Investigating Links Between Bacterial Exotoxins and Unexplained Fish Kills: Phase 3

R&D Technical Report W2-075/TR

J.W. Lewis

Research Contractor:
School of Biological Sciences
Royal Holloway
University of London

Publishing Organisation:
Environment Agency
Rio House
Waterside Drive
Aztec West
Almondsbury
Bristol BS32 4UD Tel: 01454 624400 Fax: 01454 624409

 ISBN 1844321657

Dissemination Status
Internal: Released to Regions
External: Publicly Available

Statement of Use

This report (following R&D Technical Report P2-284) is intended to raise awareness and understanding among Environment Agency staff of fish kills caused by natural toxic events. The findings of these reports will be enhanced by further research, which will be incorporated into policy guidance.

Keywords
Fish mortalities, gill pathology, hyperplasia, bacterial exotoxins, Actinomycetes, Oscillatoria.

Research Contractor
This document was produced under R&D Project W2-075 by:

School of Biological Sciences
Royal Holloway
University of London
Egham
Surrey
TW20 0EX

Tel: 01784 434455
Fax: 01784 443182

Environment Agency Project Leader
The Environment Agency Project Leader for R&D Project W2-075:
Ros Wright, Ecological Appraisal, Kelvedon, Anglian Eastern

Further copies of this report are available from:
Environment Agency R&D Dissemination Centre, c/o
WRc, Frankland Road, Swindon, Wilts SN5 8YF

tel: 01793-865000 fax: 01793-514562 e-mail: publications@wrcplc.co.uk

CONTENTS Page

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF FIGURES (CONTINUED) Page

LIST OF FIGURES (CONTINUED) Page

LIST OF FIGURES (CONTINUED) Page

LIST OF FIGURES (CONTINUED) Page

LIST OF FIGURES (CONTINUED) Page

LIST OF FIGURES (CONTINUED) Page

LIST OF FIGURES (CONTINUED) Page

EXECUTIVE SUMMARY

There is strong evidence that bacterial and algal toxins are responsible for many unexplained and often large scale fish mortalities in UK rivers, canals and lakes. Recent R&D (Project P2-284 (Phases1&2)) demonstrated significant pathological changes to the gills of experimental fish. Hyperplasia and fusion of secondary lamellae were caused by several groups of bacteria isolated from a lake suffering a toxic event, and these bacteria included species of *Actinomycetes* and *Oscillatoria*. The damage caused was comparable to gill pathology seen in a number of extensive fish mortalities, such as the Kennet & Avon Canal 1998 (and subsequent years) and lakes in the Anglian & Southern Regions 2002. Further research is required to improve our understanding of bacteria and algae, which are involved in natural toxic events, and also likely to cause pathology and mortality of freshwater fish.

The aims of Phase 3 of the R&D project were to extend the database of bacteria and environmental variables associated with toxic events This, apart from extending the experimental work undertaken in Phases 1&2, may lead to the identification of bacteria and algae associated with fish mortalities. Water samples for bacterial analysis and gill samples for SEM studies were taken from moribund fish from a number of sites undergoing fish mortalities. Five bacterial isolates were selected from the database for testing on 3 species of freshwater fish (rainbow trout, common carp and roach.). These included one species of *Oscillatoria* and 4 species of the Actinomycete family which produce peach coloured spores.

Carp, roach and rainbow trout fingerlings, experimentally exposed to acute concentrations of 5 bacterial isolates for up to 96hr, showed epithelial hyperplasia and fusion of the secondary lamellae on the gill filaments. Mortality in rainbow trout and roach exposed to *Actinomycete* 13 occurred after 48 and 72 hours respectively. Carp and rainbow trout exposed to *Actinomycete*15,17 and *Oscillatoria* sp were more susceptible to gill damage than roach. The latter were more susceptible to *Actinomycete* 12 than rainbow trout or carp. In conclusion, the degree of pathology and in turn fish mortality is related to the concentration and virulence of the isolate used and different responses are shown by carp, rainbow trout and roach to specific isolates and their bacterial exotoxins.

Pathological changes associated with the gills were similar to those seen in toxic field events in fish from the Hungerford Canal (1998 and subsequent years), from 5 lakes in the Anglian and Southern Regions 2002 and from 4 lakes in the Anglian Region, in early 2003. The degree of gill damage is often more extensive than in the experimental exposure studies which may be the result of a longer exposure to bacterial exotoxins or to higher concentrations. Fish species from affected lakes, including rainbow trout, carp, roach, bream, tench and rudd, showed differing degrees of gill pathology, which may lead to mortality in only a single species. No other significant pathogens were recorded in these cases.

The long term objective of this research is to improve the protocol for the assessment of fish kills and provide effective fisheries management. It is believed that natural toxic events may be responsible for many previously unexplained fish mortalities and may also produce chronic effects, which would make fish more vulnerable to infection with parasites and in turn result in fish disease.

KEY WORDS

Fish mortalities, gill pathology, hyperplasia, bacterial exotoxins, Actinomycetes, Oscillatoria.

1. INTRODUCTION AND AIMS

Decaying algal blooms, stimulated initially by nutrient rich waters and other factors, may occasionally give rise to toxic effects, which may be linked with bacterial activity and in turn associated with fish mortalities.

There is growing awareness and concern about unexplained, widespread and often large scale fish mortalities throughout England and Wales. In the Spring of 1998, 150 tonnes of fish died at the Berkshire Trout Farm (Hungerford) and the adjacent Kennet and Avon Canal; this recurred to a lesser degree in Spring 1999, 2000, and 2001. Another two recent unexplained fish kill events on Kennet & Avon Canal near Devizes occurred in September 2001 showing some similarities with previous incidents.

There were extensive fish mortalities (believed to be almost 100%) at Hintlesham Trout Lake in 1999 and there was unusual behaviour of fish in the following year indicating the likelihood of a toxic event. There have been numerous unexplained mortalities in canals, lakes and rivers in recent years involving all fish species where the causative agent is unknown, although in many cases symptoms include gill hyperplasia and the fusing of secondary gill lamellae.

In many cases the methodology used following outbreaks of fish mortalities has focused on analytical chemistry to locate conventional pollution sources such as a chemical spill or farm slurry. Investigations have widened only when these more usual sources of pollution have been ruled out. There is now increasing evidence that many such mortalities are associated with bacterial or algal toxins or a combination of both. Extensive research and biological monitoring have already taken place in respect of the incidents on Kennet and Avon Canal by the Thames Region of the Environment Agency, WRC and British Waterways (Johnson *et al* 1998, Barnard *et al* 1999, Johnson *et al* 2000); and on Hintlesham Lake by the Anglian Region of the Environment Agency (Parry, 2000,2001).

Pathological symptoms exhibited by the fish were similar in all of these incidents and no toxic chemical was involved. In addition Parry (2001) revealed that a possible cause could be an exotoxin produced by bacteria isolated from the toxic water from Hintlesham Trout Fisheries, sampled prior to dosing with hydrogen peroxide.

The toxicity of up to 9 bacterial isolates, cultured from Hintlesham Trust Fisheries by Parry (2001), were tested following experimental exposure to carp fingerlings for 24 hours (Lewis 2001). Samples of carp exposed to 8 of 9 isolates tested showed evidence of epithelial hyperplasia and fusion of secondary lamellae on the gill filaments (primary lamellae). A link was demonstrated between isolates of *Actinomycetes* and *Oscillatoria* and significant pathological changes to the gills of experimental fish (Lewis 2002, R&D project P2-284)
These changes included varying degrees of collapsing, hyperplasia and fusion of secondary lamellae with loss of microridging, distortion and erosion on the filamental epithelium of primary gill lamellae leading to the loss of respiratory surface. This serves as a defence mechanism to limit the uptake of toxin, but if exposure continues this may lead to hypoxia and death.

Environment Agency Fisheries Officers collected fish samples from 5 lakes in the Anglian and Southern Regions during February to March 2002 following incidents of fish mortalities and these showed similar gill pathology. Similar symptoms were also seen in gill samples

from the Kennet and Avon Canal in 1998 and subsequent years. A database has been established at the University of Lancaster to record occurrence of microbiological organisms, which may lead to identification of the most potent or widespread toxin-producing bacteria (Parry 2002). *Actinomycete spp*, which produce peach coloured spores, have occurred in a number of water samples taken during toxic events and may be responsible for causing gill damage and fish kills.

Further experimental approaches are needed to further our understanding of the relationship between gill pathology, and possible bacteria-induced fish mortality, using acute exposures of cyprinid and salmonid fish to potentially toxic isolates of *Actinomycetes* and *Oscillatoria.*

Therefore the objectives of the present investigation are:- (a) to test the acute toxicity of selected bacterial isolates ie 4 species of *Actinomycete* (producing peach coloured spores) and *Oscillatoria sp* to carp, roach and rainbow trout fingerlings for up to 96 hours and (b) to relate any toxic effects associated with these isolates to gill pathology (and mortality) of the three fish species using scanning electron microscopy (SEM). Ultrastructural changes to the gill epithelial surface will be examined in detail and any relationship between specific gill pathology and bacterial exotoxins assessed both in experimental fish and fish samples collected from potentially toxic waters in the Anglian Region of the Environment Agency during February/ March 2003.

These objectives will contribute further to our understanding of the relationships between algae and bacteria in eutrophic waters. The ultimate aim is to predict and manage the effects of toxic incidents on fish populations thus minimising environmental and economic costs.

2. METHODOLOGY

2.1 Bacterial Isolates

Parry (2001) has previously described the methods used for isolating the bacteria, which were derived from toxic waters. The 5 bacterial isolates used in the present investigation include 4 species of the Actinomycete family (*Actinomycetes* 12,13,15 and 17) and the filamentous blue-green cyanobacterium, *Oscillatoria sp*.

2.2 Fish Maintenance and Exposure to Bacteria Isolates

Carp and roach fingerlings (0+) were maintained in laboratory aquaria containing filtered (activated charcoal), dechlorinated and aerated water at a constant temperature of 20^0C as previously described by Lewis (2002). Rainbow trout fingerlings (0+) were maintained under similar conditions except the temperature was maintained at a constant 10^0C. Batches of 10 carp, roach and rainbow trout fingerlings were exposed to each of the five isolates at acute concentrations for up to 96 hours. The concentrations of isolates were expressed as cells per ml made up in 5 litres of dechlorinated waters as follows:- acute exposures at concentrations of 1x106 cells/ml for *Actinomycete 12* and 17, 1.08 x 105 cells/ml for *Actinomycete* 13, 3.3 x 105 cells/ml for *Actinomycete* 15 and 5.44 x 104 cells/ml for *Oscillatoria* sp. These concentration levels are based on the availability of bacterial isolate material at the time of the investigation and in the present study the concentration levels were lower than those used by Lewis (2002).

Batches of fish were sacrificed 96 hours after exposure, except for fish, which may have died before this time. Those fish exhibiting unusual behaviour and stress were sacrificed immediately by severing the brain stem and spinal cord with a scalpel. Similar procedures were used on control unexposed fish samples. The gill arches of each exposed and control fish were carefully removed, washed in fresh dechlorinated water, fixed in 3% glutaraldehyde in 0.1M phosphate buffer at 4^0C for 2 hours and washed with buffer. Gill specimens were then dehydrated through a graded ethanol series, critical-point dried, mounted on aluminium stubs and gold coated for ultrastructural examination using a Hitachi S2400 SEM.

