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Impacts of Suspended Sediments on Fertilization, Embryonic Development, and Early Larval Life Stages of the Pacific Herring, *Clupea pallasi*

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Abstract. Pacific herring reproduce in the San Francisco Bay estuary during times of the year when suspended sediment loads are highest due to freshwater input, yet little is known about the effects of sediment on herring early life stages. During the first 2 h after eggs contacted water, embryos were adhesive and susceptible to having sediment particles attach permanently to the chorion. Treatment with suspended San Francisco Bay dredged sediments at ecologically relevant concentrations of 250 or 500 mg/l during this time period increased self-aggregation of the eggs and led to sublethal and lethal effects. After the first 2 h in water, sediments that contacted embryos did not attach to chorions and did not have an observable impact. Sediment treatment during the first 2 h was not linked statistically to declines in fertilization or total larval hatch rate, but it did produce significant sublethal effects that included increases in precocious larval hatch and higher percentages of abnormal larvae, as well as an increase in larval mortality.

Introduction

Pacific herring, *Clupea pallasi* Valenciennes, 1847, eggs and embryos provide a convenient model for investigating the effects of sediment loads on sessile or attached organisms. The species utilizes coastal shallow waters and estuaries for spawning during times of the year when freshwater input and resultant sediment loads are elevated (Alderdice and Velsen, 1971; Spratt, 1981; Hay, 1985; Barnhart, 1988; see also Potter *et al.,* 1990; Ruhl *et al.,* 2001; Ruhl and Shoellhamer, 2004; Watters *et al.,* 2004). At spawning, eggs released by females settle on and attach to biotic and abiotic substrata where they remain throughout embryonic development. This time period is 8–10 days in southern latitudes (San Francisco Bay), but may reach 30 or more days in more northern latitudes (Alderdice and Velsen, 1971; Eldridge and Kaill, 1973; Hay, 1985; Gillis *et al.,* 1990; Griffin *et al.,* 1998). Thus, the adhesive eggs and embryos are captive to localized sediment conditions for periods from a week to more than a month.

The input of sediment is a key component in estuarine productivity because it supplies nutrients and replenishes substrata (Potter *et al.,* 1990). High sediment loads, however, can reduce light penetration and thus primary productivity (both in the water column and on the benthos) and can cover and smother benthic organisms, including eggs and embryos, due to particle settling (Bruton, 1985; Cloern, 1987; Newcombe and McDonald,1991). These problematic high loads of suspended sediment derive from anthropogenic activity in either estuaries or the watersheds of estuaries (Conomos and Peterson, 1977; Bush, 1989; Howarth *et al.,* 1991). A prime example is San Francisco Bay. This bay is a heavily urbanized estuary that is dramatically altered and impacted by human disturbance throughout its watershed (*e.g.,* by placer mining, agriculture, urbanization) as well as within the bay itself (Krone, 1979). Suspended sediment loads in San Francisco Bay can reach 500–600 mg/l during the herring reproductive season, with the potential of localized higher concentrations due to activities like dredging of harbors and channels, and nearshore devel-

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Abbreviations: CaMgF, calcium and magnesium-free; GLM, General Linear Models regression analysis in SYSTAT software; HPF, hours post-fertilization; PF, post-fertilization; *S,* practical salinity; SF Bay = San Francisco Bay.

opment projects (Krone, 1979; Ingram and DePaolo, 1993; Ingram *et al.,* 1996; Watters *et al.,* 2004; McKee *et al.,* 2006).

Several laboratory studies using *Clupea* species (both Atlantic and Pacific species) have generally concluded that sediment levels of 1000 mg/l or greater were required to produce significant mortality in embryos or larvae (Boehlert *et al.,* 1983; Kiorboe *et al.,*1981; Morgan and Levings, 1989). These studies, however, were conducted with eggs that were fertilized in sediment-free water, after which embryos were transferred to sediment-containing water and allowed to develop. These studies did not investigate whether fertilization was affected. They also did not test the impacts of the presence of sediments during the early post-fertilization period when eggs and embryos are sticky and still forming the adhesive layer that will anchor them to substrata (Laale, 1980; Gillis *et al.,* 1990). Here we show that development of the adhesive layer of herring embryos requires at least 2 h and sediment particles that attach to embryos during this 2-h window remain bound throughout embryonic development. We also show that attachment of sediment particles leads to retarded development and reduced larval survival rates at sediment concentrations as low as 250 mg/l. Furthermore, sediment particle attachment increases egg aggregation, resulting in the formation of egg clusters, and this also leads to retarded development or mortality of embryos—an effect attributed to hypoxia within the under-layers of these aggregates (Stacey and Hourston, 1982; Hay, 1985; Haegle and Schweigert, 1985).

Materials and Methods

Solutions

The optimum salinity for fertilization and embryonic development of San Francisco Bay Pacific herring is a practical salinity (*S*) of 16; thus all experiments were conducted at this value (Griffin *et al.*, 1998). Seawater at $S = 16$ was made by diluting 0.45-μm-filtered Bodega Marine Laboratory flowing seawater with distilled water. Seawaters were stored at 12 °C. Salinity was monitored with a salinometer (refractometer). Calcium and magnesium-free (CaMgF) seawater of $S = 16$ was made by diluting $S = 32$ CaMgF seawater (Cavanaugh, 1956) with equal parts distilled water. CaMgF polyvinyl alcohol (PVA) was made by adding 0.25% PVA to $S = 16$ CaMgF seawater and stored at 4 °C. All chemicals were obtained from Sigma Chemical (Sigma-Aldrich, St. Louis, MO).

Sediment handling and storage

We used sediments from two sources: (1) dredged San Francisco Bay (SF Bay) sediment from the Port of Redwood City (SF Bay sediment) supplied by the U.S. Army Corps of Engineers, San Francisco, California; and (2) Fuller's earth, a commercially available control sediment (F-200, Sigma-Aldrich, St. Louis, MO). Prior to processing, SF Bay sediment was stored in lidded glass jars (250 ml) at −20 °C, and Fuller's earth was stored dry as packaged by the supplier. Fuller's earth was used in pilot experiments to determine appropriate final concentrations of sediment, resolve effective times of sediment treatment, refine laboratory techniques with sediments, and delineate whether observed effects with SF Bay sediments were caused by sediment particles alone.

The composition of SF Bay sediment was 18% clay (<4 μm), 75% silt (4–63 μm), and 7% sand and gravel (>63 μm) as determined from a 2005 analysis (USACE, 2005). Chemical analyses to determine the presence of contaminants were available from five sediment samples; chromium was the only toxicant present for which the average of the five analyses exceeded background levels; the chromium average was 130 ppm (130 mg/l) compared to the San Francisco Bay ambient sediment concentration values of 112 ppm (112 mg/l). Biological toxicity testing utilized an amphipod bioassay; survival of *Rhepoxynius abronius* and *Ampelisca abdita* ranged from 84% to 100% and averaged 94.71% ± 4.92% standard deviation (Source: USACE, 2005. United States Army Corps of Engineers, Redwood City Harbor Channel toxicity data. An internal report by the United States Army Corps of Engineers, San Francisco, CA).

Suspended sediments were processed for experiments by suspending, washing, and adjusting concentration to 2 g dry weight sediment/liter. To obtain a final concentration of 2 g/l we began with 4 g/l of Fuller's earth and 6 g/l of SF Bay sediment; the difference in initial weights was because Fuller's earth was obtained as dry sediment, while SF Bay sediment was dredged and contained water. Weighed sediments were suspended in 1 l of seawater, $S = 16$, at 4 °C, stirred vigorously with a magnetic stir bar for 60 min, and the larger particles allowed to settle for 30 min. The resultant supernatant was decanted and allowed to settle overnight at 4 °C. Settled particles were resuspended in 1 l of clean $S = 16$ seawater, stirred for 60 min, and again allowed to settle overnight; this was done twice. After the second wash, resuspended sediment particles were gravity-filtered twice through Whatman #2 filters. Sediment concentration of the filtered suspended sediments was determined by obtaining sediment dry weight for a known volume, as follows. A 100-ml aliquot was removed and suction-filtered onto a pre-weighed 0.45-μm analytical filter. The filter was dried overnight in a 60 °C drying oven and the weight of the dried sediments determined. A stock sediment concentration of 2 g/l was stored frozen at −20 °C in 200-ml aliquots until use. We also developed a standard curve of suspended sediment concentration by recording absorbance at 546 nm with a Biomate spectrophotometer (Thermo Spectronic, Madison, WI) and known concentrations of suspended Fuller's earth or SF Bay sediment. These standard curves were

used as a second method of determining concentration to validate that the dry weight concentration determinations were accurate.

The accepted method of storage in previous studies has been sub-zero storage, either frozen intact at −20 °C or −70 °C, or freeze-dried (Norton *et al.,* 1999). However, a more recent study questioned the efficacy of using previously frozen contaminated sediments without a post-freeze wash, due to the accumulation of ammonia during freeze storage (Geffard *et al.,* 2004). To address this concern, freeze-stored sediments were thawed and washed once before use in experiments. Sediment particle size after the final wash and resuspension averaged 7.6 μm in diameter with a range of 2–47 μm.

