Impacts of Suspended Sediments in San Francisco Bay on Pacific Herring Larval Survival and Condition

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EXECUTIVE SUMMARY

This report describes the results of a laboratory study on the effects of suspended sediment particles on early post-hatch Pacific herring, *Clupea pallasi,* larval survivability and condition. The research was commissioned by the San Francisco Bay LTMS Environmental Windows Science Work Group and funded through the United States Army Corps of Engineers. The project adds to the scientific data regarding suspended sediment impacts on Pacific herring reproduction, thus providing a scientific platform on which to base regulation of dredging in San Francisco Bay during the herring reproductive season. A previous project demonstrated that if herring eggs were exposed to 250 mg/l of suspended sediment during the first two hours after eggs contacted water, embryo development was affected and larval survival and growth declined (Griffin et al. 2008; 2009). The current project examined direct sediment effects on larvae when present after hatching has occurred. This report presents evidence that supports a conclusion of no effect on hatched larvae from the presence of 400 mg/l or less of suspended sediment for 16 hr; neither 10-day survival nor condition of larvae subsequent to that exposure was impaired. The report also describes a suspended sediment delivery and testing system that was designed and used to maintain sediment particles in suspension for extended time periods while not harming larval herring. This sediment treatment system should be of use with other sensitive planktonic organisms.

San Francisco Bay is an important nursery area for marine fish, including Pacific herring; it supports reproduction of the southernmost stock of herring in the eastern Pacific Ocean. Pacific herring reproduce during Winter/Spring when input from freshwater sources is greatest. Freshwater input delivers sediments and dissolved nutrients into these bays and estuaries and thus the herring reproductive season coincides with times of high sediment input. It would not be unreasonable to suggest that although Pacific herring early life stages may have adapted to their presence, suspended sediments can, at some level, impact growth and survival of embryos or larvae. At question is whether environmental sediment levels in San Francisco Bay, which may exceed 500-600 mg/L at least on short, daily to hourly time scales, are deleterious to herring reproduction. The previous report (Griffin *et al.* 2008; 2009) concluded that the first two hours after eggs first contact water (adhesion development period) was a critical time. This first two hrs is the adhesion development period, a time when the embryos remain “tacky or sticky” and sediment particles can irreversibly adhere to embryos. Effects of adherence of sediment particles to embryos included precocious larval hatch, smaller size at hatch, and reduced larval survival. In addition, hatched larvae from sediment exposed embryos were more likely to be abnormal; abnormalities included larvae that did not survive because they were exhibited bent spines (scoliotic) or did not exit from the tightly coiled posture of pre-hatch larvae. These sediment effects were not seen if Pacific herring embryos were exposed for 2 hrs after the adhesion development period.

As in the initial project that determined the 2 hr window of susceptibility for embryos, sediments from two sources were utilized in the current project, commercially available Fuller’s earth and San Francisco Bay sediments obtained through the U.S. Army Corps of Engineers from the dredging of the Port of Redwood City (PRC); the latter were analyzed by the U.S. Army Corps of Engineers and found to be relatively uncontaminated. Procedures for sediment preparation, handling and storage followed

those used in the earlier project (Griffin et al. 2008; 2009). Fuller's earth was used during modification and testing of the sediment treatment system

Two important variables that influence the effects of suspended sediments are particle concentration and duration of exposure. To investigate sediment effects on herring larvae we used environmentally relevant particle concentrations for San Francisco Bay of 200-400 mg/l and exposures of 16 hr. The upper limits of particle concentrations in San Francisco Bay rarely exceed 500 mg/l and the duration of elevated levels is thought to be less than one tidal cycle (~12 hrs) due to reversal of tidal currents within a cycle. Post-hatch herring larvae were incubated in suspended sediments (Fuller's earth or PRC sediment) for 16 hrs after which they were transferred to sediment-free water and static cultured for up to 10 days. There were no mortalities during the 16 hr suspended sediment incubation. During the post-sediment treatment culture period survival, growth, and condition were measured. Survival was not decreased by prior sediment treatment. In addition, larval condition and growth were not affected by sediment treatment. Three criteria for larval condition were used: heart rate, prey capture, critical swimming velocity.

The sediment treatment apparatus was a temperature controlled re-circulating system in which sediment suspension and sediment delivery to larvae was accomplished with independent submersible pumps. Sediment was suspended from the bottom of the sediment treatment tank, a round, conical bottom tank that contained 48 liters of suspended sediment water. A sediment suspension pump was situated at the bottom of the tank and drew in those sediment particles that were settling and redistributed and resuspended them. A ½ x ½ inch square plastic grid baffle was placed above the sediment suspension pump. The second pump was situated above the baffle, but below three larval test chambers, and connected to the larval chambers via a flow-control manifold and airline tubing. The larval test chambers were 2 liter Nalgene graduated beakers with Nitex mesh bottoms. The chambers were suspended from the top of the water column in the treatment tank. Sediment water was delivered from the second pump to the top of each of the larval chambers. Thus, sediment water entered the top of each larval chamber and exited the bottom, reducing the need for mixing within the chambers.

ACKNOWLEDGEMENTS

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INTRODUCTION

Previous laboratory research demonstrated that suspended sediments do have an impact on embryonic development of Pacific herring, *Clupea pallasi,* if they are present during the first two hours after release of eggs into water (Griffin *et al.,* 2008; 2009). These first two hours correspond to the period of time when the egg/embryo adhesive layer is forming and when sediment particles attach permanently to eggs/embryos. In laboratory experiments, sediment presence during this time caused eggs to aggregate into multiple layers and increased mortality. Less dramatic, yet significant, effects were also seen in eggs that did not cluster into multiple layered aggregates. When sediment particles were present during the initial 2 hr period at concentrations equal to or greater than 250 mg/L, larvae hatched sooner, were smaller, exhibited more abnormalities, and experienced reduced survival (Griffin *et al.,* 2008; 2009). Treatment of embryos for two-hour periods of time or longer subsequent to the initial two-hour period had no significant effect on embryos or larvae. In addition, two-hour pulse sediment treatment of hatched larvae did not reduce larval survival to 15 days post-hatch. The current project extends our understanding of suspended sediment effects on Pacific herring early life stages by investigating whether sediment treatment of hatched larvae over a prolonged period of time reduces condition, as evidenced by impacts on growth and behavior, and thus future survival. This project, in response to an RFP from the San Francisco Bay LTMS Environmental Windows Science Assessment and Data Gaps Work Group, Herring Subgroup, was directed at the following objective: Determine effects, if any, of suspended sediments on survival, growth, and condition of early stage Pacific herring larvae (up to 2 weeks post-hatch).

