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# ACUTE EFFECTS - PACIFIC HERRING ROE IN THE GULF OF ALASKA

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and

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## I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

The objective of this project was to evaluate the potential impact of OCS oil development activities on a single species of Alaskan marine fish, the Pacific herring (*Clupea harengus pallasi*). This species occurs commonly in the Gulf of Alaska and Bering Sea, is of commercial importance in Alaskan waters and is particularly vulnerable to surface spills during its embryonic development. Pacific herring spawn in the intertidal and shallow subtidal at depths from 0-20m. Eggs are adhesive and demersal. Typically, eggs are spawned onto seagrass blades or kelp fronds, and remain in a fixed location for the duration of development to hatching. Since they are in shallow water and cannot escape, herring eggs will be adversely affected by hydrocarbon contamination of Alaskan surface waters. This project sought to simulate conditions of a crude oil spill to test the effects of low boiling point, water soluble hydrocarbon components of Prudhoe Bay crude on developing herring larvae. Initial hydrocarbon concentrations in the experimental containers were less than 1 ug/gm $H_2O$ (1 ppm). Exposure for as little as 48 hrs. led to a significantly higher incidence of gross morphological abnormalities. Exposure for six days resulted in 100% mortality in the fertilized embryos. Gross abnormalities usually consisted of flexures in the body which reduced or prevented locomotion. Results of scanning electron microscopy reveal other defects, such as improperly formed mouth, which adversely affect biological fitness, yet are difficult to detect.

These results should be viewed in light of two important facts. First,

natural mortality of herring embryos and larvae is very high. Any factor in the environment which tends to increase this already high wastage will have devastating results on herring populations. Second, the very toxic water-soluble fractions of crude oil may remain in seawater for several months following a spill. Since embryonic and larval development in Alaskan waters may take several months, this means that oil spills over a four month period in the vicinity of herring roe or larvae will have dramatic results.

The findings of this study, if corroborated by other related studies, should suggest a re-evaluation of water quality standards for Alaskan marine waters. Clearly, herring roe cannot tolerate continued exposure to ppm levels of hydrocarbon contaminations.

## II. INTRODUCTION

### A. General Nature and Scope of Study

This was an experimental study of limited scope. We examined the effect of exposure to oil-equilibrated seawater on hatching success and prevalence of morphological abnormalities in herring larvae.

### B. Specific Objectives

1. We calculated hatching success of herring eggs maintained for 4, 8, 12, 24, 48 and 144 hours in crude oil-equilibrated seawater. These success rates were compared statistically to those of the control groups (no exposure).

2. We evaluated the frequency of gross morphological abnormalities in experimental vs. control larvae.

3. We sought to measure uptake of hydrocarbon components in larvae and unhatched eggs.

C. Relevance to Problems of Petroleum Development

As discussed below, herring spawn in a habitat which is particularly susceptible to the influence of crude oil. Many of the roe are deposited in the intertidal, the larger usually being deposited highest on the beach. Since the larger eggs normally produce the larvae with the greatest. chance of reaching adulthood, the presence of oil on the water and on the beach will select against the highest quality of eggs in particular and will cause an increased mortality in general. Spills or seepage during the three to four week reproductive period could have significant impact on egg and larval mortality. These mortality rates are already high in nature. Therefore, development activities could have a major impact on the herring fishery in Alaska.

## V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

### A. Source of Experimental Material

Leonard Weimer, IMS, Seward, made arrangements for us to pick up freshly harvested kelp laden with herring roe, shortly after it was spawned in Prince William Sound. Algal fronds were collected by Mr. Weimer and other "kelpers" on April 21, 1976 at Ellamar Cove near the village of Tatitlick. Fronds with roe were made available by the personnel of Seward Fisheries, Inc. We selected only fronds of sieve kelp, Thalassiophyllum clathrus, with their attached burden of herring roe. The fishermen and personnel at Alaska Department of Fish and Game, Seward, stated the eggs had been spawned two to three days before we made our collection. Experimental material was returned to the Seward Marine Station by float plane in several 5 gal. plastic buckets with lids.

### B. Experimental Design

Herring eggs were maintained in seawater at temperatures ranging from 3.5°C to 6.0°C until the experiment actually began on April 24, 1976. Since embryonic development of herring is temperature regulated, we chose this low temperature to insure that little development took place before the initiation of exposure to oil-equilibrated seawater.

We chose to expose the embryos to oil-equilibrated seawater rather than to seawater with globules of oil in it. Our rationale was to avoid the complication of oil coating the eggs since uniformity of coating would be impossible to achieve. Seawater equilibrated with oil contained the water soluble low boiling point components of the Prudhoe Bay crude.

These rather volatile components are known to be highly toxic to biological systems and persist in seawater for several months after an oil spill (Blumer et at., 1973).

The oil-equilibrated water was mixed in three clean 12 gal. glass reservoirs which had been previously flushed with sea water. Each reservoir contained 42 l of sea water upon which was poured 500 ml of the crude oil to make a film about 1/4 in. thick. These solutions were mixed with stainless steel stirring rods for 24 hrs. and allowed to stand an additional 12 hr. for the oil droplets to rise to the surface. These reservoirs remained tightly capped during the entire experimental period to minimize evaporation.

Rather than attempting to vary the hydrocarbon levels to which we exposed different experimental groups, we chose to vary exposure time. Exposure times of4 hr, 8 hr, 12 hr, 24 hr, 48 hr, and 6 day (144 hr) were employed. Three wide mouth gallon glass jars were used for each exposure period including three jars for the controls. Into each jar was placed a small algal frond to which were attached approximately 200 eggs.

A semi-static sea water system was used to maintain the eggs. Using the method of Blaxter (1968) the water was changed in all containers every 48 hours. Aeration was avoided due to the increased loss of hydrocarbons from aeration. According to other research using aereated systems (Linden, 1975; Anderson, et.al., 1974a) approximately 90% of the aqueous hydrocarbons are lost in a 24 hour period with alkanes disappearing more rapidly than aromatics. Benzene has been found to decrease by between 70% and 75% in that same time period (Struhsaker, et.al., 1974). Care was taken to jostle the fronds and eggs as little as possible. Water bath temperature

was maintained at 8-9°C after referring to other research (Rice, pers. comm.) arid determining that hatching time at this temperature would be 15-20 days. This provided a reasonable time scale within which to work.

Eggs were observed with a dissecting microscope each day and development was noted. Total lengths of the individual larval herring were determined upon hatching for the first six days, days 12-17. Measurements were made while observing the larvae under the dissecting microscope.

Preparation and scanning microscopy was performed by Al Soeldner at the Oregon State University Electron Microscopy Laboratory. The samples were prepared by fixation in gluteraldehyde and storage in cacodolate buffer for transport. The samples were then placed in a fluid displacement series of 30, 50, 70, 80 and 100% acetone for approximately 10 minutes each and then a trichloro-trifloro-ethane (TF) series of 30, 50, 70, 85, and 100% for the same time periods. This procedure was followed by critical point drying in an Omar SPC-900 dryer. Monochloro-trifloro-ethane was used as the transition fluid. After drying the samples were mounted on stubs and glued down with colloidal silver paint. Scanning microscopy was performed on a MSM-2 Mini-SEM.

C. Analytical Procedures

Samples of hatched larvae and unhatched eggs were kept for hydrocarbon analyses. So also were samples of uncontaminated seawater, oil-equilibrated seawater at the initiation of the experiment and oil-equilibrated seawater at the end of each exposure period. Procedures for sample extraction and gas chromatographic analyses were the same as those reported by D. G. Shaw (1976), except that no integrator was employed. Neither was any comparison

with an internal or external standard. Sample extracts were not evaporated to a constant volume and no evaluation of total hydrocarbon burden was provided by the associate investigator, Dr. Pearson.

## III. CURRENT STATE OF KNOWLEDGE

Hydrocarbons in the world's oceans derive from a number of sources. Ahearn (1974) lists the following sources and estimates the percentage of the total hydrocarbon load contributed by each source:

| Source | % of total |
|---|---|
| decaying organisms | 50 |
| normal shipping operations | 18 |
| terrestrial runoff | 17 |
| atmospheric fallout | 8 |
| natural seepage | 4 |
| accidents in shipping and production | 3 |

He estimates the total annual hydrocarbon input at 6-12 million metric tons. Clearly, some of these inputs, such as decaying organisms and natural seepage, have acted over geologic history to affect the evolution and distribution of marine organisms. Others, such as those associated with normal shipping operations and with accidental spills, are not typical in the environment and may dramatically affect marine organisms by accumulating vast quantities of pollutants in relatively small areas. Particularly sensitive areas include enclosed bays, especially those used for shipping and drilling. Other coastal areas are also vulnerable, particularly the intertidal region. The sea surface, where hydrocarbons accumulate, should be a sensitive area worldwide.

Of all oil components the low boiling point, aromatic hydrocarbons are generally the most toxic to marine organisms. This toxicity is a result of the greater volubility of the aromatics, enabling organisms to concentrate aromatics more readily than saturated hydrocarbons (Warner, 1976; Struhsaker et al., 1974; Moore and Dwyer, 1974; Kuhnhold, 1972). The aromatics are also retained in mollusc, crustacean, and fish tissue for

greater periods of time than the alkanes (Anderson, et al., 1974a). Lirl-den (1975), working with Baltic herring, found that aromatic hydrocarbons interfere with and disrupt fatty membranes and can destroy the larval primordial fin. In adults, aromatics stimulate copious secretion of thick mucus. Long term toxicity is thought to be a result of higher boiling point aromatic hydrocarbons; those above n-$C_{22}$ (Blumer et al., 1973).