Animal collection and acquisition of gametes

Gravid Pacific herring were collected by hook and line or mid-water trawl from SF Bay and transported on ice to the Bodega Marine Laboratory. Ovaries and testes were dissected and kept moist in culture dishes at 4 °C for up to 4 days until needed for experiments (Yanagimachi *et al.,* 1992). Some ovaries and testes were obtained from fish collected in Puget Sound, Washington. These were dissected from freshly caught fish and sent on ice to the Bodega Marine Laboratory *via* overnight express.

Fertilization and embryo sediment treatments

Fertilizations and development of embryos to larval hatch were conducted inside a 12 °C incubator in 10 × 5 cm circular glass dishes (≈200 eggs) or in 1-l rectangular glass dishes. Sperm from 2–3 testes were combined and added at a final concentration of 10^5 cells/ml into $S = 16$ seawater that contained 0–500 mg/l suspended sediment. Eggs from ovaries were either directly distributed into the sperm/sediment suspension or placed into CaMgF $S = 16$ seawater containing 0.25% polyvinyl alcohol (1/2 CaMgF PVA) and then distributed to experiments *via* pipette (Griffin *et al.,* 1998). Eggs from 2–4 females were pooled for each experiment. A final sperm concentration of 10^5 sperm/ml was used to ensure that fertilization rates would be below 100% and on the log portion of the sperm concentration-fertilization curve as described by Griffin *et al.* (1998). Use of these sperm concentrations ensured that we did not mask any inhibitory effects of sediment on fertilization by having elevated numbers of sperm (Vasquez, 2003). In all but the aggregation experiments (see *Sediment-induced egg aggregation experiments* below), eggs were distributed or broadcast into water evenly to prevent aggregation of eggs into multiple layers; eggs that aggregated into multiple-layered clusters were removed to eliminate aggregation effects on experimental results. To determine sediment effects on fertilization, eggs were co-incubated with sperm and sediments for 15 min (normal time for maximum fertilization; see Yanagimachi *et al.,* 1992). In experiments to determine sediment effects on embryo development, gametes and sediments were co-incubated for 2 h (this was the approximate time frame required for adhesive hardening). Controls consisted of eggs fertilized and treated like experimental cultures except that sediments were not present. After the predetermined sediment treatment time (15 min or 2 h), cultures were washed (3 times) in sediment-free $S = 16$ seawater to remove unattached sediments and sperm. The initial 2-h period after eggs were dispensed into water was denoted as 0–2 h post-fertilization (0–2 HPF). Embryos that were treated with sediment at all time periods after 0–2 HPF were fertilized in sediment-free water and then exposed to sediment from 2 to 4 HPF, 4 to 6 HPF, or 24 to 26 HPF, or for 2 h at 5 days post-fertilization.

We scored fertilization at 2 HPF (elevated and translucent chorion; Yanagimachi *et al.,* 1992); however, to ensure that spontaneous egg activation was not erroneously interpreted as fertilization, only embryos that progressed to the epiboly stage of development (about 24 h after fertilization) were deemed fertilized.

Developing embryos were incubated through larval hatch (6–12 days) in sediment-free water. Cultures were monitored and provided with 100% daily exchanges of $S = 16$ water. For the chronic sediment-treatment experiments, $S = 16$ seawater containing the appropriate sediment concentration was added at the daily exchanges. Attempts to delineate the timing of developmental stages prior to hatching proved inconclusive; sediment particles that adhered to embryos in the 0–2 HPF sediment treatments precluded accurate determination of developmental progression. Thus, although embryonic developmental stages were monitored, larval hatch was deemed the best measure of successful embryonic development and was expressed as percentage of fertilized eggs that hatched.

Beginning at first sign of hatching, larvae were removed with daily water changes and counted. Normal and abnormal larvae were scored; abnormal larvae were those that did not uncoil or straighten out after hatch or were scoliotic. Random aliquots of hatched normal-appearing larvae (10 individuals per culture) were photographed for length and yolk-sac measurements through a digital camera mounted on an Olympus stereo-zoom microscope (see *Microscopy and image analysis* below).

For chronic sediment treatments (duration of embryonic development), eggs were added to tissue culture flasks, and the flasks were capped and placed on a rocker table at 12 °C for 2 h. The cultures were then washed free of sperm after 2 h and subsequently received water and sediment changes daily.

Sediment-induced egg aggregation experiments

During pilot experiments with Fuller's earth, we observed a greater tendency for eggs to adhere to one another

(aggregate) in the presence of suspended sediments. To document this phenomenon, we exaggerated egg-egg contact during egg settling as follows. The water in culture dishes was swirled as eggs were dispensed; swirling was maintained until eggs began to adhere to the bottom of a dish (≈5 s). Embryos were then cultured as described above. To quantify egg aggregation, we tested the hypothesis that as aggregation increased, spatial distribution of eggs would decline and egg/egg cluster "footprints" would become significantly greater than that of an individual egg. The average "footprint" was determined using imaging software to determine egg/egg cluster area in digital images of cultures (see *Microscopy and image analysis* below).

Larval culture

Larval age was measured in days post-hatch. Larvae designated as day 1 post-hatch were those that hatched during the first 24-h period after larvae were first observed in a culture. Ages of larvae that hatched after the initial 24-h period were then designated as day 2 post-hatch, day 3 post-hatch, *etc.;* thus, day 2 post-hatch larvae were larvae that had hatched 24–48 h after first observation of larval hatching. Larvae from 0–2 HPF sediment-treated embryos began hatching one day before no-sediment controls (see Results); therefore, in experiments on larval size, growth, and survival, we utilized larvae that hatched on the same calendar date in order to compare larvae of the same post-fertilization age.

About 200 larvae were pooled from replicate embryo dishes. Larvae were transferred into clean 1-l rectangular glass dishes containing 400 ml of sediment-free $S = 16$ seawater and cultured as described by Griffin *et al*. (2004). The larvae were kept at 12 °C, and after daily water exchanges of 50%, larvae were fed 0.5 ml of a concentrated rotifer solution (300–500 rotifers). Dead larvae were removed from cultures daily and mortalities recorded. Aliquots of live larvae were removed and photographed for measurement of larval growth and development (total length and yolk-sac diameter) as described in the next section.

Microscopy and image analysis

Eggs, embryos, and larvae were assessed after images were collected through an Olympus SZH stereo-zoom microscope using a PixelLink CCD camera (PLA-662) and capture software (ver. 1.3). Spatial distribution of embryos (egg aggregation) in cultures was analyzed by determining area of embryo "footprints" using ImageJ software, ver. 1.41 (National Institutes of Health). Larval length and yolk-sac area were measured with ImageJ software measurement functions and calibrated against collected images of a 2-mm stage micrometer.

Statistical analysis

Data were analyzed using SIGMASTAT, ver. 3.5 (Jandel Scientific) or SYSTAT 12 (SYSTAT Software, Inc) software. Unless otherwise identified, data in figures are presented as means ± standard deviations (minimum $n = 5$). Where data were presented as percentages, arcsine square root transformation was performed to achieve normality before statistical analysis. GLM (General Linear Models, SYSTAT) regression analysis was run on transformed data to identify relationships between dependent and independent factors. When relationships were indicated, a Student's *t*-test (SIGMASTAT) was employed to determine if there were differences between individual dependent and independent variables. To eliminate group effects on *t*-tests, the significance level was set at $\alpha = 0.05/k$, where k is the number of comparisons; *t*-tests that were not assigned to a GLM regression analysis utilized a significance level of $\alpha = 0.05$.

Survival Analysis (SYSTAT) of larval survival was used to compare control and sediment-treated cultures (Muenchow, 1986). Data were presented as number of days post-hatch that larvae survived during 14 days of the experiment; larvae that remained alive at experiment termination were denoted as surviving 15 days and were defined as right-censored data. Mean survival times as well as Kaplan-Meier survival probabilities for individual post-hatch days were recorded. In addition, GLM regression analysis was employed on three segments of transformed data (2–5, 6–10, and 11–15 days post-hatch) to discern if decreased larval survival in sediment-treated cultures was due to higher mortality during a particular time segment of the experiment. The segments were chosen because they represented early, middle, and late periods of the larval culture and were time frames within which the relationship between controls and sediment treatments appeared to be constant. GLM was conducted to test this assumption after cumulative mortality data were converted to cumulative percentage mortality (based on 200 larvae per culture at experiment initiation) and subjected to arcsine square root transformation.

