvae were individually imaged following counting, then fixed in 4% PF in PBS. This procedure worked well when few embryos had hatched, but proved to be very labor-intensive when large numbers of embryos had hatched. Thereafter, representative images of larvae were collected. In addition, the low numbers of live larvae in the first few days of hatching allowed observation of larvae for abnormal motor activity and cardiac function. Increased numbers of hatched larvae in subsequent days resulted in an increase in the time needed to assess morphology, such that cultures were not counted daily, and larvae were transferred to bowls for anesthesia in MS-222 prior to evaluation for motor activity.

Total hatching rates were highest for embryos exposed to clean gravel with little difference between UVB ($95 \pm 3\%$) or UVT ($93 \pm 2\%$) treatments (Fig. 1). Embryos exposed to urban gravel had slightly lower total hatching rates, again with little difference between UVB ($88 \pm 5\%$) or UVT ($89 \pm 1\%$) exposure. Total hatch rates for embryos exposed to ANS 0.1, 0.3, and 1.0 and UVB were similar to clean or urban gravel ($92 \pm 2\%$, $88 \pm 6\%$ and $91 \pm 4\%$ respectively). For ANS 0.1, UVT exposure resulted in comparable total hatching to UVB ($91 \pm 1\%$ vs 92%), while exposure to UVT and ANS 0.3 and 1.0 resulted in lower total hatch rates ($80 \pm 8\%$ and $66 \pm 6\%$ respectively). Total hatch rates for CBO 0.1 UVB and UVT were similar to that for urban gravel ($85 \pm 5\%$ and $87 \pm 0.4\%$ respectively). Total hatch rates for UVT exposure and CBO 0.3 and 1.0 were much lower than for the UVB exposed embryos ($94 \pm 3\%$ vs $44 \pm 13\%$ for CBO 0.3, and $66 \pm 15\%$ vs $1 \pm 0.5\%$ for CBO 1.0).

Normal hatch rates were also highest for clean gravel UVB ($91 \pm 4\%$) and UVT ($88 \pm 0.6\%$). Urban gravel exposure resulted in slightly lower normal hatch rates whether exposed to UVB ($79 \pm 5\%$) or UVT ($79 \pm 4\%$). Normal hatch rates for embryos exposed to ANS 0.1 and ANS 0.3 were lower than for the clean gravel, but no difference between UVB or UVT exposure was observed ($82 \pm 2\%$ for both ANS 0.1 UVB and UVT; $61 \pm 6\%$ and $58 \pm 7\%$ for ANS 0.3 UVB and UVT respectively). Embryos exposed to ANS 1.0 had lower normal hatch rates in both UVB and UVT treatments ($28 \pm 12\%$ and $10\% \pm 2\%$ respectively). For all three CBO treatments, normal hatch rates were lower than the clean and urban gravel, and all three treatments had lower hatch rates for UVT vs UVB exposed embryos; CBO 0.1 UVB ($74 \pm 4\%$) > CBO 0.1 UVT ($58 \pm 0.4\%$); CBO 0.3 UVB ($23 \pm 10\%$) > CBO 0.3 UVT ($7 \pm 3\%$); and CBO 1.0 UVB ($3 \pm 2\%$) > CBO 1.0 UVT (0%). No normal larvae hatched in the CBO 1.0 UVT treatment.

Observation of morphological abnormalities in herring larvae is summarized in Table 2. Edema (Figure 2B,C) was the abnormality most observed, ranging from 0% in clean gravel (UVB) to 75% in CBO 1.0 (UVB). High rates of scoliosis (Fig 2B) were also observed, ranging from 3% in clean gravel (UVB) to 72% in CBO 1.0 (UVB). Jaw abnormalities (Fig 2B,C) and opaque yolks were also frequently observed (Fig 2B,C), jaw abnormalities ranging from 0.5% in clean gravel (UVB) to 58% in ANS 1.0 (UVT) and opaque yolks ranging from 1% in clean gravel (UVB) to 41% in CBO 0.3 (UVT). Less frequently observed abnormalities included opaque heads (Fig 2C) (0 % in ANS 0.1 UVB and UVT and ANS 1.0 UVB to 4% in CBO 1.0 UVB) and bent heads (Fig 2D) (0% in ANS 0.1 and 1.0 UVB to 17% in CBO 0.3 UVB). In general, the incidence of abnormalities was higher in UVT treatments, however, in some cases, rates were similar or less than those for UVB treatments (Figs. 3-5). A dose response was observed for ANS and CB gravel under UVB and UVT exposure (Figs. 3-5).

Figure 1: Total and normal hatching of herring embryos exposed to gravel coated with Alaska North Slope Crude Oil (ANS), Cosco Busan bunker oil (CB), clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 1). Blue (UVB) or white (UVT) bars represent normal hatching, and red bars represent abnormal hatching (means \pm SE). Total hatch rates are represented by the combination of blue or white bars with corresponding red bars.

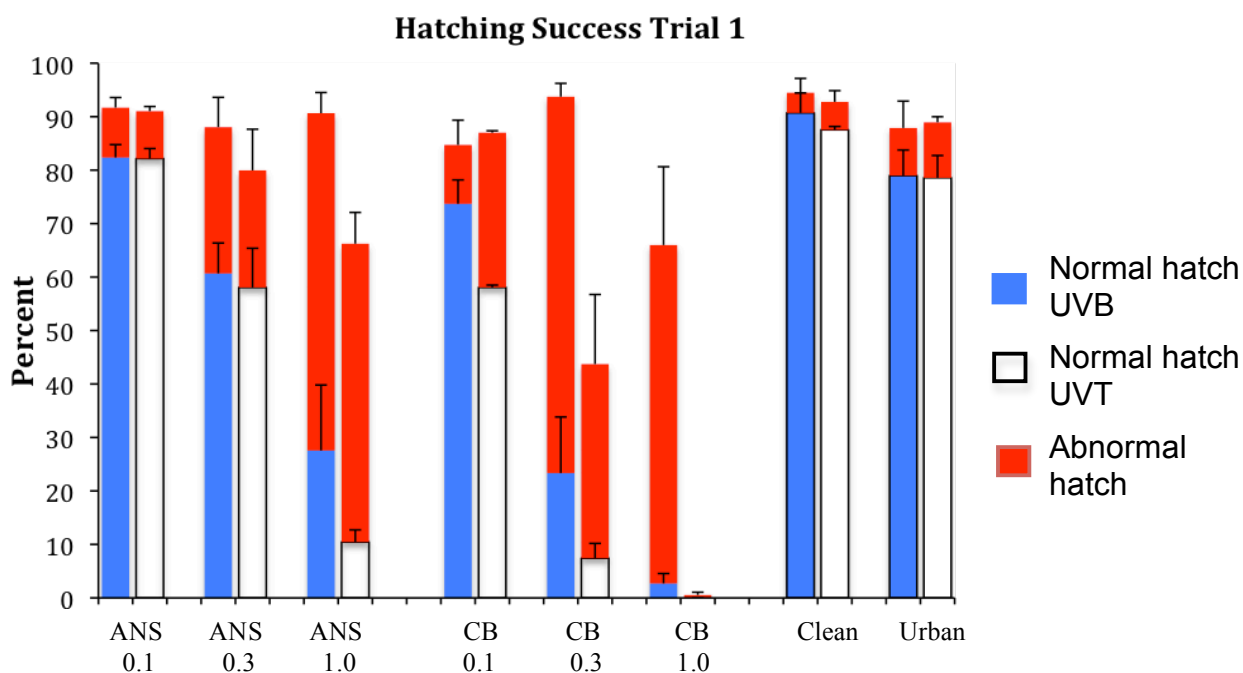
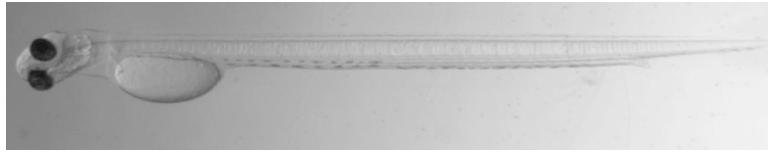
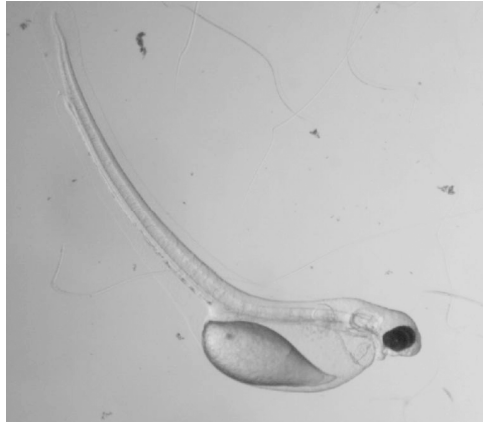


