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Effects of Suspended Sediment on Eggs and Larvae of Lingcod (*Ophiodon elongatus*), Pacific Herring (*Clupea harengus pallasi*), and Surf Smelt (*Hypomesus pretiosus*)

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EFFECTS OF SUSPENDED SEDIMENT ON EGGS AND LARVAE OF LINGCOD (*Ophiodon elongatus*), PACIFIC HERRING (*Clupea harengus pallasi*), AND SURF SMELT (*Hypomesus pretiosus*)

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*Raw data referred to in this document may be found in the appendices of Morgan (1987), an unpublished contractor's report prepared for the Ocean Dumping Advisory Committee - Pacific Region.

ABSTRACT

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. 1729: 31 p.

Fertilized eggs and newly hatched larvae of lingcod (Ophiodon elongatus), Pacific herring (Clupea harengus pallasi), and surf smelt (Hypomesus pretiosus) were exposed to different concentrations of a contaminated (False Creek) and relatively uncontaminated (Roberts Bank) marine sediment. Each bioassay test was conducted with the sediments in suspension. Effects measured included egg mortality during development, timing of development, percent hatchability, and larval survival after hatching.

Exposure of fertilized herring and surf smelt eggs to False Creek sediment suspensions resulted in lower hatching success than exposure to Roberts Bank sediments at similar concentrations. Differences in hatching success of eyed lingcod eggs between the test sediments were not significant. Premature hatching of lingcod eggs were observed at higher concentrations of both test sediments, while hatching of herring eggs was delayed at 10 g/L of False Creek sediment. Survival of newly hatched lingcod, herring and surf smelt larvae to yolk sac exhaustion was higher in Roberts Bank sediments than in False Creek sediment suspensions. Larvae of all three species were more sensitive to suspended sediment exposure than the egg stage. The toxic response of suspended sediments to Pacific herring and surf smelt was concentration-dependent, as egg hatchability and larval survival decreaded with an increase in concentration of both test sediments. Trends in egg hatchability and larval survival between concentrations of test sediment were not observed for lingcod. Early life history stages of the surf smelt were the most sensitive to suspended sediment exposure, followed by Pacific herring and lingcod, respectively.

Key words: marine fish, eggs, larvae, suspended sediment, mortality, hatchability, development, ocean dumping

RÉSUMÉ

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. 1729: 31 p.

Des oeufs fécondés et des larves nouvellement écloses de la morue longue du Pacifique (Ophiodon elongatus), du hareng du Pacifique (Clupea harengus pallasi) ainsi que de l'éperlan argenté (Hypomesus pretiosus) ont été exposés à des sédiments marins contaminés (False Creek) et relativement non contaminés (Roberts Bank) à différentes concentrations. Chaque essai biologique a été fait avec les sédiments repris en suspension. La mortalité des oeufs en cours de développement, la vitesse du développement, le pourcentage des oeufs qui éclosent et le pourcentage de survie des larves après l'éclosion ont été les paramètres mesurés.

Moins d'oeufs d'éperlan et de hareng exposés aux sédiments en suspension de False Creek ont éclos qu'il n'y a eu d'oeufs éclos qui ont été exposés aux sédiments de Roberts Bank à de mêmes concentrations. Il n'y avait pas d'écart significatif entre les pourcentages d'éclosion d'oeufs oeillés de morue exposés à un type ou l'autre de sédiment. Une éclosion prématurée d'oeufs de la morue longue a été observée lorsque les oeufs étaient exposés à des sédiments des deux types en concentration élevée tandis que les oeufs de hareng exposés aux sédiments de False Creek à 10 $g.L^{-1}$ ont eu une éclosion retardée. La survie des morues, des éperlans et des harengs nouvellement éclos jusqu'à l'épuisement du sac vitellin était supérieure chez ceux exposés aux sédiments de Roberts Bank qu'elle ne l'était chez les sujets exposés aux sédiments de False Creek. Les larves des trois espèces se sont révélées être plus susceptibles à l'exposition aux sédiments que les oeufs. La réponse toxique du hareng et de l'éperlan aux sédiments repris en suspension dépend de la concentration puisqu'on a observé que l'éclosion des oeufs et la survie des larves diminuaient à mesure qu'augmentait la concentration des sédiments. Chez la morue longue du Pacifique, la variation de la concentration en sédiments n'a pas eu d'effet sur l'éclosion des oeufs et la survie des larves. Les premiers stades d'évolution de l'éperlan argenté se sont révélés être les plus sensibles à l'exposition aux sédiments repris en suspension; venaient ensuite le hareng du Pacifique et la morue longue du Pacifique, dans l'ordre.

Mots clés: poisson de mer, oeufs, larves, sédiments en suspension, mortalité, éclosion, développement, rejet en mer

INTRODUCTION

BACKGROUND

Biological effects of ocean disposal of dredged spoils have been studied extensively in recent years (e.g., Windom 1976; Conlan and Byers, 1979; Engler et al., 1981; Levings 1982; Kester et al., 1983). However, most of the published data on effects of dredged material disposal upon fish have dealt with adult life stages, and little information is available regarding effects on egg and larval stages of marine species.

Sherk et al. (1974) reviewed the effects of suspended and deposited sediments on estuarine fish species, and concluded that eggs and larvae were the most sensitive life history stages tested. Auld and Schubel (1978) also determined relatively high sensitivities of demersal fish eggs and larvae to adverse impacts from spoil disposal. In British Columbia, concerns have been expressed about the possible effects of ocean dumping on the sensitive egg and larval stages of a wide range of commercially important fish species. Of particular interest are the Victoria Dumpsite (near the Quarantine Buoy in the Juan de Fuca Strait) and the Point Grey Dumpsite, both of which receive substantial quantities of dredged material and both of which are proximate to important commercial groundfisheries.

In response to these concerns, E.V.S. Consultants conducted a preliminary study in 1982 for the Canadian Department of Fisheries and Oceans (McGreer and Munday, 1982) to investigate the effects of suspended and settled sediment upon the eggs and larvae of the Pacific cod (*Gadus macrocephalus*). Fertilized eggs and newly hatched larvae were exposed to an uncontaminated silt, and a contaminated sediment from a heavily industrialized marine embayment. Tests with fertilized eggs examined mortality during egg development, hatching success, and time to hatch for 50% of the eggs. Tests were run with different concentrations of sediment overlying the eggs (quiescent condition), and with sediments in suspension. Tests with cod larvae were designed to assess acute toxicity of sediments to larvae, and the degree to which larvae would ingest the different sediment types. Results of the 1982 study indicated that successful hatching in fertilized eggs of Pacific cod was virtually eliminated when eggs were covered to a depth of 1 mm with contaminated marine sediments, and no successful hatching of larvae occurred when fertilized eggs of Pacific cod were exposed to concentrations of suspended contaminated sediments of 7.5 g/L or greater. The study also showed that fertilized eggs which did not survive to hatching generally died at a very early stage of development; time to 50% hatch for fertilized eggs increased with the depth of overlying, uncontaminated sediment; and, survival and feeding behaviour of newly hatched larvae were not affected by short-term (4 h) exposure to contaminated sediment.