Benzene is among the most toxic of all the aromatic hydrocarbons. It is relatively soluble and comprises about 20% of the total aromatics in many crude oils (Struhsaker, et al., 1974). Benzene can contribute both lethal and sublethal effects. The sublethal effects of benzene on yolk absorption, growth, and respiration of Pacific herring and northern anchovy have also been noted by the above authors. According to their study these effects show that benzene influences metabolic rate and energy utilization, a low concentration of benzene causes an acceleration of metabolic rate. Thomas and Rice (1975) have also found an increased metabolic rate to be a normal response of fish to pollutant stress. A long term effect of oil pollution would be to create a higher energy requirement which could be decremental to survival and reproductive potential (Thomas and Rice, 1975).

In comparing the effects of benzene on mortality of herring eggs and larvae, Struhsaker et al., (1974) found that eggs were more resistant than larvae. This is in agreement with other pollutant studies and points out that teleost eggs are much more impermeable to most substances than are larvae (Blaxter, 1969). Although eggs are more resistant on a short-term basis, exposure may result in hatched larvae with abnormalities severe enough to cause death eventually (Struhsaker, et a?., 1974).

The survival of oil-contaminated marine organisms depends largely on

how the oil is introduced into the system. Oil dispersions, suchashave resulted from the use of detergents to clean up oil spills, may have toxicity levels 10-100X higher than the unemulsified oil (Kuhnhold, 1972). In adult fishes both toxicity and the effect of tiny oil droplets on the gill apparatus appear to be important (Mironor, 1972). Coating and smothering effects of an oil film are important only when considering a weathered oil from which the soluble aromatic components have evaporated. If these aromatics are still present, their toxic effects mask the coating effects (Moore and Dwyer, 1974).

According to Blumer (1973) degradation of oil in the marine environment is accomplished by evaporation, dissolution, microbial action, and chemical degradation. Evaporation and dissolution are primarily of concern in the depletion of the lower boiling point, more soluble hydrocarbons. These, as was mentioned previously, are generally the more toxic to marine life. Microbial degradation occurs over a wider range of molecular weight. Hydrocarbons in the same homologous series are generally attacked at the same rate (Blumer, et al., 1973). This degradation proceeds most readily on n-alkanes as is apparent from the decrease of these n-alkanes in the natural environment in comparison to the decrease of compounds having similar boiling point and similar volubility compounds (Blumer, et al., 1973). Chemical degradation, according to Blumer, et al., (1973) is apparent at advanced stages of the weathering process. These processes result in oxidation of the medium and higher molecular weight aromatics leading to an increase o-F high molecular weight polar compounds known as asphaltenes. Formation of alcohols, alkyl- and araylethers, carbonyl -compounds and sulfoxides have been noted by Kawahara (1969, as quoted in Blumer, et al., 1973) as additional reactions that affect chemical degradation.

After studying three major sites of crude oil spills Blumer has determined the fates of these oils in the natural environment. Lower boiling point components of the crude oils are lost within a few months after the spill occurs. The oil that remains stabilizes and retains approximately 10% of the hydrocarbons with boiling points near n-heptadecane to n-octodecane and about 50% of those with boiling points in the range of n-nonadecane to n-heneicosane. Hydrocarbons above n-$C_{22}$ were retained for the length of the studies; up to 16 months.

## IV. STUDY AREA

This was an experimental study conducted at Seward Marine Station, Seward, Alaska. Herring roe were obtained from Ellamar Cove, near Tatitlick, in Prince William Sound, Alaska.

## VI. RESULTS

### A. Hydrocarbon Analyses

This project was to include hydrocarbon analyses of seawater used in the experiments, the oil-equilibrated seawater, samples of hatched larvae and unhatched eggs from the various exposure periods. We were to report findings on total hydrocarbon burden and, hopefully, on the levels of specific contaminants incorporated into the experimental organisms. These chemical analytical procedures and the results we expected from them were the responsibility of the Associate Investigator, Dr. John G. Pearson. In training the technician who actually performed the GC analyses, Dr. Pearson apparently failed to instruct him in the importance of including a reference standard in the samples and/or evaporating the solvent extract to a constant volume. The actual GC traces were not all run under the same conditions, i.e., different gains were used on different runs. Therefore, it. is impossible to compare hydrocarbon levels from one sample to the next. Dr. Pearson did not supply any quantitative information on hydrocarbon burden.

I arranged to have our control seawater and the oil-equilibrated seawater analyzed by Dr. David Shaw's group. They supplied us with the following data:

| sample | ug hydrocarbon/gin $H_2O$ |
|---|---|
| control H20 | 0.013 |
| experimental H20 | 0.679 |

Chromatographic analysis of the control water revealed virtually nothing. (Fig. 1), The experimental water (oil-equilibrated) contained a number

of contaminants (Fig. 2). Of the total hydrocarbon load in this sample, the bulk consisted of napthalene, 16.82%, methyl napthalenes 13.00%, and dimethyl napthalenes, 4.59%.

Chromatographic traces developed by Dr. Pearson's group, although of no use quantitatively, do supply useful qualitative information. Six components have been identified on these traces: branched $C_9H_{20}$, n-$C_9H_{20}$, $C_3$ benzenes, $C_4$ benzenes, napthalene, and $C_5$ benzenes. The first two components occurred in every experimental group plus the controls. Therefore, I assume that these components are either organic compounds naturally occurring in herring eggs and larvae, are contaminants in the solvents used to extract the samples, or were picked up in the natural environment.

Both unhatched eggs and hatched larvae from the control tank were examined with GC techniques. No other hydrocarbon components appeared on the traces. The 4 hr. exposure, tank #l, yielded both hatched and unhatched material for analysis. The unhatched eggs contained $C_4$ benzenes, napthalene, and $C_5$ benzenes while the hatched larvae contained the $C_4$ benezenes only. Traces of the hydrocarbons from 8 hr. exposure, tank #1 show the same basic pattern for unhatched eggs and hatched larvae. Both groups contained all four of the above mentioned hydrocarbon groups, with the hatched group having slightly lower levels of $C_3$ benzenes.

Two comparisons can be made in the 12 hr. experiments. The 12 hr. tank #1 yielded eggs and larvae with the same contaminants (all four groups) at roughly the same levels. The 12 hr. tank #3 comparisons showed that no $C_5$ benzenes were incorporated into the experimental tissue. Further, no napthalene was in evidence in the hatched larvae.

Three hatched groups were examined from the 48 hr. exposure period. All these pooled samples of larvae contained significant levels of all four hydrocarbon groups identified in this study.

Clearly, all experimental groups examined, whether consisting of unhatched eggs or hatched, viable larvae contained hydrocarbon contaminants. In contrast, the control organisms did not contain any of the three benzene groups or napthalene. This is evidence that herring eggs exposed to the hydrocarbon levels used in this study, 0.679 ppm, incorporate hydrocarbon material into their tissues. This occurs even when exposure time is limited to 4 hrs.

B. Hatching success

Table 1 contains the relevant data on hatching success. Included are values for total numbers of eggs in each container, the number hatched and the percent hatched for each container and experimental regime. Statistical analysis of percent hatching values was performed using a single factor analysis of variance in conjunction with Dunnett's Test for testing a control against all other groups (Zar, 1974). The only significant difference in hatching success was between control and six day exposure groups ($P<0.01$).

Hatching began on day 12 of the experiment and proceeded through day 24. There were no obvious differences between controls and experimental with regard to duration of the hatching period except for the six day group in which no larvae hatched. Similar hatching patterns were observed in all groups with modes of hatching frequency on day 14 and day 17 or 18 (Fig. 3).

Maintenance of individual experimental jars was ended two days after the last viable larvae appeared. Inspection of unhatched eggs in the container revealed that development was not progressing, indicated by lack of movement and lack of heart beat. Several nonviable larvae were collected in the last few days of the experiment. These larvae were highly contorted, lacked the transparency of living larvae and showed no signs of movement or heart beat (Fig. 4-). We concluded that these embryos were liberated from eggs which had disintegrated, and counted them as unhatched.

C. Morphological abnormalities

Table 2 presents the information we gathered on the occurrence of morphological abnormalities in the control and experimental regimes. Statistical analyses show that the only experimental regime with a significantly higher percentage of abnormal larvae than the control group was the 48 hr. exposure period ($P<0.01$).

Most of the abnormalities detected were bent spines. These bends lent an L, S or helical configuration to the larvae (Figs. 5, 6). The affected larvae were unable to swim normally, if they could swim at all. Conversely, normal larvae were capable of straight line locomotion and easily traversed the confines of their containers (Fig. 7).

Another abnormality easily recognized when viewing the larvae under a dissecting microscope was an enlarged pericardial cavity. Fig. 8 shows this abnormal condition in a larva from the 48 hr. exposure group.