San Francisco Bay is a nursery and/or migration corridor for several marine and anadromous fish species, including Pacific herring, but it is also a heavily urbanized estuary where anthropogenic activity can and has impacted life stages of the species that use the estuary. Dredging of shipping lanes, channels, and harbors is thought to be one such activity. Dredging supports commercial ship and recreational boat traffic by maintaining depths of commercial shipping lanes, boat channels, and harbors that fill with sediment over time. San Francisco Bay is a shallow estuary (current average depth of < 14 m; USGS, 2010) with much of the substratum the result of anthropogenic sediment input that began over 150 years ago with Sierra Nevada placer mining (Krone, 1979; van Geen and Loma, 1999). Fine silt & clays at concentrations thought to be as high as 5×10^6 metric tons per year entered and settled out in the estuary during 1856-1887 (van Geen and Loma, 1999; Kondolf, 2000). Since that time re-suspension and redistribution of existing sediments as well introduction of new sediments have made dredging a necessity.

The highest loads of suspended sediments occur during the Winter/Spring, the Pacific herring reproductive season, due to increased freshwater flows from the watersheds and storm driven mixing within the estuary (Krone, 1979; Ingram and DePaolo, 1993; Ingram *et al.,* 1996; Watters *et al.,* 2004; McKee *et al.,* 2006). Ambient suspended sediment loads due to re-suspension of resident sediments and/or introduction of new sediments vary depending on weather and freshwater outflow to the Bay, but range from 50 mg/L to 500-600 mg/L (Ruhl and Shoelhamer, 1998, 2004; Ruhl *et al.,* 2001; Ganju *et al.,* 2004; McKee *et al.,* 2006). Dredging activity locally adds to this

background ambient turbidity by re-suspending fine sediments. Concentrations of suspended sediment due to dredging can reach 800-1,000 mg/L in dredge plumes over a distance of up to 1,000 m depending on dredge type and environmental conditions (Wilber and Clarke, 2001). Within San Francisco Bay the persistence of a dredge plume is hypothesized to be in part dependent on tidal currents that reverse every 6 hrs (Wilber and Clarke, 2001).

The input of sediment from watersheds is important to estuarine productivity because it replenishes nutrients and substrata, but it can also be problematic (Cloern *et al.,* 1983; Bruton, 1985; Cloern, 1987; Potter *et al.,* 1990; Newcombe and McDonald, 1991). High sediment loads can: 1) reduce light penetration which affects primary productivity (both in the water column and on the benthos) and can alter the vertical distribution and behavior of planktonic organisms, phytoplankton and zooplankton; and 2) cover and smother benthic organisms, including eggs and embryos, due to particle settling (Bruton, 1985; Cloern, 1987; Potter *et al.,* 1990; Newcombe and McDonald, 1991). In addition to direct effects, suspended sediments either entering from watersheds or from re-suspension of resident particles can contain toxicants (Conomos and Peterson, 1977; Bush, 1989; Howarth *et al.,* 1991).

Pacific herring eggs and embryos attach to biological and non-biological substrates at spawning and remain attached until hatching, usually 10-14 days after fertilization in San Francisco Bay (Eldridge and Kaill, 1973; Hay, 1985; Griffin *et al.,* 1998). Blaxter (1962) recognized eleven larval stages that Atlantic herring pass through, based on body length and definable changes in the larval skeleton. Humphrey *et al.,* (1995) simplified larval staging for Pacific herring by recognizing four stages, along with intervening substages: Stage 1 is the yolk-sac period; Stage 2 includes the preflexion, dorsal fin development period; Stage 3 is the period when flexion occurs and the caudal fin is not yet forked; Stage 4 is the post-flexion period during which the caudal fin is forked. The current study focused on the first two, Stage 1 and Stage 2. Yolk-sac larvae acquire the ability to feed before the end of Stage 1. Herring larvae do not have functioning jaws at hatching and it is during Stage 1 that the ability to capture and ingest prey is developed. In British Columbia herring, Stage 1 persists for 4-5 days post-hatch during which time the yolk-sac is absorbed as larvae utilize yolk for nutrition; larvae feed by the 5th to 8th day post-hatch (Alderdice and Hourston, 1985; Middaugh *et al.,* 1998). Once feeding does commence, copepods are the primary prey taken by herring larvae (Grosse and Hay, 1988), however, other invertebrate larvae and tintinid ciliates are also prey for herring larvae (Hourston, 1958, 1980; Middaugh *et al.,* 1998, Bollens *et al.,* 2004). Growth during Stage 1 is around 0.2 mm/day and larvae may reach 10 mm in length by the end of the Stage (Erlich *et al.,* 1976; McGurk, 1984, 1985). During Stage 2 the dorsal fin develops from a primordial structure extending from just behind the head and larvae continue to grow and change morphologically. Growth rates and morphological changes associated with growth, such as length/weight ratio changes and eye height/head height ratio changes have been published (Ehrlich *et al.,* 1976). Larval growth averages 0.48-0.52 mm per day in British Columbia herring and larvae can reach up to 4 cm in length within a month (Hourston, 1958; Alderdice and Hourston, 1985).

The impact of suspended sediment on hatched larvae after they have left the protection of the chorion has received little attention. Although our previous study focused on sediment exposure of eggs and early embryos, we did show that a 2 hr

exposure of hatched larvae to sediments, either 1 day or 10 days after hatch, had no effect on 15-day survival (Griffin *et al.,* 2008). There have been studies that showed direct exposure of hatched-larvae to suspended sediments and toxicants can affect larval behavior and condition as measured by larval position in the water column, larval swimming ability, and prey capture by larvae (Brett 1964, 1967; Swenson and Matson, 1976; Boehlert and Morgan, 1985; Fox *et al.,* 1999; Auld and Schubel 1978; Morgan *et al.,* 1973; reviewed by Wilber and Clarke, 2001; Colby and Hoss, 2004; Utne-Palm, 2004). In most of these studies sediment loads of 500 mg/L either had no effect or had a positive effect. For example, suspended sediment concentrations of 500 or 1,000 mg/L enhanced prey capture by Pacific herring larvae (Boehlert and Morgan, 1985; see Colby and Hoss, 2004). However, Fox *et al.,* (1999) reported that Atlantic herring larvae reduced the depth at which they fed when turbidity was increased and this can impact survival. Although studies linking suspended sediments and larval condition have been conducted, to our knowledge there have been no reports that link sediment exposure after hatching directly to survival of herring larvae. The overall question addressed by the current study was: Does the presence of suspended sediments during early post-hatch stages decrease larval survival, growth, and/or condition? We report that up to 20 hrs of exposure to environmentally relevant suspended sediments did not affect mortality, growth, or measurable larval condition for up to 10 days.

MATERIALS AND METHODS

Solutions

Half-strength seawater at 16 practical salinity units (psu) was made by diluting 0.45 µm filtered seawater 1:1 with distilled water. Seawaters were stored at 12°C. Salinity was monitored with a salinometer. Calcium and magnesium-free (CaMgF) half-strength seawater made by diluting full-strength CaMgF seawater was made according to Cavanough (Cavanough, 1975) with equal parts distilled water. CaMgF polyvinyl alcohol (PVA) was made by adding 0.25% PVA to 16 psu CaMgF water and stored at 4°C. All chemicals were obtained from Sigma/Aldrich (St. Louis, MO).

