Effects of Volcanic Ash and Estuarine Sediment on the Early Life History Stages of the Pacific Herring, Clupea harengus pallasi

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Water Resources Research Institute
Oregon State University
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ABSTRACT

Volcanic ash from the eruption of Mt. St. Helens was an important component of the sediment which caused shoaling in the Columbia River and downstream in the estuary. The present research was conducted to assess the potential effects of volcanic ash upon the early life history stages of the Pacific herring, Clupea harengus pallasi, a species which spawns demersal eggs in the lower Columbia River estuary. These effects were compared with effects of uncontaminated estuarine sediment. The stages considered in this work were developing eggs, yolk-sac larvae, and newly feeding larvae.

Experiments conducted with eggs used both static and dynamic systems. In the static experiments, ash or sediment suspensions were allowed to settle on the developing eggs; increasing concentrations resulted in slowed development times and mortalities approaching 100%. The effects were consistent with oxygen deprivation from smothering and were more dramatic with estuarine sediment than with equivalent concentrations of volcanic ash. In the dynamic experiments, suspensions were maintained nearly constant throughout incubation. Although a fine layer of sediment or ash accumulated on the eggs, mortality rates and development times did not differ from controls, suggesting that the chorion prevented abrasion and protected the developing embryos.

Experiments with newly hatched yolk-sac larvae were based upon a 24 hour exposure to suspensions. At the end of this period, mortalities based upon lack of heartbeat were no greater in experimental than in control groups of larvae. Maintenance of these larvae in clean water beyond the 24 h period, however, suggested that slight increases in mortality were apparent in higher concentrations, with a more marked effect in the volcanic ash trials.

Qualitative assessment of the larval finfold suggested that abrasion was occurring and was more apparent in the ash experiments. This was confirmed through histological analysis.

Experiments with feeding larvae showed interesting effects of both types of suspension upon feeding incidence and the mean number of food items consumed. In sediments resuspended periodically, control values were below those of virtually all suspensions, suggesting that the suspensions stimulate feeding. In experiments with continually suspended ash and sediment, control values were greater than all but 500 mg/l estuarine sediment suspensions and 500 and 1000 mg/l volcanic ash suspensions. It is suggested that the suspensions provide contrast between the prey items and the surrounding water, promoting feeding.

Overall, the effects of volcanic ash and estuarine sediment were not severe upon herring larvae at environmentally realistic suspension concentrations. The greatest potential effect of increased suspended ash or sediment in the lower Columbia River estuary will be upon the egg stage, where smothering may result in increased mortalities.

FOREWORD

The Water Resources Research Institute, located on the Oregon State University campus, serves the State of Oregon. The Institute fosters, encourages and facilitates water resources research and education involving all aspects of the quality and quantity of water available for beneficial use. The Institute administers and coordinates statewide and regional programs of multidisciplinary research in water and related land resources. The Institute provides a necessary communications and coordination link between the agencies of local, state and federal government, as well as the private sector, and the broad research community at universities in the state on matters of water-related research. The Institute also coordinates the inter-disciplinary program of graduate education in water resources at Oregon State University.

It is Institute policy to make available the results of significant water-related research conducted in Oregon's universities and colleges. The Institute neither endorses nor rejects the findings of the authors of such research. It does recommend careful consideration of the accumulated facts by those concerned with the solution of water-related problems.

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INTRODUCTION

The eruption of Mt. St. Helens had far-reaching effects on aquatic resources from the headwaters of the Toutle River to the Columbia River estuary, and was even noted offshore in the Columbia River Plume waters (Baker and Curl 1981). Virtually all of the fish in the Toutle River, and the Cowlitz River below its confluence with the Toutle, were killed. In addition, volcanic mud and debris buried large areas of the Toutle River watershed under 10 to 450 feet of mud, destroying fish habitats and adversely altering the physical properties of the flowing waters (Martin et al. 1982). Shoals of ash and mud up to 25 feet deep were found in the Columbia River; these deposits have also been carried to the estuary where shoaling has been noted near the port of Astoria. It has been estimated that 14 million cubic yards of mud must be dredged to return the river to its original dimensions.

The effects of the eruption had important, direct effects upon the biota of area rivers and the Columbia River estuary through direct habitat alteration, particularly for fishes. The direct habitat modification may have an impact on fish distribution (through habitat selection), but further effects of sedimentation and higher suspended particulate loads may have effects upon the fishes' physiology (Redding and Schreck 1982), behavior (Gardner 1981; Swensen and Matson 1975), and other indirect effects (Muncy et al. 1979). Increased particulate load, and therefore certain of these effects, may continue in the Columbia River as ash and sediment are added to the water by erosion and disturbance from dredging. Particularly, fine particles of volcanic ash flow downstream as suspended

particulates, continuing to settle in the waters of the lower estuary. The Columbia River estuary is a nursery area for many marine species, and increased sedimentation rates may affect reproduction, hatching, and larval survival, as has been noted in other habitats (see Muncy et al. 1979 for a review). Ash particles may have further detrimental effects on epidermal and gill tissues of juvenile chinook salmon; more devastating effects might occur in delicate tissues of early larvae, which are more subject to sublethal effects than are later life stages. It is the intent of this research to focus on the effects on early life history stages of a Columbia River estuary species, the Pacific herring (Clupea harengus pallasi).

The effects of suspended ash upon larvae has not been studied; effects on salmonids were started almost immediately after the eruption, but the results are not completely clear. The Toutle and Cowlitz River systems had large runs of economically important salmonids before the volcanic eruption. The immediate effect on these fish was devastating and research has been conducted to determine the concentrations of volcanic ash which impact salmonids. Early work centered on live-box bioassays, which showed values of LC50 of about 480 to 1300 mg/l of suspended ash and sediment, with lower levels generally apparent for smolts as compared to presmolts (Stober et al. 1982). Laboratory bioassays by the same investigators, however, showed a drastic increase (15 to 55-fold) in LC50, with higher values characterizing smolts. The discrepancy in laboratory and field trials may be attributed to the variable nature of water quality parameters (temperature, velocity, and organic compounds, among others) in the live box bioassays, while only suspended ash and sediments varied in the laboratory bioassays.

The sublethal effects of volcanic ash have also been examined in salmonids. Volcanic ash is composed primarily of silica (Fruchter et al. 1980), that has sharp, angular characteristics which might enhance abrasion. Although early reports detected abrasion and puncturing damage by ash particles (Amos 1980), Stober et al. (1982) found no effect of suspended ash on the structure of gill tissue or upon the subsequent ability of the smolts to enter seawater or perform in swimming trials. Under short term conditions, Redding and Schreck (1982) also found no mechanical abrasion of the gills, but found that an exposure to 2000 to 3000 mg/l of sediment or ash causes physiological responses associated with sublethal stress, including increased hematocrit and plasma corticosteroid levels. Volcanic ash may thus lower the chances of subsequent survival of fish, and its sublethal effects may become more pronounced when other stressful factors, such as increased water temperature or velocity, are evident.

These studies have concentrated on juvenile and adult stages of salmonids. Adult fish populations may not be as susceptible to discharges of suspended sediments as fish eggs and larvae. In both the upper and lower Columbia River estuary, several species deposit demersal eggs which eventually hatch and, as small pelagic larvae, feed and develop in the estuary. Demersal eggs are, by their nature, stationary on sediment or gravel and are thus subject to direct abrasion and smothering by settling ash or sediments, which may inhibit gas exchange across the chorion to the developing embryos. After hatching, larvae lack the protection of the egg chorion, and the free swimming yolk sac larvae are subject to direct abrasion and other detrimental effects of the ash. After depletion of yolk reserves, larvae must feed; both turbidity and suspension of particles similar in size to food items may alter the ability of fish to

discern and consume normal prey. Also, alterations in the penetration of light from increased turbidity may result in changes in the vertical distribution of larvae and subsequent separation of larvae from appropriate food sources.

We know that survival of the egg and larval stages of marine fishes is important to population dynamics; it has been suggested that critical periods of heightened mortality at these stages may result in the determination of year class strength (Hjort 1914; Hunter 1976). While some factors may be severe enough to cause larval mortalities, other, less direct factors may cause sublethal effects, leading to a decrease in survival potential later in life. Even a brief exposure to a toxin may cause an energy deficit which the larva must overcome to survive (Rosenthal and Alderdice 1976).

A great deal of research has been conducted on the effects of suspended sediments on fish eggs, but little is known of the actual concentrations at which lethal or sublethal effects occur. Concentrations of 1000 mg/l were found to have an effect on hatching success of striped bass and white perch (Auld and Schubel 1978), and also on herring (Rosenthal 1971). The same concentrations had no effect, however, on yellow perch, alewife, or American shad (Auld and Schubel 1978). Similarly, Kiorboe et al. (1981) observed an effect of 500 mg/l suspended sediment on the development of herring eggs. Although Auld and Schubel (1978) and Kiorboe (1981) described no sublethal effects, Rosenthal (1971) observed increased malformations and retarded development in Atlantic herring hatching in 1250 mg/l of red mud. Morgan et al. (1973) observed similar effects in striped bass.

After larvae hatch, habitat selection and limited avoidance behavior

may occur. Lack of the egg chorion will allow more rapid oxygen exchange, but direct epidermal abrasion due to ash may cause lethal or sublethal stress. Swenson and Matson (1976) found that growth and survival of lake herring larvae were not influenced by suspended sediments at naturally occurring concentrations (1-28 mg/l). At higher concentrations, short term exposures to suspended sediment increased larval mortality in studies by Auld and Schubel (1978), Rosenthal (1971), and Sherk et al. (1975).

Another major sublethal effect on fish larvae is restricted food availability; appropriately-sized food must be present in high concentrations for significant survival and growth (Hunter 1972). Large-scale mortalities often occur among captive larvae immediately following yolk absorption, implicating starvation as the major cause of death. Turbidity decreases feeding rates in juvenile bluegill (Gardner 1981). The effects upon larval fish feeding, however, remain unknown. Larvae are generally visual feeders (Hunter 1981) and small larvae typically feed on particles about 50 µm wide. The the possibility exists for consumption of suspended particles with food. This could result in either damage to intestinal tissues or to blockage of the esophagus (Rosenthal 1971).

The goals of this research were to determine the effects of suspended ash and estuarine sediment on the early life history stages of the Pacific herring, Clupea harengus pallasi, a marine species which spawns demersal eggs in the lower Columbia River estuary. This species has adhesive, demersal eggs, a common characteristic among estuarine-spawning fishes. It is an important resource in the northern Pacific, both as commercial catch and as forage for other important species. Its range is from southern California to Alaska, across the Bering straits, and south to

Honshu Island, Japan (Hart 1974). Spawning occurs in estuaries in Oregon and Washington in January through early May (Steinfeld 1972; Pearcy and Myers 1974). Prior to migrating to nearshore coastal areas, larvae develop and feed, remaining in the estuarine nursery grounds through the juvenile stage. Our specific objectives with this species are to determine the following:

-- lethal concentrations (mg/l) of volcanic ash and of uncontaminated estuarine sediment on the hatching success of herring eggs;

-- developmental anomalies and abnormalities associated with larvae hatched from ash and sediment-exposed eggs as compared to controls;

-- lethal concentrations of volcanic ash and of estuarine sediments on yolk-sac larvae after hatching;

-- effects of ash and sediment upon feeding success in healthy larvae;

-- mechanical effects of volcanic ash upon epidermal structure in larvae.

MATERIALS AND METHODS

Volcanic ash and estuarine sediments

Volcanic ash from Mt. Saint Helens was obtained from the Oregon State University Soil Science Department. The original source was from airfall ash at Moses Lake, Washington. This ash was dominated (weight percent fraction) by 20-45 µm particles (Fruchter et al. 1980). Uncontaminated estuarine sediment was collected at low tide from a specific area near Sally's Bend on Yaquina Bay, Oregon. Both sediment and volcanic ash were standardized with respect to size by settling techniques. Particles larger than 24 µm were removed by settling over 10 cm. This resulted in a particle size distribution smaller than that used in other experimental studies (Stober et al. 1982; Redding and Schreck 1982), but was necessary for the work on fish larvae. Maintenance of suspensions of larger particles would not have been compatible with the relatively low turbulence necessary for larval fishes; for flow-through systems, moreover, larger particles would have clogged the screens necessary to segregate the larvae. Secondly, the smaller particles may be more realistic with respect to what larvae may encounter in the lower Columbia River estuary. Particles remaining in suspension (those smaller than 24 µm) were allowed to settle over 48 hours. After this time, the ash and sediment were treated differently. For both, the supernatant was siphoned off the settled material. For the volcanic ash, the remaining material was dried and the ash subsequently dispersed. The sediment was autoclaved and maintained in a liquified state to maintain particle separation; this solution typically contained about 80% water. Final particle sizes of

volcanic ash and estuarine sediment were analyzed with a Coulter counter and were slightly different (Table 1). Standardized suspensions (mg/l) of sediment and ash were used to develop calibration curves of optical density; these curves were used to check the concentrations of suspensions during experiments.

Specimen collection and maintenance

All experiments were conducted at the Oregon State University Marine Science Center. The seawater system for these facilities draws water from Yaquina Bay during high tides to maintain salinities in excess of 27 ppt. This water flows through settling and storage tanks into the laboratories. Adult herring, when maintained in the laboratory, were held in this seawater at ambient temperature. For experimental use, the water was passed through a sand and gravel filter to remove particulate matter and then irradiated with ultraviolet light to eliminate pathogens. It was mixed with the appropriate amount of dechlorinated fresh water to bring the final salinity to 15 ppt, and held in a large aerated reservoir in a constant temperature room to allow equilibration to the desired temperature and ambient dissolved oxygen levels. Light in the constant temperature room, where all egg incubation, larval rearing, and experiments were conducted, was maintained at an intensity of 150 ft-candles at the water surface; photoperiod was adjusted to reflect seasonal changes in daylength.

Pacific herring (Clupea harengus pallasi) were collected either as adults, which were spawned in the laboratory, or as naturally spawned eggs. This species spawns in Yaquina Bay during winter and early spring months. In 1982, all work was conducted with naturally spawned eggs collected along the north jetty of Yaquina Bay on 6 March, 14 April, and

Table 1: Particle size distribution of volcanic ash and estuarine sediment after settling and preparation for egg and larval experiments.

Particle Size	SEDIMENT		ASH	
µm	% by number	% by weight	% by number	% by weight
19.0 - 23.9	0.1	3.9	0.1	3.8
15.1 - 19.0	0.3	8.0	0.6	8.7
12.0 - 15.1	1.0	12.1	2.9	18.8
9.5 - 12.0	2.4	14.3	6.0	21.4
7.5 - 9.5	5.0	15.1	10.5	18.4
6.0 - 7.5	9.9	15.1	14.6	12.9
4.8 - 6.0	19.2	14.6	18.9	8.3
3.8 - 4.8	29.5	11.0	21.8	4.9
3.0 - 3.8	32.7	6.0	24.6	2.8

16 April after natural spawns. After bringing the eggs to the laboratory, they were placed in static 40 liter aquaria with aeration at 10°C in a constant temperature laboratory. Approximately 50% of the water was changed daily. As larvae hatched, they were transferred in small beakers to 250 liter tanks prior to use in experiments. Those used for yolk-sac larval experiments were used within two days of hatching. Larvae to be used for feeding experiments were monitored for yolk utilization and feeding ability. When specimens were capable of feeding, the rotifer(Brachionus plicatilis),along with algal culture, was added to the tanks in densities (see feeding experiments, below) which allowed a high rate of feeding.

Adult herring were captured for egg and yolk-sac larval experiments in 1983. Adults were captured in February during a commercial roe fishery in Yaquina Bay and in March by fishing from local piers. Gravid individuals from the fishery were immediately spawned onto glass microscope slides; the adhesive eggs remained in monolayers on the slides and were fertilized by immersing in small containers containing freshly extruded sperm. After several minutes, which insured virtually 100% fertilization, slides were washed and suspended in aquaria from styrofoam floats. Water and egg handling was subsequently conducted as described above. The adults captured live in March were maintained in the laboratory for short periods in running seawater at ambient bay temperatures prior to spawning.