Scanning electron microscopy revealed some additional abnormalities although we cannot even estimate their frequency in the control and

experimental populations. The pectoral fin is an example. Fig. 9 illustrates a normal fin from a control larva. It shows no evidence of erosion. Fig. 10 shows the eroded pectoral fin from the 48 hr. exposure group. Fig. 11 illustrates the lack of fin development seen in some 48 hr. larvae.

The mouth is abnormally developed in a number of larvae examined. Normal configuration can be seen in Fig. 12. A missfit lower jaw is illustrated in Fig. 13, a very poorly differentiated jaw is seen in Fig. 14, and a larva with missing premaxillary bone is seen in Fig. 15.

The branchiostegal region is another problem area. The normal branchiostegal membranes, (Fig. 16) may be missing, as seen in Fig. 17.

D. Embryonic development

The eggs of both control and oil-exposed groups developed a coating of microorganisms on the external surface (Fig. 18). These microorganisms include bacteria (Fig. 19) and diatoms (Fig. 20). No observations were made on the prevalence of bacteria on the egg surface with respect to the length of exposure to oil-equilibrated seawater. We began an experiment on tomcod, *Microgadus proximus*, in which fertilized eggs were exposed to the same oil-equilibrated seawater. Although we were unable to carry it through to hatching, we did observe that the exposed embryos were much more heavily coated with bacteria and diatoms than were the controls.

Microscopic comparison of embryos showed a retardation in embryonic development in long exposure groups. Fig. 21 shows a control embryo on the tenth day of the experiment. The head and eyes are large and

well developed. The body is well differentiated, making two circuits of the egg interior. An embryo from the 24 hr exposure group (Fig. 22) has a smaller head and shorter body. Fig. 23 illustrates the very retarded state of development seen on the tenth day of the experiment in the six day exposure group. Cephalization has taken place but not to the extent seen in the two previous figures. The body is very short and very little of the tail has separated from the yolk sac. Further evidence of retardation of metabolic processes can be seen in Table 3, which lists the lengths of hatched larvae from each control and experimental container. Statistical tests indicate that the control and 48 hr groups are significantly different (P 0.01).

## VII. DISCUSSION

The results of our hydrocarbon analyses are extremely limited in usefulness. However, they suggest that experimental material, whether hatched or unhatched, picked up hydrocarbon contaminants from the oil-equilibrated seawater. This uptake occurred even for short exposure periods of 4 hr. Total hydrocarbon levels in the oil-equilibrated seawater were less than 1 ppm.

Hatching success of herring eggs was significantly affected in this experiment. It should be mentioned that in our experiment the control groups suffered a notable incidence of unhatched eggs. Other experimental studies had similar results. Herring eggs in the natural environment are known to exhibit the same sort of prehatching mortality. Therefore, the presence of hydrocarbon pollutants significantly aggravates a natural

tendency toward embryonic mortality.

In statistical comparisons, the control and 48 hr exposure group were found to differ with respect to incidence of gross abnormalities and mean total length of hatched larvae. Both these factors are very strongly related to overall biological fitness. Abnormalities of body configuration cause difficulty in swimming. Indeed, some abnormal larvae could not swim at all. Typically, herring larvae are planktonic and may initially require swimming to remain up in the water column. Swimming is also undoubtedly essential for effective feeding since these larvae begin feeding on zooplankton organisms early on. Since herring larvae are small they are preyed upon by other zooplankters and also nectonic animals. To the extent that oil reduces larval length it prolongs larval life and prolongs the time period during which larvae are subject to predation. In the natural environment., without the detrimental effects of oil, larval herring wastage is estimated to be between 90% and 95%.

Insufficient material was examined with scanning electron microscope techniques to evaluate the relative incidence of hard-to-find abnormalities. I assume that the incidence of abnormal mouths, pectoral fins and branchiostegal membranes increases with exposure to oil-equilibrated seawater for periods of 48 hr or longer.

Clearly, these less obvious abnormalities can have a dramatic impact on larval fitness. A well-formed mouth is probably critical for effective feeding. Functional, well-formed fins are important for gross swimming movements as well as for delicate adjustments of attitude and position.

All the above-mentioned irregularities in embryonic development have important impacts on larval fitness. If the embryos are not killed outright,

they may hatch into larvae which may die almost immediately or persist and be more likely to fall prey to other organisms in the pelagic environment.

## VIII. CONCLUSIONS

1. The herring reproductive cycle is vulnerable to oil pollution for at least two months, possibly more.
2. Very low hydrocarbon levels ( 1ppm) produced significant effects in terms of hatching success, gross morphological abnormalities, total larval length, and presence of hydrocarbons in hatched larvae and unhatched embryos.
3. Deleterious effects produced by oil contamination reduce the fitness of larvae which, at best, had little chance to survive to adulthood.

## IX. NEEDS FOR FURTHERS TUDY

Additional studies are needed to evaluate the effects of oil pollution on herring larvae and on the eggs and larvae of other common Alaskan marine organisms. Ancillary studies on the effects of siltation on herring hatching success are needed in inshore areas likely to feel the impact of oil exploration and development. Other studies are needed to examine the physiological and biochemical effects of oil on adult marine organisms. Clearly, reproduction is dramatically affected. What happens to growth, food assimilation, and digestive processes?

## X. SUMMARY OF LAST QUARTER OPERATIONS

### A. Laboratory Activities

1. Work done

Hydrocarbon analysis were performed on two samples collected during our aquarium experiments. One sample was of control seawater, the other was a sample of the oil-equilibrated seawater used in all the experimental jars at the initiation of the experiment.

2. Scientific party

Ronald L. Smith, IMS Principal Investigator
Jane Anne Cameron, IMS Research Assistant
Barbara Baker, IMS Research Technician

3. Methods

The methods employed for the hydrocarbon analyses were those employed by Dr. David Shaw, IMS. In fact, the analyses were performed in Dr. Shaw's lab by his personnel.

4. Sample localities

Not applicable

### B. Problems Encountered

All the possible problems in this project have already been encountered in previous quarters.

Table 1. Number and Percent Hatched in each experimental group. The last column is the average percent hatched for each exposure regime.

| Container | | Initial Number | Hatched Number | Hatched % | Average % |
|---|---|---|---|---|---|
| Control | 1 | 210 | 81 | 38.5 | |
| | 2 | 211 | 82 | 38.9 | 53.4 |
| | 3 | 191 | 158 | 82.7 | |
| 4hr | 1 | 201 | 109 | 55.7 | |
| | 2 | 209 | 115 | 55.0 | 53.3 |
| | 3 | 197 | 97 | 49.2 | |
| 8hr | 1 | 214 | 95 | 44.4 | |
| | 2 | 212 | 109 | 51.4 | “43.7 |
| | 3 | 199 | 70 | 35.2 | |
| 12 hr | 1 | 217 | 65 | 30.0 | |
| | 2 | 209 | 122 | 58.4 | 53.9 |
| | 3 | 202 | 148 | 73.3 | |
| 24 hr | 1 | 199 | 83 | 41.7 | |
| | 2 | 198 | 39 | 19.7 | 32.1 |
| | 3 | 218 | 76 | 34.9 | |
| 48 hr | 1 | 207 | 28 | 13.5 | |
| | 2 | 200 | 55 | 27.5 | 27.6 |
| | 3 | 199 | 83 | 41.7 | |
| 6 day | 1 | 217 | 0 | 0 | |
| | 2 | 201 | 0 | 0 | 0 |
| | 3 | 199 | 0 | 0 | |

Table 2. Number and Percent Abnormalities in each experimental group. Also presented is the overall percentage for each exposure regime.

| Container | | Initial Number | Abnormal Number | % | Overall % |
|---|---|---|---|---|---|
| Control | 1 | 210 | 8 | 10 | |
| | 2 | 211 | 12 | 15 | 10.9 |
| | 3 | 191 | 12 | 7.6 | |
| 4hr | 1 | 201 | 6 | 5.5 | |
| | 2 | 209 | 18 | 15.7 | 13.9 |
| | 3 | 197 | 20 | 20.6 | |
| 8 hr | 1 | 214 | 15 | 15.8 | |
| | 2 | 212 | 19 | 17.4 | 26.5 |
| | 3 | 799 | 18 | 25.7 | |
| 12 hr | 1 | 217 | 17 | 26.2 | |
| | 2 | 209 | 20 | 16.4 | 18.7' |
| | 3 | 202 | 20 | 13.5 | |
| 24 hr | 1 | 199 | 18 | 21.7 | |
| | 2 | 198 | 9 | 23.1 | 20.6 |
| | 3 | 218 | 13 | 17.1 | |
| 48 hr | 1 | 207 | 12 | 40.0 | |
| | 2 | 200 | 23 | 41.8 | 50.6 |
| | 3 | 199 | 58 | 69.9 | |
| 6 day | 1 | 217 | -- | --- | |
| | 2 | 20? | -- | --- | |
| | 3 | 199 | -- | --- | |

Table 3. Lengths of hatched larvae from the control and experimental containers.