Results

Suspended sediment–egg chorion interactions

Suspended sediment particles permanently attached to the adhesive layer of eggs and embryos if the sediments were present during the first 2 h after the eggs were dispensed into water. Eggs adhered to glass culture dishes both in the presence and absence of sediments, and 6 days later remained bound (Fig.1). Six-day post-fertilization (PF) control embryos were translucent and easily viewed through "clean" chorions. Embryos that had been treated with 500 mg/l of suspended SF Bay sediments at 0–2 h post-fetilization (HPF; Fig. 1B) were coated with particles, whereas

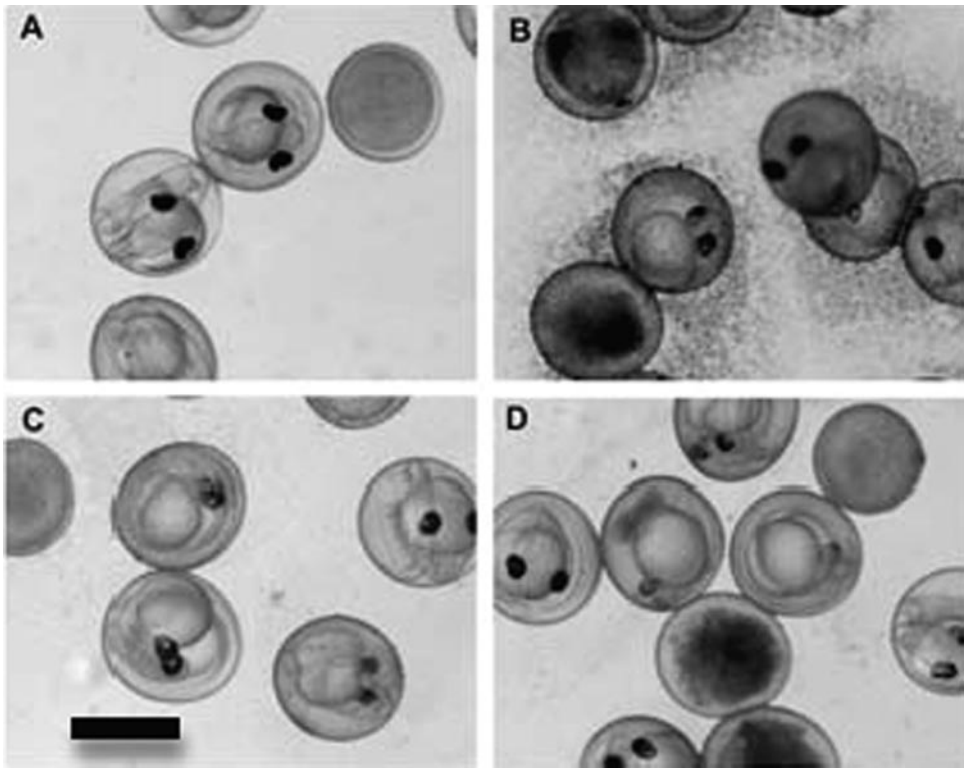

Figure 1. Suspended sediment particles are retained if chorions are treated at 0–2 HPF. (A) Control embryos had translucent chorions at 6 days PF through which developing larvae could be seen. Embryos that had been treated with 500 mg/l suspended SF Bay sediment at 0–2 HPF (B) retained sediment particles at 6 days post-fertilization, while embryos treated at 2–4 HPF (C) and at 4–6 HPF (D) were particle-free. Scale = 1 mm.

embryos that had been treated with suspended sediment at 2–4 HPF and 4–6 HPF were sediment-free (Fig. 1C, D). In the 0–2 HPF cultures, sediment particles also remained attached to the substrata immediately surrounding embryos. At 6 h PF we noted particles on embryos in the 2–4 HPF cultures that were no longer present at 6 days PF (data not shown).

Suspended sediments and spatial distribution of eggs and embryos

Eggs showed a greater tendency to aggregate or cluster into multiple layers in the presence of suspended sediments. When egg-egg contact was artificially enhanced during fertilizations by swirling of culture dishes as eggs were dispensed, no increase in egg-egg aggregation was observed in controls, but aggregation increased when suspended SF Bay sediment was present (Fig. 2A, B). In controls and cultures that were treated with 65 mg/l of suspended SF Bay sediment, aggregation was minimal; the average "footprint" was close to the area of an individual embryo, diameter about 1 mm, area about 1.57 mm^2 (Fig. 2 graph). Embryos treated with 250 and 500 mg/l of sediment aggregated into clusters that attached to the substratum, creating "footprints" that were on average significantly larger than that of an individual embryo; 500 mg/l of suspended SF Bay sediment produced an average "footprint" that was 8 times the size of an individual embryo (Fig. 2 graph).

Fertilization and embryonic development

Suspended sediments did not significantly reduce fertilization or total larval hatch (completion of embryo development). We observed no decline in fertilization with Fuller's earth; fertilization in controls averaged 60.6% ± 13.6%, while sediment treatments ranged from 59.9% ± 13.0% (65 mg/l sediment) to 58.0% ± 12.3% (500 mg/l sediment). Fertilization in the presence of suspended SF Bay sediment ranged from 66.2% ± 8.9% (65 mg/l sediment) to 58.6% ± 8.5 (250 mg/l) compared to 68.4% ± 8.6% for controls. Differences were not statistically significant (*t*-test $P > 0.01$, $\alpha = 0.01$). Lastly, fertilization rate did not vary between sediment types. We statistically compared 0–2 HPF suspended sediment treatments using SF Bay sediment and Fuller's earth and found no interaction of sediment source and fertilization (GLM: $P = 0.393$).

Likewise, total larval hatch was not affected by sediment source nor by sediment concentration. There was no statis-

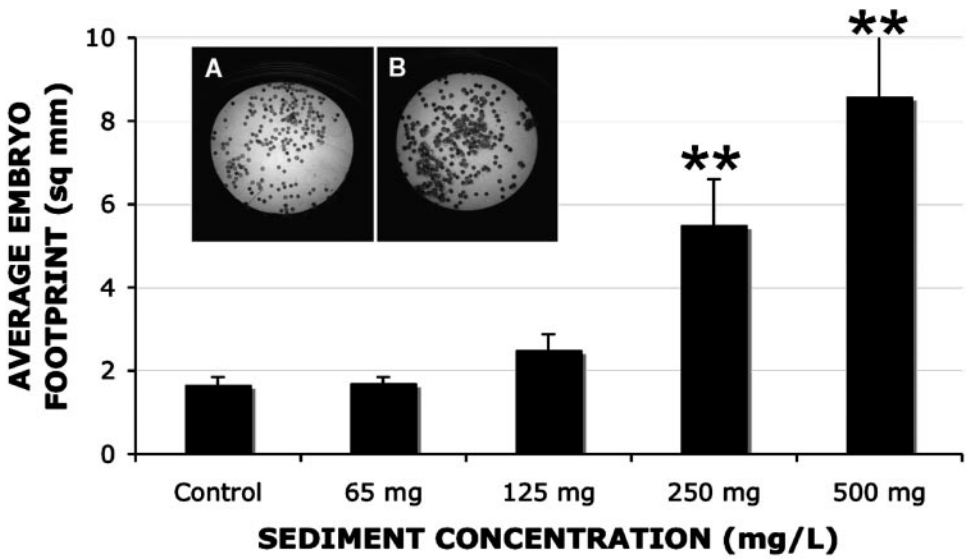

Figure 2. Treatment with suspended SF Bay sediment at 0–2 HPF induces aggregation of eggs. (A) Control eggs that settled on and adhered to the bottom of culture dishes provided embryo "footprints" slightly larger than an individual egg. (B) Aggregated eggs that were co-incubated with 500 mg/l of sediment formed footprints larger than individual eggs. The 250 mg/l and 500 mg/l treatments yielded increased aggregation (*t*-test, $P < 0.001$**); $n = 5$, with 20 measurements per n. Scale = 5 mm.

tical interaction between sediment source (Fuller's earth and SF Bay sediment) and total hatch up to 500 mg/l suspended sediment treatment at 0–2 HPF (GLM: $P = 0.566$). Three different sediment treatment protocols, all of which contained a 0–2 HPF sediment treatment, failed to show a statistical connection between SF Bay sediment concentration and total larval hatch. In two of the experiments (short-term sediment treatment at 0–2 HPF and a 2-h short-term treatment at different developmental times) GLM analysis did suggest an interaction between sediment concentration and total larval hatch when sediments were present 0–2 HPF; however, low r^2 values implied high variability and suggested that other factors were influencing declines in total larval hatch. For example, there was an interaction in the constant short-term treatment experiments (GLM: $F(1,33) = 13.418$, $P = 0.001$); however, $r^2 = 0.289$. In a third experiment comparing short-term *versus* long-term sediment treatment it was clear there was no interaction between total hatch and sediment concentration in any treatments, including one where sediment was present at 0–2 HPF (GLM: $F(4,40) = 1.359$, $P = 0.265$).