Egg experiments

All experiments on the effects of suspended volcanic ash and estuarine sediment upon herring eggs were conducted during 1983, when adult fish were available, allowing controlled timing for fertilization

and controlled substrate for incubation. Two sets of experiments were conducted, static and dynamic, as described below. All egg experiments were conducted at a constant temperature of 12°C, which is associated with a hatching time of 10.6 days (Alderdice and Velsen 1968). The static experiments were conducted in 4 liter glass vessels filled with 2.5 liters of ash or sediment suspension, resulting in a water column height of 15 cm. Other than gentle aeration, there was no disturbance in these vessels during the experiments to maintain the ash or sediment in suspension. Eggs for the experiments were spawned on 7 February from fish captured by commercial fishermen. Slides were allowed to remain in clean seawater for 24 hours at 12°C. At this time, eggs were observed and all unfertilized eggs were removed from the slides; the slides were then placed horizontally in small holders on the bottoms of the experimental vessels and the suspension was uniformly mixed. Two slides, with 201 to 443 eggs each, were placed in each of the following treatment vessels:

Estuarine sediment: 0, 500, 1000, 2000, 4000, and 8000 mg/liter.

Volcanic ash: 0, 500, 1000, 2000, 4000, and 8000 mg/liter.

During the experiments, water and ash suspensions were changed at 72 h intervals. At 48 h intervals, the dead or deformed eggs were removed from the slides with forceps and dissecting needles and enumerated; prior to returning the slides to the appropriate experimental vessel, the suspensions of ash or sediment were thoroughly stirred. This procedure was continued until 216 hours post-fertilization, when the first sign of hatching was apparent. To prevent loss of larvae hatching under sediment

and not being recovered, all slides were removed from the suspensions and floated vertically from small styrofoam floats in one-liter beakers of clean seawater. This allowed hatching to proceed and the larvae to be recovered and recorded from individual replicate slides. Hatching was monitored until 288 hours post-fertilization, or approximately 32 hours after the time of median hatching as predicted by Alderdice and Velsen (1968). At this time, all slides were preserved in 10% formalin for later enumeration of the total numbers and stage of development of the remaining eggs.

For the dynamic dosing experiments with herring eggs, a different apparatus was necessary to maintain the volcanic ash and estuarine sediment in continuous suspension and to circulate it over the eggs. This apparatus was designed to eliminate the need for manual suspension of sediments and to create more uniform exposure of the suspensions to each larval container. The actual device (Figure 1) was designed to test three replicate groups of eggs or larvae at six concentrations. Three 1-liter experimental chambers were cut from 4 inch (I.D.) black ABS plastic pipe covered at one end with 335 µm nylon mesh screen and connected in a rosette pattern to a center PVC pipe which supported both the chambers and an airlift device. The rosette was partially submerged in a round 10 liter black plastic tank containing the suspension of ash or sediment which was kept in suspension outside the chambers by a variable-speed stirrer. The nylon screen was small enough to keep larvae and eggs contained and still allow suspensions (and for later experiments, larval food) to be recycled back into the 10 liter container. A concave deflector was placed in the fourth position (Figure 1) to create a mixing area within the larger container. Two airstones placed beneath the center pipe airlifted the suspensions through three glass tubes (9 mm I.D.). The

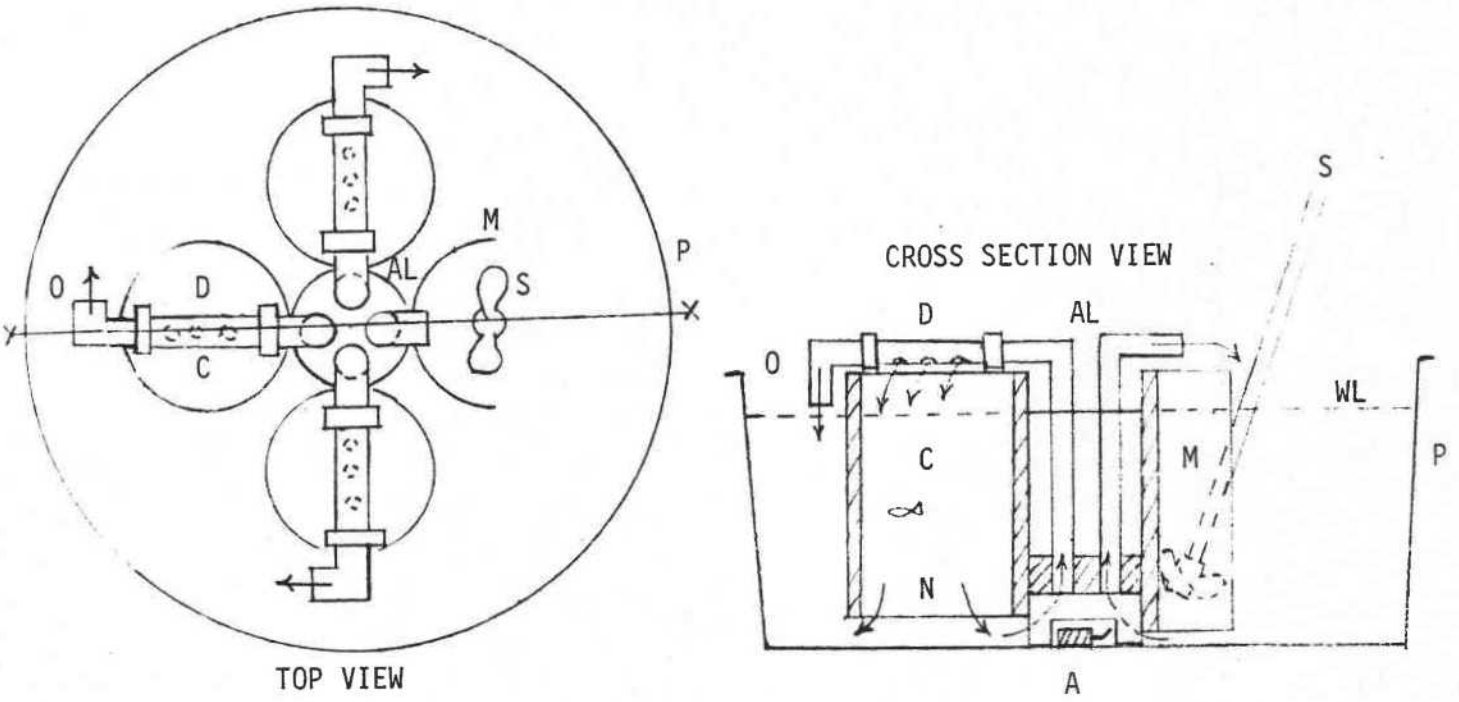


Figure 1: The three-chambered dynamic dosing device used in the eggs, yolk-sac larvae, and feeding larvae experiments with Pacific herring. Total volume of the containers holding the larvae was one liter, the volume of the larger tank 10 liters. The flow rate into the smaller chambers was maintained at approximately 240 ml/min. A, airstone; AL, airlift system; C, dosing chamber; D, flow regulator; M, mixing area; N, 335 μm nylon screen; O, overflow; P, 10 liter tank; S, stirrer connected to variable speed motor; WL, water level. Arrows indicate the direction of the flow of suspension through the apparatus.

tubes passed through a rubber stopper within the center pipe. These glass tubes were bent at right angles at the top and directed into the three dosing chambers. The airlift system above the test containers was continued with 1.3 cm (O.D.) plastic tubing with three 0.140 inch holes bored along one side to allow control of the trickle of suspensions into the test containers. Flow rates were maintained at approximately 240 ml/minute.

The dynamic bioassays were conducted with herring eggs spawned on 7 February (concurrent with the static bioassays) for trials with volcanic ash and with eggs spawned 21 March for the estuarine sediment trials. Protocol for spawning eggs onto slides was handled as described previously. Briefly, 247 to 490 eggs (ash trials) or 99-140 eggs (sediment trials) were spawned in a monolayer onto cleaned glass microscope slides and fertilized with sperm from several males. After washing in seawater, they were suspended from styrofoam floats in 40 liter tanks. After 24 hours, the slides were examined and unfertilized eggs were removed prior to initiating experiments. Four replicate slides were introduced to each of six suspension concentrations (0, 500, 1000, 2000, 4000, and 8000 mg/l); during the trials, the labelled slides were suspended from styrofoam floats within the dosing chambers of the dynamic device (Figure 1). Slides were removed from the device each 48 hours to count and remove dead or deformed eggs and embyros. The seawater and suspensions were changed once during the trials, which lasted until larvae began to hatch. At this time (216 h post-fertilization in the ash experiments and 192 h post-fertilization in the sediment trials) slides were moved to individual 1-liter beakers with clean, aerated seawater. These were checked daily, with hatched larvae enumerated as deformed or normal (following Barahona-Fernandes 1982) and dead eggs removed. For

both experiments, termination occurred at 288 h post-fertilization, when remaining eggs were classified as dead, arrested development, or normal development (the latter category may have hatched given sufficient time). Voucher specimens from each treatment were preserved in 10% formalin.

Yolk-sac larvae experiments

Two types of experiments were conducted to evaluate the effects of continually suspended sediment and ash on newly hatched herring larvae. The first set of experiments was carried out in 1982 and was designed to determine the immediate effects of a 24 h exposure on larvae. The second set was carried out in 1983; after a 24 h exposure to the suspensions, larvae were transferred to separate containers and mortality was followed over time for unfed larvae.

The first set of experiments was carried out on 19 March with eggs which had been naturally spawned on the north jetty of Yaquina Bay. After hatching, larvae were counted to individual 1-liter containers held within larger containers. The small containers had a nitex false bottom to allow free flow of sediment and ash suspensions. Larvae were introduced from small beakers into duplicate containers at the six levels of suspended ash or sediment; each two hours the suspension was replaced with a new one liter volume slowly siphoned into the vessel. After 24 hours, the yolk-sac larvae were removed to petri dishes and enumerated as either dead or alive. The criterion of death was lack of a heartbeat at the end of the 24 h period. Live larvae were subsequently preserved for later microscopic analysis.

The 24 h dose itself may not have resulted in direct mortalities as measured by the absence of a heartbeat, but rather may have induced

sublethal effects, with later mortality. The 1983 experiments were designed to address both the constant introduction of sediment and ash suspensions as well as the subsequent development and survival of the larvae beyond the 24 h term of the experiment. These experiments were conducted on 4 and 5 March with larvae hatched on the previous day. Again, approximately 20 larvae were counted out and introduced to each experimental vessel; three replicates were run for each concentration of the appropriate suspension. After 24 h of exposure in the dynamic dosing device, the stirrers and aeration keeping the ash or sediment in suspension were turned off and the sediment and ash allowed to settle. Larvae were individually pipetted out and enumerated; live larvae were placed in 1-liter containers of clean seawater. Larval mortalities were monitored over the subsequent nine days.

Feeding experiments

Larval feeding trials were conducted with the prey source consisting of the rotifer Brachionus plicatilis. Methods of mass culture of the rotifer followed Theilacker and McMaster (1971). The culture required daily maintenance to provide Brachionus with adequate food for growth. The marine phytoplanktonic alga, Isochrysis sp. (Ewart and Epifanio 1981), was cultured as food for the rotifers. Plexiglass tanks containing 50 liters of algae at a density of approximately one million cells per ml were innoculated with between 150,000 and 200,000 Brachionus. Aeration and constant illumination were provided at a temperature of 18°C. Algae were added as necessary to the increasing populations of rotifers. Maximum densities of 100 rotifers per ml were attained before the cultures began to decline. Mass cultures were maintained for approximately 30 days with minimal harvesting. The average size of Brachionus was .220 mm

long and .125 mm wide, a size acceptable to first feeding herring larvae. Rotifers were harvested for experiments by seiving through a 65 µm screen, washed in clean seawater, and placed in a larger vessel overnight to acclimate to the 12°C experimental temperature.

Two types of experiments were conducted with feeding larvae. The first was based upon experiments which minimized turbulence and disturbance to the larvae during feeding trials. First-feeding planktonic larvae are visual feeders with stereotyped behavioral sequences necessary for feeding (Hunter 1972; Blaxter and Staines 1971). Therefore the excessive disturbance necessary for constant maintenance of given suspensions might inhibit feeding capabilities, resulting in very low feeding incidence even in controls (Brownell 1980). The two types of experiments were therefore based upon periodic suspension of ash or sediment in the first set and constant maintenance of he suspensions in the second set. This latter set of experiments resulted in higher turbulence but maintained mean suspension concentrations within 10% of the nominal value; thus lower feeding incidence was expected a priori.

The first set of experiments was conducted in 10-liter black circular tanks containing a total volume of 5 liters during the experiments. Larvae were chosen at times when all had initiated feeding; experiments were run on 24 and 25 March. On the night prior to the experiment, 100 to 200 larvae were introduced into three liters of water in the experimental vessel without food to allow evacuation of stomach contents. Preliminary experiments demonstrated that this period of time would result in evacuation of identifiable food items. The following morning, an additional 2 liters of suspended ash or sediment was added to make final concentrations of 0, 500, 1000, 2000, 4000, and 8000 mg/l. The prey

(Brachionus plicatilis) was then added, followed by thorough stirring with a paddle, to begin the experiments. Food concentrations were provided at 6 rotifers per ml, a density which would initiate a well-defined feeding response in the larvae. The duration of the experiment was two hours with resuspension of ash or sediment concentrations at 15 min intervals. While this treatment kept turbulence low, it also resulted in some loss of suspension concentration. Separate trials were conducted to determine the change in suspension concentration at the surface, middle, and bottom of the experimental vessel at the end of the 15 min stirring interval. Monitoring the concentrations showed that the surface layer (top 0.5 cm) densities decreased, averaging 32% lower than the nominal concentrations. In the middle depth, there was an average 16% decrease in concentration, and at the bottom of the containers, a 50% increase in concentration. A similar trend of changes in the densities of food organisms was apparent, with increasing abundance of food particles in the denser ash or sediment at the bottom of the experimental vessels at the end of the 15 min. At the end of the 2 h feeding period, experiments were terminated by quickly seiving the suspensions and larvae through a .335 mm nylon screen and immediately preserving the larvae in 10% formalin to minimize potential regurgitation of food. Although this handling may cause regurgitation in this species, it was consistent among treatments and therefore resulted in no bias in the results.

In the 11 and 13 May feeding experiments, the 3-chamber dosing device described above (under dynamic egg dosing experiments) was used. Three replicates of about 20 larvae were tested per treatment, in which the suspensions were continually maintained. Again, larvae were preserved at the termination of experiments for later enumeration of feeding incidence

and number of food particles. Preserved larvae were examined individually under a dissecting microscope for presence of food. Discrete food particles were enumerated and recorded for each larva. This could be done without dissection as the gut is straight in herring larvae and the transparent gut wall makes observation possible.

Histological preparation and analysis

After 24-h yolk-sac and larval feeding experiments, selected larvae were preserved in 2% buffered glutaraldehyde in teleost saline. Preserved larvae were randomly selected from these experiments and subsequently processed for histological analysis. Larvae were dehydrated with alcohol, cleared with toluene, and embedded in paraffin (Paraplast-plus, 56-57°C). Within the limits of larval body configuration in the paraffin, larvae were embedded for sagittal and longitudinal sections. Larvae were serially sectioned at a thickness of 6 µm on a rotary microtome. Sections were mounted on glass slides and stained with Harris' hematoxylin and eosin-phloxine B. Sectioned specimens were examined under a microscope for evidence of abrasion to the epidermis in both yolk-sac and feeding treatments.

Specimens from the yolk-sac larval experiments were also prepared for scanning electron microscopy. Larvae were prepared for observation following the procedures of Dobbs (1974). Briefly, larvae were preserved in 2% glutaraldehyde, dehydrated through a graded series of alcohols, and put in two changes of liquid freon. Larvae were placed in small porous capsules and processed in a critical point dryer (Bomar). They were mounted on studs using double-stick tape and coated with a thin layer of gold-palladium in a vacuum evaporator. These specimens were examined on an AMR 1000 scanning electron microscope at the Oregon State University

Electron Microscopy Facility. Particular attention in these specimens was paid to the epidermal tissues and the presence of ash or sediment-related damage.

RESULTS

Egg experiments

The static experiments were conducted in a manner similar to those described by Rosenthal (1971) with horizontal slides; in the current experiments, however, the volume of water (and therefore the suspension) in the experimental vessels was approximately four times that in Rosenthal's experiments. With a water column height of 15 cm, full settlement of the volcanic ash or estuarine sediment would therefore result in 0, 7.5, 15, 30, 60, and 120 mg/cm^2, coating the eggs in the six different treatments. Since the slides were kept in the suspension until hatching began, they remained in the suspension vessels for 216 h, when all were removed and slides placed individually in 1-liter beakers of clean, aerated seawater, where they were maintained until 288 h post-fertilization. Larvae were removed daily, enumerated, and preserved. At the end of the experiment, many of the eggs remained unhatched but apparently contained viable, developed larvae. The numbers of eggs per slide, dead eggs removed, normal and abnormal hatched larvae, and viable and non-viable embryos remaining at the termination of the experiment are shown in Table 2. Since unfertilized eggs were removed in the first 24 h after development (when the experiments began), mortality is calculated as the percentage of the sum of dead eggs removed, moribund or deformed larvae removed at hatching, and eggs which had not developed to the eyed larval stage by 288 h post-fertilization. This latter category, which showed no further development after transfer to clean water, represented the majority of the mortalities (Table 2). The

Table 2: Herring egg experiments in the static egg incubation system with doses of ash or sediment suspensions. Column legends are as follows: A, total number of eggs per slide; B, dead eggs removed during incubation; C, larvae hatched normally; D, deformed, hatched larvae; E, eggs unhatched at termination of experiment, but viable, developed; F, non-viable, undeveloped eggs at termination of experiment.