| Container | | Mean length (mm) | S | n | Exposure Group Average |
|---|---|---|---|---|---|
| control | 1 | 8.21 | 1.33 | 50 | |
| | 2 | 8.47 | 0.90 | 47 | 8.34 |
| | 3 | 8.34 | 0.66 | 50 | |
| 4hr | 1 | 8.59 | 0.52 | 50 | |
| | 2 | 8.44 | 0.55 | 50 | 8.26 |
| | 3 | 7.74 | 1.26 | 50 | |
| 8hr | 1 | 8.04 | 1.45 | 50 | |
| | 2 | 8.21 | 0.83 | 43 | 8.01 |
| | 3 | 7.77 | 1.34 | 36 | |
| 12 hr | 1 | 7.94 | 0.93 | 48 | |
| | 2 | 7.09 | 1.31 | 50 | 7.56 |
| | 3 | 7.65 | 1.33 | 50 | |
| 24 hr | 1 | 7.83 | 0.70 | 50 | |
| | 2 | 7.73 | 1.30 | 21 | 7.49 |
| | 3 | 6.92 | 1.00 | 38 | |
| 48 hr | 1 | 6.43 | 2.07 | 25 | |
| | 2 | 6.22 | 1.69 | 48 | 6.10 |
| | 3 | 5.66 | 1.54 | 50 | |
| 6 day | 1 | 0 | 0 | 0 | |
| | 2 | 0 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 | |

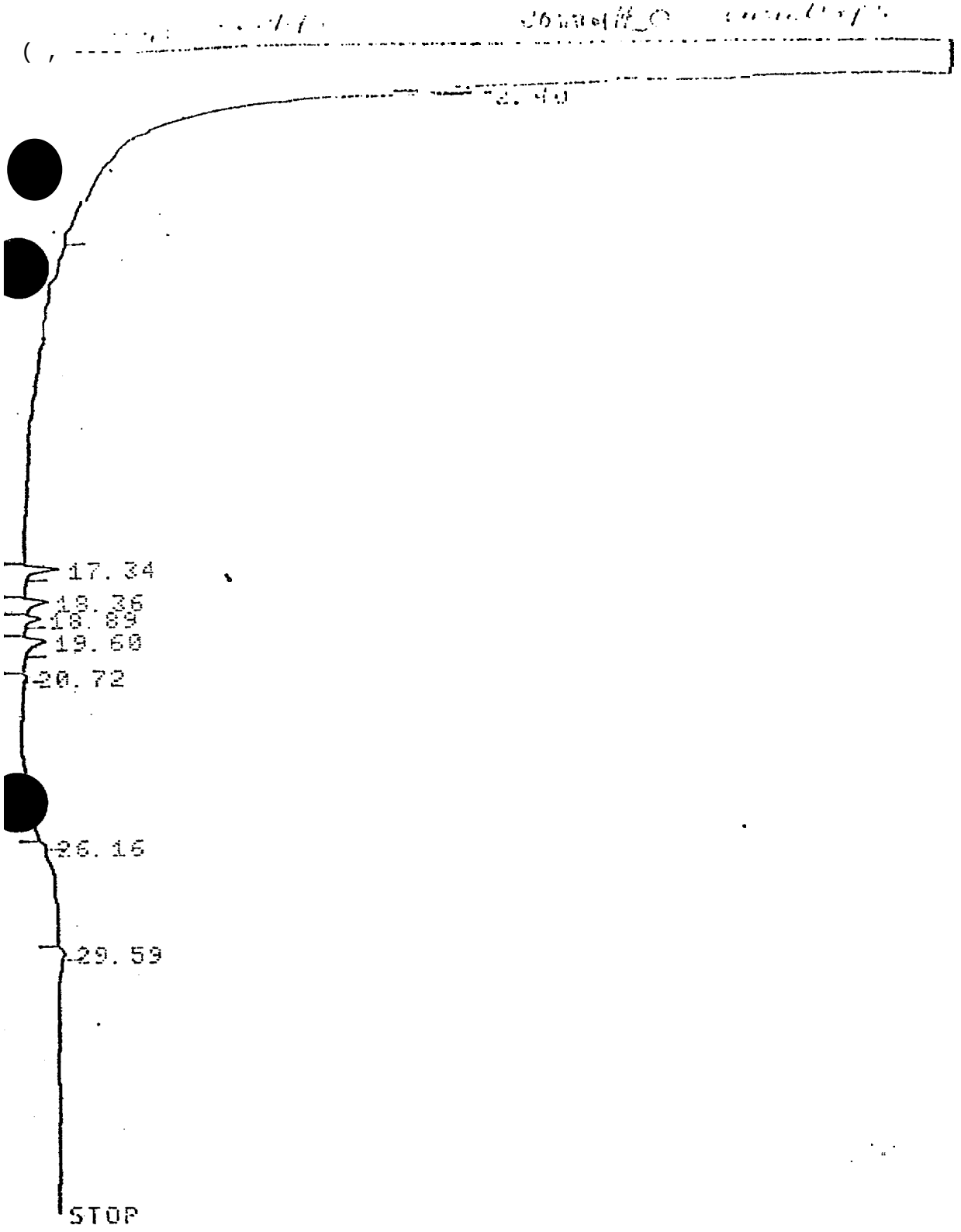


Figure 1. Gas chromatographic trace of a sample of seawater from Resurrection Bay. This was the control seawater and shows virtually no hydrocarbon contaminants.

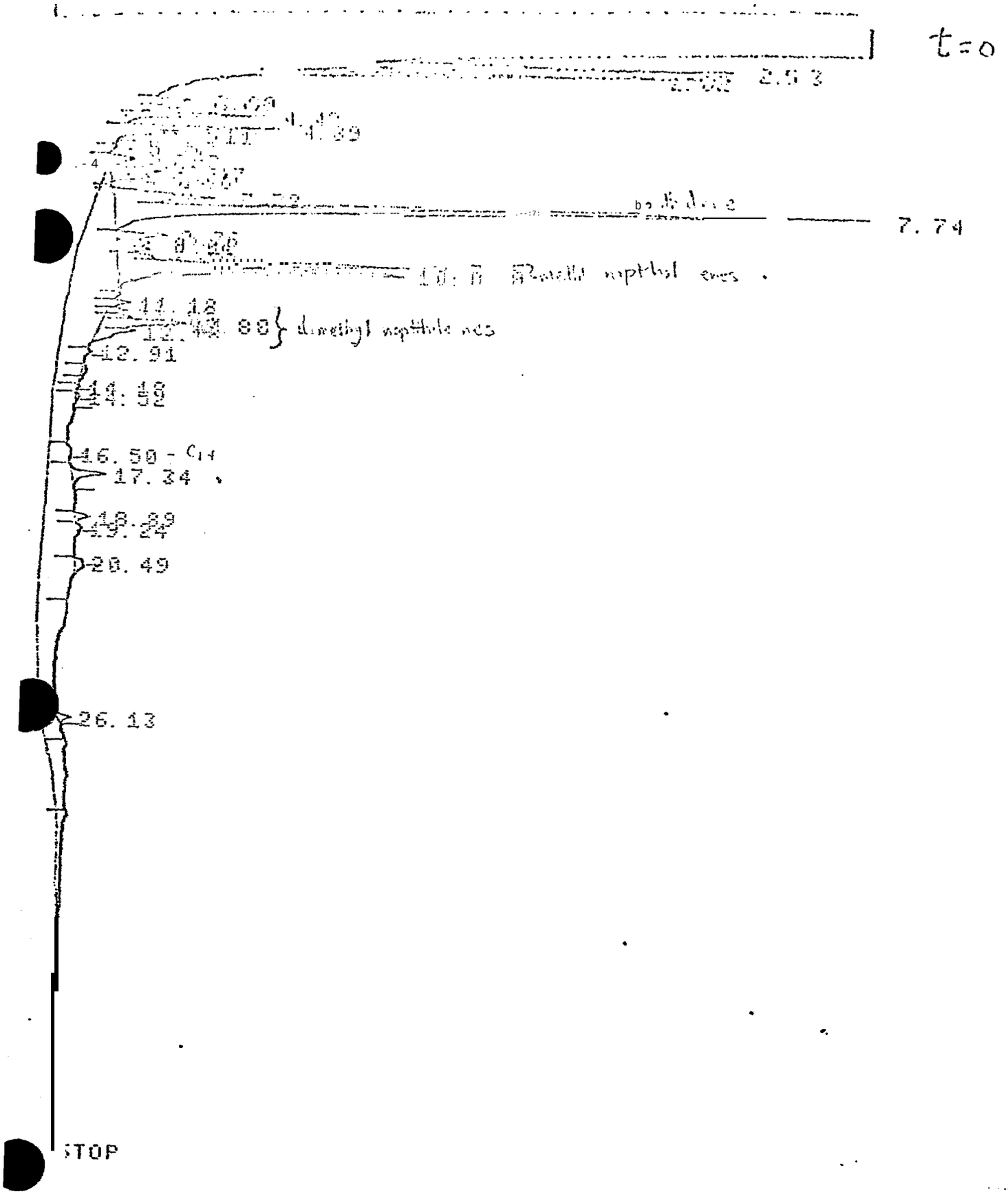


Figure z. Gas chromatographic trace of oil -equil - brated seawater used in the experimental containers. Major peaks are identified as naptha lene, methyl naptha lenes, and dimethyl napthalenes.

