EFFECTS OF PRUDHOE BAY CRUDE OIL ON

PACIFIC HERRING (CLUPEA HARENGUS PALLASI, V.) DEVELOPMENT

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EFFECTS OF PRUDHOE BAY CRUDE OIL ON PACIFIC HERRING (*CLUPEA HARENGUS PALLASI*, V.) DEVELOPMENT

A

THESIS

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ABSTRACT

Herring eggs were exposed to Prudhoe Bay crude oil for various exposure times to evaluate the potential impact of Alaskan oil on development in marine fishes. Larvae were collected upon hatching and observations were made on morphological effects of the exposure.

Most abnormalities noted were associated with flexures of the spine, forcing the larvae into arched, bent, or corkscrew positions. Other abnormalities included partially formed mouth parts and frayed pectoral and caudal fins.

Statistical differences in hatching success were found between the controls of the 6 day exposure group ($P < 0.05$). Analyses of total length and occurrence of abnormalities showed a significant difference between the controls and the 48 hr group ($P < 0.05$). Electron micrographs indicated external and cellular effects of exposure to the crude oil. Cellular effects consisted, principally, of vacuole formation in larvae and decomposition in eggs.

ACKNOWLEDGMENTS

Sincere appreciation is expressed to Dr. Ronald L. Smith for his assistance throughout the project and my educational program. Thanks are also given to the personnel of the University of Alaska's Seward Marine Station, the Artificial Upwelling Project, Seward Fisheries personnel, and Mr. A. Paulson for assistance during the experimental period of the project; to Dr. T. Gosink, personnel of the Hydrocarbon Laboratory, Institute of Marine Science, University of Alaska, Mr. C. Ackert, and Mrs. Eva Ackerman for analytical and technical assistance; and to M. A. Smith of the Electron Microscopy Laboratory, University of Alaska, and A. Soeldner, Electron Microscopy Laboratory, Oregon State University for their technical expertise in the areas of transmission and scanning electron microscopy. Special thanks for assistance with my program of study and the reviewing of this report go to Dr. David Shaw and Dr. R. Ted Cooney. This project was funded through the Bureau of Land Management's Outer Continental Shelf Office under contract No. 03-5-022-56, task order No. 18.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURE ABBREVIATIONS

B . . . . . bacteria

C . . . . . chorion

CY . . . . . cytoplasm

D . . . . . debris

DS . . . . . dorsal surface

E . . . . . eye

EY . . . . . enlarged yolk sac

G . . . . . gelatinous coat

GA . . . . . gill apparatus

H . . . . . head

J . . . . . jaws

L . . . . . lamella

LL . . . . . lateral line

LLO . . . . . lateral line organ

M . . . . . mitochondrion

N . . . . . nucleus

P . . . . . perivitelline space

PF . . . . . pectoral fin

S . . . . . normal spacing between cells

V . . . . . vacuole

Y . . . . . yolk

YP . . . . . yolk particle

YS . . . . . yolk sac

LIST OF TABLES

INTRODUCTION

Construction of the trans-Alaska pipeline has generated concern as to the possible environmental impact of an oil spill in Alaskan waters. The pipeline terminus is at Valdez, Alaska, with shipping operations to proceed from there through Prince William Sound. This route makes the Sound a high risk area for the possibility of an oil spill. Extensive study of the species that inhabit the Sound and the effects of this oil on them is necessary to understand the environmental impact likely to occur.

Of all oil components the lower boiling point aromatic hydrocarbons are generally the most toxic to marine organisms. This toxicity is a result of the greater solubility of aromatics, enabling organisms to concentrate aromatics more readily than saturated hydrocarbons (Kuhnhold, 1972; Moore and Dwyer, 1974; Struhsaker, et al., 1974; Warner, 1976). The aromatics are also retained in mollusc, crustacean, and fish tissue for greater periods of time than the alkanes (Anderson, et al., 1974a). Linden (1975), working with Baltic herring, has 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 crude oil (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 Struhsaker, et al. According to their study, benzene influences metabolic rate and energy utilization. A concentration of 5 ppm benzene causes an acceleration of metabolic rate with a resultant energy cost. Thomas and Rice (1975) have also found an increased metabolic rate to be a normal response of fish to pollutant stress. They speculate that a long term effect of oil pollution would be to create a higher energy requirement which could be detrimental to survival and reproduction potential.

The survival of marine organisms when contaminated by oil depends largely on how the oil is introduced into the system. Greater damage occurs from oil emulsified in sea water than occurs from an oil film on the water surface. In adult fishes both the toxicity and the effect of tiny droplets of oil on the gill apparatus appear to be important (Mironov, 1972). Coating and smothering effects of an oil film are important only when considering a weathered oil in which the soluble aromatic derivatives have evaporated. If

these aromatic hydrocarbons are still present the mortality due to coating effects may be negligible. Intertidal sessile species, however, are particularly susceptible to coating (Moore and Dwyer, 1974).

According to Blumer, et al., (1973) degradation of an 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. Both evaporation and dissolution are logarithmically related to the molecular volume of the hydrocarbons because of the logarithmic dependence of boiling point to molecular volume. As a result, losses from evaporation and dissolution decrease rapidly for higher molecular volume members of homologous series.

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 relative to more resistant components of similar boiling point and solubility (Blumer, et al, 1973).

Chemical degradation, according to Blumer, is apparent at advanced stages of the weathering process. These effects include oxidation of the medium and higher molecular weight

aromatics leading to an increase of high molecular weight polar compounds, known as asphaltenes. Formation of alcohols, alkyl- and arylethers, carbonyl-compounds and sulfoxides have been noted by Kawahara (1969, 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 (b.p. 301.8°C) to n-octodecane (b.p. 316.1°C) and about 50% of those with boiling points in the range of n-nonadecane (b.p. 329.7°C) to n-heneicosane (b.p. 365.5°C). Hydrocarbons above n-C_{22} were retained for the length of the studies; up to 16 months. These higher boiling aromatics include phenanthrene, anthracene, their substituted homologues, and higher ring number aromatics. These compounds are thought to be responsible for the long term toxicity of oil.

Almost without exception the most highly sensitive life stages to environmental stress are the developmental stages of the egg and larva. Eggs tend to be less resistant to oil pollutants (Mironov, 1972), as the larval stage is able to

recover to a certain degree while eggs are irreparably damaged (Struhsaker, et al., 1974). Investigations into the effects of oil in the marine environment should be focused on the most susceptible life stages to understand what the full impact of the oil will be.

One habitat likely to be affected by an oil spill is the inshore area for this is where the oil would probably accumulate. Pacific herring (Clupea harengus pallasi, V.) spawn in Prince William Sound in April and May each year. Eggs are spawned demersally, remaining attached to the substratum or algae in intertidal areas. This species, therefore, could be particularly vulnerable to oil.

Another consideration in the choice of C. h. pallasi for study is the importance of this species in commercial fisheries. Adult herring have been harvested in the past by the American fishing fleet for reduction, oil, and bait. The fleet is now principally interested in harvesting the eggs. These eggs are collected both attached to algae and as sac roe. The eggs are then packaged in brine and transported for sale on the Japanese market as a delicacy food item. Commercial harvesting of the adult is still continuing, though to a lesser extent than in the past, primarily for use as bait in salmon and crab fisheries.

Taking these points and the proposed shipping route into consideration this project was directed towards some

of the effects of the water soluble fractions of Prudhoe Bay crude oil on Pacific herring development. As was noted previously the water soluble fractions of oil produce a greater degree of toxicity than would an oil film. The toxic effects were determined by examining hatching success, occurrence of abnormalities, total lengths of the hatched larvae, and the ultrastructure characteristics of both eggs and larvae. Transmission and scanning electron microscopy were used to study the ultrastructure and the exterior abnormalities.

METHODS AND MATERIALS

A. Seward Study

The experimental set-up consisted of twenty-one wide mouth gallon glass jars. Approximately 200 C. h. pallasi eggs attached to sieve kelp (Thalassiophyllum clathrus) were placed in each of these jars and were exposed to water equilibrated with Prudhoe Bay crude oil for exposure times of 4, 8, 12, 24, and 48 hrs, and 6 days (144 hrs). Three of the gallon jars were designated for each exposure time. Three were also set up as control jars and were filled with uncontaminated sea water.

The oil equilibrated water was mixed in three 12 gallon 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 ¼ in thick. These solutions were mixed with stainless steel stirring rods for 24 hrs and allowed to stand for an additional 12 hrs to allow time for the oil droplets to rise to the surface. These reservoirs remained tightly capped during the entire experimental period to minimize evaporation.

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. According

to other research using aerated systems (Linden, 1975; Anderson, et al., 1974b) 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 the 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.) and determining that hatching time at this temperature would be 15-20 days. This provided a reasonable time scale within which to work.

The herring roe were collected by Seward Fisheries personnel on April 21, 1976. It was estimated by these gentlemen that the roe had been spawned 2-3 days previously. The roe were picked up in Tatitlek Narrows, Prince William Sound, and transported back to Seward by float plane. The sea water in the buckets containing the eggs was replaced by water from Resurrection Bay upon return to the lab. Water was changed at regular intervals until the eggs were counted and placed in their respective experimental jars on April 24. The eggs were in late neurula or early tail bud stages. This was counted as day 0.

Eggs were observed with a dissecting microscope each day and development was noted. Heart rates were calculated prior to hatching on days 6, 8, and 11. No heart rates were

determined for the larval herring as they moved about too quickly to be counted. Total lengths of the individual larval herring were determined upon hatching for the first 6 days, days 12-17. Measurements were made while observing the larvae under the dissecting microscope. Abnormalities were also noted at this time.

All larvae, regardless of appearance or mobility, were considered to be hatched until the last two days of the experiment in each individual container. The experiment was concluded separately for each container when no viable larvae had appeared in a two day period. Viability was determined by mobility and general appearance; viable larvae being more transparent. The non-viable larvae that appeared during the final two days were counted as non-hatched larvae. This decision was made after it was noticed that the eggs had begun to deteriorate. It was felt that these non-viable larvae were produced from disintegrating eggs and not from actual hatching. Before the final decision was made to terminate a particular container the remaining eggs were examined with a dissecting microscope. If any viable eggs remained all previous larvae were considered to have hatched and the eggs were replaced to continue developing. Viability, in this instance, was determined from transparency, movement, and heart beat. At the conclusion of the experiment, containers Control 3, 8 hr 2, and 8 hr 3 still had

viable embryos. Time did not permit continuing the experiment until these embryos either hatched or died. It was assumed that these eggs would have hatched if time had permitted and were, therefore, counted as hatched larvae but were not included in the abnormality calculations.

Water samples were collected from the control and the initial oiled water for gas chromatographic analysis. These samples were prepared by hexane extraction and drying with Na_2SO_4. Preparation and analysis were performed by B. A. Baker, Hydrocarbon Laboratory, University of Alaska.

B. Electron Microscopy

1. Transmission

Egg and larva samples for electron microscopy were taken from the first few that hatched in each jar. The larvae that were kept for analysis had no body distortions and swimming ability was not impaired.

Standard procedures were used for fixation. Egg and larva samples were fixed for 1-2 hours in gluteraldehyde and then transferred to cacodolate buffer and refrigerated for return to Fairbanks. Postfixation was in 1% OsO_4 for 1 hour. The samples were then dehydrated in an alcohol-acetone series and embedded in plastic. Sections were stained with uranyl acetate and lead citrate for 1 hour and 10 minutes, respectively. The transmission electron microscope used was a

JEM-6AS located in the Geophysical Institute of the University of Alaska.

2. Scanning

Preparation and scanning microscopy were 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.

RESULTS

A. Seward Study

Results from gas chromatographic analysis of the water samples indicate the hydrocarbon content of the experimental water was 0.67 ppm above the control sample. The major constituents of this water soluble fraction were napthalene (17%), methyl napthalenes (13%), and dimethyl napthalenes (5%). Chromatographs of the uncontaminated sea water, the experimental water, and a standard are located in Appendix I.

Table 1 (Appendix II) contains the pertinent data from the experiment including: numbers of eggs in each container, number and percent successfully hatched, and number and percent of the hatched larvae that were abnormal. The number and percent of hatched larvae for each twenty-four hour period and abnormal larvae for each twenty-four hour period are listed in Tables 2 and 3 (Appendix II), respectively. Graphical representations of the percent hatched ($Y = 54.31\ e^{-.01573t}$) and percent abnormal ($Y = 0.79X + 9.83$) for all exposure times are shown in Figures 1 and 2, where bars indicate the range per exposure group. Figure 3 is a representative portrayal of the percent hatching per day as seen in the 8 hr exposure group. This type of hatching occurred in all exposure groups with modes appearing on days 14 and 17 or 18. No differences in the total time to

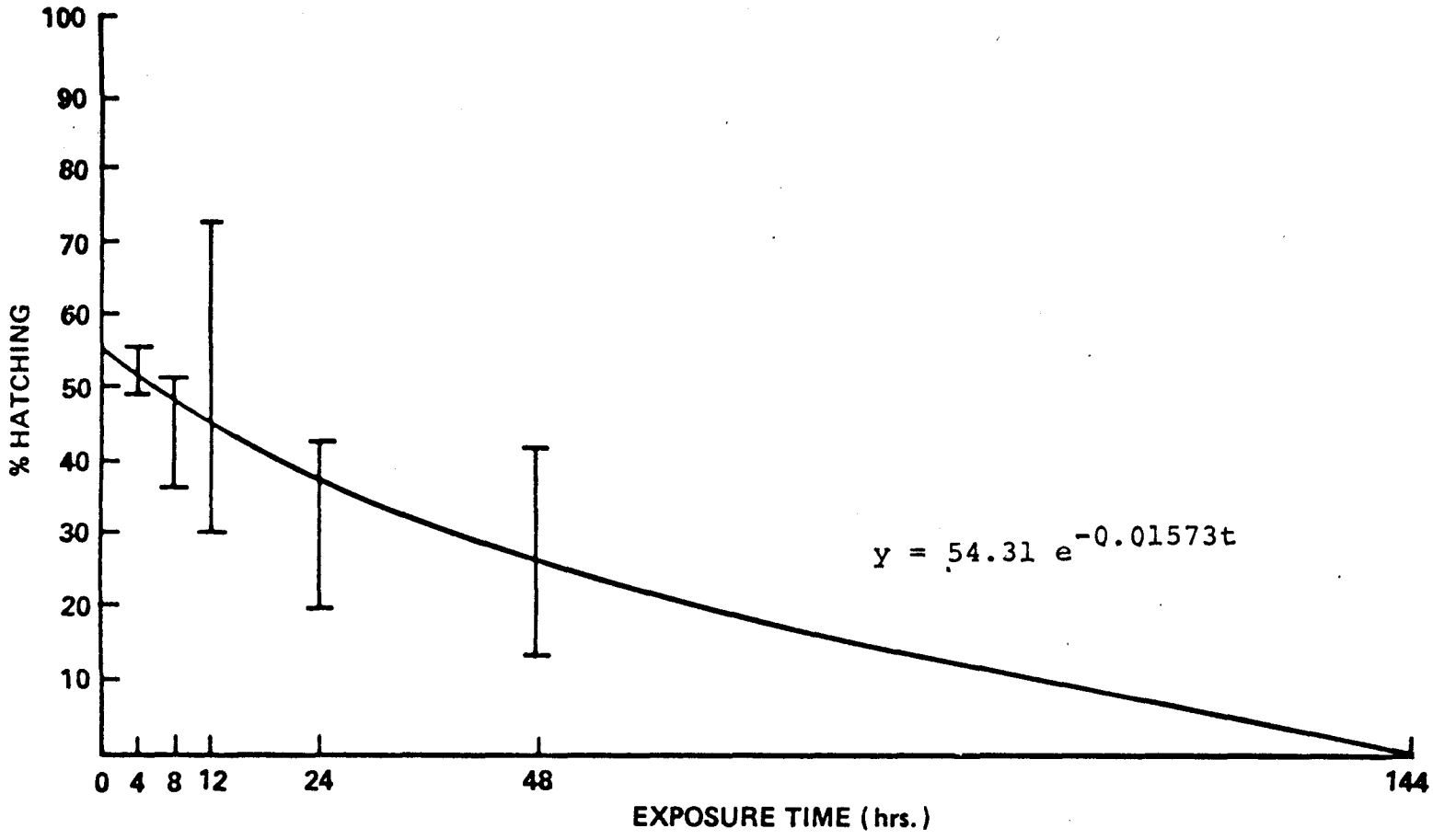


Figure 1. Hatching success vs. exposure time.