OBJECTIVES

The purpose of the present study was to extend the information generated through the 1982 study to the eggs and larvae of three other marine fish species of local commercial importance, viz. lingcod (Ophiodon elongatus), Pacific herring (Clupea harengus pallasi), and surf smelt (Hypomesus pretiosus). These species spawn in proximity to the Point Grey and Victoria Ocean Dumpsites. Bioassay tests using eggs and larvae of each species were conducted to investigate the toxic effects of contaminated and uncontaminated sediment suspensions upon hatching success, timing of development, and larval survival after hatching. Based on these data, recommendations are made regarding the use of toxicity testing with early life history stages of marine fish species to determine the quality of dredged spoils designated for ocean dumping.

MATERIALS AND METHODS

TEST SEDIMENTS

An uncontaminated sediment (clean silt), and a contaminated sediment were collected from the Fraser River estuary (1 km north of the Roberts Bank coal port) and the east basin of False Creek, Burrard Inlet, respectively, on January 20, 1987. Sediment sample collection sites were consistent with the locations used in the previous study using Pacific cod (McGreer and Munday, 1982), to facilitate comparison of the test results. Sediment samples were collected using a ponar grab and transported to the laboratory in 20 L plastic containers. Sediments were sieved through a 63 µm sieve and the fraction passing through the sieve was retained for testing.

Prior to testing, a portion of each sediment was frozen for analysis of percent organic carbon, copper, lead, zinc, cadmium, mercury and total polychlorinated biphenyls (PCB's) content. Organic carbon content was determined as volatile residue on ignition at 550^{0}C. Metals (except cadmium and mercury) were determined by ICAP (Inductively Coupled Argon Plasma). Cadmium was analysed by low-level graphite furnace atomic absorption spectrophotometry (AAS), and mercury by cold vapour, flameless AAS. PCB's were analysed by gas-liquid chromatography with electron capture detection after extraction by hexane/acetone. All analyses were carried out by the EPS/DFO Environmental Laboratory, West Vancouver, B.C. Procedures followed were those described in the Environmental Laboratory Manual of the Department of Environment (1979). Appropriate BCSS-1 and MESS-1 reference materials were analysed concurrently for quality assurance/quality control.

EGG COLLECTION

Fertilized lingcod eggs were collected by SCUBA divers from Copper Cove near Horseshoe Bay, B.C. on January 29, 1987. Three lingcod egg nests were collected from the crevices of boulders (diameter=1.5 m) on the bottom, located approximately 10 m from shore and at a depth of about 8 m. One of the nests was being actively guarded by a male lingcod during the collection period. Surface water temperature and salinity during collection were 7^{0}C and 27 ppt, respectively. The egg nests were transported to the laboratory in 70 L plastic buckets filled with seawater into which oxygen was slowly bubbled. The eggs were then held in 100 L aquaria containing aerated seawater at 8^{0}C and 28 ppt salinity, prior to testing.

Adult Pacific herring, captured by purse seine in Northumberland Channel during March, were obtained from the Pacific Biological Station in Nanaimo, B.C. on April 8, 1987. Natural spawning of Pacific herring in the Nanaimo area occurred from approximately March 18-20, 1987 (K. Slater, DFO, pers. comm.), and spawning of the captured fish was therefore delayed about 3 weeks. This holding period was considered acceptable, and should have prevented detrimental effects on the viability of eggs and larvae of Pacific herring, which may be caused by delaying spawning by 4 weeks or more (Hay, 1986).

Adult herring can be very susceptible to mortality during transport (Reid and McGreer, 1982; Siddens et al., 1985) and were therefore spawned at the Pacific Biological Station. Two mature females (length=25 cm) and two males (length=20 cm) were sacrificed and descaled prior to spawning. The females were massaged to induce egg deposition into two separate 4 L plastic trays filled with seawater (25 ppt) at 9^{0}C. The trays were lined with 63 µm mesh nitex screen which served as a spawning substrate. Fertilization was accomplished by massaging each male herring to release milt into the containers. The water was mixed with a glass rod during spawning to ensure an even distribution of eggs and sperm on the substrate. Each container was aerated with a battery-operated air pump, cooled with ice-packs, and incubated for 2 hours to allow complete fertilization. After 2 hours, the water in each container was replaced with fresh seawater and the containers were sealed and transported to the laboratory. Upon arrival at the bioassay laboratory, the two sections of mesh with fertilized eggs attached were transferred to a 100 L aquarium containing aerated seawater at 28 ppt salinity and 10^{0}C. The fertilized eggs were held for 24 hours to ensure viability prior to testing.

Fertilized surf smelt eggs were collected from the Wreck Beach area of Point Grey on August 12, 1987. This sandy beach has been previously documented as a surf smelt spawning area by Levy (1985). Naturally deposited smelt eggs were collected according to the technique of Misitano (1977). Four litres of high intertidal (10' tide level) pea gravel containing fertilized eggs were removed to a depth of 2 cm and transported to the laboratory in a moist state using a sealed plastic bucket. Prior to testing, the egg-laden gravel was held in a continuous-flow incubator capable of simulating high and low tide conditions found on the natural spawning beach. The advantages of this method over artificial spawning of mature adults to obtain fertilized eggs are that eggs used from a natural spawning are generally more viable, and the number of eggs exposed per treatment can be determined more precisely.

TEST PROCEDURES

The bioassay chambers used in the present study were 20 L "V" shaped glass aquaria with an aeration stone extending the full length of the bottom. Total test volume per tank was 12 L for lingcod and herring, and 8 L for surf smelt. Suspended sediment concentrations used for testing were 0.5, 4.0 and 10.0 g/L, plus a seawater control. The tests were run in duplicate to allow for natural variation in hatchability. These concentrations reflect sediment suspensions found in the vicinity of dredging and spoil disposal sites, where concentrations may frequently approach 10 g/L (Auld and Schubel, 1978). They are also comparable to concentrations of suspended sediments used in previous toxicity testing with marine fish eggs (Moore, 1977; Kiorboe et al., 1981; McGreer and Munday, 1982).

Dilution seawater for the tests was obtained from the DFO Laboratory in West Vancouver, B.C. which operates a 60 ft. deep-water intake providing seawater of optimum water quality. The seawater was passed through a 1 µm (nominal rating) filter and aged for 48 h prior to testing.

Test suspensions were established by adding appropriate amounts of uncontaminated (Roberts Bank) or contaminated (False Creek) sediment so that gentle, aeration-induced circulation uniformly maintained the desired suspended particulate concentrations. Any settled sediment was resuspended every 24 h using a large pipette and bulb apparatus. Every five days during testing, and before resuspension of settled sediment, 100 mL of test solution were removed from the bioassay chambers, filtered through preweighed 0.45 µm diameter membrane filters, dessicated, and weighed in order to document suspended sediment concentrations after settling.

For each bioassay test, approximately 100-300 viable eggs were placed into incubation baskets. Each basket consisted of a 16 cm long x 12 cm wide x 10 cm deep plastic frame open at the top with sides, ends and bottom covered with 0.5 mm Nitex mesh. These incubation baskets allowed maximum circulation of water and sediment yet permitted minimum sediment trappings. The screen also effectively dampened the mixing motion from aeration, thereby avoiding physical damage to the eggs. The baskets were randomly distributed among the control and test tanks, and were suspended to about mid-depth using metal hooks. Each tank was covered with a glass lid during the incubation period.

Lingcod eggs used for testing were obtained from the egg nest that was being guarded by a male during collection, as it appeared to be in the best condition (i.e. fewer dead eggs observed). The lingcod eggs were very adhesive to each other. Therefore, individual sections (4 x 3 x 1 cm and weighing 5 g each, equivalent to approx. 300 eggs) were removed from the surface of the nest using a stainless steel scalpel. The lingcod eggs had reached the optic stage of development prior to testing.