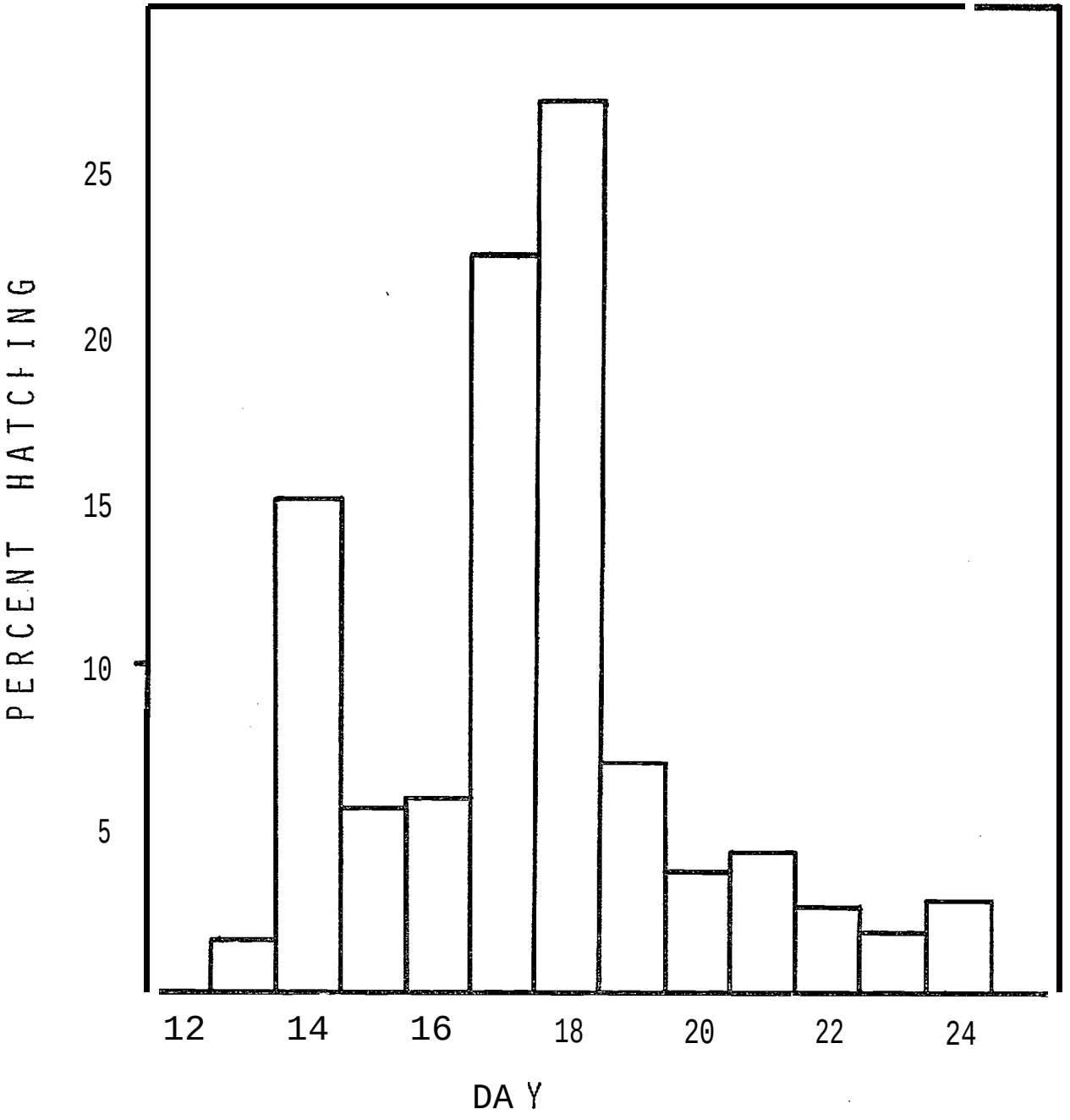


Figure 3. Percent hatching per day of herring eggs exposed for 8 hr to oil-equilibrated sea water.

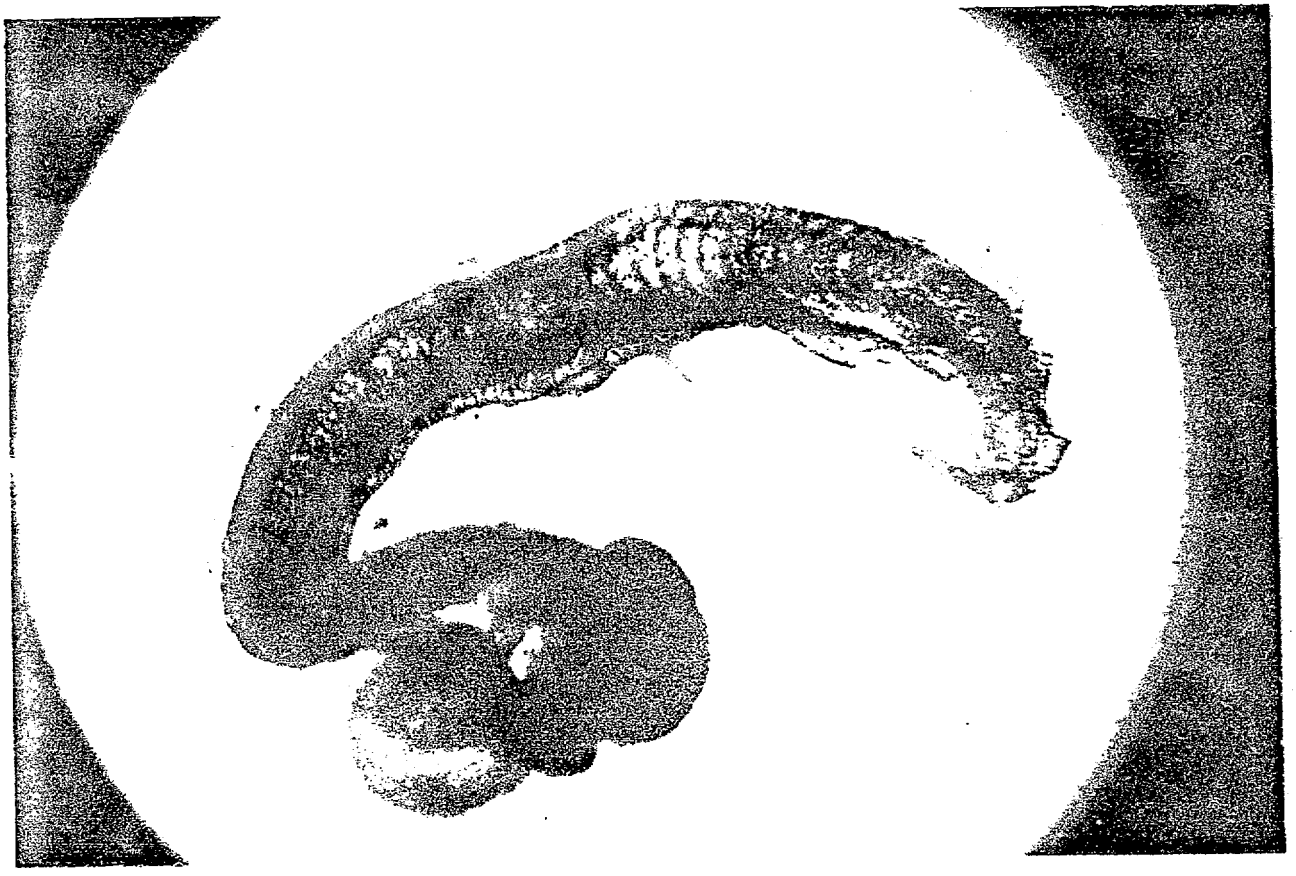

Figure 4. Dead herring larva with opague body. Contrast the appearance of this larva with living larva in Figs. 5, 6, and 7.

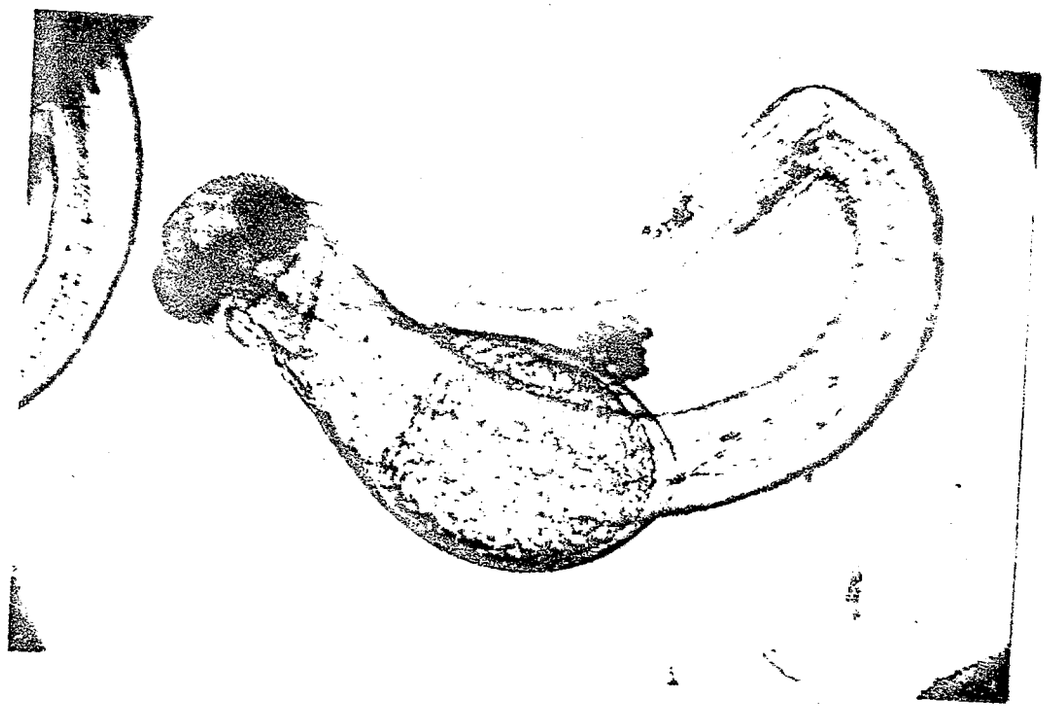

Figure 5. Hatched larva from 48 hr exposure experiment. Note dramatic bend in Spine and malformed mouth.