Table 2	Type of Abnormality	Scoliosis	Edema	Opaque head	Bent head	Opaque yolk	Jaw
Treatment	UV exposure	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Clean	UVB	3.3 \pm 1.4	0	2.0 \pm 1.3	0.5 \pm 0.5	1.0 \pm 0.5	0.5 \pm 0.5
	UVT	4.2 \pm 2.3	0.5 \pm 0.5	1.1 \pm 1.1	0.5 \pm 0.5	1.6 \pm 1.6	1.4 \pm 0.7
Urban	UVB	6.3 \pm 1.3	1.3 \pm 0.7	2.4 \pm 0.6	3.0 \pm 0.5	1.5 \pm 1.1	0.9 \pm 0.5
	UVT	3.4 \pm 1.0	2.3 \pm 1.5	0.8 \pm 0.8	6.1 \pm 4.6	3.6 \pm 2.2	2.5 \pm 0.4
ANS 0.1	UVB	6.6 \pm 0.5	5.3 \pm 1.8	0.0 \pm 0.0	0.0 \pm 0.0	0.7 \pm 0.7	4.4 \pm 2.5
	UVT	7.2 \pm 2.7	5.8 \pm 3.3	0.0 \pm 0.0	1.5 \pm 1.5	1.5 \pm 1.5	4.7 \pm 1.2
ANS 0.3	UVB	10.1 \pm 3.5	21.5 \pm 7.5	0.6 \pm 0.6	7.4 \pm 3.8	6.2 \pm 1.6	16.5 \pm 7.1
	UVT	12.0 \pm 0.5	15.3 \pm 3.1	1.6 \pm 0.9	6.0 \pm 3.3	2.9 \pm 1.5	11.7 \pm 5.6
ANS 1.0	UVB	34.6 \pm 9.3	64.2 \pm 15.7	0.0 \pm 0.0	0.0 \pm 0.0	2.9 \pm 2.9	52.2 \pm 17.2
	UVT	68.8 \pm 5.1	62.5 \pm 7.1	3.4 \pm 3.4	3.4 \pm 3.4	18.4 \pm 3.2	57.6 \pm 2.0
CB 0.1	UVB	6.0 \pm 2.2	6.3 \pm 1.1	0.6 \pm 0.6	3.7 \pm 2.5	2.0 \pm 1.0	4.4 \pm 1.3
	UVT	19.7 \pm 3.9	19.6 \pm 3.6	2.2 \pm 2.2	6.5 \pm 2.7	13.3 \pm 5.3	15.7 \pm 2.1
CB 0.3	UVB	34.0 \pm 12.0	65.8 \pm 12.3	1.1 \pm 1.1	16.8 \pm 9.5	35.0 \pm 17.4	56.2 \pm 18.3
	UVT	58.2 \pm 4.3	56.0 \pm 5.3	1.4 \pm 1.4	6.1 \pm 3.1	41.1 \pm 11.9	54.6 \pm 4.6
CB 1.0	UVB	71.6 \pm 10.7	75.2 \pm 9.5	3.8 \pm 2.1	6.9 \pm 6.9	40.1 \pm 3.1	56.5 \pm 5.4
	UVT	100*	100*	0*	0*	0*	100*

Table 2: Incidence of morphological abnormalities in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 1). Numbers in red indicate highest percent for each abnormality. *n = 1 live hatchling out of 168 embryos



2A



2B



2C



3D

Figure 2: Morphology of herring larvae. A) Normal herring larva. B) Herring larva exhibiting scoliosis, edema, yolk opacity, and abnormal jaw. C) Herring larve exhibiting opaque head, edema and yolk opacity. D) Herring larvae exhibiting bent head.

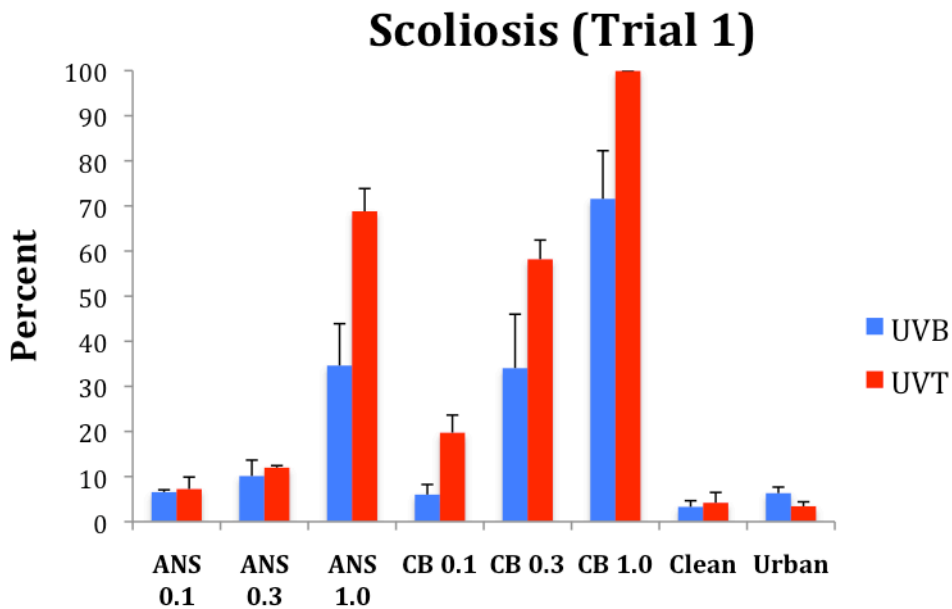


Figure 3: Percent scoliosis in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (means + SE).

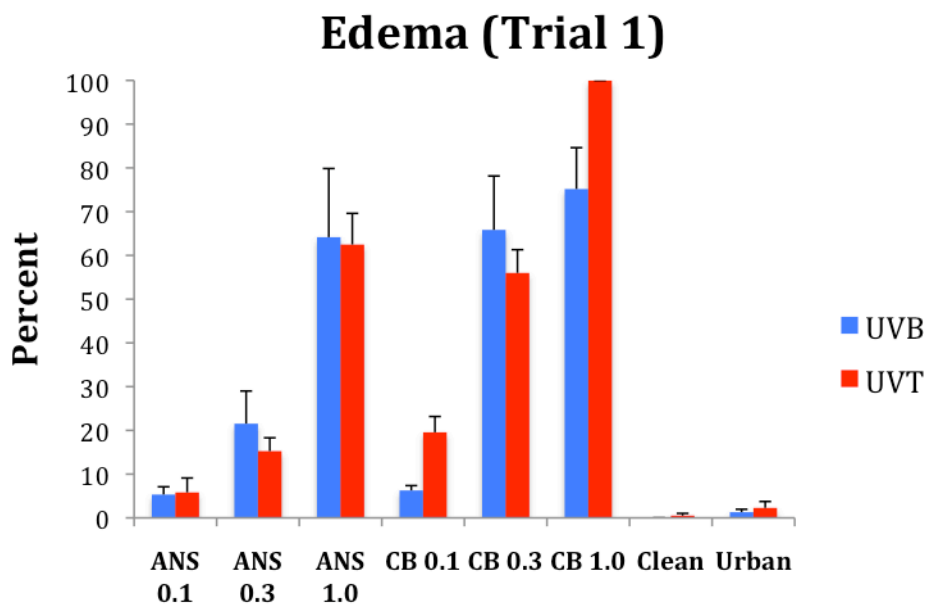


Figure 4: Percent edema in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (means + SE).

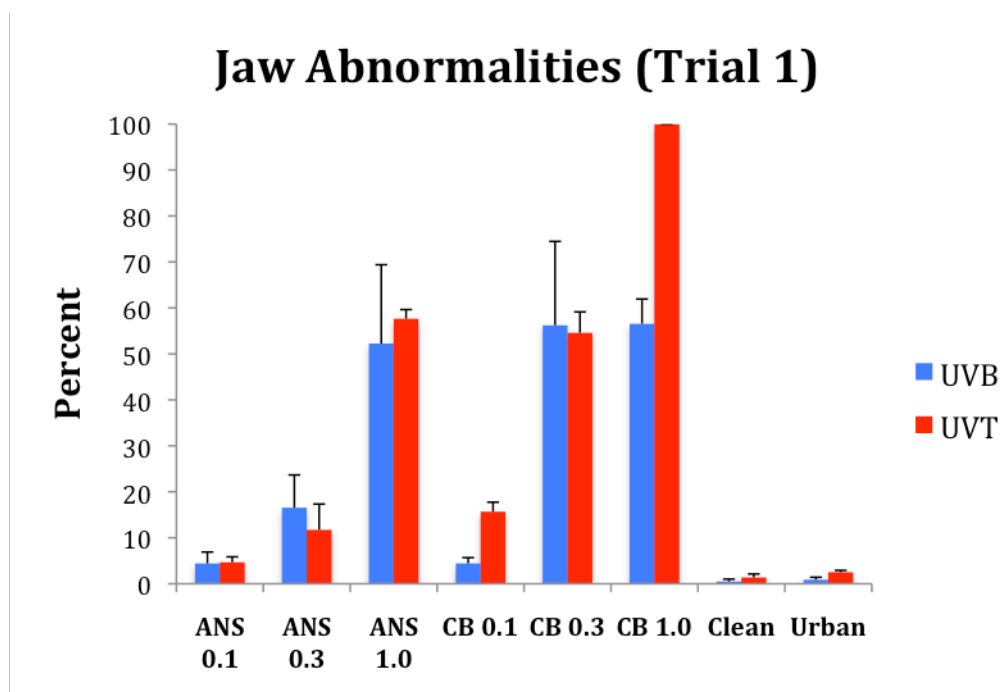


Figure 5: Percent jaw abnormalities in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (means + SE).

