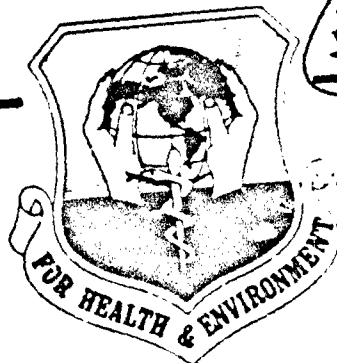


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STS-5 FISH KILL  
KENNEDY SPACE CENTER, FLORIDA  
JANUARY 1983

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*William E. Mabson*  
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Other toxic substances. Water temperature and dissolved oxygen concentrations were found to be normal both pre- and post-launch. Water and sediment heavy metal analyses both pre- and post-launch were unremarkable. A moderate parasite load was found in the fish population by histopathology, but not believed to be a contributing cause of death. The only other pathologic changes were limited to the gills. These changes, including swollen erythrocytes, epithelial and endothelial dilatation and goblet cell prominence, were consistent with edematous change. In addition, post-launch water pH was significantly lower than pre-launch. These impressions were confirmed by laboratory bioassay procedures. The conclusion was that the fish died from ionic imbalances and fatal anoxia resulting from severe gill damage caused by a rapid decrease in the water pH. The change in water pH was a transitory phenomenon and not perceived to be a long-term environmental threat.

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## I. INTRODUCTION

Since fish kills were observed following previous Space Transport System (STS) launches, the USAF Occupational and Environmental Health Laboratory (~~USAF OTHL~~) was requested to conduct an on-site investigation of any possible fish kill associated with STS-5 on 11 November 1982 at Kennedy Space Center (KSC). Due to the acuteness of the fish kills and close association with time of launch, STS launch exhaust products, such as HCl and/or aluminum oxide, were suspected as the cause. Other man-made and natural causes, however, could not be ruled out.

This report presents the possible causes of fish kills, the investigative procedures employed and tests conducted before and after the launch of STS-5. It also presents the results obtained, observations made, a diagnosis of the problem and recommendations concerning immediate and long-term environmental impact.

## II. BACKGROUND

### A. Causes of Fish Kills

Fish are easily stressed by environmental factors, either man-made or natural. These stresses, singly or in synergism, can be a direct or contributing cause of death. The various causes of fish kills include infectious diseases, parasites, mechanical interference with respiration, insufficient oxygen, trauma, exposure to toxic substances, and changes in water temperature and pH.

### B. Diseases and Parasites

Most fish, whether in the wild or reared in captivity, harbor parasites. Likewise, all fish are subject to naturally occurring diseases. Death from diseases and parasites in fish occurs continually, but generally goes unnoticed because only a few individuals die in a given area at one particular time. Occasionally, however, epizootic conditions occur in large concentrated populations and result in many deaths in a short period of time. Man can sometimes trigger an epizootic kill by accidental introduction of pathogens into bodies of water where they are not normally found, or by imposing stress factors that lessen or eliminate natural tolerance, or immunity to existing pathogens.

### C. Mechanical Interference With Respiration

Materials such as silt, petroleum compounds or even depositions of insoluble metal compounds, such as aluminum oxides, can interfere with normal respiratory functions if introduced in sufficient quantity to coat gill surfaces and, therefore, prevent oxygen uptake.

#### D. Insufficient Oxygen

At least four to six parts per million (ppm) of dissolved oxygen (DO) in water is necessary to support a fish population. Increased biochemical oxygen demand resulting from decomposing organic materials will decrease DO. Likewise, increased chemical oxygen demand resulting from spontaneous, oxygen-requiring, chemical reactions will lower DO. If the DO level falls below that required to support fish life, a fish kill occurs.

#### E. Trauma

Traumatic causes of fish kills are usually catastrophic events such as hurricanes, tornadoes and earthquakes. However, in small bodies of water, physical injury and subsequent death of fish may result from such things as dumping of solid wastes, construction, explosions and missile blasts.

#### F. Exposure to Toxic Substances

Direct introduction of toxic substances, in sufficient concentration, will cause an immediate or acute kill. However, death can also occur as a result of chronic exposure to lower concentrations of the given toxin over a period of days, weeks, or even months. This dilemma of acute versus chronic toxicity can be further complicated by seasonal mixing and periods of water turnover. In this case, sublethal amounts of a toxin may enter the water and settle out in the bottom sediment, only to be freed at a later time in lethal amounts during mixing periods.

#### G. Temperature and pH Changes

Some fish species can tolerate wide ranges of water temperature and pH. Other species can survive only within very narrow ranges of these environmental factors. Regardless of tolerance, however, most species are adversely affected by sudden, drastic changes in water temperature or pH.

#### H. Fish Kills Associated With the STS

Fish kills associated with the STS launches have been acute kills observed shortly after the launch. These kills, according to the definition in "Standard Methods" (9), can be characterized as moderate kills (100 to 1000 dead or dying fish of various species within 1 to 2 km of stream or equivalent area of a lake or estuary). These kills at KSC have been limited to small species (<5 cm length) in a brackish lagoon immediately north of Launch Pad 39A in an area impacted by the Solid Rocket Booster (SRB) exhaust plume (Fig 1). The objectives of the fish kill investigation before and after the launch of STS-5 were to rule out natural environmental stresses as the cause, identify a specific man-made STS-related cause and assess the impact of that event on the environment.

### III. MATERIALS AND METHODS

#### A. Water and Sediment Sampling

One-liter water samples were collected, both pre- and post-launch, at three sites in the lagoon (Fig 2, Sites A, B, and C) in accordance with the USAF OEHL Water Sampling Guide (6). Temperature, pH and DO were measured on-site using a YSI 51-B DO Meter (Yellow Springs Instrument Co., Yellow Springs OH) and a Fisher Accumet pH Mini-Meter (Fisher Scientific, Pittsburgh PA). Culturette swab subsamples were collected from each sample using a Culture Collection and Transport System (Precision Dynamics Corp., Burbank CA) and submitted to the Epidemiology Division, USAF School of Aerospace Medicine (USAFSAM) for bacterial culture and identification. The one-liter water samples were then preserved with nitric acid and submitted to the Analytical Services Division, USAF OEHL, for heavy metal analysis. Lagoon bottom sediment samples were also collected, both pre- and post-launch, at the same sites and submitted for heavy metal analysis. An additional water sample was collected post-launch at the site of eventual fish kill (Fig 2, Site D) and tested as described above. Temperature, pH and DO were also determined for the covered and uncovered bucket water (as described in Section III.B below).

