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THE EFFECT OF PETROLEUM AND SELECTED FRACTIONS ON THE GILL MORPHOLOGY OF RAINBOW TROUT

by

Marc Emil Duey

A thesis presented to the University
of Ottawa in partial fulfillment
of the requirements for the degree
of Master of Science in Biology

Ottawa, Ontario, 1984

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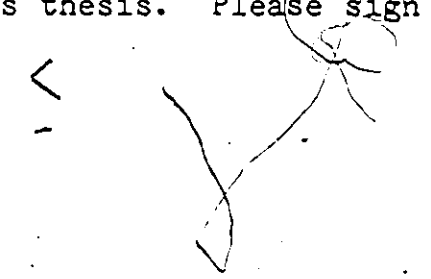

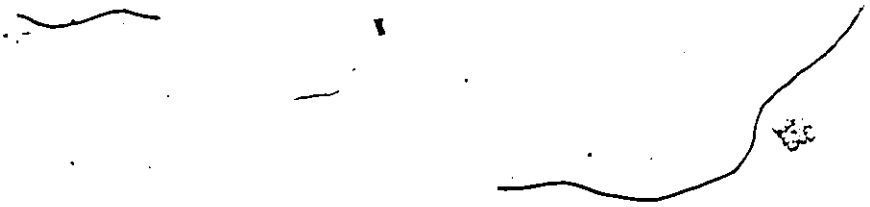

ABSTRACT

Petroleum hydrocarbons are important contaminants in the aquatic environment and a considerable amount of information is available on the general toxicity of crude oils. Relatively little work has been done, however, to characterize the morphological effects of crude oils. This study examined the effects of two crude oils (Norman Wells, Venezuelan) and certain hydrocarbon fractions on the gill structure of rainbow trout (Salmo gairdneri) using light transmission and, scanning electron microscopy.

The effects of exposure to mechanical dispersions of fresh (100 µl/L) and weathered (200 µl/L) concentrations of two crude oils were examined over seven days, and showed severe and varied pathology including fusing of lamellae, epithelial lifting, and chloride cell proliferation and alterations.

Physical effects of oil were simulated by a non-toxic paraffin oil at two different concentrations, and were not found to be contributory to the effects observed. The water soluble fraction of the two crude oils was tested and indicated that while this fraction had an effect on the surface of epithelial cells, it was not responsible for the changes produced by crude oil dispersions.

The contribution to morphological effects of an internal burden of hydrocarbons was tested by intraperitoneal injections of crude oil in both fresh and sea water adapted fish. The results showed few morphological changes at the gill level.

The effects of specific aromatic hydrocarbons were tested by exposing fish to five selected compounds (benzene, naphthalene, phenathrene, benzo(a)anthracene, benzo(a)pyrene). The results indicated that especially higher molecular weight aromatic hydrocarbons may contribute to chloride cell damage.

RESUME

Les hydrocarbures de pétrole constituent d'importants agents de contamination de l'environnement aquatique. Même s'il existe une quantité d'information importante sur la toxicité des pétroles bruts, peu d'effort ont été consacrés à décrire les effets morphologiques des pétroles bruts. Cette étude examine les effets de deux pétroles bruts (Norman Wells, Venezuelan) et de certaines fractions d'hydrocarbures de pétrole sur la structure des branchies de truites arc-en-ciel; (Salmo gairdneri), à l'aide de la microscopie optique et la microscopie électronique à balayage optique.

Les effets d'une exposition à des dispersions mécaniques de deux pétroles bruts, avec des concentrations de 100 ul/L en huile fraîche et de 200 ul/L en huile altérée observés pendant sept jours, révèlent une histopathologie sévère et variée, y compris la fusion de la lamelle, le soulèvement de l'épithélium et des altérations de la cellule de chlorure.

On observe que les effets physiques du pétrole, simulés par une paraffine non-toxique à deux concentrations différentes, ne contribuent pas aux changements décelés. La vérification de la fraction soluble à l'eau des deux

pétroles bruts permet de constater que même si cette fraction a un effet sur la surface des cellules épithéliales, elle n'est pas responsable des changements produits par les dispersions.

La contribution d'une charge interne d'hydrocarbures aux changements morphologiques est verifiée au moyen d'injections intrapéritonéales de pétrole brut dans des poissons d'eau douce et dans des poissons adaptés à l'eau salée. Les résultats ne révèlent qu'un nombre réduit de changements morphologiques au niveau des branchies.

Quant aux changements attribuables aux hydrocarbures aromatiques, ils sont vérifiés en exposant des poissons à une sélection de cinq composés (benzène, naphthalène, phénanthrène, benzo(a)anthracène, benzo(a)pyrène). Les résultats indiquent que les hydrocarbures aromatiques d'un poids moléculaire plus élevé pourraient contribuer à endommager les cellules de chlorure.

ACKNOWLEDGEMENTS

I extend my sincere gratitude to my colleagues, Dr. Rainer Engelhardt and Michael Wong. Dr. Engelhardt, as my thesis supervisor, provided continual support and indispensable guidance during the experimental and writing stages of this work. Michael Wong, a friend and close collaborator, provided advice and laughter during the many hours of sampling.

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I am particularly grateful to my family for providing enduring moral support and a place to come home and rest.

This thesis is dedicated to my best friend and wife, Maureen. Her smile is a constant reminder of the beauty of nature.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter I

INTRODUCTION

The impact of oil development activities on the environment has received considerable scrutiny. Environmental scientists from around the world have carried out studies to assess the effects of petroleum contamination on fish. From a Canadian perspective, careful consideration of the adverse effects of oil in Canadian waters is important in developing sound policies for protecting a valuable natural resource. Canada enjoys the world's largest coastline, parts of which are constantly travelled by tankers carrying large amounts of crude oil. A major oil spill is considered a slight but distinct possibility.

Numerous studies have examined the effect of the water soluble component of oil on fish. An interpretation of the impact of dispersed oil on fish is still difficult because of a lack of adequate information. It is recognized that oil originating from a spill is frequently dispersed in particles. In fact, dispersion is often the objective of chemical countermeasures to oil spills.

A comprehensive and well controlled study of physiologically-relevant gill morphology changes, comparing the effects of various fractions, components, and environmental conditions is needed.

The gills of teleosts have a delicate epithelium exposed to the environment. This thin tissue functions in respiration and in the maintenance of salt balance. The gill is prone to damage by water-borne pollutants, including hydrocarbons (Solangi and Overstreet, 1982). As such it is reasonable to employ branchial tissue as a sensitive index to pollutant action.

The purpose of this study was to examine the relative structural effects of various hydrocarbons on the gills of both fresh water and sea water adapted rainbow trout. The test organism was selected because of the abundant physiological and morphological information available on it, and for its ability to survive in various salinities. In addition, rainbow trout is a model salmonid, a genus important in the food industry, and a fish found at depths relevant to oil contamination.

Fresh water fish lose salt to their hypotonic external medium by diffusion and by the excretion of large amounts of urine. Therefore, an uptake of ions via the gills is necessary in the fresh water environment to maintain osmotic balance. Sea-water-adapted fish by comparison, show an influx of ions from the hypertonic medium and maintain their ionic balance by drinking salt water and excreting excess ions via the gills.

SOURCES OF PETROLEUM IN AQUATIC SYSTEMS

Petroleum is a natural and very complex organic mixture containing more than 10,000 different compounds. Hydrocarbons are the predominant component of this mixture, formed through molecular modification of plant and animal material over geological time. The chemical composition of crude oil varies depending on the nature and location of the reservoir in the world.

The potential threat of oil pollutants in the aquatic environment has increased as the use and demand for fossil fuels has increased. An important source of water-borne oil pollution can be attributed to the hydrocarbon industry, encompassing the drilling, production and transportation of oil. It is of interest, however, that oil pollution from land-based sources (e.g., river outflows) greatly exceeds that attributed to the oil production industry. Two other sources of hydrocarbons are geochemical and biosynthetic. The former may be a very important contributor through natural oil seeps, occurring in many marine regions.

Much of the oil spilled is dispersed (Gordon et al., 1973). The aerosol effect of underwater blow-outs and wave action on surface oil results in about 90 percent of the oil introduced to the seas to be in the mechanically

dispersed form in the water column. A number of events occur when oil is spilled. It can be mechanically dispersed, and the volatile fraction may evaporate; a slick on the surface will form, and certain components will dissolve in the water, part may form tarballs, some may be modified chemically by microorganisms, and photochemical reactions may occur (Clark and MacLeod, 1977). The mechanically dispersed elements of crude oil spills have been chemically and physically analyzed by Shaw and Reidy (1979), but little is known about their toxic mode of action in fish. It must be noted that this dispersed phase can remain persistent for weeks within the water column (Parker et al., 1971).

The physical characteristics of the particulate hydrocarbons employed in the experiments were similar to those described by Forrester (1971) following an oil spill. He reported that surf and wave action formed mechanically dispersed particles in the 5 µm to 10 mm range, and that they could be detected in the water column to depths of 80 m and distances as far away as 250 km from the site of the spill. The fresh 100 µl/L and the weathered 1000 µl/L exposures are not dissimilar to concentrations which one might expect to find in the aftermath of a spill in a large volume of water. The weathered 1000 µl/L exposure corresponds to 200 µl/L and 40,000 particles/ml after

homogenizing and weathering for 3 days (Engelhardt et al., 1981). The particle size of the oil-in-water dispersions ranged from 1 μm to 2 mm in diameter, with a mean of 5 μm. As can be expected, the particle numbers and total hydrocarbon concentrations declined as the experiments progressed through the standard seven day exposure regime.

Weathering causes the loss of low end volatiles. Norman Wells is a light-weight crude with a high concentration of volatile and soluble components; upon weathering, much of these are dissipated even in a matter of hours (Engelhardt, 1978). Venezuelan crude has a high proportion of medium and high medium weight hydrocarbons which persists in the water. Volatile, low-boiling hydrocarbons in fresh oil can rapidly penetrate and solubilize the lipid components of membranes. The higher-boiling hydrocarbons in weathered oil would penetrate more slowly.

GENERAL GILL STRUCTURE

This study made extensive use of scanning electron microscopy as a tool in the visual assessment of gill morphology and ultrastructure. A number of workers have examined the gills of fish using this sophisticated technique (Olson and Fromm, 1973; Vogel et al., 1976; Hunter and Nayudu, 1978; Hughes, 1979; Kendall and Dale, 1979). These

studies were all of unstressed fish. Under high magnification it is clear that gill morphology differs among species. The work of Kendall and Dale (1979) serves as a good reference point for the ultrastructure of rainbow trout gills specifically.

Hart and Ogelsby (1979) used the scanning electron microscope to test the effect of high concentrations of iron on the gills. The effect of high temperatures on gill structure was examined by Jacobs et al. (1981) using similar techniques.

Scanning techniques permit the examination of changes at the gill surface first at low magnification to determine frequency and then at high magnification to describe and characterize the disturbance. In addition, it is easy to correlate the surface structure examined with the overall gill morphology. In fish, surface morphology may well determine the efficiency of the respiratory function. Scanning electron microscopy provides an effective means of visualizing the gill tissue.

Branchial Tissue

The fish gill is a specialized organ, and the main site for gaseous exchange and the maintenance of ionic and osmoregulatory balance (Maetz, 1971). The gill tissue is

heterogeneous, containing numerous specialized cell types. The branchial structure represents the largest permeable surface on fish in continuous contact with the external media, and is an obvious target site for water borne toxicants (Hughes, 1966). Gill browning has been used for monitoring point sources of petroleum pollution in lobster habitats (Payne et al., 1983). As such, the gill structure can be regarded as a valuable tool to evaluate the effect on fish of toxic components in the water. In fact, since the micellar layer can absorb water borne hydrocarbons, the gill may be the primary site of hydrocarbon entry in fresh water adapted fish (Lee et al., 1972). It has been shown that the gills can accumulate substantial amounts of individual hydrocarbons as well as crude oil (Rice et al., 1977). Oil-in-water dispersions may have a major morphological and functional effect on the sensitive gill structure. Because of the complex nature of crude oil, a significant question to answer would involve a physiochemical examination of which parts and what factors are important in the development of gill disturbances.

The secondary lamellar distance can theoretically accommodate a range of particulate hydrocarbon sizes available in the water column after a spill. This close proximity of dispersed oil and epithelial cells may be an important factor since Anderson et al. (1974b) found that

oil-in-water dispersions often retain the chemical composition of the parent oil.

Chloride Cells

Rainbow trout chloride cells are similar to those of other species, but do not contain an apical pit (Morgan and Tovell, 1973). The chloride-type cells are normally situated beneath the epithelium and are attached to the basal lamina. Chloride cells were first described by Keys and Willmer in 1932. They are columnar and acidophilic cells which extend from the free surface of the filament to the membrane adjacent to the blood supply (Kessel and Beams, 1962). Morgan (1974) found that chloride cells are more numerous in the developing gill than in the adult trout. When the cells lift away from the basal lamina an epithelial lymphoid space is formed. Chloride cells characteristically have numerous mitochondria and a highly developed agranular endoplasmic reticulum (Pétrik, 1968) and are the primary site of gill Na^+, K^+-ATPase (Kannaky et al., 1976). The variation in morphological changes, as revealed by scanning electron micrography of chloride cells induced by exposure to selected components of crude petroleum in conjunction with plasma ion information, has not been studied previously.

DISTURBANCE OF GILL FUNCTIONS

Numerous toxic substances may cause osmoregulatory disturbances in fish (Lewis and Lewis, 1971; Wedemeyer and Yasutake, 1974; Schreck and Lorz, 1978; Sugatt, 1980), and an altered state of the branchial transport epithelium was suggested. The appearance of multiple lesions in the gill tissue is a common response to toxic exposure of a variety of compounds (Mitrovic et al., 1968; Skidmore and Tovell, 1972; Smith and Piper, 1972; Brenniman et al., 1979). While considerable work has been carried out on the effect of lethal concentrations of petroleum compounds, there is relatively little work that differentiates the effect of sub-lethal oil-in-water dispersions. It is postulated that certain gill functions were impaired in those fish captured in the area of a spill demonstrating gross structural gill damage (Blanton and Robinson, 1973). Histological changes were evident on exposure to Bunker C oil for 96 hours, in both fresh water and sea water adapted trout (McKeown and March, 1978). Individual components such as naphthalene have been shown to cause gill damage in Fundulus heteroclitus, including hyperplasia and hemorrhage (DiMichele and Taylor, 1978).

Observations such as these prompted the research incorporated in this thesis. They raised the possibility

that certain hydrocarbons were involved in the morphological changes of the gill structure, which in turn may have contributed to the observed plasma ionic changes.

Of particular interest in this study were the epithelial and chloride cells of trout gills. Organic pollutants have been demonstrated to disrupt membrane transport (Kinter et al., 1972). Since petroleum hydrocarbons have a high affinity for lipids, they may affect the structure of the epithelial cell membrane (Roubal, 1974). The chloride cells are specific loci for electrolyte exchanges (Karnaky, 1980). In addition, petroleum may modify chloride cell morphology (Blanton and Robinson, 1973; Lopez et al., 1981). Studies that have demonstrated ionic imbalance in oil exposed fish have suggested chloride cell damage to be a contributing factor (Wong, 1982; McKeown and March, 1978).

HYPOTHESES

Most of the work on gill tissues in association with the evaluation of contaminant stress has been done with the paraffin histological techniques and as such has been limited to the description of gross histological changes of low resolution. Only recently have higher resolution techniques been used. Cell surface and chloride cell

change may occur on exposure to oil and can be monitored. Such changes can thus be used as an indicator of the existence and relative severity of the effects due to the contaminant. Using light microscopy of thinly-sectioned plastic embedded tissue and scanning electron microscopy, Kendall and Dale (1979) revealed the many specialized cell types and intricately organized cell surfaces of healthy rainbow trout gill tissue. Similar techniques were employed in this hypothesis-oriented study, with an analysis of physiological data provided by colleagues carried out to evaluate the effect and overall significance of morphological changes induced by exposures. Numerous tests, using two crude oils and various hydrocarbon fractions in association with other manipulations, were carried out to lend support to the hypothesis that the dispersed particulates are the main vectors of gill disturbances.

Several hypotheses are addressed in this investigation.

Hypothesis 1: the important morphological alterations of the gill are precipitated specifically by the physical contact of the secondary lamella with the emulsion or mechanically dispersed phase of crude oil.

Previous studies have not isolated the relative importance of the components of crude petroleum, at

relevant concentrations, in causing physiologically significant abnormalities. As mentioned earlier, dispersions are an important element of oil spills, and when oil is dispersed in micron-sized droplets, the surface area of the oil is increased by a million fold (Berridge et al., 1968). Because emulsion particles and the average distance between adjacent secondary lamella are of the same order of magnitude, the trapping of oil particles is a possibility. It has been suggested that the suspended dispersions may have a greater impact than dissolved hydrocarbons (Nelson-Smith, 1972).

Hypothesis 2: the type of crude oil contaminating the water modulates the degree of cell damage incurred. The effects of two types of crude oils, Norman Wells and Venezuelan, were tested.

The weathering of petroleum can substantially modify certain characteristics of the crude oils, including water solubility, dispersibility, and chemical composition (Shaw and Reidy, 1979). Both fresh and weathered crude petroleums were examined in this study.

Hypothesis 3: the physical effects of a dispersed oil are important in causing epithelial cell damage.

Hebert and Merkens (1971) demonstrated that suspended inert particles such as diatomaceous earth, for instance, could change gill morphology. To differentiate

the purely physical effects of a dispersion from toxic ones, two paraffin oil exposures were evaluated.

Hypothesis 4: the water soluble fraction of crude oil contributes to the morphological changes observed.

Hawkes (1977) showed that the water soluble fraction can cause cellular disruption, and so this study also examined the effect of the water soluble fraction of two crude petroleums.

Hypothesis 5: the external hydrocarbon load is of prime importance in causing cell damage.

Evaluation of the relative importance of the internal hydrocarbon load on gill morphology was carried out by intraperitoneal injections of petroleum.

Hypothesis 6: stress-related responses in the cortisol balance of fish, exposed to hydrocarbons, play a role in the manifestation of morphological and hydromineral imbalances.

An early stress response to petroleum contamination in the American eel, Anguilla rostrata (Nava, 1980), and in the mummichog, Fundulus heteroclitus (Levitan and Taylor, 1979), was an elevation in plasma cortisol. An experiment was carried out on rainbow trout to evaluate the potential contribution to gill perturbations by a general stress reaction mediated by cortisol.

Hypothesis 7: a final hypothesis states that

aromatic hydrocarbons, at environmentally realistic concentrations cause gill abnormalities.

Part of the toxicity of petroleum has been attributed to aromatic hydrocarbons which can be selectively accumulated and metabolized by fish. Aromatic hydrocarbons are hydrocarbons with one to six benzene rings. To examine the contribution of this category of crude oil compounds, a series of five aromatic hydrocarbns were tested.

Certain observed plasma ionic imbalances can be explained by morphological changes at the gill level, especially at the sites of epithelial cells along the secondary lamella (Engelhardt et al., 1981; Wong, 1982). An examination of the effect of hydrocarbons on the ultrastructural surface detail of epithelial cell and chloride cell were carried out in this study for eventual comparison with plasma parameters examined in the same fish under the same exposure conditions.

The present work represents one part of a two-part study on the effect of crude oil and certain fractions on rainbow trout. The two studies were linked by the use of the same experimental animals, and treatment regimes. Examination of the morphological changes due to hydrocarbon exposure was the main objective of this study. The

influence of hydrocarbons on the mechanisms regulating ion balance were examined; plasma from control and experimental animals was sampled to measure Cl^-, Na^+, K^+, Ca^{++}. This latter work was performed by M.P. Wong (Wong, M.Sc. Thesis, 1982, University of Ottawa) and some of the results have been published (Engelhardt et al., 1981).

Chapter II

METHODS AND MATERIALS

EXPERIMENTAL ANIMALS AND FACILITIES

Juvenile rainbow trout, Salmo gairdneri, (Richardson), weighing between 50 and 200 grams, all of the same genetic strain, were obtained from the Thistle Springs Trout Farm, a commercial fish hatchery (Ashton, Ontario). For fresh water experiments, the trout were held in large fiberglass tanks, with a constant flow through of dechlorinated tap water from the city of Ottawa (8-12°C, pH 7.4-7.8). For salt water experiments, the fish were held in a recirculated, filtered artificial sea water system with a salinity of 21 percent (ppt) (Instant Ocean Inc.); and one third of the water was replaced weekly with water of the same salinity. The water was continuously aerated, and monitored regularly for quality. Chlorine and ammonia were always found to be below levels of detection. Dissolved oxygen was kept above the 75 percent saturation level (Yellow Springs Instruments). A photoperiod of 12D/12L was held constant with wide spectrum fluorescent lights. Trout were acclimated for over four weeks prior to experimentation. During this time the fish were fed ad libitum once daily with dry commercial trout pellets (Purina Chow, No. 4

Developer).

After this acclimation period, groups of eight fish were placed in glass aquaria for one week prior to experimentation, with the water held at 10°C±1, and recirculated at 4 to 6 L/minute through a filter (Dynaflo Power filter 150). During this period the fish were fed every other day. Fish were not fed once the treatment phase of the experiments began.

The level of ammonia in the tanks was monitored regularly, and was found not to deviate beyond acceptable levels. This was done because Smart (1976) showed that chronic exposure to ammonia caused serious gill histopathology, including hematomoas and epithelial lifting.

EXPERIMENTAL PROCEDURES

Each group of fish was exposed to various regimes and modes of impact. All were seven-day exposure tests using methods similar to those recommended by LaRoche *et al.* (1970) for the evaluation of oil toxicity. In all cases, each exposure regime used its own control group to allow for any seasonal changes.

Paraffin

To test the mechanical effects of oil-in-water dispersions, and to help differentiate them from the toxic effects, a light to medium weight paraffin oil (14 to 18 carbons) was employed. For the purposes of comparison, a fresh 100 µl/L and a 200 µl/L concentration which had been weathered for three days (initial concentration of 1000 µm/L) were used. A homogenizer was used to mechanically disperse the oil into oil-in-water particles in size from 1 µm to 2 mm, with a mean of 5 µm (Wong, 1982).