Herring eggs used for testing were obtained from the spawning substrate which contained the highest percentage of viable eggs. Egg density on the substrate was estimated by area (no. of eggs/cm^2) and individual sections (16 cm^2, equivalent to 200-300 eggs) were removed with stainless steel scissors. The fertilized herring eggs were one day old before testing.

Surf smelt eggs used for testing were separated from the gravel held in the incubator. This was accomplished by examining a petri dish containing gravel and seawater under a dissecting microscope (6X magnification) using a cold light source. Live smelt eggs were identified and removed using forceps. Each egg was attached to a piece of small gravel which aided in removal as the forceps could grasp the gravel, leaving the egg untouched and therefore physically undamaged. Egg viability was determined by the presence of a clear perivitelline space, and only eggs where organ rudiments were not yet visible and which had not yet reached the optic stage (i.e. <3 days old) were used for testing. The eggs were separated into batches of 100, which were placed into plastic weigh-boats filled with seawater and kept at incubating temperature prior to use.

Surf smelt eggs require a daily cycle of water immersion and exposure for best hatching success (Misitano, 1977), and therefore a diurnal combination of 4 hours immersion to 20 hours of air exposure was maintained for this species. During periods of air exposure, the incubation baskets were suspended just above the water surface to keep the eggs moist. Herring and lingcod eggs remained immersed throughout the tests.

The eggs tests were run at water temperatures similar to the collection sites for each species (lingcod - 8^{0}C, herring - 10^{0}C, smelt - 15^{0}C). The tests were conducted in a constant environment room that maintained water temperature at $\pm$1^{0}C and controlled light intensity over a 12-h photoperiod.

Each test series was conducted until hatching was completed in all incubation baskets and through to yolk sac exhaustion of surviving larvae (a total of 18 - 33 days depending on the species). Eggs and larvae were therefore exposed to a "worst case" condition, as continuous exposure to suspended sediments at a dump site would occur over a shorter time period.

Daily observations were made on egg development and mortalities. Observations were made by temporarily removing the incubation baskets from the aquaria and examining the eggs in a tray of clean seawater, or under a Wild M5A dissecting microscope if necessary. Egg mortality was based on the lack of movement and total opaqueness of the embryo. The degree of development was based on optic development (except lingcod), pigmentation and hatching. Hatchability was based on the rupturing of the egg chorion by the embryo and subsequent larval survival at the time of hatch.

Newly hatched larvae were gently removed from the incubation baskets with a wide bore pipette and placed into 1 L glass beakers containing the same concentration of sediments as the respective incubation tanks, and with a test volume of 800 mL per beaker. The sediments were kept in suspension by constant aeration. The light sensitive larvae were kept in darkness, except for about 1-2 h daily during observations.

Daily counts of healthy, stressed and dead larvae were performed by gently pouring test solutions and larvae through a 250 µm mesh nitex screen. The retained larvae were quickly resuspended in a tray of clean seawater and the number of healthy (actively swimming), stressed (alive but on the bottom and exhibiting sublethal effects) and dead larvae were determined. Larval mortality was determined by lack of body movement, rigidity, mouth gaping, opaqueness and absence of heart beat. Sublethal effects exhibited by stressed larvae included abnormal swimming behaviour, lethargy, and physical abnormalities such as curled tails. Healthy and stressed larvae were returned to the beakers using a wide bore pipette, while dead larvae were discarded. Lingcod, herring and smelt larvae were maintained for a minimum of 10, 4 and 7 d after hatching, respectively, and survival was measured over these periods. Surviving lingcod, herring and smelt larvae reached yolk sac exhaustion after 12, 3 and 4 d, respectively, and they were not fed. At test termination, wet weight (to the nearest 0.1 mg) and standard length (to the nearest 0.5 mm) measurements were made on subsamples of surviving larvae from each suspended sediment concentration.

Water temperature, salinity, dissolved oxygen and pH were also recorded daily in all bioassay chambers during the tests. At the end of each bioassay, the sediments were composited according to site and frozen for chemical analyses in order to determine the possible effects of testing (e.g., aeration) on the chemical composition of the sediments.

Mean percent values were calculated from the total numbers of eggs or larvae in replicates A and B, rather than averaging the two percentages. Significant differences in hatching success and larval survival between the test sediments and the control were determined statistically by Dunnett's procedure (Steel and Torrie, 1960). One-tailed Dunnett t-tables ($P=0.05$) were used to determine if mean percent hatch and mean larval survival in each test sediment concentration was significantly different from the control values. Significant differences between similar concentrations of Roberts Bank and False Creek sediments were determined by paired t-tests ($P=0.05$). Prior to statistical analysis, the data were transformed using the Arcsin percentage transformation, a procedure recommended for binomial data expressed as percentages (Steel and Torrie, 1960).

RESULTS

SEDIMENT CHEMISTRY

Results of analyses of the Roberts Bank ("uncontaminated") and False Creek ("contaminated") sediments prior to testing for heavy metals, PCB's and organic carbon content are summarized in Table 1. Concentrations of most heavy metals and PCB's in the Roberts Bank sediments were similar to those recorded in 1982 (McGreer and Munday, 1982), with the exception of cadmium and mercury which were significantly higher and lower, respectively, than found in the previous study. Values of heavy metals and PCB's recorded for False Creek sediments in the present study were slightly lower than documented in the 1982 study. However, the levels of heavy metals and PCB's measured in the False Creek sediments were an order of magnitude higher than found in Roberts Bank sediments and approached ODCA limits in most cases, with cadmium exceeding limits allowed for ocean disposal. According to results of the chemical analyses, therefore, Roberts Bank and False Creek sediments can still be classified as being relatively uncontaminated and contaminated, respectively.

Results of chemical analyses of the test sediments after the bioassays (Table 1) indicated that levels of cadmium decreased by about 50% during the tests. The concentrations of mercury increased significantly, while the other parameters were generally 5-10% higher after the bioassays than before. The reference materials showed no differences in the concentration of each parameter before and after testing. The reasons for the observed differences in the levels of heavy metals in the test sediments before and after the bioassays are not known.

LINGCOD EGG BIOASSAY

Egg Survival and Hatchability

Results of the lingcod egg hatching tests are summarized in Table 2. Egg mortality began in both test sediments after 2 days of exposure. Mortalities over the 33-day incubation period ranged from 1-14% in Roberts Bank sediments, 3-9% in False Creek sediments, and was 12% in the control. Trends in egg mortality between concentrations and test sediments were not evident.

The mean percent hatch of lingcod eggs is shown in Figure 1. Hatching success was quite high in both test sediments, ranging from 86-99% in Roberts Bank and 91-97% in False Creek exposures. Hatchability was not significantly (P=0.05) affected by increased concentration of suspended sediment. The percentage of larvae which died upon emergence (i.e., dead hatch) was slightly higher in Roberts Bank (5-19%) than in False Creek (2-5%) sediments. The majority of larvae hatching dead occurred in the last 4-5 days of the incubation period. Several larvae that hatched dead were curled up and opaque, suggesting that these dead larvae were liberated from eggs which had disintegrated. This late dead hatch has been observed by other studies with marine fish eggs (Reid and McGreer, 1982; Smith and Cameron, 1979).