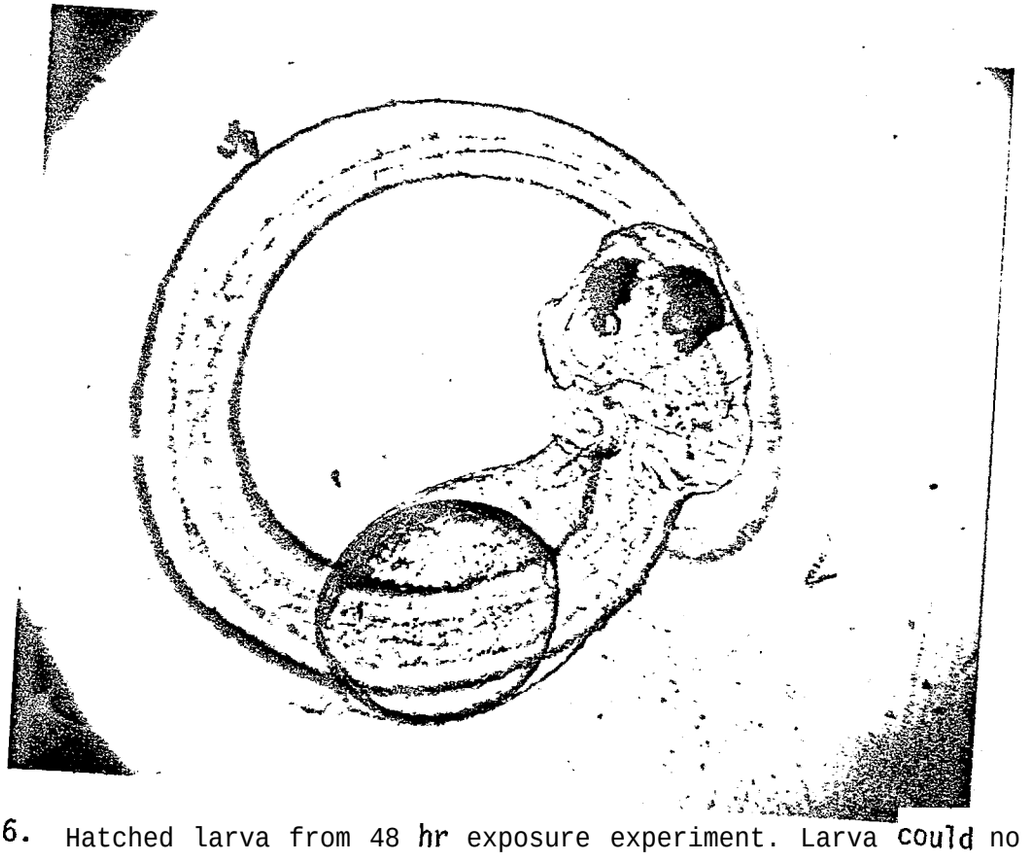

Figure 6. Hatched larva from 48 hr exposure experiment. Larva could not straighten body.

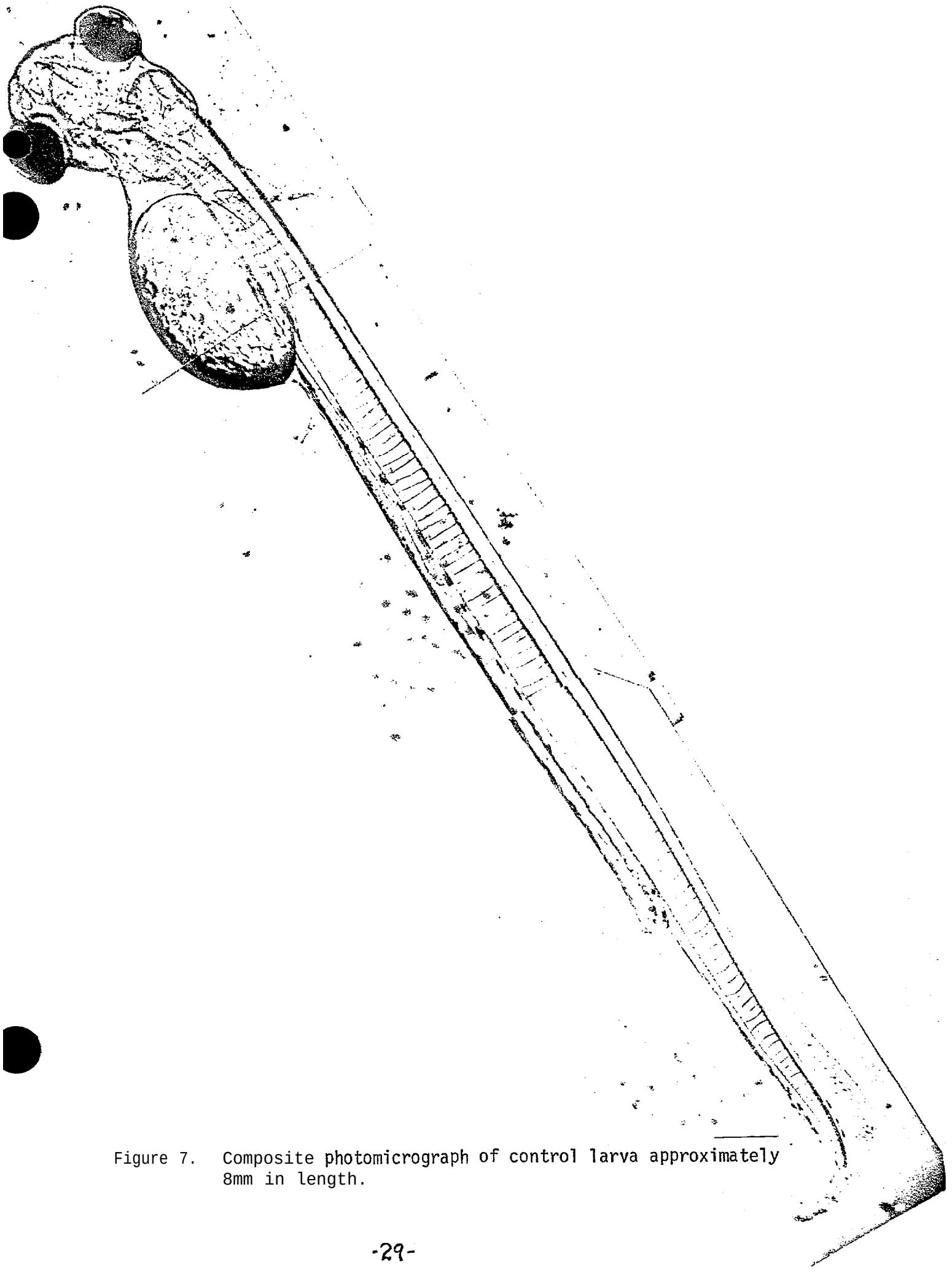

Figure 7. Composite photomicrograph of control larva approximately 8mm in length.

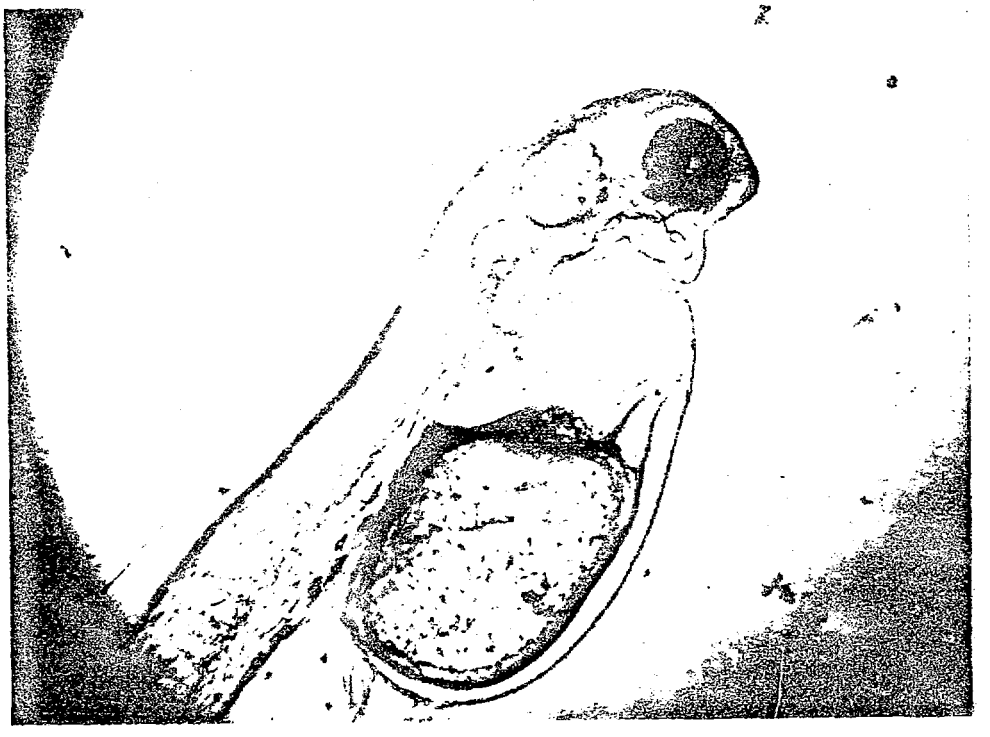

Figure 8. Larva from 48 hr exposure group exhibiting enlarged pericardial cavity anterior to yolk sac. (About 100X).