2.3 Fish Samples From 'Toxic' Sites, Anglian Region

Samples of cyprinids and salmonids from unexplained fish kill incidents in four sites from the Anglian Region of the Environment Agency were submitted to the National Fisheries Laboratory at Brampton for investigation. The gill samples were fixed in 70% alcohol and subsequently sent to Royal Holloway, University of London for SEM analysis and prepared as previously described for the experimental fish exposures. These samples included gills from roach and bream, One House Pond, Stowmarket (10/3/03), rainbow trout from Highfield Farm Reservoir, Essex (28/2/03), carp from Bovington No 1 Pit, Hatfield Peverel, Essex (31/3/03) and carp from Cockaynes Pit, Alresford, Essex (31/3/03).

3. RESULTS

3.1 Fish Mortality

Three of the isolates, namely *Actinomycete* 13 and 15 and *Oscillatoria* species induced fish mortalities during the period of investigation (Fig 1, Table 1). Following exposures to *Actinomycete* 13, 20% and 40% of rainbow trout died after 48 hr and 72 hr respectively whereas 20% of roach succumbed after 72 hr. The rainbow trout were also susceptible to exposure with *Actinomycete 1*5 and *Oscillatoria* sp, both isolates of which induced mortality in 20% of fish after 72 hr (Fig 1, Table 1).

3.2 Gill Pathology of Fish Exposures

Pathological changes associated with exposure to the 5 bacterial isolates tested, included collapsing of secondary gill lamellae, hyperplasia of epithelial tissues leading to lamellar fusion and in some cases swelling and erosion and loss of microridging on the filamental epithelium of primary gill lamellae. (Tables 2 – 6).

The degree of gill pathology in fish is more severe with *Actinomycete 1*3, followed by *Oscillatoria* sp, *Actinomycete 1*5 and 17 and to a lesser extent *Actinomycete* 12. The extent and rate of pathological changes are similar in the salmonid (rainbow trout) and cyprinid (carp, roach) fish species tested and with the exception of *Actinomycete* 12 and 17. Rainbow trout appeared to be more susceptible than either carp or roach to exposure with *Actinomycetes* 13 and 15 and *Oscillatoria* sp. Both carp and roach were able to withstand extensive changes in gill pathology, including lamellar fusion, during the 96 hr experimental period except for a 20% mortality in roach exposed to *Actinomycete 1*3 after 72 hr.

Control fish (Figs 2.0 – 2.7)

Secondary lamellae in the gills of carp, roach and rainbow trout are more or less equally spaced and little or no distortion/swelling is observed on the filamental epithelium of primary gill lamellae. The microridging is also intact.

Exposure of carp, roach and rainbow trout to bacterial isolates

Actinomycete 12 (Table 2, Figs 3.0 – 3.15).

In carp fingerlings (Table 2, Fig 3.0 – 3.5), apart from a moderate degree of distortion to the filamental epithelia of primary lamellae, up to 30% of secondary lamellae had collapsed after 24 hours exposure but with no evidence of lamellar hyperplasia and fusion. However after 48 hours, hyperplasia leading to fusion of secondary lamellae occurred in 10% of carp increasing to 25% after 96 hours, with a moderate degree of distortion to the filamental epithelia but a minimum loss of microridging.

Similar pathological changes were observed in roach (Table 2, Fig 3.6 – 3.9) except that lamellar fusion (10%) occurred earlier after 24 hours exposure increasing to 35% after 96 hours, accompanied by moderate degrees of distortion and loss of microridging on the filamental epithelium. Of the three fish species under investigation, rainbow trout appeared to be the least affected (Table 2, Figs 3.10 – 3.15) and by 48 hours there was no evidence of lamellar fusion and little distortion or loss of microridging on the filamental epithelium. By

96 hours only 15% of lamellar fusion and moderate degrees of distortion and loss of microridging on the filamental epithelium occurred.

***Actinomycete* 13** (Table 3, Figs 4.0 – 4.16)

The rate and extent of pathological changes in all three fish species exposed to *Actinomycete* 13 were greater than in the remainder of the isolates used to such an extent that 20% of rainbow trout and roach fingerlings died after 48 hours and 72 hours respectively (Table 1, Fig 1.0). Hyperplasia leading to fusion of secondary lamellae occurred in 10% of carp, 10% of roach and 15% of rainbow trout after 24 hours exposure. Fusion had increased to 50% in carp (Table 3, Figs 4.0 – 4.5) and to 60% in both roach (Table 3, Figs 4.6 – 4.10) and rainbow trout (Table 3, Figs 4.11 – 4.16) by 96 hours. This was accompanied by a high degree of distortion/erosion of the filamental epithelium and a moderate loss of microridging.

***Actinomycete* 15** (Table 4, Figs 5.0 – 5.14)

Rainbow trout fingerlings appeared to be more susceptible to exposure with *Actinomycete 1*5 than the two cyprinid fish species with 20% mortality occurring by 96 hours (Table 1, Fig 1.0) and hyperplasia and fusion occurring in up to 50% of secondary lamellae. This was also accompanied by severe distortion/erosion on the filamental epithelia of primary lamellae (Table 4, Figs 5.9 – 5.14). Carp fingerlings also displayed extensive hyperplasia and lamellar fusion which increased from 10% to 50% after 24 hours and 96 hours respectively, although no mortalities were recorded (Table 4, Figs 5.0 – 5.5). Roach on the other hand was the least susceptible species to exposure with *Actinomycete* 15 with little or no hyperplasia occurring within 48 hours and only 15% lamellar fusion was evident after 96 hours (Table 4, Figs 5.6 – 5.8).

***Actinomycete* 17** (Table 5, Figs 6.0 – 6.12)

No mortalities were recorded in fish exposed to *Actinomycete* 17, although similar pathological changes were recorded to those described for *Actinomycete* 15 except for more severe distortion on the filamental epithelium of primary lamellae (Table 5). Apart from a high proportion of collapsing of secondary lamellae, carp and rainbow trout again appeared to be more susceptible to gill damage than roach, with 15% lamellar fusion being observed in both carp (Fig 6.1) and rainbow trout (Fig 6.9) after 48 hours. Roach fingerlings again appeared to resist the effects of *Actinomycete* 17 with no lamellar fusion occurring after 48 hours. However by 72 hours and 96 hours, up to 15% and 40% of secondary lamellae respectively were fused (Table 5, Figs 6.5, 6.7, 6.12), accompanied by a high degree of distortion/erosion on the filamental epithelium with the loss of microridging.

***Oscillatoria* sp** (Table 6, Fig 7.0 – 7.15)

Rainbow trout fingerlings were particularly susceptible to exposure with *Oscillatoria* sp with a 20% mortality occurring after 72 hours (Table 1, Fig 1.0). Hyperplasia leading to lamellar fusion occurred in 50% of secondary lamellae, with severe distortion/erosion of the filamental epithelium (Table 5, Figs 7.14 – 7.15). Carp fingerlings were slightly less susceptible than rainbow trout as no mortalities occurred although 30% of secondary lamellae of carp became fused by 96 hours accompanied by a moderate degree of distortion/erosion of the filamental epithelium (Table 5, Figs 7.3, 7.4). As in the case of *Actinomycetes* 15 and 17, roach appeared to resist the toxic effects of *Oscillatoria* sp and although up to 50% of

secondary lamellae had collapsed after 48 hours, there was no evidence of any lamellar fusion (Table 6, Fig 7.5). The latter did, on the other hand, occur in roach after 72 hours and 96 hours where up to 25% and 30% of secondary lamellae respectively demonstrated hyperplasia and fusion/erosion (Table 6, Figs 7.6 – 7.8). This was together with a high degree of distortion/erosion of the filamental epithelium (Fig 7.8) and an extensive loss of microridging (Fig 7.9).

3.3 Gill Pathology of Fish Samples From 'Toxic' Sites in the Anglian Region (Figs 8.0, 8.11)

The gills of bream examined from One House Pond, Stowmarket, showed extensive hyperplasia leading to 100% fusion of secondary lamellae (Fig 8.0). Parts of the secondary lamellae showed evidence of prominent swellings or cysts, which may have arisen from a low infection with the myxosporidian protozoan parasite, *Myxobolus* sp, previously reported on these fish by the National Fisheries Laboratory at Brampton. The pathology associated with roach samples examined from One House Pond was similar to that of bream but there was less extensive fusion and more collapsing of secondary lamellae.

The gills of rainbow trout from the Highfield Farm Reservoir also showed a high proportion of collapsing, with up to 20% of lamellar fusion near the tips to produce a 'clubbing' effect (Fig 8.4). Furthermore a 100% of secondary lamellae were fused along their length (Fig 8.5) and the majority of the filamental epithelium was covered in a mass of mucus plugs (Fig 8.6). Carp gill samples from Bovington No 1 Pit, Hatfield Peverel, showed collapsing and severe hyperplasia leading to a total fusion of secondary lamellae (Fig 8.7, 8.8). In addition telangiectasis (or swellings) was observed on the surface of 50% of filamental epithelia of primary lamellae (Fig 8.8).

Pathological changes in carp gills examined from Cockaynes Pit, Alresford were similar but less severe than in carp examined from the Bovington Pit. Nonetheless up to 70% of lamellae had collapsed and 20% showed hyperplasia and lamellar fusion mainly near the tips to produce a 'clubbing' effect (Fig 8.9, 8.11). Telangiectasis was also observed on up to 30% of filamental epithelia. A more detailed assessment of the filamental epithelia from fish samples examined from the 4 'toxic' sites in the Anglian Region was not possible due to poor fixation of the gills.

4. DISCUSSION AND CONCLUSIONS

Previous histopathological studies of fish exposed to pollutants have shown that fish gills are efficient indicators of water quality (Roncero *et al* 1990, Kirk and Lewis 1993). Fish gills are vulnerable to pollutants in water because of their large surface area and external location. Furthermore gills perform numerous functions which include respiration, osmoregulation, excretion of nitrogenous waste products and acid-base balance. Therefore functional impairment of gills caused by pollutants can significantly damage the health of fish and induce mortality. For this reason fish gills are considered to be most appropriate indicators of water pollution levels (Satchell, 1984).

Irritant-induced gill lesions have previously been investigated at Royal Holloway University of London (Kirk & Lewis 1993; Alazemi, Lewis & Andrews 1996; Johnson *et al* 1998; Barnard *et al* 1999 and Johnson *et al* 2000) using the SEM. These studies have shown that SEM can make an important contribution in recognising specific damage caused by different pollutants and in the case of deployed fish exposed to toxic water in the Kennet and Avon Canal during 1998-2000, and carp fingerlings experimentally exposed to bacterial isolates (Lewis 2001,2002 *a*). This specific damage included gill hyperplasia, collapsing and fusion of the secondary gill lamellae.