Time for initial hatch of eyed lingcod eggs in Roberts Bank sediments ranged from 6-20 days, while peak hatch generally occurred from 19-20 days in all concentrations. Eggs in False Creek sediments began hatching after 18-21 days, while peak hatch occurred 20-21 days after initial exposure. The times to 5, 50 and 95% hatch for lingcod eggs are given in Table 3. Times to 5% and 50% hatch were 1-2 days earlier in Roberts Bank and False Creek sediments than in the controls, while times to 95% hatch were an average of 4.5 and 5 days earlier, respectively. Hatching times were generally shorter as concentration of both test sediments increased, indicating that premature hatching (larvae hatching before development was complete) may have been induced at higher suspended sediment concentrations.

Water quality parameters measured in all incubation tanks during the bioassay are provided elsewhere (Morgan, 1987). Water temperature (8 ± 1°C), salinity (28 ppt), pH (7.8 ± 0.2), and dissolved oxygen (9.5 ± 0.5 mg/L) remained at acceptable levels throughout the incubation period. Suspended sediment concentrations during testing ranged from 0.5-0.7, 2.8-4.0, and 8.8-10.0 g/L for the three sediment concentrations set up (0.5, 4 and 10 g/L).

Larval Survival

Results of lingcod larvae survival are presented in Table 4. Larval survival over 10 days was highest in the control (82%). In contrast, Roberts Bank and False Creek sediments had significantly (P=0.05) lower larval survivals than the control, ranging from 4-10% and 2-7%, respectively. Roberts Bank sediments had slightly higher larvae survival rates than False Creek sediments; however, the differences were not statistically significant (P=0.05). Trends in larval survival between suspended sediment concentrations were not evident.

Length and weight measurements of surviving lingcod larvae at test termination are provided in Morgan (1987). Control larvae averaged 9.9 mm in length and 4.3 mg in weight at yolk sac exhaustion. Mean length and weight of Roberts Bank and False Creek larvae were 9.3 mm and 7.1 mg, and 9.2 mm and 6.6 mg, respectively. Higher weights obtained for larvae exposed to test sediments compared to the controls may have been the result of sediment ingestion, as documented for Pacific cod larvae by McGreer and Munday (1982); however, the larvae were not examined to verify this hypothesis.

Ranges in water quality parameters during larvae testing were: temperature, 8-9°C; salinity, 28-30 ppt; pH, 7.3-7.9; DO, 7.8-9.7 mg/L.

HERRING EGG BIOASSAY

Egg Survival and Hatchability

Results of the herring egg hatchability tests are summarized in Table 5. Egg mortality began in the highest concentration (10 g/L) of False Creek and Roberts Bank sediments after 1 and 2 days of exposure, respectively. Egg mortalities during the incubation period ranged from 6-10% in Roberts Bank sediments, 6-18% in False Creek sediments, and was 5% in the control. Egg mortality was slightly higher in False Creek than in Roberts Bank sediments, and generally increased with increasing concentration of both test sediments.

The mean percent hatch of herring eggs is shown graphically in Figure 2. Viable hatch ranged from 77-87% in Roberts Bank sediments, 55-84% in False Creek sediments, and was 92% in the control. In general, hatching success was higher in Roberts Bank than in False Creek sediments, and decreased with increasing suspended sediment concentration. The percentage of larvae which hatched dead was higher in False Creek (10-27%) than in Roberts Bank (7-13%) sediments, and increased with an increase in concentration of both test sediments. A statistically significant difference (P=0.05) in percent hatch compared to the control was observed only in the highest concentration of False Creek sediment (10 g/L).

Data on the timing of embryo development are given in Morgan (1987). The eyed stage was reached in both test sediments and the control on day 5, with the exception of 10 g/L of False Creek sediment, where development of the eyed stage was not observed until after 6 days of exposure. Melanophore pigments along the spinal chord of the herring embryos were observed in all test tanks on day 9, and hatching began 1 day later after 10 days of exposure. The times to 5, 50 and 95% hatch for herring eggs are presented in Table 6. The time to 5% hatch was similar (10 d) in both test sediments at all concentrations, while the times to 50% and 95% hatch were 2-3 days longer in 10 g/L of False Creek sediment, compared to Roberts Bank sediments and the control. The delay in hatching at the highest concentration of False Creek sediment may have been caused, in part, by a layer of silt which covered the eggs throughout the incubation period. This physical barrier may have restricted the amount of oxygen supplied to the eggs, thereby slowing the development of the embryos. A similar increase in hatching time due to overlying sediment was observed for Pacific cod eggs by McGreer and Munday (1982).

Water quality parameters measured in all incubation tanks during the bioassay are provided in Morgan (1987). Water temperature (10^0C), salinity (28 ppt), pH (7.8 ± 0.1), and dissolved oxygen (9.6 ± 0.2 mg/L) remained at acceptable levels throughout the exposure period. Suspended sediment concentrations ranged from 0.5-0.7, 3.5-4.0, and 8.5-10.0 g/L during testing in each of the three pre-set concentrations (0.5, 4 and 10 g/L).

Larval Survival

Results of herring larvae survival are shown in Table 7. Larval survival after 4 days was highest in the control (76%) and was significantly (P=0.05) lower in the presence of both test sediments. This may indicate physical effects of the sediments on larval survival, in addition to mortality which may be caused by chemical contamination. Herring larvae exposed to Roberts Bank sediments had higher survival rates (0.3-9.8%) than with False Creek sediments (0.4-0.7%). Survival of herring larvae generally decreased as the concentration of both test sediments increased.

Length and weight measurements of surviving herring larvae at test termination are included in Morgan (1987). Control larvae averaged 9.4 mm in length and 2.3 mg in weight at yolk sac exhaustion. Overall mean lengths and weights of herring larvae exposed to Roberts Bank and False Creek sediments were 9.4 mm and 2.7 mg, and 8.7 mm and 2.4 mg, respectively. Although surviving False Creek larvae were smaller than Roberts Bank and control larvae, the small sample size of surviving False Creek larvae (n=3) precludes a definitive comparison of the results.

Water quality parameters during larvae testing ranged as follows: temperature, 9.5-10^{0}C; salinity, 28 ppt; pH, 7.7-7.8; DO, 9.0-9.4 mg/L.

SURF SMELT EGG BIOASSAY

Egg Survival and Hatchability

Results of the surf smelt egg hatchability tests are summarized in Table 8. Egg mortality began in all test tanks after 1 day of exposure and was quite high throughout the incubation period, ranging from 90-95.5% in Roberts Bank sediments, 94.5-98.5% in False Creek sediments, and 69.5% in the control at test termination. Egg mortality was slightly higher in False Creek than in Roberts Bank sediments and generally increased with increasing concentrations of both test sediments.

The mean percent hatch of surf smelt eggs is shown in Figure 3. Hatching success was quite low in both test sediments, ranging from 4.5-10% in Roberts Bank and 1.5-5.5% in False Creek exposures. Percent hatch in all concentrations of both test sediments was significantly (P=0.05) lower than the control value (30.5%). Total hatch was generally higher in Roberts Bank than in False Creek sediment and tended to decrease with an increase in suspended sediment concentration. The percentage of larvae which hatched dead was also higher in Roberts Bank (4-6.5%) than in False Creek (1.5-4.5%) sediments.