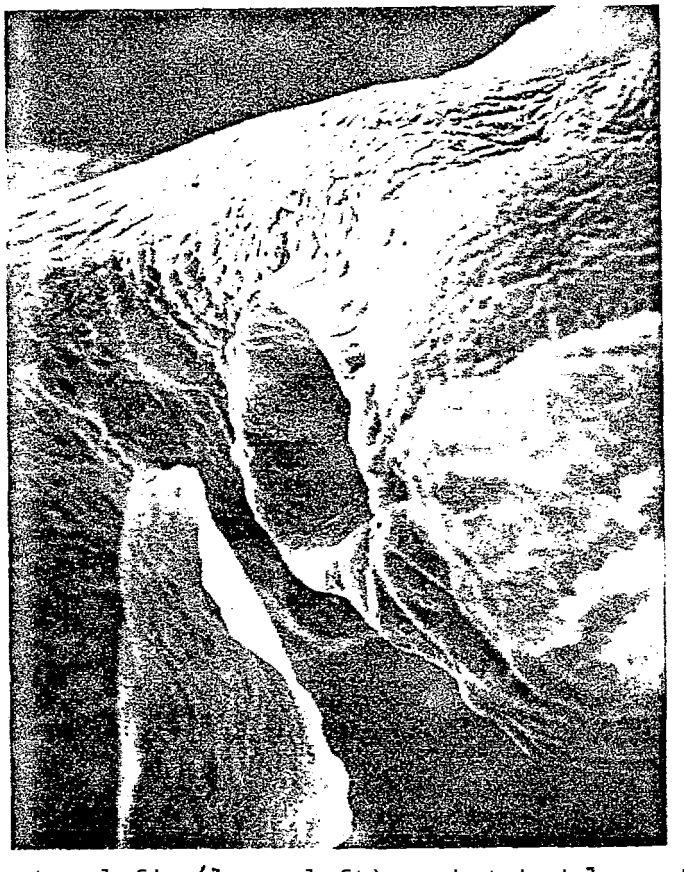

Figure 9. Normal pectoral fin (lower left) on hatched larva from 4 hr exposure group. Head of larva is to right. in photo. (200X)

Figure 10. Eroded larval pectoral fin from 48 hr exposure group. **(200X)**

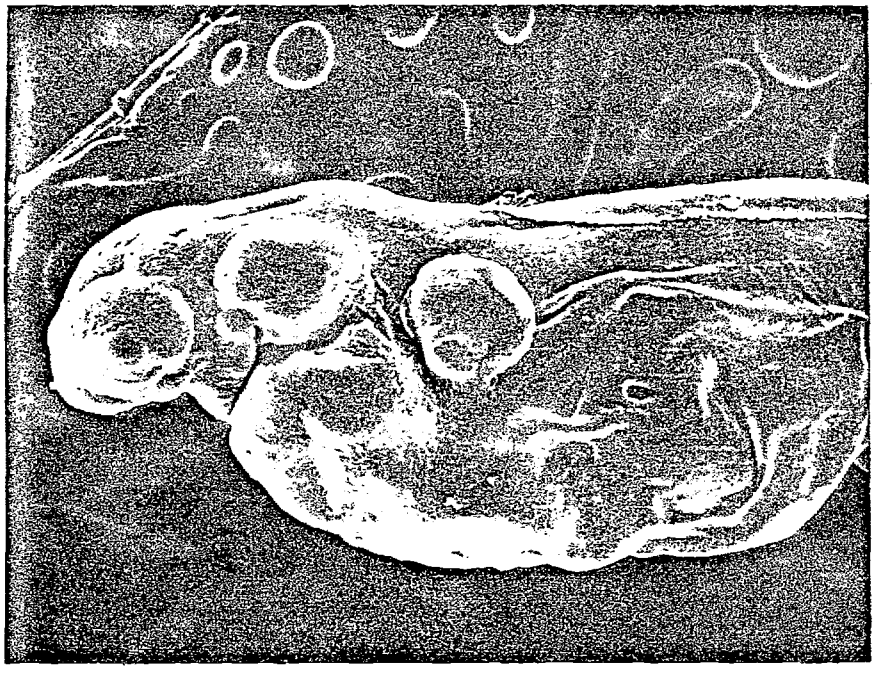

Figure 11. Larva from 48 hr. exposure group showing lack of pectoral fin development, enlarged pericardial cavity and poor eye development.. (100X)

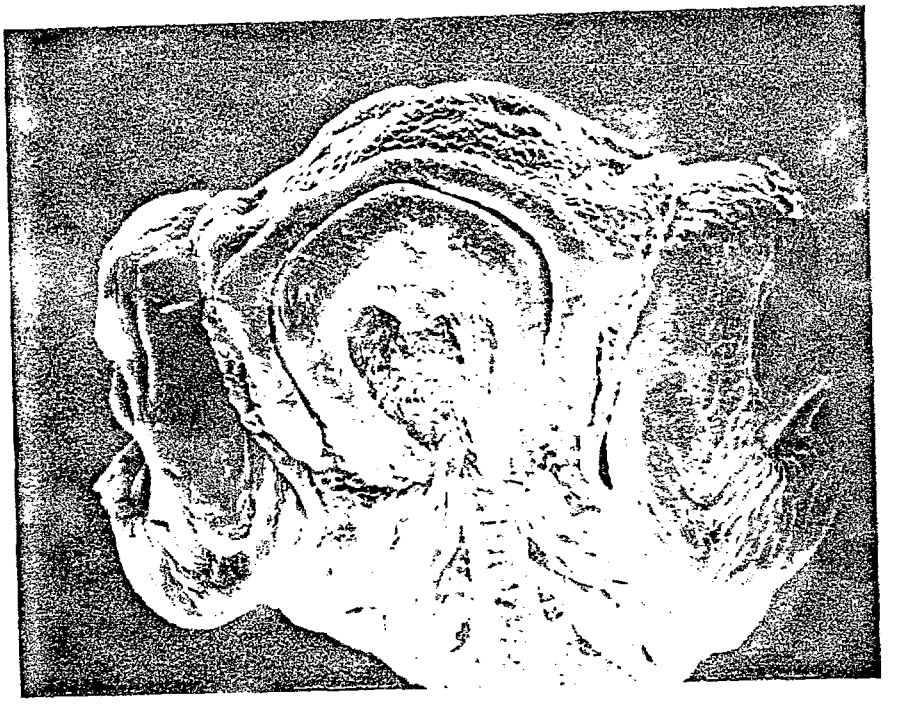

Figure 12. Head of larva exhibiting normal development (4 hr exposure group, 200X).

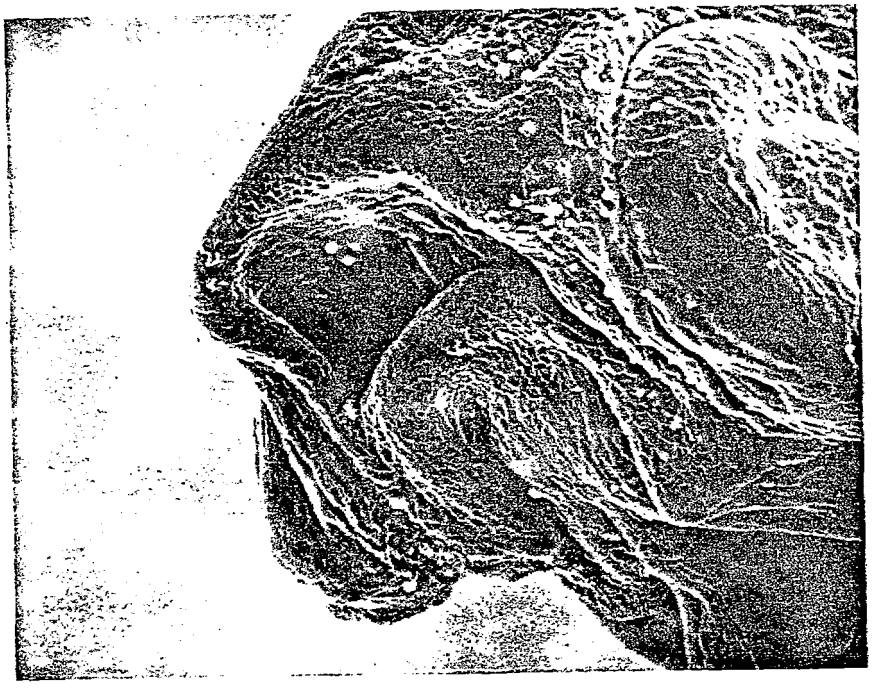

Figure 13. Malformed mouth in 48 hr exposure group larva. (200X)

Figure 14. Poorly differentiated head region and mouth (48 hr exposure 200X).