Trial 2, February 13-22, 2009

This trial was aborted due to low normal development in the controls. However, one slide for each treatment was analyzed as for the other trials.

Hatching success in the clean gravel was similar between UVB and UVT exposures, with 55% total hatch for UVB and 58.3% total hatch for UVT (Fig. 6). Normal hatch rates were lower for the UVT treatment (55% normal for UVB vs 45.6% normal for UVT). Hatch rates for the urban gravel were lower than for the clean gravel, with a difference between UVB and UVT exposure. Total hatch rates for UVB and UVT urban exposures were 32.8% and 22.2% respectively, while normal hatch rates were reduced to 24.6% and 14.8% respectively. No dose response was observed for total hatch rates for the ANS or CB exposures under UVB exposures, and in fact the highest total hatch rate for all treatments occurred with the ANS 1.0 UVB exposure (67.5%) > ANS 0.1 (23.2%) > ANS 0.3 (6.4%). A similar pattern was seen for CB UVB exposure with CB 1.0 (65.3%) > CB 0.1 (52.1%) > CB 0.3 (50.0%). Normal hatch rates showed the same pattern as for total hatch, with ANS 1.0 (37.3%) > ANS 0.1 (11.6%) > ANS 0.3 (2.1%) and CB 1.0 (41.1%) > CB 0.1 (35.2%) > CB 0.3 (18.4%). Hatching rates for UVT exposures were all much lower than for UVB exposures, and in contrast to UVB exposure, a dose response was observed for normal hatch success in both ANS and CB treatments, and for total hatching in the CB treatments. Total hatch rates for CB and ANS treatments were <18% and normal hatch rates were all <7%. No embryos hatched live from the CB 1.0 UVT exposure.

Morphological abnormalities were observed in all treatments and are summarized in Table 3. Cumulative abnormalities were lowest for clean UVB exposure (4.6%) and highest for CB 0.1 UVT (166.7%). Edema was the most common abnormality observed, ranging from 0% (urban UVT, ANS 0.3 UVB) to 44.7% (CB 0.3 UVB). The incidence of yolk opacity was also quite high, ranging from 0% (clean UVB, ANS 0.3 UVT) to 50% (CB 0.3 UVB). Bent heads were lowest (0%) in clean UVB, ANS 0.3 UVB, and CB 0.3 UVT, and highest (30%) in ANS 0.1 UVT. The incidence of scoliosis ranged from 0% (clean UVB, urban UVT, ANS 1.0 UVT) to 44.4% (CB 0.1 UVT). Jaw abnormalities were observed less often, ranging from 0% (clean UVB, urban UVB and UVT, ANS 0.1 UVB, ANS 0.3 UVB, ANS 1.0 UVT), to 50% in CB 0.3 UVT.

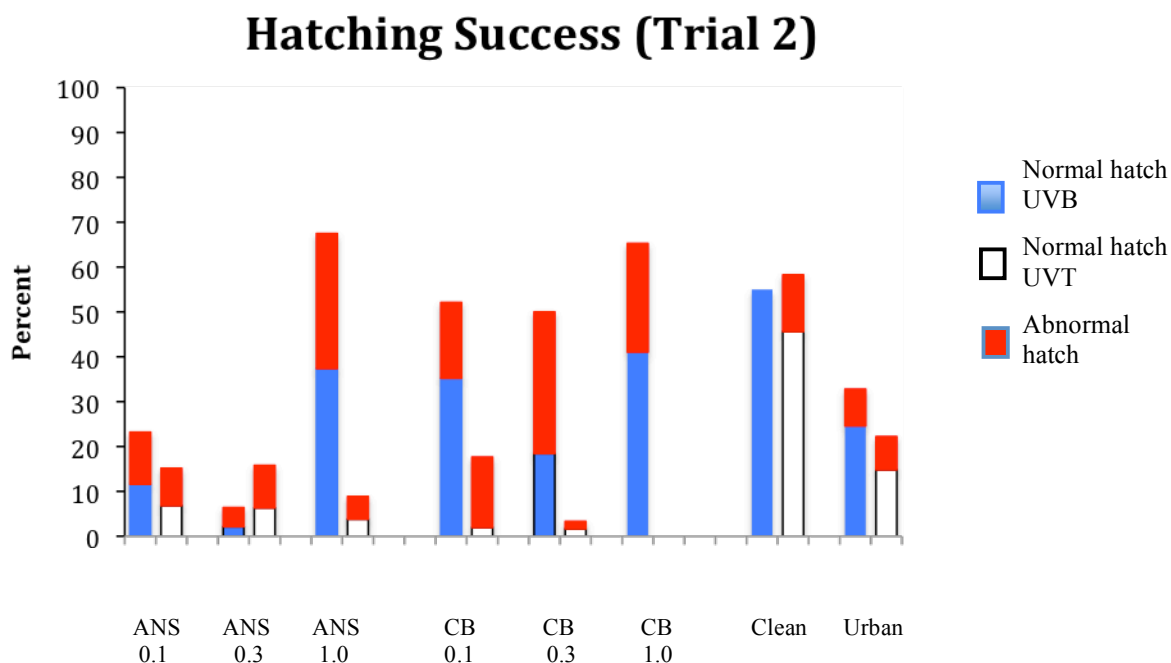


Figure 6: Total and normal hatching of herring embryos exposed to gravel coated with Alaska North Slope Crude Oil (ANS), Cosco Busan bunker oil (CB), clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 2). Blue (UVB) or white (UVT) bars represent normal hatching, and red bars represent abnormal hatching. Total hatch rates are represented by the combination of blue or white bars with corresponding red bars. Note: no embryos hatched from the CB 1.0 UVT treatment.

Table 3

	Type of Abnormality	Scoliosis	Edema	Bent head	Opaque yolk	Jaw	Cumulative
Treatment	UV exposure	Mean	Mean	Mean	Mean	Mean	
Clean	UVB	0	4.6	0	0	0	4.6
	UVT	10.0	5.0	10.0	8.3	3.3	36.7
Urban	UVB	5.0	5.0	10.0	5.0	0	25.0
	UVT	0	0	16.7	16.7	0	33.3
ANS 0.1	UVB	18.8	25.0	18.8	18.8	0	81.3
	UVT	5.0	35.0	30.0	20.0	20.0	110.0
ANS 0.3	UVB	33.3	0	0	33.3	0	66.7
	UVT	20.0	40.0	6.7	0	13.3	80.0
ANS 1.0	UVB	12.5	35.7	8.9	7.1	12.5	76.8
	UVT	0	14.3	28.6	42.9	0	85.7
CB 0.1	UVB	10.8	16.2	5.4	5.4	2.7	40.5
	UVT	44.4	44.4	22.2	22.2	33.3	166.7
CB 0.3	UVB	34.2	44.7	13.2	15.8	21.1	128.9
	UVT	25.0	25.0	0	50.0	50.0	150.0
CB 1.0	UVB	8.1	25.8	4.8	4.8	8.1	51.6
	UVT	NA*	NA*	NA*	NA*	NA*	NA*

Table 3: Incidence of morphological abnormalities in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 2). Numbers in red indicate highest percent for each abnormality. Only one replicate per treatment was analyzed for this trial. NA* = no live embryos hatched from this exposure.

Trial 3 (2-26-09 to 3-7-09)

Total and normal hatch rates for all treatments were all lower than the lab control (total hatch = 86%; normal hatch = 77%) (Fig. 7). Total hatch rates for the clean and urban UVB treatments were 52 +/- 3% and 56 +/- 6% respectively, and normal hatch rates were 37 +/- 3% and 40 +/-

1% respectively. Total and normal hatching rates were reduced for both clean and urban gravel treatments under UV transmitting (UVT) conditions (17 +/- 3% total hatch and 9 +/- 2% normal hatch in the clean gravel, and 13 +/- 2% total hatch and 6 +/- 2% normal hatch in the urban gravel). Total hatch rates for the ANS UVB treatments did not show a dose response: ANS 0.3 (47 +/- 3%) > ANS 1.0 (35 +/- 6%) > ANS 0.1 (34 +/- 8%). Normal hatch rates for the UVB treatments also did not show a dose response, although in this case, the ANS 0.1 treatment had a higher normal hatch rate than the ANS 1.0 treatment (ANS 0.3 = 22 +/- 7% > ANS 0.1 = 17 +/- 4% > ANS 1.0 = 6 +/- 2%). With the exception of total hatch for the ANS 0.3 UVT treatments (15 +/- 4%), embryos exposed to ANS 0.1, 0.3, and 1.0 under UV transmitting conditions had hatch rates < 7%. Exposure to CBO 0.1, 0.3, and 1.0 under UVB or UVT conditions did result in a dose response. Total hatching rates for CBO 0.1 and CBO 0.3 were slightly lower than those for the clean and urban gravel (51 +/- 15% and 47 +/- 5% respectively), while CBO 1.0 embryos had the lowest total hatch rate of all treatments (30 +/- 8%). Normal hatch rates also followed a dose response: CBO 0.1 (26 +/- 13%) > CBO 0.3 (15 +/- 3%) > CBO 1.0 (3 +/- 2%). All CBO treatments exposed to UV (UVT) had total hatch rates < 7% and normal hatch rates < 1%. No live embryos hatched from the CBO 1.0 UVT treatment.