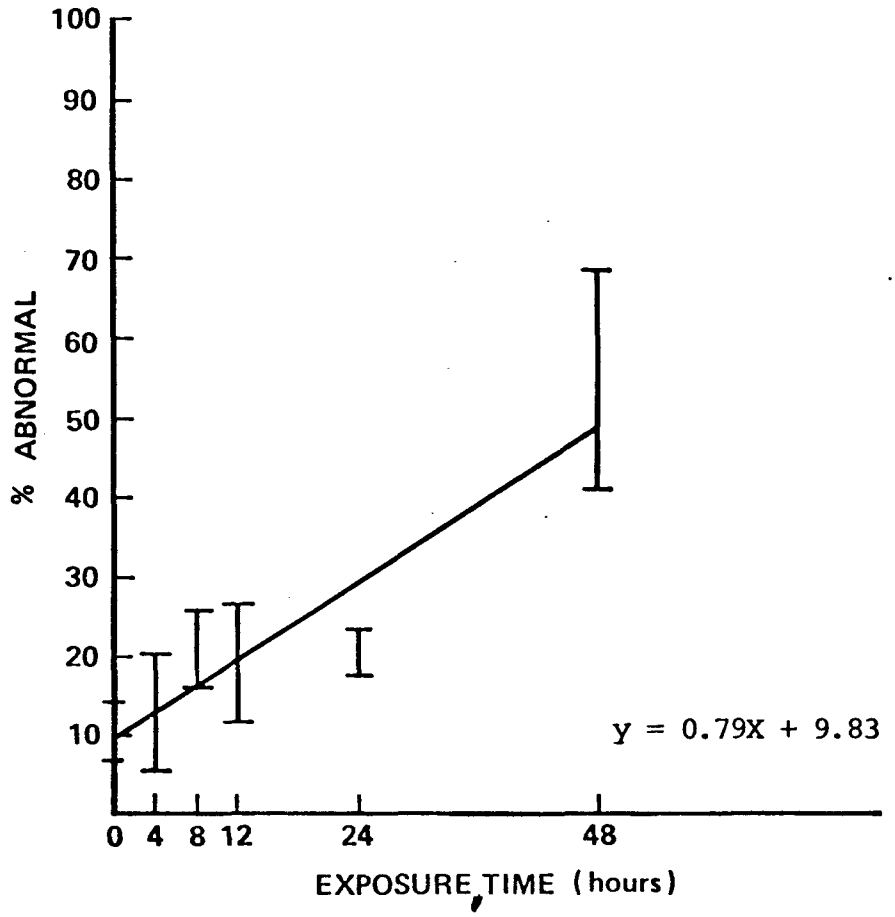


Figure 2. Percent abnormal vs. exposure time.

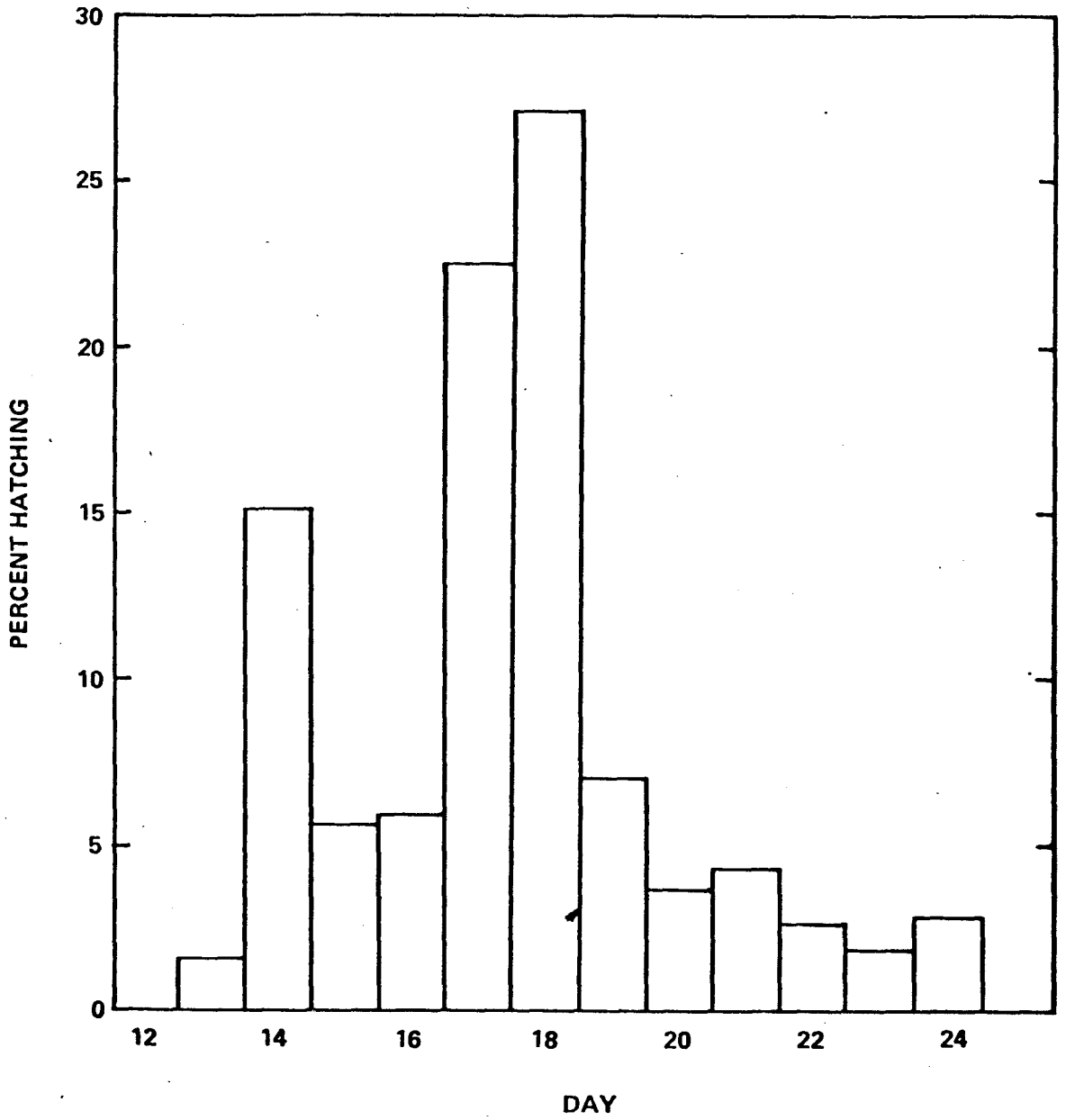


Figure 3. Typical hatching pattern as seen in the 8hr exposure group.

hatching were noted between exposure groups.

Structural abnormalities appeared in all exposure groups. The majority of these were abnormalities in flexures of the spine. Conditions varied between curved spines forcing the larvae into an arched position, L and S shaped spines, and corkscrew positions. These larvae had difficulty swimming with many totally unable to swim and remaining on the bottom of the containers. Normal larvae tended to swim directly to the surface of the containers upon hatching.

Another abnormality that appeared quite frequently was an enlarged yolk sac region extending anteriorly to the ventral head area. This type of abnormality appeared only with the longer exposure times of 24 hours and 48 hours. The abnormal larva collected from the 48 hr exposure group (Figure 4) was representative of this class of abnormality. As can be seen, the yolk sac enlargement appears to be part of the larva that was not incorporated properly into the body before budding off from the yolk during development. Additionally, the pectoral fins have not formed but appear as knobs on the side of the larva. Another knob is anterior to the location of the pectoral fin and appears to be in the area where the gill apparatus should be. The mouth parts, also, have not formed properly and the eyes are malformed as the pupil is extruded. In normal larva the pupil was sunken.

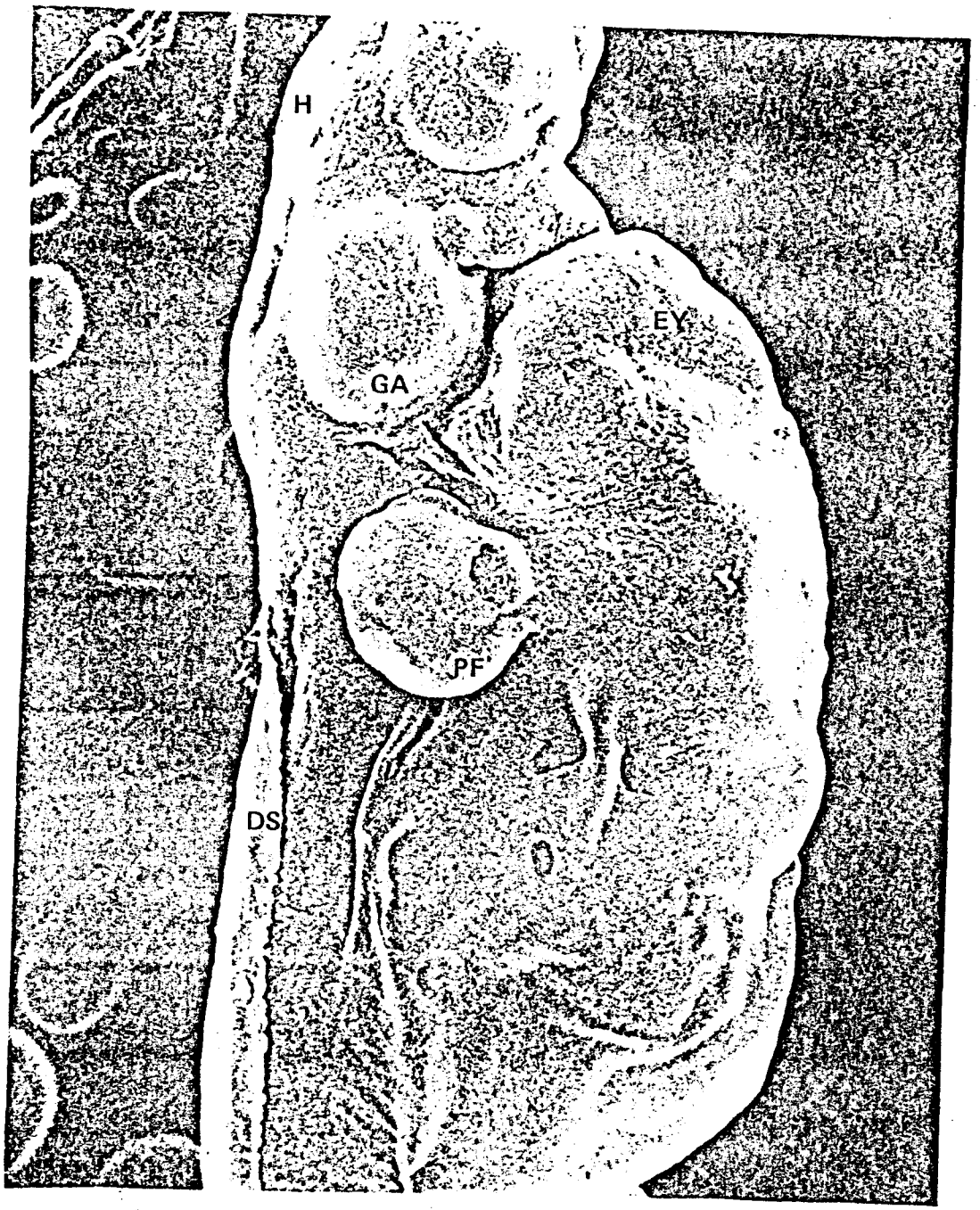


Figure 4. Scanning Electron Micrograph - 48 hr abnormal larvae showing enlarged yolk sac and undeveloped pectoral fin, head area, and gill apparatus (400X).

Another common abnormality was that of incomplete formation of the lower jaw. This feature was readily apparent in many of the collected larvae when viewed by scanning electron microscopy. Figures 5 and 6 show both normally and abnormally formed lower jaws.

Another of the external features noticed was the fraying of the pectoral fins. Normal fins appeared very delicate. Their edges were continuous with no signs of fraying (Figure 7). The abnormal fins did not appear as delicate. The edges were ragged and the texture of the rest of the fin seemed coarser (Figure 8). Statistical analyses of percent hatching and percent abnormal figures were performed using an analysis of variance in conjunction with Dunnett's Test for testing a control against all other groups (Zar, 1974). According to these tests the percent hatching figures were statistically different between the control and the 6 day exposure groups ($P < .05$). Percent abnormal figures varied significantly between the control and 48 hour exposure groups ($P < .05$). The results from these tests are shown in Tables 4 and 5.

Heart rate counts were taken on several embryos on days 5, 8, and 11. Counts were not taken after hatching began, day 12, in an effort to disturb the eggs as little as possible. These counts are listed in Table 6 (Appendix II). Whenever possible calculations were made on five embryos and

Figure 5. Scanning Electron Micrograph - normal jaw structure (400X).