Dispersions

The effect of the mechanical dispersions (emulsions) of two crude oils, Venzuelan and Norman Wells, were studied. These two oils have different characteristics. Norman Wells is a light crude originating from the North-West Territories in Canada. Venezuelan crude is transported regularly to eastern Canada. Both samples of crude oil were stored in amber containers and kept at -20°C until used. Oil-in-water dispersions were prepared in a manner previously described by Wong (1982). Both fresh and weathered treatments of each crude oil were carried out. Concentrations of 1000 µl/L were weathered for three days

in aquaria under constant circulation. At the time of introduction of the fish, this treatment corresponded to an exposure of 200 µl/L and 4 x 10^4 particles/ml, which simulated conditions and concentrations after an oil spill (Clark and Findlay, 1977). For comparison, fish were introduced to aquaria with 100 µl/L amounts of fresh oil. In all four of the above mentioned treatments, oil was emulsified mechanically with the help of an homogenizer into oil-in-water dispersions.

Water Soluble Fraction

To evaluate the effects of the soluble component alone, trout were exposed to the water soluble fractions of Norman Wells and Venezuelan crudes. Fish were exposed to the water soluble fraction by being placed in aquaria, partially covered by a slick of an initial concentration of 1000 µl/L, and aged for three days prior to experimentation. The trout and crude oil were physically separated by a mesh barrier which did not permit particulates to enter the water column or the trout to contact the oil. Hydrocarbon concentrations at the time of introduction were measured spectrofluorometrically (Engelhardt et al., 1977), and found to be 35 µl/L and 45 µl/L for Norman Wells and Venezuelan crude oils repectively.

IP Injections

To study whether hydrocarbon-induced morphological changes might be due in part to an internal hydrocarbon load, trout were treated with intraperitoneal injections of Norman Wells oil. The crude was weathered for three days and injected at a dose of 100 µl/kg fish/day for seven days. The experiment was repeated, for comparison, with salt water adapted trout. The recirculating system (Living Streams, Model LS 700. Frigid Units, Inc., Toledo, Ohio) was equipped with a high capacity charcoal filter and circulator (Min-o-cool, Frigid Units, Inc.) to remove any excreted hydrocarbons and products of metabolism. Control fish were sham injected with corn oil and kept in a similar system.

Cortisol Experiment

The contributory role of a cortisol-mediated stress reaction in causing morphological gill abnormalities was evaluated. A series of eight fish were injected with 80 µg cortisol/100 g fish. Controls were sham injected with corn oil.

Aromatics

The involvement of the aromatic fraction of crude oils in the production of gill disturbances was examined. Five individual aromatic hydrocarbons were tested. A nominal dose of 100 µl/L of paraffin oil was spiked with the tested compound in the following concentrations: 3.0 mg/L of benzene, 3×10^{-1} mg/L of naphthalene, 3×10^{-2} mg/L of phenanthrene, 3×10^{-3} mg/L of benzo(a)anthracene, and 3×10^{-4} mg/L of benzo(a)pyrene. These concentrations were chosen to approximate realistic levels of these compounds found in the water after an oil spill. In each case, the mixture was mechanically dispersed with the use of an homogenizer. As in all other experiments the exposures were for seven days.

Table 1 is a summary of the exposure treatments used in this study.

SAMPLING AND TISSUE PREPARATION

Sampling of fish was conducted in the forenoon to minimize diurnal effects. Fish were sedated in less than one minute using 0.1 percent ethyl-m-aminobenzoate methane sulfonate (MS-222, Sigma) as the anaesthesia (1 g/L). Blood samples were taken from the caudal vein into ammonium

Table 1 Summary of Treatments Employed during this Study

A: External Exposures

Treatment	Concentration at start of experiment (µl/L)	# of runs	# of fish
Fresh Water Control	--	6	48
Salt Water Control	--	2	16
Paraffin (FW)[a]	100 (fresh)	1	8
Paraffin (FW)	200 (weathered)[b]	1	8
Norman Wells (FW)	100 (fresh)	1	8
Venezuelan (FW)	100 (fresh)	1	8
Norman Wells (FW)	200 (weathered)	1	8
Venezuelan (FW)	200 (weathered)	1	8
Norman Wells (SW)[c]	200 (weathered)	1	8
Venezuelan (SW)	200 (weathered)	2	16[d]
WSF[e] NW (FW)	35 (weathered)	1	8
WSF VEN (FW)	45 (weathered)	1	8

Table 1 cont.

B: IP Injections

Treatment	Dose[f] (µl/kg fish/day)	# of runs	# of fish
IP (FW)	100	1	8
IP (SW)	100	1	8

C: Cortisol

Treatment	Dose (µg cortisol/100 g fish)	# of runs	# of fish
Cortisol injected	0	1	8
Sham injected	80	1	8

D: Aromatics

Treatment§	Concentration (mg/L)	# of runs	# of fish
Benzene	3	1	8
Naphthalene	3×10^{-1}	1	8
Phenanthrene	3×10^{-2}	1	8
Benzo(a)anthracene	3×10^{-3}	1	8
Benzo(a)pyrene	3×10^{-4}	1	8

Notes

[a] Fresh water adapted trout.
[b] initial concentration of 1000 µl/L, weathered for 3 days prior to start of experiment.
[c] Sea water adapted trout.
[d] Experienced 50 percent mortality in both runs.
[e] Water soluble fraction of crude oil.
[f] Dose of Norman Wells crude oil weathered for 3 days.
§ All aromatics, in 100 µl/L of paraffin.

heparinate tubes.

The second gill arch on the right side was removed and examined under the dissecting microscope for gross pathological changes. Gills from half the fish were further processed for light and scanning electron microscopy by fixing minced tissue in 4.0 percent glutaraldehyde buffered with phosphate, and 5.5 percent sucrose for two hours on ice.

Tissues destined for light microscopy were washed three times in the phosphate buffer (10 minutes each), then dehydrated through a seven-stage ethanol series and left overnight in anhydrous ethanol. The next morning, the tissues were embedded with plastic (Spurr, 1969). Thin sections (0.5 μm) were cut on a Sorvall ultramicrotome and stained wih azure blue. Slide preparation continued until three slides of a series of secondary lamellae were achieved. Observations were recorded on fusing phenomena, epithelial separation and chloride cell exposure, location and presence of vacuolation.

For scanning electron microscopy, the mucous layer was removed to increase the surface structural detail (Kendall and Dale, 1979), by placing the tissues on a gentle shaker during the fixation period. Tissues were washed once in 0.1 M phosphate--two percent sucrose buffer (20 minutes), then two times in a sucrose free buffer.

Post fixation was in a two percent osmium tetroxide-phosphate buffer solution for 1.5 hours on ice. The samples were then washed three times in an 0.1 M phosphate buffer (15 minutes each), then dehydrated through an acetone series. The gill pieces were critical point dried and coated with carbon and gold before observation with an AMR 1000 A scanning electron microscope. A systematic procedure was used to observe and evaluate the condition of the gill of each sample. Table 2 is a resume of this procedure.

The observations from light microscopy and scanning electron microscopy were compared for each treatment to check for consistency. Relative changes were identified and accumulated from multiple examinations of the gill tissue samples.

ANALYSIS

The decision was made to employ a subjective evaluation of morphological changes rather than a quantitative morphometric evaluation. Based on preliminary experiments it was evident that the gill changes focused on were distinct from control morphology. In addition it was considered more valuable to examine additional variables relevant to the question of exposure to hydrocarbons rather

than to undertake a detailed statistical evaluation of a limited number of parameters which would necessarily suffer in terms of breadth of investigation.

Nevertheless, the total number of observations or characterizations that support the results of this study is large. The sequence of sample preparation and morphological analysis indicating the number of characterizations made for each experimeht is as follows:

8 fish

Scanning electron microscopy	Light transmission microscopy
4 fish	4 fish
8 gill preparations	20 plastic capsules/fish
36 scans/preparation	80 capsules
288 systematic scans	1 slide/capsule
3 characterizations/scan	80 slides
864 characterizations	5 characterizations/slide
	400 characterizations

The five major morphological criteria characterizing the hydrocarbon effects were: 1. degree of secondary lamellae fusing; 2. epithelial separation; 3. chloride cell exposure; 4. chloride cell location; and 5. presence of vacuoles in the chloride cells. Light transmission microscopy provided information on all 5 of the criteria.

Scanning electron microscopes provided information on 3 (degree of fusing, chloride cell exposure, and chloride cell location) of the criteria in addition to providing vital information on the state of epithelial cell surfaces.

Table 2 Summary of the Protocol Employed to Observe and Evaluate the Gill Samples of Each Fish under the Scanning Electron Microscope

Area of Gill	Minimum # Examined	# of Pictures Taken	Magnification Range
Primary lamella	5	2	150-200x
Primary lamella surface tip	5	1	high
Primary lamella mid-point	5	1	high
Primary lamella base	5	1	high
Series of secondary lamellae	5	2	350-450x
Secondary lamellae surface detail	5	2	high
Gill raker	2	1	150-250x
Gill raker	2	1	high
Gill arch	1	1	medium
Gill arch	1	1	high

Chapter III

RESULTS

Controls, which were repeated for each test run, displayed a consistent behavioural and morphological profile. There were no behavioural abnormalities or gross pathology to note. The results of light and scanning electron microscopy were consistent, providing a detailed picture of both the internal and surface structure of the gill tissue.

The three main areas of the gill examined by scanning electron microscopy were the primary lamella (Figure 1), secondary lamella (Figure 2-1), and the gill raker. The relationships between the primary and the secondary lamellae can be seen in Figure 2-2. The surface of the primary lamella contains numerous single elongated repetitive ridges and intervening grooves resembling a maze-like pattern. Pores break the lamellar surface and the ridges and grooves continue into the pore (Figure 3). Adjacent cell boundaries can be observed at the periphery of each whorl. Large mucus containing goblet cells can be seen protruding from the surface at the tips of the primary lamella. This area may be the primary site for and source of mucilaginous secretions. The secondary lamella, containing the respiratory epithelial surface, resembles

the primary lamellar surface since it consists of ridges with intervening wide grooves. The gill raker surface has low ridges with wide intervening expanses, often with a locus and demarcated by a single continuous ridge at the periphery. In some areas, the gill raker surface is perforated by deep pores (Figure 4).

Figures 5-1 and 5-2 show scanning electron micrographs of the primary lamella of fresh water control trout. The surface epithelium of the tip has ridges that are well defined. Mucous cells are not prominent. The mid-region of the primary lamella has a characteristic depression.

The sections of secondary lamella examined by light microscopy concurred with previous studies. The secondary lamellae resemble sheet-like processes that contain parallel banks of capillaries running traversely to the long axis of the lamella. Pillar cells are present between capillary loops. Details of the epithelial surface of the secondary lamella, as seen by the scanning electron microscope can be seen in Figure 6.

PARAFFIN OIL

Paraffin treated fish, when examined under light and scanning electron microscopy, revealed a picture similar to controls (Figures 7, 8, 9). The cellular layers were

intact and little or no fusing was in evidence (Table 3). Plasma ions for these same fish showed little response (Table 4). Chloride cells were usually below the epithelium and attached to the basal lamina. Chloride cell exposure was minimal in these fish. The location of the chloride cell was always basal, with the exception of the fresh 100 µl/L paraffin exposure where chloride cells extended part way up the secondary lamella. The degree of chloride cell vacuolization was mininal.

DISPERSIONS

Examination under the dissecting microscope for gross pathology revealed a picture consistent with controls for all runs except the dispersion regimes. All fish exposed to emulsions had observable oil particles at the base and tips of the primary lamellae. Substantial amounts of mucous material and localized hemorrhaging was seen. These changes were more pronounced in the experiments using Venezuelan oil.

Varied and at times severe histopathology was displayed among the six oil-in-water dispersion experiments. Curling of the secondary lamella was a common observation, as illustrated in Figure 10, in fish exposed to 200 µl/L weathered petroleum. Fusing of lamella was more pronounced

in fish exposed to weathered Venezuelan oil. The effect of 100 µl/L dosages of fresh oil-and-water dispersions on the secondary lamella were less dramatic than the effect of weathered oil-and-water dispersions. Fresh Norman Wells oil (100 µl/L) exposed fish displayed a thickening of the secondary lamella and prominent pores along the primary lamella. Scanning electron microscopy of fresh Venezuelan oil (100 µl/L) exposed fish revealed secondary lamella that were equally spaced and in good condition (Figure 11).

The effects of weathered Venezuelan oil (200 µl/L) in fresh water were more pronounced on other parts of the branchial tissue than those produced by Norman Wells oil. Figure 12 is exemplary of the gill raker surface of fish exposed to either Venezuelan or Norman Wells weathered oil-in-water dispersions. In the case of weathered Venezuelan oil exposed fish, bulging cells showed reduced ridge definition, and empty pores. These empty pores correlate well with the physical observation that more mucous covered the gills of fish exposed to weathered Venezuelan oil in fresh water.

Also characteristic of fish exposed to a weathered Venezuelan oil-in-water dispersion in fresh water was the proliferation of chloride cells and an increase in their exposed apical cell surface. Fortunately, chloride cells can be easily identified because of distinctive ridges on

their cell surface (Figure 13).

Both sodium and chloride ion levels in the plasma of fish exposed to weathered and fresh oil-in-water dispersions were significantly lower than their control counterparts (Table 4).

Exposure to mechanically dispersed oil in salt water also resulted in gill abnormalities with fusing evident and occasional entrapping of oil droplets. Fusing was extensive and variable with Venezuelan oil. However, there was little epithelial detachment. Chloride cells were located mainly at the base of the secondary lamella, and their surfaces were only minimally exposed. Some vacuolation of chloride cells was evident in the salt water oil-in-water dispersions.

Figure 14 shows the general appearance of salt water adapted primary lamellae exposed to weathered (200 µl/L) oil-in-water dispersions. Extensive multiple fusions were common. Closer examination of the secondary lamella along the length of the primary lamellae revealed more extensive fusing in the case of weathered Venezuelan oil exposed fish in salt water (Figure 15).

Figure 16 provides a comparison of the information provided by light and scanning electron micrographs. In this case, the evidence indicates that multiple fusions may contain hydrocarbon particulates trapped between the

lamellae.

The structure of microridges on the epithelial cells near the tip of the secondary lamella often showed some degeneration. This degeneration appeared more pronounced in fish exposed to weathered Venezuelan oil in salt water (Figure 17).

Figure 18 provides a comparison of light micrographs of the effect of dispersions on the secondary lamella of trout. Curling of the primary lamellae, epithelial separation and chloride cell damage were prominent in the fresh water weathered experiments. With Venezuelan oil treatment (1000 µl/L, weathered to 200 µl/L), the trout gills showed an abnormal secondary lamellar epithelium, dislodged from the pilar cell system. Detachment of chloride cells from the underlying epithelial layer was seen. In the weathered Norman Wells emulsion experiments in fresh water, the epithelial lifting was not as marked. There was evidence of lamellar fusion and of vacuolation of the chloride cells. Figure 19 provides corroborating evidence based on information provided by scanning electron microscopy. It is interesting to note that the sodium, potassium and chloride ion levels in the plasma of salt water adapted fish exposed to weathered oils (200 µl/L) were significantly higher than in the salt water control fish (Table 4). The calcium ion concentration was significantly lower

than the controls.

WATER SOLUBLE FRACTION

Figure 20 provides light micrographs and Figure 21 provides information based on scanning electron micrography for this treatment group. The epithelial cell surfaces of fish exposed to the water soluble fraction often showed extensive reduction of the ridge patterns. This reduction in cell surface may be responsible for the reduction in potassium and chloride ion concentration in the blood of these fish (Table 4).

INTERNAL HYDROCARBON LOAD

In the intraperitoneal injection experiments, in both fresh and salt water, gill morphology approximated that of the appropriate control condition. There was no fusing of secondary lamellae in the two water soluble fraction runs and very rare fusing (<1%) in the IP treatments. The layer of epithelium was always secure and chloride cell exposure was minimal. The chloride cells were located at the base of the secondary lamella, only rarely displaying vacuolation. In both regimes three out of four ion levels measured were not significantly

different from the corresponding controls (Table 4). Figure 20-1 and Figure 22 provide examples of gill tissue sampled.

CORTISOL EXPERIMENT

Cortisol injected rainbow trout did not display gill abnormalities similar to those observed upon exposure to hydrocarbons, but an apparent increase in chloride cell numbers along the length of the lamella was observed Figure 23-2). Control fish sham injected with corn oil displayed undisturbed gill structure (Figure 23). The plasma ion profiles for cortisol injected fish did not differ significantly from those of the sham injected control fish.

AROMATIC HYDROCARBONS

The effect of aromatic hydrocarbons at the concentrations tested was, in general, not severe. Fusing was rarely observed (<1%) with benzo(a)anthracene. Epithelial separation was rare to non existent, except with benzo(a)-pyrene. Chloride cell exposure was always present to a degree. Chloride cells were located along the length of the secondary lamellae, except with benzene where they were most often at the base. Vacuolation seemed to increase

with the molecular weight of the aromatic hydrocarbon. Only rare vacuoles were observed with benzene while vacuolation was extensive with the benzo(a)pyrene exposure.

Figures 24 and 25 are light and scanning electron micrographs representative of the aromatic hydrocarbon exposed fish analyzed.

Table 3 Summary of Gill Morphology following Seven Days of Treatment, Comparing Different Modes of Exposure

Exposure Mode	Degree[a] of Fusing	Epithelial Separation	Cl-Cell[c] Exposure	Cl-Cell Location	Cl-Cell Vacuoles
Controls					
Fresh water control	none	none	none	basal	rare
Salt water control	rare	none	none	basal	none
Experimental Controls					
Paraffin 100[d]	none	none	little	(basal)[b]	little
Paraffin 200[e]	none	none	rare	basal	none
Crude Oil Dispersions					
Fresh Water:					
NW 100[f]	rare	rare	some	length	common
VEN 100[g]	none	none	little	basal	some
NW 200[h]	some	some	extensive	length	extensive
VEN 200[h]	extensive	extensive	extensive	length	some
Salt Water:					
NW 200[h]	some	none	little	(basal)	some
VEN 200[h]	variable	none	some	(basal)	common
Water Soluble Fraction					
WSF - NW (0.45 µl/L)	none	none	rare	basal	rare
WSF - VEN (0.35 µl/L)	none	none	little	basal	none
Intraperitoneal Injection[i]					
Fresh Water	little	none	some	(basal)	rare
Salt Water	rare	none	none	basal	none
Cortisol Experiment					
Sham injected	none	none	some	basal	rare
Cortisol injected	none	none	common	length	rare

Table 3 Continued

Exposure Mode	Degree[a] of Fusing	Epithelial Separation	Cl-Cell[c] Exposure	Cl-Cell Location	Cl-Cell Vacuoles
Aromatic Hydrocarbons					
Benzene[j]	none	none	some	basal	rare
Naphthalene[k]	none	rare	some	length	some
Phenanthrene[l]	none	rare	some	length	common
Benzo(a)anthracene[m]	rare	none	little	length	common
Benzo(a)pyrene[n]	none	little	some	length	extensive

Notes to Table 3

[a] Use of terms: none (0%) < rare (1%) < little (10%) < common (30%) <extensive (50%).

[b] Chloride cells found in basal region and extending part way up secondary lamella.

[c] Chloride-type cell.

[d] Fresh, 100 µl/L exposure of paraffin.

[e] Paraffin, 1000 µl/L exposure, weathered for 3 days 200 µl/L.

[f] Norman Wells, fresh 100 µl/L exposure.

[g] Venezuelan, fresh 100 µl/L exposure.

[h] crude oil, 1000 µl/L exposure, weathered for 3 days to 200 µl/L.

[i] Injection of Norman Wells, 100 µl/kg fish weight/day.

[j] 3.0 mg/L of benzene in 100 µl/L of paraffin.

[k] 3×10^{-1} mg/L of naphthalene in 100 µl/L of paraffin.

[l] 3×10^{-2} mg/L of phenanthrene in 100 µl/L of paraffin.

[m] 3×10^{-3} mg/L of benzo(a)anthracene in 100 µl/L of paraffin.

[n] 3×10^{-4} mg/L of benzo(a)pyrene in 100 µl/L of paraffin.

Table 4 Summary of Plasma Ion Levels Following Seven Days of Treatment (same fish as in Table 3)

Exposure Mode	Plasma Ion			
	Na^+	K^+	Ca^{++}	Cl^-
Controls				
Fresh water	C[a]	C	C	C
Salt water	C	C	C	C
Experimental Controls				
Paraffin 100[d]	C	–	–	C
Paraffin 200[e]	C	–	–	C
Crude Oil Dispersions				
Fresh Water:				
NW 100[f]	L[b]	–	–	L
VEN 100[g]	L	–	–	L
NW 200[h]	L	–	–	L
VEN 200[h]	L	–	–	L
Salt Water:				
NW 200[h]	H[c]	H	L	H
VEN 200[h]	H	H	L	H
Water Soluble Fraction				
WSF – NW (0.45 µl/L)	C	L	C	L
WSF – VEN (0.35 µl/L)	C	L	C	L
Intraperitoneal Injection[i]				
Fresh Water	C	C	C	L
Salt Water	C	C	L	C
Cortisol Experiment				
Sham injected	C	C	C	C
Cortisol injected	C	C	C	C
Aromatic Hydrocarbons				
Benzene[j]	L	–	–	L
Naphthalene[k]	C	–	–	C
Phenanthrene[l]	C	–	–	C
Benz(a)anthracene[m]	C	–	–	L
Benzo(a)pyrene[n]	C	–	–	C

Notes to Table 4

[a] Controls or not significantly ($p \leq 0.05$) different from controls.

[b] Significantly ($p \leq 0.05$) lower than controls.

[c] Significantly ($p \leq 0.05$) higher than controls.

[d] Fresh, 100 µl/L exposure of paraffin.