Sublethal sediment effects on embryo development

SF Bay sediment treatment correlated with several sublethal effects. The percentages of hatched larvae that were morphologically normal, duration of embryonic development (time from fertilization to hatching), larval size at hatching, post-hatch larval growth, and larval survival all declined with suspended SF Bay sediment treatment of eggs or embryos at 0–2 HPF. Abnormalites included scoliotic larvae and larvae that did not straighten from their pre-hatch tightly coiled posture (Fig. 3). There were also abnormalities that precluded hatching—instances of larvae that did not completely exit the chorion and died during hatch as well as embryos that had developed to the larval stage but did not hatch. These non-hatch abnormalities were not included in abnormal larval counts. Increase in larval abnormality (decrease in normal larvae) was linked to suspended sediment concentration (Fig. 4; GLM: $F(1,66) = 23.198$, $P = 0.001$, $r^2 = 0.778$). The percentage of normal larvae that hatched from sediment-treated embryos (250 or 500 mg/l of suspended sediment at 0–2 HPF) was significantly less than total hatch and also less than the percentage of normal larvae that hatched from control embryos (*t*-test, $P < 0.0125$). In controls, 68.7% ± 0.4% of the total hatch was normal, while normal hatch after exposure to concentrations of 250 mg/l was 45.3% ± 3.9% and 37.3% ± 4.1% with 500 mg/l treatment at 0–2 HPF (Fig. 4).

Larvae from sediment-treated eggs or embryos also hatched earlier than controls, and hatching was protracted. Larvae from both 0–2 HPF and chronic SF Bay sediment-treated cultures hatched one day before control larvae. In two sets of experiments, sediment-treated cultures began hatching on day 6 PF, while controls commenced hatching on day 7 PF. In a third experiment, sediment-treated cultures hatched beginning on day 7, while controls did not begin to hatch until day 8. Figure 5 depicts the pooled data from experiments. Instead of grouping larval hatch by day

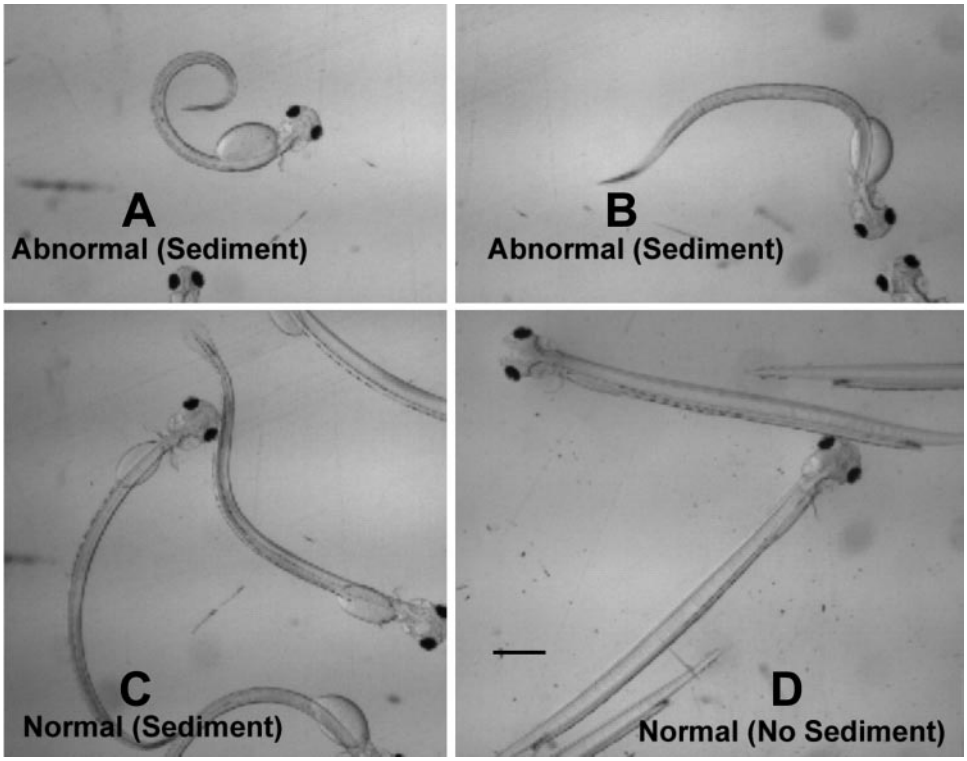

Figure 3. Abnormal hatched larvae. (A) Larva whose tail did not completely uncoil from around the head and yolk sac; and (B) scoliotic larva with one or more kinks in the spinal column. (C) Normal-appearing larvae from an embryo treated at 0–2 HPF with 500 mg/l SF Bay sediment; photographed while swimming. (D) Normal larva from a no-sediment control culture. Scale =1 mm.

post-fertilization, we designated the first day that controls hatched as day 1; thus, day 1 equated to day 8 PF for the third set of experiments, and to day 7 for the other two.

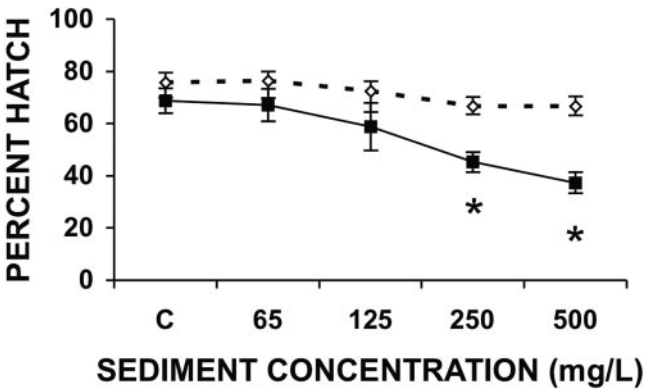

Figure 4. Suspended sediment impacts on larval hatching: total larval hatch and normal larval hatch. Percentages of hatched larvae that were normal declined with increasing suspended sediment concentration. Percentage total larval hatch (white diamond) and normal larval hatch (black square) after treatment of embryos at 0–2 HPF with 0–500 mg/l SF Bay suspended sediment. Total larval hatch differed from normal larval hatch (GLM: $F(1,66) = 23.198$, $P < 0.001$, $r^2 = 0.778$). Percent normal larvae decreased over both no-sediment controls and total hatched larvae in both 250 and 500 mg/l treatments (t-test, $P < 0.0125$*). Graph = mean ± std dev; $n = 8$.

There was a strong interaction between hatch day and sediment treatment (Fig. 5; GLM: $F(10,144) = 11.104$, $P < 0.001$, $r^2 = 0.767$). On day −1 the number of larvae that hatched from both sediment-treated cultures was greater than from the controls (t-test, $P < 0.001$, $\alpha = 0.01$). On day 1, when control larvae began to hatch, and on day 2 there were no differences in number hatched between sediment treatments and controls ($P > 0.01$). On day 3, both sediment-treated cultures produced fewer larvae than did controls, and by day 4 hatching in the chronically treated culture was below that of controls ($P < 0.001$). Total accumulated hatch in these experiments was not reduced by sediment treatment. Total mean hatch was 118 larvae ± 13 in 0–2 HPF sediment-treated cultures and 120 ± 16 in chronic sediment cultures compared to 129 larvae ± 15 ($P = 0.119$ and $P = 0.229$) in controls. In both sediment treatments there was a longer hatch period, 5 days as opposed to 4 days in controls.

At hatch, normal larvae from sediment-treated cultures were shorter (total length) than controls (Fig. 6). They also possessed larger yolk sacs and grew at a slower rate over the first 5 days post-hatch. Controls averaged 5.74 mm ± 0.33 total length at hatch and grew to 9.32 mm ± 0.80 by day 5 PH. Larvae from sediment-treated embryos averaged 4.85

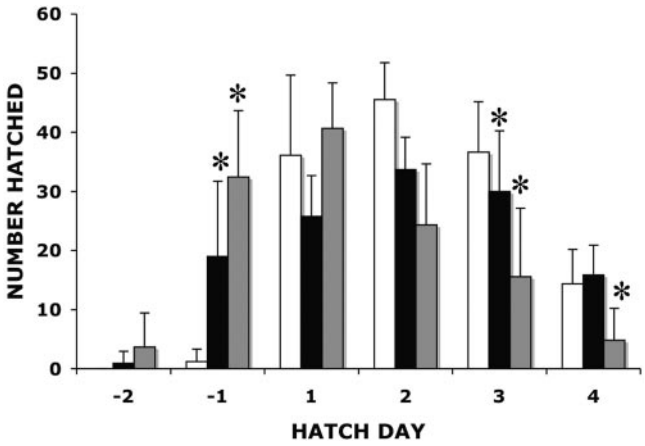

Figure 5. Daily larval hatch. Hatch per day of larvae from embryos treated with 500 mg/l suspended SF Bay sediment at 0–2 HPF (black bars), embryos treated chronically with sediments throughout embryonic development (gray bars), or embryos that were no-sediment controls (white bars). Hatch Day 1 was designated as the first day for which the hatch per day of control larvae was greater than 10% (≈20 larvae) of embryos in the culture. There was an interaction between hatch day and sediment treatment (GLM: $F(10,144) = 11.104$. $P < 0.001$, $r^2 = 0.767$). Larvae in chronic and 0–2 HPF cultures began hatching one day earlier than did larvae in controls (*t*-test, $P < 0.001$*). There was no difference between either sediment treatment and controls on Hatch Days 1 and 2 (*t*-test, $P > 0.008$). On Hatch Day 3, hatching in both sediment treatments was less than in no-sediment controls, and on Hatch Day 4, only hatching in chronic sediment treatment was below that of no-sediment controls (*t*-test, $P < 0.001$*). Graph data = mean ± std dev, $n = 9$.

mm ± 0.23 at hatch and grew to 6.42 mm ± 0.72 total length by day 5 PH. Total lengths of control and sediment-treated larvae were significantly different for all three post-hatch time points (Fig. 6A, $P < 0.001$). Yolk-sac area of larvae from control embryos averaged 0.55 mm^2 ± 0.08 while those from sediment-treated embryos averaged 0.64 mm^2 ± 0.08 at hatch, again a significant difference (Fig. 6B, $P < 0.001$). Early hatch, shorter total length, and larger yolk sacs indicate precocious hatch in sediment-treated larvae (Williams *et al.,* 2003).