Sediment Storage and Handling

Fuller's earth (Sigma/Aldrich) and San Francisco Bay dredged sediment from the Port of Redwood City (U.S. Army Corps of Engineers, San Francisco, CA) were utilized in different experiments. Both sediments were processed as described by Griffin *et al.,* 2009. Fuller's earth was stored dry as packaged by the manufacturer. Sediments from the Port of Redwood City (SF Bay), obtained prior to the 2006 reproductive season, were stored in glass-lidded jars (250 ml) at -20°C. Chemical analysis of the San Francisco Bay sediment showed three compounds or elements that were above SF Bay background levels (chromium, mercury, and silver were less than 125% of background (unpublished USACE, 2005). Biological toxicity testing utilized the amphipods, *Rhepoxynius abronius* and *Ampelisca abdita*, with survival at greater than or equal to 90%. (unpublished USACE, 2005).

Sediments were prepared for larval experiments by suspension and washing as described by Griffin *et al.,* (2008, 2009) except that the final stock concentration was 4 g/L Six liters of 4 g/L suspended sediment were required to supply a targeted concentration of 500 mg/L in the 48 liters of water in the experimental treatment tank. Eight grams of Fuller's earth or 12 grams of SF Bay sediment provided a final stock solution of 4 g/L. Weighed sediments were suspended in 1 L of 16 psu seawater at 4°C, stirred vigorously with a magnetic stir bar for 60 minutes, 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 then washed by re-suspension in 1 liter of clean 16 psu seawater followed by stirring for 60 minutes and overnight settling of particles; this was done twice. After the second wash re-suspended sediment particles were gravity filtered twice through Whatman #2 filters. Sediment concentration of the filtered suspended sediments was determined by obtaining sediment dry weight in a known volume. A 100 ml aliquot of filtered suspended sediment was removed and suction filtered onto a 0.45 µm analytical filter. The filter was dried overnight in a 60°C drying oven and the weight of the dried sediments determined. We also used a standard suspended sediment concentration curve based on absorbance at 546 nm in a Biomate spectrophotometer (Thermo Spectronic) of known concentrations of suspended Fuller's earth. This standard curve was used to validate dry weight concentration determinations.

Acquisition of Gametes, Fertilization and Embryo Culture

Pacific herring were obtained by hook and line, throw net, or otter trawl from San Francisco Bay and transported on ice to the Bodega Marine Lab during the 2008-2009 and 2009-2010 San Francisco Bay herring reproductive season (January to March). Ovaries and testes were dissected from gravid animals and kept refrigerated in petri dishes at $4^{o}C$ until used (up to 4 days; Yanagimachi *et al.,* 1992). In preparation for fertilizations, sperm were pooled from 2-3 males diluted into 16 psu seawater and adjusted to a stock suspension of 10^{8} sperm/ml. Eggs were either removed from dissected ovaries with a metal spatula and directly distributed into experimental containers or distributed into 16 psu Ca^{2+} and Mg^{2+} free seawater containing 0.25% polyvinylalcohol (1/2 CaMgF PVA) and then distributed to experiments via pipette (Griffin *et al.,* 1998). Eggs from 2-4 females were pooled.

Fertilizations were conducted in 10 x 20 cm rectangular PYREX®dishes in which the bases of the dishes served as the substrate for egg attachment. After addition of sperm (10^{5} cells/ml) to 400-500 ml of 16 psu sediment-free seawater, about 500 herring eggs were distributed evenly into the dishes to ensure that eggs attached to the bottoms of dishes in a monolayer. This enhances successful embryo development to larval hatch (Griffin *et al.,* 2009). The eggs were co-incubated with sperm for 2 hr in a $12^{o}C$ incubator after which two water changes were made to remove excess sperm. The embryos then were cultured in the $12^{o}C$ incubator until hatching (7 -12 days). The dishes were monitored, dead embryos removed, and water was changed (100% exchange) daily.

Larvae were transferred to clean PYREX® dishes within 24 hr of hatching. After transfer, 75% of the water was replaced with clean water. To prevent loss of larvae during water changes, larvae were induced to congregate into one end of the dishes with a fiber optics light, and water was removed from the opposite end by vacuum siphoning using a disposable pipette that was attached to a vacuum pump. Hatching proceeded for 2-3 days, during which time larvae were pooled based on the embryo dish from which they hatched. Although the number of hatched larvae varied with experiment, larval density was maintained in the PYREX® dishes at 50 larvae per 100 ml.

Sediment Treatment System

The Sediment Treatment System was built and first utilized during the 2008-09 herring reproductive season. During that season the numbers of available reproductive herring were much reduced over previous years and gamete quality was low. At no point during the season did we have sufficient numbers of larvae to conduct full experiments, however, we were able to obtain enough larvae to test modifications and refinements to the design of the sediment treatment system that corrected two major problems. One problem was that a mixing current that was high enough to maintain sediment in suspension also generated currents in the larval chambers that damaged larvae. The other problem arose when larvae were transferred out of chambers and into post-sediment treatment culture dishes. Initially this was done with plankton nets, however, because of the turbidity in the larval treatment chambers, not all larvae were collected and netting also damaged larvae.

The larval chambers used during the 2009-10 season were 2 L plastic beakers with the bottoms removed and replaced with 500 micron Nitex mesh. Chambers were placed in a plastic ring-like jacket equipped with an arm that hung over the side of the

treatment tank and suspended the larval chambers inside the sediment treatment tanks such that approximately ¾ of each larval chamber was submerged (**Fig. 1**). Three larval chambers were placed inside each tank. Sediment treatment tanks were re-circulating conical tanks that contained either 48 liters of suspended sediment or sediment-free water. Sediment was kept in suspension and water circulated with a 17 liter/min Aqua Clear 50 submersible sediment suspension pump that was glued to the bottom of the treatment tank. Output from the pump was through a PVC T-connector that created two oppositely directed circular currents at and parallel to the bottom of the tank. This configuration re-suspended sediment as it collected near the bottom of the tank, but also created a vortex; in the original testing (2008-09 season) we found that the vortex's downward current pulled larvae onto the Nitex screen bases. To neutralize the vortex without reducing sediment mixing, a ½ x ½ inch square plastic grid baffle was placed above the mixing pump. This baffle deflected the vortex, which reduced the downward current through the chambers such that larvae were able to maintain in the water column of the chambers. A manifold supply submersible pump (Aqua Clear 30) was mounted on the tank wall above the baffle. The output of this pump was directed via aquarium airline tubing to multiport manifolds at the top of each treatment tank which supplied re-circulated treatment tank water to the top of each larval chamber Flow rates to each larval chamber were adjustable with the manifold. Thus, there was a constant flow of water and suspended sediment, entering at the top and exiting through the 500 micron mesh bottom in each larval chamber. The sediment treatment tanks were in turn placed in a square 4 ft x 4 ft x 2 ft deep tank that contained flow-through seawater for temperature control (**Fig. 2**).