#### B. Specimen Collection

Representative live specimens of native fish species were collected from the lagoon (Fig 2, Site C) on the day prior to launch using a seine net. These specimens were identified as Cyprinodon variegatus (sheepshead minnow), Gambusia affinis (mosquitofish), Peocilia latipinna (sailfin molly) and Lucania parva. Five specimens of each species (ethanized with halothane in water) were necropsied as controls, and gross appearance observed and recorded on 35 mm color slides. In addition, two blood smears were made from each specimen and observed for dyscrasias. The blood smears were fixed with methanol. The fish were preserved with formalin and submitted to the Veterinary Sciences Division, USAFSAM, for histopathologic examination. Five live specimens of each of the three predominant species (minnow, mosquitofish, molly) were placed in an uncovered 20-liter bucket of brackish water adjacent to the lagoon (Fig 2, Site E) on the day prior to launch. Five of each species were also placed in a covered bucket at the same site. At approximately one and one-half hours post-launch, a fish kill was observed in the lagoon (Fig 2, Site D). Two mosquitofish, one minnow, one Anchova mitchilli (bay anchovy) and one Microgobius gulosus were collected at the site. These fish kill specimens, as well as the fish from the buckets, were necropsied post-launch and subjected to the same examinations as described above. Another 100+ dead fish were discovered the following day at Site F (Fig 2), in shallow water, but were not examined due to postmortem decomposition.

#### C. Bioassay

In an attempt to confirm, in the laboratory, the suspected cause of the fish kill in the lagoon, a bioassay was conducted with Pimephales promelas (fathead minnow). Five fish were placed in a 500 mL beaker of deionized distilled water adjusted to pH 2.4 with HCl. Another five fish were sacrificed as controls with halothane in water. All fish, following death, were necropsied, preserved in formalin and submitted for histopathology.

#### D. Histopathology

All fish specimens were preserved in 10% buffered formalin. The lagoon fish killed by the STS-5 launch and the bucket fish were not preserved in formalin until L+3:45-5:15 hours. The control fish and the fish used in the bioassay were immediately placed in formalin after their deaths. Whole body decalcification and tissue processing were done using standard techniques. The paraffin blocks were cut at 4  $\mu$ m and stained with hematoxylin and eosin. Special stains and electron microscopy were also done using standard techniques.

### IV. RESULTS AND DISCUSSION

#### A. Water Samples

Pre-launch water samples were collected on 10 Nov 82, at 1010 hours, under clear skies, at 26°C ambient temperature, and NE winds at 15-20 MPH. Post-launch samples were collected on 11 Nov 82, at 0853 hours (L+1:34), under clear skies, at 21°C ambient temperature, and ENE winds at 6-10 MPH. Water depths at the sampling sites were: Site A, 45 cm; Site B, 45-50 cm; Site C, 30 cm; Site D, 7-10 cm.

##### 1. Temperature, pH, DO

The results of the on-site measurements of water temperature, pH and DO are presented in Table 1.

TABLE 1. ON-SITE MEASUREMENTS OF WATER TEMPERATURE, pH AND DO

Location Time <sup>a</sup>	SITE A		SITE B		SITE C		SITE D	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Parameters								
pH	8.0	8.0	8.0	8.5	8.1	8.3	—	6.2
DO, ppm	7.5	7.2	7.4	6.6	7.4	6.9	—	7.4
Temperature, °C	20.5	21.0	21.0	20.0	21.0	21.0	—	21.0

<sup>a</sup>Pre = pre-launch; Post = post-launch

a. There were no significant temperature differences in the various samples and those temperatures were compatible with fish survival. These data disprove the theory that the fish kills could be due to excessive temperatures generated during the launch.

b. There were also no significant DO differences in the various samples and those DO concentrations (6.5-7.5 ppm) were above the minimum (4.0-6.0 ppm) necessary to support fish life.

c. There were no significant pre- and post-launch pH differences at the three primary lagoon sampling sites (Fig 2, Sites A, B, and C). At the

fish kill area along the lagoon edge, water pH was notably lower (pH 6.2) than the normal range (pH 8.0-8.5) measured in this lagoon. In addition, the water pH in the uncovered bucket (pH 2.4) was greatly reduced from that in the covered bucket (pH 7.2). These data indicate that a significant acid deposition is resulting from the SRB exhaust. The deep water areas are probably resistant to this acid influence due to the inherent dilution effect of a large water volume combined with natural wave action mixing. The shallow water of the grass-protected lagoon edge, however, probably experiences a sudden and drastic reduction in pH, similar to that measured in the uncovered bucket. This sudden and drastic pH change would be sufficient in itself to cause an acute fish kill.

## 2. Heavy Metals

The lagoon water samples were analyzed for Al, Fe, Cd, Pb and Mn. The concentrations of these metals reported to be toxic to fish (2) are as follows:

Al	5000 µg/L (dissolved Al in acidic water)
Fe	900 µg/L at pH 6.5-7.5; >1900 µg/L at <pH 6.5
Cd	4000-20,000 µg/L (dissolved Cd)
Pb	300 µg/L (dissolved Pb in acidic water)
Mn	0.1-1.5 g/L

The results of the water analysis for heavy metals in the pre- and post-launch lagoon samples from Sites A, B and C (Fig 2) are presented in Table 2. The two predominant metals present, Al and Fe, range from 160-830 µg/L and 100-360 µg/L, respectively. Statistical analysis of that data is presented in Table 2. There was no significant difference between pre- and post-launch means. All of the metals analyzed were present in concentrations far below those reported to be toxic in fish.