To summarise Phase 3 results, carp, roach and rainbow trout fingerlings exposed to acute concentrations of 5 bacterial isolates showed epithelial hyperplasia and fusion of secondary lamellae on the gill filaments (or primary lamellae). Fusion occurs at the outer margin and base of secondary lamellae and progresses to fill the interlamellar spaces with hyperplastic tissue primarily along the anterior third and tips of the gill filaments. Hyperplasia serves as a defensive mechanism in fish, leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance. This response takes place at the expense of the respiratory efficiency of the gills and eventually the respiratory impairment outweighs any protective effect against pollution uptake (Abel and Skidmore, 1975). Therefore in the later stages of acute poisoning increasing hypoxia can lead to fish mortality, by impairing the oxidative activity of the gills through disruption of their cellular organisation. Furthermore erosion or loss of the microridging on the filamental epithelial cells may adversely affect the retention of mucus protecting the gill surface; it may also reduce the effectiveness of the exchange processes, including gaseous exchange, over the surface epithelia. These pathological changes were more pronounced in all three fish species exposed to *Actinomycete* 13 and resulted in rainbow trout and roach mortalities after 48 hours and 72 hours respectively. The two isolates *Actinomycete* 15 and *Oscillatoria* sp also caused extensive hyperplasia and fusion of secondary lamellae with the carp and rainbow trout fingerlings being more susceptible to gill damage than roach; rainbow trout mortality occurred after 72 hours exposure to both isolates. The least active isolates included *Actinomycete* 17 followed by *Actinomycete* 12 as no mortalities were reported. However carp and rainbow trout again showed more pathological gill changes than roach on exposure to *Actinomycete* 17 but conversely the extent of gill damage in roach exposed to *Actinomycete* 12 was greater than in carp and rainbow trout.

Thus in acute toxicity testing, the degree of pathology, and in turn mortality, is related to the concentration and virulence of the strain/species of isolate used and also to the different responses shown by the 3 fish species to specific isolates and their bacterial exotoxins. Rainbow trout and to a lesser extent carp were found to be susceptible to 4 of the isolates tested ie *Actinomycete* 13, 15, & 17 and *Oscillatoria* sp, with mortalities occurring in rainbow

trout exposed to *Actinomycete* 13, 15 and *Oscillatoria* sp and mortalities reported in roach exposed to *Actinomycete* 13. Apart from the latter isolate, roach appeared to resist, at least for 72 hours, severe gill damage when exposed to *Actinomycete* 15 and 17 and *Oscillatoria* sp.

On the other hand roach showed an increasing susceptibility to gill damage when exposed to *Actinomycete* 12, unlike carp and rainbow trout.

However in general, lamellar hyperplasia and fusion usually occurs within 48 hours of acute exposure to the bacterial isolates producing fusion at the tips of secondary lamellae ie a 'clubbing' effect together with degrees of disruption to the filamental epithelium of primary lamellae. Following 96 hours of exposure, a high proportion (up to 60%) of gill damage does occur in the fish species under investigation in the form of lamellar fusion and erosion, together with distortion, erosion and loss of microridging on the filamental epithelium.

It should be emphasised, however, that surface changes on the gills of fish exposed to bacterial exotoxins are not consistent, for example, with specific alterations to gill ultrastructure associated with heavy metals (copper, chromium, cadmium), cyanide, atrazine phenal or ammonia previously described by Kirk and Lewis (1993) and Alazemi, Lewis and Andrews (1996). This supports the possibility of naturally occurring toxins associated with algal or bacterial growth contributing to unexplained fish kills in the Kennet and Avon Canal during 1998-2001 and at Hintlesham Trout Lake in 1999. The gills appear to be the focus of toxic activity resulting in respiratory impairment in the fish, and the responses by various fish species will vary due to heterogeneity within the fish population and the degree of virulence exhibited by the algal/bacterial strains.

In the present study, the pathological changes described for gill samples of bream, roach, rainbow trout and carp collected from four 'toxic' sites in the Anglian Region during February and March 2003 demonstrated distinct similarities to those described for carp, roach and rainbow trout fingerlings exposed to the 4 isolates of *Actinomycete spp* and *Oscillatoria* sp. These pathological changes are also similar to those observed by Lewis (2002) on samples of carp, roach, bream and tench taken from lake and gravel pit sites in the Southern and Anglian Regions of the Environment Agency during the Spring of 2002. The degree of gill damage and especially hyperplasia and lamellar fusion in fish samples taken from the 4 sites in the Anglian Region during February/March 2003 are more extensive than in experimental fish exposures described in the present study. This includes the occurrence of telangiectasis on the filamental epithelia of primary lamellae. It is likely that the unexplained fish kills from these 'toxic' sites are related to the presence of microbial exotoxins, the concentrations of which in these toxic waters were likely to be higher than those used in experimental exposures in the present study. There appeared to be no evidence of any other potential pathogens in the fish samples examined from these field sites, except for light infections of gill parasites such as the myxosporidian protozoan, *Myxobolus* sp.

5. FURTHER WORK

The overall objective should be to develop an operational tool for predicting fish kills caused by microbial toxins and to develop procedures for the prevention and treatment of toxicity in field sites used as fisheries. Initially a comprehensive database of fish kill incidents should be established to simultaneously include microbial analysis of water and sediment samples with the aim of identifying the microbial isolates and their toxins. Variability in the virulence shown by different microbial strains should also be considered.

Further SEM analyses of the gills of a wider range of fish species, relative to age and gender, from 'toxic' field sites need to be undertaken and further comparative investigations made with fish species experimentally exposed to selected microbial isolates and their toxins. A SEM guide to gill pathology could then be produced to aid the diagnosis of unexplained fish kills and to improve our understanding of natural toxic events.

6. ACKNOWLEDGEMENTS

Grateful and sincere thanks are extended to Dr Jackie Parry and her Research Group at the University of Lancaster for kindly providing the bacterial isolates. The excellent technical assistance of Dr Neil Morley of the School of Biological Sciences and Ms Patricia Goggin of the Electron Microscope Unit, Royal Holloway, University of London are gratefully acknowledged and also to Mrs Jenny Reid of the School of Biological Sciences for her first rate secretarial support.

7. REFERENCES

Abel P D and Skidmore J F (1975). Toxic effects of an anionic detergent on the gills of rainbow trout. *Water Research*, *9* , 759-765.

Alazemi B M, Lewis J W and Andrews E B (1996). Gill damage in the freshwater fish *Gnathonemus petersii* (family Mormyridae) exposed to selected pollutants: an ultrastructural study. *Environmental Technology*, *17*, 225 – 238.

Barnard S, Conrad A and Sims I (1999). Biological Monitoring of the Kennet & Avon Canal, Hungerford, Spring 1999. *R & D Technical Report for Environmental Agency Thames Region.*

Johnson I, Barnard S, Sims I, Conrad A, Parr W, Hedgecott S & Cartwright N (1998).Technical investigation of the Hungerford fish mortality. *Report to the Environment AgencyNo CO 4626*.

Johnson I, Pyke Y and Sims I (2000). Biological Monitoring of the Kennet & Avon Canal, Spring 2000. *R & D Technical Report for Environment Agency Thames Region.*

Kirk R S and Lewis J W (1993). An evaluation of pollutant induced changes in the gills of rainbow trout using scanning electron microscopy. *Environmental Technology, 14*, 577-585.

Lewis JW (2002). Investigating links between bacterial exotoxins and unexplained fish kills Phases 1 and 2. *Environmental Agency, R&D Technical Report P2-284/TR*

Lewis JW (2002 *b*). SEM investigation on fish gill samples from lake/gravel pits in the Southern and Anglian Regions of the Environment Agency, Feb – May 2002. *Report to the Environmental Agency, Anglian Region*.

Parry J (2000). Examination of the bacterial Components of Toxic Water from Hintlesham Trout Fisheries. *Report to Environment Agency Anglian Region.*

Parry J (2001). Examination of the bacterial Components of Toxic Water from Hintlesham Trout Fisheries by MAST ID. *Report to Environment Agency Anglian Region.*

Parry J (2002). Review of the potential toxic agents responsible for six unexplained fish kills. *Report to the Environment Agency Anglian Region*.

Roncero V, Vincente J A, Redondo E, Gazquez A and Duran E (1990). Experimental lead nitrate poisoning: microscopic and ultrastructural study of the gills of tench (*Tinca tinca L.*). *Environmental Health Perspectives, 89,* 137-144.

Table 1 **Survival (%) of rainbow trout and roach exposed to isolates of *Actinomycetes* 13 and 15 and *Oscillatoria* sp for up to 96 hr.**

Isolate	Survival of fish (%) over time			
	24 hr	**48 hr**	**72 hr**	**96 hr**
(a) Trout				
Actinomycete 13	100	80	60	60
Actinomycete 15	100	100	80	80
Oscillatoria sp	100	100	80	80
(b) Roach				
Actinomycete 13	100	100	80	80

Table 2 Pathological changes on the gills of carp, roach and rainbow trout following exposure to *Actinomycete 12* for up to 96 hr.

Time of exposure (hr)	Fish species	Pathological changes to the gill			
		Collapsing of secondary lamellae (%)	Fusion / erosion of secondary lamellae (%)	Distortion of filamental epithelium	Loss of microridging
24	carp	30	-	+	+
	roach	40	10	++	+
	trout	30	-	-	+
48	carp	40	10	+	+
	roach	50	20	++	++
	trout	50	-	+	+
72	carp	50	20	+	+
	roach	60	30	++	++
	trout	70	10	+	+
96	carp	60	25	++	+
	roach	60	35	++	++
	trout	70	20	++	++

+++ high; ++ moderate; + low; - absent

Table 3 Pathological changes on the gills of carp, roach and rainbow trout following exposure to *Actinomycete 13* for up to 96 hr.

Time of exposure (hr)	Fish species	Pathological changes to the gill			
		Collapsing of secondary lamellae (%)	Fusion / erosion of secondary lamellae (%)	Distortion of filamental epithelium	Loss of microridging
24	carp	30	10	+	+
	roach	40	10	+	++
	trout	40	20	+++	++
48	carp	40	30	+++	++
	roach	50	15	++	++
	trout**	50	40	+++	++
72	carp	40	40	+++	++
	roach*	50	30	+++	++
	trout***	40	50	+++	++
96	carp	50	50	+++	++
	roach	30	60	+++	++
	trout	20	60	+++	++

*20% mortality of roach by 72 hr; ** 20% mortality of rainbow trout by 48 hr;
*** 40% mortality of rainbow trout by 72 hr

+++ high; ++ moderate; + low; - absent

Table 4 Pathological changes on the gills of carp, roach and rainbow trout following exposure to *Actinomycete 1*5 for up to 96 hr.

Time of exposure (hr)	Fish species	Pathological changes to the gill			
		Collapsing of secondary lamellae (%)	Fusion / erosion of secondary lamellae (%)	Distortion of filamental epithelium	Loss of microridging
24	carp	20	10	+	+
	roach	20	-	+	+
	trout	30	10	++	+
48	carp	30	20	++	+
	roach	30	5	+	++
	trout	40	25	++	+
72	carp	40	30	++	++
	roach	30	10	++	++
	trout	50	35	++	++
96	carp	40	50	++	++
	roach	40	15	++	++
	trout*	40	50	+++	++

* 20% mortality of rainbow trout by 96 hr;

+++ high; ++ moderate; + low; - absent

Table 5 Pathological changes on the gills of carp, roach and rainbow trout following exposure to *Actinomycete 17* for up to 96 hr.