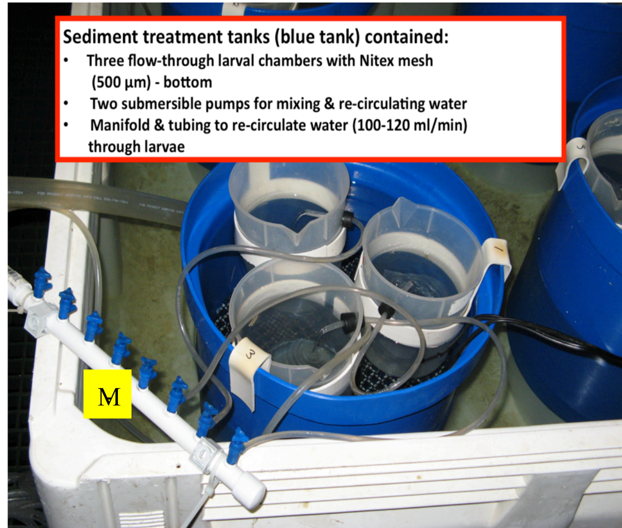

Figure 1. Sediment treatment tank and larval chambers. Each of the three larval chambers was supplied at the top of each chamber with recirculating water through a flow-control manifold (M) that in turn was supplied from a pump situated below the larval chambers.

Figure 2. Sediment Treatment System. Four treatment tanks, each containing 3 of the larval chambers were inserted into a 4'x4'x2' temperature control tank.

The ability of the system to maintain sediment in suspension was tested without larvae present. Forty-eight liters of 500 mg/ml suspended Fuller's earth were monitored over 20 hrs after addition of 6 liters of 4 g/l sediment water to 42 liters of sediment-free water. Beginning at 15 min after sediment addition aliquots of water were withdrawn by pipette from each larval chamber hourly and assayed for sediment concentration with a Biomate spectrophotometer (Thermo Spectronic, Madison, WI), absorbance at 546 nm, using a standard curve developed with Fuller's earth (Griffin *et al.,* 2008; 2009)

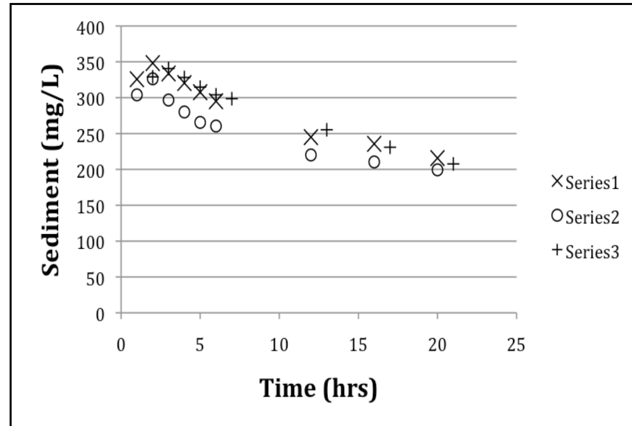

Figure 3. Test suspended sediment concentrations in sediment treatment system. Six liters of 4 g/L of Fullers earth was added to 42 L of water (expected final concentration of 500 mg/L) and sampled hourly for 20 hr. Three replicates were conducted in the absence of larvae (X, O, +).

Sediment concentration increased within larval chambers during the first 2 hrs of sediment system tests with a decline over the subsequent 20 hrs (**Fig. 3**). At no time did the concentration within larval chambers reach 500 mg/ml. Since the 2 hr sediment concentration was the highest measured, it was denoted as the starting suspended sediment concentration. For the three test runs, starting concentration averaged 338.37 mg/l $\pm$ 11.12 s.d. The decrease in suspended sediment particles through hour 5 was 45.98 mg/l $\pm$ 12.96 s.d. of the starting concentration (average rate of loss was 15 mg/l/hr) and particle settling over the next 15 hrs (5-20 hrs) totaled 84.81 mg/l $\pm$ 16.3 s.d. of starting sediment concentration (average loss of 5.7 mg/l/hr). Ending suspended sediment concentration was 207.58 mg/l $\pm$ 8.2 s.d. at hr 20 (61.34% + 0.55 s.d. of the starting concentration). In all of the test experiments the lowest sediment concentration occurred at experiment termination (20 hrs). Accumulation of sediment that had come out of suspension was observed in two areas of the system. Sediment accumulated around the bottom perimeter inside the larval chambers where the Nitex mesh was glued to the chamber; this did not impact the Nitex mesh or the flow of water through it and the chambers. Sediment also collected on the walls of the treatment tanks at or near the bottom and probably represented the major cause for decrease in suspended sediment concentration.

Larval Sediment Treatment

The sediment treatment apparatus was equilibrated prior to each experiment. Sediment-free water was added to the control and sediment treatment tanks 24 hrs prior to an experiment. Both the mixing and re-circulating pumps were activated to insure that the system was working properly and that temperature was stabilized. Five hours prior to each experiment, 6 liters of stock suspended sediment (4 g/l) was added to the experimental treatment tank after removal of 6 liters of sediment-free water. Mixing was allowed for 2 hrs, after which samples of water were removed from each of the 3 larval chambers by pipette for sediment concentration determination; this sample was denoted

as the beginning suspended sediment concentration. Samples of water were also removed at experiment termination and denoted as ending suspended sediment concentration.

Larvae were transferred from culture dishes to larval chambers in the sediment treatment apparatus by gently pouring the larvae into the chambers (numbers of larvae added varied between experiments, 200-400, but were similar between replicate larval chambers). Larvae were incubated in the system for 16 hrs after which they were removed to clean glass culture dishes. Larvae were transferred out of the chambers by first enclosing each larval chamber from the bottom with a plastic freezer bag; this allowed us to remove larval chambers, water and larvae from the treatment tank without handling the larvae. The larvae and water from each chamber were gently poured into culture dishes. Water was exchanged twice via vacuum siphoning and addition of sediment-free water. The larvae were cultured at a density of 50 larvae per 100 ml, 12°C, to monitor growth and survival. Mortalities were removed daily (**Fig. 4**).

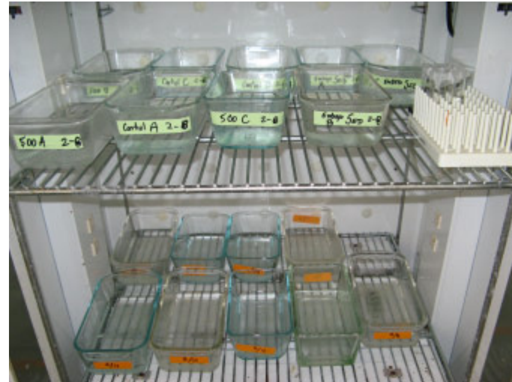

Figure 4. Larval culture. After sediment treatment larvae were removed to glass larval culture dishes and incubated in a temperature controlled chamber at 12°C for 10 days.