Data on the timing of surf smelt embryo development are presented in Morgan (1987). The eyed stage was reached in the controls and in the 0.5 g/L concentration of both test sediments on day 4, and in 4 and 10 g/L on day 5. Pigmented embryos were observed in all incubation tanks after 7 days of exposure. Eggs in Roberts Bank and False Creek sediments and the control began hatching after 9-13, 10-12 and 11 days, respectively. The times to 5, 50 amd 95% hatch for surf smelt eggs are given in Table 9. The time to 5% hatch was similar in both test sediments compared to the control, while the times to 50% and 95% hatch were 2-3 days shorter in both test sediments compared to the control. Definitive comparisons of the timing data cannot be made, however, owing to the low numbers of hatched eggs involved and the variability in the age of the embryos used to initiate the test (1-2 days old).

Water quality parameters measured in all incubation tanks during the bioassay are provided in Morgan (1987). Water temperature (15 $\pm$ 1^{0}C), salinity (28 ppt), pH (7.9 $\pm$ 0.1), and dissolved oxygen (8.0 $\pm$ 1.0 mg/L) remained at acceptable levels throughout the exposure period. Suspended sediment concentrations ranged from 0.5-0.6, 3.1-4.0, and 9.4-10.0 g/L during testing for the three nominal sediment concentrations (0.5, 4 and 10 g/L).

Larval Survival

Results of surf smelt larvae survival are presented in Table 10. Larval survival to 7 days was observed only in the control (41%) and in the 0.5 g/L concentration of Roberts Bank sediment (43%). One hundred percent mortality was recorded in all other concentrations of both test sediments after only 2-3 days of exposure. Surviving surf smelt larvae averaged 5 mm in length at test termination.

Water quality parameters during larvae testing ranged as follows: temperature, 15-16^{0}C; salinity, 28 ppt; pH, 7.9-8.0; DO, 7.5-8.6 mg/L.

DISCUSSION

LINGCOD EGG AND LARVAE BIOASSAY

Lingcod spawn in the Strait of Georgia during January and February, with hatching taking place from March to early June. The average incubation period appears to be about 7 weeks (Low and Beamish, 1978). As a result of this relatively long incubation time, it was necessary to use eyed lingcod eggs for toxicity testing in the present study to keep exposure times to hatching at about 30 days. False Creek and Roberts Bank sediments were less toxic to eyed lingcod eggs in the present study than recorded for fertilized Pacific cod eggs in McGreer and Munday (1982), where most egg mortality occurred at a very early stage of development. The eyed egg stage of most marine and freshwater fish species is known to be less sensitive than newly fertilized eggs (Peters et al., 1976; Rosenthal and Alderdice, 1976), and this may explain the difference in toxicity observed between the two studies.

Exposure to the higher concentrations of the two test sediments resulted in premature hatching of lingcod eggs, compared to lower concentrations and the control. Premature hatching related to toxicant exposure has been recorded in previous hatchability studies with surf smelt, herring, coho salmon (Oncorhynchus kisutch) and pike (Esox lucius) eggs (Chapman et al., 1983; Rosenthal and Sperling, 1974; Reid et al., 1983; Westernhagen et al., 1975). A possible explanation for this response is an alteration of the egg surface due to toxicants, decreasing the permeability of the chorion to oxygen and thus inducing early hatching (Halter and Johnson, 1974).

Survival of lingcod larvae exposed to False Creek and Roberts Bank sediments was significantly lower than the controls, and the test sediments were substantially more toxic to larvae than to the eyed-egg stage. These results are consistent with previous studies conducted by Halter and Johnson (1974), Klaverkamp et al. (1977), Peters et al. (1976) and Reid et al. (1983), where the hatched larval stage was found to be more sensitive to toxicant exposure than the egg stage.

HERRING EGG AND LARVAE BIOASSAY

Pacific herring eggs incubated in control tanks during the present study showed optimal rates of development and survival, when compared to data at similar levels of temperature and salinity provided in Alderdice and Velsen (1971). Results of the herring bioassay indicated that False Creek sediment suspensions were more toxic to eggs and larvae than Roberts Bank sediments, and this is consistent with the higher levels of contaminants found in the False Creek sediments. The biological responses were also concentration-dependent, as egg hatchability and larval survival decreased with an increase in concentration of both test sediments.

Newly fertilized herring eggs exposed to the test sediments were more sensitive in terms of survival and hatchability than eyed lingcod eggs (e.g., 55 versus 84% viable hatch in 10 g/L of False Creek sediment). This may be attributed to the difference in sensitivities between newly fertilized eggs and the eyed egg stage previously mentioned, and a variation in species sensitivities. Herring larvae were also more sensitive than lingcod larvae in the present study, lending further support to the latter hypothesis.

Herring larvae survival in the presence of False Creek and Roberts Bank sediments was substantially lower than the control, and both test sediments were significantly more toxic to herring larvae than to the egg stage. These data are consistent with the results obtained for lingcod in the present study.

SURF SMELT EGG AND LARVAE BIOASSAY

Surf smelt eggs incubated in control tanks during the present study had lower hatching success (mean percent hatch 31%) than obtained for lingcod (88%) and Pacific herring (95%). Previous work with surf smelt in the laboratory has also shown relatively low hatching success in controls, ranging from 14-52% (Chapman et al., 1983; Hawkes and Stehr, 1982; Malins et al., 1982). Under natural conditions, where fertilization appears to be virtually 100% successful, often less than 10% of the eggs survive to hatching (Pentilla, 1978).

The surf smelt bioassay indicated that False Creek sediments were more toxic to developing embryos than Roberts Bank sediments and that the toxic response was concentration-dependent. These data are consistent with results obtained for Pacific herring in the present study.

The surf smelt was the most sensitive species tested during the present study, with hatching success in similar concentrations of both test sediments (1-10%) being significantly lower than observed for lingcod (86-99%) and herring (82-94%) eggs.

Surf smelt larvae survival in the control (41%) was also substantially lower than observed for lingcod (82%) and herring (76%) larvae in the present study. Survival rates of laboratory-reared surf smelt reported in the literature have also been rather low, ranging from 42-68% (Chapman et al., 1983; Malins et al., 1982). Surf smelt larvae in the present study were highly sensitive to False Creek sediments, and to Roberts Bank sediment suspensions above 0.5 g/L. Similar results were also obtained for lingcod and Pacific herring.

GENERAL

Results of the present study indicate that marine fish egg and larvae toxicity tests could be used, in conjuction with other bioassay tests (e.g., amphipod) and established ODCA chemical criteria, as a monitoring tool to determine the quality of sediments under application for ocean dumping. Egg hatchability and larval survival for the three species were generally found to be higher in the relatively uncontaminated sediments than in the chemically contaminated sediment suspensions, indicating that the test responses were affected by sediment quality.

Comments on the suitability of the three marine fish species used in the present study for possible use in toxicity testing of dredge spoils are given below.

Lingcod eggs and newly hatched larvae are located in relatively close proximity to established B.C. dump sites (e.g., Point Grey) from December to June, and are therefore susceptible to effects of contaminated suspended sediments from ocean dumping. Unfortunately, the long incubation period of this species makes it impractical for routine egg hatchability tests, as resulting turn-around time could take several months and a decision of whether the material is suitable for ocean dumping may be required much sooner.

Pacific herring eggs and larvae have been used extensively in laboratory studies and appear to be relatively sensitive to toxicants (Struhsaker et al., 1974; Eldridge et al., 1978; Rice and Harrison, 1978; Smith and Cameron, 1979; Reid and McGreer, 1982). Their relatively short incubation time (about two weeks) and high survivorship of embryos and yolk sac larvae cultured in the laboratory make them very suitable for early life history toxicity testing of sediments considered for ocean disposal. The major disadvantages of using Pacific herring for routine testing include a restricted spawning season (February to March) and the intolerance of adult herring to capture and transport (Siddens et al., 1985). Spawning of mature herring immediately upon capture at the spawning grounds and rapid transportation of the fertilized eggs to the laboratory would overcome the latter problem.