[e] Paraffin, 1000 µl/L exposure, weathered for 3 days 200 µl/L.

[f] Norman Wells, fresh 100 µl/L exposure.

[g] Venezuelan, fresh 100 µl/L exposure.

[h] crude oil, 1000 µl/L exposure, weathered for 3 days to 200 µl/L.

[i] Injection of Norman Wells, 100 µl/kg fish weight/day.

[j] 3.0 mg/L of benzene in 100 µl/L of paraffin.

[k] 3×10^{-1} mg/L of naphthalene in 100 µl/L of paraffin.

[l] 3×10^{-2} mg/L of phenanthrene in 100 µl/L of paraffin.

[m] 3×10^{-3} mg/L of benzo(a)anthracene in 100 µl/L of paraffin.

[n] 3×10^{-4} mg/L of benzo(a)pyrene in 100 µl/L of paraffin.

- information not available.

Information for this table was provided courtesy of Mr. M.P. Wong (M.Sc. Thesis, 1982, University of Ottawa). Statistical comparisons of test and control data was made using one-way analysis of variance (ANOVA). For further details please refer to Appendix A.

Figure 1

Scanning electron micrograph of one primary lamella (PL) with numerous branching equally spaced secondary lamellae (SL), representative of the control condition, (x600).

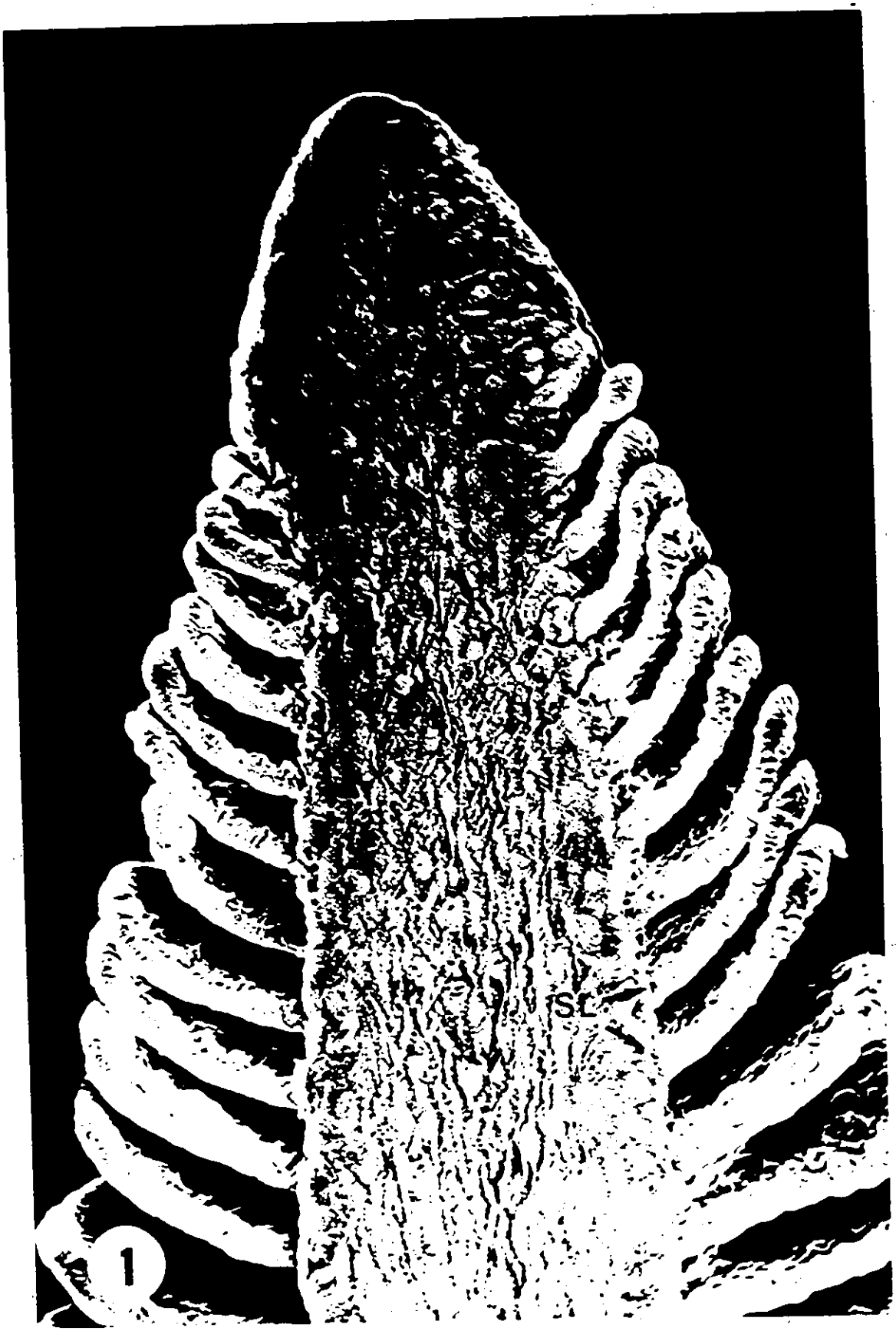
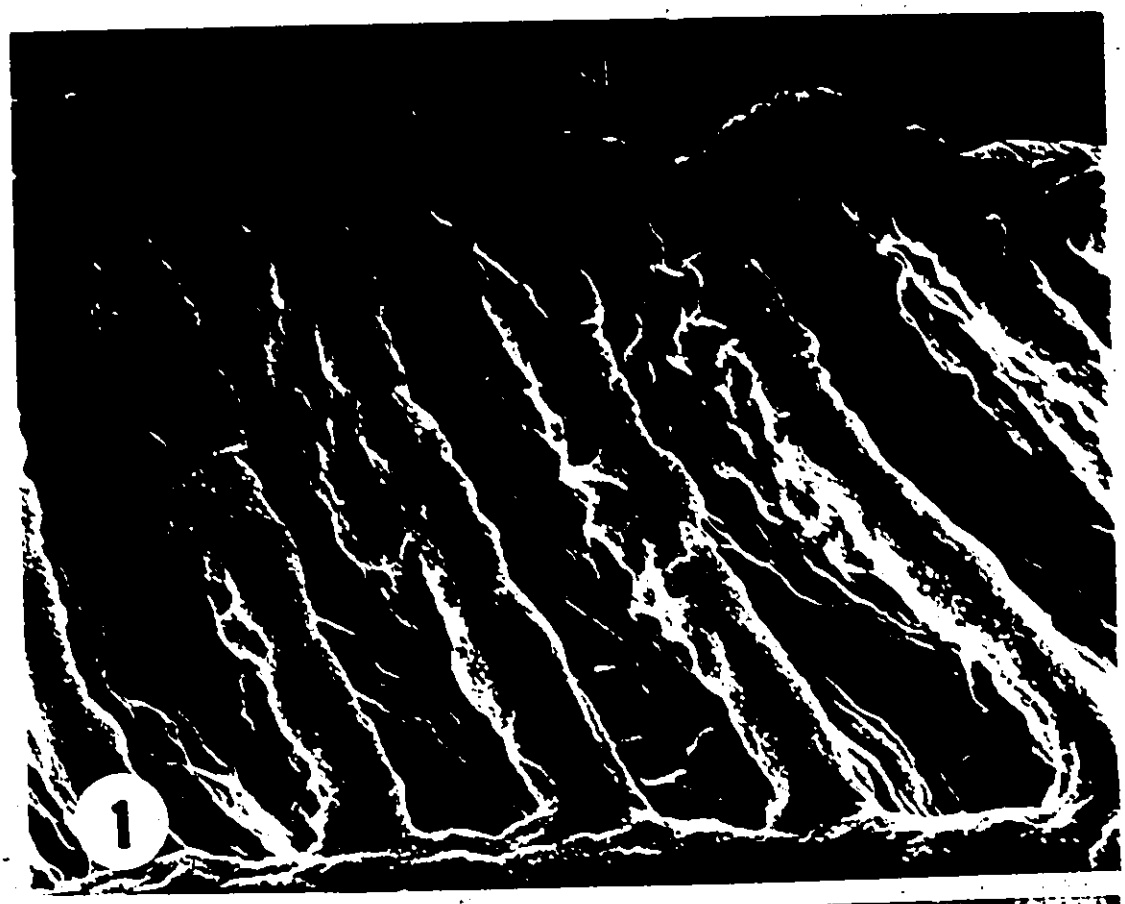

SL
1

Figure 2

Scanning electron micrograph of secondary lamellae of the control trout.

Figure 2-1. Equally spaced nature of the healthy secondary lamellae (SL), (x800).

Figure 2-2. Overview of the entire secondary lamellar structure (arrows) and its relationship to the primary lamella (PL), (x375).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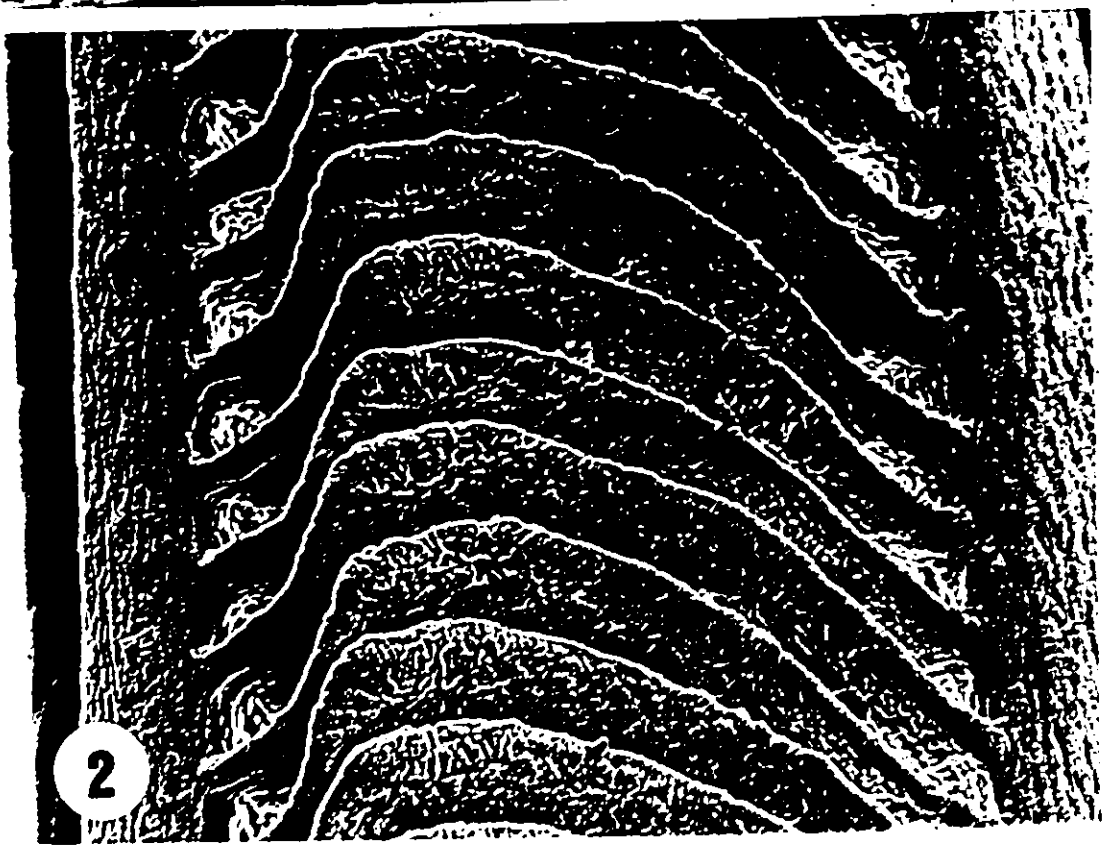
2

Figure 3

Scanning electron micrographs of salt water adapted rainbow trout.

Figure 3-1. Primary lamella (PL), (x172).

Figure 3-2. Series of secondary lamellae (SL) with the presence of numerous chloride cell types at their bases, (x423).

Figure 3-3. Pore with chloride cell emerging (arrow), (x1910).

Figure 3-4. Higher magnification of the chloride cell-type (CC), (x6800).

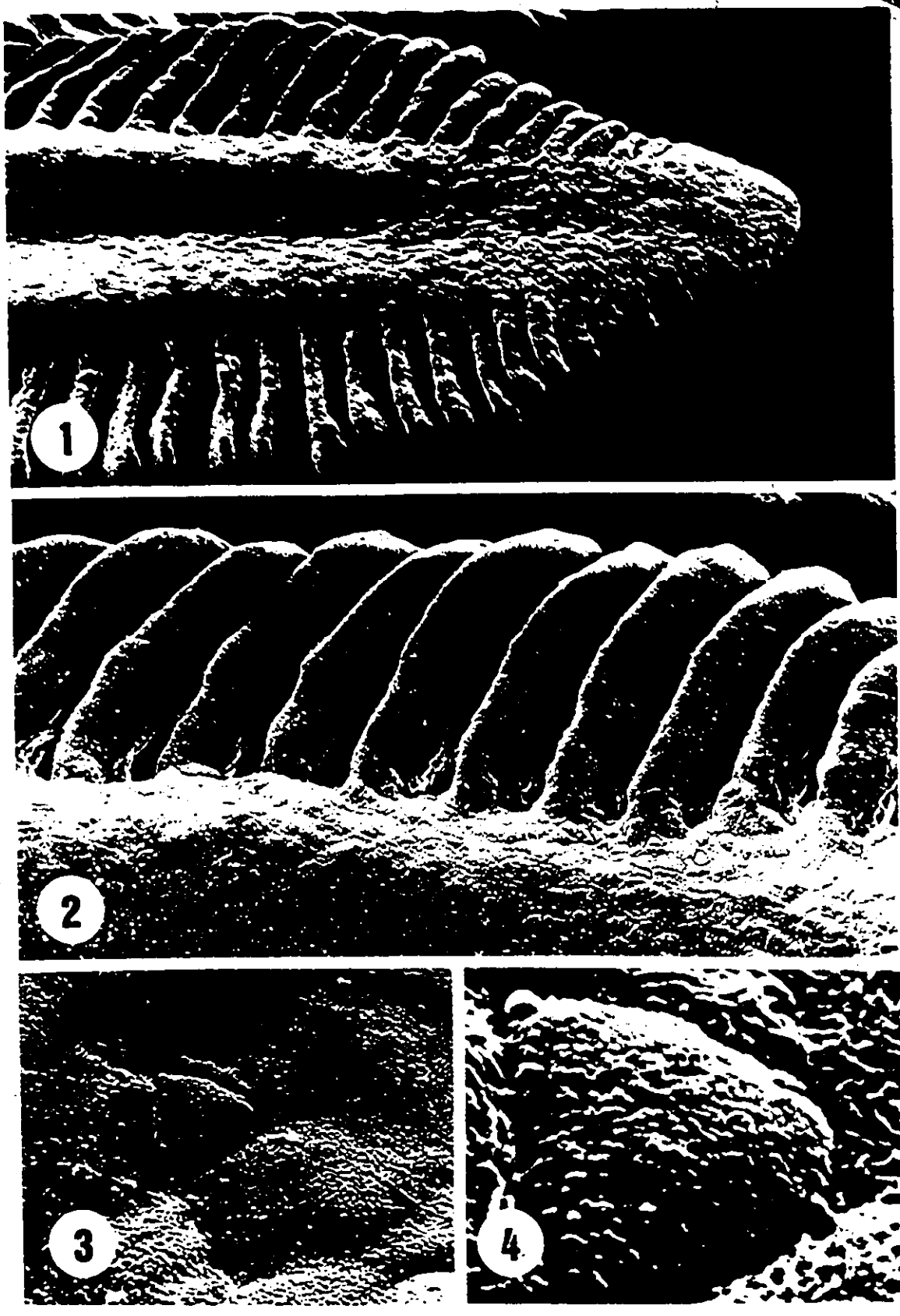
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Figure 4

Scanning electron micrographs of the gill raker of fresh water control trout.

Figure 4-1. Gill raker (gr) tip in healthy condition, (x250).

Figure 4-2. Gill raker surface showing prominent epithelial cell ridges and pores (arrows), (x4240).


1

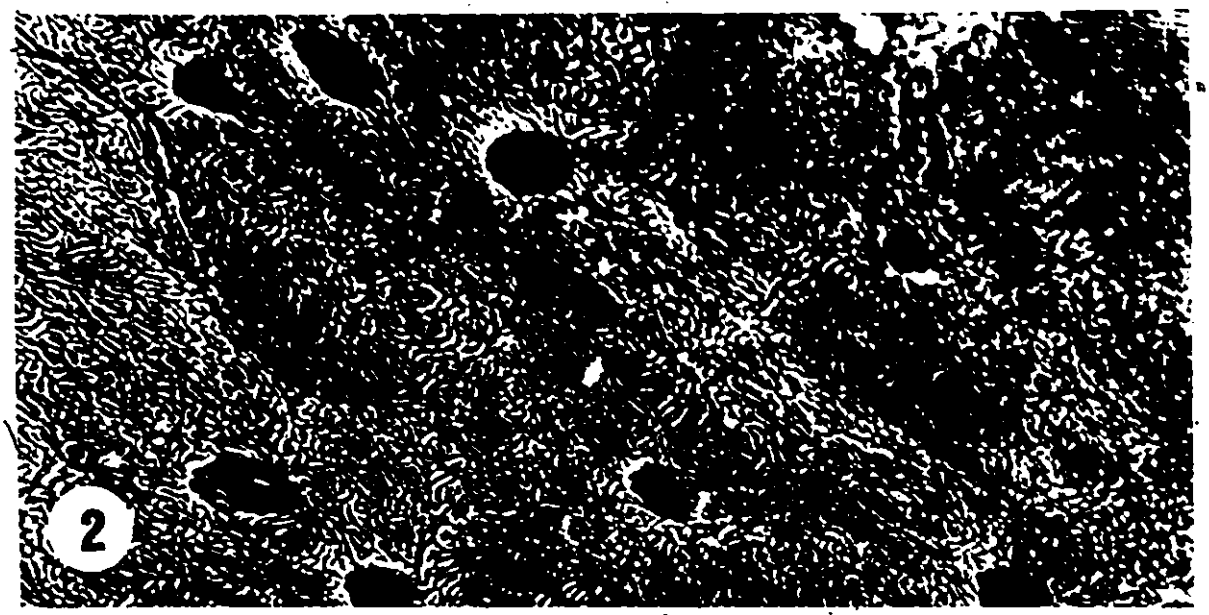
2

Figure 5

Scanning electron micrographs of the primary lamella of fresh water control trout.

Figure 5-1: Surface epithelium of the tip of the primary lamella. Ridges are well defined and mucous cells are not seen, (x3040).

Figure 5-2: Mid-region of the primary lamella showing characteristic depression, (x1520).


1


2

Figure 6

Scanning electron micrographs of the secondary lamella surface of fresh water control fish.

Figure 6-1. Tip of secondary lamella, no chloride cells seen, (x2010).

Figure 6-2. Higher magnification of previous figure, showing well defined ridges (arrows) and micro-ridges between the ridges, (x6000).

Figure 6-3. Secondary lamella (SL) surface along the length of the lamella, showing cell demarcation, (x2760).