Yolk opacity was the most common morphological abnormality observed in Run 3 (Table 3, Figure 8). While the incidence of yolk opacity in lab controls was 7 %, the incidence in the clean and urban embryos with UVB exposure was 22% for both. The incidence in UVB and ANS 0.1, 0.3, and 1.0 was higher than the clean and urban embryos, at 48%, 38%, and 70%, respectively. A dose response was seen with UVB and CB 0.1, 0.3, and 1.0 exposure, with 41%, 52%, and 72 % exhibiting yolk opacity. UVT embryos in all treatments except for CB 1.0 had a high incidence of yolk opacity. Clean and urban gravel exhibited an incidence of 46% and 53% respectively. ANS and UVT exposure exhibited a dose response, with ANS 1.0 = 72 % > ANS 0.3 = 56 % > ANS 0.1 = 38%. For CB 0.1 and 0.3, the incidence of yolk opacity was 57 % and 72% respectively. No live hatchlings were present in the CB 1.0 treatments exposed to UVT.

Other abnormalities (scoliosis, edema, bent heads, jaw abnormalities) were found at lower levels. These four abnormalities were observed in less than 8% of the lab controls. Scoliosis, edema, and jaw abnormalities occurred in 10% or less of UVB or UVT exposed embryos in clean or urban gravel, while bent heads were observed in 9 and 17% of UVB and UVT clean gravel, and 11 and 27% of UVB and UVT urban gravel. Scoliosis occurred at relatively low levels in UVB exposed ANS 0.1 embryos (13%), ANS 0.3 (5%) and ANS 1.0 (14%), and higher rates in UVT exposed ANS 0.1 embryos (13%), ANS 0.3 (29%), and ANS 1.0 (29%). A dose response was observed in UVB and UVT CB exposed embryos, with UVB CB 0.1 (12%) < CB 0.3 (15%) < CB 1.0 (35%), and UVT CB 0.1 (27%) < CB 0.3 (31%). The incidence of edema in ANS exposed embryos followed a dose response, in both UVB and UVT treatments. For UVB exposed embryos ANS 0.1 (7%) < ANS 0.3 (17%) < ANS 1.0 (31%), and for UVT exposed embryos ANS 0.1 (13%) < ANS 0.3 (24%) < ANS 1.0 (40%). UVB exposed CB embryos also showed a dose response in the incidence of edema, with CB 0.1 (15%) < CB 0.3 (25%) < CB 1.0 (57%). The incidence of edema in UVT exposed CB 0.1 and 0.3 was approximately the same (33%). Exposure to UVB and ANS 0.1 and 0.3 resulted in a higher incidence of bent heads (27% and 28% respectively) than embryos exposed to UVT and ANS 0.1 and 0.3 (4% and 17% respectively). UVB and UVT exposure with 1.0 ANS had similar incidences of bent heads (35% and 36% respectively). Bent heads in CB and UVB exposed embryos were relatively similar (21%, 26% and 21% for CB 0.1, 0.3 and 1.0 respectively). In the case of UVT and CB exposure, bent head incidence was higher than for UVB (33% and 45% for CB 0.1 and 0.3 respectively). Jaw abnormalities for the UVB exposure and ANS and CB exposure followed a dose response; ANS 0.1 (5%) < ANS 0.3 (6%) < ANS 1.0 (14%), and CB 0.1 (4%) < CB 0.3

(11%) < CB 1.0 (41%). Except for ANS 0.3, jaw abnormalities were of lower incidence in UVT exposed embryos than in UVB exposures. ANS 0.1 (4%) < ANS 1.0 (12%) < ANS 0.3 (19%), and CB 0.1 (3%) < CB 0.3 (7%).

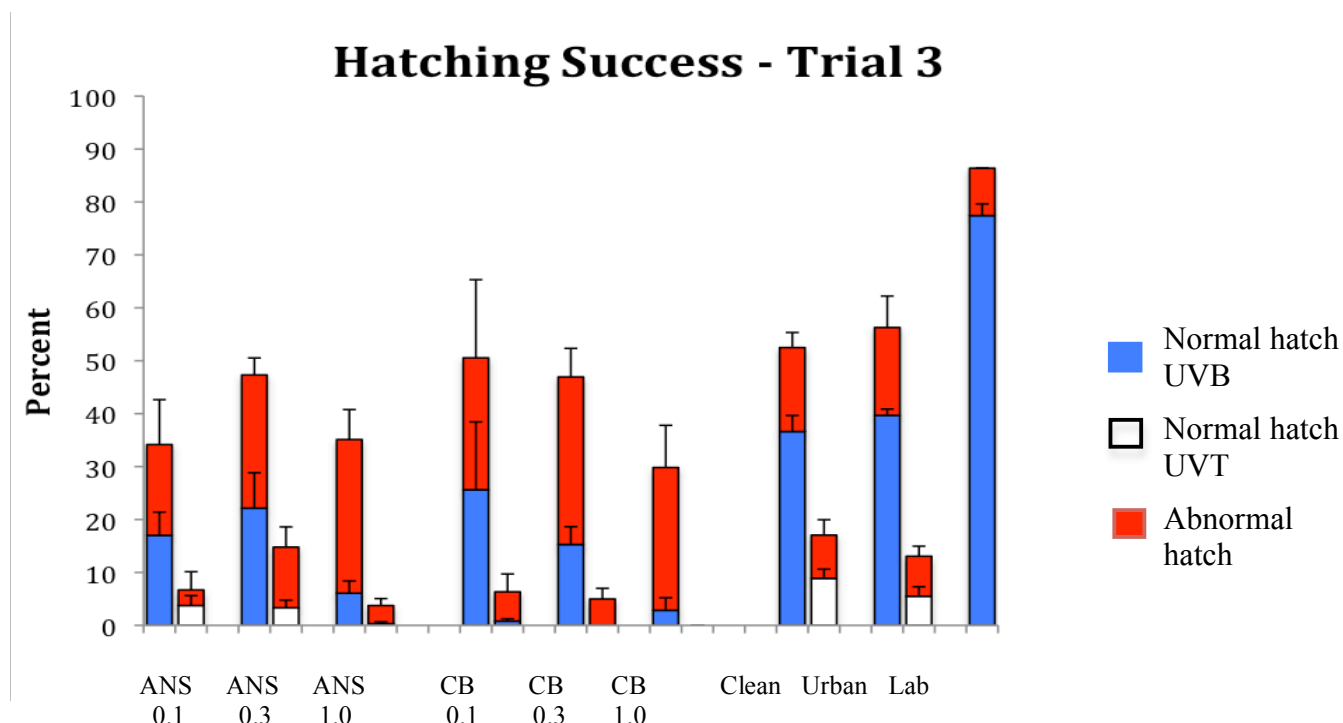


Figure 7: Total and normal hatching of herring embryos exposed to gravel coated with Alaska North Slope Crude Oil (ANS), Cosco Busan bunker oil (CB), clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Run 3). Blue (UVB) or white (UVT) bars represent normal hatching, and red bars represent abnormal hatching (means \pm SE). Total hatch rates are represented by the combination of blue or white bars with corresponding red bars.

Table 3	Type of Abnormality	Scoliosis	Edema	Opaque head	Bent head	Opaque yolk	Jaw
Treatment	UV exposure	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Clean	UVB	2.7 \pm 1.9	3.3 \pm 1.7	2.6 \pm 2.6	9.1 \pm 5.1	22.2 \pm 7.4	0.6 \pm 0.6
	UVT	7.1 \pm 0.9	0.0 \pm 0.0	9.9 \pm 3.5	17.3 \pm 1.8	46.2 \pm 8.1	0
Urban	UVB	3.9 \pm 1.2	4.8 \pm 2.7	3.5 \pm 1.9	10.7 \pm 3.1	21.6 \pm 3.7	0.8 \pm 0.8
	UVT	10.5 \pm 4.0	9.4 \pm 1.4	13.3 \pm 1.7	27.1 \pm 9.6	53.0 \pm 7.0	2.8 \pm 2.3
ANS 0.1	UVB	13.2 \pm 6.0	7.3 \pm 3.8	15.8 \pm 10.8	26.6 \pm 6.3	47.8 \pm 3.6	5.2 \pm 3.8
	UVT	12.9 \pm 3.1	13.3 \pm 4.0	9.1 \pm 7.4	4.2 \pm 3.4	38.3 \pm 16.4	4.2 \pm 3.4
ANS 0.3	UVB	4.7 \pm 2.6	17.1 \pm 1.5	2.9 \pm 1.5	27.6 \pm 6.4	37.5 \pm 11.0	6.4 \pm 5.6
	UVT	28.7 \pm 5.7	23.6 \pm 2.6	20.3 \pm 5.5	16.9 \pm 6.3	55.8 \pm 9.4	19.2 \pm 0.4
ANS 1.0	UVB	14.2 \pm 8.0	30.5 \pm 14.8	11.3 \pm 5.7	34.8 \pm 9.8	70.0 \pm 4.2	13.7 \pm 6.0
	UVT	28.9 \pm 10.6	40.0 \pm 10.0	20.0 \pm 20.0	35.6 \pm 9.9	72.2 \pm 27.8	12.2 \pm 6.2
CBO 0.1	UVB	11.9 \pm 6.6	14.7 \pm 7.1	4.8 \pm 4.8	20.7 \pm 9.3	40.6 \pm 17.4	4.0 \pm 1.7
	UVT	26.7 \pm 12.0	33.3 \pm 12.0	6.7 \pm 6.7	33.3 \pm 17.6	56.7 \pm 28.5	3.3 \pm 3.3
CBO 0.3	UVB	14.5 \pm 5.4	24.7 \pm 7.4	9.6 \pm 5.9	25.9 \pm 5.8	51.6 \pm 3.8	11.1 \pm 3.6
	UVT	30.6 \pm 19.5	32.8 \pm 4.3	6.7 \pm 6.7	45.0 \pm 22.9	72.2 \pm 14.7	6.7 \pm 6.7
CBO 1.0	UVB	34.7 \pm 15.5	57.3 \pm 9.3	18.2 \pm 8.7	20.5 \pm 3.1	72.4 \pm 12.0	41.0 \pm 9.8
	UVT	NA*	NA*	NA*	NA*	NA*	NA*
Lab		2.2 \pm 0.9	0	3.3 \pm 0.1	4.4 \pm 1.9	7.6 \pm 1.0	0.5 \pm 0.4