Time of exposure (hr)	Fish species	Pathological changes to the gill			
		Collapsing of secondary lamellae (%)	Fusion / erosion of secondary lamellae (%)	Distortion of filamental epithelium	Loss of microridging
24	carp	30	5	++	+
	roach	30	-	++	++
	trout	60	10	+++	++
48	carp	50	15	++	+
	roach	50	-	++	++
	trout	60	15	++	++
72	carp	60	30	++	++
	roach	60	15	+++	++
	trout	50	20	++	++
96	carp	40	50	++	++
	roach	50	40	+++	++
	trout	50	30	+++	++

+++ high; ++ moderate; + low; - absent

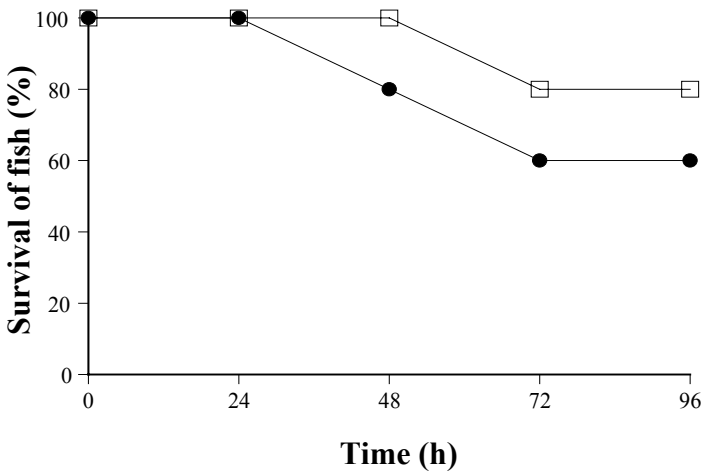
Table 6 Pathological changes on the gills of carp, roach and rainbow trout following exposure to *Oscillatoria* sp for up to 96 hr.

Time of exposure (hr)	Fish species	Pathological changes to the gill			
		Collapsing of secondary lamellae (%)	Fusion / erosion of secondary lamellae (%)	Distortion of filamental epithelium	Loss of microridging
24	carp	30	5	++	+
	roach	30	-	-	++
	trout	50	10	++	+
48	carp	60	20	++	+
	roach	50	-	++	++
	trout	50	20	++	+
72	carp	60	25	++	+
	roach	60	10	++	++
	trout*	60	50	++	+
96	carp	50	30	++	++
	roach	50	40	+++	++
	trout	30	60	+++	++

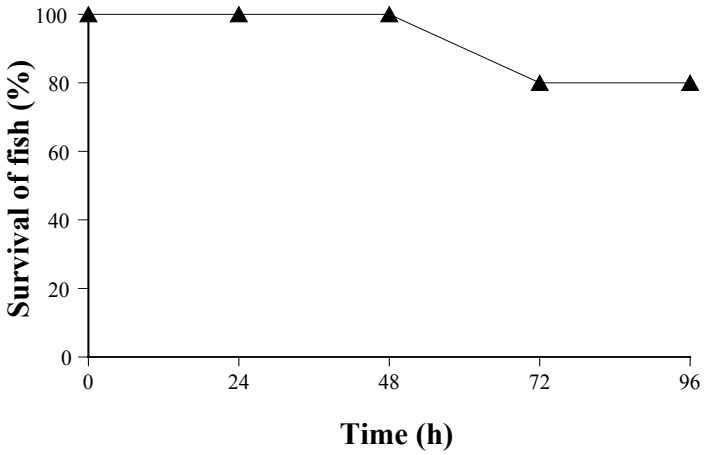
* 20% mortality of rainbow trout after 72 hr;

+++ high; ++ moderate; + low; - absent

a.



b.



c.

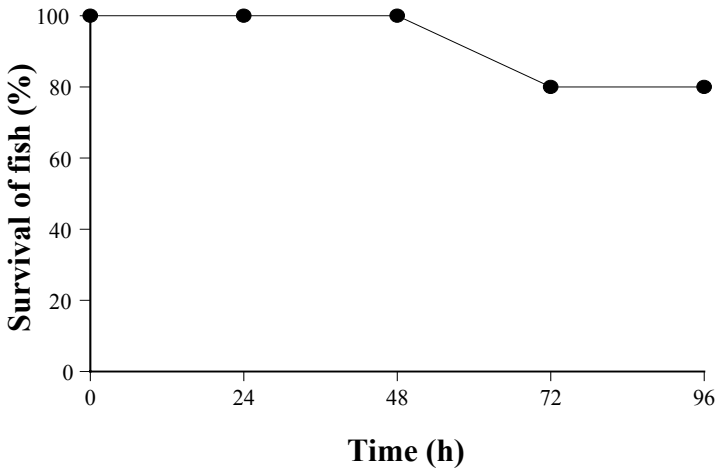


Fig 1.0 **Survival (%) of rainbow trout (a, b) and roach (c) exposed to isolates of *Actinomycete* 13 (●) and 15 (▲) and *Oscillatoria* sp (▲) for up to 96 hr.**

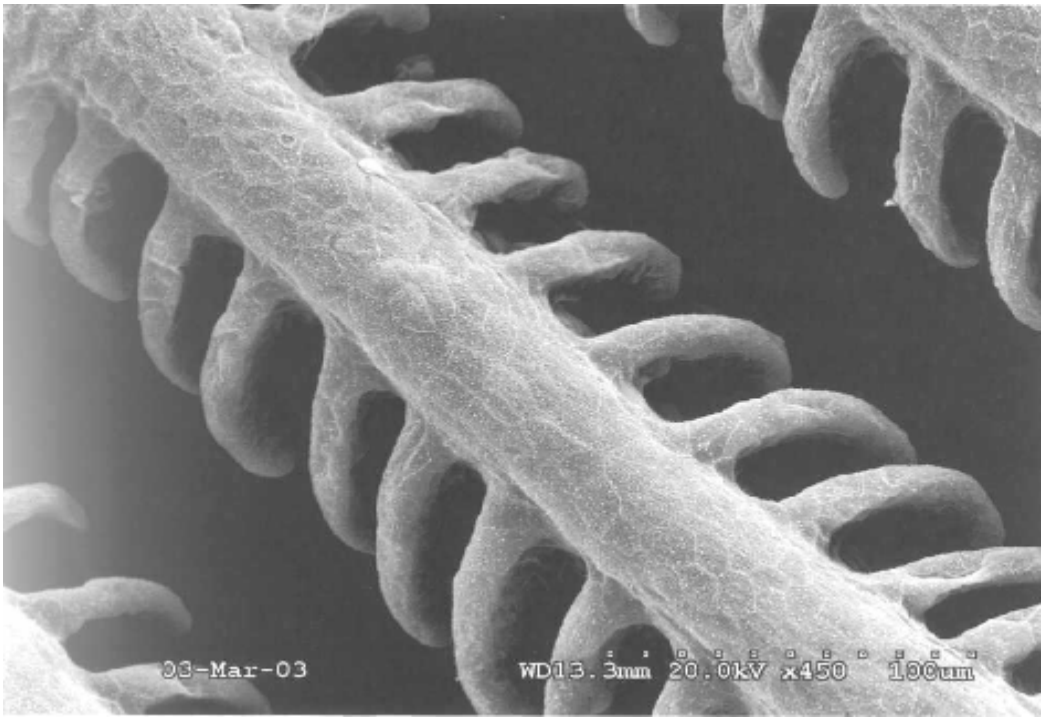


Fig 2.0 **Gills of unexposed control carp to show equal spacing of secondary lamellae with neither lamellar collapsing nor fusion.**

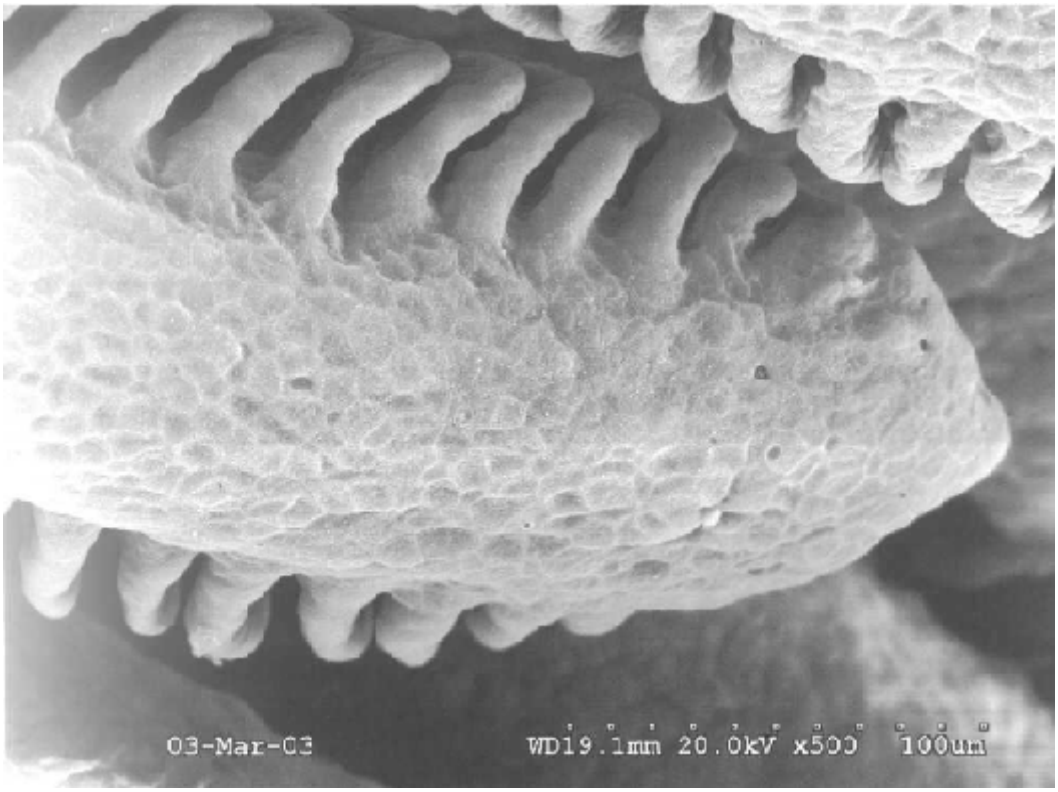


Fig 2.1 **Gills of unexposed control carp to show equal spacing of secondary lamellae with neither lamellar collapsing nor fusion.**