Larval survival was reduced when eggs and embryos were treated with 500 mg/l suspended SF Bay sediment at 0–2 HPF, and this decrease was due to early post-hatch mortality (Fig. 7). Survival analysis (SYSTAT) revealed a mean larval survival time for controls of 14.64 days PH, while mean survival was 11.86 days PH for sediment-treated cultures (Fig. 7A). The Kaplan-Meier probability of survival to day 5 PH was 0.955 in controls and 0.819 in sediment-treated cultures, indicating that survival difference began during the early post-hatch period. The overall Kaplan-Meier probability of survival to 14 days was 0.744 for no-sediment controls and 0.496 for sediment-treated cultures. GLM regression analysis of cumulative mortality after separating the data into three discrete blocks of time (2–5 days PH, 6–10 days PH, and 11–14 days PH) supported survival analysis (Fig. 7B). Cumulative mortality in sediment-treated cultures for days 2–5 PH was greater than that in controls (GLM: $F(1,66) = 21.79$, $P < 0.001$, $r^2 = 0.812$). After day 5 post-hatch, difference in cumulative mortality between controls and sediment treatments was not due to sediment treatment (GLM: days 6–10: $F(1,104) = 0.016$, $P = 0.899$; GLM: days 11–15: $F(1,86) = 0.319$, $P = 0.574$). As in other experiments, the 0–2 HPF time period was important; addition of 500 mg/l suspended SF Bay sediment to cultures after larval hatch for 2 h either on the first day of larval hatching or on day10 PH had no effect on larval survival (data not shown). Mean cumulative mortality in the day 1 PH sediment treatment was 55 larvae ± 7 and was 50 larvae ± 8 for the day 10 PH treatments, while mortality averaged 53 larvae ± 9 in the no-sediment controls. This compared to a mean cumulative mortality in 0–2 HPF treated cultures of 87 larvae ± 7.

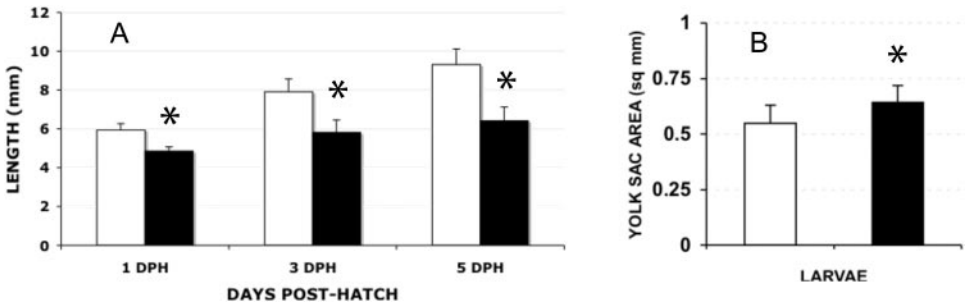

Figure 6. Larval size measurements. (A) Hatched larvae from embryos that had been incubated in 500 mg/l suspended SF Bay sediment at 0–2 HPF (black bars) and hatched larvae from controls (white bars). Sediment-treated embryos produced shorter larvae (A) with larger yolk sacs (B). Sediment-treated larvae remained shorter through 5 days post-hatch (A). ($P < 0.003$*, *t*-test for all sediment/no-sediment comparisons.) Sediment-treated larvae did not increase in length by day 3 (*t*-test, $P > 0.05$), but had done so by day 5 (*t*-test, $P = 0{,}009$); $n = 5$, with 10 measurements per n.

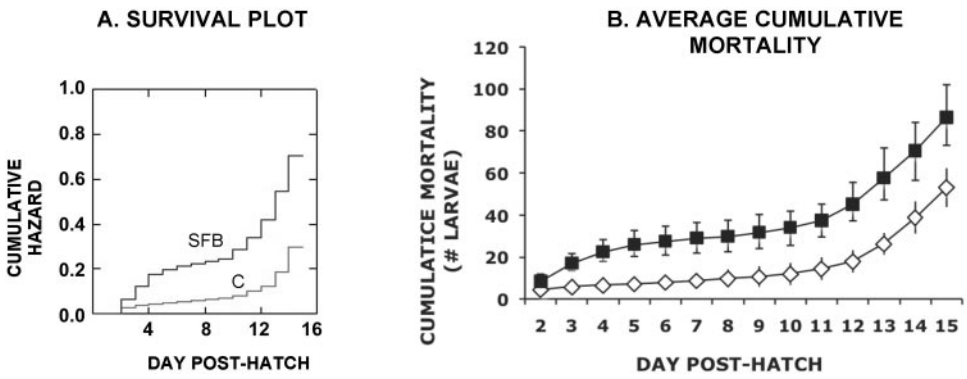

Figure 7. Larval mortality as a function of sediment treatment of eggs at 0–2 HPF. (A) Survival plot of cumulative hazard comparison for larvae from eggs treated with 500 mg/l of suspended SF Bay sediment at 0–2 HPF (SFB) and larvae from no-sediment control embryos (C). (B) Average cumulative numbers of post-hatch larval mortality in no-sediment control cultures (white diamond) and after treatment with 500 mg/l of suspended SF Bay sediment at 0–2 HPF (black square). Larval mortality in 0–2 HPF sediment-treated embryo cultures was higher than in no-sediment controls on days 2–5 post-hatch (GLM: $F(1,66) = 21.79$, $P < 0.001$, $r^2 = 0.812$). Between days 6 to 10 and days 11 to 15 there was no difference in new mortalities between 0–2 HPF sediment-treated and no-sediment cultures (GLM: Days 6–10: $F(1,86) = 0.016$, $P = 0.899$. Days 11–15: $F(1,86) = 0.319$, $P = 0.574$). Mean ± std dev; $n = 9$.

Discussion

In this study, previously reported no-effect concentrations of suspended sediments produced sublethal developmental effects in Pacific herring when the sediments were incubated with eggs and embryos during the first 2 h of egg/embryo contact with water. Furthermore, these effects had lethal outcomes. If suspended sediments were present at concentrations of 250 or 500 mg/l during this initial 2-h window of time, we found that (a) particles attached permanently to eggs; (b) eggs could aggregate into multiple layers; and (c) even in the absence of egg aggregation, significant developmental effects occurred that included precocious hatching of larvae, increases in larval abnormalities, and a decrease in survival of yolk-sac larvae after hatching. Our results contradict the literature concerning suspended sediments and herring reproductive biology, which collectively suggest that embryos and larvae tolerate concentrations of suspended sediments up to 1000 mg/l (Kiorboe *et al.,*1981; Boehlert *et al.,* 1983; Morgan and Levings, 1989). The discrepancy arises because previous studies did not test the effects of suspended sediments during these initial 2 h and either did not examine sublethal effects or, if they did so, did not follow the developmental outcome. In prior studies, eggs were fertilized in sediment-free water, on glass slides or Nitex mesh, and then transferred to sediment-containing water after periods of time greater than 2 h (Kiorboe *et al.,*1981; Boehlert *et al.,* 1983; Morgan and Levings, 1989). When we treated embryos with suspended sediment after the initial 2-h period (after 0–2 h post-fertilization), regardless of exposure time, our results paralleled the previous studies. Sediment particles did not permanently bind, and at concentrations of up to 500 mg/l had no significant effect on embryonic development, larval hatching, or larval survival.

The fact that sediment particles adhered to chorions only when presented during the first 2 h supports the suggestion that development or "maturation" of the adhesive layer requires about 2 h. The Pacific herring egg, approximately 1 mm in diameter, is negatively buoyant and is contained within a thick (≈50 μm) extracellular envelope, the chorion (Hay and Miller, 1982; Grosse and Hay, 1988). Most of the surface of the chorion is adhesive (Gillis *et al.,* 1990); only the micropyle region at the animal pole of the egg is not. Gillis *et al.* (1990) described a two-step hydration that took place after eggs contacted seawater, during which eggs "became strongly adhesive," and debris and sperm attached to the outer surfaces of chorions. The time required for this hydration has not been reported; however, it would not be unreasonable to expect that the hydration process and complete formation of this adhesive layer are connected.