Surf smelt spawn during most months of the year in Burrard Inlet (Hart, 1973; Levy, 1985) and therefore embryos are relatively easy to obtain from local beach gravels for routine toxicity testing. Siddens et al. (1985) recommended surf smelt over 10 other marine fish species as a primary canditate for use in embryo - early larvae chronic exposure testing in Washington and Oregon. The major problem with using surf smelt for routine testing of dredge spoils is a lower hatching success and larvae survival than has been obtained for other species in the laboratory.

A literature review on the effects of suspended sediments on eggs and larvae of marine fish species was previously reported by McGreer and Munday (1982). A computer search of the post-1982 literature indicated that little work has been done in the last five years on the response of fish eggs and larvae to suspended sediments, and that most studies have dealt with the effects of uncontaminated estuarine sediments rather than contaminated sediments from industrial embayments.

Morgan et al. (1983) exposed eggs and larvae of striped bass (Morone saxatilis) and white perch (Morone americana) to uncontaminated estuarine sediments from Maryland. Percent hatch was not significantly affected by up to 5 g/L of suspended sediment; however, the rates of egg development were significantly slower than the control values. Hatching delay generally increased with an increase in suspended sediment concentration. Mortalities of striped bass and white perch larvae exposed to 1.6-5.4 g/L of suspended sediment ranged from 25-57% and 23-49%, respectively.

Boehlert (1984) exposed Pacific herring larvae for 24 h to suspensions of uncontaminated sediment from the Columbia River estuary at concentrations of 0-8 g/L. Examination of the epidermis of the yolk sac larvae by scanning electron microscopy showed that physical abrasive effects were apparent with increasing concentrations of suspended sediment. In a related study, Boehlert and Morgan (1985) found that when Pacific herring larvae were exposed to suspensions of uncontaminated Columbia River estuary sediment at concentrations ranging from 0-8 g/L, maximum feeding incidence and intensity occurred at 0.5 g/L and decreased at higher concentrations. It was concluded that low concentrations of uncontaminated suspended sediment may actually enhance feeding by providing a visual contrast of prey items.

Devonald and Ausubel (1984) determined the spatial and temporal distribution of planktonic Atlantic croaker larvae in Chesapeake Bay near the Norfolk, Virginia, disposal site. Based on available data, the authors recommended a restricted dumping period during September and October to minimize impacts of ocean disposal on planktonic fish larvae.

CONCLUSIONS

The following conclusions can be made from the results of the present study.

1. Chemical analyses indicated that Roberts Bank and False Creek sediments can still be classified as being relatively uncontaminated and contaminated, respectively, as previously documented by McGreer and Munday (1982).

2. False Creek sediment suspensions were generally more toxic to marine fish eggs and larvae than Roberts Bank sediments, which was consistent with the higher level of chemical contaminants found in the False Creek sediments.

3. The toxic response of suspended sediments to Pacific herring and surf smelt was concentration-dependent, as egg hatchability and larval survival decreased with an increase in concentration of both test sediments. Trends in egg hatchability and larval survival associated with increasing concentrations of test sediment were not observed for lingcod.

4. Premature hatching was induced at higher suspended sediment concentrations of both test sediments for lingcod eggs. In contrast, hatching of herring eggs was delayed at the highest concentration (10 g/L) of False Creek sediment tested. No consistent differences in the timing of embryo development were observed for surf smelt.

5. Newly hatched larvae of all three species tested were more sensitive to suspended sediment exposure than the egg stage.

6. In terms of relative sensitivities of the egg and yolk-sac larva life history stages to suspended sediment, the test species can be ranked in the following order, from most to least sensitive.

   surf smelt > Pacific herring > lingcod

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Table 1. Results of chemical analyses of Roberts Bank and False Creek sediment samples before and after testing.

		Before Testing		After Testing		
Parameter[a]	Units	Roberts Bank	False Creek	Roberts Bank	False Creek	ODCA limits
Cadmium	µg/g, dry wt.	1.67	4.10	0.72	2.56	0.60
Mercury	µg/g	0.06	0.69	0.30	1.07	0.75
Lead	µg/g	20	318	24	333	500
Zinc	µg/g	101	657	111	591	1000
Copper	µg/g	48	222	58	225	1000
Tin	µg/g	4	14	8	21	1000
PCB's	µg/g	<0.002	0.567	n/a	n/a	1 (PCB+PCP)
Organic Carbon	%	3.3	8.9	3.6	9.5	-

a. Concentrations of metals shown are means of triplicate analyses. PCB's are the result of single analyses.

Table 2. Survival and hatching success of eyed lingcod eggs during 33 days of exposure to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Rep.	Total Eggs	Dead Eggs No.	Dead Eggs %	Larvae Hatched Live No.	Larvae Hatched Live %	Larvae Hatched Dead No.	Larvae Hatched Dead %	Mean Values Total Eggs	Mean Values % Dead Eggs	Mean Values Larvae Hatched % Live	Mean Values Larvae Hatched % Dead
Roberts Bank	10	A	309	3	1	290	94	16	5				
		B	328	3	1	307	94	18	5	319	1	94	5
	4	A	115	46	40	42	37	27	23				
		B	279	10	4	239	86	30	10	197	14	72	14
	0.5	A	192	5	3	184	95	3	2				
		B	225	25	11	127	57	73	32	209	7	74	19
False Creek	10	A	388	28	7	345	89	15	4				
		B	256	29	11	194	76	33	13	322	9	84	7
	4	A	382	10	3	358	94	14	3				
		B	416	12	3	396	95	8	2	399	3	95	2
	0.5	A	342	11	3	315	92	16	5				
		B	278	9	3	257	93	12	4	310	3	92	5
Control	0	A	142	10	7	123	87	9	6				
		B	79	15	19	44	56	20	25	111	12	75	13

Table 3. Time (in days) to 5, 50 and 95% hatch of eyed lingcod eggs exposed to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Time (d) to Hatch[a] 5%	50%	95%
Roberts Bank	10	18	19.5	23
	4	19.5	20.5	30
	0.5	18.5	21	28
False Creek	10	18.5	20	26.5
	4	19.5	21	26
	0.5	20	21.5	27
Control	0	20	21	31.5

a. Mean of two replicates.