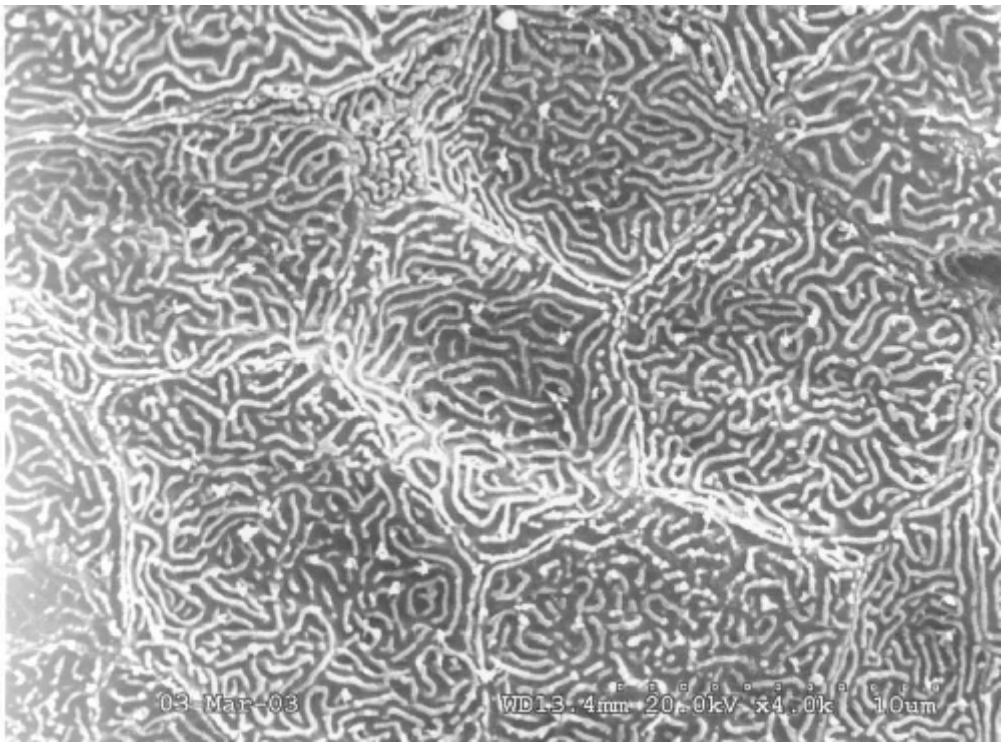


Fig 2.2 **Gills of unexposed control carp to show intact microridging on the filamental epithelium of the primary gill lamella.**

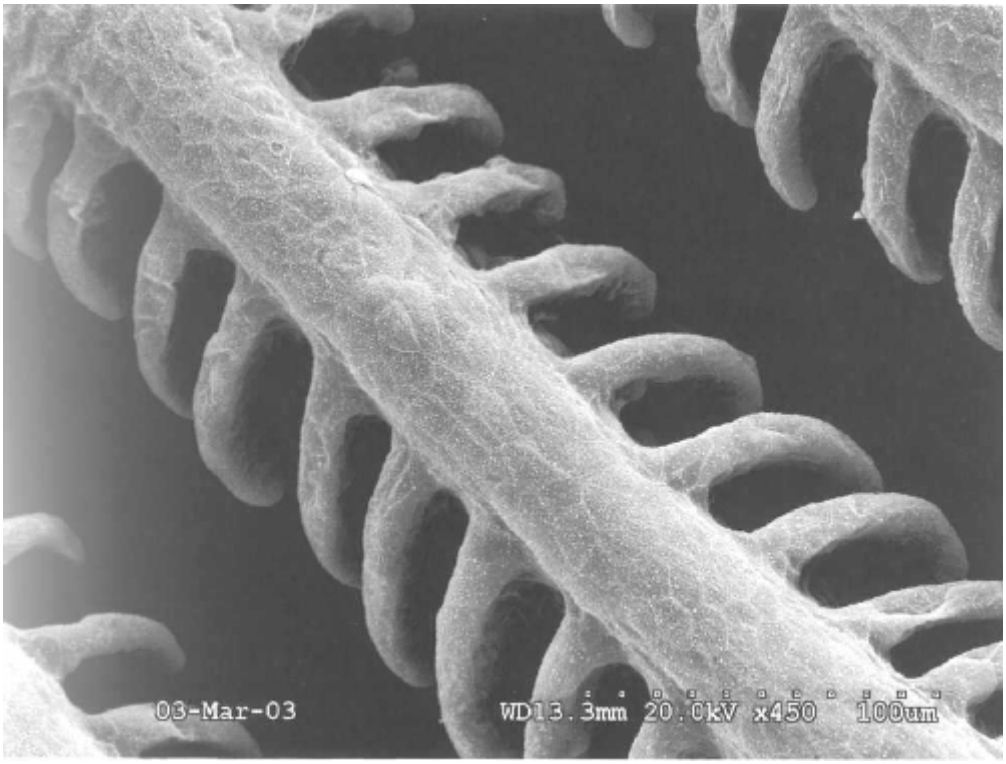


Fig 2.3 **Gills of unexposed control roach to show equal spacing of secondary lamellae with neither lamellar collapsing nor fusion.**

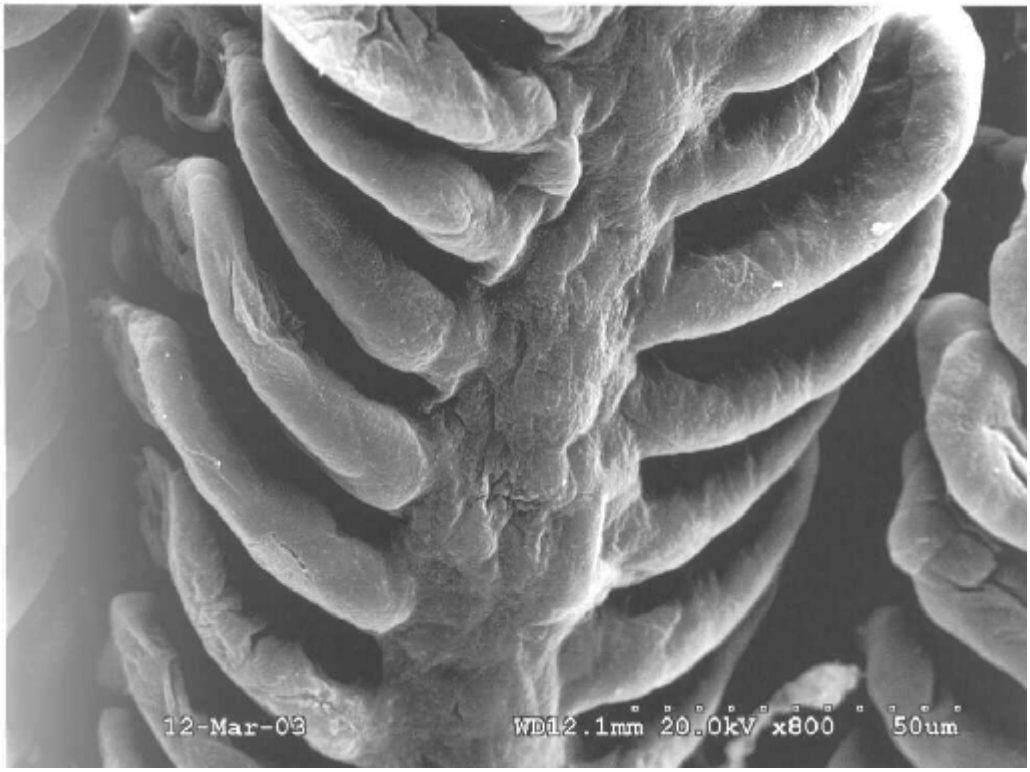


Fig 2.4 **Gills of unexposed control roach to show equal spacing of secondary lamellae with neither lamellar collapsing nor fusion.**

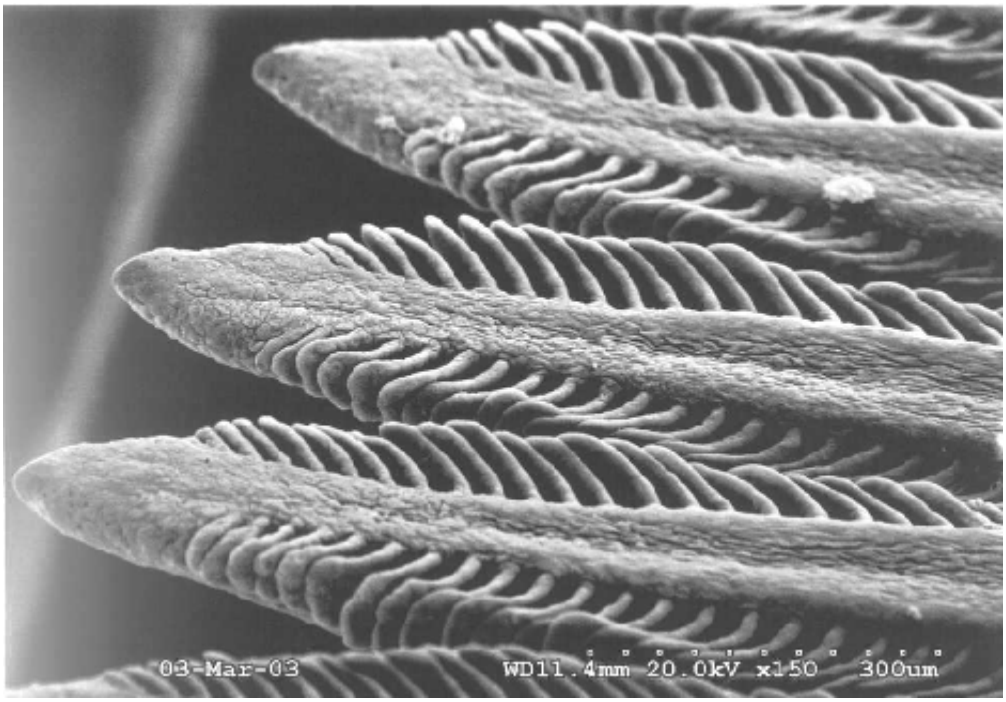


Fig 2.5 **Gills of unexposed control rainbow trout to show equal spacing of secondary lamellae with neither lamellar collapsing nor fusion.**

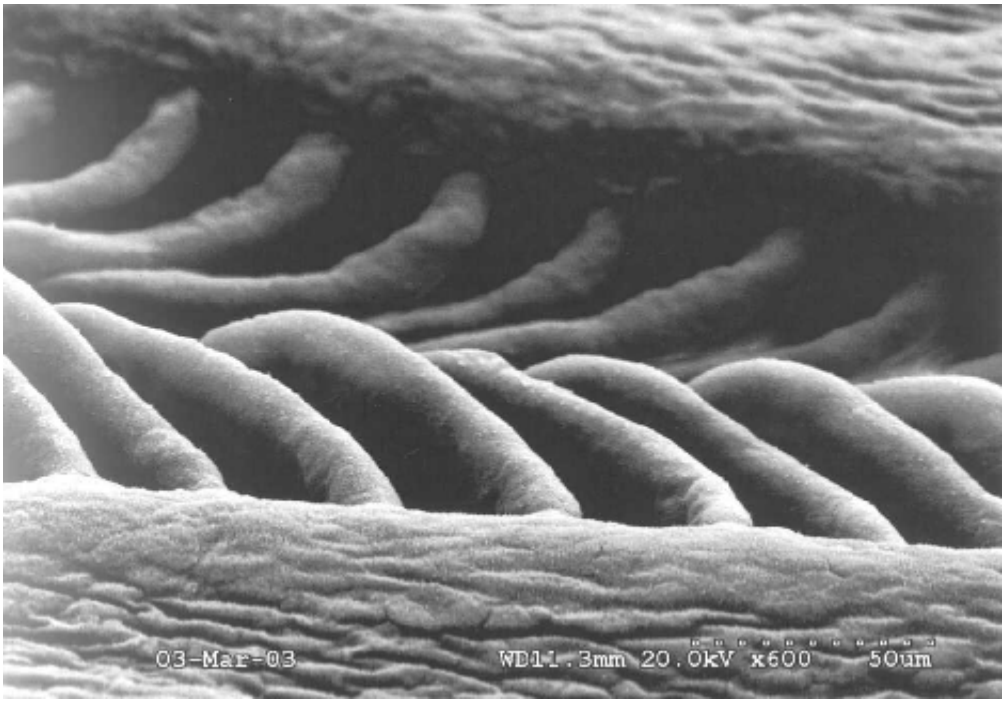


Fig 2.6 **Gills of unexposed control rainbow trout to show equal spacing of secondary lamellae with neither lamellar collapsing nor fusion.**