Suspended sediments, if present in the water column as eggs descend, enhance egg aggregation, which could have implications for natural spawns. Spawning begins with some males releasing milt into the water at a spawn site, providing a pheromone that triggers both females and other male fish to spawn (Hay, 1985; Carolsfeld *et al.,* 1997). Females and males exhibit a characteristic behavior involving substrate selection and spawning in which females release eggs in ribbonlike fashion on or close to selected substrata (Stacey and Hourston, 1982; Haegle and Schweigert, 1985; Carolsfeld *et al.,* 1997). Females may, however, swim away from substrata during spawning and release eggs into the water column (Stacey and Hourston, 1982; Aneer *et al.,* 1983; Hay, 1985). When this occurs,

eggs settle and attach to substrata or onto other eggs in a less organized manner, leading to aggregations of multiple layers or clusters. As layers increase in thickness so does hypoxia, microbial growth, and retardation of embryonic development (Stacey and Hourston, 1982; Hay, 1985). Sediment-induced aggregation of eggs in the water column would exacerbate overall aggregation and clustering.

Whether or not sediment particles that attach to herring egg chorions decrease the strength of egg-substratum adhesion is not known. The adhesive layer and its ability to bind herring eggs to solid biotic or abiotic substrata throughout the embryonic period is essential to successful reproduction: eggs that do not attach or embryos that are dislodged can be transported out of shallow, protected nursery areas by currents and tides, thus losing the developmental benefits of those nursery areas. To counteract this problem, the spawning behavior of female herring and the negative buoyancy of eggs facilitates attachment to substrata. Surprisingly little information is available regarding sediment effects on adhesion strength of aquatic eggs or embryos to substrata, regardless of the species. Wilbur and Clarke (2001) reviewed the literature but described no studies on the effects of suspended sediment on adhesion. Chapman and Fletcher (2002) reported that the development of embryos of the marine alga *Fucus* can be impacted by sediments. Although not directly addressing adhesion, these authors suggest that sediments can result in embryo removal through burial and scouring processes. The egg chorion of the sturgeon (*Acipenser* spp.), like that of Pacific herring, possesses an outer layer that facilitates egg attachment to the substratum at spawning (Cherr and Clark, 1985). Although no studies have been published on how suspended sediment affects egg-substratum adhesion, techniques have been published on use of suspended sediments to prevent the adhesion of sturgeon eggs in aquaculture settings (Kowtal *et al.,*1986). The presence of sediment either in suspension or settled onto substrata could be hypothesized to negatively affect egg-substratum adhesion strength. This subject deserves quantitative study because a reduction in adhesive strength could make eggs more susceptible to being dislodged by tidal or wave action. Thus two sediment effects on egg adhesion—induced egg aggregation (current study) and changes in egg-substrata adhesion strength (hypothesized)—could predispose fertilized eggs to reduced developmental success.

The functional morphology of eggs and sperm/egg interaction supports our findings and argues against an effect of suspended sediment on fertilization. The region of the chorion through which sperm gain access to the egg, the micropyle, is not adhesive. This 150-μm-diameter area of the chorion contains a narrow channel or canal (termed the micropylar channel) that is about the same diameter as a sperm and is the only access to the egg plasma membrane and thus fertilization (Gillis *et al.,* 1990; Yanagimachi *et al.,* 1992; Griffin *et al.,* 1996). The non-adhesive micropyle region contains the major sperm-activating molecule, sperm motility initiating factor (SMIF), and this glycoprotein induces sperm motility and functions to maintain sperm in the micropyle region, thus enhancing chances for fertilization (Yanagimachi *et al.,* 1992; Pillai *et al.,* 1993; Griffin *et al.,* 1996; Vines *et al.,* 2002; Cherr *et al.,* 2008). Therefore, it is unlikely that sediment particles adhering to the adhesive portions of the chorion would interfere with sperm access to the micropyle and micropylar channel. Since the relatively low sperm concentrations used in our study ensured that fertilization rates were on the log portion of a fertilization-response curve (Griffin *et al.,* 1998; Vazquez, 2003), it is unlikely that any sediment effect was masked by excess sperm.

Treatment of eggs or embryos with suspended sediments at 0–2 HPF affected embryo development. We did not, however, demonstrate a decline in total larval hatch. Experimental results demonstrated enough variability to mask any supposed interaction between sediment treatment and total larval hatch. Potential sources of this variability include quality differences in eggs from different females, fungal infections in cultures, or sediment-related hypoxia (Kiorboe *et al.,* 1981; Alderdice and Hourston, 1985; Hay, 1985; Laine and Rajasilta, 1999). Contaminants are another possible source of variability; however, we found no difference in total hatch due to sediment type (control Fuller's earth and SF Bay sediment). The fact that there were significant increases in the percentage of abnormal larvae as well as several sublethal effects suggests that sediment affected embryo development.

The sublethal effects were indicative of stress and may have been causative factors in reduced larval survival; they included precocious hatch, smaller larval size with larger yolk sac at hatch, and reduced growth rate. Stress from a variety of sources, including sediments, has been reported to initiate all of the sublethal effects that we observed. Mortalities of Atlantic herring embryos and precocious hatching of herring and whitefish larvae after embryo hypoxia have been reported (Rankine *et al.,* 1990; Morrison *et al.,* 1991; Czerkies *et al.,* 2001). Studies with Atlantic herring have shown that embryo aggregation into multiple layers increases length variability at hatch, indicating stress (Rankine *et al.,* 1990). Temperature stress can also lead to precocious hatch of reduced-size larvae in herring (Ojaveer, 2006); however, embryos in the current study were cultured at a constant temperature within the normal range for herring embryos and larvae (12 °C; Griffin *et al.,* 1998). In an exception to those results, Morgan and Levings (1989) reported that sediment exposure resulted in an increase in time to hatch that, according to the authors, was most likely due to low oxygen. Even in the absence of specific stressors, larval hatching can occur over 3–4 days, and those larvae that hatch early are smaller, with larger yolk sacs, than those

that hatch toward the end of the hatch period (Geffen, 2002). Some authors have suggested that although these precocious larvae attain the same size as their cohorts that hatch later, early hatch alone can have negative implications for survival; these include reduced swimming ability, which could lead to loss of position in the nursery areas or to increased predation (Rosenthal and Alderdice, 1976; Blaxter and Fuiman, 1990; Batty and Blaxter, 1992; Geffen, 2002; Williams *et al.,* 2003). Precocious hatch from sediment-treated embryos coupled with a reduced post-hatch growth rate could compound any negative effects of precocious hatch.

Sublethal and lethal developmental effects could have arisen from one or both of two sediment-linked sources: (1) physical binding of sediment particles to an egg could hinder diffusion across the chorion during development; and (2) sediment particles could deliver contaminants to embryos, and it could be the actions of the contaminants that leads to decline in oxygen consumption. Exposure to crude oil hydrocarbons (Carls *et al.,* 1999, 2002; Incardona *et al.,* 2009), Orimulsion 400 (Williams *et al.,* 2003), and creosote (Vines *et al.,* 2000) all produce precocious hatching. In the Orimulsion 400 study, Williams *et al.* (2003) reported that high concentrations produced larvae that hatched prematurely, were smaller, and had larger yolk sacs. Several deformities, including coiled larvae, cardiac edema/arrythmya, yolk-sac edema, and several varieties of spine flexion, were also described as a result of hydrocarbon-induced stress (Vines *et al.,* 2000; Williams *et al.,* 2003; Incardona *et al.,* 2004, 2009). Toxicants are present in SF Bay sediment at low levels; however, as described above, they did not impact total hatch and it is unlikely that they caused the observed sublethal effects. Although yolk sacs were larger in larvae that hatched precociously, there was no evidence of the yolk-sac or cardiac edema that is seen in embryos exposed to polycyclic aromatic hydrocarbons (Vines *et al.,* 2000; Incardona *et al.,* 2004).

The ranges of suspended sediments used in the current study were ecologically relevant. Although estimates of suspended-sediment concentration prior to anthropogenic disturbance of San Francisco Bay (which began in about the 1850s) are not available, sedimentation rates due to input of suspended sediments are thought to have varied spatially and temporally as they do today (Nichols *et al.,* 1986). For the pre-anthropogenic disturbance period, sedimentation rates ranging from 0.7 to 2.6 mm per year have been hypothesized, along with delta flow rates that varied from 500 to 3000 m^3/s; the highest flows and sediment input were during the winter/spring, during the herring reproductive season (Cloern *et al.,* 1983; Ingram and DePaolo, 1993; Conomos *et al.,* 2004). Beginning in the 1850s, placer mining and subsequent additional anthropogenic disturbances to the Sacramento/San Joaquin River and local San Francisco Bay watersheds significantly altered the quantity and composition of sediment entering San Francisco Bay; input into the Bay increased from a pre–gold rush estimate of about 1×10^6 metric tons per year, consisting primarily of sand and fine gravel, to over 5×10^6 metric tons per year with a large concentration of fine silt and clays (Kondolf, 2000). We know from more recent estimates that ambient suspended sediment levels routinely fluctuate from less than 50 mg/l up to 500–600 mg/l (Ruhl and Shoelhamer, 2004; McKee *et al.,* 2006).