TABLE 2. HEAVY METAL CONCENTRATIONS IN LAGOON WATER SAMPLES

Metal (µg/L)	Al		Fe		Cd		Pb		Mn	
	Pre <sup>a</sup>	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Location										
Site A	830	830	202	100	<10	58	<50	<50	<50	<50
Site B	160	760	200	116	10	<10	<50	<50	<50	<50
Site C	180	320	223	360	<10	<10	<50	<50	<50	<50
Mean <sup>b</sup>	390	637	208	192						
(± S.E.) <sup>c</sup>	(269)	(195)	( 9)	(103)						

<sup>a</sup>Pre = pre-launch; Post = post-launch

<sup>b</sup>No significance

<sup>c</sup>Standard Error of the Mean

The Al and Fe concentrations in the water sample collected from the fish kill site (Site D) were 1980 µg/L and 803 µg/L, respectively. Although these levels are also below toxicity levels, they are much higher than deepwater site concentrations. Statistical validity, of course, cannot be determined on a sample size of one. However, in view of expected mixing effects between launch and collection, it would not be unreasonable to infer that this higher trend indicates a near-toxic or toxic concentration of Al and/or Fe may have existed in the grassy-area shallow water immediately post-launch. On the other hand, it should be noted that reported toxic concentrations of metals usually refer to concentrations of soluble metals. The analysis performed on this sample measured insoluble as well as soluble Al and Fe. The Al increase, for example, is at least partially attributable to the expected deposition of insoluble Al oxides present in the SRB exhaust plume. Furthermore, an independent analysis of water in the closed and open buckets, post-launch, reveal <200 and 1300 µg/L Al and 200 and 1600 µg/L Fe, respectively. These "worst case" data support the latter theory that soluble heavy metal concentrations do not reach high enough levels in the lagoon, post-launch, to cause acute death in fish.

### 3. Bacterial Cultures

Results of the lagoon water bacterial cultures are shown in Table 3. All of the organisms cultured are known to occur widely in water. None of these organisms are reported pathogens in fish (3). Therefore, water culture results do not support the presence of an infectious disease as the cause of death.

TABLE 3. LAGOON WATER BACTERIAL CULTURE IDENTIFICATION

Time	Bacteria Present	
	Pre-launch	Post-launch
Location		
Site A	No growth	<u>Aeromonas (Vibrio) proteolytica</u> <u>Acinetobacter calcoaceticus</u> <u>var. Lwoffii</u> <u>Pseudomonas sutida</u>
Site B	<u>Acinetobacter calcoaceticus</u> <u>var. Lwoffii</u> <u>Aeromonas Hydrophilia</u>	No growth
Site C	<u>Pseudomonas maltophilia</u> <u>Pseudomonas sp.</u>	<u>Bacillus subtilis</u> <u>Pseudomonas sp.</u> <u>Staphylococcus epidermidis</u>

## B. Sediment Samples

The results of the lagoon bottom sediment sample analyses for heavy metals are reported in Table 4. The results of these analyses are unremarkable and do not support the theory of heavy metal intoxication as the cause of death.

TABLE 4. HEAVY METAL CONCENTRATIONS IN LAGOON SEDIMENT SAMPLES

Location	Site A		Site B		Site C	
	Pre	Post	Pre	Post	Pre	Post
Metal ( $\mu\text{g/L}$ )						
Al	242.5	80.1	85.6	59.1	115.7	57.3
Fe	322.8	96.8	86.0	75.3	130.2	59.2
Cd	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Pb	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mn	1.9	1.1	0.8	0.8	1.0	0.7
Ni	1.5	0.2	0.2	<0.1	0.2	<0.1
Si	25.0	32.5	60.0	37.5	42.5	35.0

<sup>a</sup>Pre = pre-launch; Post = post-launch

## C. Fish Pathology

### 1. Gross Pathology

The external pathology in the fish collected at the launch site was difficult to evaluate with assurance due to the postmortem decomposition. The impression was that there was less redness in the gills of the control fish examined prior to the launch than in the killed lagoon fish examined after launch. The external body pigmentation and internal organs of the killed fish were perceived to be lighter in color than those of the controls. The fish in the bioassay had marked differences in the gill color between the test and controls. The gills of the test fish were light red with occasional foci of dark redness, and the fine gill structure was discernible due to loss of the protective mucous layer (Fig 3). The epidermal pigmentation and internal organ colors were not remarkably different in the test or control bioassay fish.

### 2. Microscopic Pathology

The morphologic differences between controls and fish killed by the SFS-5 launch were difficult to describe with assurance due to postmortem decomposition. The morphologic impression of the gills was that there was marked tissue separation by clear spaces consistent with edematous change. Also, that

gill epithelium or endothelium were markedly dilated, and that the goblet cells were prominent. The goblet cells in the pharyngeal epithelium were also considered to be enlarged. The erythrocytes in the lamellar capillaries were considered to be markedly swollen. Moderate swelling of erythrocytes were recognized in capillaries at the base of lamellae and deep within the gill filaments. These changes collectively increased the width of lamellae approximately five to ten times. The morphologic examination of tissues from test and control fish used in the bioassay confirmed these impressions with marked differences between test and control fish (Fig 4-7). These changes were further documented with scanning and transmission electron microscopy.

The scanning electron microscopic examination of control gills showed smooth exterior mucous surfaces which covered and concealed the fine clumps of the mucous coating, and the gill structure was easily visualized (Figs 8 and 9). The test gill lamellae, unlike the control gill lamellae had marked swelling of cells which extended above the lamellar surface (Figs 10 and 11).

The transmission electron photographs of the control gill lamellae showed essentially normal cellular architecture (Figs 12 and 13). The photograph of the test gills illustrated the marked morphologic alteration in the cells. The major change was the marked distention of cell cytoplasm, organelles, nuclei, erythrocytes, and endothelial and epithelial cells (Figs 14 and 15). Edematous change was also recognized.