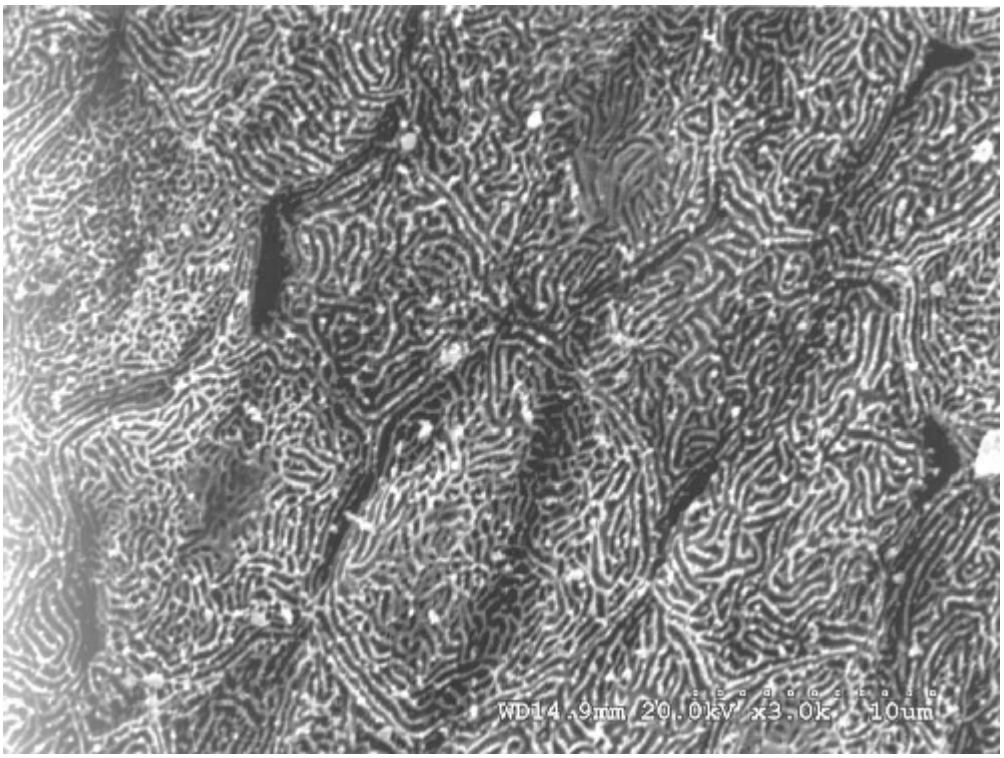


Fig 2.7 **Gills of unexposed control rainbow trout to show intact microridging on the filamental epithelium of the primary gill lamella.**

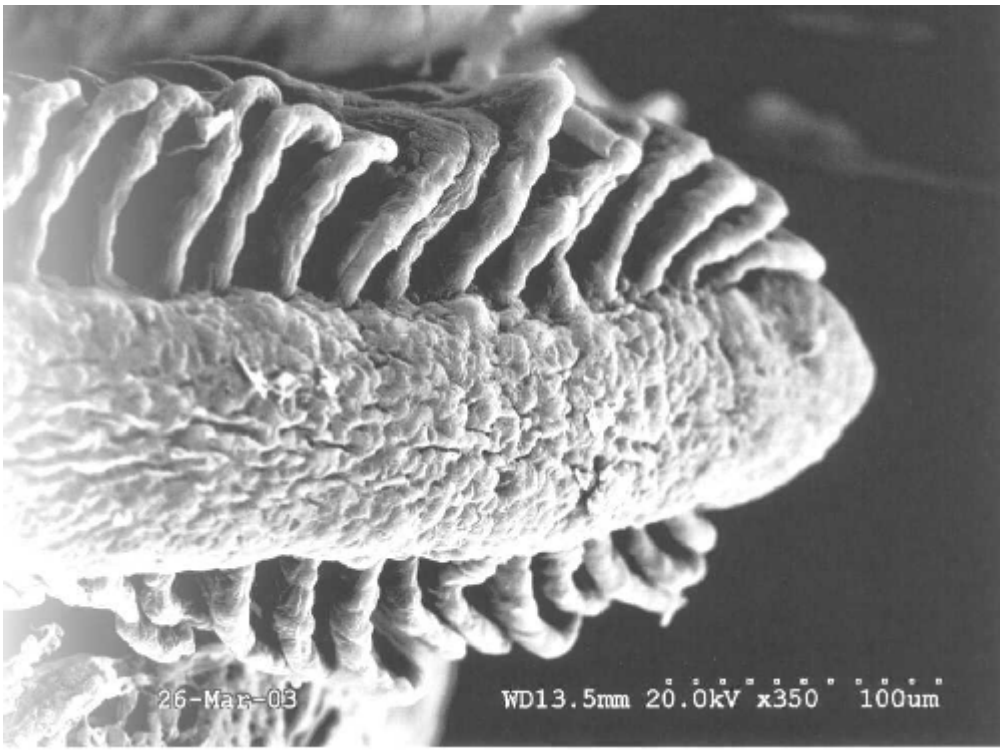


Fig 3.0 **Gills of carp following exposure to *Actinomycete 12* for 24 hours to show some collapsing of the secondary lamellae but no fusion.**

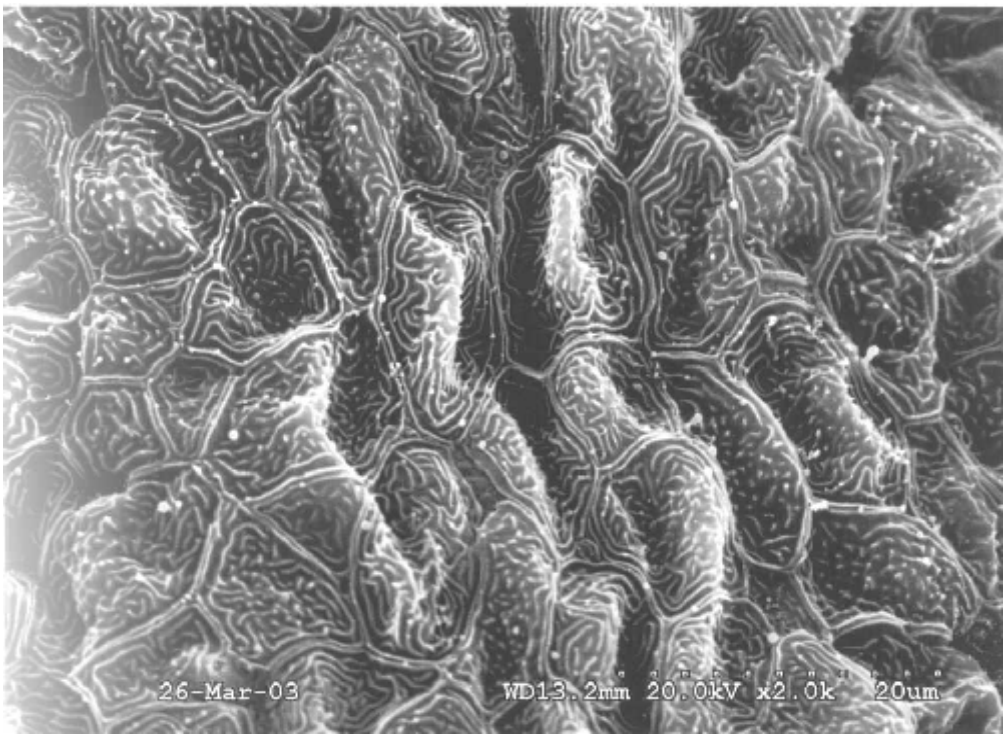


Fig 3.1 **Gills of carp following exposure to *Actinomycete 12* for 24 hours to show a low amount of distortion of the filamental epithelium and the microridging mainly intact.**

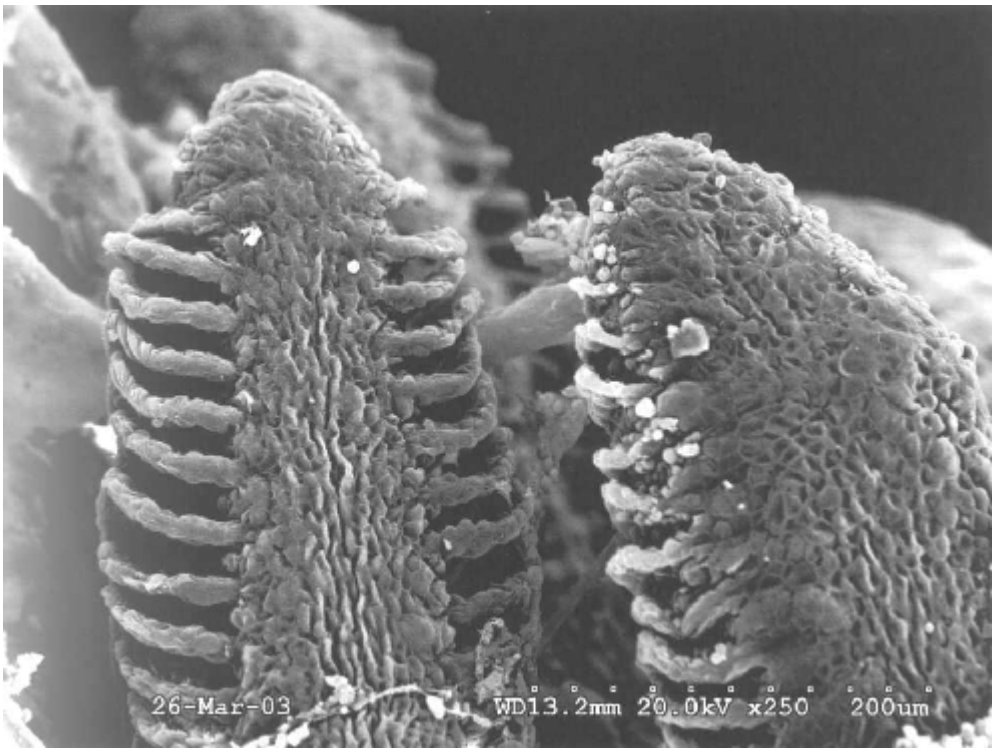


Fig 3.2 **Gills of carp following exposure to *Actinomycete 12* for 48 hours to show some fusion and erosion of the secondary lamellae especially at the tips.**