Table 3: Incidence of morphological abnormalities in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 3). Numbers in red indicate highest percent for each abnormality. NA* No live hatchlings were present in the CB 1.0 UVT treatments.

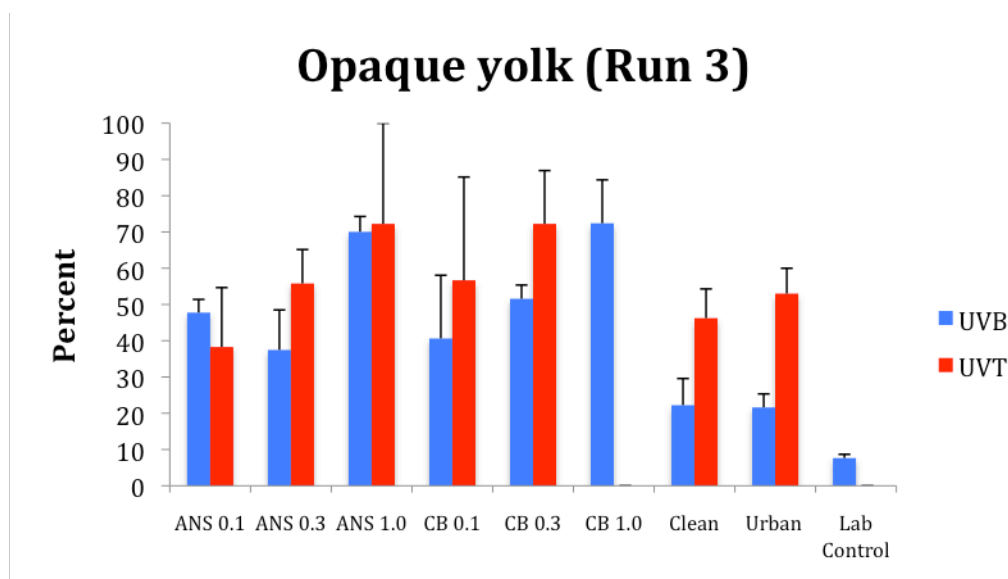


Figure 8: Percent yolk opacity in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (means + SE). Lab controls were incubated in a 12°C incubator.

Trial 4 (3-18-09 to 3-27-09)

Total and normal hatching rates were lower than for the previous trial runs. Total hatching for the lab control was 63% and normal hatching 57% (Figure 9). Total and normal hatch rates for all treatments were lower in the UVT exposures than in the UVB exposures. For the clean gravel, total and normal hatch rates were 52% and 30% respectively for the UVB treatment, and 20% and 11% respectively for the UVT treatment. Total hatch for all CB treatments under UVB exposure were lower than the clean gravel, with CB 0.3 (32 +/- 9%) > CB 0.1 (26 +/- 8%) > CB 1.0 (22 +/- 15%). Total hatch rates for the UVT exposures were much lower, with CB 0.3 (8 +/- 4%) > CB 0.1 (7 +/- 3%) > CB 1.0 (0%). Normal hatch rates followed a similar pattern, with CB 0.3 (13 +/- 7%) > CB 0.1 (10 +/- 2%) > CB 1.0 (8 +/- 7%). UVT exposure resulted in much lower total and normal hatch rates. Total hatch rates were 7 +/- 3% for CB 0.1 and 8 +/- 4% for CB 0.3, while normal hatch rates were < 1% for both 0.1 and 0.3 CB. No live hatchlings were present in the CB 1.0 UVT treatments.

Morphological abnormalities were observed in all treatments with the lowest incidence occurring in the lab controls (<5% for each abnormality) (Table 4). The incidence of edema, opaque heads, or jaw abnormalities in embryos exposed to UVB or UVT clean gravel was < 7%, while scoliosis was observed in < 12%. The most prominent abnormalities observed in clean gravel were bent heads (UVB = 22%; UVT = 13%) and opaque yolks (UVB = 28%; UVT = 30%). With the exception of scoliosis in CB 0.1 and bent heads in CB 0.3, morphological abnormalities were highest in UVT treated embryos and the corresponding CB dose. CB 0.3 with UVT resulted in the highest incidence of scoliosis (45%), edema (68%), opaque heads (28%), and opaque yolks (95%), while the highest incidence of bent heads (43%) and jaw abnormalities (29%) were observed in the CB 0.1 UVT treatment. All embryos from the CB 1.0 UVT treatments either died prior to hatch, or were dead at hatch.

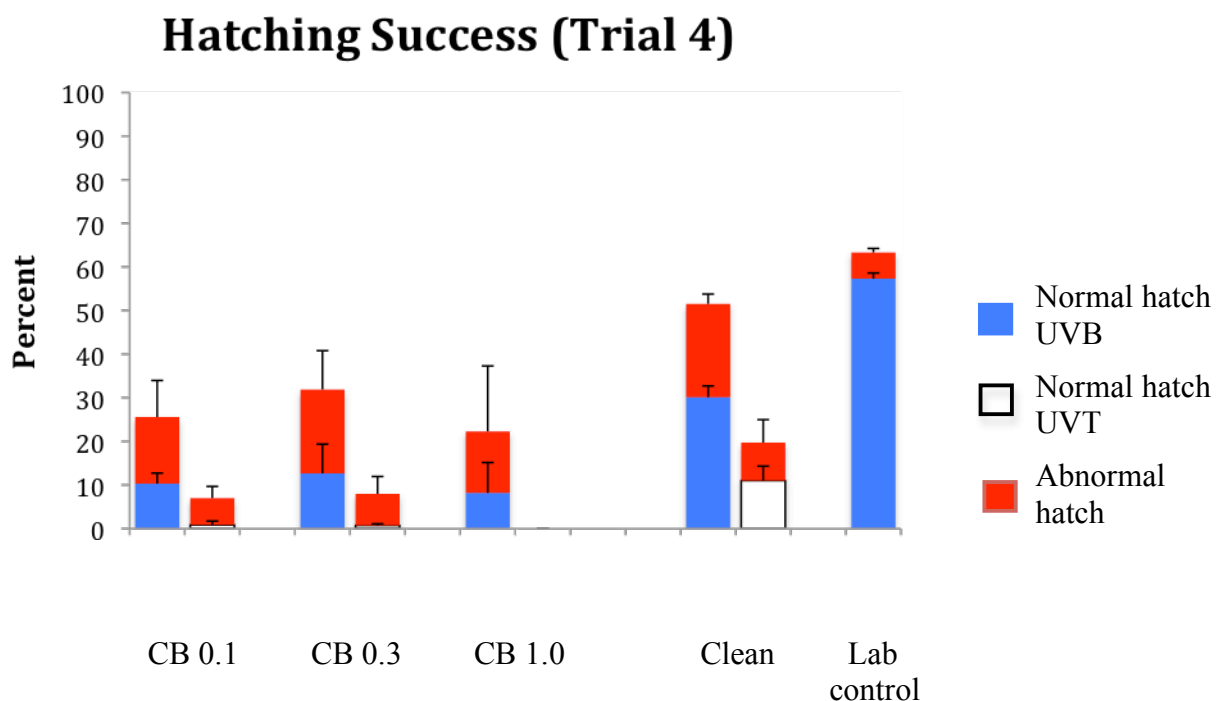


Figure 9: Total and normal hatching of herring embryos exposed to gravel coated with Alaska North Slope Crude Oil (ANS), Cosco Busan bunker oil (CB), clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 4). Blue (UVB) or white (UVT) bars represent normal hatching, and red bars represent abnormal hatching (means \pm SE). Total hatch rates are represented by the combination of blue or white bars with corresponding red bars.