Fish examined from the launch site and bioassay had moderate trematode and protozoal infestations (Figs 16 and 17). Parasites occurred in almost all fish populations examined. The parasitic lesions recognized in both the lagoon and laboratory bioassay fish were an incidental and expected finding. They did not have any significant effect on the pathogenesis of the fish kill.

No other remarkable histopathology was recognized in the fish.

## V. CONCLUSIONS

### A. Heavy Metal Toxicosis

Analysis of lagoon water and sludge samples, both pre- and post-launch were unremarkable. There was no histologic evidence of heavy metal toxicosis.

### B. Heat or Trauma

Lagoon and bucket water temperatures were identical pre- and post-launch. The expected sign of inflated swim bladders in traumatic causes of death was not observed. All dead fish sank to the bottom. There was no histologic evidence of death due to heat or trauma.

### C. Infectious Diseases

There were no gross lesions found upon necropsy, other than color changes. Lagoon water cultures were unremarkable. There was no histologic evidence of infectious diseases.

### D. Parasites

Protozoal and trematode parasites were present in many specimens, but this was an incidental and expected histologic finding. The parasite load was not heavy and did not contribute to acute death.

### E. Dissolved Oxygen

Lagoon DO levels pre- and post-launch were not significantly different and were well above minimum levels required to support fish life.

### F. Acidity

The acute deaths of the fish, in our opinion, is related directly to the low pH of the environmental water.

There has been essentially no documentation on the morphologic tissue changes in fish exposed to an acid water environment (4). However, there is considerable documentation that acid water does adversely affect fish causing population declines due to death, decreased growth and reproduction (5, 7, 8, 9). Fish are able to exist, but with decreased vitality, in pHs as low as 4.1 (9). Based on the uncovered bucket data, the fish killed by the STS-5 launch may have been exposed to a gradation of pHs as low as 2.4. The test fish used in the bioassay were also exposed to a pH of 2.4, while the controls were maintained at pH 8.4.

The morphologic changes recognized in the gills are consistent with acute death due to ionic imbalance and anoxia. Fresh water fish take up ions actively through gill epithelium. Sodium is exchanged for hydrogen ions and chloride for bicarbonate. Increased hydrogen ion activity in the surrounding medium will impede the active uptake of sodium. Severe ionic imbalance is known to affect fundamental physiologic processes such as nerve conduction and enzymatic reactions (5). Additionally, the increased width of the cells, intercellular tissue, and swelling of erythrocytes would seriously impede ion transfer and gas exchange. The observed morphologic changes are admittedly nonspecific, but laboratory data indicate no abnormalities of the environmental water at the launch site other than the low pH. The bioassay had the same water for test and control and yielded the same morphologic changes, again supporting the impression that the changes are due to the acid water.

The "bottom line" diagnosis, therefore, is: ionic imbalances and fatal anoxia resulting from severe gill damage caused by a rapid decrease in the water pH.

## VI. RECOMMENDATIONS

It can be predicted with reasonable certainty that fish kills of this type will occur in the lagoon north of Launch Pad 39A with every STS launch. The change in water pH in shallow areas is a transitory phenomenon. There is no evidence to suggest a long-term environmental impact. Due to the limited nature of these fish kills and the absence of long-term impact, it is recommended that these affected fish be considered dedicated in the interest of the mission. Corrective action does not appear to be justified. Continued environmental monitoring, however, would be prudent.