Aliquots of live larvae were photographed using a Nikon AZ100 stereo zoom microscope controlled by NIS Elements Imaging Software 3.10 (Melville, N.J.). Larval length was measured with ImageJ software (National Institutes of Health) measurement functions and calibrated against collected images of a 2 mm stage micrometer.

Heart Rate

Heart rate was determined from AVI image files of larvae. Larvae were transferred by disposable pipette to slides containing water with 100 μg/ml MS222 (in tris-buffered saline). The slides were mounted on a temperature controlled slide holder that was attached to a Nikon AZ100 stereo zoom microscope. Ten second AVI images were collected with a Photometrics CoolSNAP HQ^2 camera using NIS Elements Imaging Software 3.10. The number of heartbeats in each 10 sec segment was transcribed into beats/min.

Prey Capture Test

Larvae after treatment were co-incubated with rotifers and the number of rotifers captured was scored. Ten to twenty larvae were transferred to 100 ml circular glass dishes containing 16 psu seawater. Rotifers (1500-2000) were added and co-incubated with the larvae for 4 hrs at 13°C in ambient light. After 4 hrs, larvae were sedated with MS222 briefly and digital images of larvae were captured using dark-field optics on an Olympus stereo zoom microscope. Images of larvae were later scored for the number of rotifers in each larva's digestive tract.

Critical Swimming Test

The ability of sediment treated and control larvae to withstand incremental increases in current was tested. Brett (1964, 1967) introduced the incremental velocity (or increased velocity) test to quantify swimming performance of fish. During the incremental velocity tests, the larvae are forced to swim against a current that is incrementally increased in steps until exhaustion occurs and the fish are unable to maintain against the current; this is the fatigue or critical swimming speed (U_{crit}). Based upon recent larval swimming reports (Fisher, 2005, Bellwood & Fisher, 2001) and preliminary tests run on herring in our laboratory, three-minute time intervals were selected and used between steps or velocity increments (t_i).

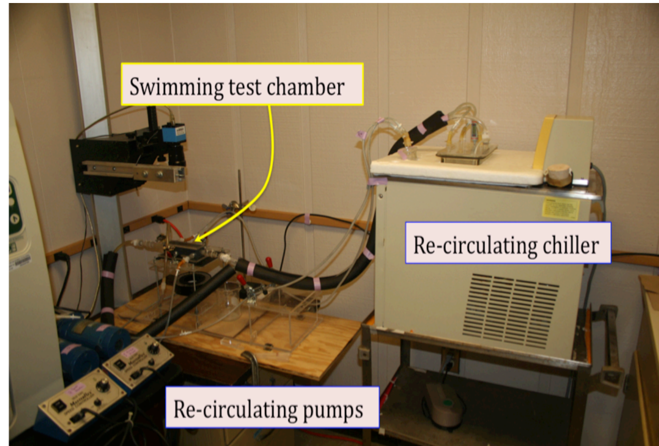

Figure 5. Critical Swimming Test System.

Test Chamber: The test system, designed and constructed by Edmund Smith, consisted of a tubular test chamber, a rectangular cooling chamber, a re-circulating temperature control 16 psu water supply, cool illumination, and a black and white digital CCD camera connected to a computer (**Figs. 5 & 6**). A tubular flow chamber was used for the swimming behavior tests. The tubular test chamber was 150 mm long and 12.6 mm in diameter. Bundles of 0.54 mm diameter microcap pipettes cut to a length of 33.8 mm and inserted into each end of the tubular test chamber provided up current and down current barriers that contained the larvae within the test chamber. These barriers also ensured a linear flow through the flow tube. Plastic tubing (6.23 mm ID) was attached to input and outlet ends of the tubular test chamber and passed through a Cole-Parmer 1-100 rpm peristaltic pump (connected to a Masterflex speed controller) to a 3 liter glass container containing 16 psu seawater. Thus the larval test chamber was a continuous flow-through system of recirculating water where the peristaltic pump pushed water into the inlet end and pulled water from the outlet end of the test chamber. The 3 liter glass container was covered with a Plexiglass plate and placed in a Neslab RTE 221 water bath for temperature control of the 16 psu seawater. An air stone oxygenated the water in the 3 liter container.

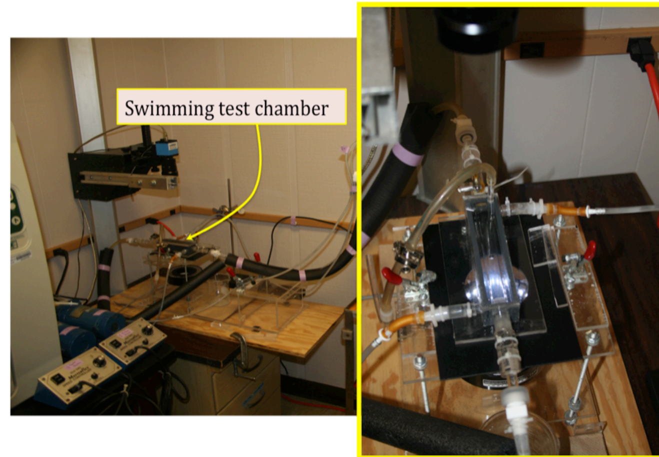

Figure 6. Swimming Test Chamber

The tubular tests chamber sat in a clear rectangular cooling water jacket in which that measured 110.5 mm long, 23.8 mm wide and 25.3 mm deep. This rectangular

cooling chamber was connected through a second peristaltic pump to a flask of cooling water that was kept in the Neslab RTE 221 water bath. Temperature was controlled at 12° C$\pm$ 0.5^{o} C within the tubular test chamber.

The characteristics of the velocity profile in the chamber are important in the consideration of swimming speed. The tubular design of the test chamber and the long fine tube bundles at each end maintained laminar flow at higher velocities. Based on dye releases in the test chamber it would appear that the linear flow and tubular construction eliminated most turbulence and wall effects along the length of the test chamber.

Lighting System: Because the herring larvae are nearly transparent it was necessary to produce near dark field images that could be detected by the digital CCD camera. An adjustable intensity, 40 LED circular light was used (120 cm diameter, Advanced Illumination, Edmunds Scientific). This lighting unit was placed 70 mm under the test chamber (clear bottom section). A black plastic plate with 60 cm hole was placed between the bottom of the test chamber and the light source. An Imaging source (DMK 31 BT03) digital black and white CCD camera with a Fuijinon TV 1:14/25 lens was located 240 mm above the bottom of the clear test chamber. The system components, camera, LED ring light, black plate and test chamber were carefully centered to assure optical alignment. The angle of each of the LEDs in the optic ring converged the light to form a near dark field image. The CCD images were monitored and recorded using a Dell Workstation running on Linux operating system.