	Replicate	A	B	C	D	E	F
CONTROL							
	1	342	6	66	4	193	73
	2	250	8	70	13	107	52
	3	231	4	150	9	68	0
	4	365	3	139	21	202	0
A. Volcanic Ash Concentration (mg/l)							
500	1	313	2	86	10	144	71
500	2	293	3	102	4	118	66
1000	1	433	2	87	13	229	102
1000	2	344	3	119	14	133	75
2000	1	442	7	110	10	148	167
2000	2	302	4	70	3	130	95
4000	1	285	4	64	2	105	110
4000	2	379	14	41	3	137	184
8000	1	201	21	10	0	84	86
8000	2	228	26	3	1	38	160
B. Estuarine Sediment							
500	1	312	15	53	8	125	111
500	2	255	2	73	6	127	47
1000	1	356	6	30	8	60	252
1000	2	274	4	26	5	92	147
2000	1	443	56	5	3	33	346
2000	2	218	3	11	3	72	129
4000	1	377	43	0	0	15	319
4000	2	293	11	0	0	20	262
8000	1	276	39	0	0	0	237
8000	2	361	19	0	0	0	342

mortality rates showed an increase with increasing sediment or ash concentration (Table 3). Percent mortalities were arcsin transformed and compared using a one-way analysis of variance with level of suspension as treatments. The estuarine sediment showed an increased effect as compared to the volcanic ash (Table 3, Figure 2). In the ash trials, the only significantly higher mortalities were in the two highest levels of suspension, whereas significantly higher mortalities were apparent in all sediment treatments except that with 500 mg/l (LSD, P =.05). The effects are apparently related to the smothering effects rather than abrasion. Although the overlying water in all treatments was continuously aerated, the embryos in the higher suspension treatments developed grey, granular yolk which is characteristic of embryos deprived of oxygen (Braum 1973). Had we analyzed the data in the manner of Kiorboe et al. (1981), considering the percent survival of embryos with time, there would have been no noticable effect except for slowed development, as the numbers of dead eggs removed, although showing a trend of increase with increasing suspension concentration, were not great (Table 2). Thus the lack of development at the end of 12 days post-fertilization, and particularly a lack of further development after removal from the suspensions, was taken as a sign of mortality in these trials.

Due to the availability of the dynamic dosing devices, the lengths of the experiments, and the availability of spawning herring, the dynamic egg bioassays were run in two sets of trials. The first was conducted with eggs spawned on 7 February (ash experiments) and the second with eggs spawned on 21 March (sediment experiments). Since these experiments were not run concurrently with eggs from the same spawning groups of herring, individual controls were run concurrently with each experiment. The numbers of eggs per slide, dead eggs removed during the experiments,

Table 3: Mean mortality rates and percent deformed, hatched herring larvae in the static dosing experiments. M = mean percent mortality, D = mean percent deformed, SE = standard error. In the mortality columns, an asterisk indicates significantly increased mortalities (ANOVA, Least significant difference, $P < .05$).

Concentration	Volcanic Ash				Estuarine Sediment			
	M	SE	D	SE	M	SE	D	SE
0	16.4	6.04	11.4	3.20	16.4	6.04	11.4	3.20
500	25.7	0.80	7.8	3.85	32.3	10.7	11.7	3.44
1000	27.0	0.14	13.4	1.59	65.8*	8.89	22.9	3.72
2000	37.7	3.93	6.7	2.40	76.7*	14.75	43.6	16.4
4000	46.9*	6.15	5.2	2.10	94.6*	1.42	--	--
8000	67.6*	14.39	16.7	16.7	100*	0	--	--

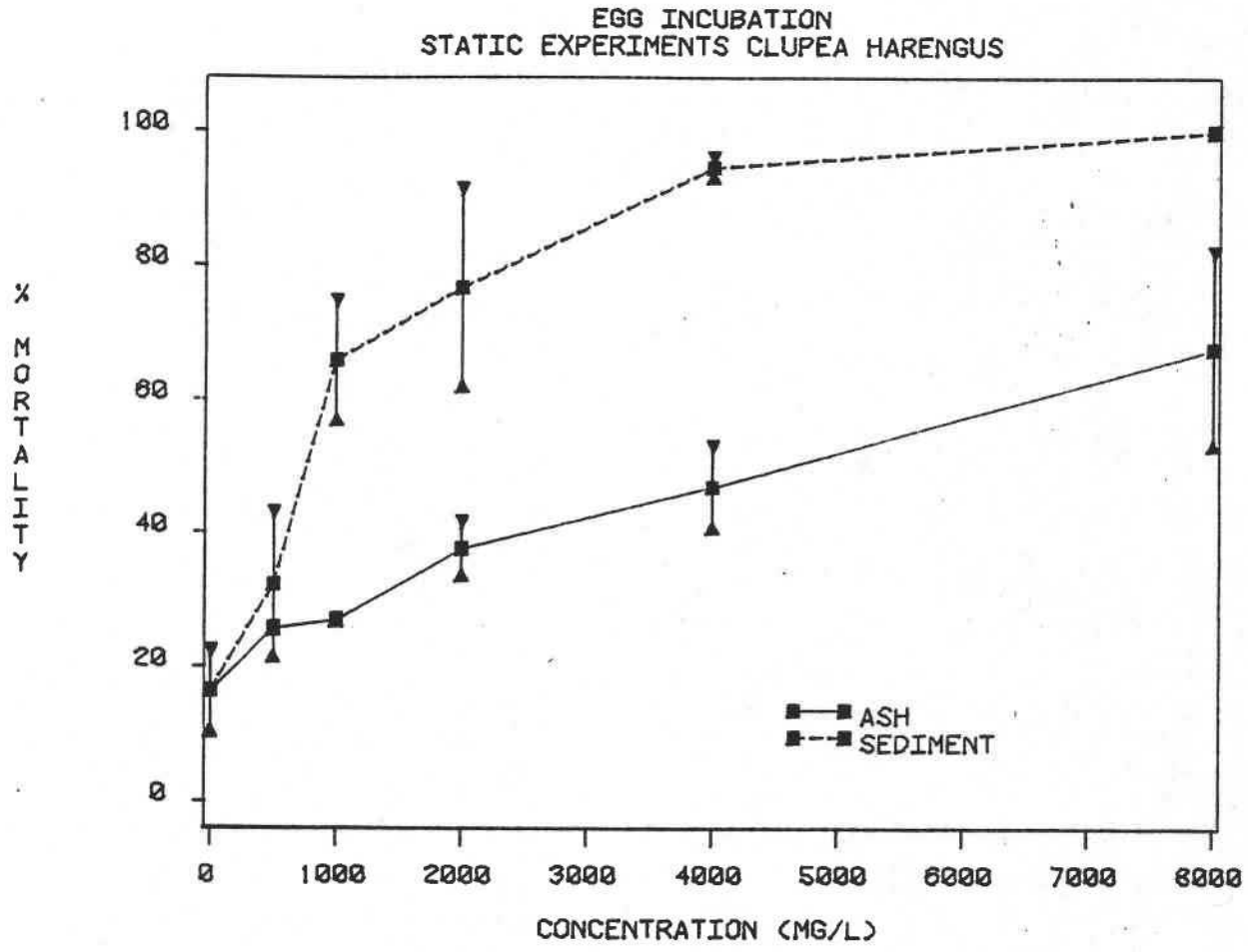


Figure 2: Effects of static estuarine sediment and volcanic ash suspensions on the mortality of Pacific herring eggs. Experiments were terminated 288 hours post-fertilization. Bars indicate ± 1 S.E.

normal hatched larvae, abnormal hatched larvae, developing, viable embryos in eggs at the end of 288 h post-fertilization, and the non-viable, undeveloped embryos at the end of the experiment are presented in Table 4 for the volcanic ash experiments and in Table 5 for the estuarine sediment experiments. Overall mortality levels in these experiments, particularly that contributed by the non-viable, undeveloped embryos (Column F, Tables 4,5) were much lower than those in the static experiments, where this category represented the majority of mortality, particularly at higher concentrations of sediment or ash (Table 2).

The mortality rates observed in the experiments were independent of the concentration of either volcanic ash or estuarine sediment (Table 6, Figure 3), as were the deformed larvae as a percentage of those hatching during the experiments. We interpret the difference between static and dynamic experiments as a function of delivery of oxygen to the developing embryos; in the static dosing device, where the settled ash or sediment could smother the eggs despite aeration of the overlying water, where the water movement in the dynamic device, along with the constant aeration from the airlift in the center of the experimental vessels (Figure 1), prevented smothering of the embyros despite a fine coating of sediment or ash. Although there was no apparent effect of sediment or ash concentration on mortality rates, the mean mortality and percent deformed larvae (Table 6, Figure 3) were significantly higher for the ash trials. This may have been a function of the difference in females from which the eggs were taken. The control eggs in the dynamic ash experiments showed mortality rates similar to the controls of the static experiments, which were spawned at the same time. The effects of sediment, in general, were greater than those of ash in the static trials (Figure 2). The dynamic sediment trials, however, showed earlier first hatching (192 hours

Table 4: Dynamic herring egg experiments with ash suspensions. Column legends are as follows: A, total number of eggs per slide; B, dead eggs removed during incubation; C, larvae hatching normally; D, deformed, hatched larvae; E, viable, developed embryos unhatched at termination of experiment; F, non-viable or undeveloped eggs at termination of experiment.

Concentration (mg/l)	Replicate	A	B	C	D	E	F
0	1	306	3	157	15	116	15
	2	440	10	56	15	266	93
	3	365	6	111	10	196	22
	4	352	10	191	35	100	16
500	1	392	20	64	22	251	35
	2	370	9	88	16	193	64
	3	418	16	60	15	225	102
	4*	--	--	--	--	--	--
1000	1	416	14	31	21	275	75
	2	319	8	75	14	189	33
	3	281	7	131	16	102	25
	4	374	7	44	14	270	39
2000	1	247	1	178	10	39	19
	2	401	8	162	15	188	28
	3	279	8	197	11	62	1
	4	317	6	182	10	108	11
4000	1	444	11	95	23	266	49
	2	490	10	70	13	235	162
	3	366	14	40	7	246	59
	4	456	10	108	12	261	65
8000	1	368	7	73	21	214	53
	2	388	4	147	11	180	46
	3	400	7	178	26	176	13
	4	392	9	134	16	176	57

*slide damaged, sample lost

Table 5: Dynamic herring egg dosing experiments with estuarine sediment suspensions. Column headings are as in the preceeding table.

Concentration (mg/l)	Replicate	A	B	C	D	E	F
0	1	137	1	97	11	25	3
	2	99	5	88	3	3	0
	3	126	3	101	6	15	1
	4	120	3	104	2	11	0
500	1	121	25	68	15	8	5
	2	122	3	74	5	40	0
	3	110	4	94	2	10	0
	4	132	5	99	4	24	0
1000	1	140	2	101	8	29	0
	2	128	29	71	13	11	4
	3	116	4	87	5	20	0
	4	135	7	98	9	20	1
2000	1	107	1	83	4	18	1
	2	121	30	66	7	13	5
	3	136	4	71	12	48	1
	4	122	3	81	2	34	2
4000	1	109	3	78	4	24	0
	2	117	3	90	5	15	4
	3	134	3	77	10	41	3
	4	126	3	61	18	43	1
8000	1	126	6	76	4	40	0
	2	137	3	56	8	70	0
	3	132	7	81	5	39	0
	4	123	2	73	4	43	1

Table 6: Mean mortality rates and percent deformed, hatched herring larvae in the dynamic dosing device. M = mean percent mortality. D = mean percent deformities of all hatched larvae, SE = standard error. Experimental values are not significantly different from the mean (ANOVA, LSD).

	Volcanic Ash				Estuarine Sediment			
Concentration (mg/l)	M	SE	D	SE	M	SE	D	SE
0	16.3	3.8	13.4	3.1	7.8	1.4	5.3	1.8
500	25.2	3.6	20.3	2.9	14.0	7.7	7.6	3.6
1000	19.2	2.4	22.8	6.5	15.9	6.8	9.2	2.2
2000	10.1	1.3	6.1	0.8	14.6	6.9	7.8	2.7
4000	24.4	4.5	15.0	2.0	11.5	2.3	11.1	4.2
8000	17.5	2.4	13.2	3.3	7.7	0.7	7.1	1.8

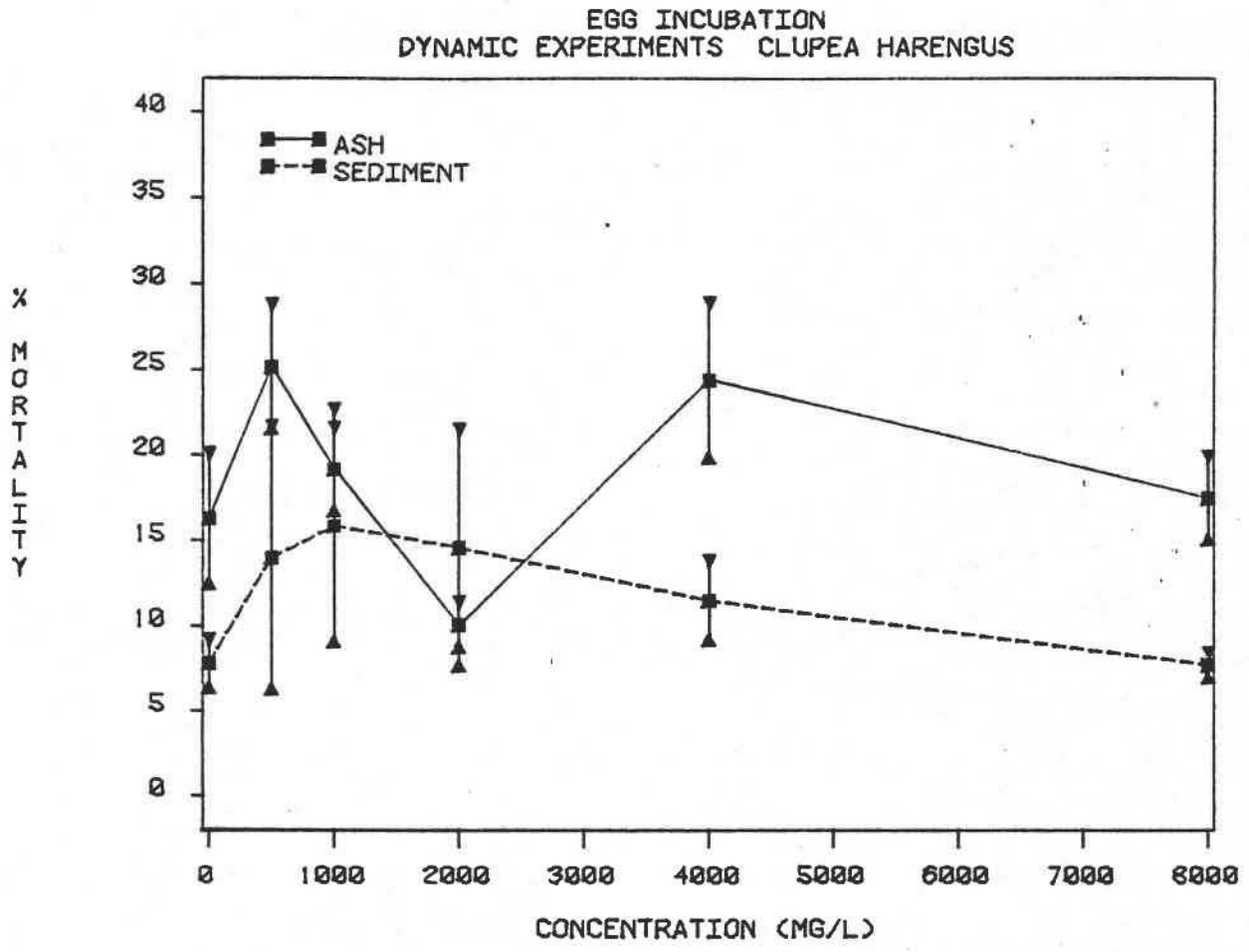


Figure 3: Effects of dynamic, continuously maintained suspensions of estuarine sediment and volcanic ash on the mortality of Pacific herring eggs. Experiments were terminated 288 hours post-fertilization. Bars indicate ± 1 S.E.

post-fertilization versus 216 hours in the ash trials) and a greater percentage hatching within the 288 h term of the experiment. We attribute this difference to different spawning groups, as the adults from which the eggs were spawned were collected some 6 weeks apart; the first group had spawned and probably departed from Yaquina Bay. Different spawning groups of herring may have different egg characteristics (Blaxter and Hempel 1966).