We have described an unreported interaction between particulates and Pacific herring eggs that can occur only during the first 2 h of egg contact with water. Significant sublethal and lethal impacts on embryonic development and larval survival were relatively small; however, taken together, these effects across developmental stages (embryo-larval stages) could enhance their ecological importance. Mortality during embryonic life stages is thought to be highly variable, ranging from 56% to 99% in British Columbia (Schweigert, 1993). Such factors as multiple-layered spawns, predation, wave action, and exposure to air during low tides can contribute to daily losses of 8% or more of spawn biomass during embryonic development (Hay and Miller, 1982; Hay, 1985; Schweigert, 1993). To elucidate the ecological impact of suspended sediments on Pacific herring embryo and larval success in the environment, questions that should be addressed include the following: (1) Do suspended sediments produce egg aggregation during natural spawns? (2) Do coatings of sediments on embryos reduce adhesion between embryos and substrates to which they are attached? (3) What are the respective contributions of particles and contaminants contained on particles to observed developmental effects? Lastly, there is a need to understand how anthropogenic input of suspended sediments (through dredging or shoreline development) adds not only to total sediment loads, but also to contaminant loads.

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Literature Cited

Alderdice, D. F., and A. S. Hourston. 1985. Factors influencing development and survival of Pacific herring (*Clupea harengus pallasi*) eggs and larvae to beginning of exogenous feeding. *Can. J. Fish. Aquat. Sci.* **42:** 56–68.

Alderdice, D. F., and F. P. J. Velsen. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). *J. Fish. Res. Board Can.* **28:** 1545–1562.

Aneer, G., G. Florell, U. Kautsky, S. Nellbring, and L. Sjöstedt. 1983. *In-situ* observations of Baltic herring (*Clupea harengus membras)* spawning behaviour in the Ask6-Landsort area, northern Baltic proper. *Mar. Biol.* **74:** 105–110.

Barnhart, R. A. 1988. *Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (Pacific Southwest)—Pacific Herring.* U.S. Fish Wildl. Serv. Biol. Rep. 82 (11.79*)* U.S. Army Corps of Engineers, TR EL-82-4. 14 pp.

Batty, R. S., and J. H. S. Blaxter. 1992. The effect of temperature on the burst swimming performance of fish larvae. *J. Exp. Biol.* **170:** 187–201.

Blaxter, J. H. S., and L. A. Fuiman. 1990. The role of the sensory organs of herring larvae in evading predatory fishes. *J. Mar. Biol. Assoc. UK* **70:** 413–427.

Boehlert, G. W., J. B. Morgan, and M. M. Yoklavich. 1983. *Effects of Volcanic Ash and Estuarine Sediment on the Early Life History Stages of the Pacific Herring,* Clupea harengus pallasi. Oregon State University, Water Resources Research Institute, Corvalis, OR.

Bruton, M. N. 1985. The effects of suspensoids on fish. *Hydrobiologia* **125:** 221–241.

Bush, G. S. 1989. Rates and patterns of estuarine sediment accumulation. *Limnol. Oceanogr.* **34:** 1235–1246.

Carls, M. G., S. D. Rice, and J. E. 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*)*. Environ. Toxicol. Chem.* **18:** 481–493.

Carls, M. G., G. D. Marty, and J. E. Hose. 2002. Synthesis of the toxicological impacts of the *Exxon Valdez* oil spill on Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, USA. *Can. J. Fish. Aquat. Sci.* **59:** 153–172.

Carolsfeld, J., M. Tester, H. Kreiberg, and N. M. Sherwood. 1997. Pheromone-induced spawning of Pacific herring. *Horm. Behav*. **31:** 256–268.

Cavanaugh, G. M. 1956. Artificial seawater solutions. Pp. 62–69 in *Formulae and Methods IV of the Marine Biology Laboratory Chemical Room.* Marine Biological Laboratory, Woods Hole, MA.

Chapman, A. S., and R. L. Fletcher. 2002. Differential effects of sediments on survival and growth of *Fucus serratus* embryos (Fucales, Phaeophyceae). *J. Phycology* **38:** 894–903.

Cherr, G. N., and W. H. Clark, Jr. 1985. An egg envelope component induces the acrosome reaction in sturgeon sperm. *J. Exp. Zool.* **234:** 75–85.

Cherr, G. N., M. Morisama, C. A. Vines, K. Yoshida, E. H. Smith, T. Matsubara, M. C. Pillai, F. J. Griffin, and R. Yanagimachi. 2008. Two egg-derived molecules in motility initiation and fertilization in Pacific herring (*Clupea pallasi*). *Int. J. Dev. Biol.* **52:** 743–752.

Cloern, J. E. 1987. Turbidity as a control of phytoplankton biomass and productivity in estuaries. *Cont. Shelf Res.* **7:** 1367–1381.

Cloern, J. E., A. E. Alpine, B. E. Cole, R. L. J. Wong, and J. F. Arthur. 1983. River discharge controls phytoplankton dynamics in the northern San Francisco Bay estuary. *Estuar. Coast. Shelf Sci.* **16:** 415–429.

Conomos, T. J., and D. H. Peterson. 1977. Suspended-particle transport and circulation in San Francisco Bay: an overview. Pp. 82–97 in *Estuarine Processes, Vol. 2, Circulation, Sediments, and Transfer of Material in the Estuary*. Academic Press, New York.

Conomos, T. J., R. E. Smith, and J. W. Gartner. 2004. Environmental setting of San Francisco Bay. *Hydrobiologia* **129:** 1–12.

Czerkies. P., P. Brzuzan, K. Kordalski, and M. Luczynski. 2001. Critical partial pressures of oxygen causing precocious hatching in *Coregonus lavaretus* and *C. albula* embryos. *Aquaculture* **196:** 151–158.

Eldridge, M. B., and W. M. Kaill. 1973. San Francisco Bay area's herring resource: a colorful past and a controversial future. MFR paper 1018, *Mar. Fish. Rev*. **25:** 25–31.

Geffard, O., E. His, H. Budzinski, J. F. Chiffoleau, A. Coynel, and H. Etcheber. 2004. Effects of storage and method and duration on the toxicity of marine embryos of *Crassostrea gigas* oysters. *Environ. Pollut.* **129:** 457–465.

Geffen, A. J. 2002. Length of herring larvae in relation to age and time of hatching. *J. Fish Biol.* **60:** 479–485.

Gillis, D. J., B. A. McKeown, and D. E. Hay. 1990. Ultrastructural observations of the ovary and eggs, and the development of egg adhesion in Pacific herring (*Clupea harengus pallasi*). *Can. J. Fish. Aquat. Sci.* **47:** 1495–1504.

Griffin F. J., C. A. Vines, M. C. Pillai, R. Yanagimachi, and G. N. Cherr. 1996. Sperm motility initiation factor is a minor component of the Pacific herring egg chorion. *Dev. Growth Differ.* **38:** 193–202.

Griffin, F. J., M. C. Pillai, C. A. Vines, J. Kääriä, T. Hibbard-Robbins, R. Yanagimachi, and G. N. Cherr. 1998. Effects of salinity on sperm motility, fertilization, and development in the Pacific herring, *Clupea pallasi*. *Biol. Bull.* **194:** 25–35.

Griffin, F. J., M. R. Brenner, H. M. Brown, E. H. Smith, C. A. Vines, and G. N. Cherr. 2004. Survival of Pacific herring larvae is a function of external salinity. Pp. 37–46 in *Early Life History of Fishes in the San Francisco Estuary and Watershed,* F. Feyrer, L. R. Brown, R. L. Brown, and J. J. Orsi, eds. Proceedings of the symposium, Early Life History of Fishes in the San Francisco Estuary and Watershed, Santa Cruz, California, 20–23 August 2003, American Fisheries Society, Bethesda, MD.

Grosse D. J., and D. E. Hay. 1988. Pacific herring, *Clupea harengus pallasi,* in the northeast Pacific and Bering Sea. Pp. 34–54 in *Species Synopses: Life Histories of Selected Fish and Shellfish of the Northeast Pacific and Bering Sea,* N. J.Wilimovsky, L. S. Incze, and S. J. Westrheim, eds. Washington Sea Grant and Fisheries Research Institute, University of Washington, Seattle, WA.