Test Protocol: Incremental increases in test chamber water flow were accomplished with the 10 step Masterflex speed controller. The actual flow through the tube was calculated for each step by measuring the time it took to fill a 100 ml graduated cylinder for each pump step. The process was repeated 10 times for each step. Velocity was calculated by using the following formula:

$$V = 1.274\ q/d^2$$

V = Velocity in mm/sec
q = Volume in ml/sec
d^2 = Tube Diameter (inside measurement) in mm

Base on these calculations the step velocities were as follows:

Step 2 = 0.75 cm/sec
Step 3 = 2.97 cm/sec
Step 4 = 4.99 cm/sec
Step 5 = 7.53 cm/sec
Step 6 = 9.66 cm/sec
Step 7 = 11.99 cm/sec
Step 8 = 13.82 cm/sec
Step 9 = 15.74 cm/sec
Step 10 = 15.74 cm/sec

Criteria for acceptance of a test: Larvae had to swim for a 10 min acclimation period without a current and for three-minutes at step 2 current velocity before the test

was begun. Velocity was then increased in steps at 3 min intervals until fatigue occurred. If a larva could not maintain at least one body length off the downstream barrier for 1 minute it was considered fatigued**.** Larvae had to be able to regain swimming ability after the current was stopped in order to be used as valid data. Only data from larvae that could activity swim, had no visible morphological abnormalities, and were able to recover from fatigue (swim in the absence of current at the end of the test) were considered valid tests.

The fatigue speed or critical speed (U_{crit}) was calculated as:

$$U_{crit} = Vp + (t_f / t_i \times Vi)$$

Vi = velocity increment (difference in velocity between steps in cm/sec)
Vp = penultimate velocity as which the larvae swam before fatigue
t_f = elapsed time from the velocity increase to fatigue
t_i = time between the velocity increments

The differences between the velocity steps (Vi) are listed below. They were not evenly spaced because of the limitations of the pump controller and pumping process. However, the incremental changes through Step 7 were consistent and this is within the U_{crit} range for Pacific herring larvae. The following values for Vi were used in the calculations of U_{crit}.

Steps	Increment Change (cm/sec)
2 -3	0.74 – 2.97 = 2.23
2 - 4	2.97 – 4.99 = 2.02
4 - 5	4.99 – 7.53 = 2.54
5 - 6	7.53 – 9.66 = 2.13
6 - 7	9.66 – 11.99 = 2.33
7 - 8	11.99- 13.82 = 1.84
8 - 9	13.82 – 15.74 = 1.91
9 - 10	15.74 – 15.74 = 0

Statistical Analysis

Data were collected on Excel spreadsheets (Microsoft Corporation) and analyzed using Excel and SYSTAT (SYSTAT Software, Inc) software. Unless otherwise identified, data in Figures are presented as means $\pm$ standard deviations (minimum n = 3). Student t-tests were used to determine significance of differences between sediment treated larvae and controls. A grand mean of four experiments was utilized to determine overall mortality and larval length differences between control and sediment-treated larvae.

RESULTS

Suspended Sediment Concentrations

Suspended sediment concentrations in the 16 hr experiments with herring larva were more variable than test runs, but showed similar declines from starting concentrations. Ending concentrations ranged from 195 to 366 mg/L. In two of the experiments using Fuller's earth, final concentration was 316 mg/L and 281mg/L respectively. In the two experiments with SF Bay sediment ending concentrations were 195mg/L and 366 mg/L. Thus, suspended sediment declined in all larval experiments and appeared to decline slightly more with SF Bay sediment. However, the ending suspended sediment concentrations across 4 experiments was 200 mg/L or greater.

Larval Survival and Growth after Sediment Treatment

In all 4 experiments Pacific herring larvae survived 16 hrs in 200-400 mg/L suspended sediment. Furthermore, larvae survived subsequent transfer to and culture in sediment-free water for 10 days. Ten-day survival of sediment treated larvae averaged 85.4% over the four experiments compared to 85.8% for controls. Mortality rates averaged 1.46% (0.6-2.9% per day) for sediment treated larvae, and 1.42% (0.8-1.9% per day) for controls. The grand mean for overall cumulative mortality on day 10 was 14.61 % $\pm$ 1.35 s.e. for sediment treated larvae compared to 14.16 % $\pm$ 1.1 s.e. for control larvae (P = 0.901; see **Fig. 7**).

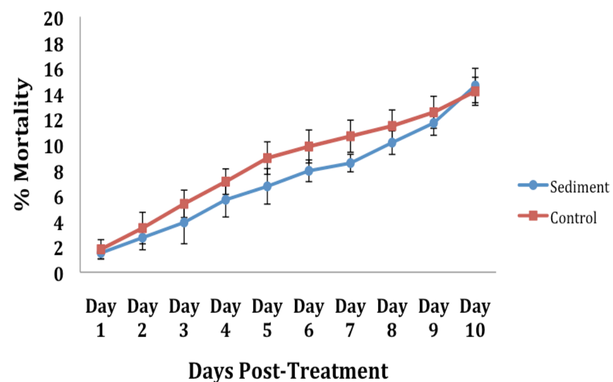

Figure 7. Grand mean of cumulative mortality. Average mortality of experiments depicted in Figure 8 was used to develop a grand mean. Cumulative mortality through day 10 for sediment treatment and controls was not significantly different. Values represent averages $\pm$ SE (P = 0.901).

Two of the four experiments utilized Fuller's earth and two experiments used SF Bay sediment. Larval mortality in experiments #4 (Fuller's earth) and #7 (SF Bay sediment) was less than 10% and remarkably similar to controls (**Fig. 8**); there was no statistical difference between sediment treated and control larvae in either (P = 0.0798 and P = 0.073 respectively). In experiment #5 (SF Bay sediment) overall mortality appeared higher for control larvae and in #6 (Fuller's earth) appeared higher for sediment treated larvae, however, there was no significant difference between controls and sediment treatments in either experiment (P = 0.076 and P = 0.084 respectively; **Fig. 8**).