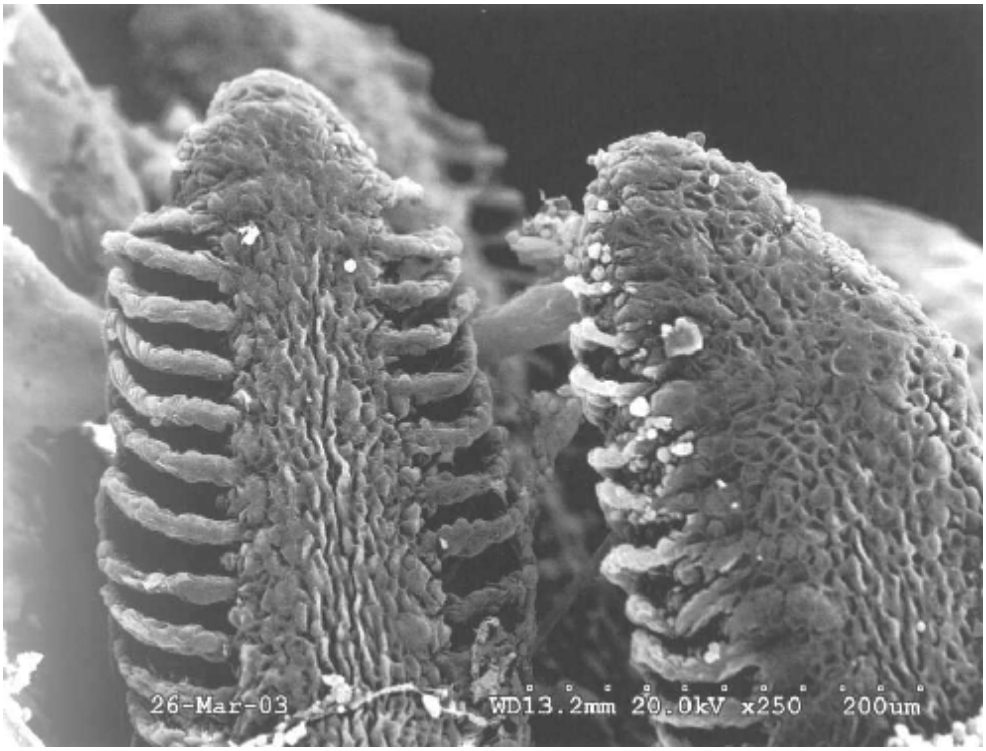


Fig 3.3 **Gills of carp following exposure to *Actinomycete 12* for 72 hours to show some distortion of the filamental epithelium but a relatively low loss of the microridging.**

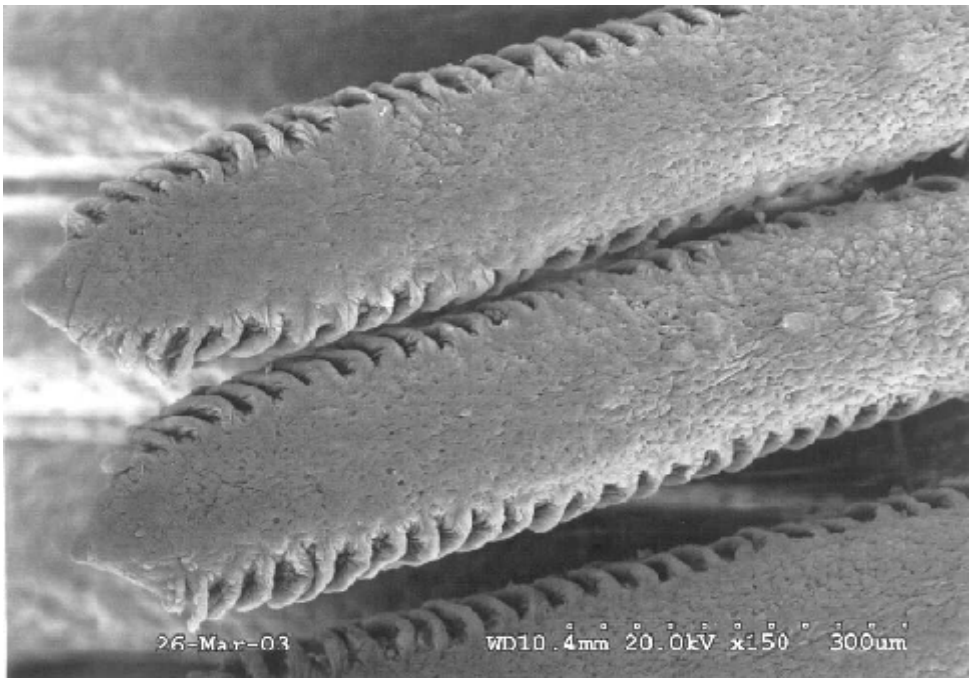


Fig 3.4 **Gills of carp following exposure to *Actinomycete 12* for 96 hours to show increasing hyperplasia leading to fusion of secondary lamellae and distortion and telangiectasis (swellings) on the filamental epithelium of primary gill lamellae.**

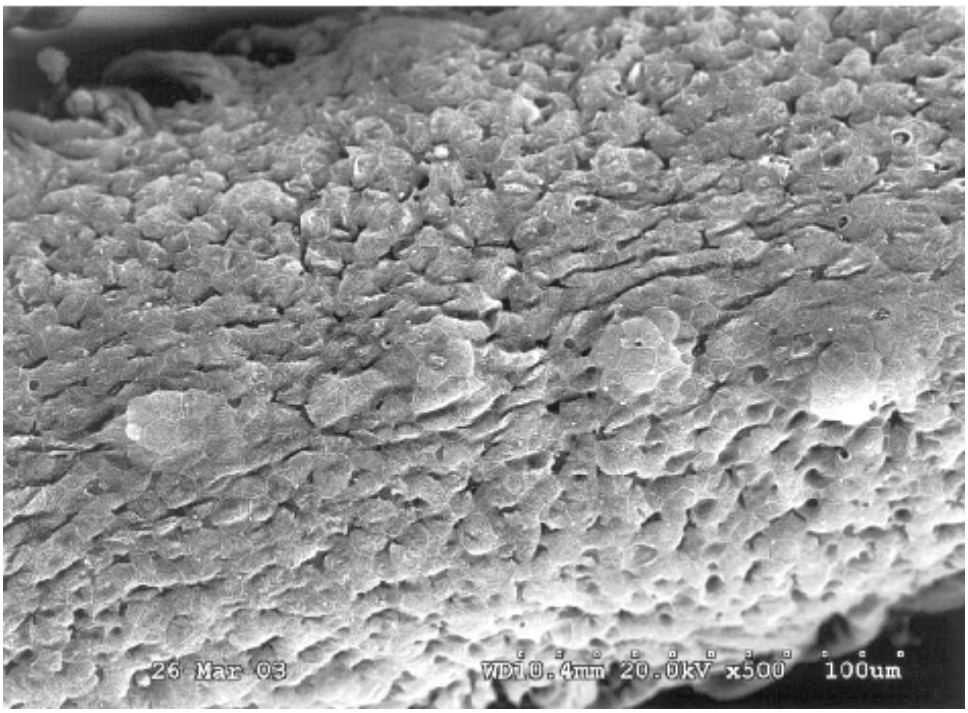


Fig 3.5 **Gills of carp following exposure to *Actinomycete 12* for 96 hours to show increasing distortion and telangiectasis (swellings) on the filamental epithelium of the primary gill lamella.**

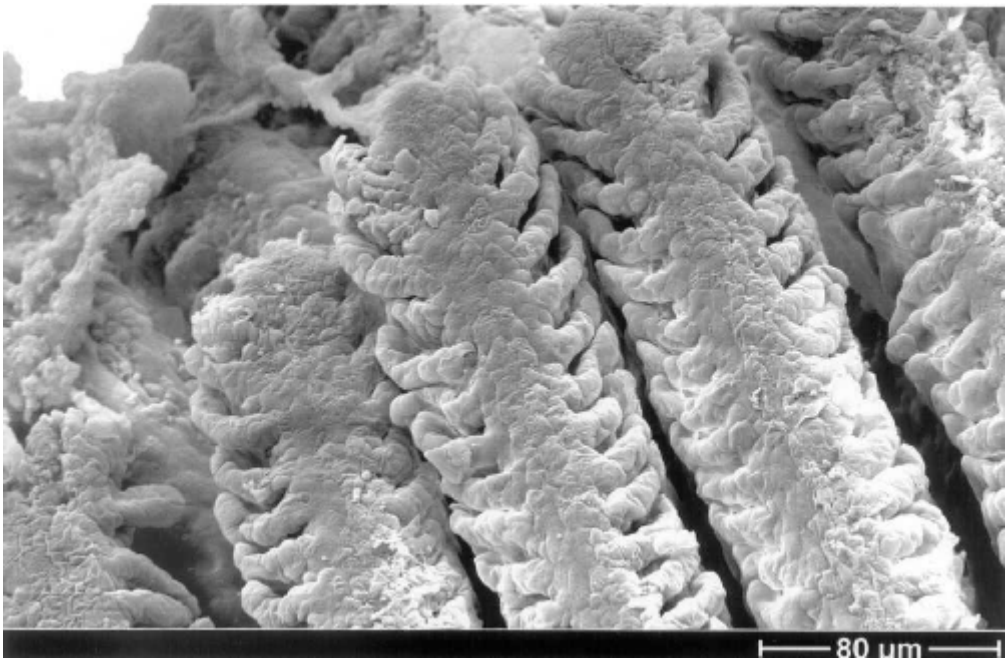


Fig 3.6 **Gills of roach following exposure to *Actinomycete 12* for 24 hr to show hyperplasia leading to fusion and erosion of secondary lamellae.**

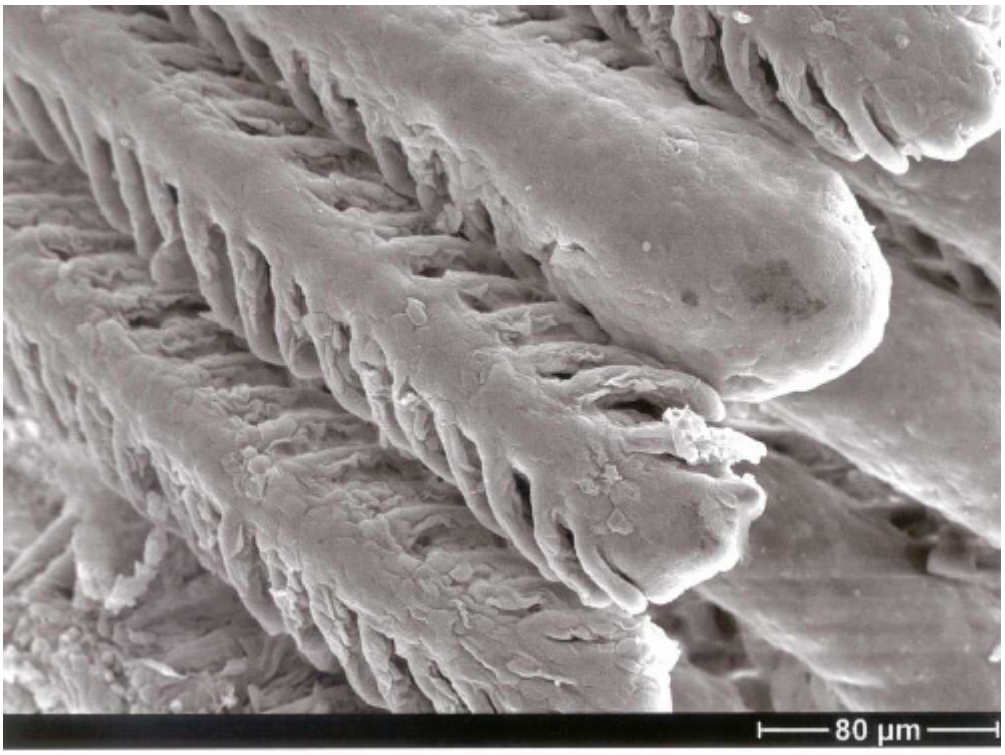


Fig 3.7 **Gills of roach following exposure to *Actinomycete 12* for 48 hr to show hyperplasia leading to fusion and erosion of secondary lamellae.**