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**APPENDIX 1**

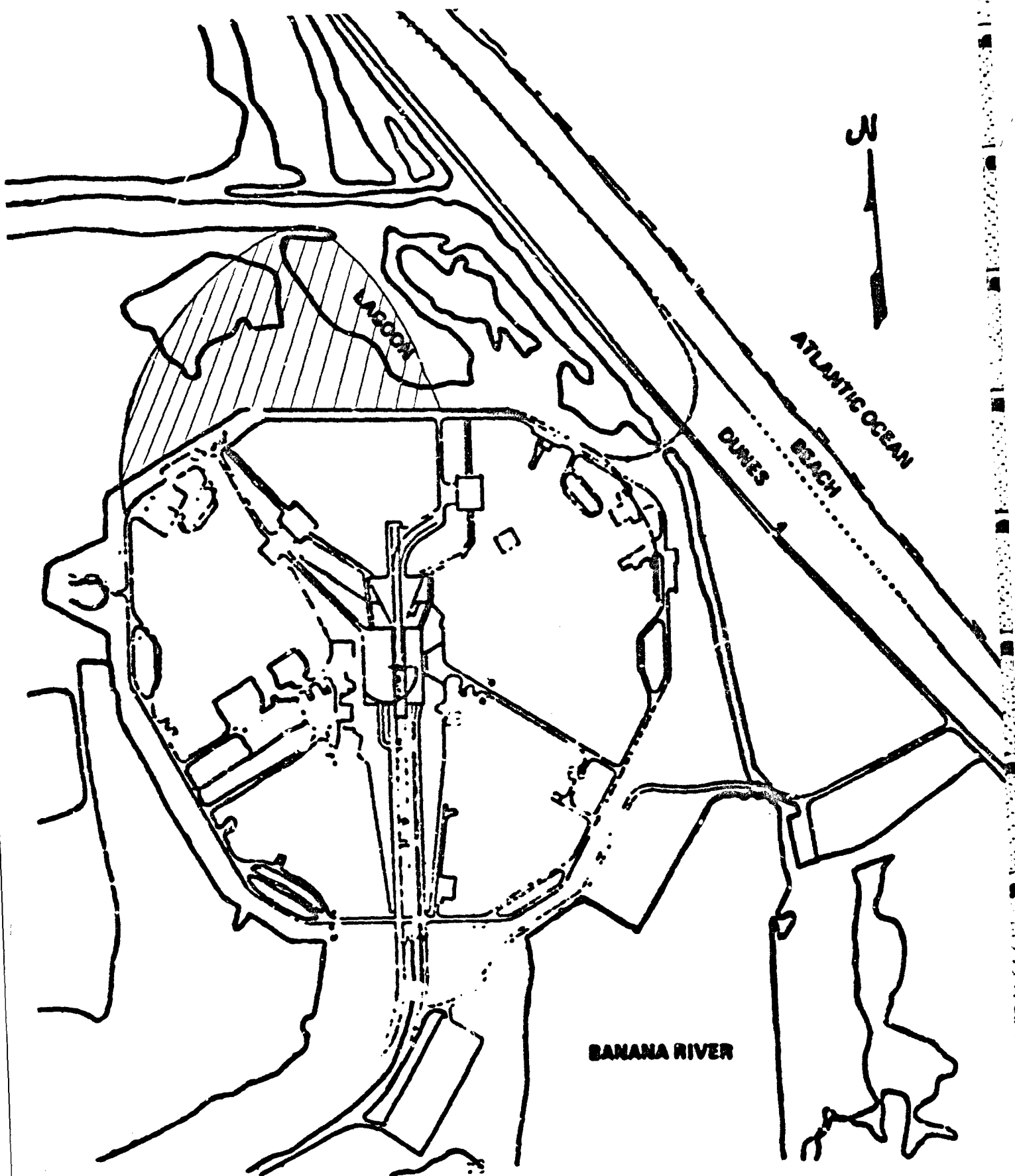
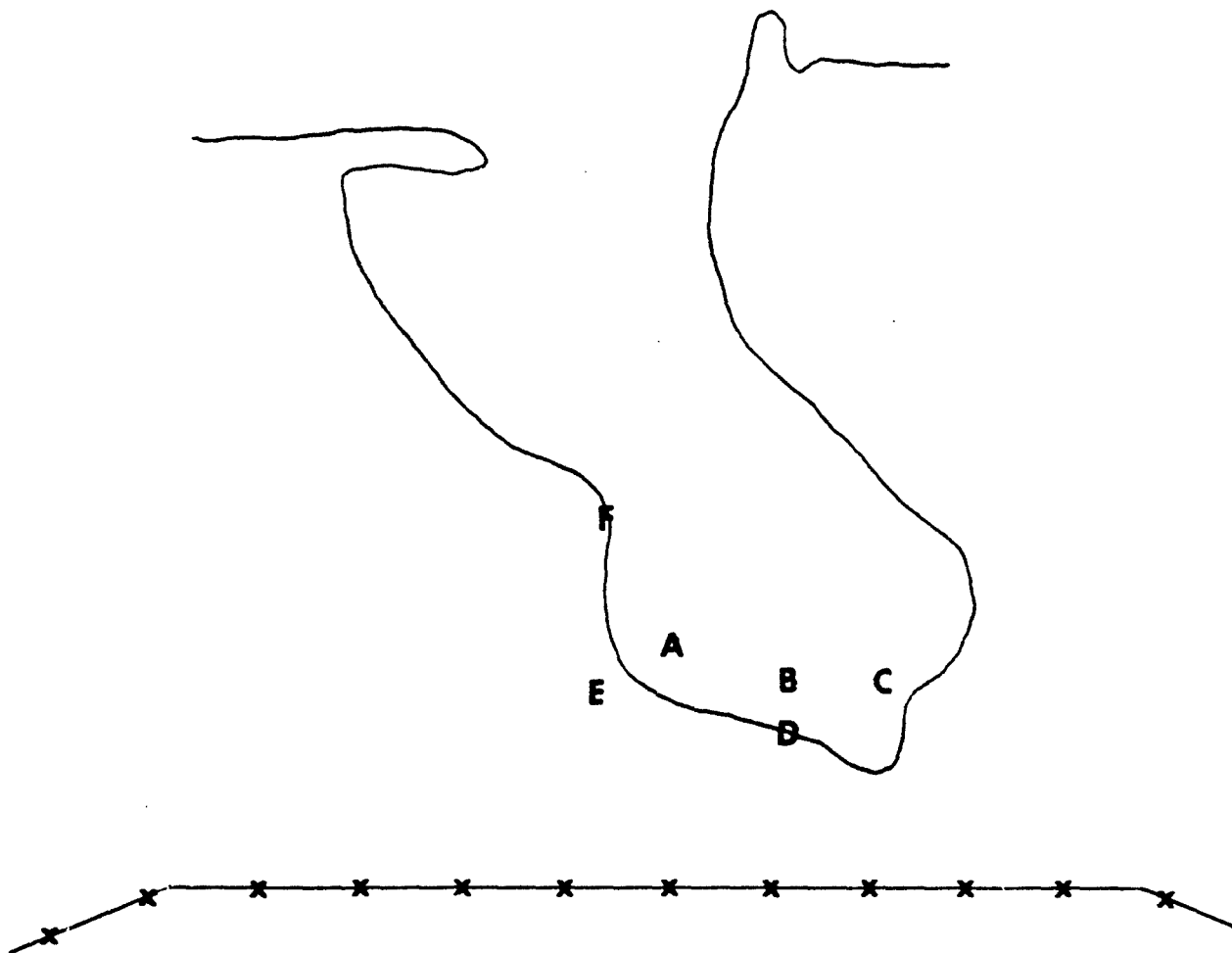


Fig 1. Launch Pad 39A and brackish lagoon to its north in area impacted by the SRB exhaust plume.



**Fig 2. Lagoon north of Launch Pad 39A.**  
Sites A, B and C are primary SIS-5 water and sediment sampling sites. Site D is the fish kill site. Site E is the site of covered and uncovered bucket placement. Site F is an additional fish kill site.