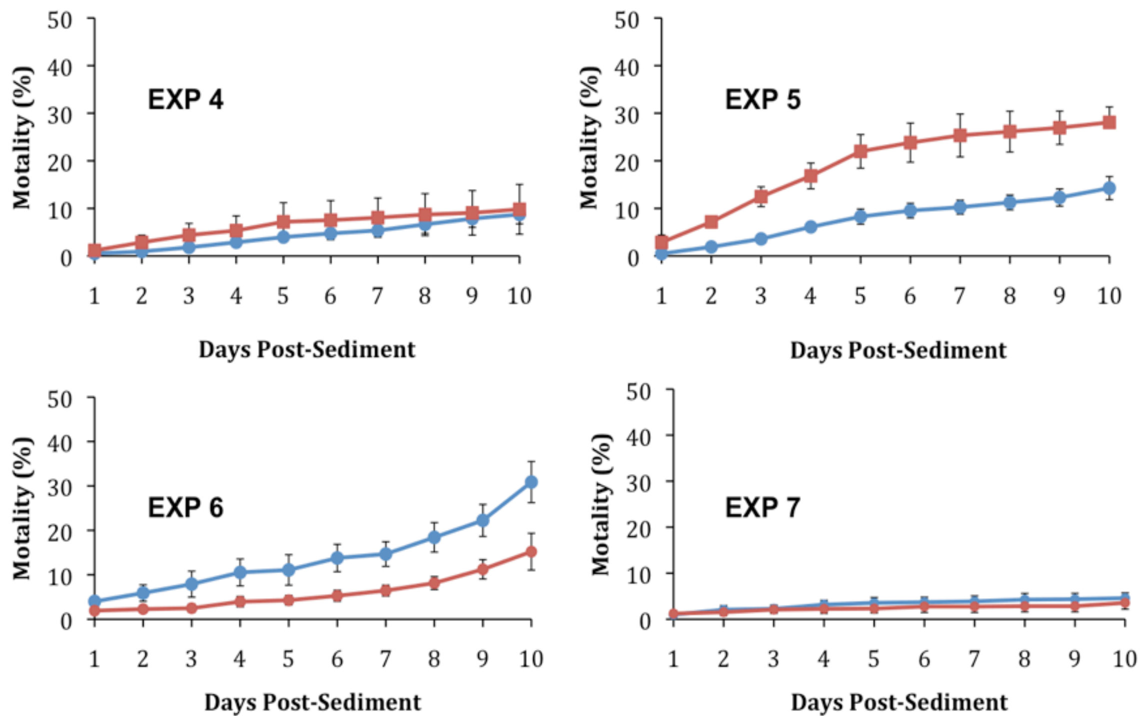

Figure 8. Cumulative mortality by experiment after treatment with suspended sediment of 200-400 mg/L. Sediment treated larval (●) mortality was not significantly different from controls (■) in any of the four experiments. Values represent average ± standard deviation (EXP #4 P = 0.079; EXP #5 P = 0.076; EXP #6 P = 0.084; EXP #7 P = 0.073).

Growth was not affected by sediment treatment. Variability occurred across experiments, but was not correlated with sediment treatment. The overriding trend in growth over the 10-day post-sediment treatment culture was that larvae continued to increase in length for the first 3-4 days after which average length did not change for either sediment-treated or control larvae. **(Fig. 9**).

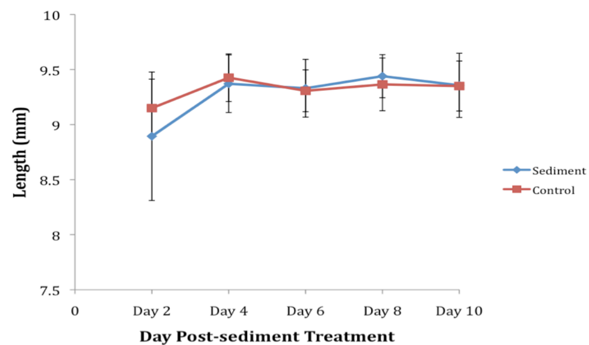

Figure 9. Grand mean of larval length. Larvae from the experiments depicted in figure 8 were sampled during the 10 day post-sediment culture and standard length determined. The grand means of sediment treated (●) and control (■) larvae from the 4 experiments were not significantly different. Values represent average ± standard deviation ($P_{day\ 10} > 0.05$).

Larval Condition

Three measures of larval condition; heart rate, prey capture, and larval critical swimming speed (U_{crit}), were not affected by with sediment treatment.

There was no difference in heart rate between experimental and control larvae. Heart rate (beats per min) for larvae the day after sediment treatment averaged 170.4 $\pm$ 12.7 s.d. for sediment treated larvae and 165.6 beats $\pm$ 8.1 s.d. for controls. Two days later heart rate had decreased to 147.6 $\pm$ 8.6 for experimental larvae and to 144.6 $\pm$ 12.8 for controls.

Prey capture results did not change with prior sediment treatment (**Fig. 10**). There was no significant difference between the average numbers of prey consumed by sediment treated and control larvae ($P > 0.05$). In addition, the number of larvae that consumed prey did not vary between sediment treated larva and controls (39.3 % $\pm$ 23.24 s.d. of treated larvae compared to 40.99 % $\pm$ 21.57 s.d. in controls). As indicated by the standard deviations, variability between replicates was high.

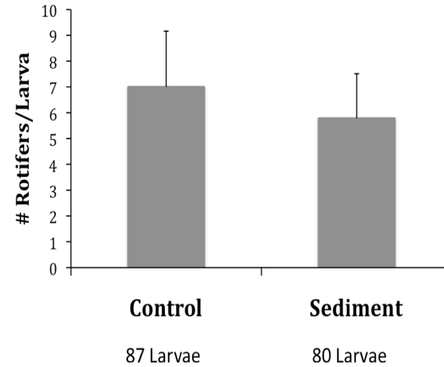

Figure 10. Larval prey capture. Larvae were co-incubated with rotifers for 4 hr after which the numbers of rotifers in larval digestive tracts was scored. There was no significant difference in the average numbers of rotifers per larvae between sediment treated and control larvae. Average $\pm$ standard deviation ($P > 0.05$).

Critical swimming speed (U_{crit}) ranged from a low of 6.3 cm/sec to a high of 12.3 cm/sec. Although U_{crit} varied between experiments and larval age, it was not dependent on whether larvae were treated with suspended sediment (**Fig. 11**). Overall, in 10 of 12 tests there was no significant difference ($P > 0.05$) between sediment treated larvae and controls. In the other two tests there were statistically significant differences between controls and sediment treated larvae, but sediment effects were not consistent. In one there was a significant difference ($P = 0.05$), where control larvae exhibited higher U_{crit} than did sediment treated larvae. There was also a statistically significant difference ($P = 0.018$) in one where the U_{crit+} was higher in the treated larvae. In this experiment, three U_{crit} values of 12.28 cm/sec, 8.11 and 7.87 were recorded for treated larvae while the maximum for the control was 6.32 cm/sec.

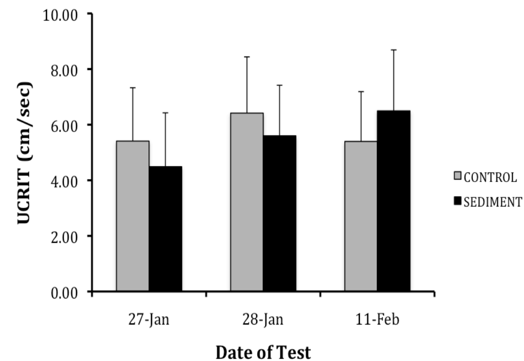

Figure 11. Larval critical swimming velocity. Three representative tests. There was no difference in the maximum current velocity that sediment treated and control larvae could withstand (U_{crit}). Values are average $\pm$ standard deviation ($n = 10$; $P > 0.05$ for all three trials).