Table 4. Survival of lingcod larvae after 10 days exposure to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Rep.	Total Larvae	10 d Survival No.	%	Mean Survival (%)
Roberts Bank	10	A	287	29	10	
		B	301	21	7	8
	4	A	42	6	14	
		B	238	4	2	4
	0.5	A	179	20	11	
		B	126	10	8	10
False Creek	10	A	343	27	8	
		B	192	13	7	7
	4	A	355	8	2	
		B	388	8	2	2
	0.5	A	313	14	5	
		B	252	22	9	6
Control	0	A	119	104	87	
		B	43	28	65	82

Table 5. Survival and hatching success of herring eggs exposed to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Rep.	Total Eggs	Dead Eggs No.	Dead Eggs %	Larvae Hatched Live No.	Larvae Hatched Live %	Larvae Hatched Dead No.	Larvae Hatched Dead %	Mean Values Total Eggs	Mean Values % Dead Eggs	Mean Values Larvae Hatched % Live	Mean Values Larvae Hatched % Dead
Roberts Bank	10	A	262	19	7	224	86	19	7				
		B	125	19	15	75	60	31	25	194	10	77	13
	4	A	201	14	7	162	81	25	12				
		B	376	22	6	332	88	22	6	289	6	86	8
	0.5	A	234	14	6	202	86	18	8				
		B	197	11	6	175	88	11	6	216	6	87	7
False Creek	10	A	251	32	13	142	57	77	30				
		B	250	57	23	134	54	59	23	251	18	55	27
	4	A	248	16	6	190	77	42	17				
		B	164	21	13	120	73	23	14	206	9	75	16
	0.5	A	341	17	5	307	90	17	5				
		B	279	20	7	211	76	48	17	310	6	84	10
Control	0	A	345	12	3	317	92	16	5				
		B	235	15	6	217	92	3	2	290	5	92	3

Table 6. Time (in days) to 5, 50 and 95% hatch of herring eggs exposed to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Time (d) to Hatch[a] 5%	50%	95%
Roberts Bank	10	10	10	11.5
	4	10	10	12.5
	0.5	10	10.5	11.5
False Creek	10	10	12.5	14.5
	4	10	10.5	12.5
	0.5	10	10	11.5
Control	0	10	11	11.5

a. Mean of two replicates.

Table 7. Survival of herring larvae after 4 days exposure to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Rep.	Total Larvae	4 d Survival No.	%	Mean Survival (%)
Roberts Bank	10	A	224	1	0.4	
		B	75	0	0.0	0.3
	4	A	162	3	1.9	
		B	332	24	7.2	5.5
	0.5	A	202	33	16.3	
		B	175	4	2.3	9.8
False Creek	10	A	142	1	0.7	
		B	134	0	0.0	0.4
	4	A	190	1	0.5	
		B	120	1	0.8	0.7
	0.5	A	307	1	0.3	
		B	211	2	1.0	0.6
Control	0	A	317	233	73.5	
		B	217	175	80.7	76.4

Table 8. Survival and hatching success of surf smelt eggs exposed to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Rep.	Total Eggs	Dead Eggs No.	Dead Eggs %	Larvae Hatched Live No.	Larvae Hatched Live %	Larvae Hatched Dead No.	Larvae Hatched Dead %	Mean Values Total Eggs	Mean Values % Dead Eggs	Mean Values Larvae Hatched % Live	Mean Values Larvae Hatched % Dead
Roberts Bank	10	A	100	95	95	1	1	4	4				
		B	100	93	93	2	2	5	5	100	94.0	1.5	4.5
	4	A	100	96	96	1	1	3	3				
		B	100	95	95	0	0	5	5	100	95.5	0.5	4.0
	0.5	A	100	87	87	6	6	7	7				
		B	99	92	93	1	1	6	6	100	90.0	3.5	6.5
False Creek	10	A	98	98	100	0	0	0	0				
		B	100	97	97	0	0	3	3	99	98.5	0.0	1.5
	4	A	100	96	96	1	1	3	3				
		B	100	97	97	1	1	2	2	100	96.5	1.0	2.5
	0.5	A	99	92	93	1	1	6	6				
		B	100	96	96	1	1	3	3	100	94.5	1.0	4.5
Control	0	A	100	74	74	23	23	3	3				
		B	100	65	65	33	33	2	2	100	69.5	28.0	2.5

Table 9. Time (in days) to 5, 50 and 95% hatch of surf smelt eggs exposed to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Time (d) to Hatch[a] 5%	50%	95%
Roberts Bank	10	10.5	10.5	14
	4	11	14.5	15.5
	0.5	11.5	12.5	15
False Creek	10	12	12	14
	4	10.5	12	14
	0.5	11.5	12	14.5
Control	0	12	15	17.5

a. Mean of two replicates.

Table 10. Survival of surf smelt larvae exposed to Roberts Bank and False Creek sediments.

Sediment	Conc. (g/L)	Rep.	Total Larvae	7 d Survival No.	%	Mean Survival (%)
Roberts Bank	10	A	1	0	0.0	
		B	2	0	0.0	0.0
	4	A	1	0	0.0	0.0
	0.5	A	6	3	50.0	
		B	1	0	0.0	42.9
False Creek	4	A	1	0	0.0	
		B	1	0	0.0	0.0
	0.5	A	1	0	0.0	
		B	1	0	0.0	0.0
Control	0	A	23	7	30.4	
		B	33	16	48.5	41.1

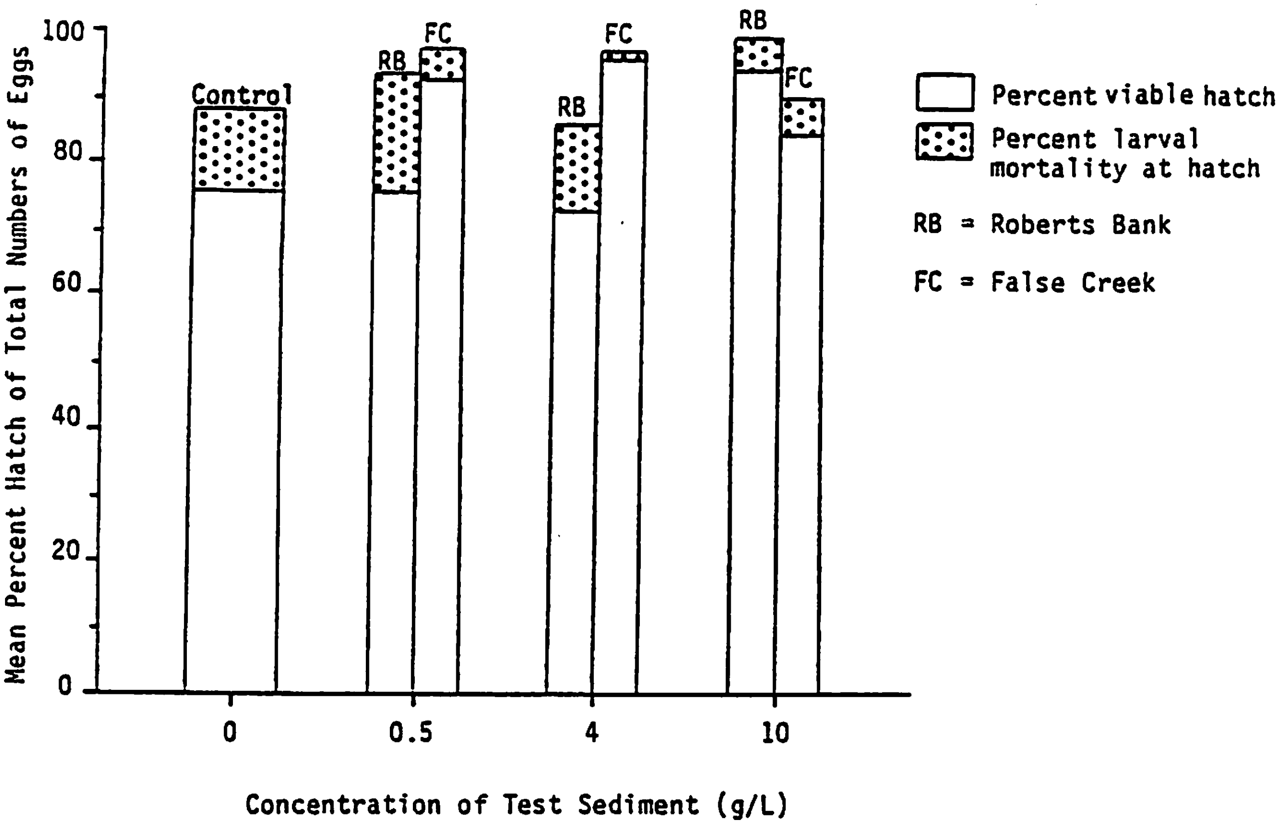

Fig 1. Mean percent hatch of lingcod eggs from the eyed stage in various concentrations of suspended Roberts Bank and False Creek sediments.