Yolk-sac larvae experiments

The initial yolk-sac larval experiments with the 1-liter containers and 2-hour suspension replacement time resulted in mortality rates which were variable but unrelated to the concentration of the suspensions used. For the trials with volcanic ash, the mean mortality over all concentrations (including controls) was 5.40% (standard deviation 5.72%, n= 12); the corresponding value for estuarine sediment was 5.28% (s= 8.15%, n= 12). We considered that the criterion for survival after 24 h, which was based upon the presence of heartbeat alone, was insufficient to determine the possible effects of the suspensions, since subjectively, larvae appeared in worse condition in the higher concentrations of ash. This assessment was based upon condition of finfold and possible epidermal damage (see discussion of histological assessment, below).

The 1983 experiments were designed to assess the effects of a 24 h exposure by monitoring survival of the remaining larvae for nine days following the tests. The surviving larvae are enumerated in Table 7 for volcanic ash and Table 8 for estuarine sediment. Although the mean mortalities are variable with time, there is a trend of decreasing survival with time in increasing volcanic ash concentration (Figure 4). These data fall into roughly two groups, with lower mortality observed in

Table 7: Effects of volcanic ash suspensions on herring yolk-sac larvae. Values presented are the percent of initial larvae surviving with time. Larvae were exposed to continual suspensions of ash from time zero to 24 hours; the value at day one represents survival at the end of the 24 h exposure. Other values were determined on successive days with larvae maintained in clean water.

Concentration (mg/l)	Replicate	Time (days) 1	2	3	4	5	6	7	10
0	1	100	85	65	50	45	40	35	35
	2	100	100	95.5	40.9	40.9	31.8	31.8	31.8
	3	95	95	80	60	55	45	45	40
	$\bar{x}$	98.3	93.3	80.2	50.3	47.0	38.9	37.3	35.6
	s	2.9	7.6	15.3	9.6	7.3	6.7	6.9	4.1
500	1	100	76.2	66.7	42.9	23.9	19.0	19.0	19.0
	2	94.7	89.5	73.7	57.9	47.4	42.1	42.1	42.1
	3	100	89.5	73.7	52.6	36.8	21.1	21.1	10.5
	$\bar{x}$	98.2	85.1	71.4	51.1	36.0	27.4	27.4	23.9
	s	3.1	7.7	4.0	7.6	11.8	12.8	12.8	16.4
1000	1	57.1	28.6	4.8	0	0	0	0	0
	2	85.7	76.2	71.4	61.9	57.1	52.4	47.6	47.6
	3	70	35	15	0	0	0	0	0
	$\bar{x}$	70.9	46.6	30.4	20.6	19.0	17.5	15.9	15.9
	s	14.3	25.8	35.9	35.7	33.0	30.3	27.5	27.5
2000	1	75	35	25	0	0	0	0	0
	2	71.4	47.6	28.6	9.5	0	0	0	0
	3	50	40	20	5	0	0	0	0
	$\bar{x}$	65.5	40.9	24.5	4.8	0	0	0	0
	s	13.5	6.3	4.3	4.8	--	--	--	--
4000	1	65	60	40	25	15	15	10	10
	2	100	94.4	83.3	55.6	50	38.9	38.9	38.9
	3	90.5	85.7	71.4	61.9	57.1	57.1	57.1	57.1
	$\bar{x}$	85.2	80.0	64.9	47.5	40.7	37.0	35.3	35.3
	s	18.1	17.9	22.4	19.7	22.5	21.1	23.8	23.8
8000	1	28.6	0	0	0	0	0	0	0
	2	68.8	50	43.8	31.3	25	18.8	18.8	18.8
	3	54.5	40.9	40.9	22.7	22.7	22.7	22.7	13.6
	$\bar{x}$	50.6	30.3	28.2	18.0	15.9	13.8	13.8	10.8
	s	20.4	26.6	24.5	16.2	13.8	12.1	12.1	9.7

Table 8: Effects of estuarine sediment suspensions on herring yolk-sac larvae. Values presented are the percent of initial larvae surviving with time. Larvae were exposed to continual suspensions of ash from time zero to 24 hours. The value at day one represents survival at the end of the 24 h exposure. Other values represent survival on successive days with larvae maintained in clean water.

Concentration (mg/l)	Replicate	Time (days) 1	2	3	4	5	6	7	10
0	1	94.7	78.9	73.7	52.6	47.4	42.1	36.8	31.6
	2	100	100	88.9	66.7	55.6	44.4	44.4	22.2
	3	95.2	71.4	38.1	23.8	23.8	19.0	19.0	19.0
	$\bar{x}$	96.6	83.4	66.9	47.7	42.3	35.2	33.4	24.3
	s	2.9	14.8	26.1	21.9	16.5	14.0	13.0	6.5
500	1	83.3	77.8	77.8	55.6	44.4	38.9	27.8	22.2
	2	100	94.7	73.7	52.6	31.6	21.1	21.1	15.8
	3	80	80	45	25	25	25	25	20
	$\bar{x}$	87.8	84.2	65.5	44.4	33.7	28.3	24.6	19.3
	s	10.7	9.2	17.9	16.9	9.9	9.4	3.4	3.3
1000	1	100	100	70	40	30	10	10	10
	2	95	95	65	50	45	45	45	40
	3	100	100	93.3	53.3	53.3	46.7	46.7	40.0
	$\bar{x}$	98.3	98.3	76.1	47.8	42.8	33.9	33.9	30.0
	x	2.9	2.9	15.1	6.9	11.8	20.7	20.7	17.3
2000	1	83.3	83.3	61.1	38.9	33.3	11.1	11.1	11.1
	2	88.9	83.3	61.1	55.6	50	50	50	50
	3	100	100	80	70	70	65	65	65
	$\bar{x}$	90.7	88.9	67.4	54.8	51.1	42	42	42
	s	8.5	9.6	10.9	15.6	18.4	27.8	27.8	27.8
4000	1	90	85	75	60	60	60	60	55
	2	90	80	65	40	40	40	40	35
	3	95	85	45	30	25	25	25	20
	$\bar{x}$	91.7	83.3	61.7	43.3	41.7	41.7	41.7	36.7
	s	2.9	2.9	15.3	15.3	17.6	17.6	17.6	17.6
8000	1	73.7	73.7	15.8	15.8	15.8	15.8	15.8	15.8
	2	77.3	68.2	63.6	40.9	36.4	36.4	36.4	31.8
	3	44.4	38.9	16.7	16.7	5.6	5.6	5.6	5.6
	$\bar{x}$	65.1	60.3	32.0	24.5	19.3	19.3	19.3	17.7
	s	18.0	18.7	27.3	14.2	15.7	15.7	15.7	13.2

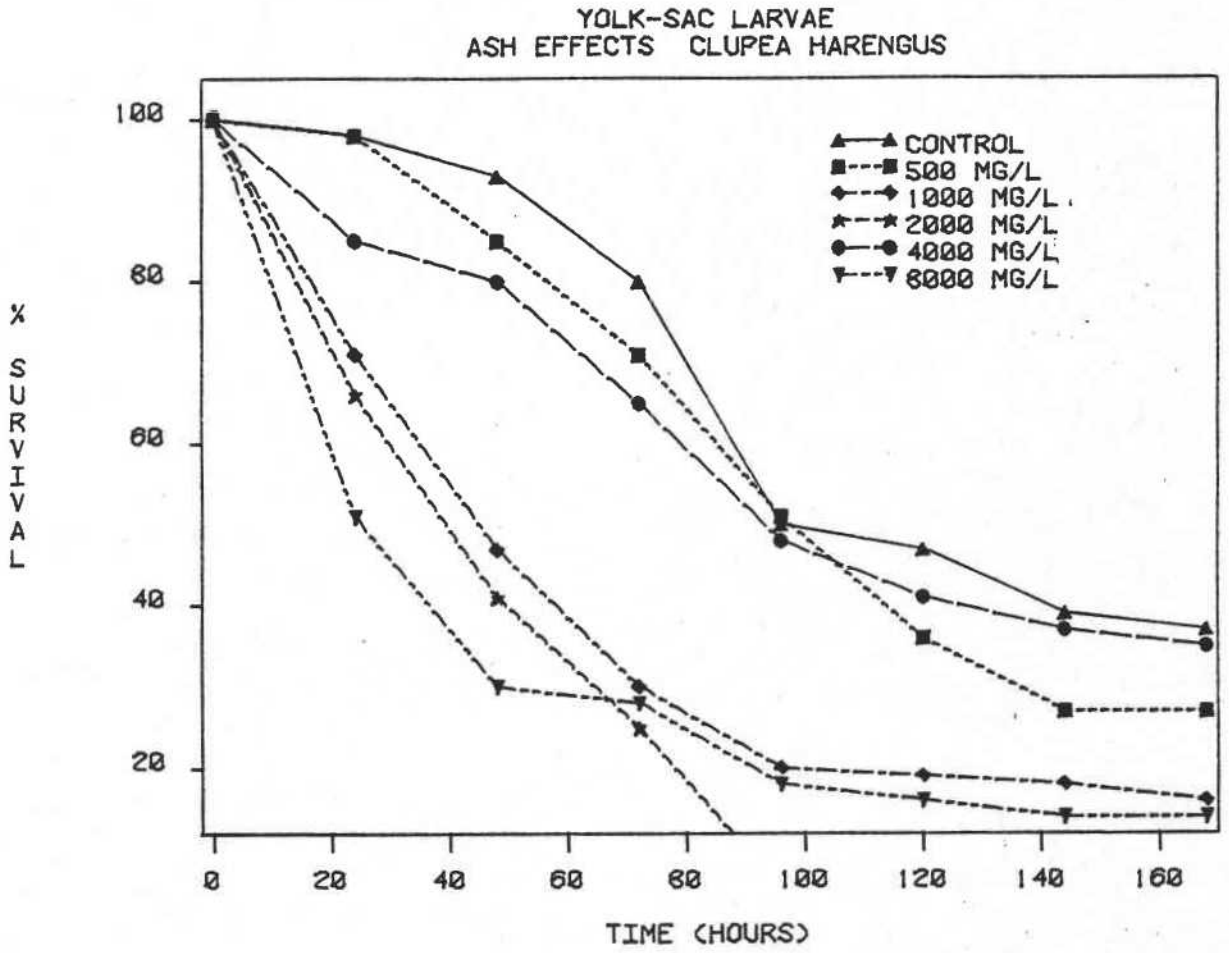


Figure 4: Effects of 24 h exposure to dynamic, continuously maintained suspensions of volcanic ash upon the subsequent survival of yolk-sac larvae of Pacific herring. Larvae were exposed to suspensions for the first 24 h, transferred to clean seawater, and survival monitored each 24 hours.

concentrations of 0, 500, and 4000 mg/l, and higher mortalities observed in 1000, 2000, and 8000 mg/l. There is thus a trend of decreased survival with increasing ash concentration with the exception of the 4000 mg/l experiments. Concentrations of estuarine sediment from 500 to 4000 mg/l show no clear effect on survival (Figure 5). All of these treatments were characterized by patterns of survival with time similar to the controls, with the exception of the 8000 mg/l concentration, which showed a survival curve similar to those with lower survival under suspensions of volcanic ash. Overall, the survival of yolk-sac larvae with time in suspensions of volcanic ash is significantly less than in suspensions of estuarine sediment ($P<.001$, Wilcoxon matched-pairs signed rank test). This result was not observed in the periodically resuspended ash and sediment trials. The observed differences when larvae were kept for extended periods of time may be due to sublethal, abrasive effects of volcanic ash.

Larval herring feeding experiments

The herring larvae used in all experiments readily fed upon the prey, the rotifer Brachionus plicatilis. The first set of experiments, conducted in the larger experimental containers, was semi-static, with stirring every 15 min to resuspend the volcanic ash or sediment. A total of 1502 larvae were examined from the volcanic ash suspension feeding experiments and 1272 from the estuarine sediment feeding experiments, with an overall average of 69% and 74%, respectively, of the larvae feeding during the 2 h duration of the experiment. The numbers of larvae in each experiment which had and had not fed, the numbers of Brachionus consumed, and the average food items consumed per larva are shown in Table 9 for both sediment and volcanic ash. In both sets of experiments, the percentage of larvae feeding increased dramatically from the control

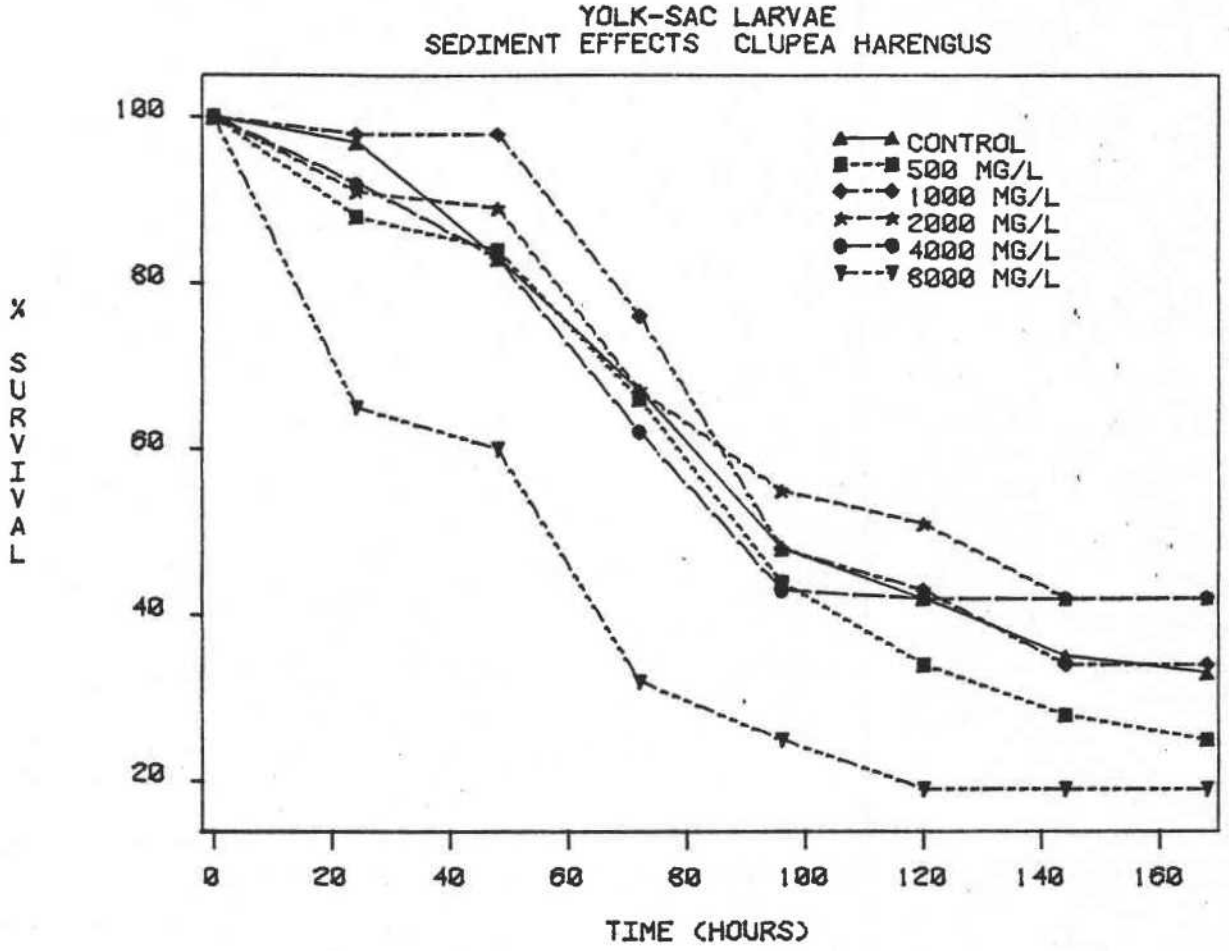


Figure 5: Effects of 24 h exposure to dynamic, continuously maintained suspensions of estuarine sediment upon the subsequent survival of yolk-sac larvae of Pacific herring. Larvae were exposed to suspensions for the first 24 h, transferred to clean seawater, and survival monitored each 24 hours.