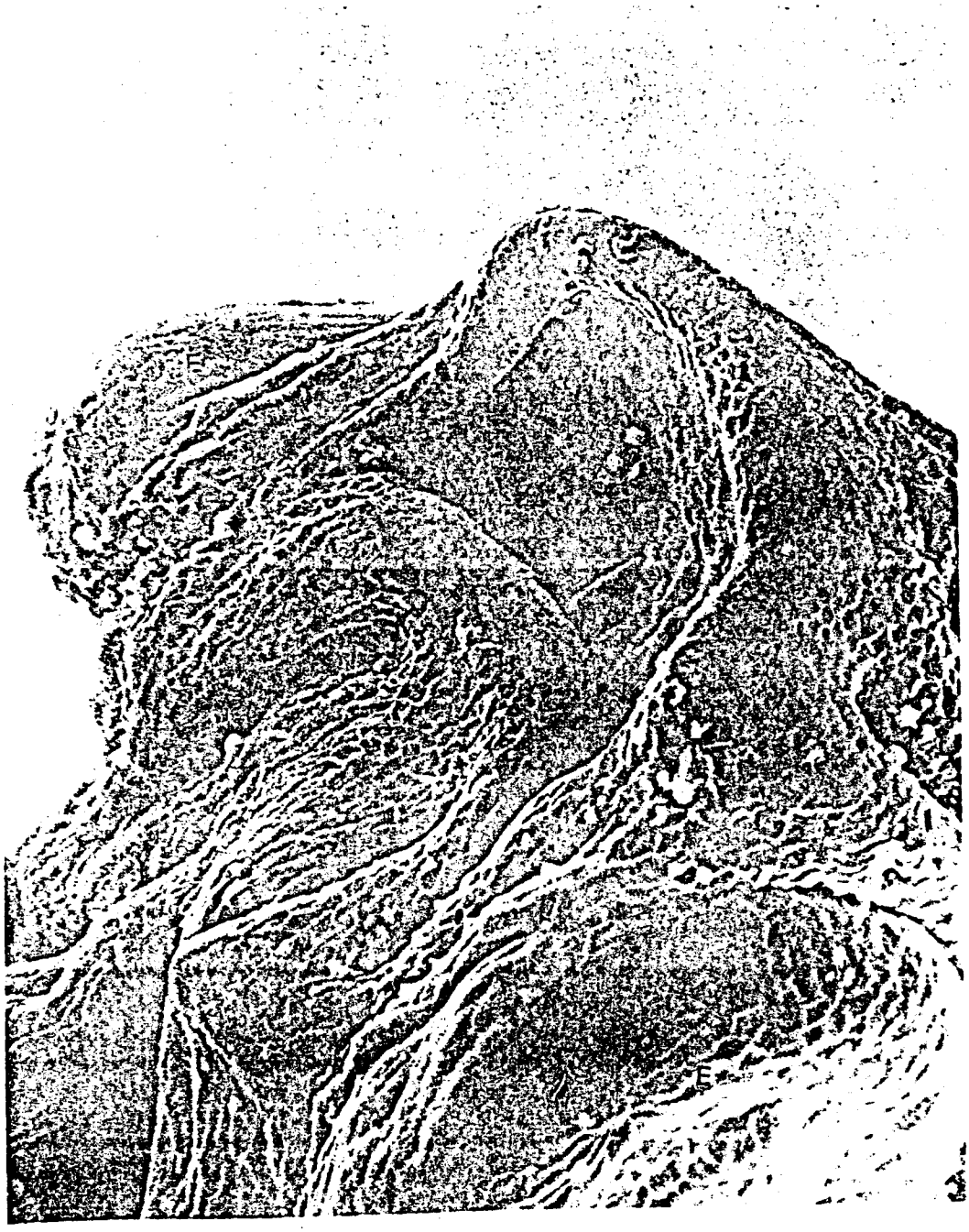


Figure 6. Scanning Electron Micrograph - abnormal jaw structure (400X).

Figure 7. Scanning Electron Micrograph - normal pectoral fin (400X).

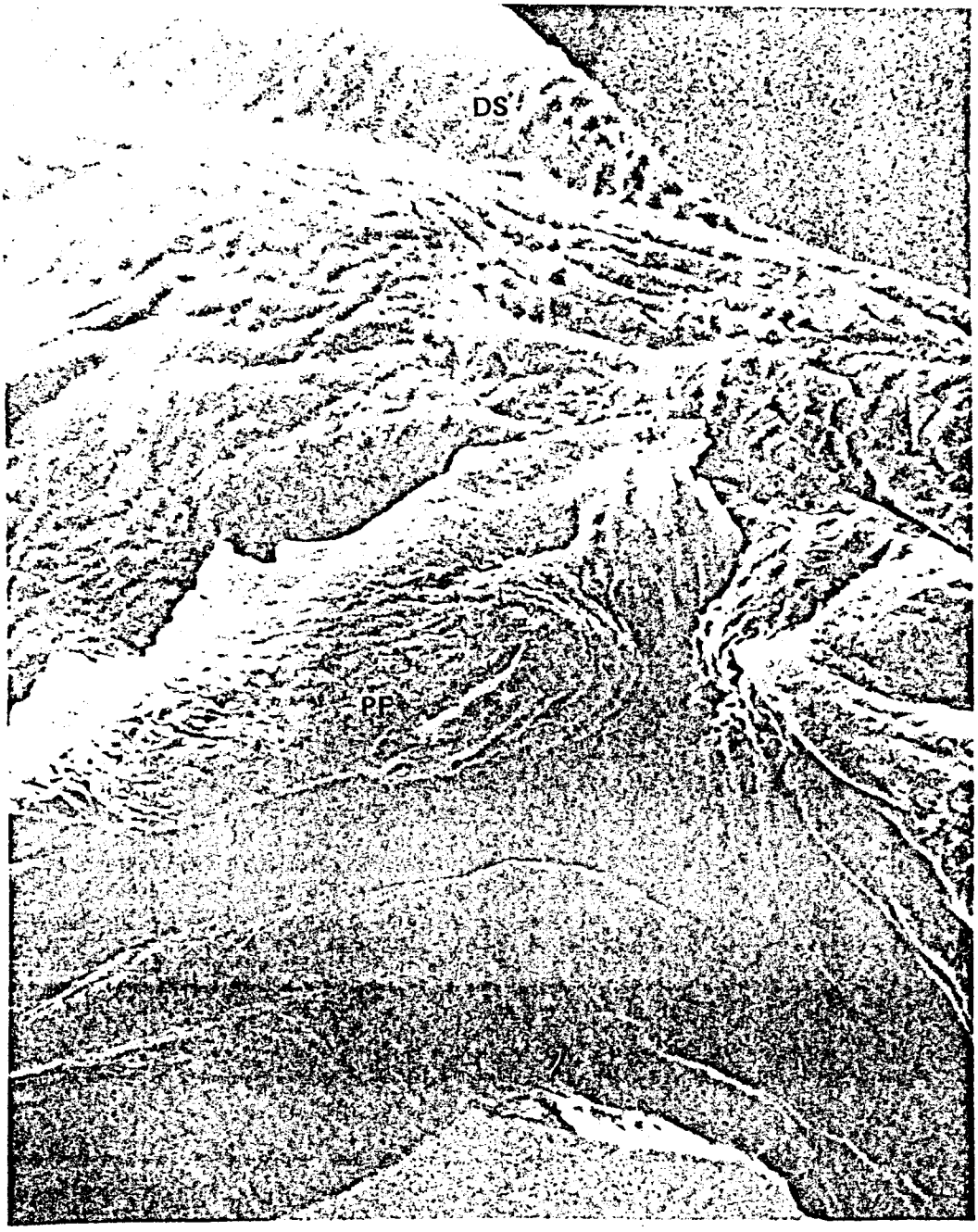


Figure 8. Scanning Electron Micrograph - abnormal pectoral fin (400X).

Table 4

Single factor analysis of variance for

A. hatching success

B. percent of larvae showing abnormalities

C. larval total length

$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7$

	Sources of Variation	Degrees of Freedom	Mean Square	F
A.	Treatments[1]	6	1163.9	
	error	14	218.5	*
B.	Treatments[1]	5	649.02	
	error	12	166.52	*
C.	Treatments[1]	5	2.04	
	error	12	0.14	*

[1]differing exposure times to crude oil

* $P < 0.05$

TABLE 5

Dunnett's test for

A. hatching success

B. percent of larvae showing abnormalities

C. larval total length

$H_0: \mu_C = \mu_A$ $\alpha = 0.05$

	Comparison	Difference	SE	$\lvert q' \rvert$	p	q'	Conclusion
A.	C vs 144	53.4	12.07	4.42	7	2.91	reject H_0
	C vs 48	25.8	12.07	2.14	6	2.84	accept H_0
B.	C vs 48	40.53	10.54	3.85	6	2.90	reject H_0
	C vs 24	9.93	10.54	0.94	5	2.81	accept H_0
C.	C vs 48	2.24	0.31	7.33	6	2.90	reject H_0
	C vs 24	0.85	0.31	2.78	5	2.81	accept H_0

averaged. The choice of embryos was determined by which were in the proper orientation to facilitate counting. Heart rates increased as development progressed in all but the 48 hr exposure group which decreased on day 8 and increased again on day 11. Heart rate decreased slightly with lengthened exposure times on all three days. By day 11 there was a less distinct pattern of decrease as seen in Figure 9. Hatching began in Control 1, 12 hr 2, 24 hr 2, and 48 hr 3 containers on day 12 and heart rate counts were subsequently discontinued.

Total lengths of hatched larvae were calculated for days 12 to 17 and are listed in Table 7 (Appendix II). There is a linear decrease in total length with increased exposure time ($Y = -0.05X + 8.36$). This is represented in Figure 10. Statistical analysis of total length data (Tables 4 and 5) showed a significant difference using an analysis of variance and Dunnett's Test between the control and 48 hr exposure groups ($P < .05$). Total lengths of these groups differed by 27%.

Another feature that appeared on the larval herring was a lateral line (Figure 11). Figures 12 and 13 show the lateral line organ at different magnifications. This feature has not been found in the literature for any life cycle stage in Pacific herring. There was no indication of adverse effects of oil on the lateral line structure.

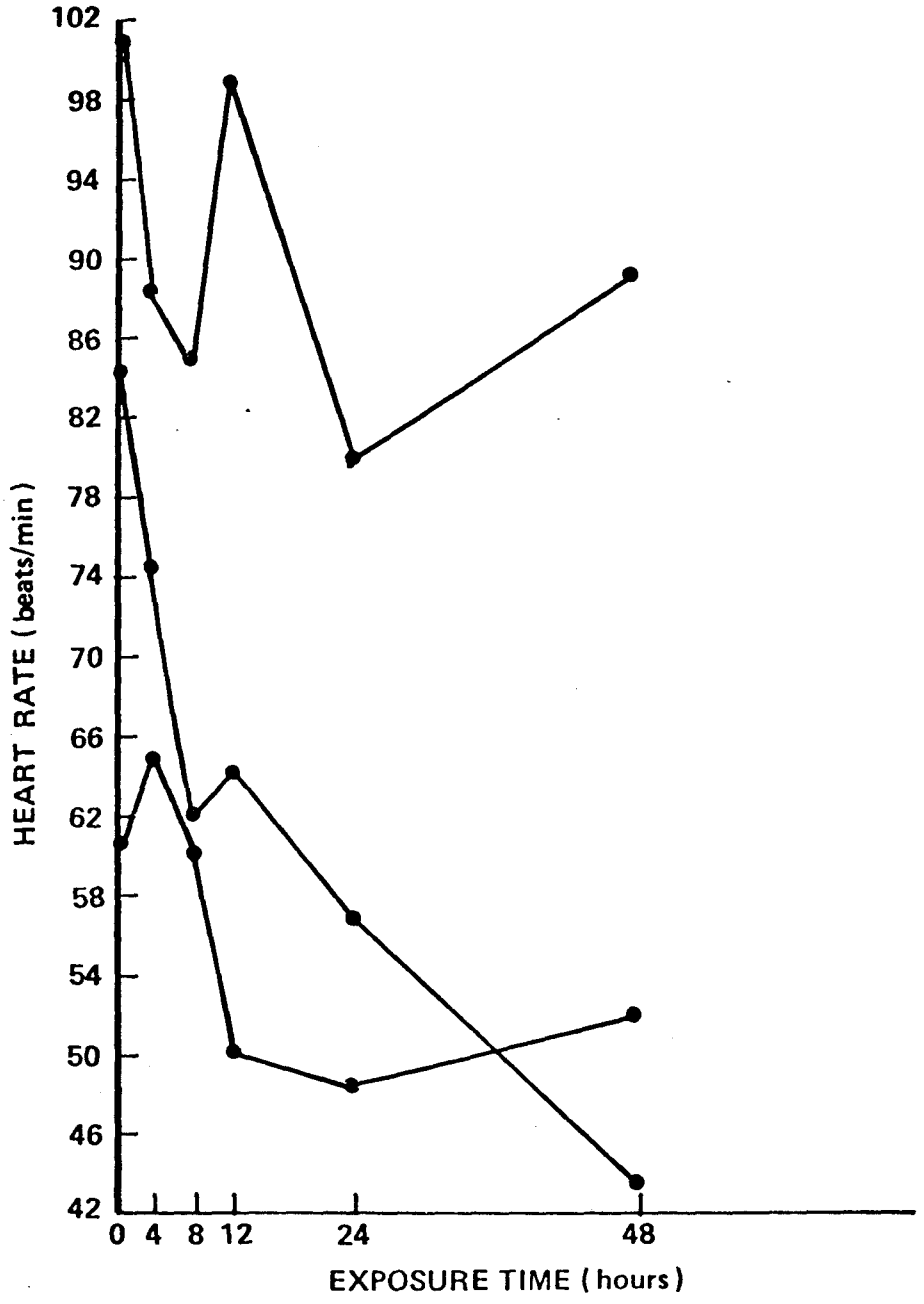


Figure 9. Embryonic heart rates vs. exposure time.

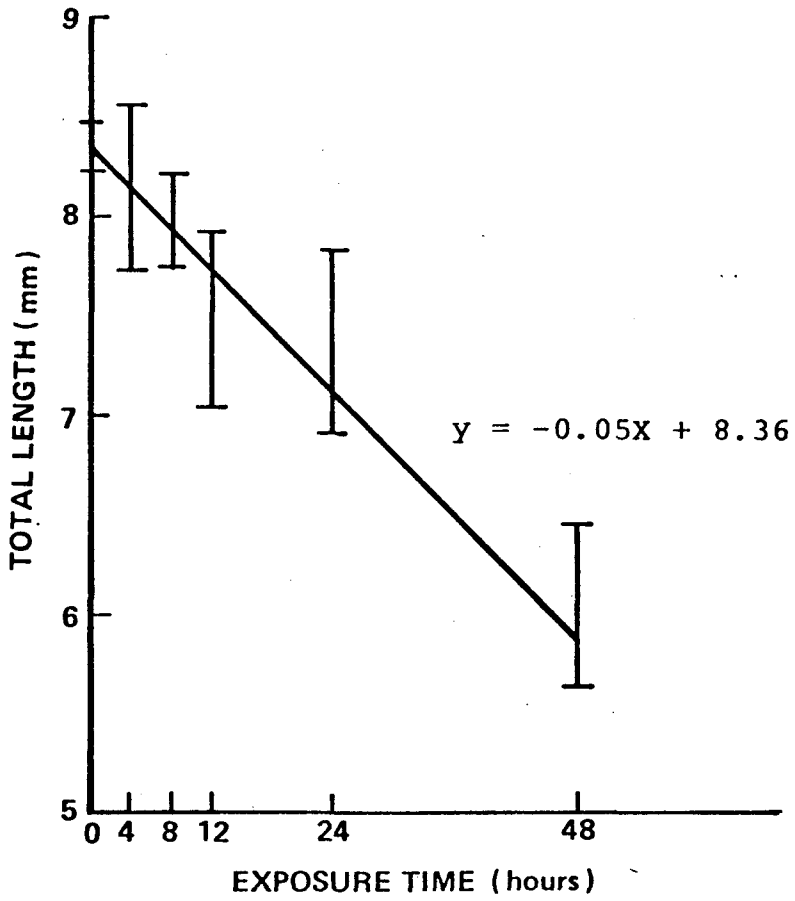


Figure 10. Larval total length vs. exposure time.

Figure 11. Scanning Electron Micrograph - lateral line system (400X).