Table 4	Type of Abnormality	Scoliosis	Edema	Opaque head	Bent head	Opaque yolk	Jaw
Treatment	UV exposure	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Clean	UVB	8.5 +/- 2.7	5.7 † 2.1	3.9 + 0.2	21.8 + 6.6	28.4 + 5.0	0
	UVT	11.5 + 3.9	6.4 + 6.4	3.9 + 2.2	12.8 + 6.8	29.5 + 3.4	0
CB 0.1	UVB	29.6 + 4.3	2.4 + 1.3	12.1 + 4.9	16.9 + 6.8	36.6 + 4.8	4.4 + 4.4
	UVT	18.3 + 9.8	39.1 + 19.5	27.0 + 20.3	43.0 + 8.5	76.7 + 14.5	28.9 + 19.8
CB 0.3	UVB	25.2 + 6.8	12.9 + 5.8	8.8 + 1.4	24.2 + 5.8	50.3 + 4.2	11.4 + 5.9
	UVT	45.0 + 4.1	68.3 + 1.4	27.5 + 2.0	15.0 + 12.3	95.0 + 4.1	16.7 + 13.6
CB 1.0	UVB	35.8 + 5.7	31.3 + 10.1	14.4 + 3.2	24.0 + 8.1	66.0 + 10.0	19.2 + 5.7
	UVT	NA*	NA*	NA*	NA*	NA*	NA*
Lab control		2.3 + 0.9	0	1.7 + 1.4	3.5 + 2.8	4.6 + 0.9	0.9 + 0.7

Table 4: Incidence of morphological abnormalities in herring embryos exposed to ANS or CB oiled gravel, clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 4). Numbers in red indicate highest percent for each abnormality. * No live hatchlings were present in the CB 1.0 UVT treatments.

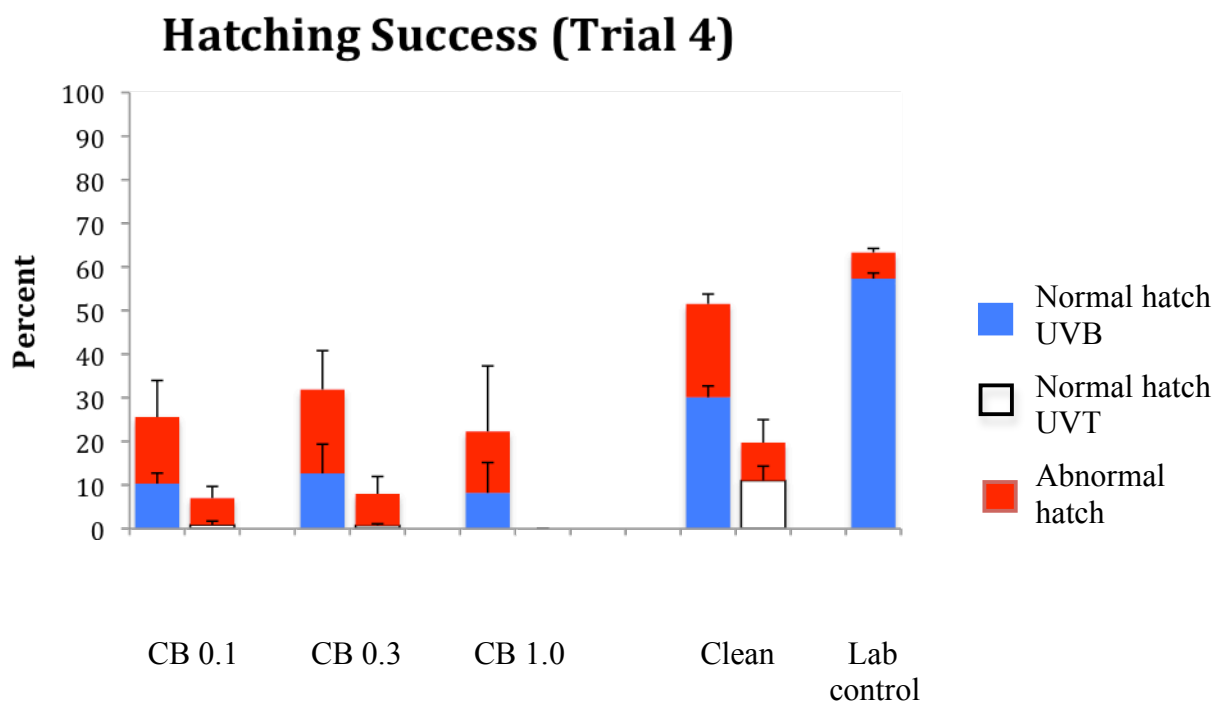


Figure 9: Total and normal hatching of herring embryos exposed to gravel coated with Alaska North Slope Crude Oil (ANS), Cosco Busan bunker oil (CB), clean gravel, or urban gravel under UV blocked (UVB) or UV transmitted (UVT) conditions (Trial 4). Blue (UVB) or white (UVT) bars represent normal hatching, and red bars represent abnormal hatching (means \pm SE). Total hatch rates are represented by the combination of blue or white bars with corresponding red bars.

Table A3-2: Tissue TPAH in embryos by wet weight, Trial 3

treatment	TPAH ($\mu\text{g/kg}$)	treatment	TPAH ($\mu\text{g/kg}$)
clean UVB	14	clean UVT	7
clean UVB	10	clean UVT	14
clean UVB	13	clean UVT	10
urban UVB	14	urban UVT	14
urban UVB	10	urban UVT	24
urban UVB	10	urban UVT	14
ANS 0.1 UVB	18	ANS 0.1 UVT	27
ANS 0.1 UVB	15	ANS 0.1 UVT	21
ANS 0.1 UVB	18	ANS 0.1 UVT	22
ANS 0.3 UVB	49	ANS 0.3 UVT	49
ANS 0.3 UVB	23	ANS 0.3 UVT	26
ANS 0.3 UVB	20	ANS 0.3 UVT	28
ANS 1 UVB	62	ANS 1 UVT	81
ANS 1 UVB	70	ANS 1 UVT	62
ANS 1 UVB	56	ANS 1 UVT	50
CB 0.1 UVB	56	CB 0.1 UVT	20
CB 0.1 UVB	32	CB 0.1 UVT	22
CB 0.1 UVB	30	CB 0.1 UVT	17
CB 0.3 UVB	89	CB 0.3 UVT	46
CB 0.3 UVB	66	CB 0.3 UVT	55
CB 0.3 UVB	68	CB 0.3 UVT	19
CB 1 UVB	174	CB 1 UVT	132
CB 1 UVB	180	CB 1 UVT	197
CB 1 UVB	172	CB 1 UVT	348
maternal	10		

Table A3-3: Aqueous TPAH in column effluent, Trial 4

treatment	TPAH (µg/l)		treatment	TPAH (µg/l)	
	day 0	day 8		day 0	day 8
UVB clean	132	47	UVT clean	83	52
UVB clean	70	33	UVT clean	110	45
UVB clean	59	39	UVT clean	44	37
UVB CB 0.1	369	100	UVT CB 0.1	974	71
UVB CB 0.1	352	69	UVT CB 0.1	525	58
UVB CB 0.1	312	39	UVT CB 0.1	348	116
UVB CB 0.3	851	144	UVT CB 0.3	816	132
UVB CB 0.3	728	135	UVT CB 0.3	788	126
UVB CB 0.3	477	144	UVT CB 0.3	579	172
UVB CB 1	1384	281	UVT CB 1	1250	298
UVB CB 1	1466	225	UVT CB 1	1247	284
UVB CB 1	779	252	UVT CB 1	1097	247

Table A3-4: Tissue TPAH in embryos by wet weight, Trial 4

treatment	TPAH (µg/kg)	treatment	TPAH (µg/kg)
UVB clean	5	UVT clean	4
UVB clean	5	UVT clean	4
UVB clean	3	UVT clean	6
UVB CB 0.1	44	UVT CB 0.1	24
UVB CB 0.1	40	UVT CB 0.1	28
UVB CB 0.1	22	UVT CB 0.1	32
UVB CB 0.3	74	UVT CB 0.3	85
UVB CB 0.3	68	UVT CB 0.3	92
UVB CB 0.3	104	UVT CB 0.3	93
UVB CB 1	170	UVT CB 1	207
UVB CB 1	125	UVT CB 1	154
UVB CB 1	143	UVT CB 1	261
maternal	7		

**Cosco Busan Fish Injury Assessment Work Plan for Herring Studies
2008-2009 Spawning Season**

Proposed by NOAA and California Department of Fish and Game Office of Spill Prevention and Response, in association with NOAA Northwest Fisheries Science Center (NWFSC) and Bodega Marine Laboratory (BML)

The following studies will be performed from approximately December 2008 through March 2009 to support continued efforts to assess potential injuries to herring in San Francisco Bay associated with the M/V Cosco Busan oil spill on November 7, 2007.

**Part 1: San Francisco Bay Herring Field Collection and Analysis of
Developmental Defects and Hatching Success**

The NWFSC and BML will implement a combined effort to collect Pacific herring natural spawn this year to assess the degree to which developmental defects documented during last year's assessment occur again, to provide further information on the potential temporal and spatial extent of such observations, and provide information on the potential association of such defects with the potential continued presence of residual Cosco Busan oil in San Francisco Bay 1+ years post-spill.