Table 9: Feeding success in herring larvae exposed to suspension of volcanic ash or estuarine sediments resuspended at 15 min intervals. The duration of the feeding trials was 2 hr. Prey in these experiments were the rotifer, Brachionus plicatilis.

A. Volcanic Ash

Concentration (mg/l)	Replicate	No. larvae	Feeding larvae	Non-feeding larvae	Total no. prey	Prey per larva	Prey per feeding larvae
0	1	87	56	31	453	5.2	8.1
	2	123	53	70	593	4.8	11.2
500	1	119	103	16	1761	14.8	17.1
	2	76	66	10	1059	13.9	16.0
1000	1	131	120	11	1693	12.9	14.1
	2	167	139	28	2180	13.1	15.7
2000	1	130	93	37	1101	7.8	10.9
	2	143	115	28	1601	11.2	13.9
4000	1	106	63	43	650	6.1	10.3
	2	154	105	49	1242	8.1	11.8
8000	1	128	55	73	452	3.5	8.2
	2	138	62	76	629	4.6	10.1

B. Estuarine Sediment

Concentration (mg/l)	Replicate						
0	1	91	40	51	287	3.2	7.2
	2	64	14	50	98	1.5	7.0
500	1	93	87	6	1406	15.1	16.2
	2	90	66	24	1023	11.4	15.5
1000	1	150	143	7	2175	14.5	15.2
	2	72	58	14	706	9.8	12.2
2000	1	110	94	16	1237	11.2	13.2
	2	137	115	22	1460	10.7	12.7
4000	1	133	102	21	1083	8.1	10.5
	2	94	71	23	801	8.5	11.3
8000	1	124	85	39	675	5.4	7.9
	2	114	66	48	562	4.9	8.5

experiments to 500 and 1000 mg/l before showing a gradual decline with successive increases in sediment or ash concentrations to near that of the controls at 8000 mg/l (Figure 6). Although there was a general trend of increased feeding incidence in larvae in sediment, it does not differ significantly from that in volcanic ash (Wilcoxon matched-pairs signed-ranks test, $P<.05$). A similar trend is seen in the mean number of prey consumed per feeding larva. In the ash suspensions, control larvae each consumed approximately 10 Brachionus whereas larvae at 500 mg/l showed the maximum number at nearly 17. Values declined until those at 8000 mg/l were near those of the controls (Figure 7). A similar pattern is seen for sediment suspensions (Figure 8). The effects of sediment and ash on the larval feeding response are not significantly different ($P<.05$).

It is apparent from these semi-static feeding trials that both sediment and volcanic ash stimulate feeding incidence and activity as compared to control fish. In these trials, the settlement of the suspended particles between stirring lowered the effective suspension concentration in the top one-half cm by 32%, in the middle of the tank by 16%, while the bottom increased by some 50%. Swenson and Matson (1976) noted that larval lake herring swim up in the water column in response to suspended sediment. This may have occurred in these experiments as well, resulting in increased visibility of prey organisms in the lowered suspensions. The lower rates of feeding in the controls, however, are not explained by the tank or experimental design. We have noted that herring larvae in glass-walled aquaria will feed even less than in black walled enclosures, a point noted with several other species of larval fishes by other investigators as well. If the black walled containers promote feeding by increasing the visual contrast between prey and background, it is possible that the suspension of ash or sediment in the water resulted

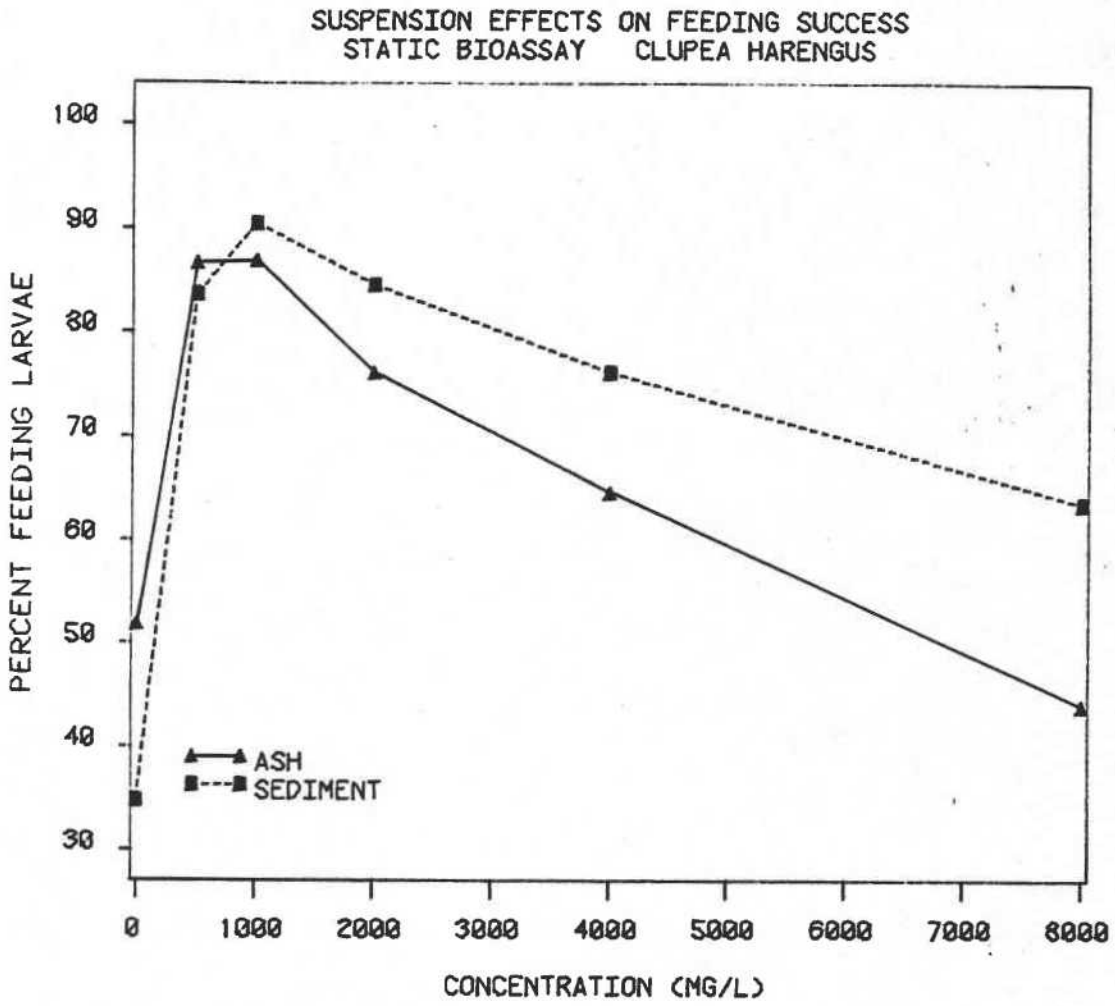


Figure 6: Effects of periodically suspended estuarine sediment and volcanic ash upon the feeding success (percent of larvae feeding) of Pacific herring larvae. Larvae were allowed to evacuate overnight and were allowed a 2-h feeding period with 6 Brachionus plicatilis per ml in the suspensions. Solutions were uniformly resuspended by stirring every 15 min during the feeding period.

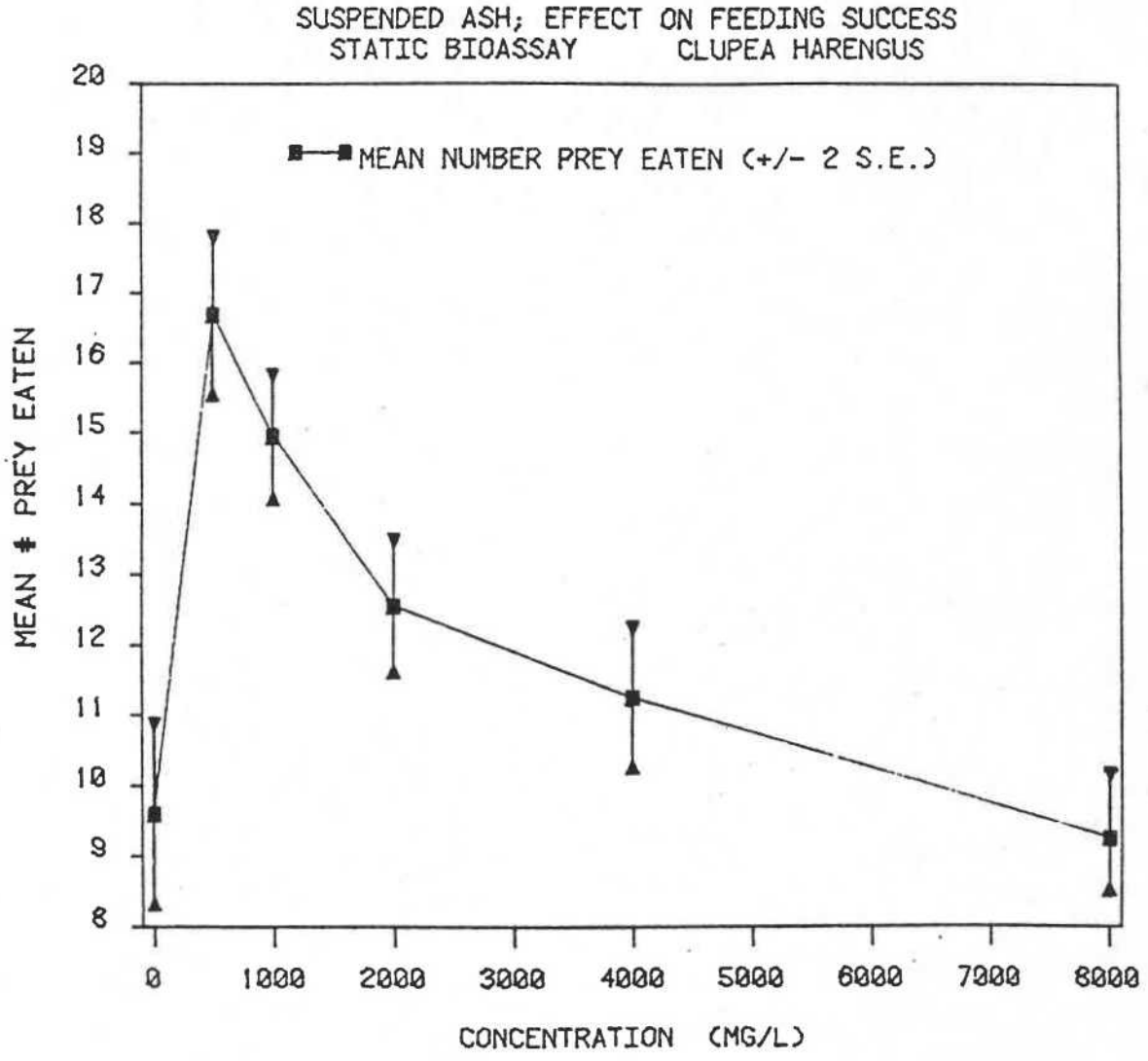


Figure 7: Effects of periodically suspended volcanic ash upon the feeding success (prey consumed per feeding larva) of larval Pacific herring.

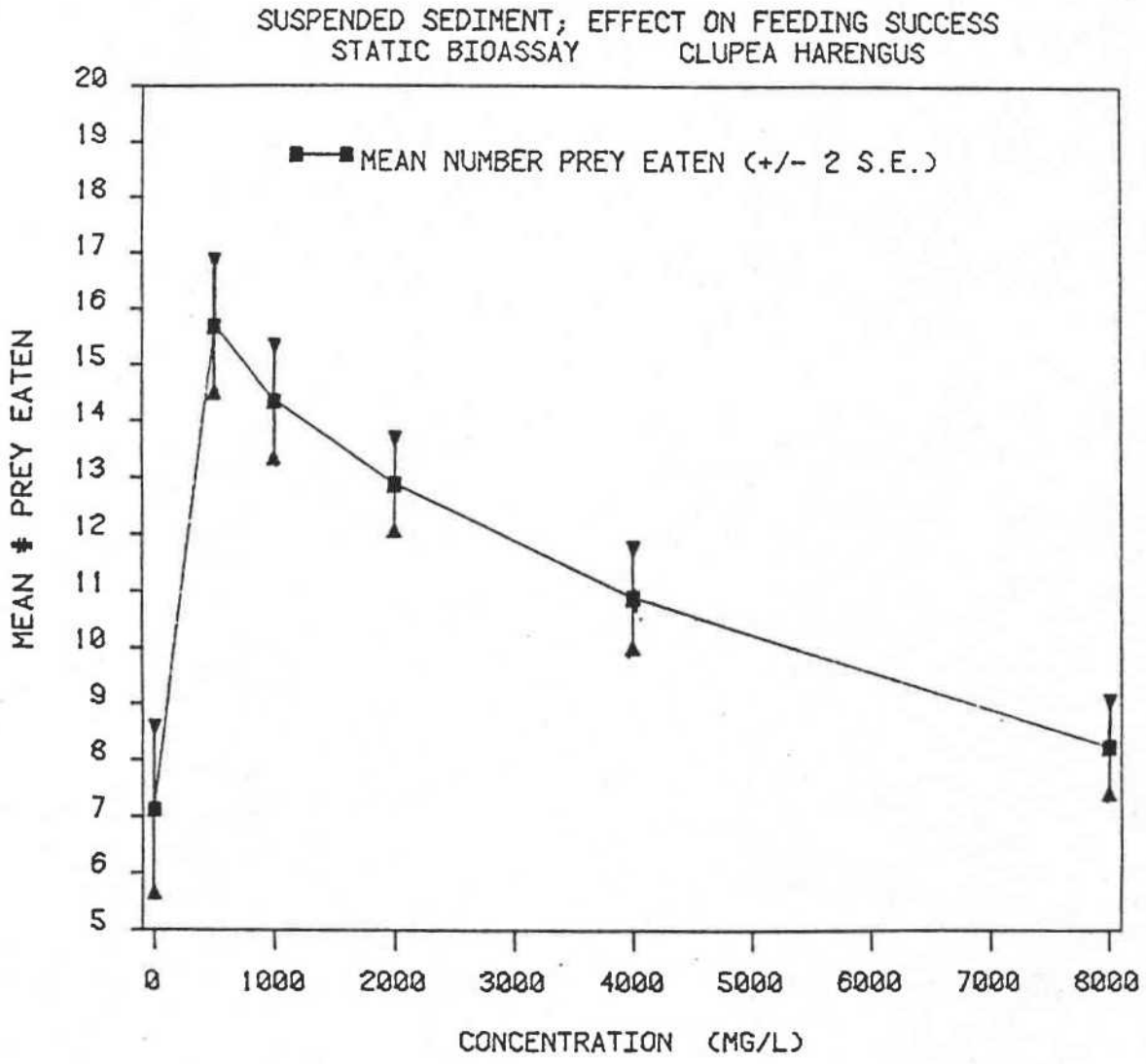


Figure 8: Effects of periodically suspended estuarine sediment upon the feeding success (prey consumed per feeding larva) of larval Pacific herring.

in an increased visual contrast at closer distances, resulting in greater availability of food organisms to the larvae.

The dynamic feeding experiments were conducted in the three-chambered dynamic dosing device, where suspensions remained within 10% of the nominal value during the course of the experiment. These experiments were run in triplicate at each suspension concentration with data analysis similar to the semi-static experiments. The container size in this experiment was 1 liter as compared to the five liter volume of he semi-static experiment. Feeding incidence was lower, averaging 31% for ash and 23% for sediment over all concentrations (Tables 10, 11). As a function of concentration, the curves of feeding incidence for ash and sediment were not significantly different ($P<.05$, Wilcoxon matched-pairs signed-rank test) but showed a different trend from the semi-static experiments (Figure 9). The percentage of larvae feeding again increased as compared to the controls, but then decreased with increasing suspension concentration, reaching nearly zero at 4000 mg/l for sediment. In these experiments the variability in the numbers of Brachionus consumed per larva within experiments as well as within replicates was high (Figures 10, 11), suggesting significant variability among individuals.