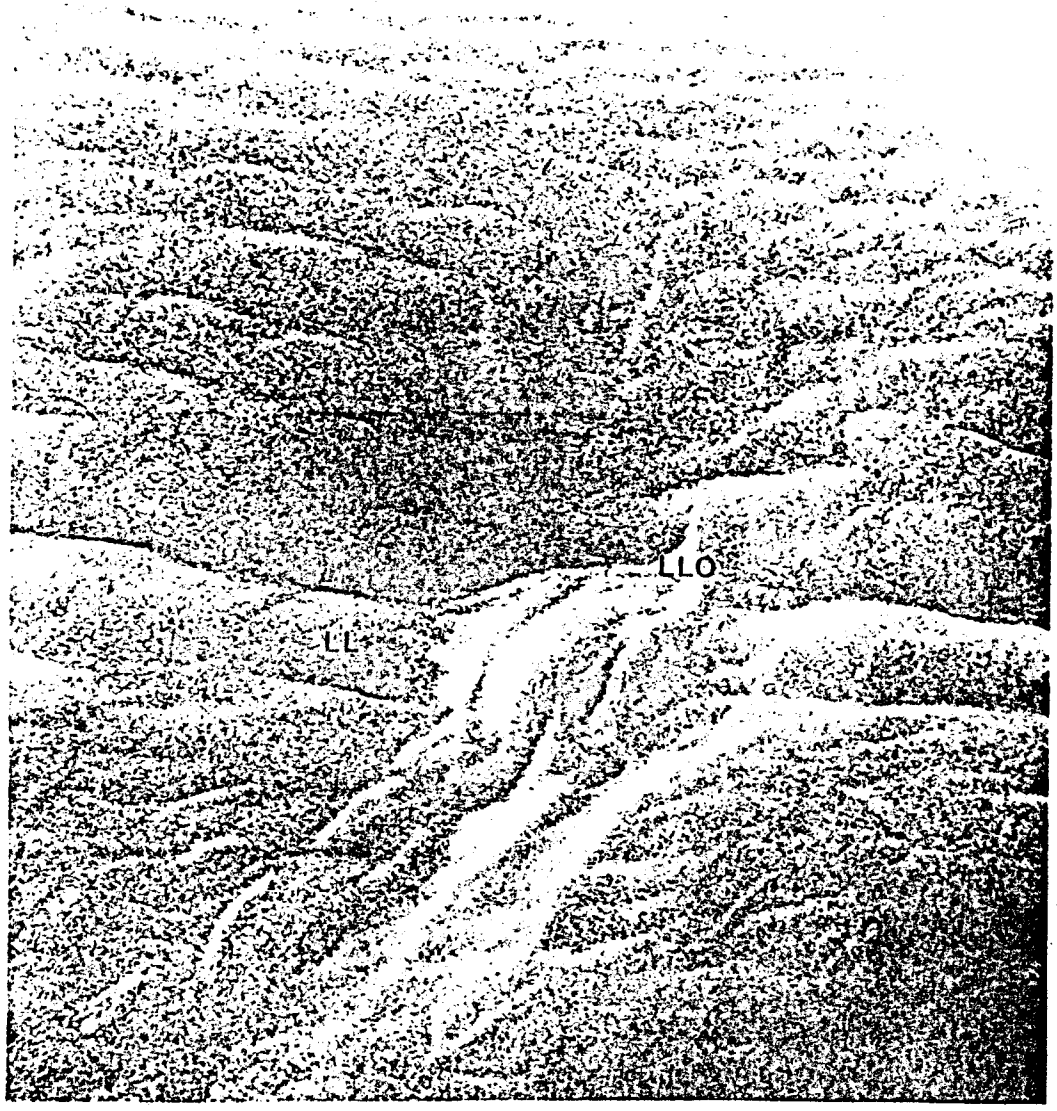


Figure 12. Scanning Electron Micrograph - lateral line organ (1,400X).

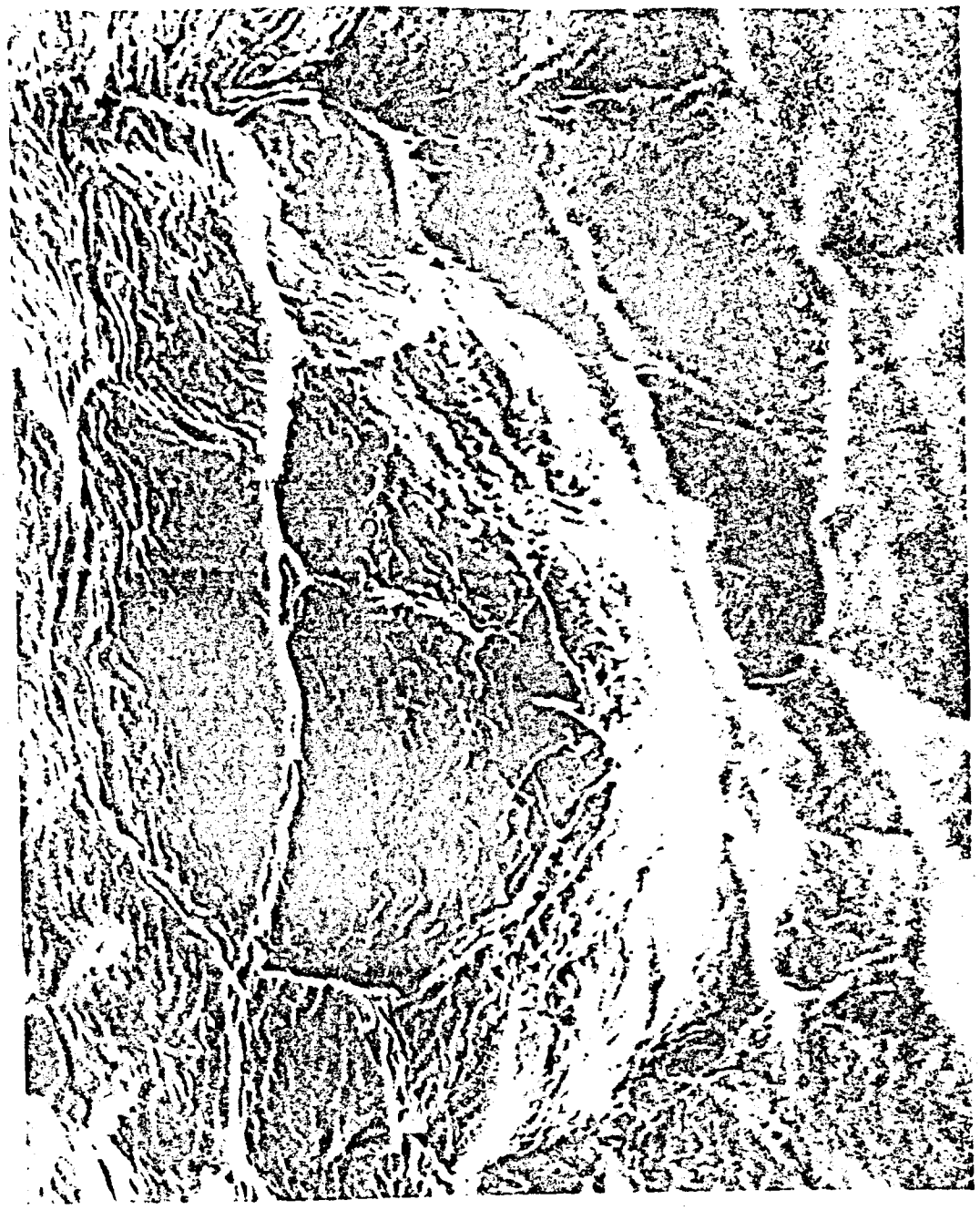


Figure 13. Scanning Electron Micrograph - lateral line organ (4,000X).

B. Transmission Electron Microscopy

The eggs from the control group and the 6 day group differed significantly. Those of the control group were fairly clean along the outer edge (Figure 14). The chorion was a lamellar structure consisting of 19 separate layers (Figure 15). The perivetilline space was quite distinct and fairly clear of debris. The boundary of the cytoplasm and the perivitelline space was somewhat irregular with small bump-like projections. The boundary between the cytoplasm and the yolk, although quite distinct, was very irregular in shape (Figure 16). There were many projections of the cytoplasm into the yolk area of varying sizes. Small globules of what would appear to be lipid material were mixed in with the cytoplasm near the boundary. These globules were possibly the means of providing nutrients to the growing organism. The yolk was granular with no distinct structural components.

The eggs from the six day exposure group appeared very different from the controls. There was 100% mortality in this exposure group and the condition of the eggs at the time of collection was one of beginning decomposition. The surface of the outer coating of the egg was covered with bacteria (Figure 17). In a few places the coating became fairly narrow and somewhat broken down. The lamellae of the chorion were still visible though they, also, were breaking

Figure 14. Transmission Electron Micrograph - control egg outer surface (31,200X).

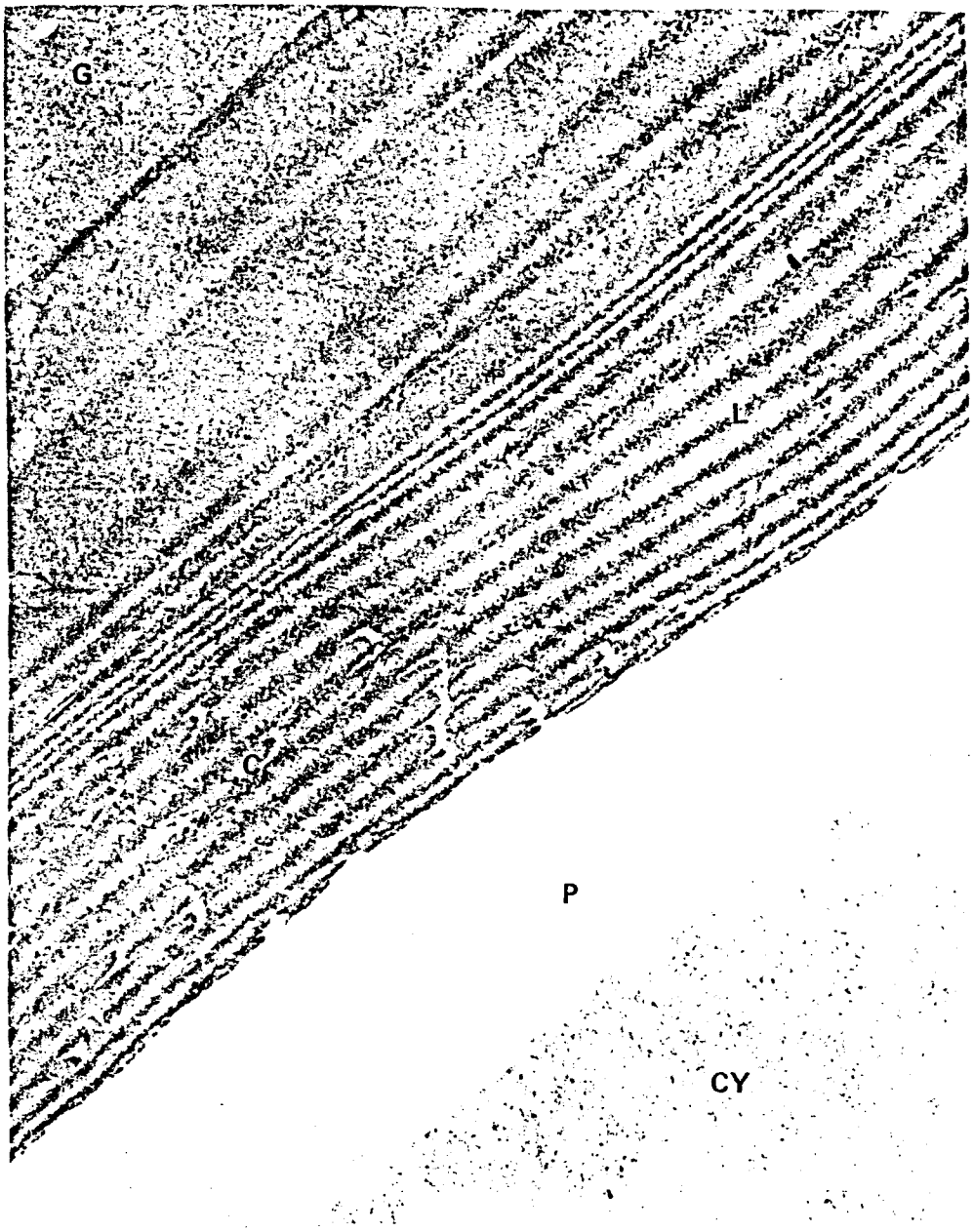


Figure 15. Transmission Electron Micrograph - control egg chorion (4,100X).

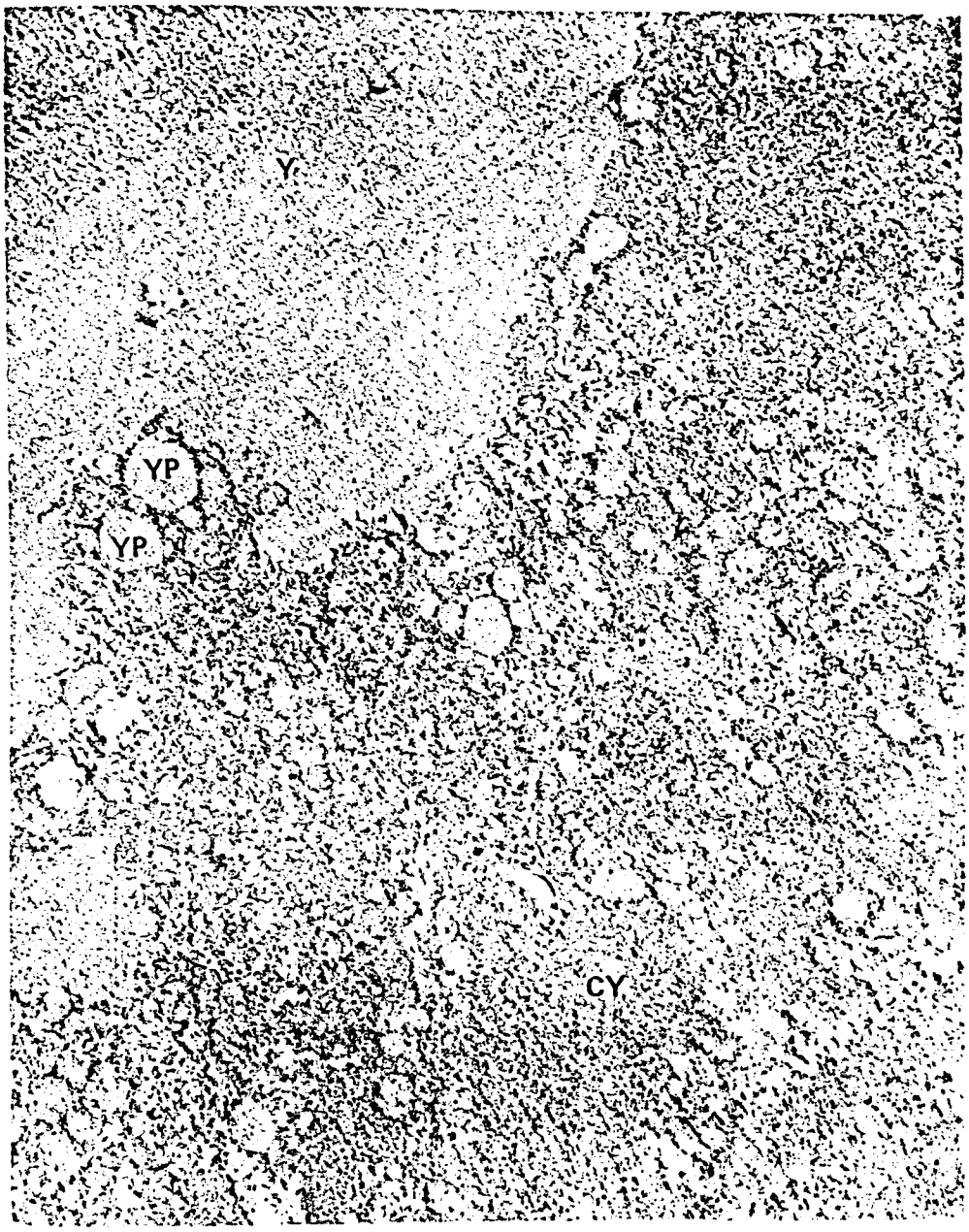


Figure 16. Transmission Electron Micrograph - control egg cytoplasm-yolk boundary (9,450X).