Haegele, C. W., and J. F. Schweigert. 1985. Distribution and characteristics of herring spawning grounds and description of spawning behavior. *Can. J. Fish. Aquat. Sci.* **42 (Suppl. 1):** 35–55.

Hay, D. E. 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasi*). *Can. J. Fish. Aquat. Sci.* **42 (Suppl. 1):** 111– 126.

Hay, D. E., and C. C. Miller. 1982. A quantitative assessment of herring spawn lost by storm action in French Creek, 1980. *Can. Manuscr. Rep. Fish. Aquat. Sci.* **1636**.

Howarth, R. W., J. R. Fruci, and D. Sherman. 1991. Inputs of sediment and carbon to an estuarine ecosystem: influence of land use. *Ecol. Appl.* **1:** 27–39.

Incardona, J. P., T. K. Collier, and N. L. Scholz. 2004. Defects in cardiac function precede morphological abnormalities in fish embryos

exposed to polycyclic aromatic hydrocarbons. *Toxicol. Appl. Pharmacol.* **196:** 191–205.

Incardona, J. P., M. G. Carls, H. L. Day, C. A. Sloan, J. L. Bolton, T. K. Collier, and N. L. Scholz. 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. *Environ. Sci. Technol.* **43:** 201–207.

Ingram, B. L., and D. P. DePaolo. 1993. A 4300 year strontium isotope record of estuarine paleosalinity in San Francisco Bay, California. *Earth Planet. Sci. Lett.* **119:** 103–119.

Ingram, B. L., J. C. Ingle, and M. E. Conrad. 1996. A 2000 yr record of Sacramento–San Joaquin river inflow to San Francisco Bay estuary, California. *Geology* **24:** 331–334.

Kiorboe, T., E. Frantsen, C. Jensen, and G. Sorenso. 1981. Effects of suspended sediment on development and hatching of herring (*Clupea harengus*) eggs. *Estuar. Coast. Shelf Sci.* **13:** 107–111.

Kondolf, G. M. 2000. Changes in flow regime and sediment budget in the Sacramento–San Joaquin river system since 1850: implications for restoration planning. CALFED Bay-Delta Program Science Conference Abstracts, 2000. Oct 3–5, 2000.

Kowtal, G. V., W. H. Clark, Jr., and G. N. Cherr. 1986. Elimination of adhesiveness in eggs from the white sturgeon, *Acipenser transmontanus*: chemical treatment of fertilized eggs. *Aquaculture* **55:** 139–143.

Krone, R. B. 1979. Sedimentation in the San Francisco Bay system. Pp. 85–96 in *The Urbanized Estuary,* T. J. Conomos, ed. American Association for the Advancement of Science, Pacific Division, San Francisco.

Laale, H. W. 1980. The perivitelline space and egg envelopes of bony fishes. *Copeia* **1980:** 210–226.

Laine, P., and M. Rajasilta. 1999. The hatching success of Baltic herring eggs and its relation to female condition. *J. Exp. Mar. Biol. Ecol.* **237:** 61–73.

McKee, L. J., N. K. Ganju, and D. H. Shoellhamer. 2006. Estimates of suspended sediment entering San Francisco Bay from the Sacramento and San Joaquin Delta, San Francisco Bay, California. *J. Hydrol.* **323:** 335–352.

Morgan, J. D., and C. D. Levings. 1989. Effects of suspended sediment on eggs and larvae of lingcod (*Ophiodon elongatus*), Pacific herring (*Clupea harengus pallasi*), and surf smelt (*Hypomesus pretiosus*). *Can. Tech. Rep. Fish. Aquat. Sci. I-VII.* **1729:** 1–31.

Morrison, J. A., I. R. Napier, and J. C. Gamble. 1991. Mass mortality of herring eggs associated with a sedimenting diatom bloom. *ICES J. Mar. Sci.* **48:** 237–245.

Muenchow, G. 1986. Ecological use of failure time analysis. *Ecology* **67:** 246–250.

Newcombe, C. P., and D. D. MacDonald. 1991. Effects of suspended sediments on aquatic ecosystems. *N. Am. J. Fish. Manag.* **11:** 72–82.

Nichols, F. H., E. Cloern, S. N. Luoma, and D. H. Peterson. 1986. The modification of an estuary. *Science* **231:** 567–573.

Norton, B. L., M. A. Lewis, and F. L. Mayer. 1999. Storage duration and temperature and the acute toxicities of estuarine sediments to *Mysidopsis bahia* and *Leptocherius plumulosus*. *Bull. Environ. Contam. Toxicol.* **63:** 157–166.

Ojaveer, E. 2006. On the external and parental effects in early development of herring (*Clupea pallasi*) at the NE Kamchatka. *Fish. Res.* **81:** 1–8.

Pillai, M. C., T. S. Shields, R. Yanagimachi, and G. N. Cherr. 1993. Isolation and partial characterization of the sperm motility initiation factor from eggs of the Pacific herring, *Clupea pallasi. J. Exp. Zool.* **265:** 336–342.

Potter, I. C, L. E. Beckley, A. K. Whitfield, and R. C. J. Lenanton. 1990. Comparisons between the roles played by estuaries in the life cycles of fishes in temperate Western Australia and Southern Africa. *Environ. Biol. Fishes* **28:** 143–178.

Rankine, P. W., L. H. Cargill, and J. A Morrison. 1990. Variation in the hatching length of spring-spawned herring larvae (*Clupea harengus* L.) on Ballantrae Bank in the Firth of Clyde. *ICES J. Mar. Sci.* 1990 **46:** 333–339.

Rosenthal, H., and D. F. Alderdice. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. *J. Fish. Res. Board Can.* **33:** 2047–2065.

Ruhl, C. A., and D. H. Schoellhamer. 2004. Spatial and temporal variability of suspended sediment concentration in a shallow estuarine environment. *San Francisco Estuary and Watershed Science* **2**(2)**:** Article 1.

Ruhl, C. A., D. H. Schoellhamer, R. P. Stumpf, and C. L. Lindsay. 2001. Combined use of remote sensing and continuous monitoring to analyze the variability of suspended-sediment concentrations in San Francisco Bay, California. *Estuar. Coast. Shelf Sci*. **53:** 801–812.

Schweigert, J. F. 1993. Evaluation of harvesting policies for the management of Pacific herring stocks, *Clupea pallasi*, in British Columbia. Pp. 167–190 in *International Symposium on Management Strategies for Exploited Fish Populations.* G. Kruse, D. Eggers, R. J. Marasco, C. Pautzke, and T. J. Quinn, eds. Alaska Sea Grant College Program Report 93(2). University of Alaska, Fairbanks, AK.

Spratt, J. D. 1981. Status of the Pacific herring, *Clupea harengus pallasi*, resource in California 1972–1980. *Calif. Dep. Fish Game Fish Bull.* **171.** 107 pp.

Stacey, N. E., and A. S. Hourston. 1982. Spawning and feeding behavior of captive Pacific herring, *Clupea pallasi*. *Can. J. Fish. Aquat. Sci.* **39:** 489–498.

Vazquez, L. C. 2003. Effect of sperm cell density on measured toxicity from the sea urchin *Tripneustes gratilla* fertilization bioassay. *Environ. Toxicol. Chem***. 22:** 2191–2194.

Vines, C., T. Robbins, F. J. Griffin, and G. N. Cherr. 2000. The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). *Aquat. Toxicol.* **51:** 225–239.

Vines, C. A., K. Yoshida, F. J. Griffin, M. C. Pillai, M. Morisawa, R. Yanagimachi, and G. N. Cherr. 2002. Motility initiation in herring sperm is regulated by reverse sodium-calcium exchange. *Proc. Natl. Acad. Sci. USA* **99:** 2026–2031.

Watters, D. L., H. M. Brown, F. J. Griffin, E. J. Larson, and G. N. Cherr. 2004. Pacific herring spawning grounds in San Francisco Bay: 1973–2000. Pp. 3–36 in *Early Life History of Fishes in the San Francisco Estuary and Watershed,* F. Feyrer, L. R. Brown, R. L. Brown, and J. J. Orsi, eds. Proceedings of the symposium, Early Life History of Fishes in the San Francisco Estuary and Watershed, Santa Cruz, California, 20–23 August 2003. American Fisheries Society, Bethesda, MD.

Wilbur, D. H., and D. G. Clarke. 2001. Biological effects of suspended sediments: review of suspended sediment impacts on fish and shellfish with relation to dredging activities in estuaries. *J. Fish. Manag.* **21:** 855–875.

Williams, J., C. Roderick, and R. Alexander. 2003. Sublethal effects of Orimulsion-400® on eggs and larvae of Atlantic herring (*Clupea harengus* L). *Environ. Toxicol. Chem.* **22:** 3044–3048.

Yanagimachi, R., G. N. Cherr, M. C. Pillai, and J. D. Baldwin. 1992. Evidence suggesting the presence of a sperm-attracting substance around the micropyles of salmonid and herring eggs. *Dev. Growth Differ.* **34:** 447–461.