The differences between ash and sediment were therefore not significant, even though the means show a significant trend of reduced feeding with increasing concentration which is more pronounced in sediment than in ash suspensions. Again, the mean number of Brachionus consumed increased as compared to the controls, to a maximum at 1000 mg/l in the ash experiments (Figure 10) and at 500 mg/l in the sediment experiments (Figure 11). In the sediment experiments, the value at 1000 mg/l was nearly zero, as were all higher concentrations. Equivalent values in

Table 10: Feeding success in herring larvae exposed to continuous suspensions of volcanic ash in the dynamic dosing device. Feeding trials were 2 h in duration. Prey was the rotifer, Brachionus plicatilis.

Concentration (mg/l)	Replicate	No. larvae	Feeding larvae	Non-feeding larvae	Total No. prey	Prey per larva	Prey per feeding larva
0	1	20	7	13	155	7.8	22.1
	2	18	5	13	53	2.9	10.6
	3	20	10	10	69	3.5	6.9
500	1	17	14	3	345	20.3	24.6
	2	20	8	12	70	3.5	8.8
	3	19	11	8	178	9.4	16.2
1000	1	22	16	6	345	15.7	21.6
	2	21	11	10	289	13.8	26.3
	3	19	2	17	11	.58	5.5
2000	1	17	0	17	0	0	0
	2	20	3	17	4	0.2	1.3
	3	20	7	13	134	6.7	19.1
4000	1	18	2	16	3	.17	1.5
	2	20	4	6	14	.7	3.5
	3	25	8	17	27	1.1	3.4
8000	1	17	0	17	0	0	0
	2	18	1	17	1	0	1
	3	16	0	16	0	0	0

Table 11: Feeding success in herring larvae exposed to continual suspensions of estuarine sediment in the dynamic dosing device. Feeding trials were 2 h in duration. Prey was the rotifer, Brachionus plicatilis.

Concentration (mg/l)	Replicate	No. larvae	Feeding larvae	Non-feeding larvae	Total no. prey	Prey per larva	Prey per feeding larva
0	1	25	6	19	66	2.6	11.0
	2	20	10	10	131	6.6	13.1
	3	25	11	14	162	6.9	14.7
500	1	22	14	8	358	16.3	25.6
	2	22	11	11	382	17.4	34.7
	3	22	17	5	432	19.6	25.4
1000	1	19	2	17	3	.2	1.5
	2	19	4	15	16	.8	4.0
	3	19	3	16	10	.5	3.3
2000	1	13	3	10	10	.8	3.3
	2	25	3	22	6	.2	2.0
	3	23	0	23	0	0	0
4000	1	22	0	22	0	0	0
	2	22	0	22	0	0	0
	3	24	0	24	0	0	0
8000	1	23	1	22	2	.1	2
	2	24	1	23	1	0	1
	3	16	1	15	1	.1	1

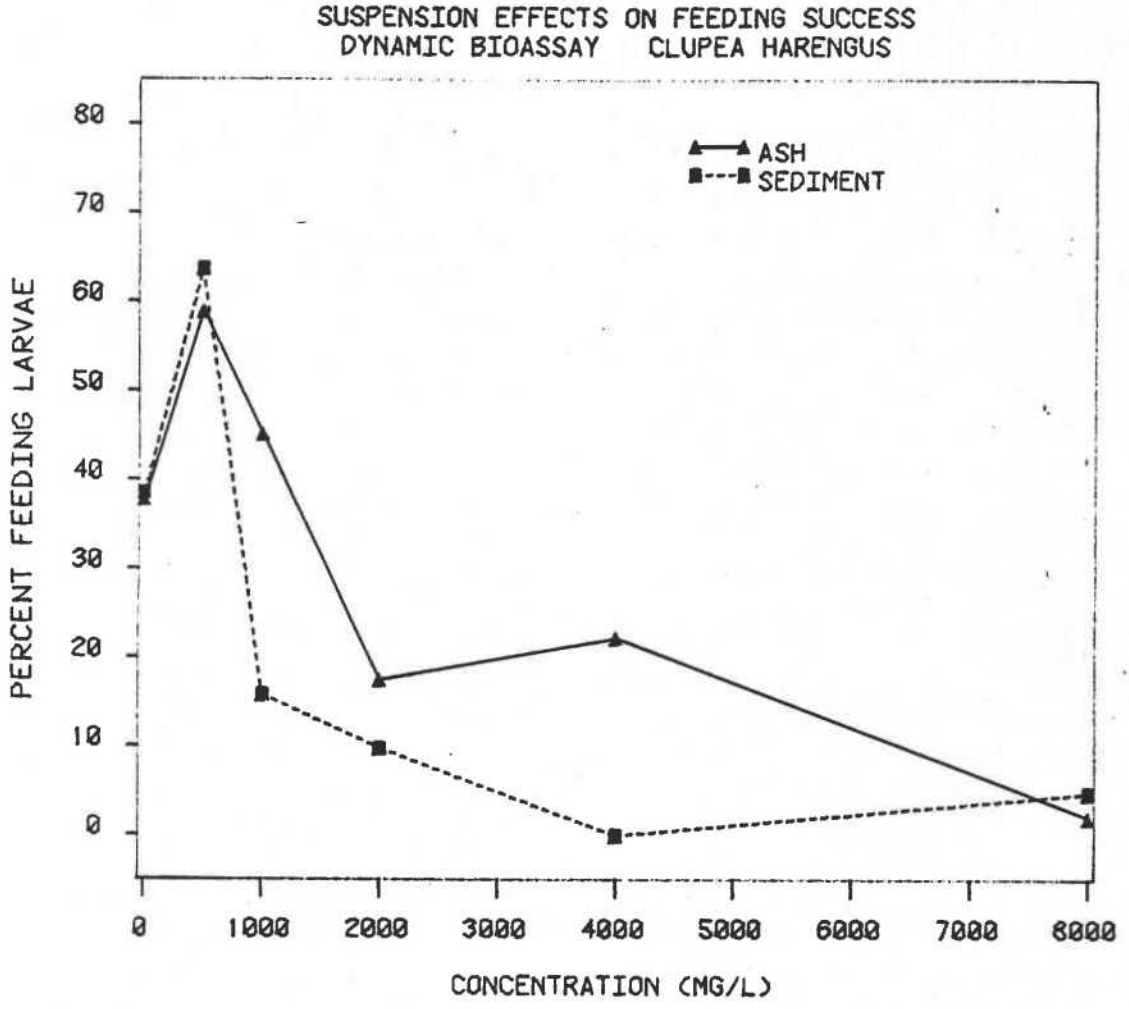


Figure 9: Effects of continuous suspensions of estuarine sediment and volcanic ash upon the feeding success of Pacific herring larvae. Larvae were allowed to evacuate overnight and were allowed a 2-h feeding period with 6 Brachionus plicatilis per ml in the suspensions. Suspensions were maintained in the dynamic dosing device.

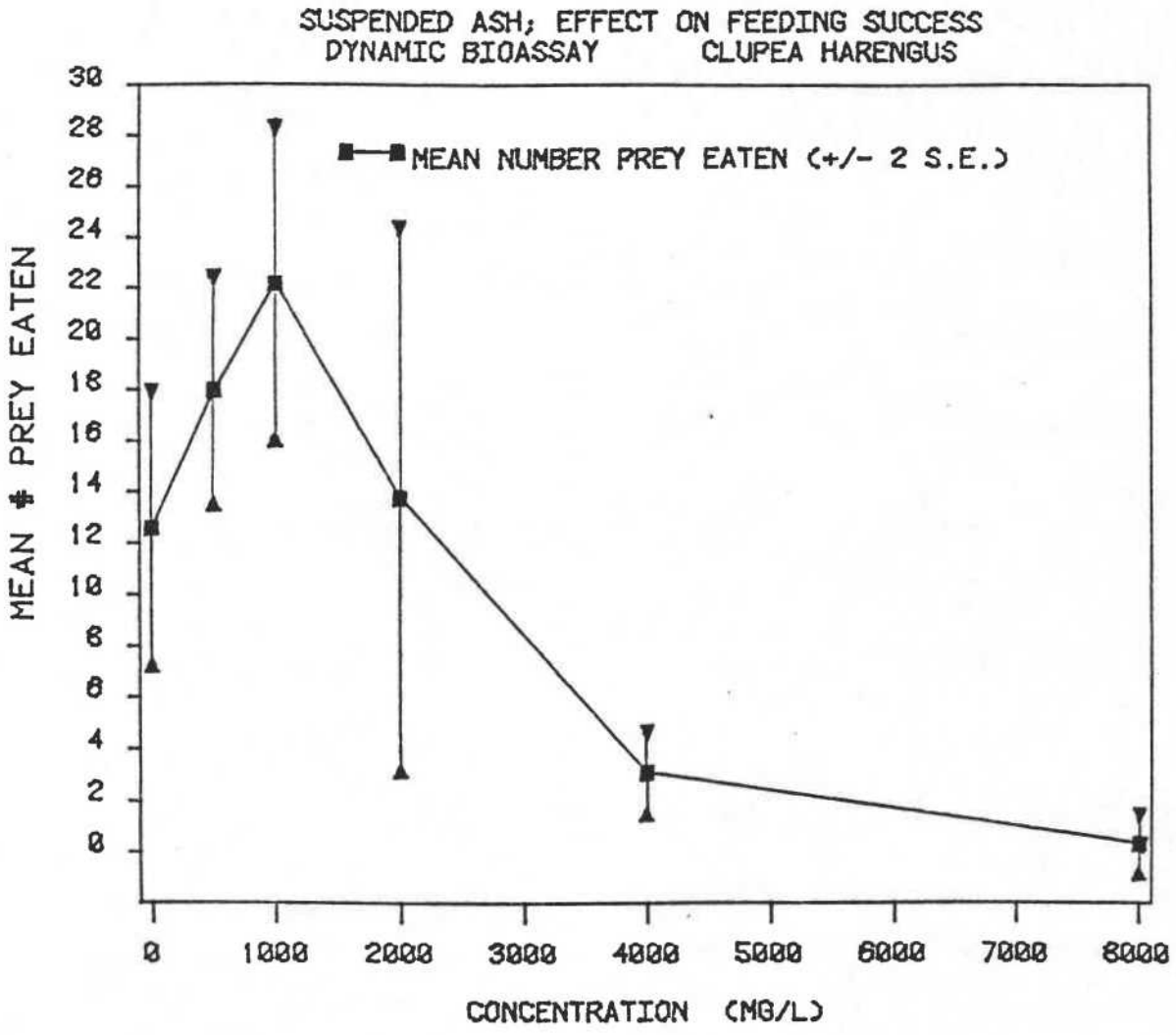


Figure 10: Effects of continuously suspended volcanic ash on the feeding success (prey consumed per feeding larva) of larval Pacific herring.

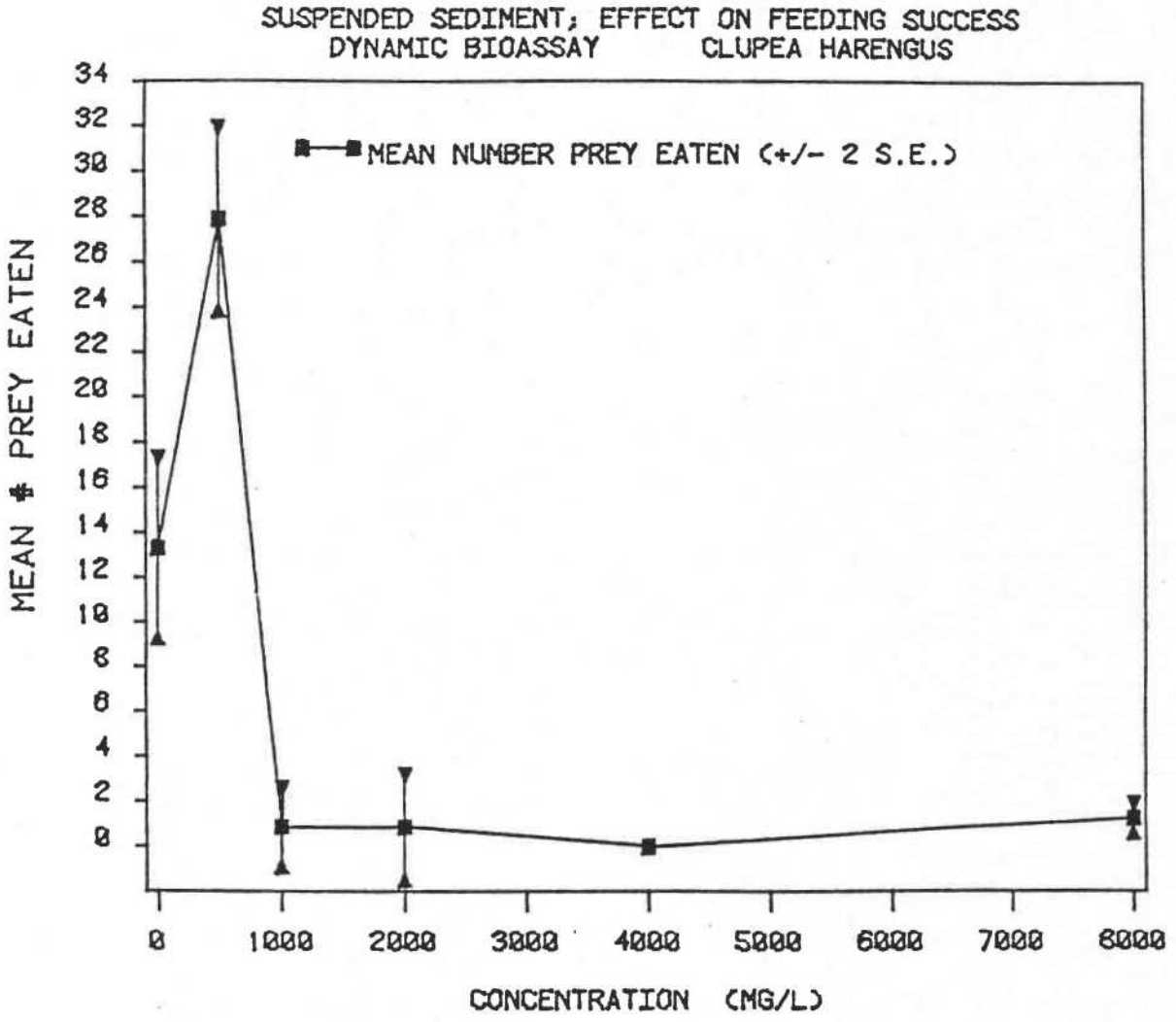


Figure 11: Effects of continuously suspended estuarine sediment upon the feeding success (prey consumed per feeding larva) of larval Pacific herring.

volcanic ash were not reached until a concentration of 8000 mg/l.

The differences between the semi-static and dynamic feeding trials, although conducted in different tanks, are apparently due to the decreasing concentrations of sediment or ash in the upper levels of the semi-static tank in the intervals between stirring., In the dynamic trials, despite a turnover of 25% of the water in the experimental vessel per minute, larvae were clearly able to feed in lower concentrations of both ash and sediment (FIgures 10, experiment). While feeding incidence was generally lower, the mean number of prey consumed per feeding larva was greater in the dynamic experiments for the controls and concentrations of 500, 1000, and 2000 mg/l in the ash suspensions (Figures 7, 10) but only in the controls and 500 mg/l in the sediment suspensions (Figures 8, 11). We thus interpret the continued feeding by the larvae in the semi-static experiments at suspension concentrations greater than 2000 mg/l in ash and greater than 1000 mg/l in sediment to be a result of the decreasing concentration in the surface of the tank and possibly the behavioral movement of the larvae to this level as well.

In the feeding trials, sediment and ash were taken into the gut in limited amounts. From the dynamic feeding experiments, groups of 20 larvae from each concentration were examined for presence or absence of ash or sediment in the intestine and also qualitatively for amount. Generally, with increased concentration of the suspension (beyond the control), the incidence of particles in the gut decreased (Table 12). Only in the highest concentration (8000 mg/l) of both ash and sediment, were some individuals present with large amounts in the gut. Generally, only traces of ash or sediment were apparent. The observation of the greatest incidence at the lowest suspension concentration would support

Table 12: Presence of ash or sediment in guts of larval herring from the feeding trials conducted 11 and 13 May, 1982. From each concentration, 20 larvae were examined.

Suspension Concentration (mg/l)	% incidence in larval guts	
	Volcanic Ash	Estuarine Sediment
0	0	0
500	95%	100%
1000	70%	80%
2000	50%	40%
4000	30%	50%
8000	25%	50%

that ingestion was associated with feeding, since feeding incidence was also highest at these concentrations (Figures 6, 9).