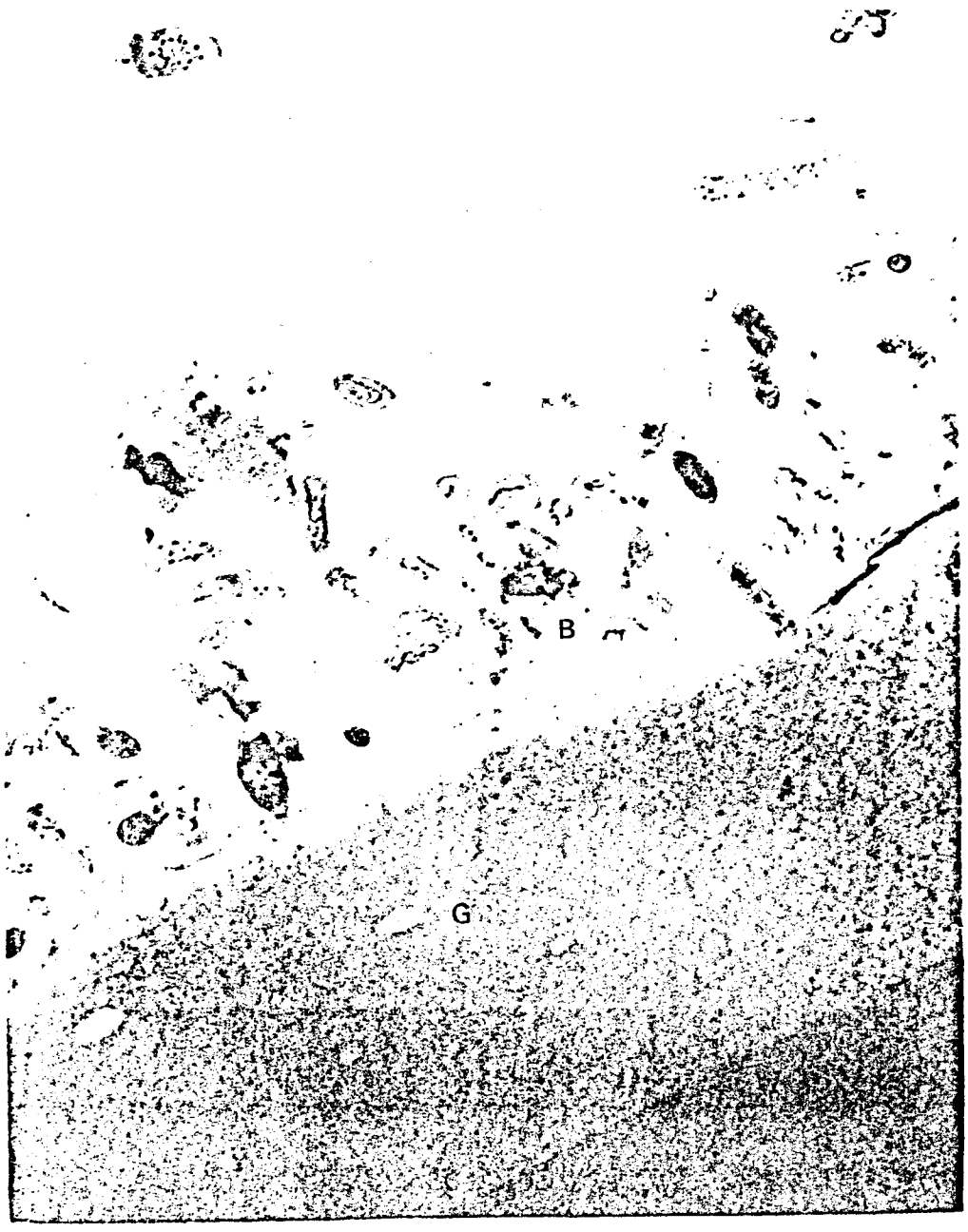


Figure 17. Transmission Electron Micrograph - exposed egg outer surface (11,100X).

down (Figure 18). It proved difficult to count the layers as they had become less distinct by this point. The yolk material was broken up into large globular fragments surrounded by cellular fragments and decaying material (Figure 19).

Viewing the surface structure of the eggs by scanning electron microscopy showed very little difference between the controls and the exposed eggs. Bacteria was found to be growing on the surface of both to approximately the same extent (Figures 20 and 21).

The larvae selected for electron microscopy showed no signs of body distortion and swimming ability was unimpaired. Sections were taken from various areas of the body. Those sections around the eye of the larvae showed no differences between the controls and the 48 hr exposure group. Size and shape of pigment granules and the cellular structure surrounding the pigment appeared similar in both groups.

Sections through the head region and those through the trunk musculature did show differences between exposure groups. Large vacuoles around nuclei and between cells appeared in the head sections from the 48 hr exposure group (Figure 22) but were not seen in the controls (Figure 23). The numbers of mitochondria associated with the trunk musculature did not vary but their appearance did. In those

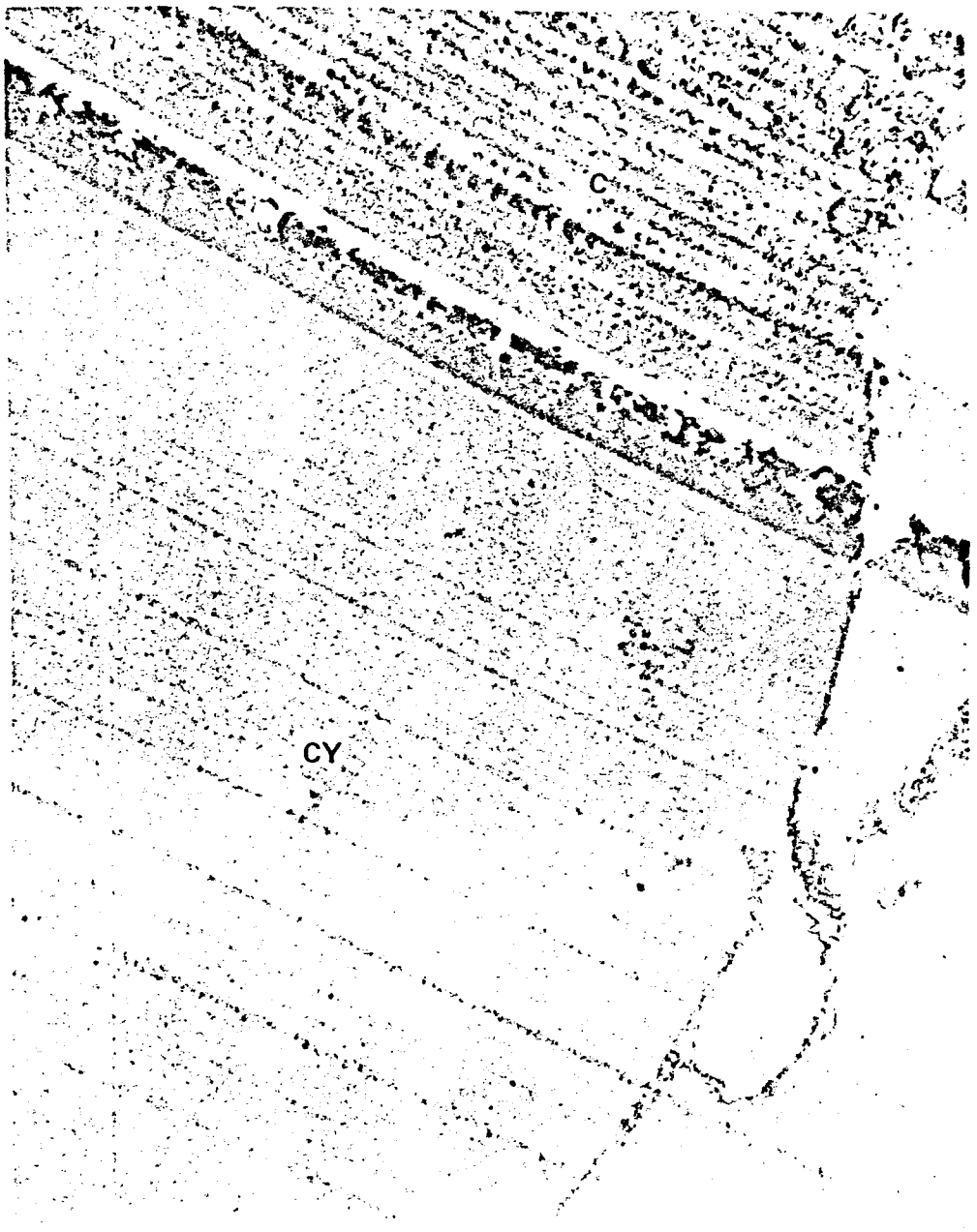


Figure 18. Transmission Electron Micrograph - exposed egg chorion (11,100X).

Figure 19. Transmission Electron Micrograph - exposed egg internal structure showing decomposition within the egg (11,550X).

Figure 20. Scanning Electron Micrograph - control egg surface (10,000X).

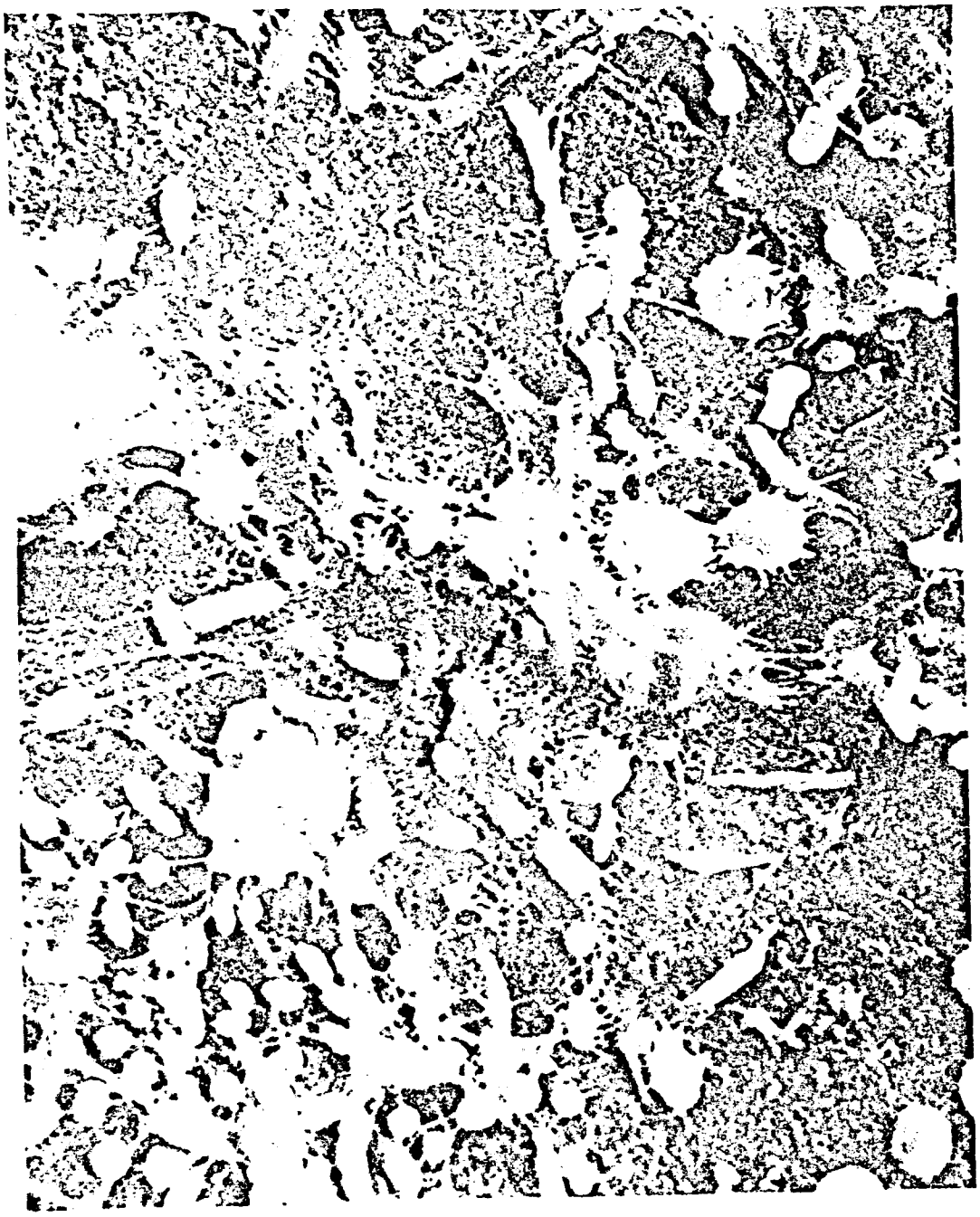


Figure 21. Scanning Electron Micrograph - exposed egg surface (10,000X).