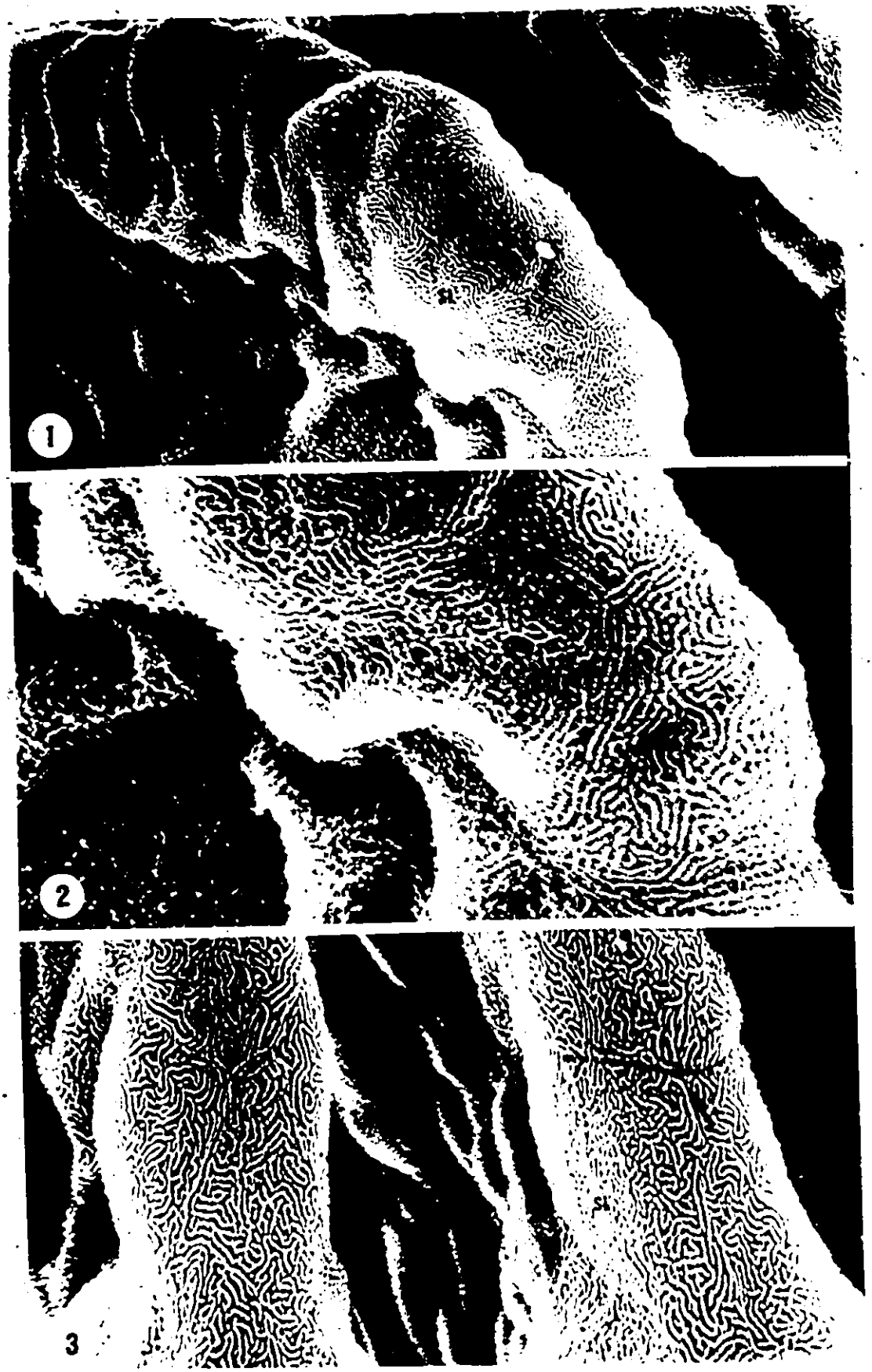
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Figure 7

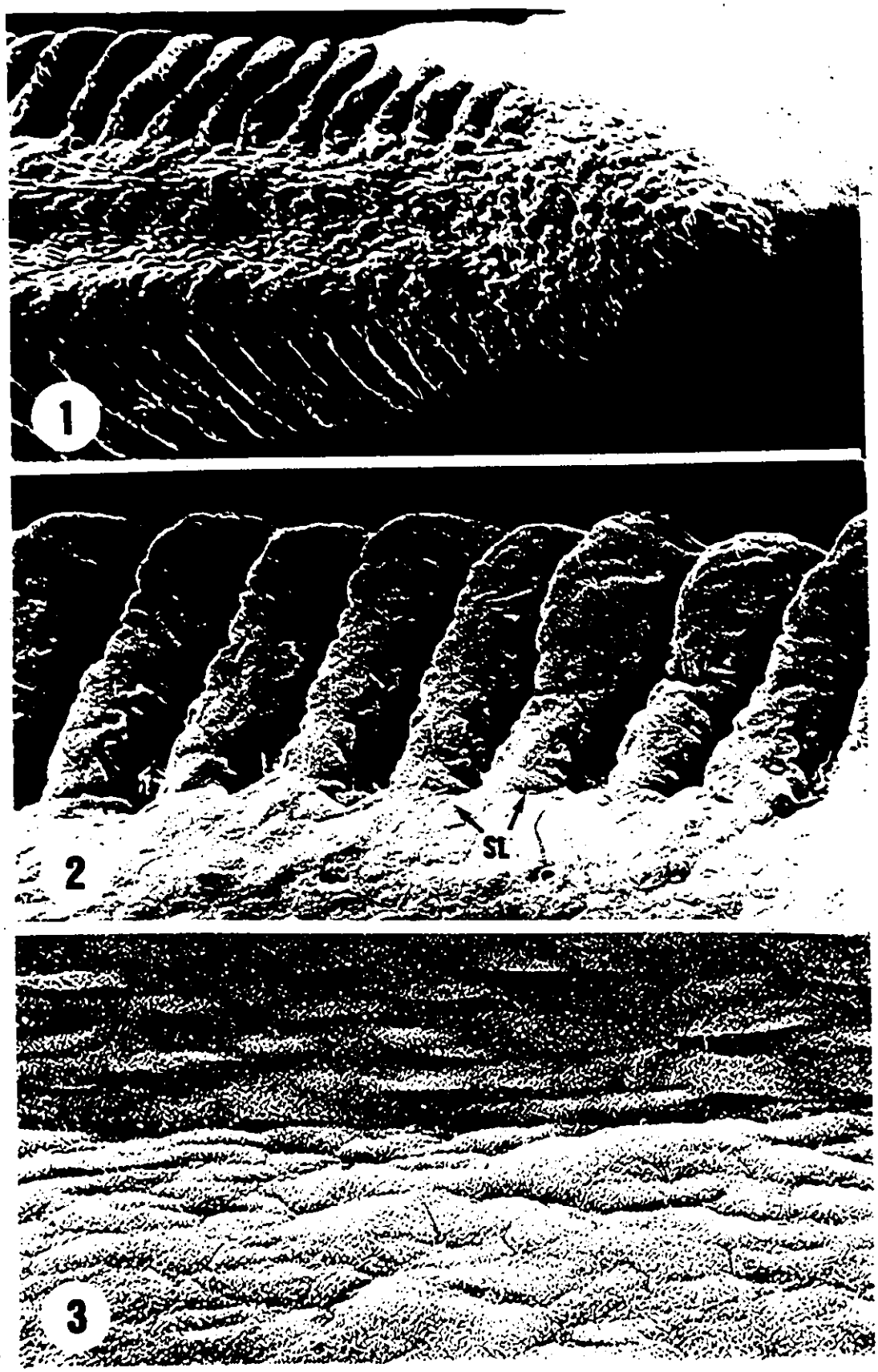
Scanning electron micrograph of trout gill tissue exposed to 100 µl/L of fresh paraffin.

Figure 7-1. Primary lamella (PL), (x220).

Figure 7-2. Series of secondary lamellae (SL), (x548).

Figure 7-3. Mid-region of the primary lamella, (x908).

Surface of gills of paraffin exposed fish are similar to controls suggesting the physical effects of the presence of an oil are not important in producing gill damage.


1
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SL
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Figure 8

Scanning electron micrographs of trout exposed to 200 µl/L of weathered paraffin.

Figure 8-1. Primary lamella (PL), (x232).

Figure 8-2. Series of secondary lamellae (SL), (x568).

Figure 8-3. Mid-region of the primary lamella, (x960).

Paraffin exposed experimental controls resemble controls, lacking significant morphological alterations.

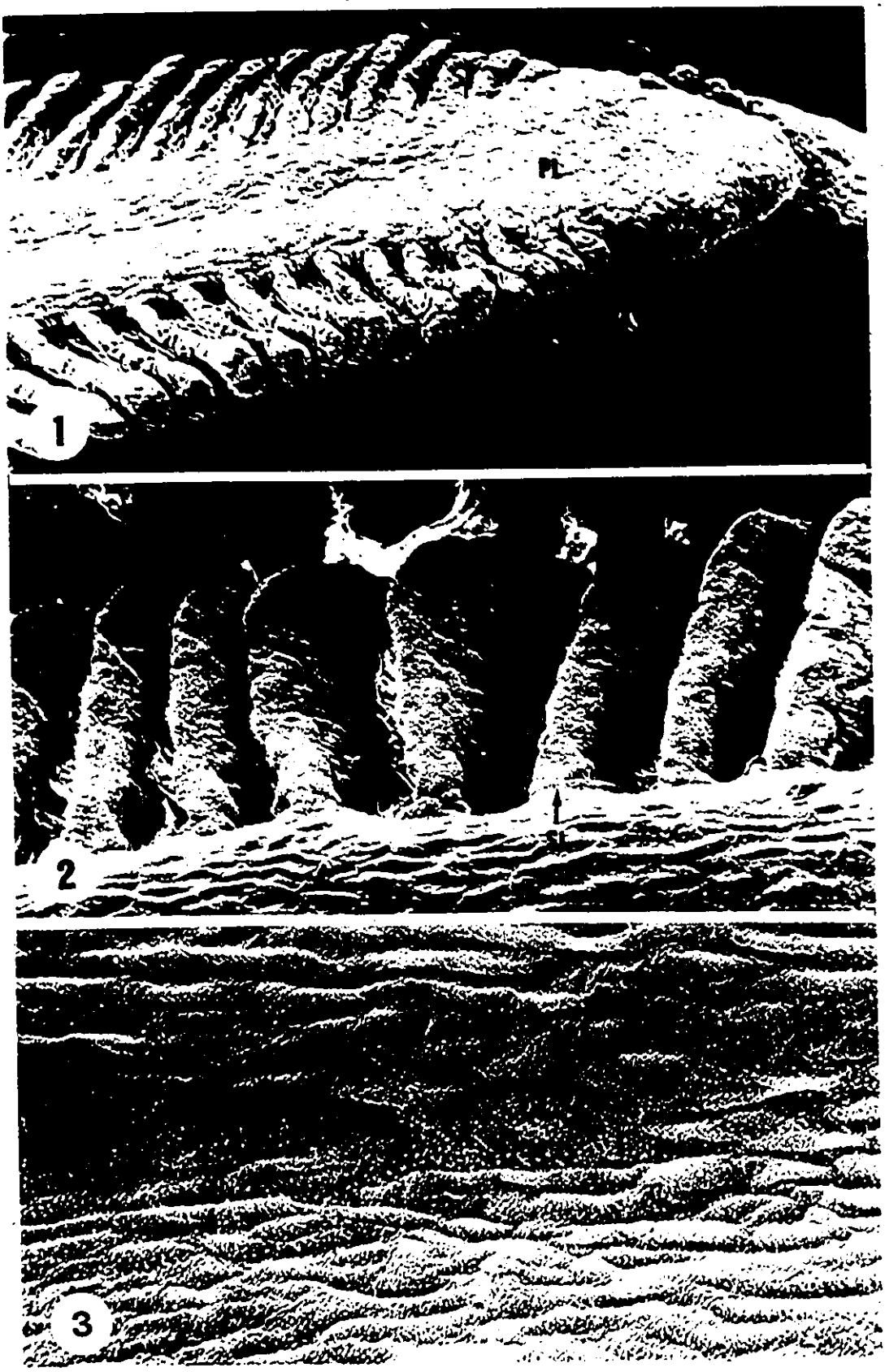
PL
1
2
3

Figure 9

Composite of micrographs of gill tissue exposed to weathered paraffin, (200 µl/L).

Figure 9-1. Scanning electron micrograph of a sliced secondary lamella showing the covering epithelial layer, a chloride-type cell (CC), and the "H" shaped pilar cell (pc), (x2840).

Figure 9-2. Scanning electron micrograph of a series of secondary lamellae (SL) and adjoining primary lamella, sliced to show internal structure, (x380).

Figure 9-3. Light micrograph of two secondary lamellae tips, as in figure 9-1, showing the pilar cell (pc) system and a covered chloride cell (CC), (x800).

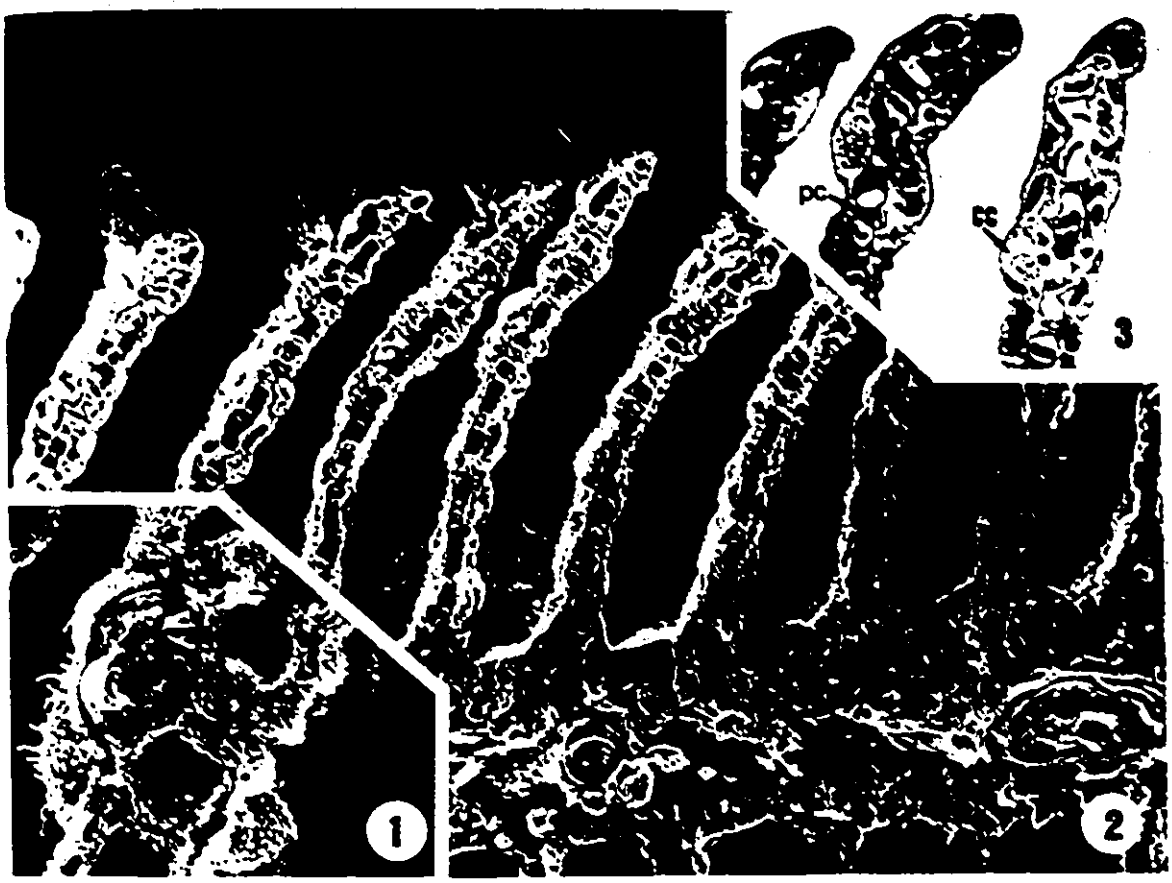
pc
fc
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Figure 10

Scanning electron micrographs of weathered 200 µl/L dosage of an oil-in-water dispersion on the secondary lamellae of fresh water acclimated trout.

Figure 10-1. Fresh water control showing evenly spaced secondary lamellae, (x496).

Figure 10-2. Norman Wells exposed fish, showing some fusing (F) of the secondary lamellae, (x413).

Figure 10-3. Venezuelan exposed fish, displaying more extensive fusing (F), (x480).

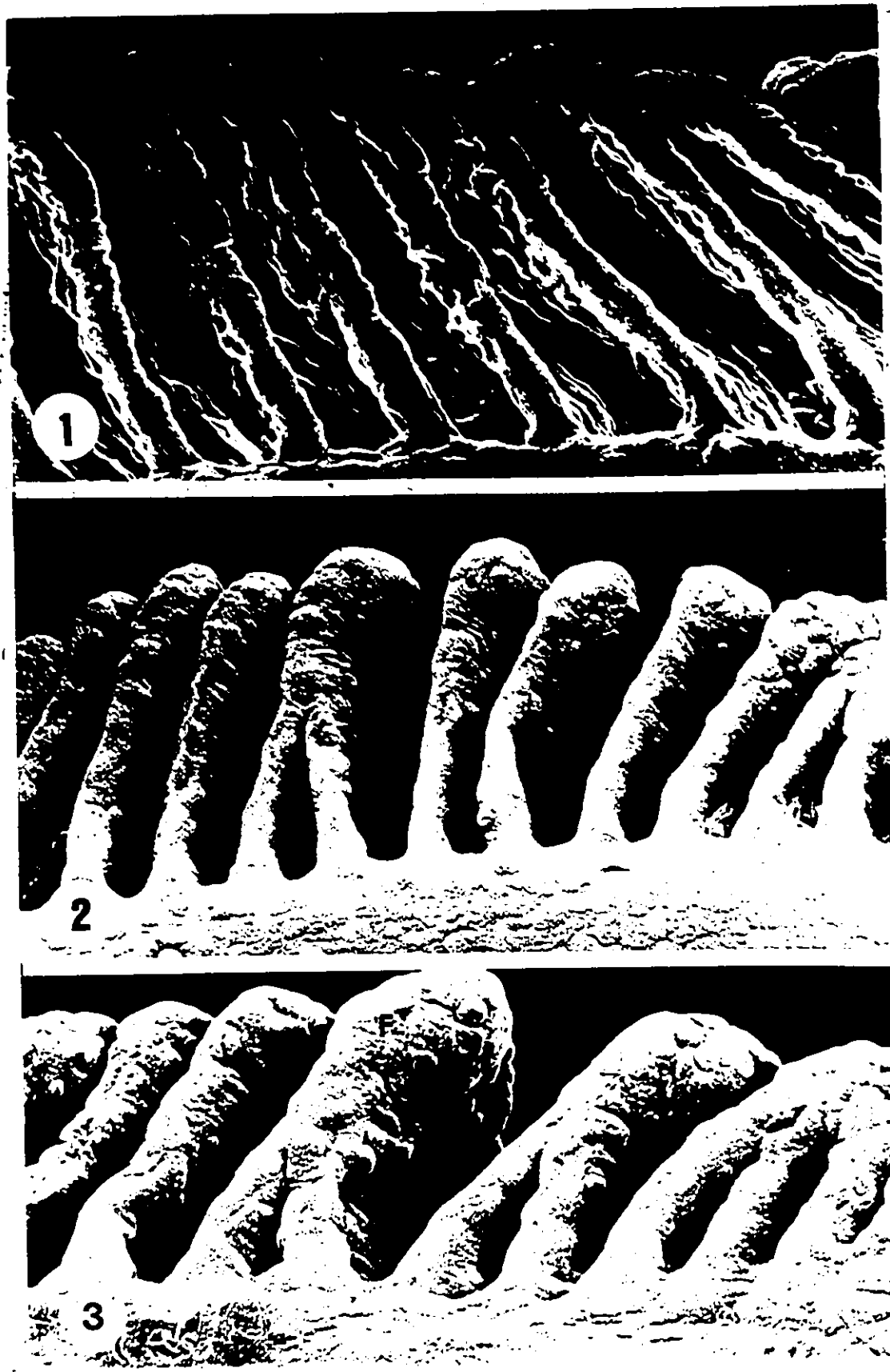
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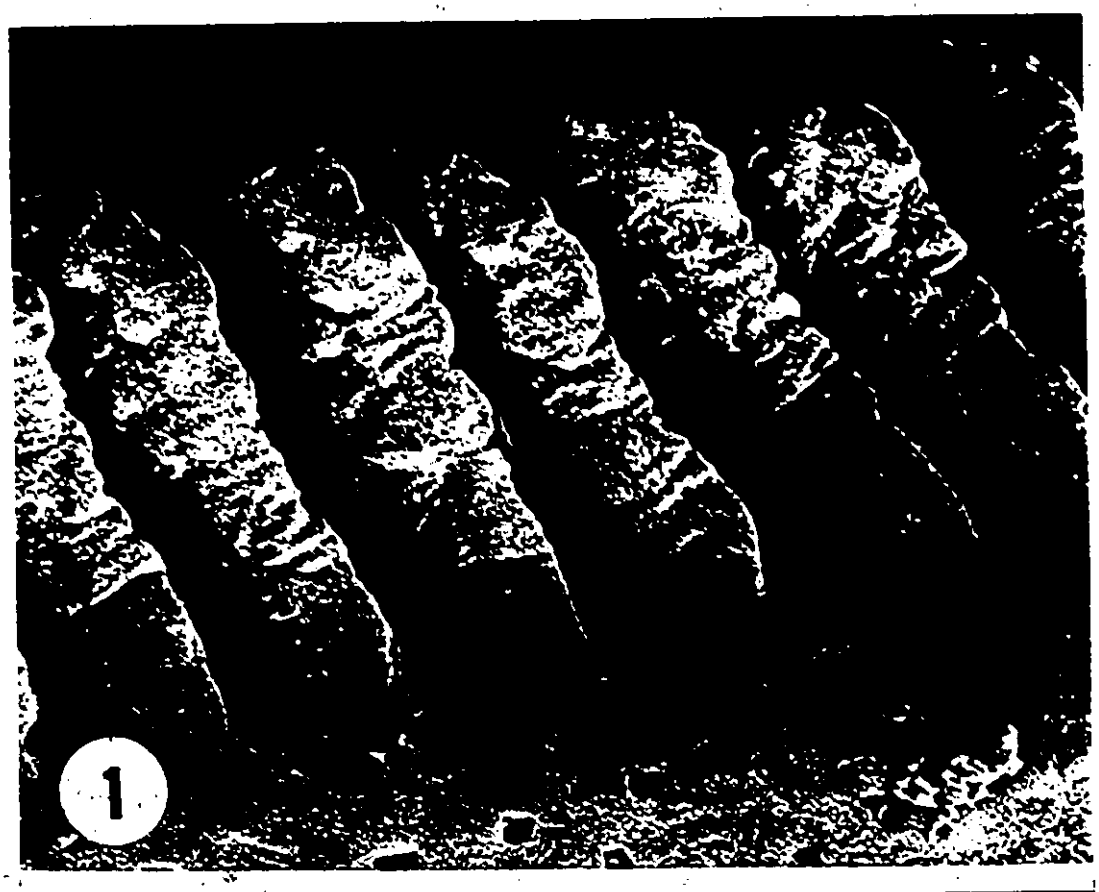
Figure 11

The effect of a 100 µl/L dosage of fresh oil-in-water dispersions on the secondary lamellae.

Figure 11-1. Norman Wells, some thickening of the secondary lamellae is apparent and pores (arrow) are more prominent, (x746).