Analysis of larval epidermis The major emphasis of the histological analysis was upon epidermal surfaces of the yolk-sac larvae. In the initial yolk-sac experiments, where the presence of a heartbeat was used as a criterion of survival, no real differences in mortality were apparent between controls and the suspensions of ash and sediment, despite an apparent difference in the subjective condition of the larvae based upon epidermal "roughness"(Figures 12A,B). To examine the abrasive effects of sediment and ash upon the epidermis, we histologically examined the epidermis of yolk-sac larvae under the light and scanning electron microscope. Live larvae were chosen from the experiments, embedded in paraffin, and sectioned in both sagittal and longitudinal orientation. Two areas were chosen for detailed analysis of epidermal structure. The first was the epidermis on the ventral surface of the yolksac, the second the epidermis on the dorsal surface in the region of the nape, just posterior to the head. In serial sections from each larvae, four sections from the areas of interest were randomly chosen and examined at 1000 magnifications. Each of these sections were graded on a qualitative basis from 1 (good, characteristic of normal fish) to 3 (poor, characterizing severely abraded epidermis). The criteria for these assessments is as follows:

1. good; epidermis smooth at surface, characterized by some small protrusions. Occasional eosinophilic vessicles, particularly in the ventral region. No separation of epidermis from underlying tissues or shrinkage apparent (Figure 12D).

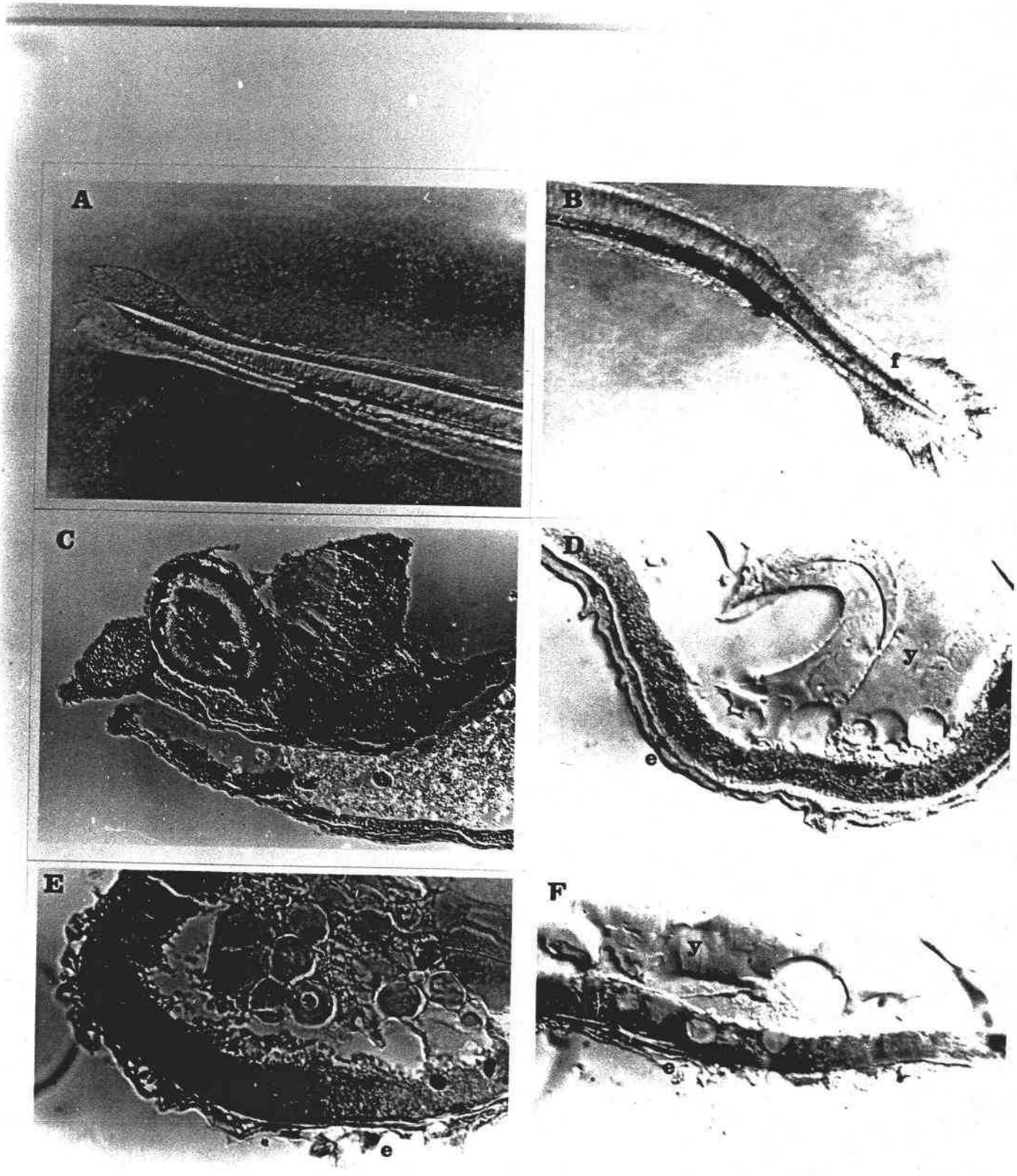
2. intermediate; epidermis smooth to slightly abraded, with thin,

irregular eosinophilic processes differing from the protrusions noted above. Some separation of epidermis from underlying tissues.

3. poor; external surface of epidermis rough, with apparent abrasion and epidermal puncturing. Frequent separation of epidermis from underlying tissues (Figure 12E,F).

In the consideration of these histological sections, care was taken to ignore damage from histological procedures, which was present as section fracturing; this was particularly apparent on the yolk-sac epidermis, where the brittle yolk is difficult to section smoothly. After the four sections were examined from each specimen and the condition assigned, a mean value for each area was determined. The mean of these mean values for a number of specimens is presented as the value for a given concentration of suspensions. Overall, about 65% of the scores within sections agreed. Mean scores showed a general trend of increase with increasing ash or sediment concentration in both dorsal and ventral epidermal areas (Table 13). In the controls, the mean score was better for the dorsal than the ventral (yolk-sac) region. Within areas and treatments, the effects of concentration were determined with one-way analysis of variance. For the ventral epidermis, there was no effect of increasing sediment concentration on the epidermal structure, whereas an effect was noted in volcanic ash, with mean scores at suspensions greater than 2000 mg/l significantly greater than those for the controls. In the dorsal nape region, effects were noted for both estuarine sediment and volcanic ash. The controls were significantly different from the sediment concentrations of 4000 and 8000 mg/l, and from all ash concentrations of 1000 mg/l and greater (Table 13). Since the same controls were considered for both ash and sediment, the abrasive effects of ash appear to be

Figure 12: Photomicrographs of the epidermis and finfold of yolksac larvae from the experiments conducted 19 March 1982. A. Caudal region from a larva in the control. The finfold is generally intact with the exception of minor damage to the most posterior region. B. Caudal region from a larva exposed to the 2000 mg/l ash suspension. Note the irregular nature of the finfold and the abrasion to the margin, particularly on the caudal finfold. C. Head region of a larva from the 1000 mg/l yolk-sac experiment. Note the sediment particles in the gullet; the gut in this yolk-sac larva is not yet open (720 x). D. Epidermis in the yolk-sac region of a larva exposed to 2000 mg/l sediment. The epidermis is smooth and uniform and was graded as condition 1 (cross section, 3500 x). E. Epidermis in the yolk-sac region of a larva exposed to 4000 mg/l sediment. The epidermis was graded as condition 3 (poor). Note the deterioration and rough surface (cross section, 2200 x). F. Epidermis from the yolk-sac region of a larva exposed to 8000 mg/l volcanic ash. The epidermis was graded as condition 3 (poor); it is discontinuous and abraded (longitudinal section, 2200 x). e, epidermis; y, yolk mass; s, sediment particles; f, finfold; r, retina.


A
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f
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e

Table 13. Histological assessment of epidermal structure in larval herring from the 13 March yolk-sac experiments. Four sections of epidermis on each specimen were graded on a subjective scale of 1 (good) to 3 (poor) for dorsal surface and ventral yolk-sac regions. Values in the table represent the mean value in each treatment. Experiments were run concurrently and controls were combined. Asterisks indicate treatment means significantly different from the appropriate controls (ANOVA, LSD, $p < .05$). N, number of specimens; $\bar{X}$, mean ranking; S, standard deviation.

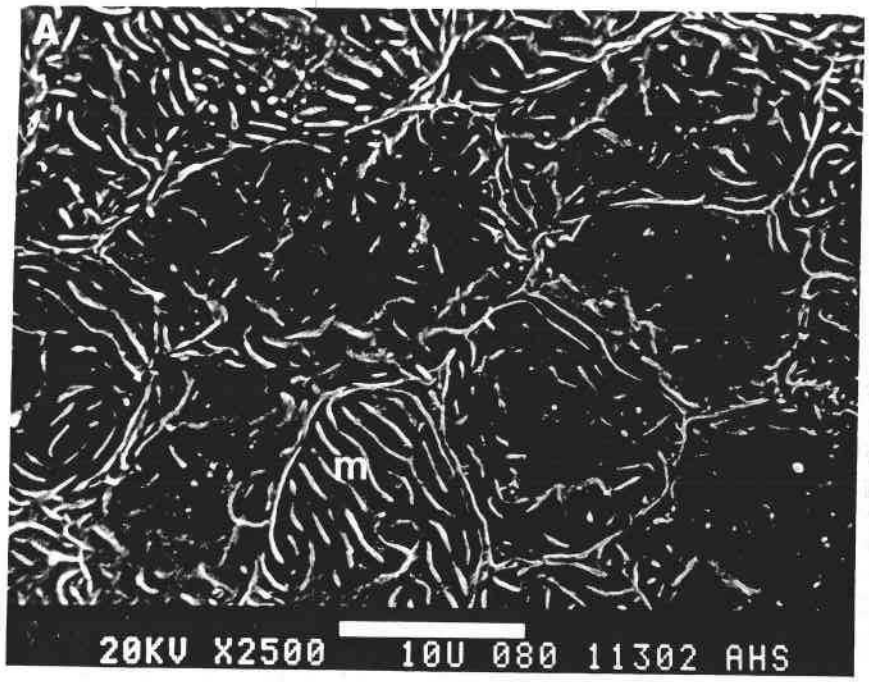
		Volcanic Ash			Estuarine Sediment		
	Concentration (mg/l)	N	$\bar{X}$	S	N	$\bar{X}$	S
A. Ventral yolk-sac region	0	10	1.35	0.36	10	1.35	0.36
	500	5	1.75	0.56	4	1.25	0.20
	1000	5	1.90	0.52	3	1.33	0.14
	2000	6	2.00*	0.72	3	1.42	0.29
	4000	6	1.96*	0.56	5	1.65	0.65
	8000	6	2.03*	0.49	4	1.63	0.32
B. Dorsal nape region	0	9	1.11	0.13	9	1.11	0.13
	500	3	1.25	0.25	4	1.13	0.14
	1000	5	1.65*	0.29	3	1.33	0.14
	2000	3	1.83*	0.29	3	1.25	0.25
	4000	6	1.96*	0.19	5	1.45*	0.21
	8000	5	1.87*	0.31	3	1.75*	0.25

greater than those of sediment.

Observations with the scanning electron microscope are in general concurrence with the histological observations. Observation of the lateral epidermis of yolk-sac larvae shows structure typical of developing teleost epidermis (Roberts et al. 1973), with distinct cellular borders and microridges (Figure 13A). Small, irregular particles, possibly associated with the processing, are irregularly apparent. The margins of the finfold are normal. Specimens examined from either ash or sediment suspensions, however, showed moderate to abundant ash or sediment particles on the epidermis. Specific abrasion is apparent in the ash experiments, which results in tear- and puncture-type damage rather than a smooth overall abrasion. Puncture wounds at 1000 mg/l ash were common over the body surface, ranging in size from about 1 to 5 µm (Figure 13B). The finfold margins are rough as compared to the controls. Similar finfold damage is seen on most larvae in ash suspensions. In larvae from 8000 mg/l, puncture wounds and ash particles are apparent on the lateral epidermis, with possibly embedded ash particles (Figure 13C). In the higher ash concentrations, the ash particles in many cases comprise a fine coating, particularly on the head region. Groups of particles seem to be maintained in some kind of coating, possibly mucous.

In the specimens from estuarine sediment experiments, the puncture-type epidermal damage is not apparent as in the ash experiments. The epidermis generally appears similar to that of the controls with the exception of less distinct microridges and increasing abundance of particles of sediment on the larvae in the higher concentrations. Again, the agglutinated groups of sediment particles are most commonly found on the head region (Figure 12D). The finfold margins were not roughened as in the ash trials.

Figure 13: Scanning electron micrographs of the epidermis from yolk-sac larvae in the 19 March 1982 experiments. A. control larva. Normal epidermis on the lateral body surface near mid-body. Microridges and cell boundaries are distinct. B. Lateral body surface epidermis from a larva exposed to 1000 mg/l volcanic ash. Note the small puncture and tear-type abrasions and the particles on the body surface. C. Lateral body surface epidermis from a larva exposed to 8000 mg/l ash. The small tears are apparent, as are ash particles. D. Head region of a larva exposed to 8000 mg/l estuarine sediment. Note the fine layer of sediment coating the head region. a, abrasive damage to epidermis; m, epidermal microridges; e, eye; s, sediment particles.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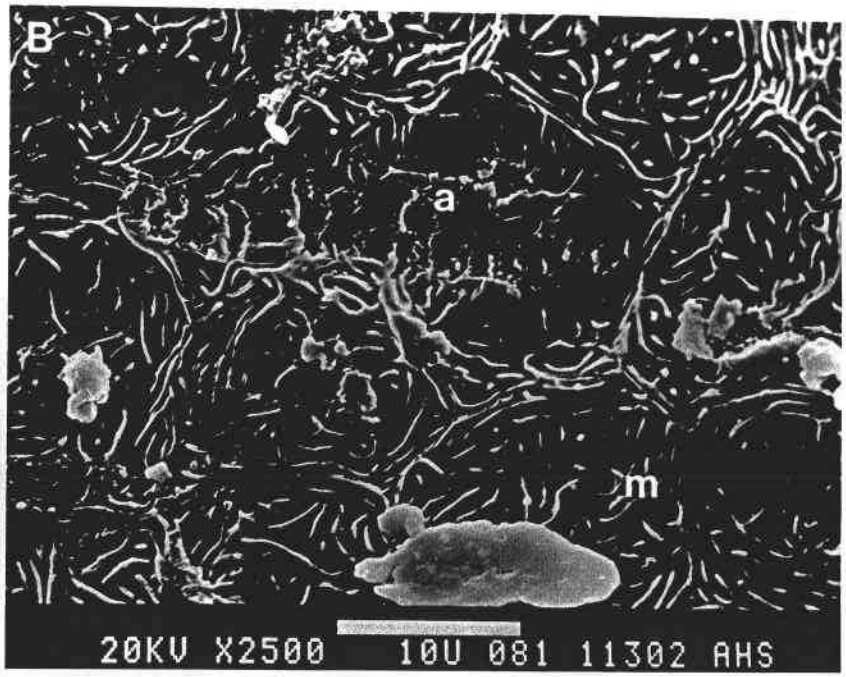
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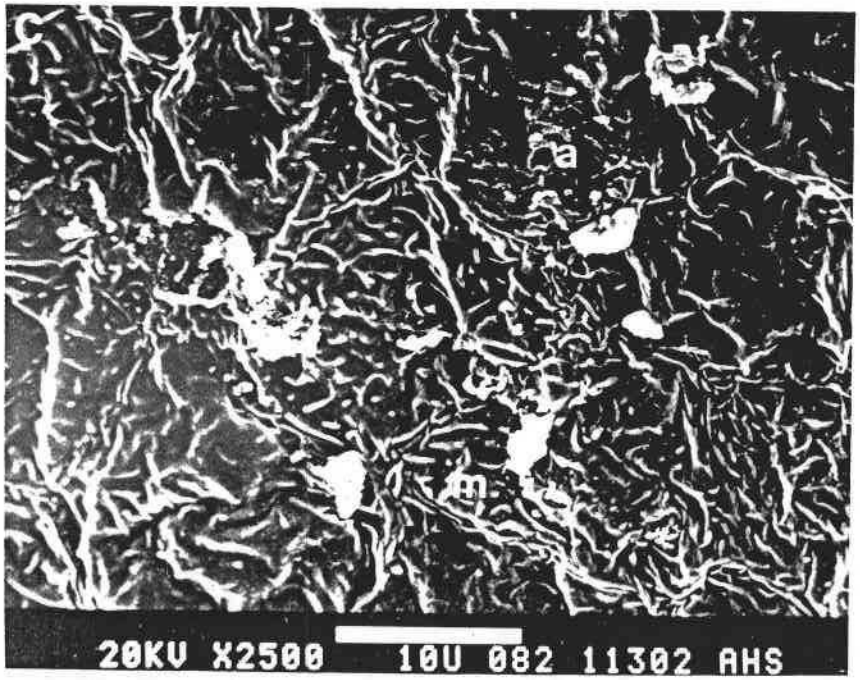
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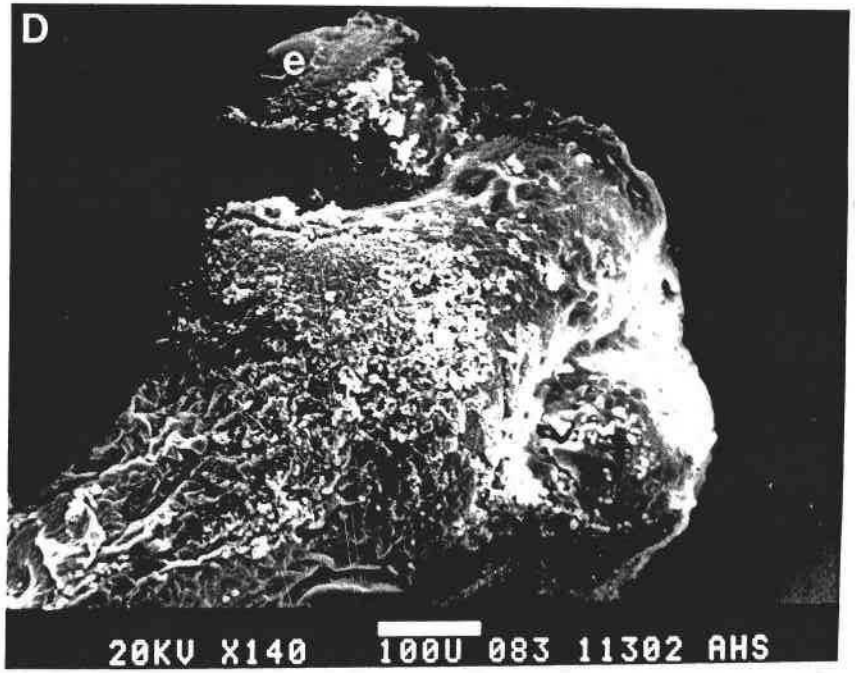
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DISCUSSION