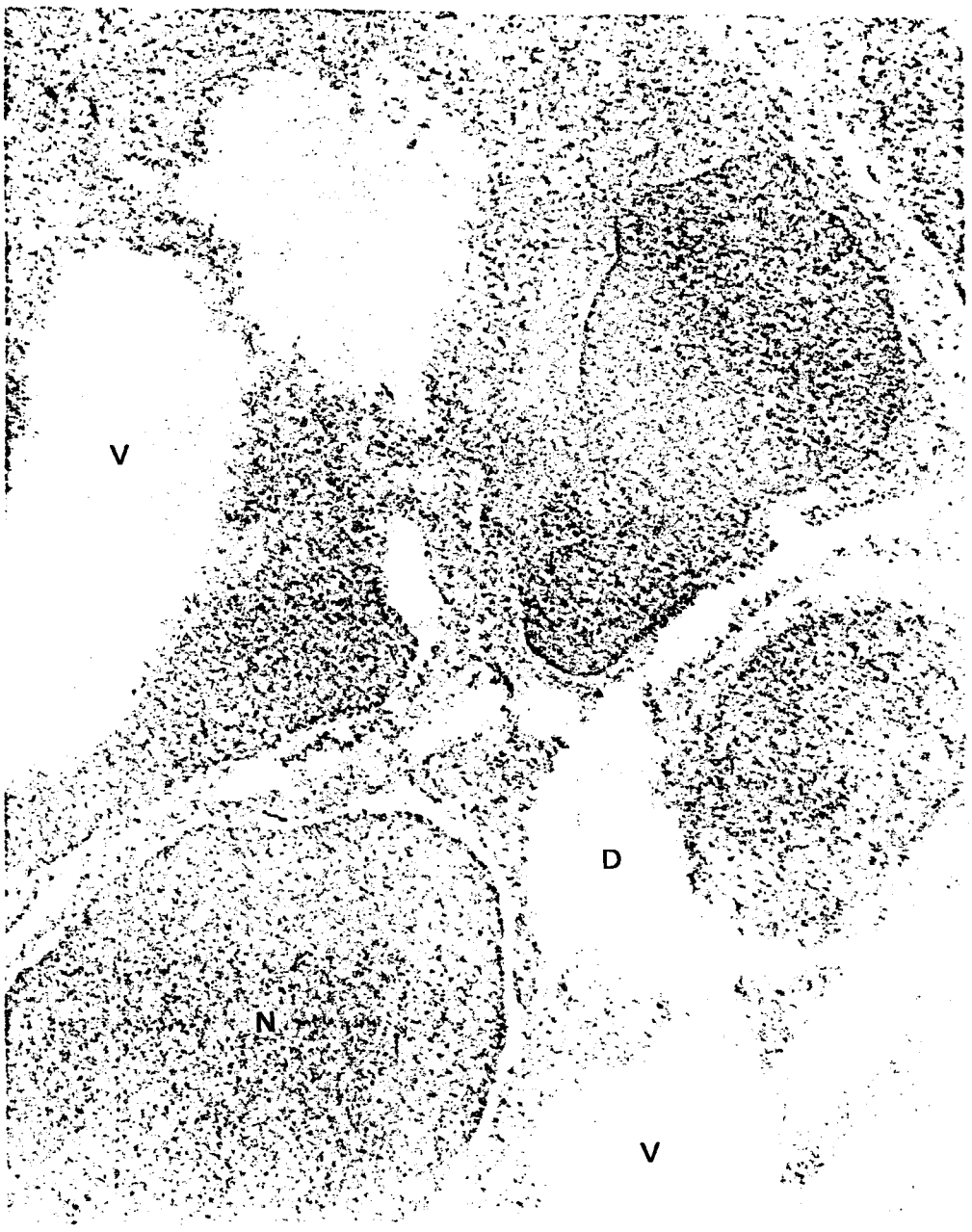


Figure 22. Transmission Electron Micrograph - 48hr head section note large vacuoles in cells (17,400X).

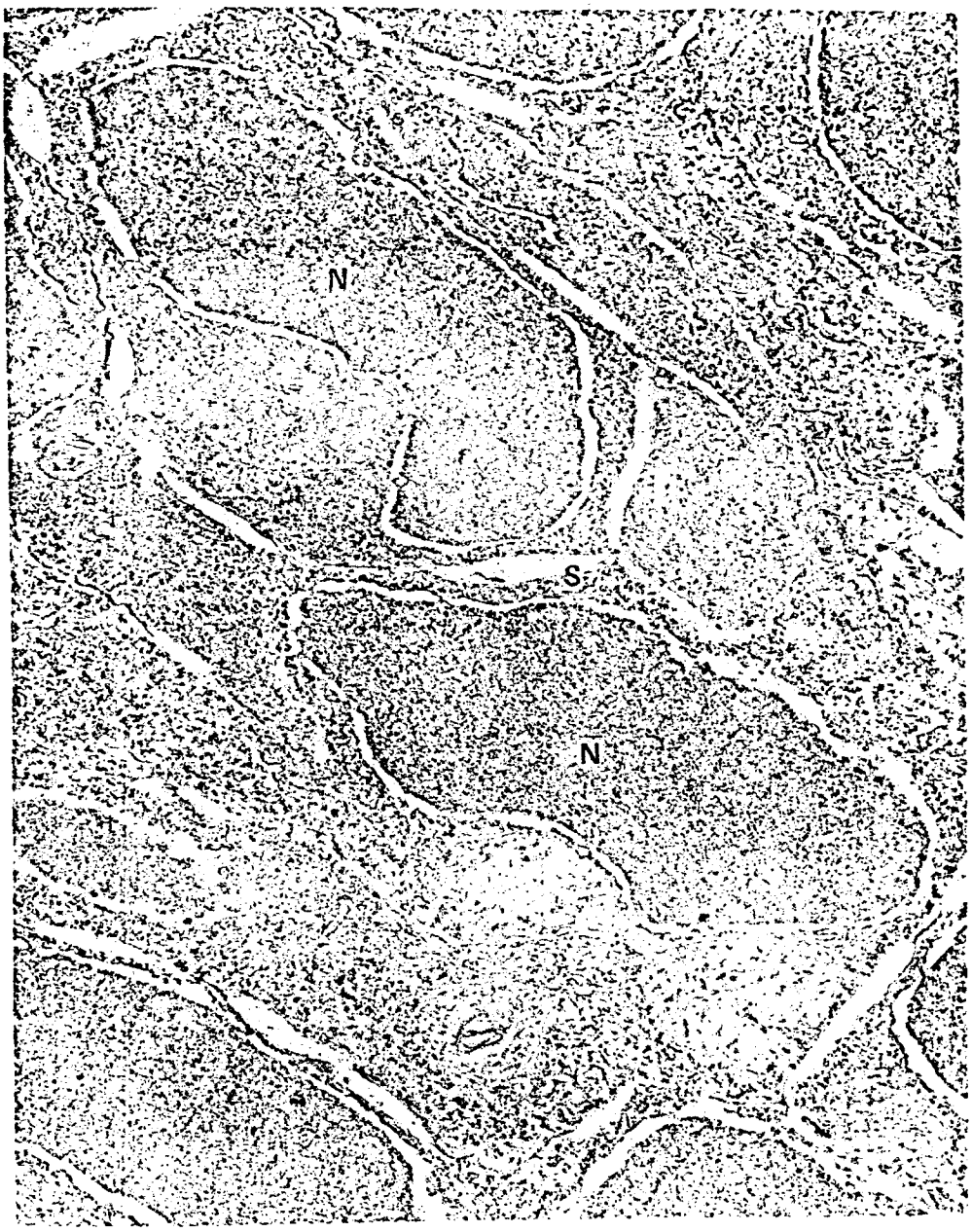


Figure 23. Transmission Electron Micrograph - control head section (15,600X).

larvae from eggs exposed for 48 hrs many of the mitochondria were partially filled with vacuoles (Figure 24). This condition is not present in the unexposed larvae (Figure 25). Vacuoles were also seen at other locations in the trunk area of the 48 hr exposure group.

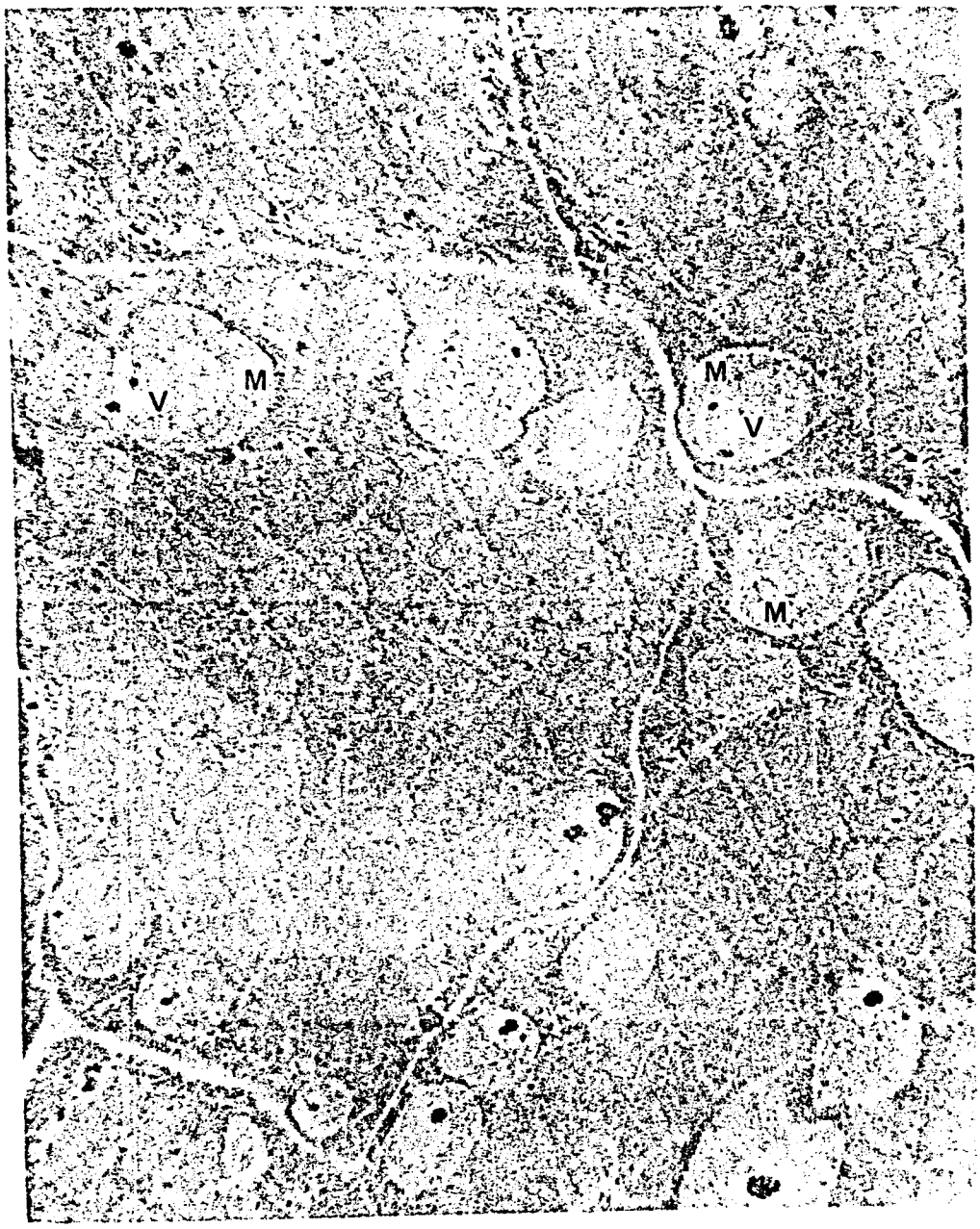


Figure 24. Transmission Electron Micrograph - 48hr trunk musculature showing vacuoles in mitochondria (15,600X).

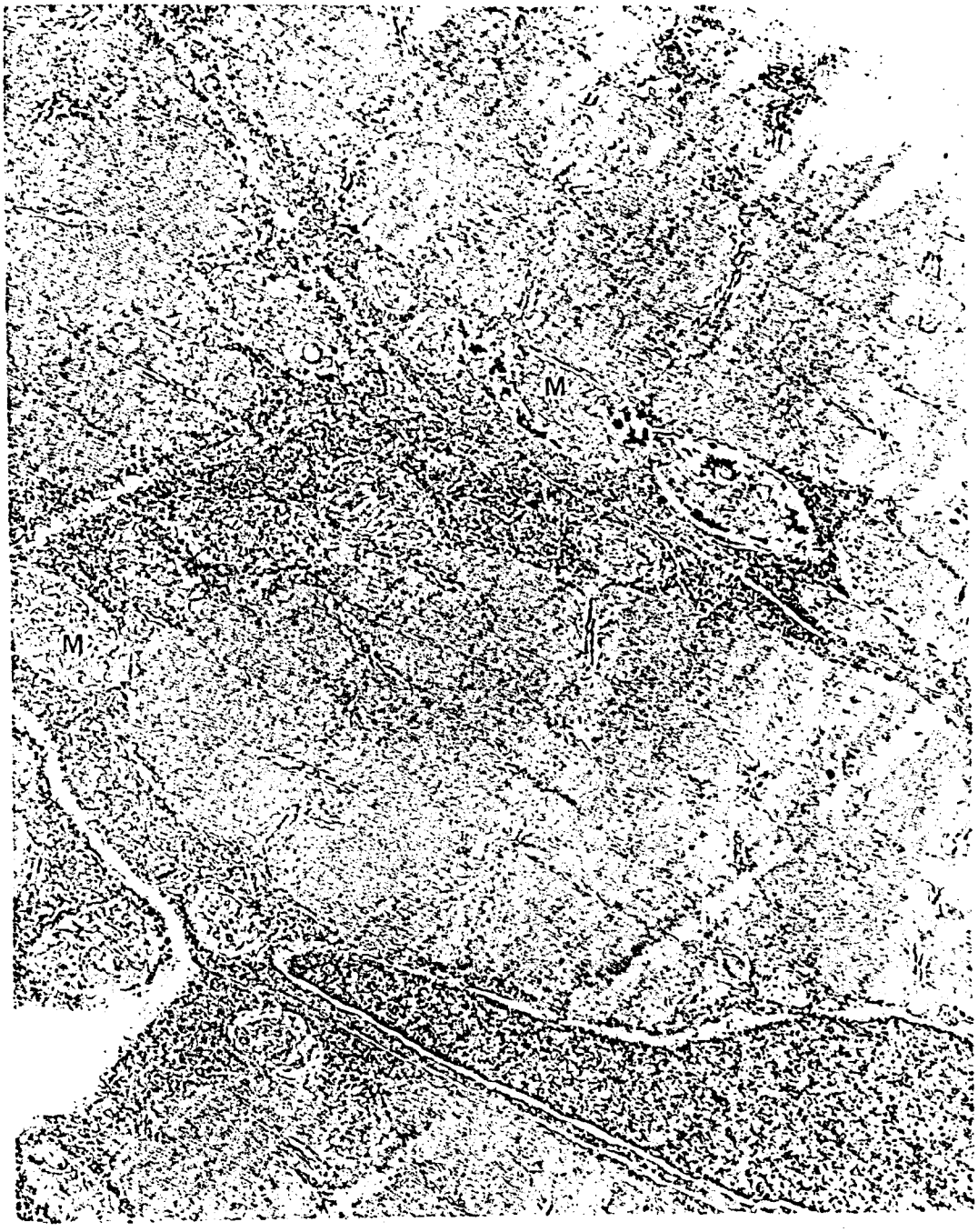


Figure 25. Transmission Electron Micrograph - control trunk musculature (15,300X).

DISCUSSION

A. Seward Study

It must be remembered that this experiment was done in a laboratory and cannot, therefore, be applied directly to an oil spill in the marine environment, just as the results from one oil spill cannot be applied directly to any other oil spill. The results, however, do give a basic understanding on which to build. They show the effects of the water soluble fraction of Prudhoe Bay crude oil on C. h. pallasi embryos at the specific concentration used. These types of effects will appear even in the natural environment, but to a different extent, either greater or lesser, depending on the amount of oil in the water, the environmental conditions, and the age of the eggs being thus affected. These results are specific for Prudhoe Bay crude oil and should not be applied to other oils, either refined or crude, as each oil will affect organisms in a different way. This is due to the varying degrees of toxicity of different oils (Anderson, et al., 1974a, 1974b; Kuhnhold, 1969, 1972).

In analyzing the effect of any oil on marine organisms it is necessary to consider both lethal and sublethal effects. Lethal effects of oil are obvious--the organism dies and the strength of the year class is thus affected. Sublethal effects are not always as obvious but can be just as

devastating to the population. These effects interfere with cellular and physiological processes. Death is not immediate but can follow due to abnormal behavior or other causes. Some examples of sublethal effects of oil include: the clogging of chemoreceptors, thus preventing the organism from avoiding contamination; structural abnormalities in developing organisms, thus causing difficulties in swimming and feeding; and hatching irregularities with hatching extending over longer periods. Any of these effects could lead to the eventual death of the organism.