Figure 11-2. Venezuelan, secondary lamellae (SL) are equally spaced and in good condition, (x481).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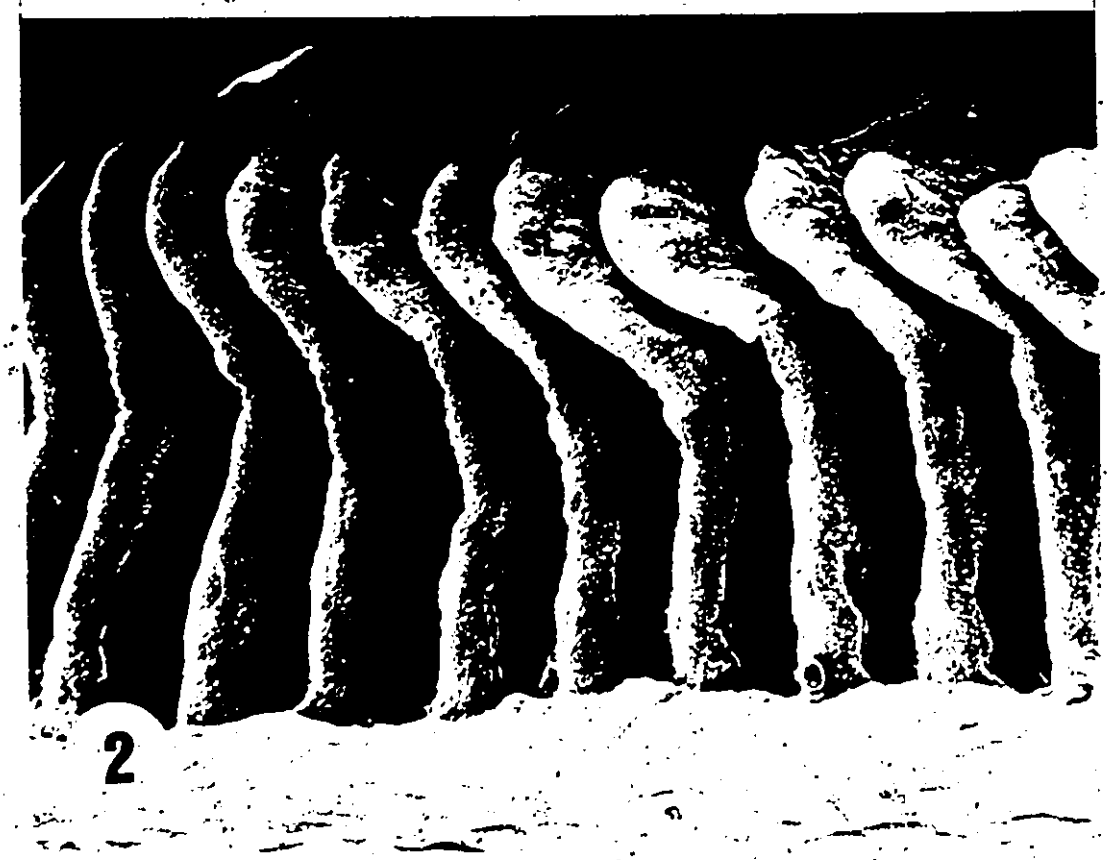
2

Figure 12

Scanning electron micrographs of the gill raker exposed to 200 µl/L of weathered oil-in-water dispersions.

Figure 12-1. Normal Wells, bulging cell has ridges, (x2500).

Figure 12-2. Venezuelan, bulging cell has reduced ridge definition, (x2500).

Figure 12-3. Norman Wells, pores (arrows) are full, (x1160).

Figure 12-4. Venezuelan, pores (arrows) empty, (x1160).

Weathered Venezuelan oil appears to have a more pronounced effect than weathered Norman Wells oil.

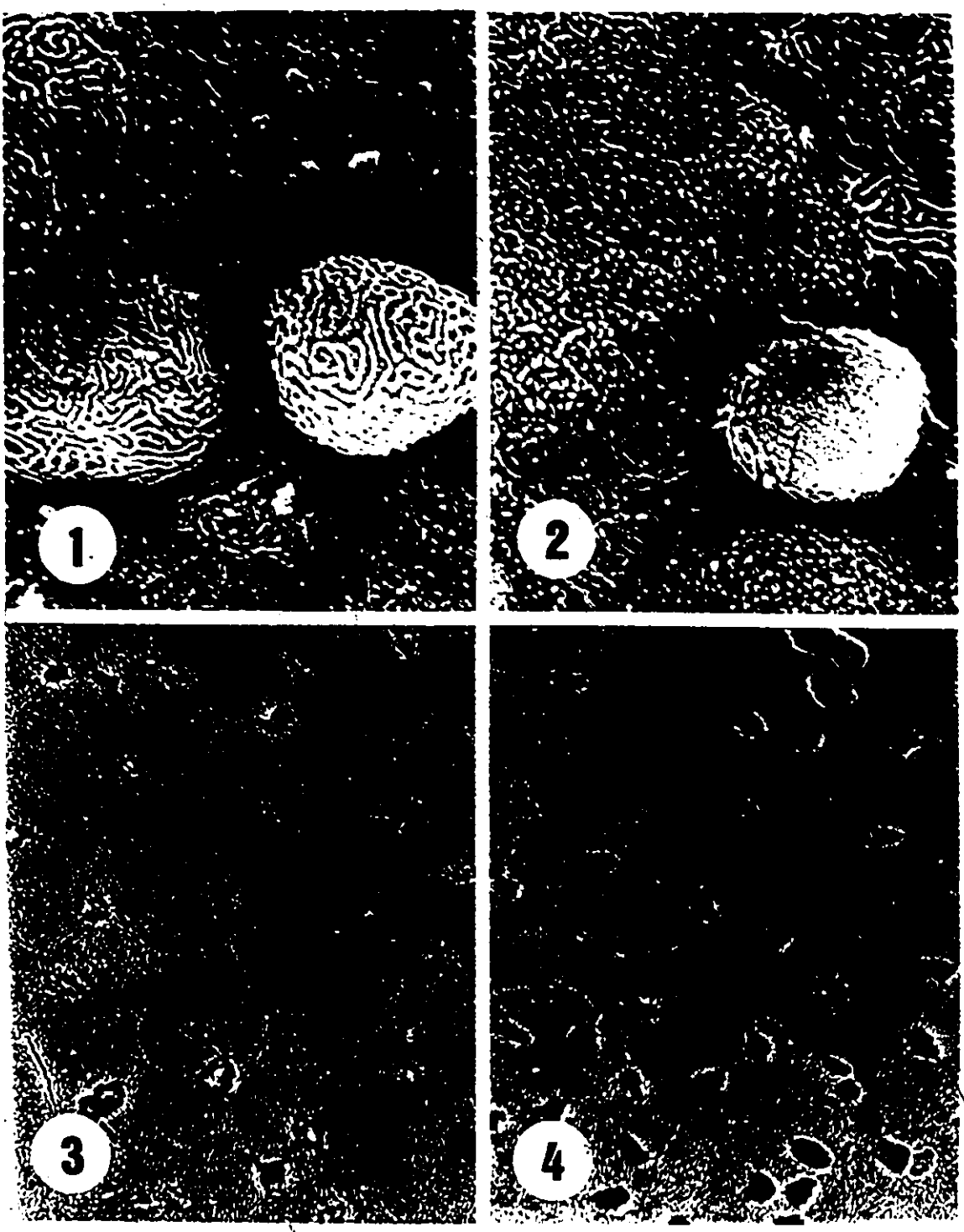
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Figure 13

Scanning electron micrographs showing the effect of the weathered 200 µl/L dispersion of Venezuelan crude oil.

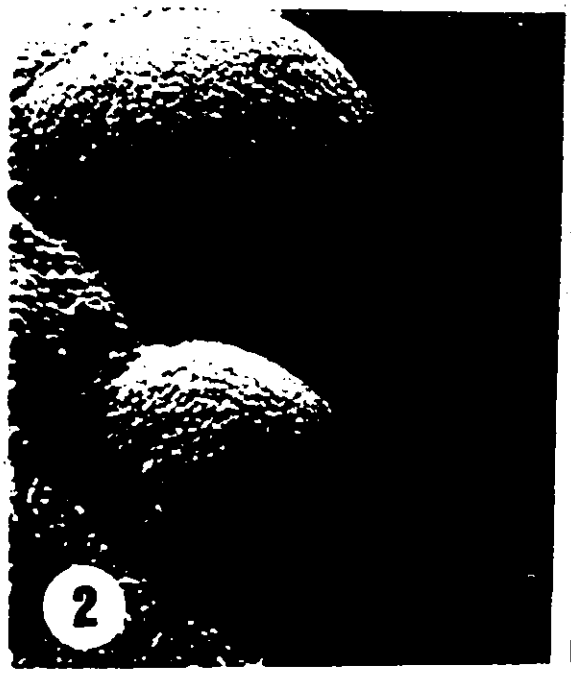
Figure 13-1. Top view of a non-fused secondary lamella (SL) showing bulging surfaces of chloride-type cells, (x1360).

Figure 13-2. Higher magnification showing a chloride cell (CC), (x4960).

Figure 13-3. View of chloride cell (CC) showing its distinctive non-ridged cell surface beside epithelial cells (E) with distinctive ridges, (x4960).

Proliferation of chloride cells and an increase in their exposed apical cell surface are characteristic of this exposure.


1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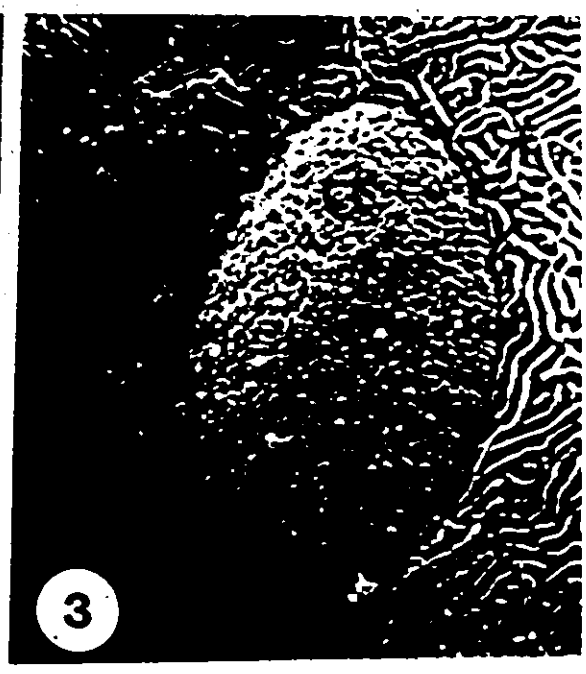

3

Figure 14

Scanning electron micrographs showing the effect of a weathered (200 µl/L) oil-in-water dispersion on salt water adapted rainbow trout.

Figure 14-1. Salt water control, primary lamella (PL), (x208).

Figure 14-2. Norman Wells exposed, showing primary lamella (PL) with multiple fusions (MF), (x200).

Figure 14-3. Venezuelan exposed, primary lamella (PL) tip displaying multiple fusions (MF), (x204).

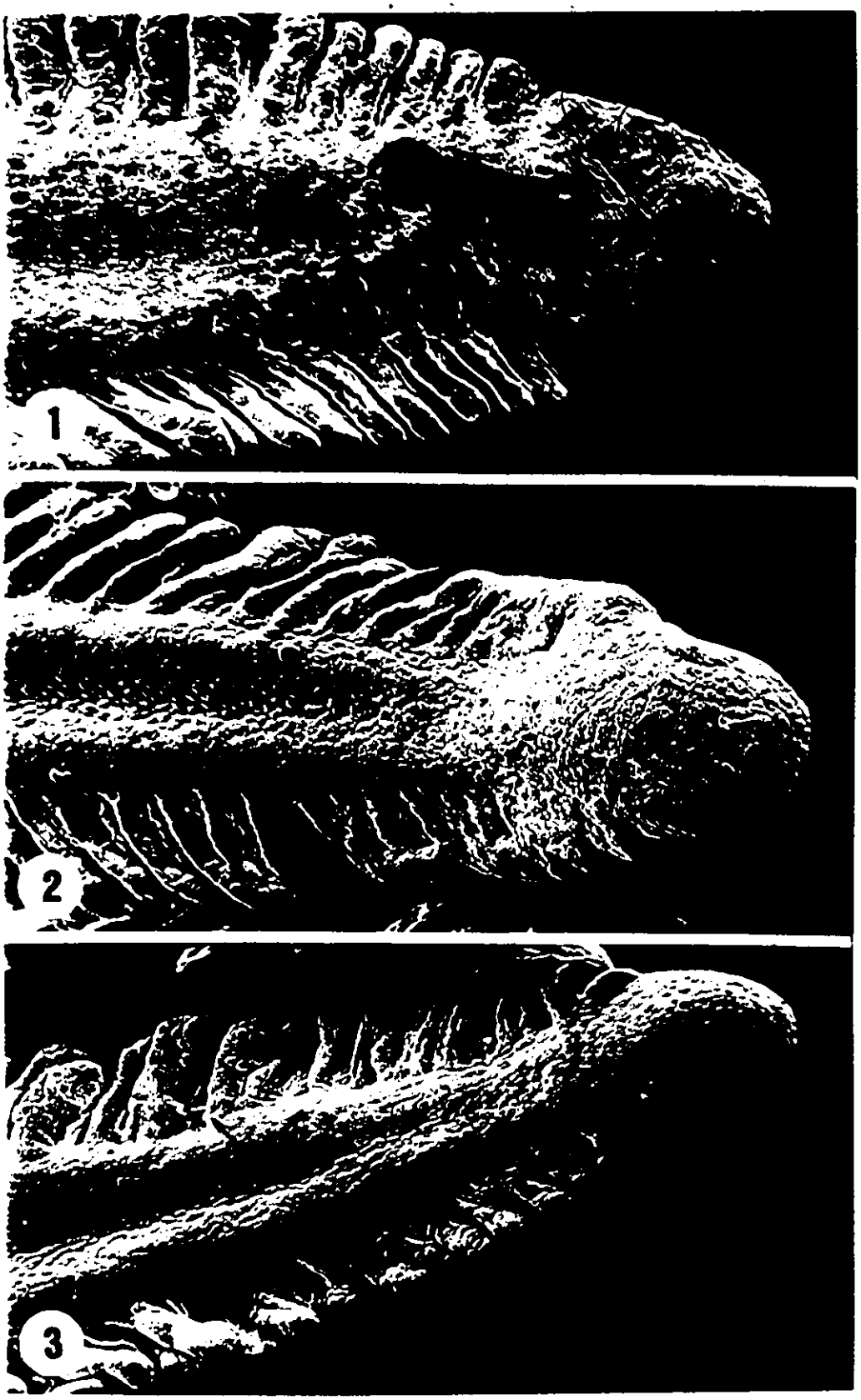

1
2
3

Figure 15

Scanning electron micrographs showing the effect of weathered 200 µl/L oil-in-water dispersions on the salt water adapted rainbow trout secondary lamellae.

Figure 15-1. Secondary lamella (SL) of salt water experimental control, (x420).

Figure 15-2. Secondary lamellae exposed to Norman Wells crude oil, showing some fusing of lamella, (x420).

Figure 15-3. Secondary lamellae exposed to Venezuelan crude oil showing extensive (MF) fusing along the length of the primary lamella, (x324).

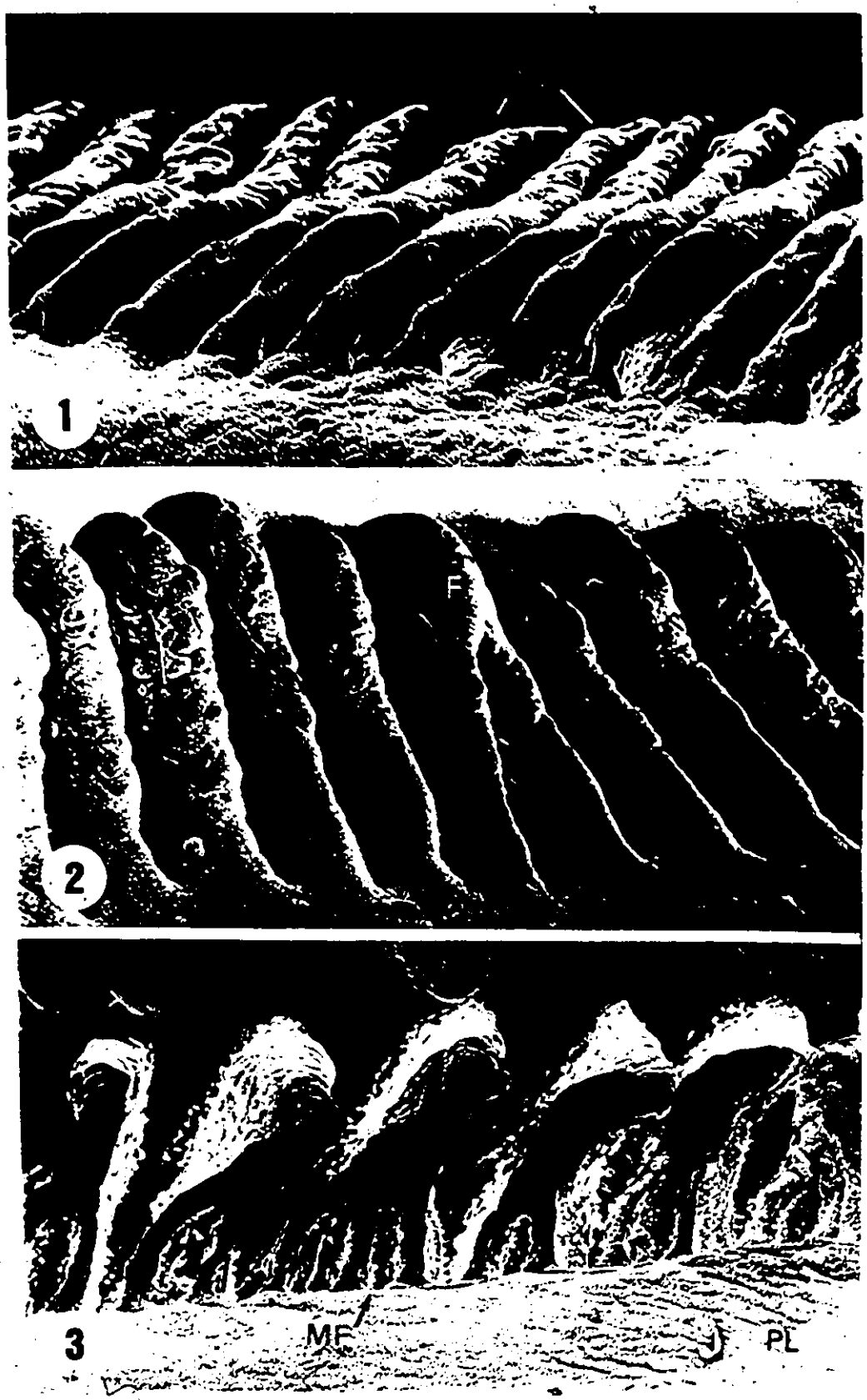

1
F
2
MF
PL
3

Figure 16

Comparison of light and scanning electron micrographs of salt water adapted trout exposed to weathered Venezuelan (200 µl/L) oil-in-water dispersions.

Figure 16-1. Series of secondary lamellae showing multiple fusions (MF), compare with figure 20-3, (x416).

Figure 16-2. Base of a secondary lamellae (SL) showing an exposed chloride-type cell (CC), compare with figure 20-4, (x240).

Figure 16-3. Light micrograph of a series of fused secondary lamellae (MF) with trapped remnants of hydrocarbons (HC), (x400).

Figure 16-4. Light micrograph of base of secondary lamellae showing exposed chloride cell, (x1000).

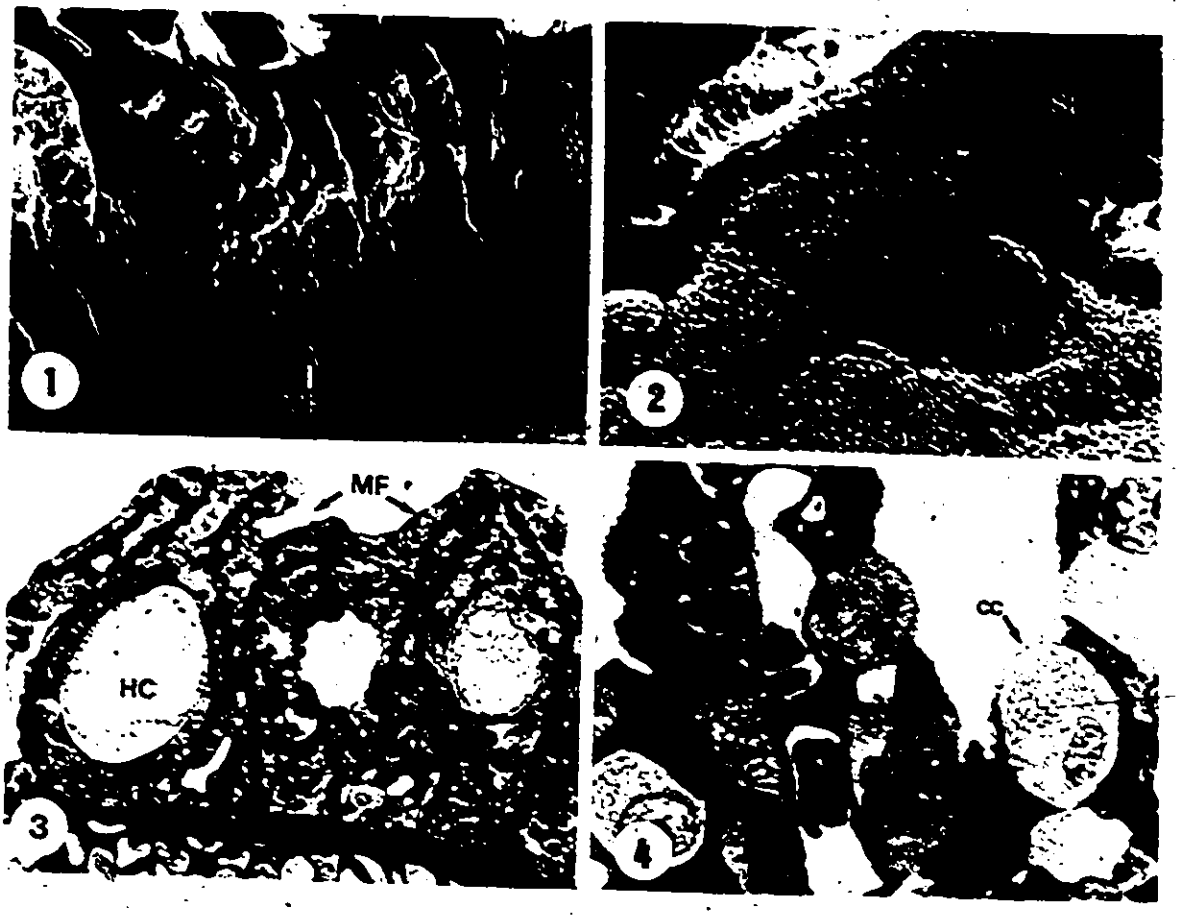

1
2
MF
HC
3
CC
4

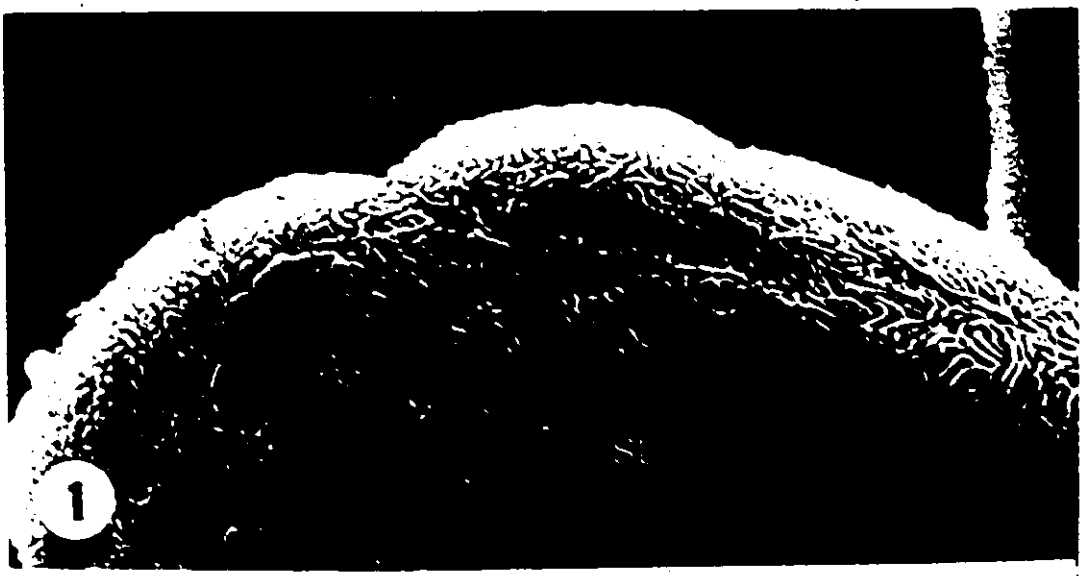
Figure 17

Scanning electron micrographs of surface epithelium of secondary lamellae tips of salt water adapted trout exposed to weathered (200 µl/L) oil-in-water dispersions.