Collection

The SOP for natural spawn collection will be essentially the same as the procedure used in the first assessment: where possible we will collect eight individual samples along a transect that follows an isobath at each site. As we did last year, we are also proposing to collect corresponding sediment samples; sediment samples will be stored but not analyzed until parties agree on the need for chemical analysis.

The highest priority is to collect at the six sites chosen for the assessment last year. Four of these are within the documented spill zone (Horseshoe Cove, Sausalito, Peninsula Point, and Keil Cove), and two are reference sites external to the spill zone but still influenced by urban inputs (Point San Quentin and San Rafael Bay). Opportunistically, we may pick an additional reference location if herring spawn near an area outside the spill zone and likely to be particularly influenced by stormwater runoff. We will also try to collect natural spawn from Angel Island and from the San Francisco waterfront, locations within the spill zone where herring spawn or may spawn and where Cosco Busan oil stranding was documented last year.

Keil Cove and San Rafael Bay are our two priority sites for collecting enough spawn for chemistry analyses. For all other sites, in the interest of facilitating faster collection and thus flexibility to visit and collect from more sites, we will collect only sufficient spawn to allow for morphological analysis and hatching success. If herring spawn multiple times this winter, we may attempt to collect from one of these sites more than once. Also, if possible, we'll collect spawn in deeper (subtidal) waters adjacent to one of the collection sites within the spill zone to look for evidence of potential differences in spawn viability based on depth.

Analysis

Morphological analysis of embryos will be carried out at BML, in the same secure wet lab we used last year. We plan to modify the existing SOP for this slightly, with the aim of being able to screen/score more embryos while still accurately documenting any developmental defects. NOAA and BML staff will work together on the embryo morphometry. The previous study focused specifically on scoring for pericardial edema and video documentation of cardiac function. The presence of necrosis and severe body axis defects in samples from oiled sites were unexpected findings. This year, embryos from the field samples will be dechorionated and examined more generally for any types of morphological or functional defects, based on what is known about normal herring development (e.g. Hill et al 1997, J Fish Biol 51:960; Incardona et al 2008, Environ Sci Technol in press). Any differences among samples will be documented by digital photo- and videomicroscopy. Depending on the estimated rates and types of abnormalities observed, sample numbers for quantification will be determined at the time of analysis, but will most likely be in the range of 20-30 embryos per sample. Imaging will involve the same microscopes and software as last year, with data backup on a duplicate pair of external hard drives. BML staff will measure hatching success (but not swimming behavior) as they did last year.

Cosco Busan Fish Injury Assessment 2008-09 Laboratory Studies: Comparative toxicity, phototoxicity, and photo-oxidation studies with CB oil

Overall approach: These experiments will address three questions pertaining to the novel lethal effects observed in herring embryos sampled from Cosco Busan oiled sites in Feb 2008: (1) Does the inherent toxicity of Cosco Busan bunker oil differ significantly from unrefined Alaska North Slope crude oil? (2) Did sunlight exposure of beached Cosco Busan bunker oil produce novel toxic compounds through photo-oxidation? (3) Was the observed necrosis in natural spawn samples due to phototoxicity of PAHs or other bunker oil constituents? Oiled gravel columns are a proven means of generating water contaminated with dissolved-phase oil constituents in a way that mimics intertidal conditions following an oil spill. Numerous studies have shown the long-term persistence of oil toxicity using this system, although primarily with unrefined Alaska North Slope crude oil. The basic methods have been used with herring embryos (1, 2), as well as salmon and zebrafish (3-5), and the kinetics are well understood (6). Typically, effluents from

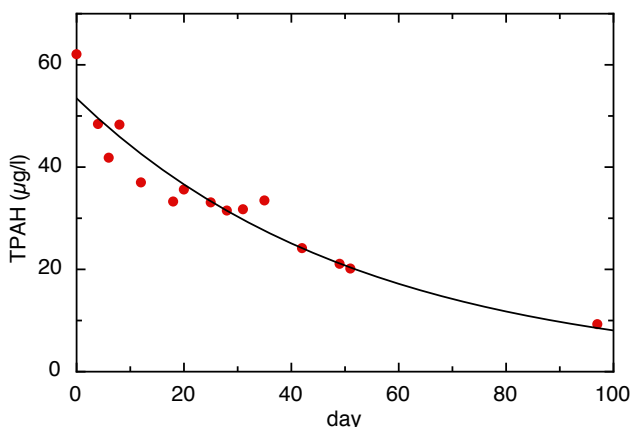


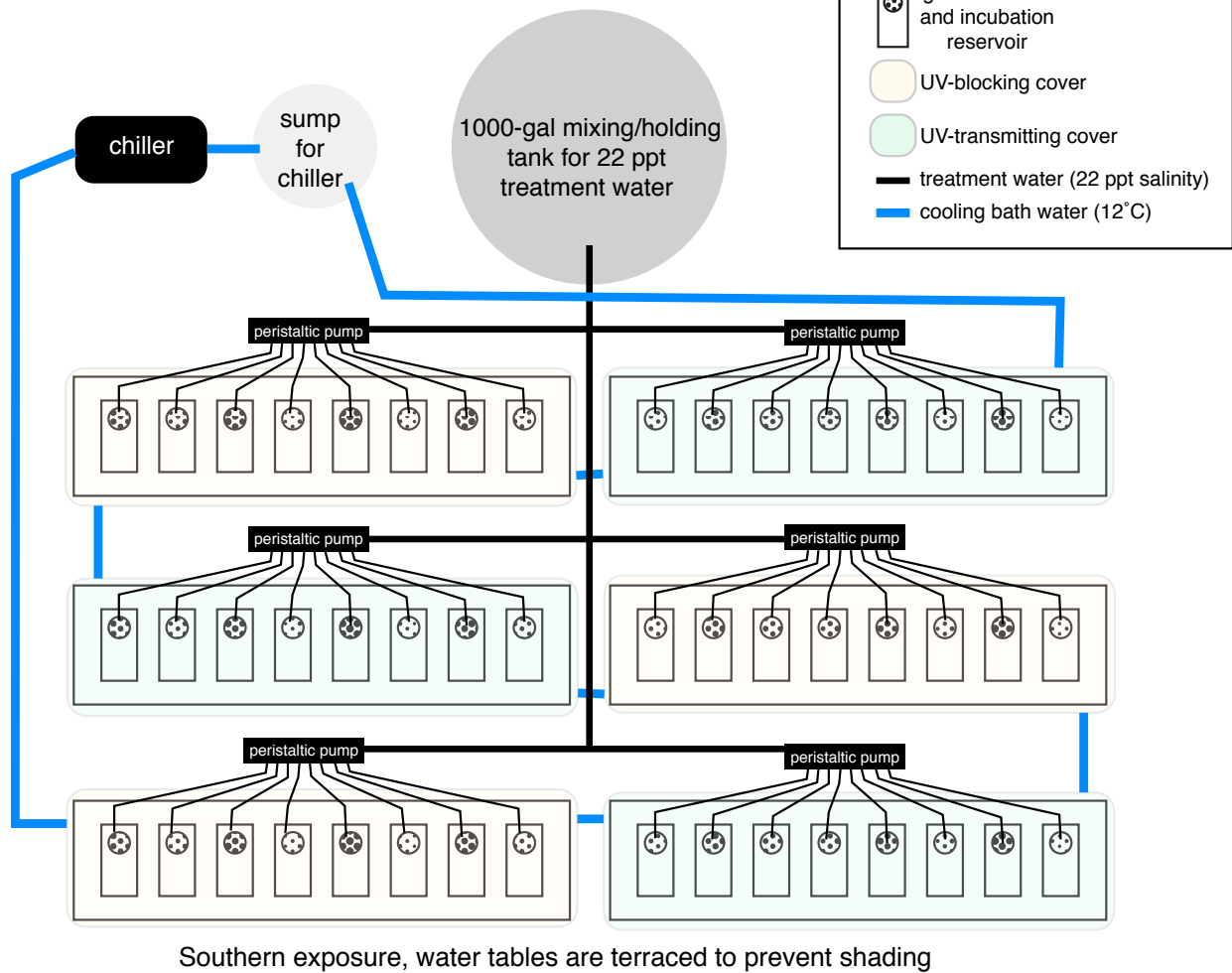
Figure 1: TPAH concentrations in water over time in oiled gravel effluent. Gravel was coated with 6 ppm oil, flow rate 30 ml/min, fresh water at 25°C.

gravel contaminated with oil in the 2000-5000 ppm range contain total PAH concentrations that are initially around 100 ppb, and that decline exponentially to around 10 ppb over a span of 2-3 months (Figure 1). Replicate columns containing gravel oiled at different doses will run continuously from December to March to mimic conditions in SF Bay potentially found after the Cosco Busan spill in winter 2007-08. Two sets of identical columns will be differentially exposed to full spectrum sunlight during weathering to determine if sun exposure generated novel toxic compounds through photo-oxidation that

subsequently leached from oiled substrates. To address PAH (or other) phototoxicity, embryos will be exposed to column effluent protected from UV exposure (but not visible light) for 7 days, then will be exposed to full spectrum sunlight for a brief period consistent with transient exposure at low tide. The ideal plan is to place clutches of embryos into column effluents on a regularly basis (e.g. weekly) for the entire duration of weathering. However, given the probable sporadic availability of ripe fish capture from SF Bay, embryos will be exposed at as many time points as possible. It is anticipated that some combination of oil dose, time, and light will reproduce the effects observed in the Feb 2008 field samples.