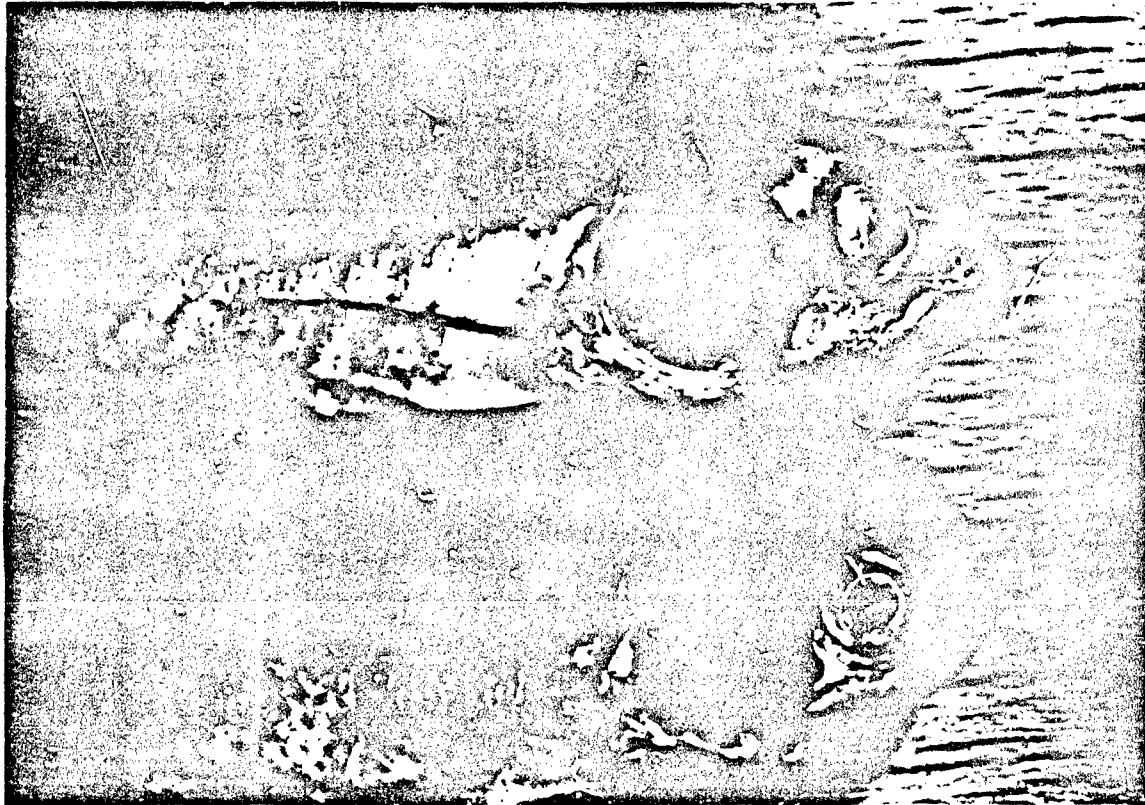


Fig 3. The gills of the bioassay test fish (pH 2.4, top) are paler than the gills of the control fish (pH 8.4, bottom). The gill structure of the test fish is very evident due to loss of the mucous coating of the gill.



Fig 4. The gill lamellae (arrows) of a bioassay control fish (pH 8.4) are delicate with inconspicuous goblet cells, intact erythrocytes and normal epithelial and endothelial cells. H&E. 310X.



Fig 5. Gill lamellae (arrows) from a bioassay test fish (pH 2.4) with prominent goblet cells (g), swollen erythrocytes and ballooned epithelial and endothelial cells. Note the increased width of the gill lamellae. H&E. 520X

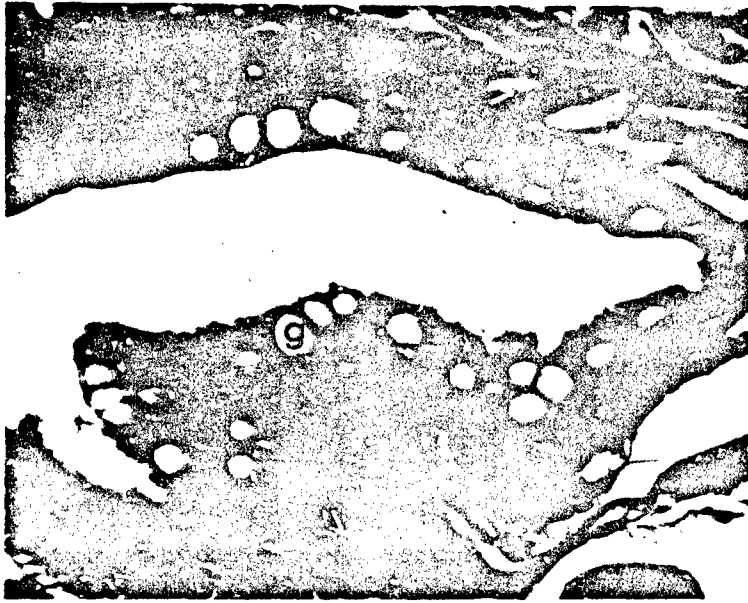


Fig 6. Pharyngeal epithelium (E) from a bioassay control fish (pH 8.4). Note the relatively small size and lack of prominence of the goblet cells (g). H&E. 490X.

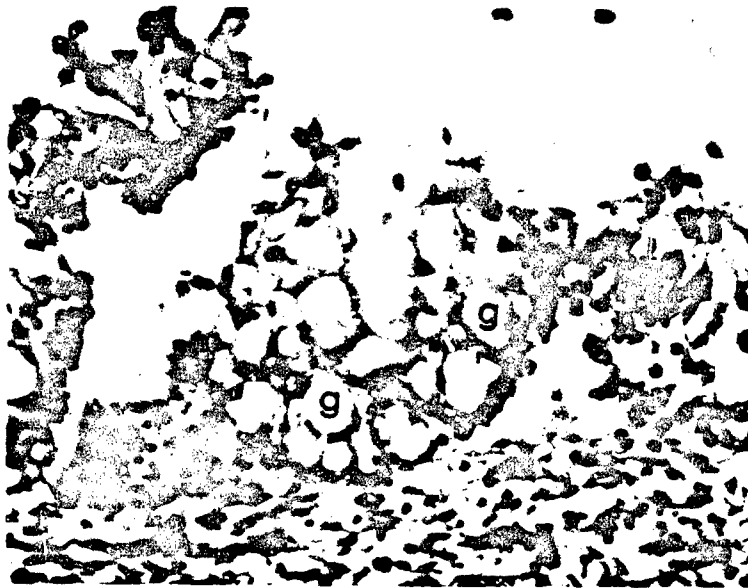


Fig 7. Pharyngeal epithelium (E) from a bioassay test fish (pH 2.4). Note the prominent, large goblet cells (g). H&E. 490X.