Figure 17-1. Salt water control, secondary lamella (SL) tip showing surface of epithelial cells (EP) intact with well defined ridges, (x4560).

Figure 17-2. Norman Wells exposure, epithelial cells (CP) display some degeneration of the ridge system, (x2840).

Figure 17-3. Venezuelan exposure, certain epithelial cells (EP) appear to be in a degenerative state, (x4320).


1


2


EP
3

Figure 18

Light micrographs of the effect of dispersions on the secondary lamellae of trout.

Figure 18-1. Fresh water control fish, showing equally spaced secondary lamellae (SL), (x400).

Figure 18-2. Salt water control, (x400).

Figure 18-3. Norman Wells exposure in fresh water, showing fusion (F) and chloride cells (CC), (x400).

Figure 18-4. Norman Wells exposure in salt water, secondary lamellae (SL), (x400).

Figure 18-5. Venezuelan exposure in fresh water, showing extensive fusing, and epithelial layer separation creating spaces (S), (x400).

Figure 18-6. Venezuelan exposure in salt water, (x400).

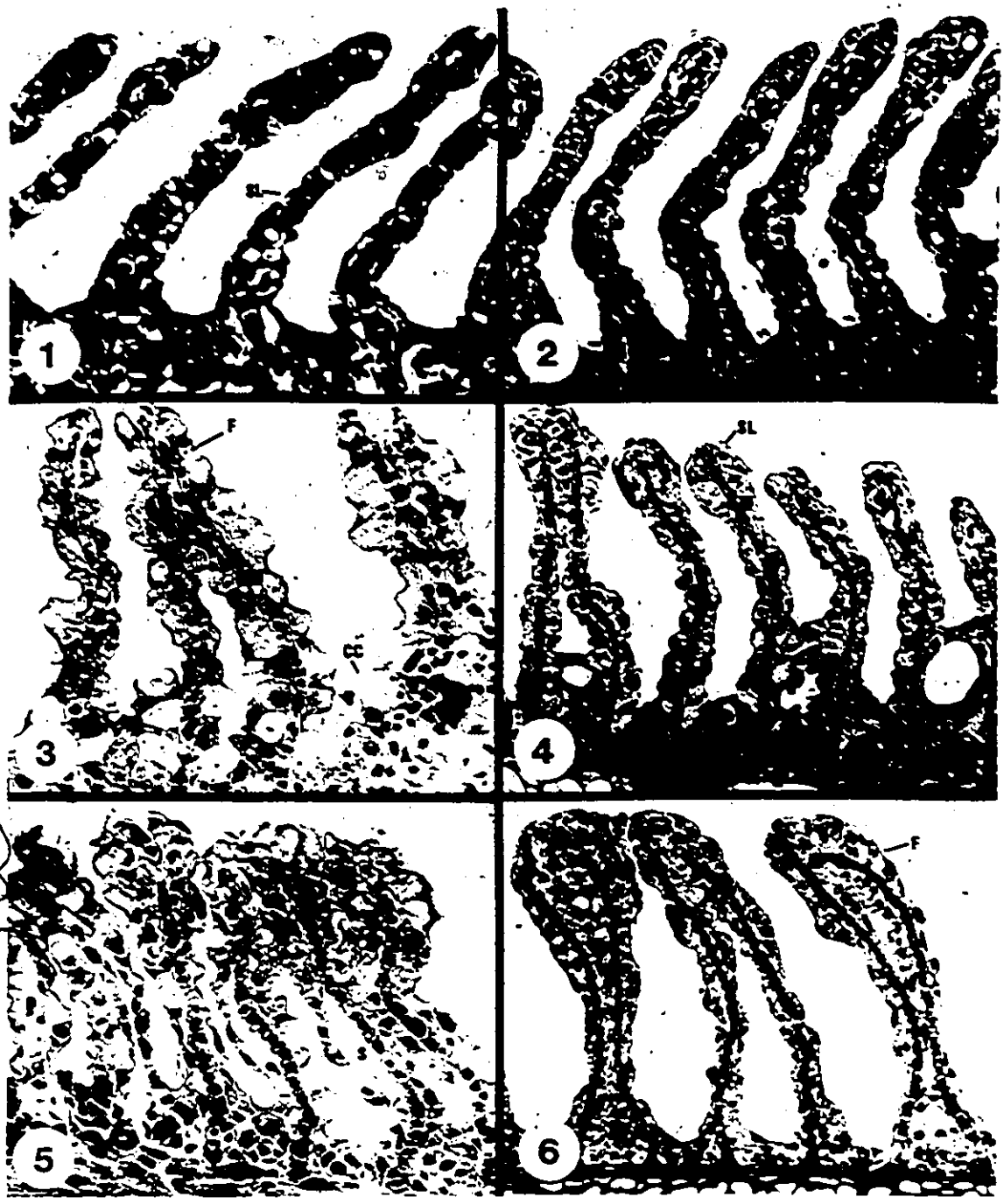

1
2
F
3
SL
4
5
F
6

Figure 19

Scanning electron micrographs of secondary lamellae of trout exposed to weathered oil-in-water dispersions of 200 µl/L.

Figure 19-1. Fresh water control, showing equally spaced secondary lamellae (SL), (x400).

Figure 19-2. Salt water control, secondary lamellae not fused, (x400).

Figure 19-3. Norman Wells, fresh water, some fusing of secondary lamellae, (x400).

Figure 19-4. Norman Wells, salt water, fusing (F) of secondary lamellae, (x400).

Figure 19-5. Venezuelan exposure in fresh water, showing multiple fusing (MF), (x200).

Figure 19-6. Venezuelan exposure in salt water, extensive fusing displayed, (x200).

Compare with figure 18.

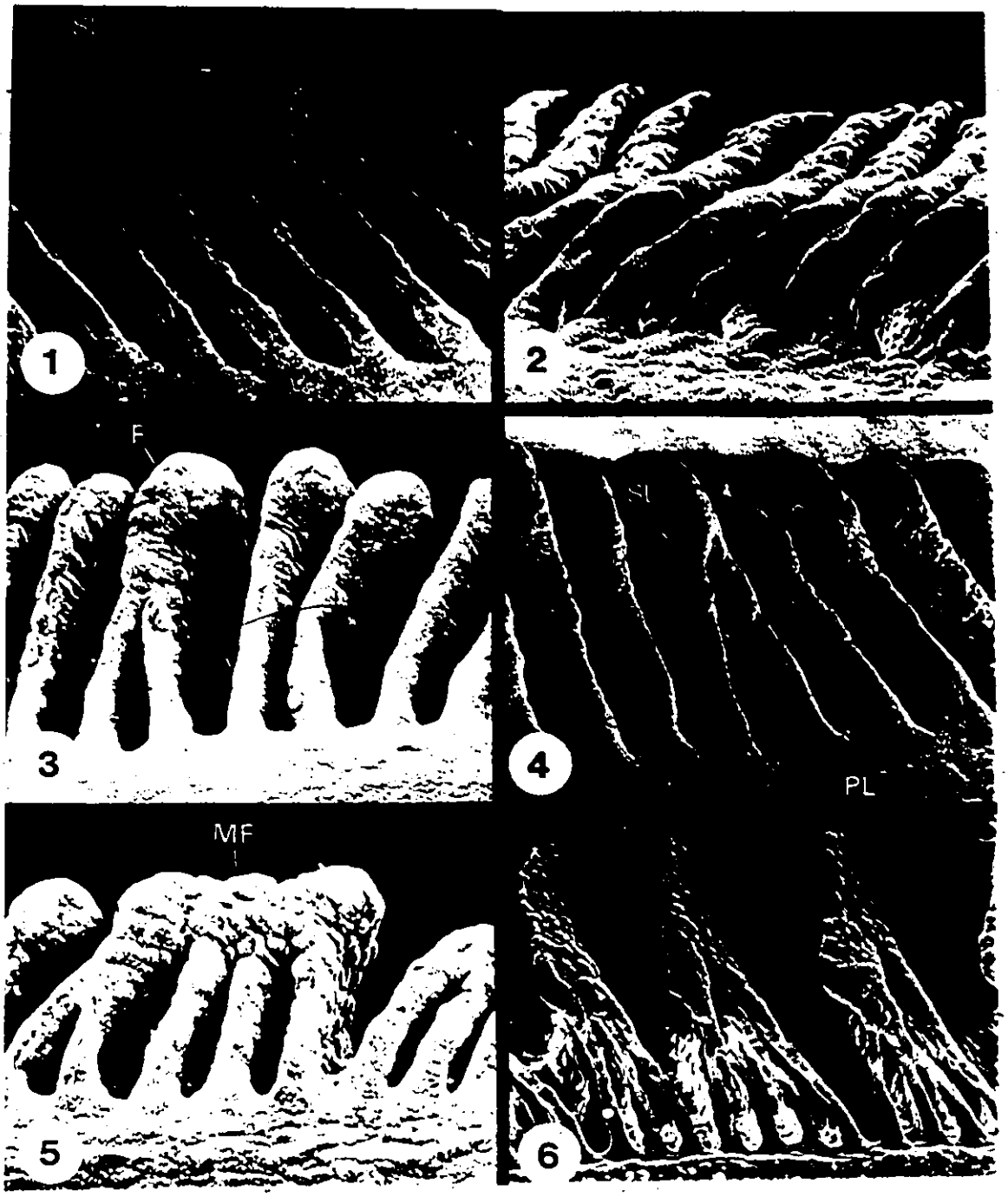

1
2
F
3
4
PL
MF
5
6

Figure 20

Composite plate of light micrographs of secondary lamellae after seven day exposure to various regimes.

Figure 20-1. Norman Wells oil intraperitoneally, in fresh water exposure, secondary lamellae (SL), (x400).

Figure 20-2. Norman Wells oil intraperitoneally, salt water exposure, secondary lamellae (SL), marginal conol (MC), (x400).

Figure 20-3. Water soluble fraction of Norman Wells oil, secondary lamellae (SL), (x400).

Figure 20-4. Water soluble fraction of Venezuelan oil, secondary lamellae (SL), red blood cell (RBC), chloride cell (CC), (x600).

Figure 20-5. Paraffin oil, 200 µl/L exposure, secondary lamellae, (x400).

Figures 20-3, 4 and 5 are of fresh water adapted trout.

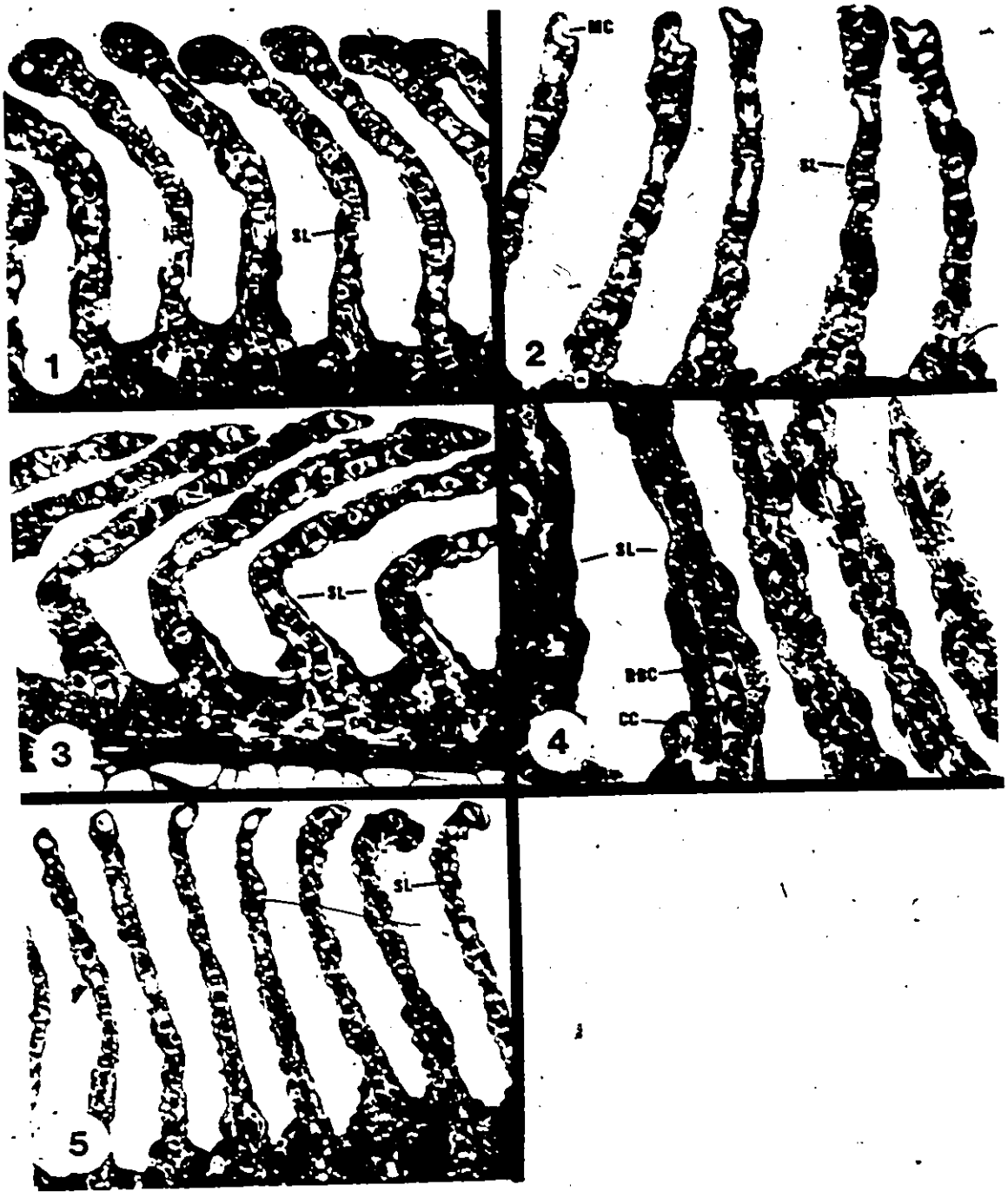

1
SL
2
MC
SL
3
SL
4
SL
RBC
CC
5
SL

Figure 21

Scanning electron micrographs of the effect of the water soluble fraction on the primary lamellae of fresh water adapted trout.

Figure 21-1. Norman Wells water soluble fraction, primary lamella (PL) compares well with the control condition, (x196).

Figure 21-2. Venezuelan water soluble fraction, primary lamella (PL) compares well with the control condition, (x200).

Figure 21-3. Higher magnification of Norman Wells exposure, near tip of primary lamella showing reduction of the ridge structure of the epithelial cells, (x4000).

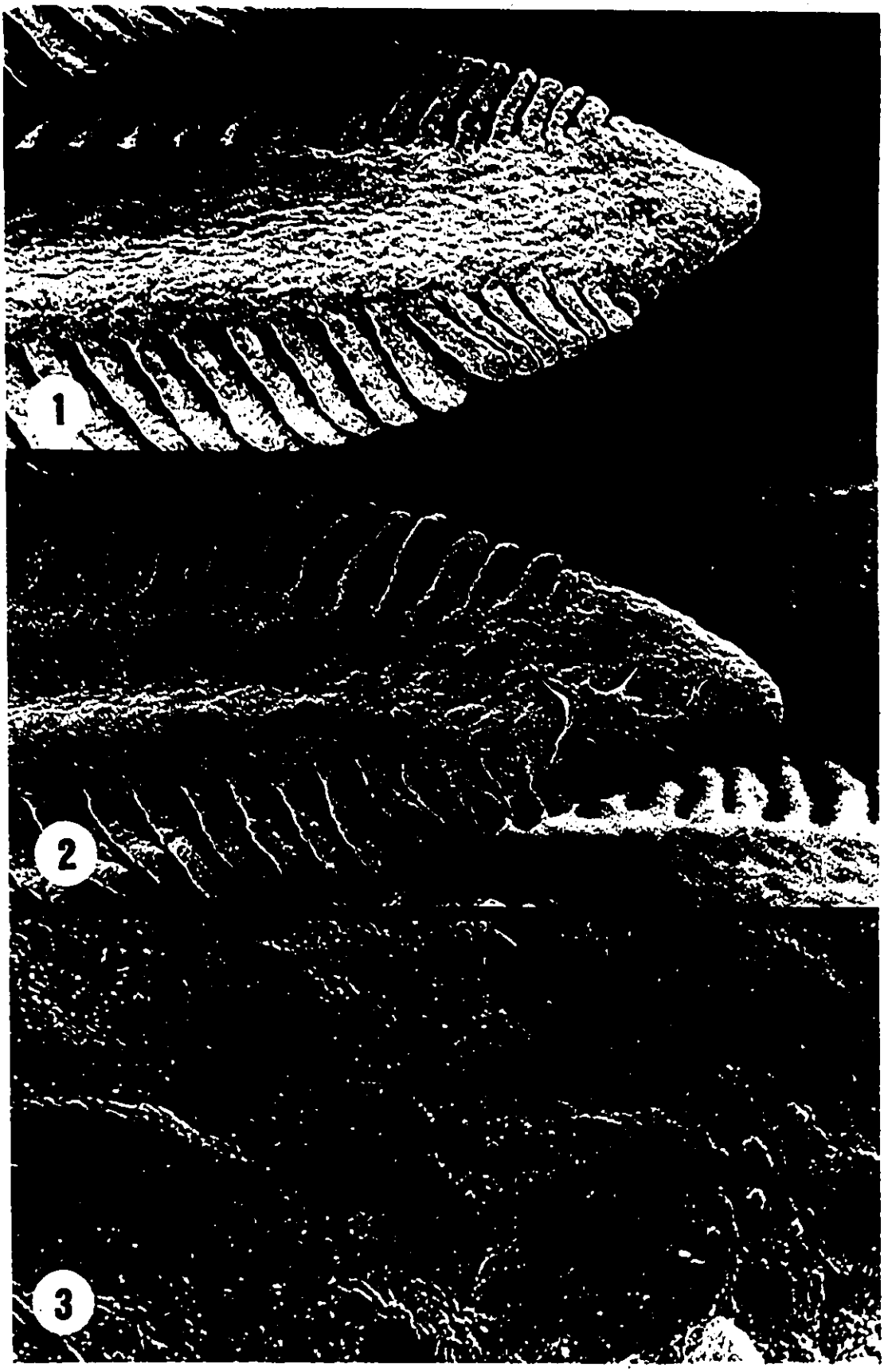

1
2
3

Figure 22

Salt water adapted trout injected IP wih Norman Wells oil.

Figure 22-1. Primary lamella (PL), compares favorably with controls which were injected with corn oils, (x152).

Figure 22-2. Secondary lamellae (SL) series, not different from controls, (x396).

Figure 22-3. High magnification of the secondary lamellar surface showing a curious circular ridge formation (arrow), (x2000).

Figure 22-4. High magnification of a similar 'doughnut' shaped ridge construction, (x5640).