DISSCUSSION

Early post-hatch Pacific herring larvae tolerated continuous suspended sediment loads of at least 366 mg/L for 16 hrs in a controlled sediment suspension system. The same larvae survived 10 days after removal from sediment exposure with no difference in mortality, growth, or condition compared to no-sediment controls. These results agree with those obtained in a previous study that investigated the effects of up to 500 mg/L of suspended sediment on embryo development and larval survival (Griffin *et al.,* 2008, 2009). Although this earlier study focused on and showed that sediments affect embryo and larval success if sediment particles are present during an initial two-hour window of time (0-2 HPF), it also suggested that exposure to sediment at later time points does not impair development or larval survival. While larvae treated at 0-2 H with sediment showed increased mortality over 15 days of larval culture, those treated for two hours with sediments after hatching (at 2 and 10 days post-hatch) had survival rates equivalent to non-sediment controls (see Figure 10, Griffin *et al.,* 2008). This result has been extended: Larvae can tolerate sixteen hours of suspended sediment with no observable effect on larval survival, growth, or condition for 10 days after exposure.

The current study attempted to address a worst-case scenario that larvae might encounter as a result of anthropogenic disturbance of sediments from, for example, dredging or other shallow water disturbance activities. We included in our design the suggestion by Wilber and Clarke (2001) that diurnal tidal current changes in San Francisco Bay dissipate suspended sediments by the end of one cycle (about 12 hrs) due to reversal of tidal current direction. To cover this time frame, sediment treatments of 16 hrs or greater were used. The sediment treatment system utilized current velocities and baffles that maintained the majority of particles in suspension, yet did not appear to stress larvae. In a prototype system current velocities that maintained sediment in suspension also created a downward current through larval chambers that trapped larvae against the Nitex mesh bottoms and caused mortality. A ½ x ½ inch square grid plastic baffle was key to reducing this downward current and associated mortality. The baffle also reduced the effectiveness of the mixing pump. There was a slow, steady decrease in suspended sediment concentration in the revised suspended sediment system, but during larval experiments greater than 50% of the original sediment load remained in suspension by experiment termination. This loss was higher than that obtained during initial test runs, but could have been due to the fact that initial test run concentrations were based on calculated values and not on actual samples taken at hour 2 (after mixing had occurred). In addition, the larval experiments included two trials using SF Bay sediments and it is feasible that these sediments were more prone to aggregation and thus coming out of suspension.

The mortality rates during post-treatment larval culture were the same for control and sediment treated larvae, and were consistent throughout 10 days of culture. The rates of 1.46 % mortality per day in sediment treated and 1.42 % in control larvae were similar to those, (< 1.5 % per day) previously seen in laboratory culture of herring larvae (Griffin *et al.,* 2004). Post-treatment static culture did by itself induce a low level stress, as evidenced by daily mortalities, and thus survival was a sensitive measure of whether sediment treatment stressed larvae. Multiple stressors can have added or synergistic impacts (Landis *et al.,* 2004; Landis, 2010) and so if sediment treatment had had a lasting

effect, post-treatment mortality rates should have been elevated. In two of the experiments (EXPs #2 & #3) there were numerical differences in mortalities that, although not statistically significant, might have indicated a trend. These were not due to sediment type since EXP #2 used San Francisco Bay sediment and EXP #3 used Fuller's earth. More importantly, control mortality was higher in EXP #2 while sediment treated larval mortality was highest in EXP #3.

During post-treatment larval culture, growth occurred that was initially in agreement with published daily values of about 2 mm per day (Erlich *et al.,* 1976; McGurk 1984). After day 4 average larval length did not change, indicating a reduction or cessation in growth. The cessation of growth could have been real and possibly due to effects of static water culture, or variable numbers of larvae in each culture dish. The standard deviations for the grand mean of larval length for each day were greater than 2 mm (the published daily growth), thus there was a high degree of variability in the length measurements for each time point. At no point during the experiments was there a difference in average length of control and sediment treated larvae, therefore decreased larval growth was not due to sediment treatment.

Three measures of larval condition, heart rate, critical swimming speed, and prey capture, were not affected by sediment treatment. Heart rate was included because it is a measure of overall physiological condition and development. Normal herring heart rate is reduced by stresses such as salinity stress, hypoxia, and toxicant stress (Holliday and Blaxter, 1960; Middaugh *et al.,* 1998; Pelster, 1999; Vines *et al.,* 2000). Critical swimming speed is a measure of larval strength, stamina, and development. The critical swimming speed test using incremental velocities allowed quantitative comparisons between sediment treated larvae and controls. In the incremental velocity tests used by other researchers, the increment times, overall test period and flow velocities vary depending on the species and age of the animals tested. Brett (1964) used incremental velocity tests up to four days long and an increment time of 1-17 hrs in juvenile salmon. More typically increments range from 2 and 200 min (Brett, 1964). Many of the earlier studies (including Brett, 1964, 1967) used juvenile fish and not larvae. Based upon recent larval swimming reports (Bellwood & Fisher, 2001; Fisher, 2005) and preliminary tests run on herring in our laboratory, three-minute time intervals were selected and used between velocity increments for this project. Prey capture was used as the final measure of condition; it reflects overall physiological condition and development since larvae must have developed the structures and capability to feed as well as possess the swimming ability to capture prey.

Critical swimming speed and prey capture are linked and deserve further exploration. Most fish larvae are visual predators (Hunter, 1981). The chain of visual predation involves prey encounter, detection, pursuit, fixation, attack and capture (Utne-Palm, 2004). For a visual predator-prey encounter, detection and capture depend on variables such as the optical regime of the water column (light level and turbidity), prey size and contrast, turbulence, prey density, and swimming ability. It would be of interest to test the link between swimming ability and prey capture after sediment treatment. This could involve testing the ability of larvae to find and capture prey while in a current during a critical swimming test. This could be investigated both in the presence and absence of suspended sediment.

The overriding goal of the two projects that have now been completed on the effects of suspended sediments on Pacific herring embryo and larval survival (Griffin *et al.,* 2009 & the current project) was to provide a scientific basis from which regulatory decisions about dredging and other sediment suspending activities in San Francisco Bay could be made. There remain gaps in information that should be addressed before the goal is realized. One gap concerns the ability of larvae to swim and capture prey while in suspended sediments. Another involves whether or not previous sediment exposure alters this ability. Especially pertinent would be whether larvae derived from eggs treated with suspended sediment during the first two hours after eggs contact water are more or less capable of tolerating suspended sediments as larvae. The possibility that this could occur and that it might constitute added stress should be examined. Lastly, achieving the overriding goal should include studies that ground-truth the laboratory studies. Translating the laboratory results and conclusions to effects in San Francisco Bay requires testing. Does increased egg aggregation occur in the Bay when suspended sediment loads are elevated during a spawn? Is subsequent larval hatch affected? Is larval survival, growth, and condition affected when these larvae from natural spawns are subjected to suspended sediments?

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