This present study has shown the major sublethal effects on the developing eggs to be those causing structural abnormalities in the larvae. The most notable of these abnormalities was a flexure of the spinal column. These abnormalities in the shape of the spine varied in severity and included: arched positions, both convex and concave positions were noted; one bend usually posterior to the yolk sac; two or three bends at various positions along the spine; S shaped spines; and a corkscrew configuration. The majority of these larvae had difficulty swimming and many died before being collected.

Abnormalities of this nature could prove disastrous to a population of fish. Although very few larvae ever become adults under optimum conditions, these types of abnormalities further decrease larval recruitment. With the ability

to swim being impaired by structural abnormalities, the chances for survival decrease. The larvae are no longer able to swim for protection or to gather food. Both of these hindrances could cause the eventual death of the larvae.

Partially formed lower jaws were another common form of abnormality. This deformity varied from larvae that had no lower jaw to those where the lower jaw was present but did not close properly. Figures 5 and 6 show normal and abnormal jaws respectively. Feeding ability would be disrupted under these conditions with the larvae eventually dying.

Another type of abnormality appeared only in the longer exposure times of 24 hrs and 48 hrs. This abnormality consisted of an enlarged yolk sac extending anteriorly to the ventral head area. The extension appeared to be part of the body that had not been totally incorporated into the larval form before budding off from the yolk during development (Figure 4). Figure 4 also shows that neither the pectoral fins nor the gill apparatus formed in this larva. As the total time to hatching was similar between groups this would indicate that one effect of Prudhoe Bay crude oil was to delay larval development, thus producing premature larvae that were unable to survive.

A less frequently noted abnormality was seen in the

fraying of the pectoral and caudal fins. In several of the larvae the edges of these fins appeared fairly ragged (Figure 8), while the controls appeared smoother (Figure 7). This finding has also been noted by Linden (1975) in studies of larval Baltic spring-spawning herring (C. h. membras, L.) exposed to Venezuelan crude oil. Oil, then, has the capacity to destroy especially delicate tissue.

Another feature found on the herring larvae was a lateral line (Figure 11). As a lateral line is not present in the adults it seems likely that these larvae lose theirs when they gain their deciduous scales. Atlantic herring do have a lateral line that becomes fully developed at 50 mm (Bamford, 1941, In: Blaxter and Holliday, 1963). Sensory papillae of the lateral line have been found in the head region of C. h. harengus (de Koch, unpub., In: Blaxter and Holliday, 1963), other members of the Clupeidae. As other members of this family have lateral lines it seems reasonable that C. h. pallasi have them as larvae. The lateral line organs on Pacific herring larvae are very similar to those shown for Atlantic herring adults by de Koch (unpub., In: Blaxter and Holliday, 1963). Pictures of the external structure can be seen in Figures 12 and 13. There was no indication of adverse effects of oil on the lateral line.

The analysis of the percent abnormal figures showed that 48 hours of exposure to the water soluble fraction of

Prudhoe Bay crude oil would deform a significant percent of the larvae above the percent found for the control group. Most of these abnormalities would lead to the eventual death of the larvae through inabilities to feed and swim. Analysis of the hatching success, or percent hatched, showed a statistically significant difference only between the control and the 6 day exposure groups ($P < .05$). This information indicates that at some time between 2 and 6 days Prudhoe Bay crude oil causes detectable mortality among developing C. h. pallasi embryos. As noted earlier, Blumer, et al., (1973) found that the lower boiling point aromatic hydrocarbons remain in the area of an oil spill for a few months before finally being removed. This would indicate that an exposure time of 6 days would be highly probable and significant mortality and abnormalities would likely occur.

Total length was found to be another characteristic that differed between exposure groups. Shorter lengths were associated with increased exposure time as shown in Figure 10. The difference in length could be a result of the oil slowing development so that the larvae did not have time to reach the longer length of normal larvae. A reduced larval length could also hamper survival in the natural environment. A shorter larva has the possibility of being a prey item for more kinds of organisms, can be preyed upon longer, and is generally less resistant to environmental stress.

Many of these larvae were also deformed, making their chance of survival even smaller.

Referring to Figure 9, it can be seen that heart rates showed an inverse relationship to exposure time on days 6 and 8. By day 11, one day prior to the first hatchings, this relationship was less apparent. This less intense decline with exposure as age increased occurred after all exposure groups had been replaced into uncontaminated sea water. It, therefore, could indicate a favorable response to the removal of stress from the oil contaminant. As development progressed heart rate increased within all exposure groups, except for the 48 hr group which decreased slightly on day 8 and increased again on day 11. Increased heart beat would be expected as the embryos develop and the circulatory system takes on greater importance. Embryos in the highest exposure groups, principally those of 24 hrs and 48 hrs, were less well developed than those in the control group. Thus, it seems reasonable that their heart rates were slower.

Struhsaker, et al., (1974) reported a heart beat range of 70-90 beats per minute for untreated C. h. pallasi embryos. This compares to a range of 60-106 beats per minute in the present study. Heart rates for herring embryos exposed to benzene in the Struhsaker study generally increased, ranging up to 110 beats per minute with irregularities in

the consistency of beating. Prudhoe Bay crude oil exposed embryos, however, had slower heart rates. This indicates that more components than benzene are affecting these embryos.

Evidence for delayed development due to exposure of Pacific herring larvae to benzene has been noted by Struhsaker, et al., (1974). Their study showed reduced larval lengths, reduced feeding, and delayed development; results similar to this study. Other preliminary results from their study indicate an increase in metabolic rate with a resultant energy cost to alleviate the stress of exposure to low (5 ppm) concentrations. A delayed metabolic rate probably due to narcotization of the larvae when exposed to high (45 ppm) concentrations of benzene was also noted. Anderson, et al., (1974a), have found increased respiration in Cyprinodon variagatus due to contamination by oils containing napthalenes and Warner (In: Anderson, et al., 1974a) found a depression of oxygen consumption in fish contaminated by bunker c oil containing napthalene and alkylphenols. As the Prudhoe Bay crude oil used in this study contained approximately 34% napthalenes, respiration could be greatly affected.

B. Transmission Electron Microscopy

Results from transmission electron microscopy are very preliminary. Most observations for this work were made on

two or three organisms per exposure group and, therefore, cannot be considered as conclusive or exhaustive. Much more work is needed in this area before any definite conclusions can be drawn. The following results are only a beginning and represent initial observations.

There appeared to be a great difference in the ultrastructure of the eggs from the two exposure groups. The general appearance of the control egg suggested a healthy organism. The cellular material was intact with no large vacuoles or any decaying material. The coating surrounding the egg appeared to be a gelatinous substance and was, presumably, the sticky material surrounding the egg that enables it to remain attached to adjoining eggs and the algae after spawning. The coating, which contained a few small vacuoles spaced at irregular intervals, showed relatively little internal structure. This coating had an irregularly shaped outer edge that was relatively clean, with little growth of any kind on it. Scanning electron microscopy showed the eggs of both exposure groups to be covered by bacteria to the same extent (Figures 20 and 21). This discrepancy is probably due to only a few eggs being examined. If more eggs had been studied a greater amount of bacteria would probably have been noted on control egg sections for transmission electron microscopy.

The chorion was a lamellar structure consisting of 19

distinct concentric layers (Figure 15). This feature has been found in other teleostean eggs (Hagström and Lönning, 1968). Their study showed that each species have a specific number of lamellae that is characteristic of the species. Yamamoto (1955, In: Blaxter and Holliday, 1963) has reported only 3 layers for Pacific herring. A 3 layered chorion has also been reported for Atlantic herring by Bower and Holliday (1961, In: Blaxter and Holliday, 1963). The original references were not available at the time of writing. Therefore, a comparison of microscopic technique and types of microscopy used is not possible to understand the discrepancy between these studies and the present one.

The differences between the control and 6 day groups in the present study were due to the death and decay of the eggs from the 6 day exposure group. A significant percent of this mortality, above that experienced by the control group, is presumably due to oil contamination.

Major cellular differences were found in the larval ultrastructure. Vacuoles around nuclei and between cells were found in sections through the head of larvae from exposed eggs. Vacuoles were also present in the mitochondria of the trunk musculature on larvae from the 48 hr exposure group. No vacuoles were found in the control larvae.

Many of these vacuoles were debris filled which indicates some cellular breakdown. This would probably deter

from the normal operation of the cell. Cellular respiration is accomplished by mitochondria. As was noted earlier, respiration of organisms is affected by oil contaminants. These results indicate that some of this effect may be caused by the destruction of mitochondria in the cell. Vacuoles within and between cells would possibly cause chronic problems within the fish later in development.

Similar results have been noted by M. A. Smith (pers. comm.) on the ultrastructure of king crab (Paralithodes camtschatica) gills. She has found large vacuoles within the epithelial cells of the gill filaments in those crab exposed to the water soluble fractions of Cook Inlet crude oil. No vacuoles appeared in the controls. These vacuoles would probably affect respiration, osmoregulation, and the ability of these animals to survive.

SUMMARY AND CONCLUSIONS

The eggs of C. h. pallasi are adversely affected by the application of 0.67 ppm of the water soluble components of Prudhoe Bay crude oil. Effects on hatching success and abnormalities, both external and cellular, have been observed.

Significant mortality differences were found between the control and 6 day exposure groups ($P < .05$). This corresponds to 53.8% hatching for the controls and 0% hatching for the 6 day group. Significant differences were also found in the numbers of abnormal larvae that hatched in the various exposure groups. These differences became significant between the control and 48 hr exposure group ($P < .05$).

The abnormalities were observed by light and scanning electron microscopy. The majority of the abnormalities observed were associated with body distortions. The distortions were generally forms of spinal flexure and included arched positions, S and L shaped spines, two or more sharp bends along the length of the larvae, circular, and corkscrew configurations. Other abnormalities were associated with partially formed lower jaws and frayed pectoral and caudal fins. These abnormalities would cause an inability to swim and feed. Thus, many of the larvae that hatch would not have lived for very long afterwards.

Transmission electron microscopy indicated that there

may be cellular forms of abnormalities caused by the oil as well. The one abnormality that was apparent in otherwise normal appearing larvae was found in cross sections of the head and trunk musculature. Larvae from the 48 hr exposure group had cells in these regions with large vacuoles around nuclei, between cells, and in mitochondria. Some of these vacuoles were debris filled signifying cellular decomposition. These vacuoles were not present in the controls and would seem to indicate an unnatural physiological state in herring larvae. These vacuoles in the mitochondria probably affect respiration. Alterations in respiration have been shown to be an effect of oil contamination. The results from the transmission electron microscope are neither conclusive nor exhaustive due to only two or three animals per exposure group being observed. These results should be considered as preliminary. They do, however, indicate that there may be some effect of oil at the cellular level which might not be observed immediately, yet might affect the herring later in development.

In conclusion, the effects of Prudhoe Bay crude oil on Pacific herring development include:

1) a significant increase in mortality after exposure for 6 days;

2) a significant increase in numbers of gross abnormalities after exposure for 48 hours;

3) the increased presence of structural abnormalities including: larvae with one or more bends along the body; arched, circular and corkscrew configurations; malformed jaw structure; frayed pectoral and caudal fins; and enlarged yolk sac areas;

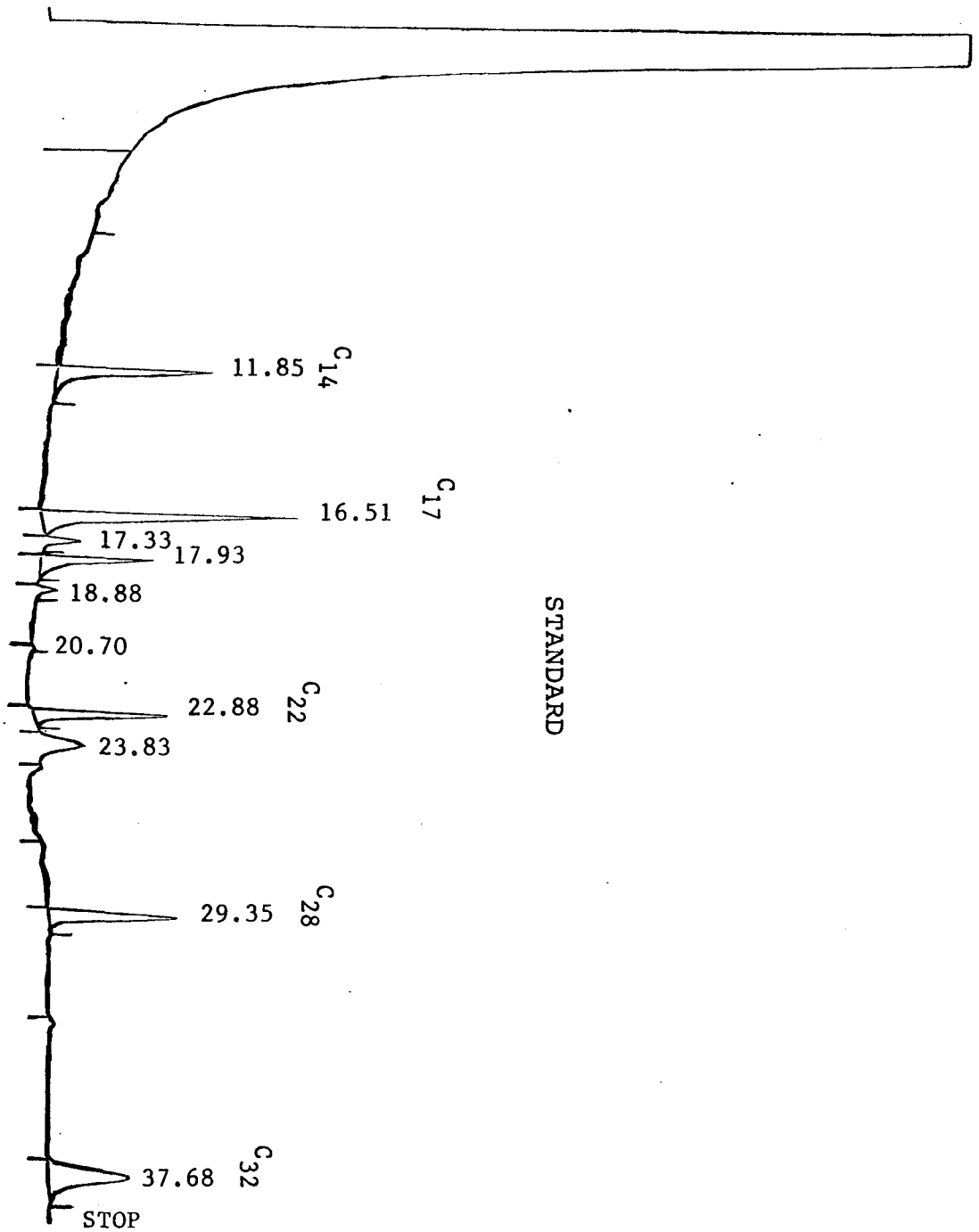
4) a significant difference in larval total length after exposure for 48 hours;