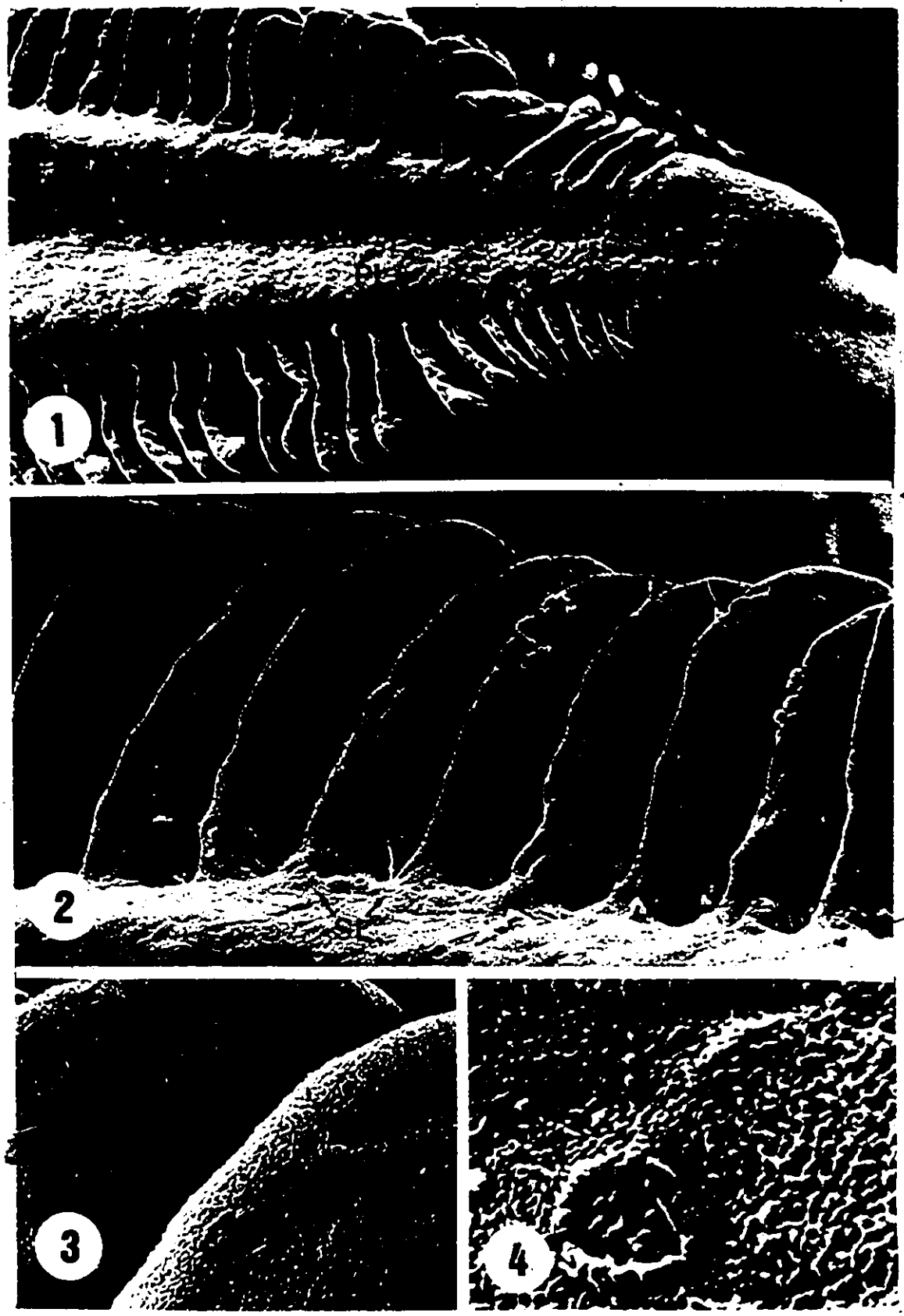

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Figure 23

Composite of light micrographs of various exposure regimes.

Figure 23-1. Sham injected trout, (x400).

Figure 23-2. Cortisol injected trout, (x400), (80 mg cortisol/100 g of fish).

Figure 23-3. Norman Wells (fresh 100 µl/L), showing numerous vacuoles (arrows) below the epithelial layer, (x400).

Figure 23-4. Venezuelan (fresh 100 µl/L), (x400).

Figure 23-5. Paraffin (fresh 100 µl/L), (x400).

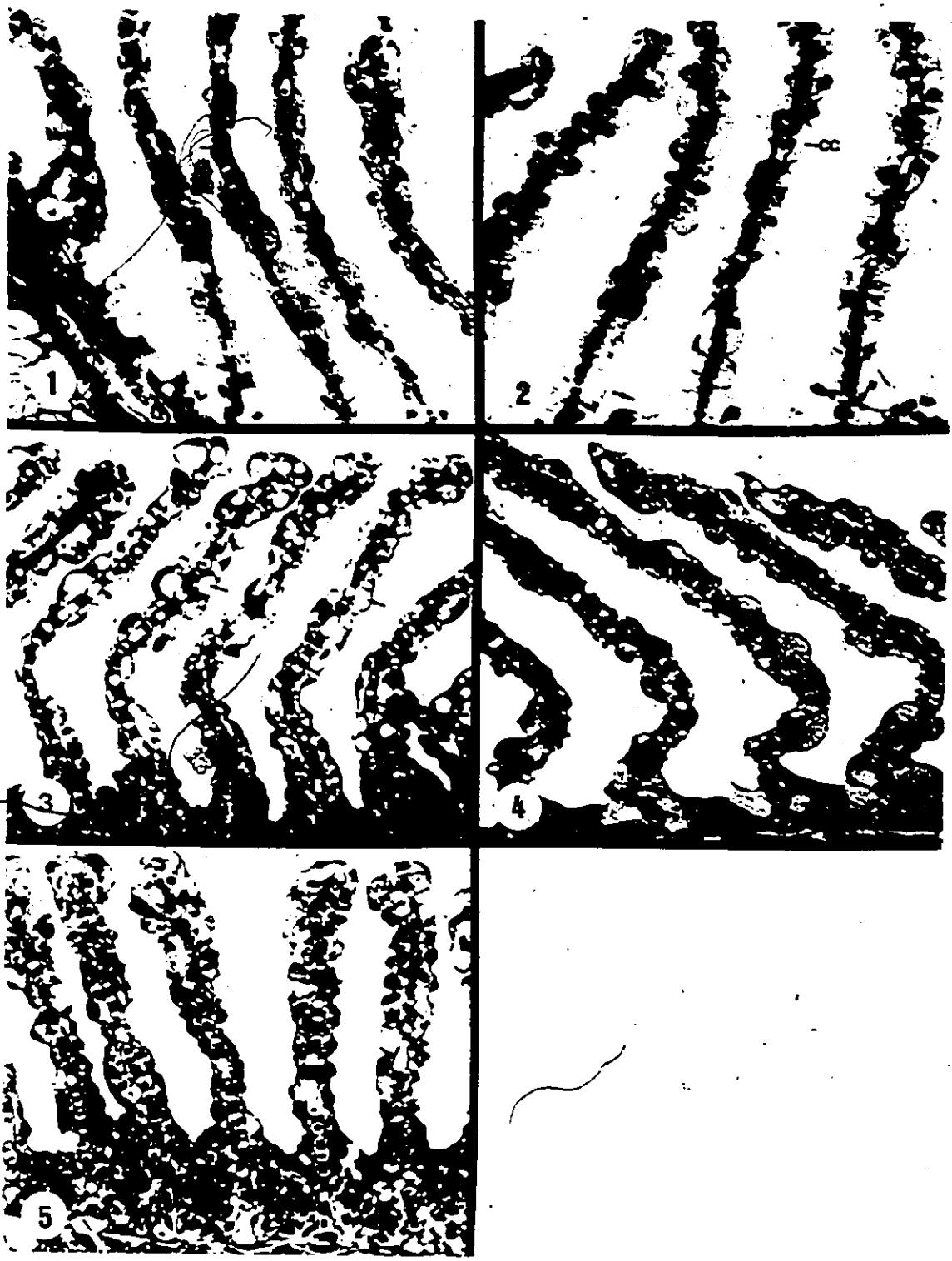
1
2
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Figure 24

Scanning electron micrographs of fresh water adapted trout exposed to various aromatic hydrocarbon regimes, primary lamellar tips, (all, x150).

Figure 24-1. Control.

Figure 24-2. Benzene exposure (3.0 mg/L in 100 µl/L of paraffin).

Figure 24-3. Naphthalene exposure (3×10^{-1} mg/L in 100 µl/L of paraffin).

Figure 24-4. Phenanthrene exposure (3×10^{-2} mg/L in 100 µl/L of paraffin).

Figure 24-5. Benzo(a)anthracene exposure (3×10^{-3} mg/L in 100 µl/L of paraffin).

Figure 24-6. Benzo(a)pyrene exposure (3×10^{-4}mg/L in 100 µl/L of paraffin).

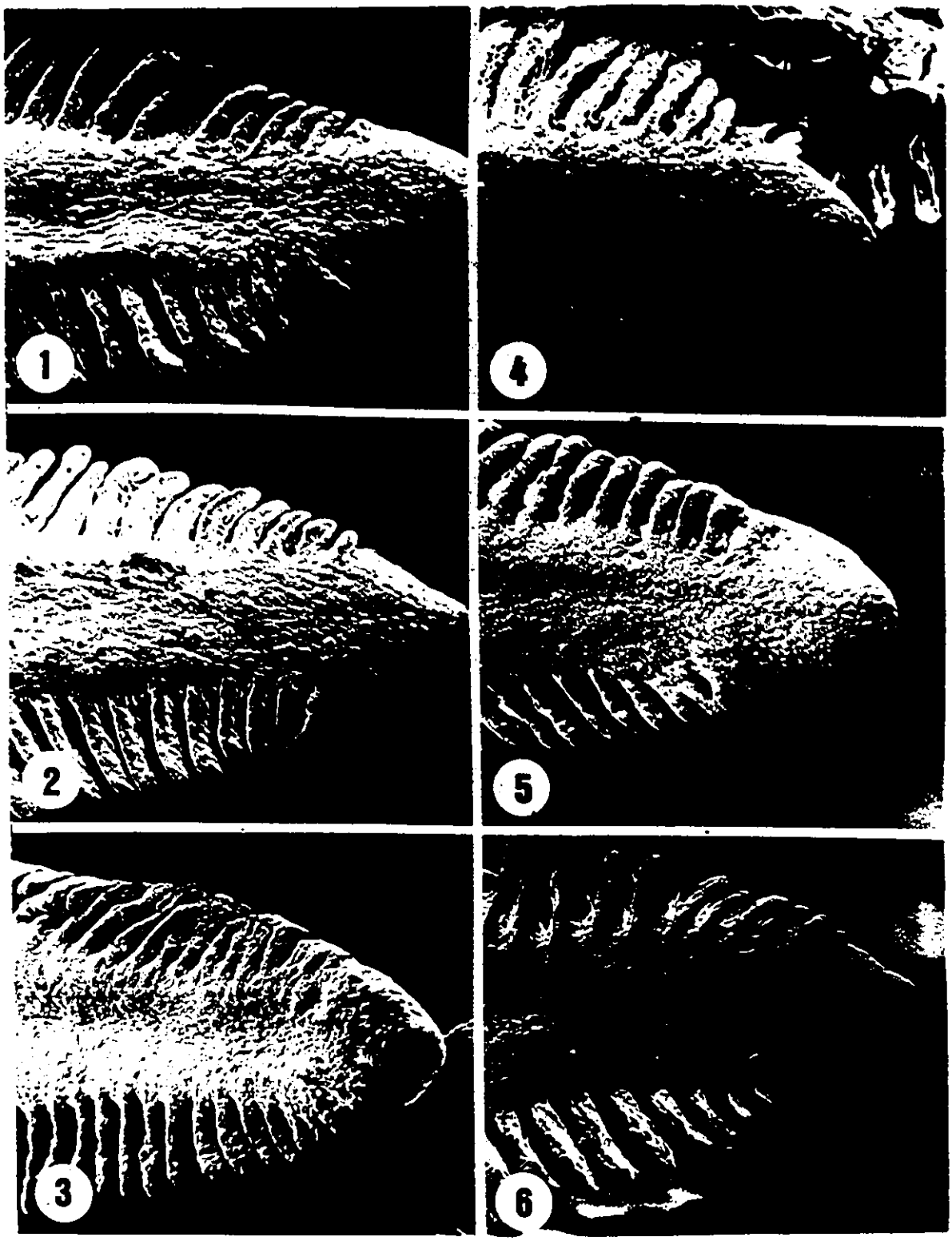

1
4
2
5
3
6

Figure 25

Light micrographs of secondary lamellae of fresh water adapted rainbow trout exposed to various regimes of aromatic hydrocarbons.

Figure 25-1. Control, (x400).

Figure 25-2. Benzene exposure, (x400), (3.0 mg/L in 100 µl/L of paraffin).

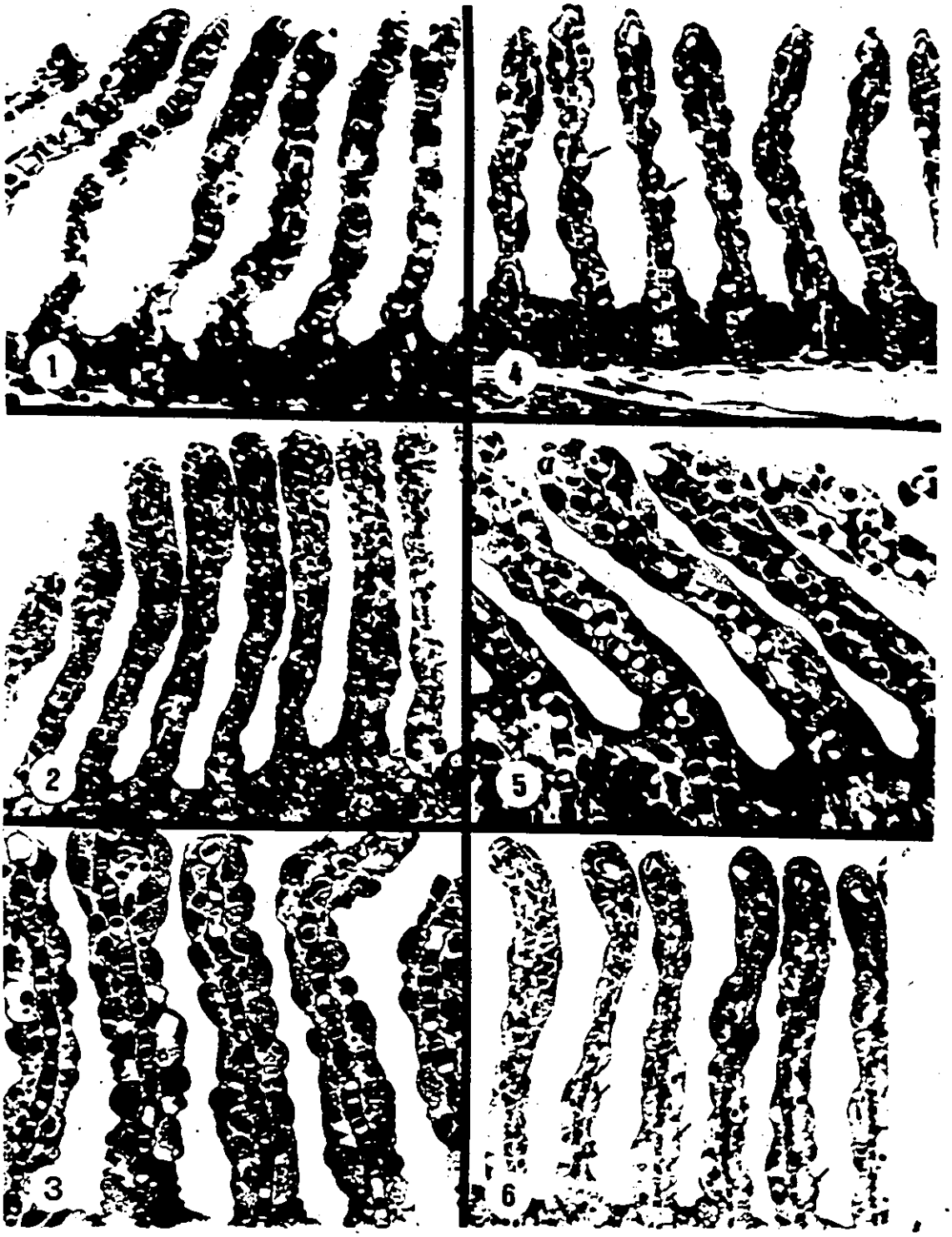
Figure 25-3. Naphthalene exposure, (x600), (3×10^{-1} mg/L in 100 µl/L of paraffin).

Figure 25-4. Phenanthrene exposure, (x400), (3×10^{-2} mg/L in 100 µl/L of paraffin).

Figure 25-5. Benzo(a)anthracene exposure, (x400), (3×10^{-3} mg/L in 100 µl/L of paraffin).

Figure 25-6. Benzo(a)pyrene exposure, (x400), (3×10^{-4} mg/L in 100 µl/L of paraffin).

Exposure regimes display a general increase in chloride cells, particularly in the naphthalene exposure, and evidence of vacuolization (arrows), particularly in the phenanthrene and benzo(a)pyrene exposures.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Chapter IV
DISCUSSION

MORTALITY

The intent of the present study was to concentrate on the sub-lethal effects of hydrocarbons on gill tissue. With one exception, no mortalities were found in the control or treatment groups in any of the experiments. Mortalities occurred only with the weathered Venezuelan exposed sea water-acclimated trout. The 50 percent mortality rate was observed again when the experiment was repeated. The salinity of the environment was probably a contributing factor. Levitan and Taylor (1979) found that the mortality of Fundulus heteroclitus increased with the salinity of the water on exposure to 6 mg/L of naphthalene. English sole in sea water exposed to sediment contaminated with Prudhoe Bay crude oil (0.2% (V/V)) for four months, experienced an 18 percent (3 of 17 fish) mortality rate (McCain and Malins, 1982).

The exposure concentrations used in all the experiments were below the LC_{50} (24-h and 96-h) values reported for rainbow trout or similar fish species. As an example, the naphthalene exposure experiment used a concentration of 0.3 µg/L, which is below the 24-h LC_{50} of 2.4 µg/L for

sheephead minnows and the 96-h LC_{50} of 2.1 µg/L for coho salmon (Anderson et al., 1974).

In general, petroleum in the water enters the fish at the level of the gills or skin, via the interfacing cellular membrane; but, in marine fish, the contaminant may enter via the intestine as these fish drink water to protect against dehydration in a hypertonic environment. Residues of crude oil were observed in the gut of sea water adapted fish exposed to dispersions, particularly in the Venezuelan exposure regime. The relative contribution of systemic toxicity in the death of these fish is not clear. Since gill pathology including extensive fusing was very evident, death may be attributed to a generalized response: gill damage modifying gas exchange, and causing hypoxia in the fish by increasing the diffusion distance for oxygen (Lloyd, 1961; Skidmore, 1970; Skidmore and Tovell, 1972; Mathiessen and Brafield, 1973; Hodson, 1974). The inhibition of Na^+, K^- ATPase at the level of the gill and a generalized metabolic stress affecting oxygen consumption may have been contributing factors (Wong and Engelhardt, 1982).

THE PHYSICAL COMPONENT

Paraffin oil was used because its viscosity approximates that of crude oil and it was thought that it would

mimic the physical effect of oil better than Sephadex beads or diatomaceous earth.

The results obtained in the paraffin exposures shows that paraffin is not an important contributor to gill damage and that the purely physical component of dispersions, as simulated by paraffin, are not responsible for the morphological disturbances associated with oil exposure. This finding agrees with previous biological studies showing the paraffinic fraction of petroleum to cause few toxic effects (Nelson-Smith, 1972; Van Gelder-Ottway, 1976).

CRUDE OIL DISPERSIONS

The gills of fish exposed to oil-in-water dispersions showed morphological alterations after seven days, as well as evidence of trapping of oil particles. Epithelial cells bulged out and lifted away from the lamellar and pillar cell system. Lamellar fusion was common, particularly in the weathered Venezuelan exposures. This description is similar to observations of the effect of other toxins on gill structure. The first sign of zinc sulphate damage is the lifting of the outer layer of epithelium, increasing the size of lymphoid spaces found between layers of gill epithelium. The lymphoid space, whose composition

is still unknown, is often found near chloride cells which often show the first signs of lifting (Skidmore and Tovell, 1972). Similar to this study, detachment of the epithelial cells, coalescing of adjacent secondary lamella, and cytoplasmic abnormalities including extensive vacuolation were observed by Matthiessen and Brafield (1973). They examined the gills of fish exposed to dissolved zinc and found active chloride cells on the secondary lamella, a common feature of exposure regimes in this study. The chloride cell proliferation, causing substantial thickening of the respiratory epithelium, and the lifting away of epithelial cells increases the water to blood diffusion distance and may make the process of gaseous exchange more difficult.

Both types of weathered oil had severe pathological effects on the gills. The Venezuelan oil showed a tendency for greater effect; and may have caused other lesions within the fish (Fletcher et al., 1982). The differences in damage seen in the gills induced by the two crude oils may perhaps be attributed to specific chemical characteristics of the oils. The Norman Wells crude oil contained high concentrations of aliphatic hydrocarbons, and aromatic hydrocarbons of low molecular weight. In comparison, Venezuelan crude oil contained very low concentrations of aliphatic hydrocarbons, and had a high concentration of

medium to high molecular weight aromatic hydrocarbons.

The epithelial separation seen in the gills of treated fish correlates well with preliminary data showing an up to 50 percent decrease in oxygen uptake efficiency in rainbow trout exposed to dispersed oil (Wong, unpublished data). The oxygen uptake capacity of fish has been shown to be disturbed by gill damage induced by toxic compounds (Skidmore, 1970; Burton, Jones and Cairns, 1972; Smart, 1976). Hypoxia may have been the cause of death in these studies with Venezuelan oil dispersions. Alternatively, the cause of death may be attributed to severe pathological changes in the gills causing osmoregulatory dysfunction (Lewis and Lewis, 1971; Smith and Piper, 1972; Wedemeyer and Yautake, 1974; Voyer et al., 1975; McKeown and March, 1978; Wedemeyer et al., 1979; Levitan and Taylor, 1979). The present results showed that dispersed hydrocarbons induced changes in gill histology and parallel research carried out showed a disrupted hydro-mineral regulatory process. The hyponatremia and hypochloremia correlated well with the gill changes seen in the 200 µl/L crude oil treatments in fresh water. Certain results of these experiments have been previously presented in descriptive and tabular form (Engelhardt et al., 1981).