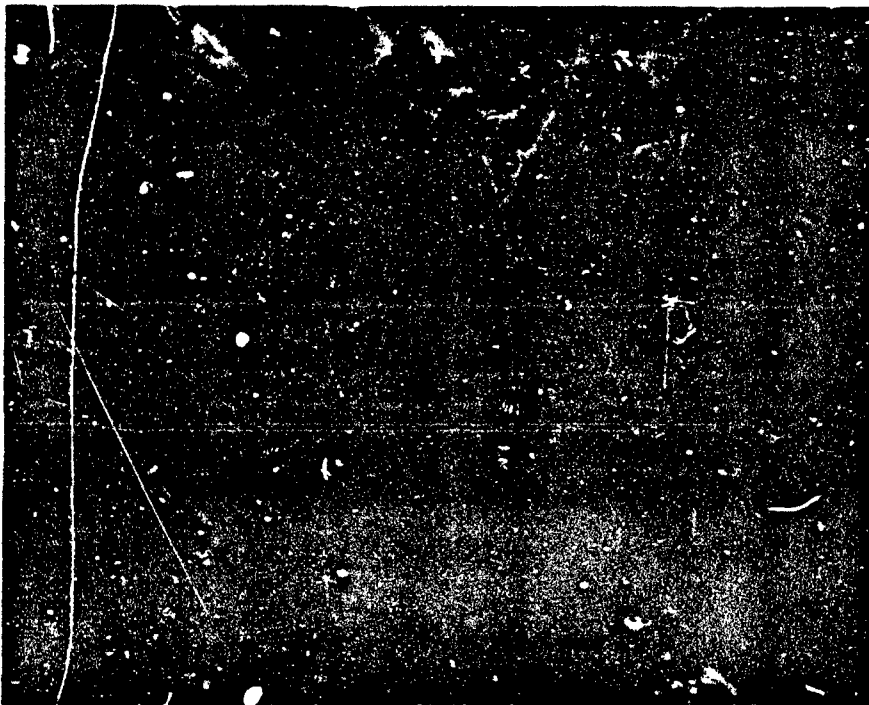


Fig 8. This scanning electron photograph of the gill of a bioassay control fish (pH 8.4) demonstrates the intact mucous protective layer (M) which masks the fine morphology of the gill lamellae (g). 160X

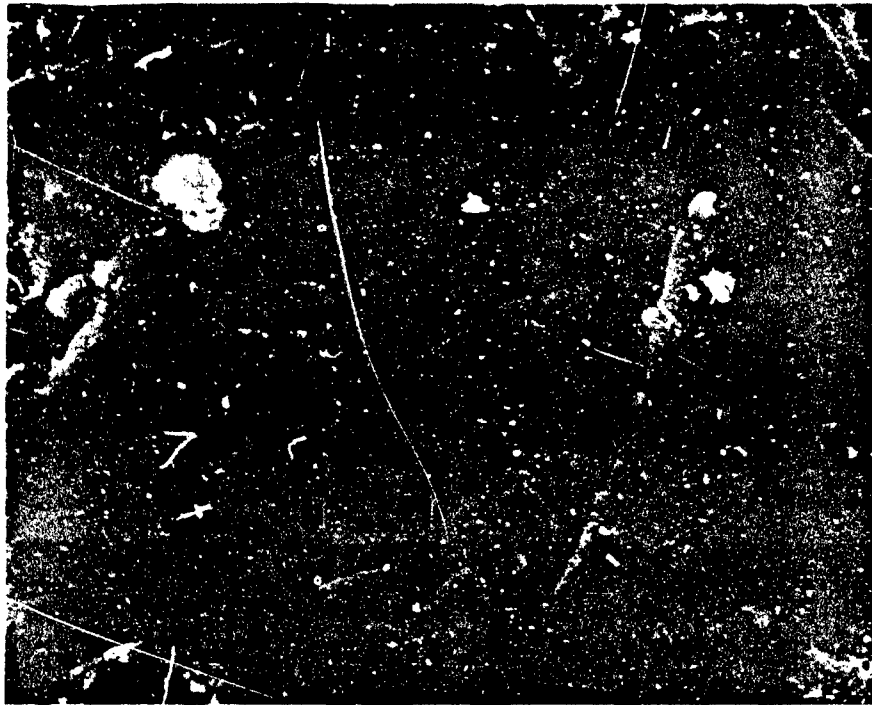


Fig 9. This scanning electron photograph demonstrates the normal gill lamellae (L) without the protective mucous layer. Note the lack of swollen epithelial cells (pH 8.4). 1400X.



Fig 10. This scanning electron photograph of the gill of a bioassay test fish (pH 2.4) demonstrates the loss of the mucous protective layer and reveals the fine structure of the gill lamellae (L). 120X.



Fig 11. This scanning electron photograph demonstrates the swollen cells (arrows) of the unprotected gill lamellae of a bioassay test fish (pH 2.4). 1600X.