Survival of fishes through early life history stages is an important aspect of population dynamics and should be studied when adverse environmental impacts may affect it. Oviparous fishes which produce large numbers of small eggs obviously suffer high mortality rates in early life history stages. That these mortalities occur at discrete periods within the larval phase has given rise to the concept of the critical period; such a "critical phase" may be defined as that stage wherein year-class strength is largely determined (Gulland 1965). The major sources of mortality for these early larvae are starvation and predation (Hunter 1976, 1981). Environmental pollution may clearly increase mortalities during these early life history stages through either direct effects (Longwell and Hughes 1981; Hunter et al. 1978) or sublethal effects (Rosenthal and Alderdice 1976).

The general effects of suspended solids upon the survival of fish eggs and larvae have received much more attention in freshwater environments than in estuarine and marine systems. A large number of studies have addressed the effects of siltation on the eggs of salmonids, particularly in response to logging activities (see Alderdice et al. 1977). Further, the effects of sediment on reproduction and early life stages of warmwater fishes have recently been reviewed by Muncy et al. (1979). A variety of effects, dependent upon species and type of sediment, is apparent. For eggs, the most consistent effect appears to be the reduction in respiratory gas exchange. Normal development and growth of fish embryos and larvae requires high levels of dissolved oxygen (Carlson

and Seifert 1974; Braum 1973); in the egg stage, sediment may serve to reduce the diffusion rate of respiratory gases across the chorion of the egg (Rosenthal 1971). The burial of eggs by sediment may result in either retarded, abnormal development or mortalities.

Egg as well as larval stages of fishes are characterized by heightened sensitivity to stress and pollution as compared to later life history stages. Rosenthal and Alderdice (1976) have reviewed the sublethal effects of pollutants and physical factors upon eggs and larvae of fishes. Within the egg, developing embryos are subject to a wide variety of pollutants, including elevated temperature, salinity, heavy metals, ultraviolet radiation, lowered dissolved gases, and other factors. The embryonic stage, moreover, may be more sensitive at selected critical periods of development (Vladimirov 1975), when cytological or genetic damage may occur.

The effects of increased sediment load and suspended particulates on the egg stages of estuarine fish eggs have been unclear and have varied with species and sediment type. Auld and Schubel (1978) used sediment concentrations from 2 to 1000 mg/l and observed varying responses from different species. Eggs of several species hatched normally under all tested concentrations, whereas others showed reduced hatching success. Rosenthal (1971) and Kiorboe et al. (1981) considered the effects of "red mud" and estuarine sediment, respectively, upon eggs of the Atlantic herring, Clupea harengus harengus. Rosenthal (1971) conducted two types of experiments. In the first, slides were held vertically so that settling sediment did not smother them; here, mortality ranged from 11% (control) to 55% (about 6000 mg/l). In the second set of experiments, slides were arranged horizontally on the bottoms of the suspension

containers so that greater settlement occurred on the eggs; here, mortality increased from 13.5% (controls) to 100% (7500 mg/l). Kiorboe et al. (1981) used concentrations of suspended sediments from 5-500 mg/l and observed no effect of sediment on hatching success and development, concluding that no adverse effects of mining or dredging near herring spawning grounds would occur.

In the present study, our experiments with the eggs of the Pacific herring, Clupea harengus pallasi, showed clear effects depending upon the type of experiment. In static systems, where the suspended solids were able to settle upon the eggs on horizontally oriented slides, mortalities showed a clear relationship with suspension concentration (Tables 2,3; Figure 2). These results relate to those of Rosenthal (1971) with horizontal slides. The doses used by Kiorboe et al. (1981), however, were below the range used in the current experiments. Had we limited our range as he did, our results would be substantially the same. Our results are consistent with smothering and lack of dissolved oxygen as the major causes of mortalities in the higher suspensions; the lack of development, grey, particulate yolk, and cessation of growth of the embryos are very similar to the results of Braum (1973), who noted the same characteristics in developing embryos of Atlantic herring deprived of oxygen. As distinct from the study of Rosenthal (1971), however, the present study did not observe an increase in the frequency of deformed, hatched larvae with increasing concentration.

The dynamic experiments in the present study would relate most closely to those of Auld and Schubel (1978), who maintained approximately constant suspensions during experiments and therefore minimized the effects of smothering. In our experiments, despite a fine coating of ash

or sediment on the eggs during development, development rate of the eggs was no different from that of the controls, and the mortalities were not significantly different as a function of suspension concentration (Table 6, Figure 3). This suggests that abrasion was not a problem for the egg chorion, which is generally thick in species with demersal eggs and therefore resistant to mechanical damage; in the herring, for example, Blaxter and Hempel (1966) have estimated that the proteinaceous chorion represents from 15 - 30% of the ash-free dry weight of the egg. We also observed no detachment of eggs from slides in the dynamic experiments. Devinny and Volse (1978) suggested that abrasive scour of sediments in moving water prevented proper attachment of gametophytes of Macrocystis pyrifera. This may have been a potential problem if the fertilizations were carried out in the suspensions.

When larvae hatch, they no longer have the protection of the chorion. Hunter (1972) and Weihs (1979) demonstrated that newly hatched Northern anchovy (Engraulis mordax) larvae depend upon the epidermis for gaseous exchange, a situation typical of many fish larvae. If larvae develop a coating of particulates over the epidermis, smothering may again become a problem for respiratory gas exchange. The epidermis in early larvae, furthermore, is only a few cells thick (O'Connell 1976, 1981; Jones et al. 1966), making larvae subject to abrasion damage and other potentially sublethal effects. The experiments with yolk sac larvae showed no specific differences in mortality after a 24 h exposure to suspensions when the presence of a heartbeat was used as a criterion of survival. When these yolk-sac larvae were transferred to clean water and survival monitored over the next several days, however, effects were apparent, particularly for volcanic ash (Tables 7,8; Figures 4,5).

While the turbulence necessary to maintain the suspensions may itself have resulted in mortalities, there was further damage noted to larvae in the suspensions. Qualitatively, the larval epidermis as observed at the finfold, was markedly damaged (Figure 12A). This damage was observed histologically as well (Figures 12 D-F). No published work shows detrimental effects of sediment or volcanic ash upon histological features of larval fishes. In juveniles and adults, however, damage has been noted. Herbert and Merkens (1961) observed increased healing time for wounds in fish exposed to sediment loads, resulting in higher probability of bacterial infection. Sherk et al. (1975) noted a change in the gill structure of white perch exposed to sediments; especially obvious was the increase in the abundance of mucous-producing goblet cells, which presumably aid in removal and sloughing of sediment from the gills. Larvae, however, may lack the ability to remove particles with mucous (Everhart and Duchrow 1976); indeed, goblet cells are not obvious features of larval herring epidermis. Unpublished findings on the histological changes in yearling chinook salmon exposed <u>in situ</u> to volcanic ash suggest marked effects. The epidermis was badly abraded, with approximately one-eighth its normal thickness and loss of all epidermal mucous cells. Pathological conditions were also noted on the gills and pseudobranch, both of which are exposed directly to the ash (T. Yasutake, U.S. Fish and Wildlife Service, personal communication). This suggests that the sharp, glass and crystalline structure of the volcanic ash (Fruchter et al. 1980) may result in cellular and tissue damage greatly beyond that caused by sediment alone.

More recent studies on the histological effects, however, are equivocal. Stober et al. (1982) and Redding and Schreck (1982) found no damage to the gill tissue of salmonids dosed with volcanic ash in the

laboratory, despite relatively high dosages. This also points out the differences between effects in the field and laboratory, since Stober et al. (1982) also noted significantly higher lethal levels of ash in the laboratory as compared to the live-box bioassays in the field shortly after the eruption. Our histological work on the yolk-sac larvae of Pacific herring show that epidermis is in significantly poorer condition, apparently from abrasion, in both dorsal and ventral areas for volcanic ash and in the dorsal area only for estuarine sediment. These effects are apparent as the frayed finfolds in these experiments (Figure 12A) and the poor condition of the epidermis in higher levels of suspension. The abrasive damage noted in these specimens under the light microscope (Figure 12E,F) correlate with the damage apparent under the scanning electron microscope, where the presence of puncture-type damage to the epidermis of larvae exposed to volcanic ash was apparent (Figure 13).

Historically the term "critical period" arose to describe the high mortalities apparent shortly after complete yolk absorption during early efforts at fish culture; Hjort (1914), however, applied a similar concept to describe the importance of this period in determining subsequent year-class strength in the cod and herring stocks of Norway. His interpretation and definition of the critical period have been used by Marr (1956) and May (1974) in reviews of evidence for a critical period in larval development. May (1974) concluded that presence of a critical period depends upon several ecological and species-specific factors; the critical evidence, based upon survival rate shortly after absorption of the yolk and the resultant relationship to year-class strength, is unavailable.

Inferential evidence strongly favors existence of a critical period.

First-feeding larvae are very small, have relatively small perceptive fields, and possess only weak swimming abilities; early larvae thus are able to search only limited volumes for food. Past research has shown search rates from 0.1 to 1.0 liters per hour in early larvae (Hunter 1972, 1981). Compounding low search volume in first-feeding larvae is a low feeding success rate. Estimated values range from 2-10% (Hunter 1981). Thus, based upon feeding success rate and volume searched it is obvious that first feeding larvae require relatively high densities of food particles to survive. Laboratory studies of food density and its effect on feeding and survival confirm that very high densities are required. Experimental food densities for 50% survival range from 199/liter for sea bream (Houde 1978) to 4000/liter (Northern anchovy, O'Connell and Raymond 1970).

For larvae to locate these high densities of food, visual orientation is critical; increased turbidity or other factors affecting visual field and perception may reduce food consumption and increase the probability of starvation. The effects of turbidity on feeding in larval fishes has not been studied previously. Gardner (1981), however, noted reduced feeding rates in juvenile bluegill exposed to concentrations of bentonite clay from 400 to 1200 mg/l; he observed no change, however, in size selectivity of available prey. Our results are thus unique and, in some respects, unexpected. In both semi-static and continual suspensions of both estuarine sediment and volcanic ash, low suspension levels actually increased feeding rates (Figures 7,8,10,11) and the percentage of larvae feeding (Figures 6, 9). We believe that this result is based upon the enhanced visual contrast allowing the larvae to better visualize the prey. In the controls, feeding incidence and the numbers of particles consumed per feeding larva were low; in these black-walled containers,

however, feeding was nonetheless higher than in other control experiments with glass-walled containers. The values for the continual suspensions, which were held nearly constant during the 2 h experiments, were significantly lower than those of the periodically resuspended experiments. It is significant to note, however, that relatively high levels of ash or sediment were necessary to reduce the feeding to levels observed in the controls.

Another effect of turbidity-induced light reductions may be a change in the vertical distribution of larvae. This may have a great effect on larvae in estuaries, where vertical distribution may have important effects upon horizontal distribution. Swenson and Matson (1976), while noting no effect of suspended sediment of 1-28 mg/l upon growth and survival of larval lake herring, observed a change in the vertical distribution within the test containers. We noted similar effects in the semi-static feeding experiments despite increased densities of Brachionus in the bottom of the tanks. Larval fishes generally use light intensity for depth regulation (Blaxter 1974). Thus light attenuation from turbidity may result in larvae occupying shallower depths. In estuarine fish larvae such as herring, depth regulation may be necessary to maintain populations within an estuary (Weinstein et al. 1980). This has been observed in larvae of Atlantic herring (Graham 1972) and is probably true of Pacific herring as well. A distribution higher in the water column could therefore result in advection out of the estuary and probable increases in mortality.

A comparison of the relative effects of estuarine sediment, which herring commonly encounter in nature, and volcanic ash shows selected differences. In the egg experiments, smothering effects were definitely

present in the static experiments; the difference between ash and sediment, show greater mortalities present in the sediment trials (Figure 2). Since the observed effects are the result of loss of respiratory gas exchange due to the layer of ash or sediment, it is probable that the smaller mean particle size of the sediment (Table 1), providing a denser barrier to oxygen diffusion, may have been the cause of the increased mortality in the sediment experiments.

In the dynamic experiments, the potential differential abrasion between ash and sediment was not apparent in the results. Mortality rates showed no trend with concentration of either ash or sediment, and the mortality rates were not significantly different among the trials (Figure 3). With the ready supply of oxygen in the dynamic dosing device and only a thin layer of ash or sediment on the vertically oriented slides, neither abrasion nor gas exchange was a problem for the developing embryos. In the field, herring typically deposit eggs in tidal areas where water is well oxygenated and characterized by tidal movements. In the Columbia River estuary, as elsewhere, it is therefore unlikely that significant smothering and resultant embryo mortality will occur with the exception of very localized dredging areas. Moreover, there are possible behavioral characteristics of the spawning adults which may prevent or inhibit spawning in highly turbid waters.

In the yolk-sac experiments, there was no difference between the mortality rates between ash and sediment at the end of the 24 h exposure, and no significant difference from the controls. While there was no significant difference in subsequent survival, there was a trend of increased mortality in ash as compared to sediment (Figures 5,6). Our examination of the gross morphology of the yolk-sac larvae suggested a

more marked effect in higher concentrations of ash (Figure 12), where the finfold was characterized by abrasion and erosion. This pattern was corroborated in the histological analysis, where the abrasive effects of the volcanic ash on the epidermis were apparent at lower concentrations as compared with the estuarine sediment (Table 13). Since it was not apparent after the 24 h experiment, we consider this to be a sublethal dose in the sense of Rosenthal and Alderdice (1976), since the probability of subsequent mortality was increased.

No great differences were apparent between ash and sediment for the larval feeding experiments. It is interesting to note, however, that the lower concentrations of both ash and sediment resulted in enhanced feeding abilities. In the dynamic experiments (Figure 9), the feeding incidence showed a peak at 500 mg/l for sediment and rapidly decreased thereafter, whereas the volcanic ash showed a peak at 1000 mg/l and decreased less markedly. Light transmission through equivalent suspensions of ash and sediment differ, with greater light transmission through the volcanic ash. For visual feeders such as fish larvae, it is this difference in available light that may cause the difference in feeding abilities.

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