Data provided on the hydromineral profiles indicates that the imbalances noted, in both fresh water and sea

water exposure, correlated consistently with gill lesions. The changes in ion concentration may be due to hydrocarbon induced breakdown of ion transport mechanisms since crude oil can alter enzyme activities (Wong and Engelhardt, 1982). Specifically, the damage or changes to chloride cells, the presumed sites of ion transport suggested by Keys and Willmer (1932), probably account for the electrolyte imbalance noted. The inhibition of Na-K ATPase activity has been correlated with the disruption of osmoregulatory processes induced by DDT (Janicki and Kinter, 1971; Leadem et al., 1974). Levitan and Taylor (1979) suggest this may also be the mode of action of petroleum hydrocarbons.

Chloride cell proliferation and greatly increased apical surface area in fresh water adapted fish exposed to weathered dispersions, may be modifications to recruit ions from the medium for the affected fish. An increased cell surface area may assist Na^+ and Cl^- uptake at a time when these ions are significantly low, as seen in Table 4. Recent studies on the gills of Pimephales promelas provides some support for this idea (Leino and McCormick, 1984).

The amounts of mucus secreted seemed to increase; this process of coating the tissue may be partially responsible for the trapping of oil particles.

Blanton and Robinson (1973) demonstrated that

petroleum hydrocarbons could alter gill morphology in fish captured near an oil spill. They reported the general sloughing of gill epithelial cells. The gills of eels captured near the 1978 Amoco Cadiz oil spill off the coast of France displayed a number of pathological changes including an increase in ionocytes (chloride cells) and mucous cells (Lopez et al., 1981). The secretory cells of the pseudobranch of the Atlantic silverside (Menidia menidia) degenerated after exposure to 0.14 µg/L of Texas-Lousiana crude oil (Gardner, 1975) Waste motor oil at a concentration of 100 ppm caused severe vacuolization of the pseudobranch in the same species (Gardner, 1975). Smith (1976) showed that Cook Inlet crude oil, which has 133 mg/L of naphthalene equivalents, caused gill damage to crabs in seawater. Not all findings point in the direction of gill damage. Fish exposed to a Venezuelan oil slick, whose concentration was unfortunately unknown, and for six months (Payne et al., 1978) resulted in no observable histological changes in the gill, which could be explained by a very low effective exposure concentration.

Studies by Haensly et al. (1982), and Solangi and Overstreet (1982) found that crude oils did cause histopathology but did not provide information on the relative importance of the component part of crude petroleum in producing physiologically relevant morphological changes.

McKeown and March (1978) observed slightly lowered serum sodium concentrations in freshwater acclimated rainbow trout exposed to Bunker C oil which displayed some morphological changes. This concurs with the correlated results of the present study. Toxic hydrocarbons in the crude oil were probably responsible for the changes in electrolytic levels because the inert paraffin particles did not cause major gill damage or significant decreases in the measured ions.

The eventual effect of a contaminant on an aquatic organism may depend on the quality of water and the salinity at the time of exposure (Gardner and Yevich, 1969b). The salt water experiments carried out in this present work serve both as a comparison and to confirm the effects of dispersions. Exposure in sea water to dispersed crude petroleum resulted in gill lesions as well, with fusing and entrapping of oil droplets evident. Epithelial detachment and chloride cell exposure were less evident in sea water experiments. The reason for this is not clear. If chloride cells function to excrete ions into the hypertonic medium, then one would predict that a dysfunction in chloride cell would result in higher plasma concentrations of ions. Coho salmon, Oncorhynchus kisutch, when acclimated to salt water at 30 ppt and exposed to components of crude oil, had higher monovalent ion levels (Morrow et al.,

1975). The fish whose gills were studied in this research had ionic profiles showing a generally inversed response as compared to fresh water treatments. Chloride, sodium, and potassium ions exhibited increased plasma levels in the sea water adapted and crude oil exposed fish.

The curling of primary gill filaments and the disarray of the secondary lamellae observed under the dissection microscope of fish gills exposed to mechanically dispersed oil may have been the result of a breakdown of the vascular skeleton. Some vascular breakdown was observed. Skidmore and Tovell (1972) suggest this phenomenon may cause a decrease in blood hydrostatic pressure within the pillar cell system.

The reduction of microridge patterns on exposure to various hydrocarbons, particularly the water soluble fraction, and a low concentration to benzo(a)pyrene, may be the result of the effect of specific toxic elements on the surface of the cell membrane. But, Jacobs et al. (1981) reported the reduction and loss of microridge patterns on exposure to severe heat shock for five days. Alternatively, the suggestion can be made that the reduction of microridge patterns, on exposure to weathered Venezuelan oil, may be explained as a general response to an environmental shock.

Trace metals like cadmium and nickel may be

associated with petroleum, and they have been shown to cause gills to lose epithelial cells in both the marine and fresh water environment (Voyer et al., 1975; Haider, 1964). Prolonged oral administration of methyl mercury caused hyperplasia in rainbow trout (Wobeser, 1975). The possible specific contributions and synergistic effects of trace metals and other compounds, present in small quantities in petroleum, on the gill morphology is not clear.

WATER SOLUBLE FRACTIONS

With the exception of some epithelial ultrastructure surface effects, the water soluble fractions of Norman Wells and Venezuelan crude oil, at the concentrations (measured to be 35 µl/L and 45 µl/L for Norman Wells and Venezuelan crude oils respectively) used in this study did not elicit important gill changes. This observation suggests that the chemically dispersed fraction of oil spills are not responsible for the deleterious effects of crude oil exposures in fresh water. Hawkes (1977) reported that coho salmon and starry flounder exposed to the water soluble fraction of Prudhoe Bay crude oil in a flow through marine system for five days resulted in localized gill lesions and loss of surface cells.

If gaseous exchange is primarily passive across the

gill surface (Hughes, 1966), then the structure of the respiratory surface is of critical importance (Newstead, 1967). The loss of ridge and microridge structure after exposure to the water soluble fraction of oil may be physiologically significant. There are two hypothesized functions for the microridges. They may aid in the retention of mucus at the cell surface (Hughes and Wright, 1979), and they may aid gaseous exchange by increasing the surface area and by producing microturbulences of water across the membrane surface (Hughes, 1978). It has been postulated that the ridges on the surface of cells help hold onto water molecules to aid in gas transfer (Rajbanshi, 1977). Minor respiratory responses might be expected from ridge and microridge loss. Thomas and Rice (1975) observed opercular movement rates in pink salmon to increase in proportion to the concentration of the water soluble fraction of Prudhoe Bay crude oil. Hart and Ogelsby (1979) postulated metaplasia as a cause for the loss of the microridge patterns in trout gill exposure to toxins. The water soluble fraction of crude oil may have a similar effect on other species. The common intertidal limpet Patella vulgata, while showing no change when exposed to a water soluble fraction extract from North Sea crude oil at 25 percent saturation, did show the loss of cilia and microvilli at 100 percent water soluble fraction after 24

hours (Nuwayhid and Davies, 1980).

INTERNAL HYDROCARBON LOAD

Hydrocarbons that enter the fish via various membranes (gills, skin, intestine) may contribute to toxicity and subsequent gill damage. Weathered Norman Wells crude oil injected intraperitoneally in both fresh and sea water adapted trout resulted in little or no gill disturbances, providing evidence that the internal hydrocarbon load (100 µl/kg fish/day) is not an important contributor, or that the fish may be able to excrete the compounds before toxic levels are accumulated. Hydrocarbon hydroxylase is the enzyme associated with the metabolism and excretion of hydrocarbons (Varanasi and Malins, 1977). The intraperitoneal injection of certain aromatic hydrocarbons results in their modification to soluble components within 24 hours in coho salmon (Roubal et al., 1977). Such rapid metabolism may explain the lack of effects observed in testing the contribution of an internal hydrocarbon load.

CORTISOL EXPERIMENT

Morphological and hydromineral imbalances observed in this and other studies may be due in part to a stress

response mediated by cortisol. For years the plasma cortisol level has been used to evaluate the stress placed on fish (Nava, 1980). Apart from an apparent increase in chloride cell number in cortisol injected rainbow trout, gill tissue was undisturbed. This observation is consistent with the findings of Boyle and Epstein (1972). They found that cortisol increases correlated with an increase in chloride cell number in salinity stressed cells. The cortisol mediated stress response experienced by fish exposed to particulate hydrocarbons may indirectly contribute to the fate of the organism by increasing the vunerability of chloride cells to toxic elements. On the other hand the increases in plasma cortisol levels and in the number of branchial chloride cells may be an adaptive response by the fish to compensate for a plasma ionic imbalance.

AROMATIC HYDROCABONS

With one exception, the concentrations of aromatic hydrocarbons used in this study reflect the levels that may be found in the water column after an oil spill in the environment. While benzo(a)pyrene is not found to be in crude oil or else is of only trace concentrations, it enters the environment predominantly as a result of pyro-

lytic reactions (e.g., "cracking" of petroleum in petrochemical processes). The aromatic hydrocarbons released in a spill in general have solubility limits (that can be obtained in moving water) above acutely toxic levels. Toxic concentrations are more likely to occur in winter and low temperature when the solubility and evaporation of aromatic hydrocarbons associated with dispersed oil particles is decreased.

The results of the five aromatics tested do not present a clear picture. The most serious effects, particularly vacuolation, were observed in phenanthrene and benzo(a)pyrene exposed fish, while the plasma ion profiles for only benzene and benzo(a)anthracene were significantly different from controls (Table 4).

The observation that only benzo(a)pyrene resulted in loss of ridge structure on certain epithelial cell surfaces suggests this compound may be particularly toxic to the sensitive tissue. Aromatic hydrocarbons cause avoidance reactions in fish (Maynard and Weber, 1981).

Fundulus heteroclitus, acclimated in sea water, exposed to naphthalene had signficant increases in sodium levels and osmolality (Levitan and Taylor, 1979). DiMichele and Taylor (1978) found histopathological evidence, including hyperplasia, in naphthalene exposures to the same species.

Phenol is an oxidation product of benzene in the marine environment. It is a minor component of crude oil (Keith, 1976) and several people have looked at its toxic effects. Phenol (0.1 mg/L) induced cytological damage (disintegration of the gill) in adult clams (Fries and Tripp, 1977), while in goldfish, (Carassius auratus), 10 ppm of phenol did not damage gills but did affect liver, kidney, and spleen (Vishnevetskii, 1961). Rainbow trout gills sloughed epithelial cells and developed a general inflammation after exposure to 6.5 to 9.6 mg/L phenol (Mitrovic et al., 1968). In the freshwater bream (Abramis brama), chronic exposure to phenol resulted in destruction of epithelial cells, damage to blood vessels and extravasation of blood in the gill lamella (Waluga, 1966). The limited field observations which are available reveal less severe damage. Fourteen species of fish sampled from the phenol-polluted Rhine and Elbe Rivers displayed discharged mucous glands and inflammation (Reichenbach-Klinke, 1975). Fathead minnows, Pimephales promelas, exposed to phenanthrene absorbed to tar balls, displayed no obvious damage to gills (Gerhart et al., 1981).

Although higher molecular weight aromatic hydrocarbons are effective in modifying gill tissue, the physiological significance of these particular changes is not clear when considering the plasma ion levels of these fish.

Exposure to benzene significantly lowered certain plasma ion levels without causing a corresponding change in gill morphology. Benzene has been shown to significantly lower the activity of several gill ATPases (Wong, 1982), and perhaps this is responsible for the low ion levels observed.

PRACTICAL IMPORTANCE

Evidence from this study supports the hypothesis that the physically dispersed elements of crude oil are particularly important in causing gill damage in fish. An oil spill in Canada's North might result in a relatively contained emulsion phase with the low temperature holding down the concentration of physically dispersed particles. The use of a chemical dispersing agent on this hypothetical spill would increase dispersion and in so doing potentiate the toxic effect of the spill to fish. The results of this study demonstrated that a dispersed oil can cause severe lamellar damage, suggesting that a decision-making process leading to the use of chemical dispersants as a counter-measure for an oil spill must take into consideration a probable increased toxic effect on fish in the spill area.

Chapter V

CONCLUSIONS

The experimental evidence presented in this thesis supports the primary hypothesis that prolonged (7 days) physical contact of the gill tissue with the physical dispersion is important in producing severe gill histopathology on exposure to crude petroleum.

The results of the experiments also supported the following conclusions:

- The morphological changes in gill tissue on exposure to petroleum hydrocarbons are synonymous with the plasma ionic disturbances observed in the same fish, and may be responsible for them;

- Weathered Venezuelan oil dispersions produce effects on gill morphology different from weathered Norman Wells crude oil dispersions;

- The purely physical component of crude petroleum (i.e., the droplets of oily substance) does not contribute significantly to gill disturbance;

- The water soluble fraction of the crude oils tested

is not responsible for the severe gill damage produced by crude petroleum, but does have the effect of reducing micro-ridge patterns at the gill surface;

- The internal systemic load of crude petroleum, as tested in this study, is not an important factor in producing gill abnormalities;

- Stress reactions mediated by cortisol may increase the vulnerability of gill tissue to the toxic effects of petroleum by increasing the number of chloride cells in the branchial tissue;

- Larger molecular weight aromatic hydrocarbons are more effective than lower molecular weight aromatic hydrocarbons in producing epithelial cell surface and chloride cell alterations.

APPENDIX A

PLASMA ION DATA

The following pages provide additional information on the plasma ion concentrations of the fish examined in this study for morphological changes at the gill level.

Figure 26

Changes in plasma ion concentrations in rainbow trout after 7 days of exposure to oil by way of dispersions (emulsion), water soluble fractions (WSF), and intraperitoneal injection (IP). Treatments compare responses between unexposed controls (C), and paraffin oil (P), Norman Wells crude oil (NW) and Venezuelan crude oil (VEN) in fresh- and sea-water acclimated trout. Vertical bars represent means of 8 fish showing standard deviation; significantly different (*) from controls at $P \leq 0.05$.

Adapted from Engelhardt, F.R., M.P. Wong, and M.E. Duey (1981). Aquatic Toxicology, 1:178.

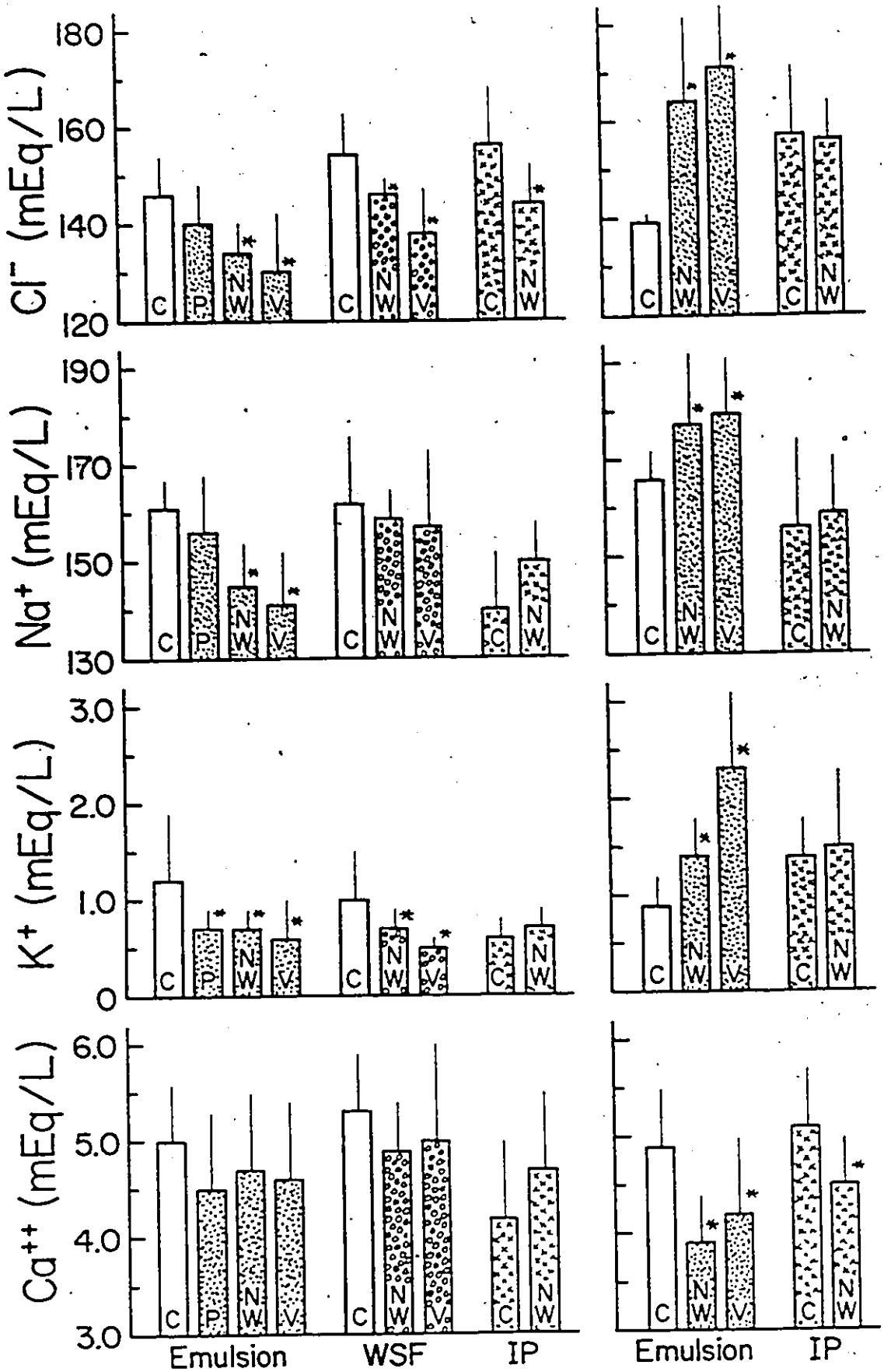
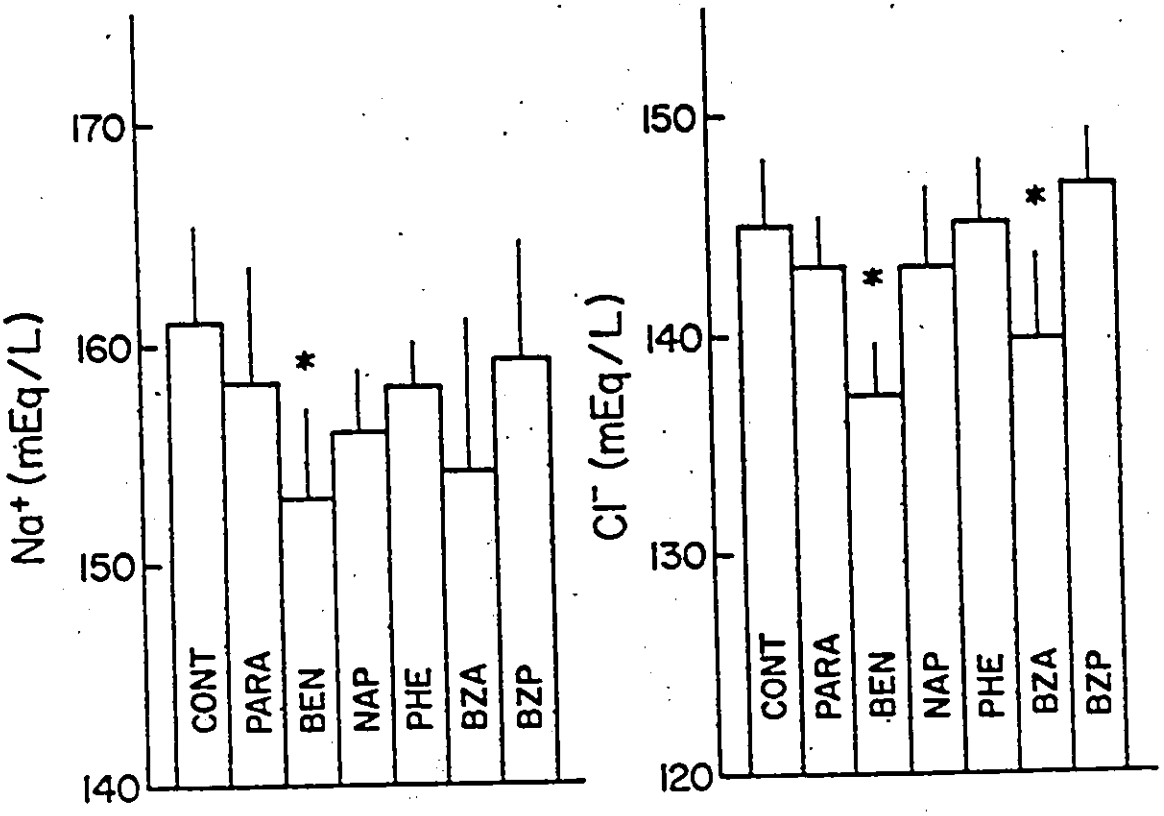

Cl⁻ (mEq/L)
180
160
140
120
Na⁺ (mEq/L)
190
170
150
130
K⁺ (mEq/L)
3.0
2.0
1.0
0
Ca⁺⁺ (mEq/L)
6.0
5.0
4.0
3.0
C
P
NW
V
Emulsion
WSF
IP
Fresh water
Sea water

Figure 27

Plasma sodium and chloride ion concentrations in freshwater rainbow trout following exposure to individual aromatic hydrocarbons in (100 µl/L) paraffin (PARA) dispersions. The aromatic hydrocarbons used were: benzene (BEN) at 3.0 mg/L, naphthalene (NAP) at 3.0×10^{-1} mg/L, phenanthrene (PHE) at 3.0×10^{-2} mg/L, benzo(a)anthracene (BZA) at 3.0×10^{-3} mg/L, and benzo(a)pyrene (BZP) at 3×10^{-4} mg/L. All values are means of 8 fish and vertical bars represent one standard deviation; "*" denotes a statistically significant difference ($P \leq 0.05$) from controls (CONT).

Adapted from Wong, W.P. (1982), M.Sc. thesis, University of Ottawa.


Na+ (mEq/L)
170
160
150
140
CONT
PARA
BEN
NAP
PHE
BZA
BZP
*
Cl- (mEq/L)
150
140
130
120
CONT
PARA
BEN
NAP
PHE
BZA
BZP
*
*

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