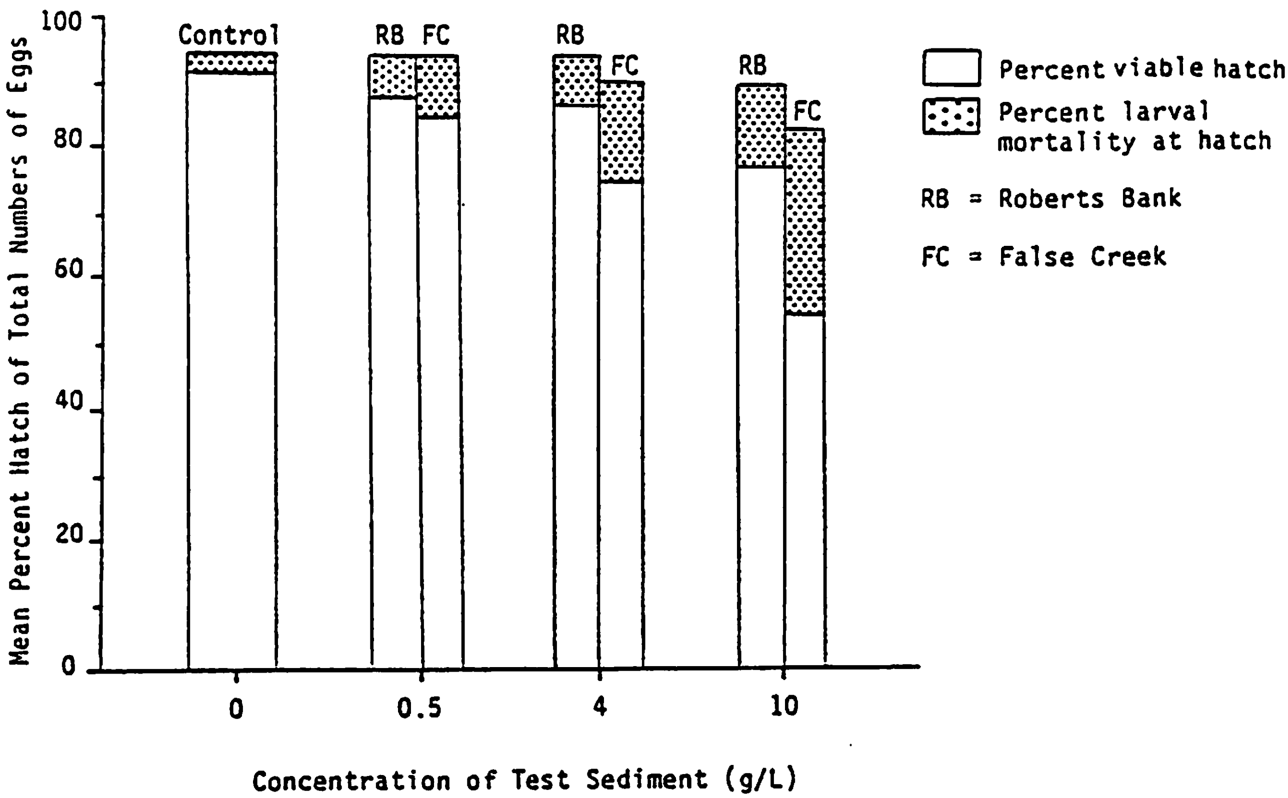

Fig. 2. Mean percent hatch of herring eggs in various concentrations of suspended Roberts Bank and False Creek sediments.

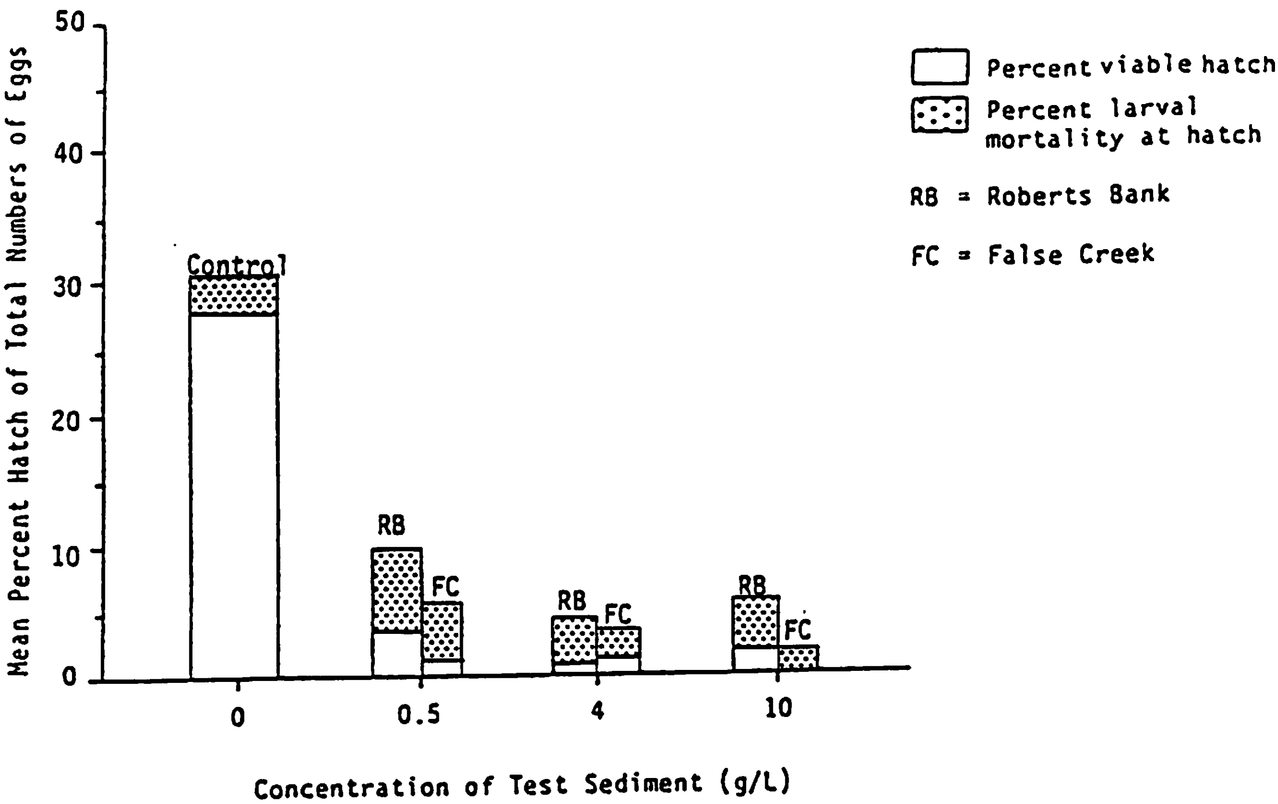

Fig. 3. Mean percent hatch of surf smelt eggs in various concentrations of suspended Roberts Bank and False Creek sediments.