Oil dosing: Three doses of oiled gravel will be prepared for each Cosco Busan bunker oil and 20% weathered Alaska North Slope crude oil. Cleaned and sized (~1 cm) crushed rock will be coated with oil at 1000, 300, and 100 ppm (1 g oil/kg gravel to 0.1 g oil/kg gravel). Each of the three doses for each oil and a control of clean gravel will be packed into six replicate columns. A second set of control columns will be packed with comparably sized gravel from a SF Bay beach

A: Plan view of oiled gravel column exposure system



B: Profile view of a single column unit

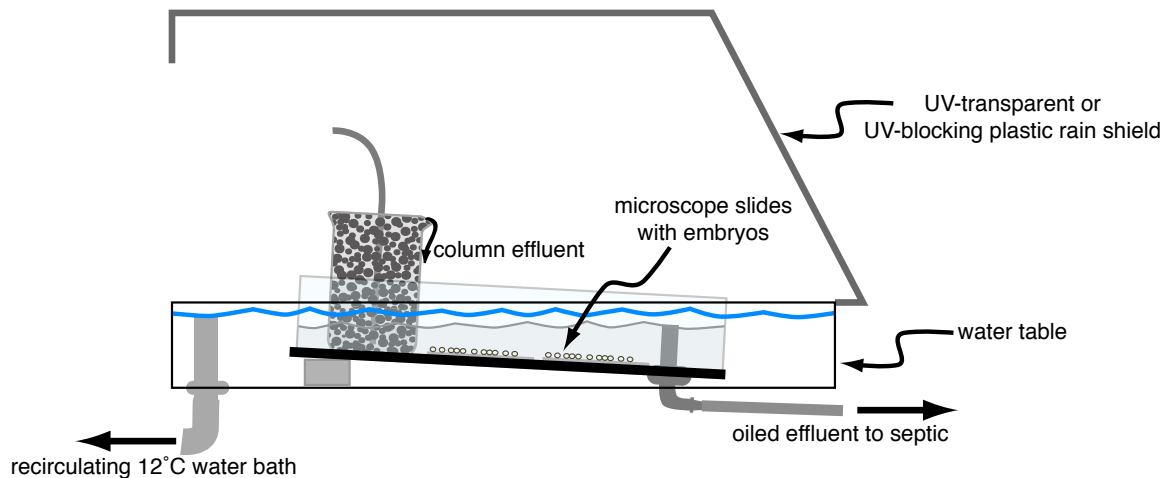


Figure 2: Oiled gravel column design

outside the spill zone (e.g. San Rafael Bay). Three replicates (8 columns total each) will be weathered under a cover of UV-transmitting plastic and three will be weathered under UV-blocking plastic (to allow exposure to visible sunlight wavelengths). Seawater at 25 ppt salinity will be distributed to the bottom of each column via glass tubing at a rate of 0.6 l/hr, and column effluent will be collected in incubation reservoirs (custom made 8" X 16" X 3" aquaria) that will hold microscope slides coated with fertilized herring eggs. Column weathering will begin in early to mid-December.

A schematic of the exposure system and its location are shown in Figures 2 and 3, respectively. Column flow will be controlled with peristaltic pumps delivering 10 ml/min to each column; the bank of 48 columns will use about 200 gal per day of 22 ppt seawater. This exposure water will be mixed and stored in a 1000-gal tank that will be plumbed with in-line flow meters for full-strength seawater and fresh water from the labs' well source. A 5-micron bag filter will be used to remove fine clay silt present in the fresh water. The aquaria used for the incubation reservoirs have a ½" diameter PVC stand-pipe to maintain a water depth of 1-2", and will be held in a water table plumbed to maintain an appropriately deep (~ 4-6") cooling bath held at 12°C by a ¾-hp Aqua-Logic Delta Star chiller. The water bath will be fresh water recirculated from a sump with a high head submersible pump. Columns will be randomly distributed by dose

and oil type within each bank, and water tables will be randomized for UV or no-UV exposure. The location of the column bank is on a concrete pad at the southeast corner of the main BML wet lab building (Figure 3). This site receives unobstructed southern exposure. Plastic covers will be constructed for each of the six water tables to keep out rain, but will be open on the northern side to permit air circulation and prevent a "greenhouse" effect. Three covers will be constructed of UV-transparent plastic (e.g. pure Plexiglas), and three will be constructed with UV-



Figure 3: Site location at BML. At top is a view of the lab from the south, at bottom is the satellite view. Red arrow indicates concrete pad where oiled gravel column bank will be installed

blocking material. UV transmission will be checked with a Macam UV203 radiometer.

Herring embryos: Ripe fish will be collected opportunistically in SF Bay. Gametes will be dissected and transported to the lab on ice as described previously (CBOS SOP manual 2007). Eggs are removed from ovaries and dispersed in half-strength seawater containing 0.25% polyvinyl alcohol to prevent adherence to each other. Eggs are then distributed onto precleaned microscope slides and incubated with milt, after which they adhere to the slides and are fertilized. Eggs will be distributed at a density of ~ 200 per slide. After overnight incubation to ensure fertilization rates, embryos will be placed in column effluent. Incubation reservoirs will hold up to 12 slides. A single slide from each column will serve as a replicate for biological observations, while groups of slides will be pooled to obtain 1-2 gram samples for analytical chemistry.

Morphological observations: Because embryos will be incubated in filtered seawater, chorions remain unobscured by sediment, biofilm, diatoms, etc. Individual slides will be removed at 7 days post-fertilization and initial observations made through the chorion. Presence of edema, necrosis, or other notable phenotypes will be scored for ~ 100 embryos on a slide. Subsamples of embryos will be dechorionated for imaging. The same temperature control and imaging system will be used as described previously (CBOS SOP manual 2007).

Exposure of herring embryos to full spectrum sunlight after oil exposure: After 6-8 days exposure in oiled (and control) gravel effluent, slides with herring embryos will be transferred to a water table in direct ambient sunlight. UV-A and UV-B irradiation will be quantified with a Macam UV203 radiometer. After up to 2 hours of ambient sunlight exposure, embryos will be transferred back to the lab for monitoring of sunlight-induced morphological changes in the same manner as described above.

Analytical chemistry: The amount of tissue required for chemistry is much larger than what is required for biological observations. Due to this limiting factor, it is possible that replicate samples will not be available for chemical analysis. At least a single 2 g sample will be extracted for each dose and time point, and PAH levels determined to verify dosing.

References:

- (1) Carls, M. G.; Rice, S. D.; Hose, J. E., 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 pallasii*). *Environ. Toxicol. Chem.* **1999**, *18*, 481-493.
- (2) Incardona, J. P.; Carls, M. G.; Day, H. L.; Sloan, C. A.; Bolton, J. L.; Collier, T. K.; Scholz, N. L., Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasii*) exposed to crude oil during weathering. *Environ. Sci. Technol.* **2008**, *in press*.

- (3) Heintz, R. A.; Short, J. W.; Rice, S. D., Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered Exxon Valdez crude oil. *Environ. Toxicol. Chem.* **1999**, *18*, 494-503.
- (4) Incardona, J. P.; Carls, M. G.; Teraoka, H.; Sloan, C. A.; Collier, T. K.; Scholz, N. L., Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. *Environ. Health Perspect.* **2005**, *113*, 1755-1762.
- (5) Marty, G. D.; Short, J. W.; Dambach, D. M.; Willits, N. H.; Heintz, R. A.; Rice, S. D.; Stegeman, J. J.; Hinton, D. E., Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Can. J. Zool.-Rev. Can. Zool.* **1997**, *75*, 989-1007.
- (6) Short, J. W.; Heintz, R. A., Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Northwestern Gulf of Alaska based on a PAH weathering model. *Environ. Sci. Technol.* **1997**, *31*, 2375-2384.

Approximate Budgets

NWFSC estimated costs for San Francisco Bay herring natural spawn collection and assessment

Labor (3.0 PYs, including John Incardona @ 0.25, Nathaniel Schulz @ 0.1): \$80,600
Per diem: \$8,200
Housing: \$6,700
Airfare: \$5,250
Vehicles: \$3,000 (truck)
Supplies: \$1,000
Equipment: \$0
Shipping: \$500
Chemistry: \$0

TOTAL: \$105,250

NWFSC estimated costs for San Francisco Bay herring laboratory studies conducted at BML

Labor (1.0 FTE, including John Incardona @ 0.25, Nathaniel Scholz @ 0.1): \$29,600
Per diem: \$3,100
Housing: \$2,200
Airfare: \$1,750
Vehicles: \$1,500 (sedan)
Supplies: \$4,000
Equipment: \$14,400
Shipping: \$500
Chemistry: \$26,700

TOTAL: \$83,750