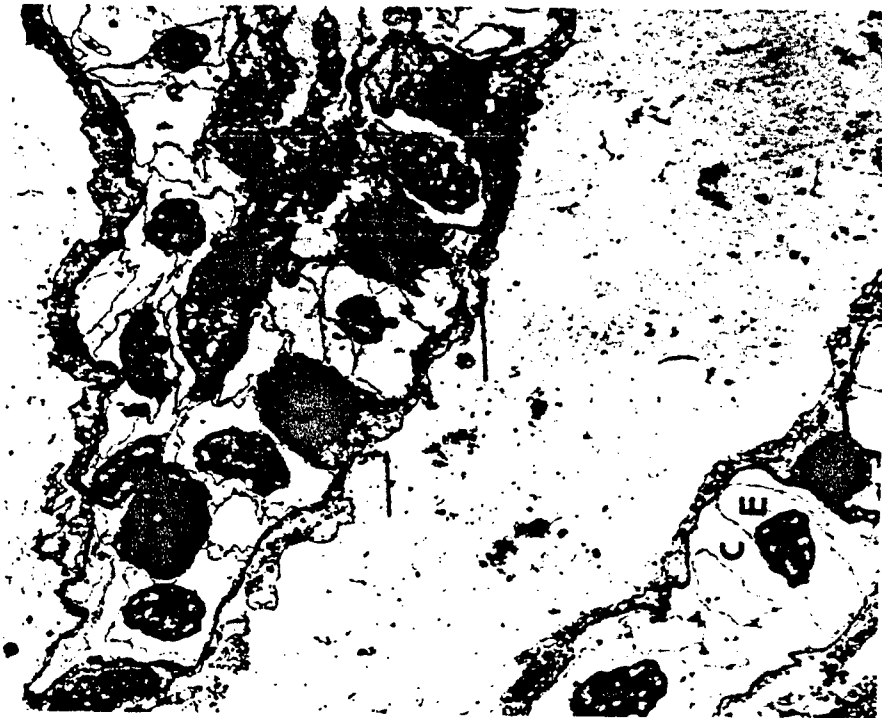


Fig 12. Transmission electron photograph of gill lamellae with normal cells and cell configurations. Note erythrocyte (E), endothelial cell (arrows), epithelial cell (c) and capillary lumen (C). 3600X.

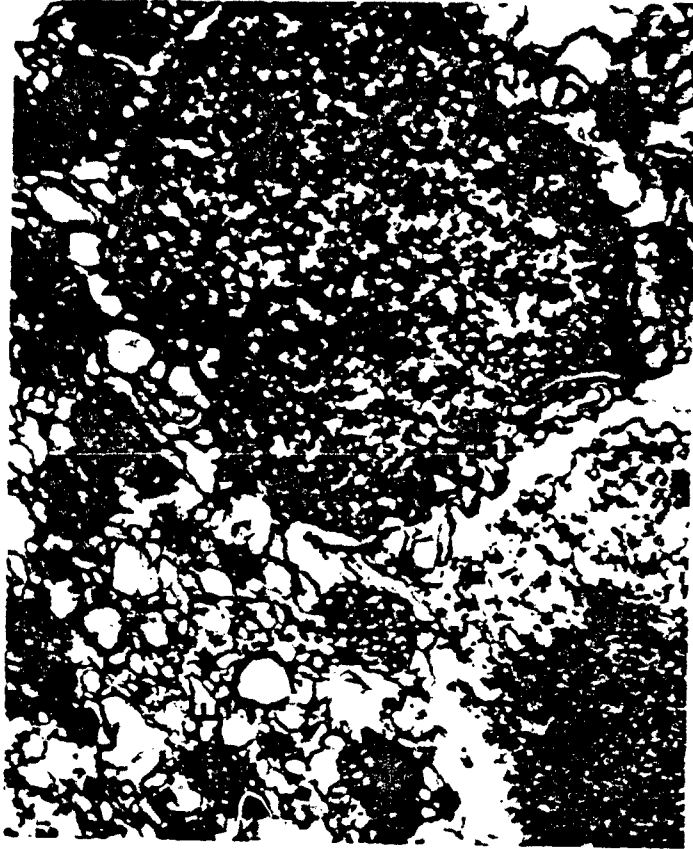


Fig 13. Transmission electron photograph of typical normal cell morphology. Note nucleus (N), mitochondria (m) and endoplasmic reticulum (E). 2400X.



Fig 14. Transmission electron photograph of gill lamellae with swollen erythrocytes (E), endothelial cells (e), epithelial cells (s) and intercellular spaces. 1800X.

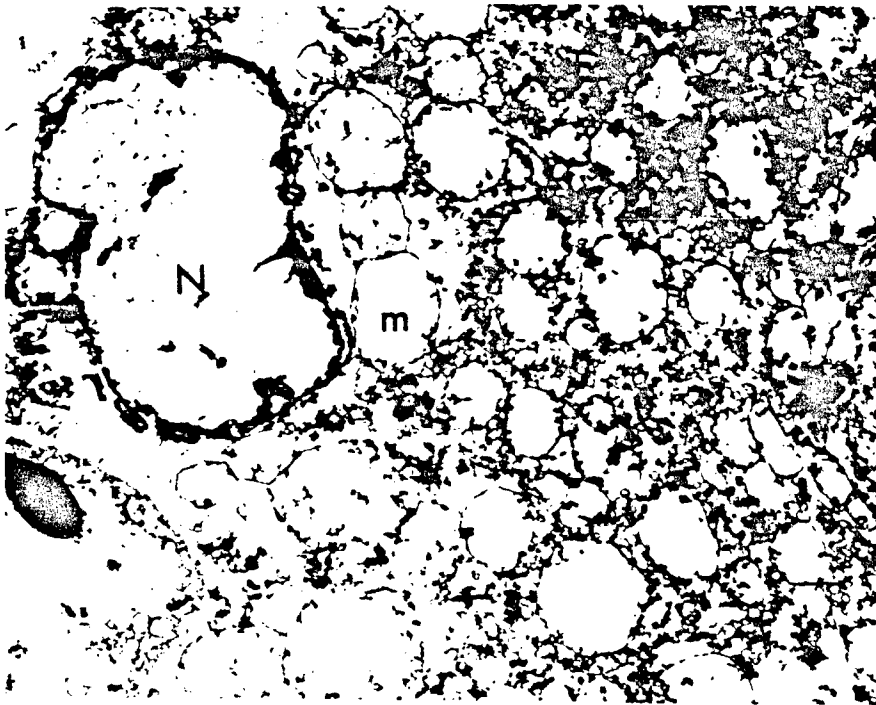


Fig 15. Transmission electron photograph of typical swollen cell with marked distortion and degeneration of the nucleus (N), mitochondria (m) and endoplasmic reticulum (E). 9000X.



Fig 16. A typical cross section of an encysted trematode parasite (T) in the lamina propria of a gill lamellae. Note the minimal inflammatory response. H&E. 290X.



Fig 17. A protozoal cyst (P) in brain of control fish which is causing disruption of the normal architecture of the central neuropile. H&E. 130X.