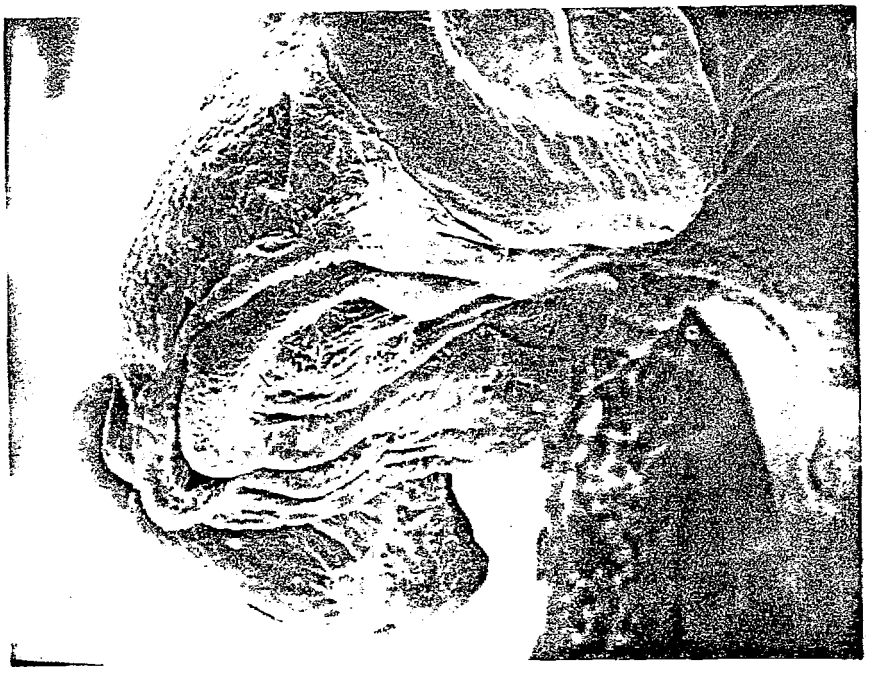

Figure 15. Upper jaw missing premaxillary bone (24 hr exposure 200X).

Figure 16. Branchiostegal membranes are complete on this larva (12 hr exposure, 200X)

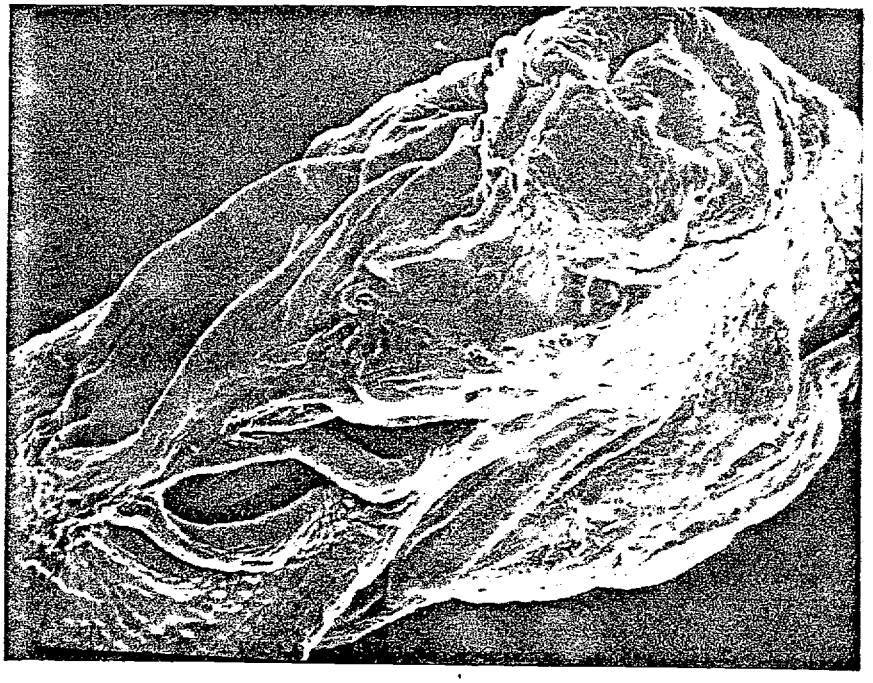

Figure 17. Branchiostegal membranes are missing on this larva from the control group (200X).

Figure 18. Herring eggs covered with microorganisms (50X).

Figure 19. Surface of egg with associated bacteria (6 day exposure group, 5000X).

Figure 20. Diatom and bacteria on surface of control egg (5000X).

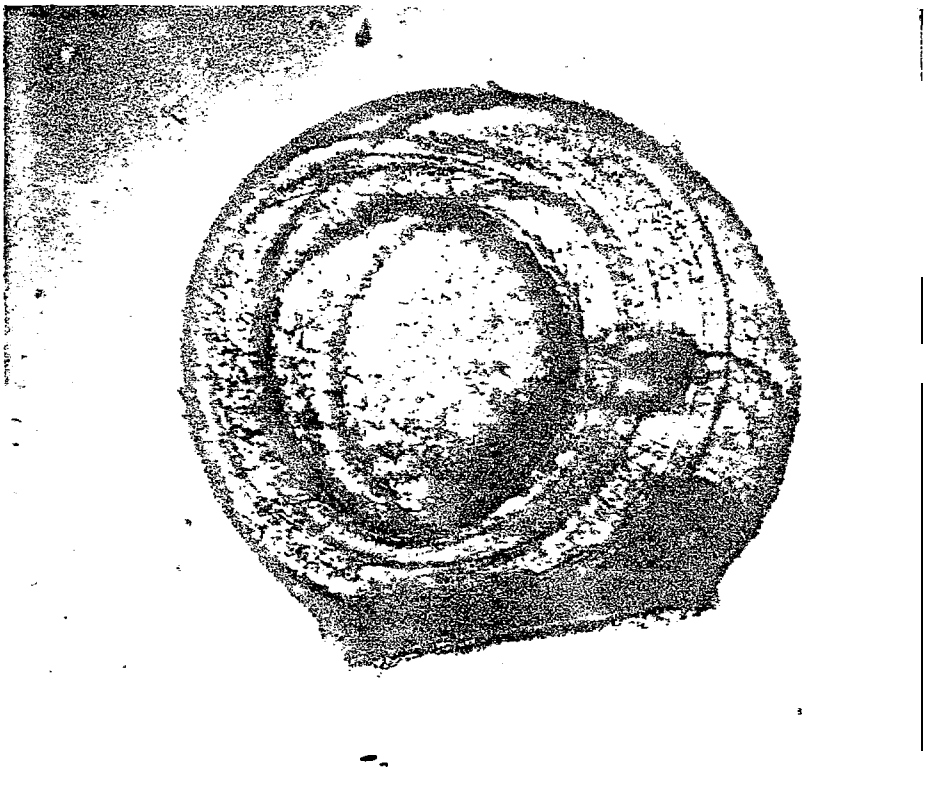

Figure 21. Unhatched control embryo on tenth dayof experiment.

Figure 22. Unhatched embryo from 24 hr exposure group, tenth day of experiment.

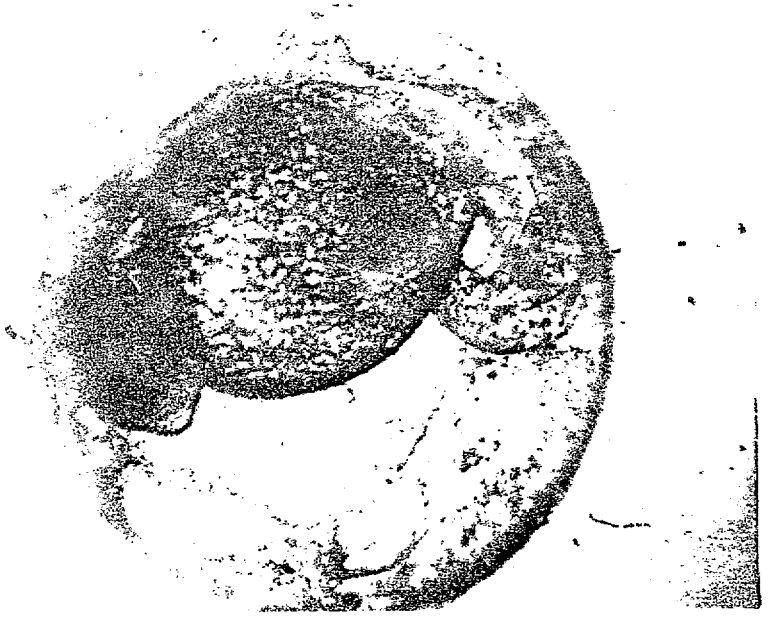

Figure 23. Unhatched embryo from 6 day exposure group, tenth day of experiment.

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