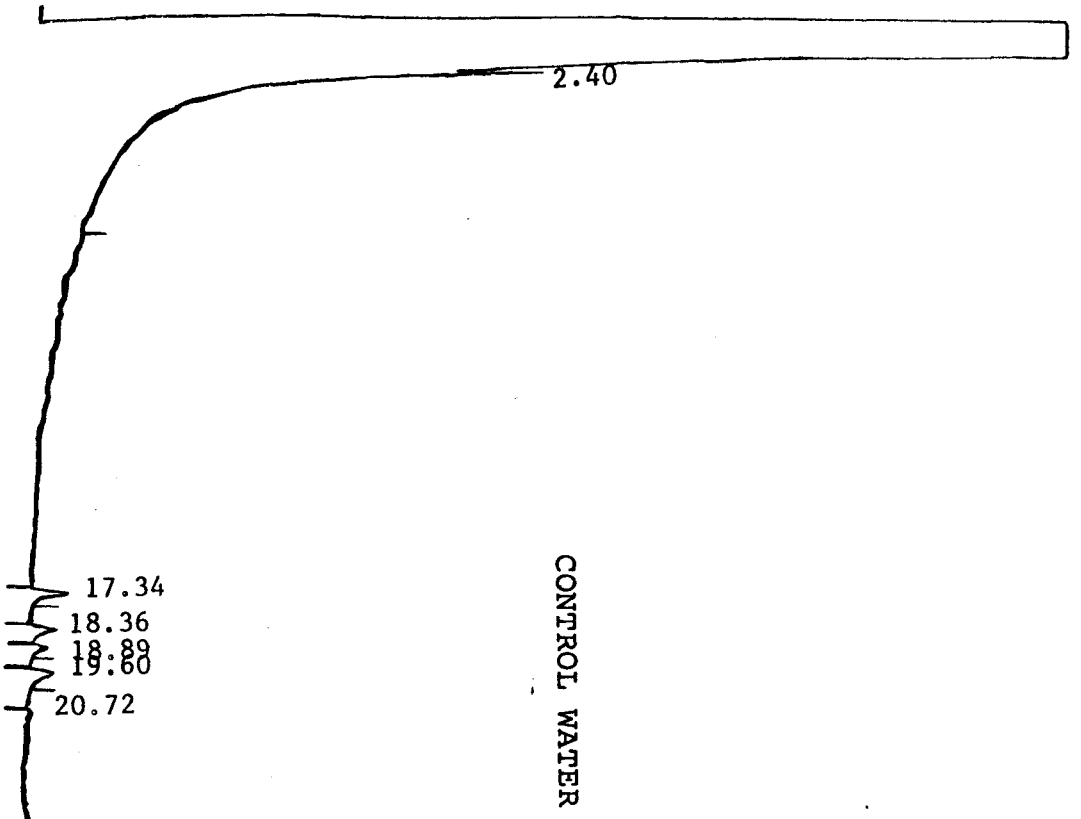
5) possible decreased survival due to inabilities to feed and swim;

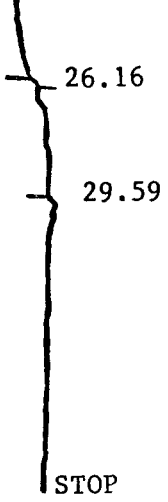
6) vacuole formation within and between cells in otherwise normal appearing larvae which will possibly affect later life stages.

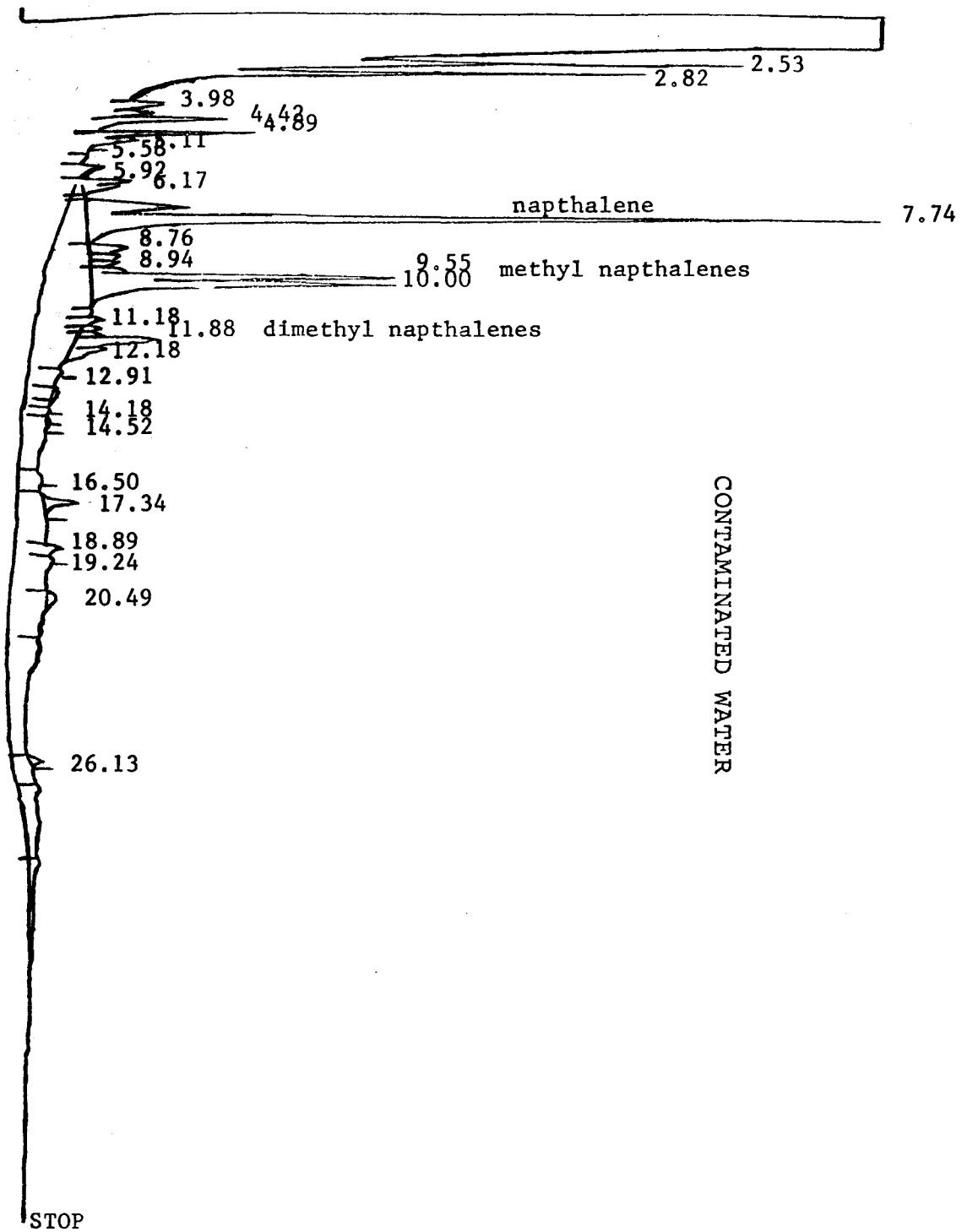
APPENDIX I

GAS CHROMATOGRAPHS


11.85
C_{14}
16.51
C_{17}
17.33
17.93
18.88
20.70
22.88
C_{22}
23.83
29.35
C_{28}
37.68
C_{32}
STOP
STANDARD


2.40
17.34
18.36
18.89
19.60
20.72
CONTROL WATER


26.16
29.59
STOP


2.53
2.82
3.98
4.43
4.89
5.58
5.92
6.11
6.17
napthalene
7.74
8.76
8.94
9.55
10.00
methyl napthalenes
11.18
11.88
dimethyl napthalenes
12.18
12.91
14.18
14.52
16.50
17.34
18.89
19.24
20.49
26.13
STOP
CONTAMINATED WATER

APPENDIX II

TABLES

TABLE 1

Number and Percent Hatched and Abnormal

Container		Initial number	Hatched No.	Hatched %	Avg. %	Abnormal No.	Abnormal %	Avg. %
Control	1	210	81	38.6		8	9.9	
	2	211	82	38.9	53.4	12	14.6	10.7
	3	191	158	82.7		12	7.6	
4 hr	1	201	109	54.2		6	5.5	
	2	209	115	55.0	52.8	18	15.7	13.9
	3	197	97	49.2		20	20.6	
8 hr	1	214	95	44.4		15	15.8	
	2	212	109	51.4	43.7	19	17.4	19.6
	3	199	70	35.2		18	25.7	
12 hr	1	217	65	30.0		17	26.2	
	2	209	122	58.4	53.9	20	16.4	18.7
	3	202	148	73.3		20	13.5	
24 hr	1	199	83	41.7		18	21.7	
	2	198	39	19.7	32.1	9	23.1	20.6
	3	218	76	34.9		13	17.1	
48 hr	1	207	28	13.5		12	42.9	
	2	200	55	27.5	27.6	23	41.8	51.5
	3	199	83	41.7		58	69.9	
6 day	1	217	0	0		-	-	
	2	201	0	0		-	-	
	3	199	0	0		-	-	

Table 2 - Number and Percent Hatching per Day

Container		12		13		14		15		16		17		18		19		20		21		22		23		24	
		#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Control	1	2	2.5	1	1.2	34	42.0	33	40.7	5	6.2	4	4.9	2	2.5	0	0	0	0	0	0	End					
	2					9	11.0	3	3.7	18	22.0	17	20.7	21	25.6	8	9.8	5	6.1	1	1.2	End					
	3					28	17.7	2	1.3	7	4.4	37	23.4	45	28.5	8	5.1	7	4.4	8	5.1	6	3.8	2	1.3	8	5.1
4 hr	1					19	17.4	1	0.9	8	7.3	43	39.4	22	20.2	11	10.1	2	1.8	2	1.8	1	0.9	End			
	2					32	27.8	3	2.6	24	20.9	30	26.1	17	14.8	5	4.3	3	2.6	1	0.9	0	0	End			
	3					21	21.6	2	2.1	3	3.1	26	26.8	37	38.1	5	5.2	3	3.1	0	0	0	0	End			
8 hr	1			2	2.1	17	17.9	4	4.2	10	10.5	21	22.1	34	35.8	3	3.2	4	4.2	0	0	End					
	2					8	7.3	6	5.5	3	2.8	26	23.9	21	19.3	18	16.5	6	5.5	8	7.3	7	6.4	3	2.8	3	2.8
	3			2	2.9	14	20.0	5	7.1	3	4.3	15	21.4	18	25.7	1	1.4	1	1.4	4	5.7	1	1.4	2	2.9	4	5.7
12 hr	1			1	1.5	19	29.2	4	6.2	5	7.7	19	29.2	8	12.3	9	13.8	0	0	0	0	End					
	2	2	1.6	0	0	8	6.6	3	2.5	5	4.1	40	32.8	39	32.0	15	12.3	7	5.7	3	2.5	0	0	End			
	3			2	1.4	18	12.2	5	3.4	8	5.4	39	26.4	51	34.5	19	12.8	4	2.7	0	0	0	0	1	0.7	1	0.7
24 hr	1					20	24.1	15	18.1	9	10.8	15	18.1	14	16.9	8	9.6	2	2.4	0	0	End					
	2	1	2.6	0	0	9	23.1	13	33.3	2	5.1	8	20.5	6	15.4	0	0	0	0	0	0	End					
	3					9	11.8	2	2.6	2	2.6	25	32.9	17	22.4	12	15.8	9	11.8	0	0	End					
48 hr	1					11	39.3	3	10.7	8	28.6	3	10.7	3	10.7	0	0	0	0	0	0	End					
	2			1	1.8	12	21.8	14	25.5	4	7.3	17	30.9	4	7.3	3	5.5	0	0	0	0	End					
	3	1	1.2	0	0	38	45.8	4	4.8	3	3.6	23	27.7	5	6.0	9	10.8	0	0	0	0	End					
6 day	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	End							
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	C	0	End							
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	End							

Table 3 - Number and Percent Abnormal per Day

Container		12		13		14		15		16		17		18		19		20		21		22		23		24	
		#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Control	1	0	0	1	12.5	2	25.0	3	37.5	1	12.5	0	0	1	12.5	-	-	-	-	-	-	End					
	2					0	0	1	8.3	0	0	1	8.3	4	33.3	4	33.3	1	8.3	1	8.3	End					
	3					0	0	0	0	0	0	2	16.7	1	8.3	2	16.7	0	0	3	25.0	2	16.7	1	8.3	1	8.3
4 hr	1					0	0	0	0	0	0	2	33.3	1	16.7	1	16.7	1	16.7	1	16.7	0	0	End			
	2					1	5.6	0	0	2	11.1	11	61.1	2	11.1	1	5.6	1	5.6	0	0	-	-	End			
	3					1	5.0	0	0	0	0	10	50.0	7	35.0	1	5.0	1	5.0	0	0	-	-	End			
8 hr	1			0	0	0	0	1	6.7	0	0	6	40.0	5	33.3	0	0	3	20.0	0	0	End					
	2					0	0	1	5.3	0	0	3	15.8	1	5.3	0	0	2	10.5	4	21.1	5	26.3	3	15.8	0	0
	3			0	0	0	0	1	5.6	1	5.6	4	22.2	5	27.8	0	0	1	5.6	3	16.7	1	5.6	2	11.1	0	0
12 hr	1			0	0	0	0	0	0	2	11.8	1	5.9	5	29.4	9	52.9	-	-	-	-	End					
	2	0	0	-	-	0	0	1	5.0	0	0	9	45.0	3	15.0	4	20.0	2	10.0	1	5.0	-	-	End			
	3			0	0	1	5.0	0	0	0	0	8	40.0	1	5.0	6	30.0	3	15.0	0	0	0	0	1	5.0	0	0
24 hr	1					2	11.1	1	5.6	2	11.1	1	5.6	3	16.7	8	44.4	1	5.6	-	-	End					
	2	0	0	-	-	2	22.2	2	22.2	1	11.1	0	0	4	44.4	-	-	-	-	-	-	End					
	3					3	23.1	0	0	0	0	6	46.2	1	7.7	1	7.7	2	15.4	-	-	End					
48 hr	1					8	66.7	0	0	3	25.0	0	0	1	8.3	-	-	-	-	-	-	End					
	2			1	4.3	1	4.3	11	47.8	0	0	5	21.7	3	13.0	2	8.7	-	-	-	-	End					
	3	0	0	-	-	32	55.2	3	5.2	3	5.2	14	24.1	0	0	6	10.3	-	-	-	-	End					

TABLE 6

Embryonic Heart Rates (beats per minute)

Container		Day 6	Day 8	Avg. Day 8	Day 11	Avg. Day 11
Control	1		78.5		105.8	
	2		94.2	84.3	103.4	101.0
	3	60.7	80.3		93.8	
4 hr	1		64.0		83.6	
	2		83.2	74.6	100.0	88.2
	3	65.8	76.5		81.1	
8 hr	1		63.0		82.7	
	2		59.8	62.2	97.2	85.1
	3	60.2	63.8		75.5	
12 hr	1		62.4		109.1	
	2		65.6	64.2	97.5	99.2
	3	50.0	64.6		91.0	
24 hr	1		55.7		78.7	
	2		56.4	56.2	92.8	80.9
	3	48.7	56.4		71.2	
48 hr	1		*34.3		103.6	
	2		53.7	43.8	84.1	89.5
	3	52.0	43.3		80.9	
6 day	1		0		0	
	2		0	0	* 43.5	43.5
	3	0	0		0	

* - indicates less than 5 embryos sampled.

TABLE 7

Larval Total Length

Container		Average length (mm)	S	Number sampled	Avg. per exposure group
Control	1	8.21	1.33	50	
	2	8.47	0.90	47	8.34
	3	8.34	0.66	50	
4 hr	1	8.59	0.52	50	
	2	8.44	0.55	50	8.26
	3	7.74	1.26	50	
8 hr	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	

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