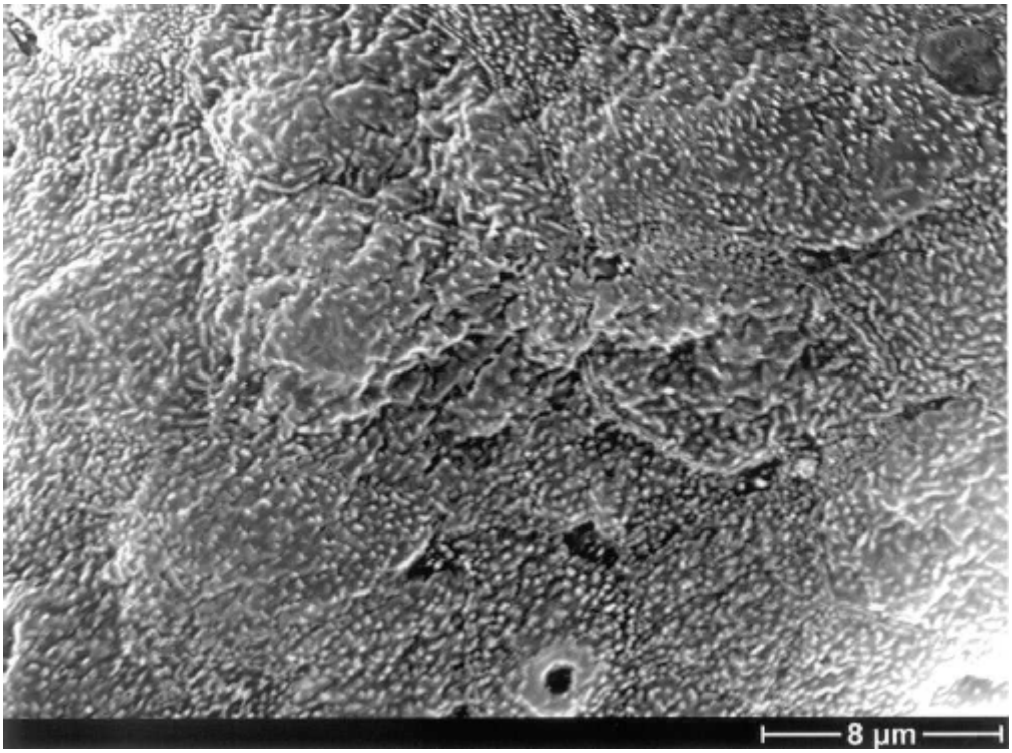


Fig 3.8 **Gills of roach following exposure to *Actinomycete 12* for 48 hr to show a moderate loss of microridging on the filamental epithelium of the primary gill lamella.**

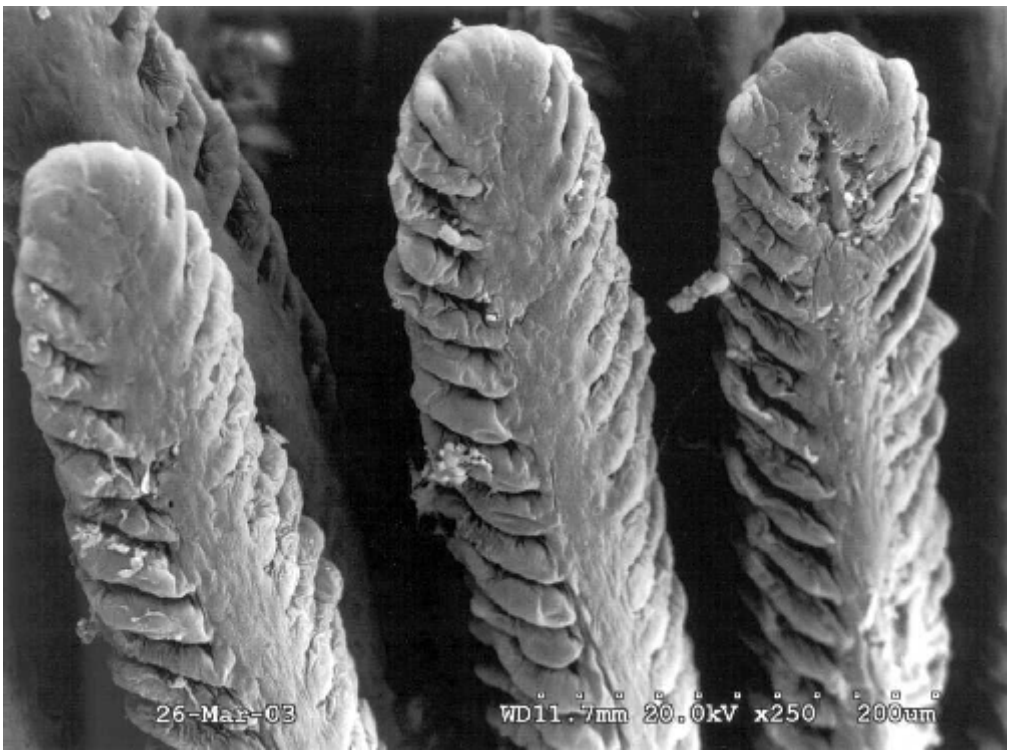


Fig 3.9 **Gills of roach following exposure to *Actinomycetes 12* for 96 hr to show hyperplasia leading to fusion and erosion of secondary lamellae.**

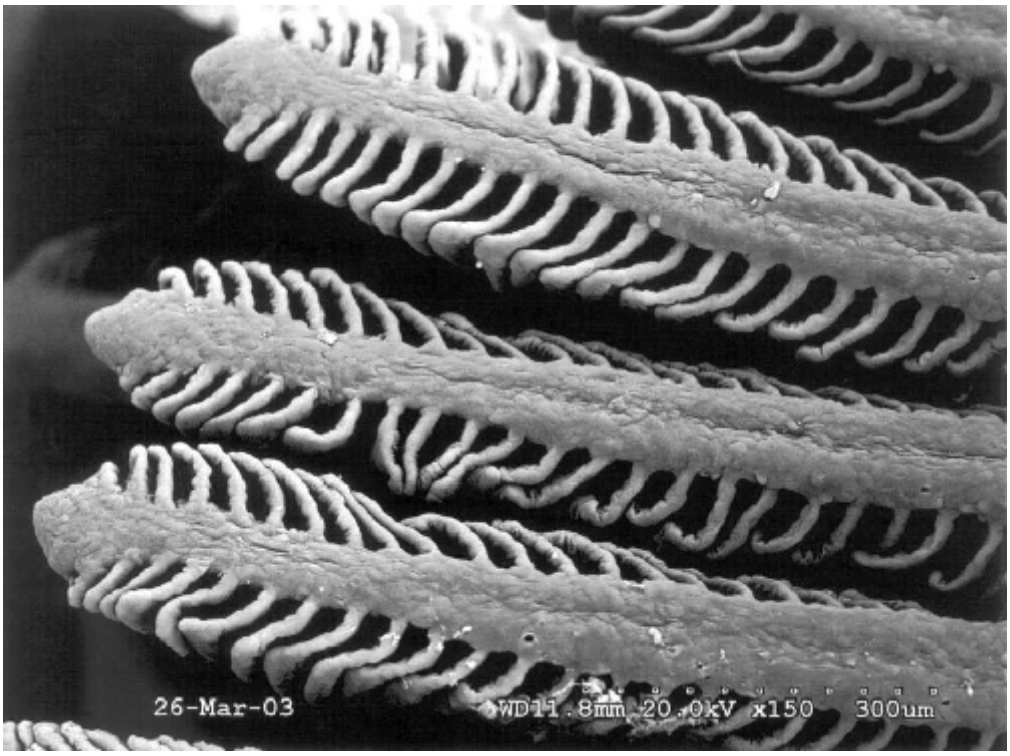


Fig 3.10 **Gills of rainbow trout following exposure to *Actinomycetes 12* for 24 hours to show collapsing but no fusion of the secondary lamellae.**

Fig 3.11 **Gills of rainbow trout following exposure to *Actinomycetes 12* for 24 hours to show intact microridging on the filamental epithelium of the primary lamella.**

Fig 3.12 **Gills of rainbow trout following exposure to *Actinomycetes 12* for 48 hours to show collapsing of secondary lamellae.**

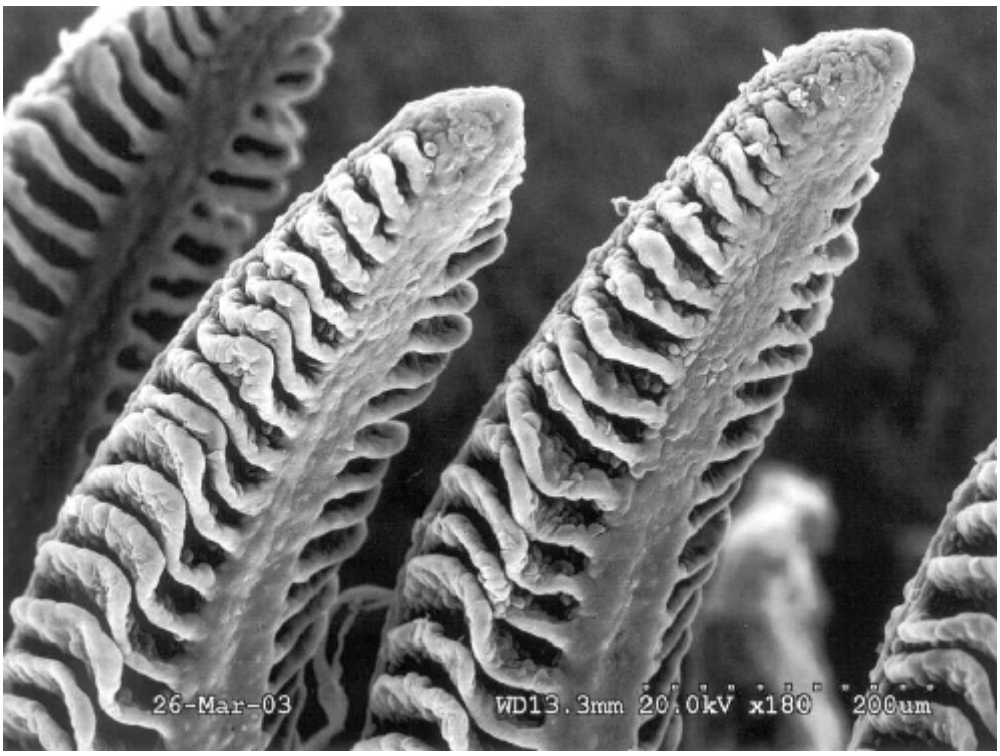


Fig 3.13 **Gills of rainbow trout following exposure to *Actinomycete 12* for 72 hours to show collapsing and some fusion/erosion near the tips of secondary lamellae.**

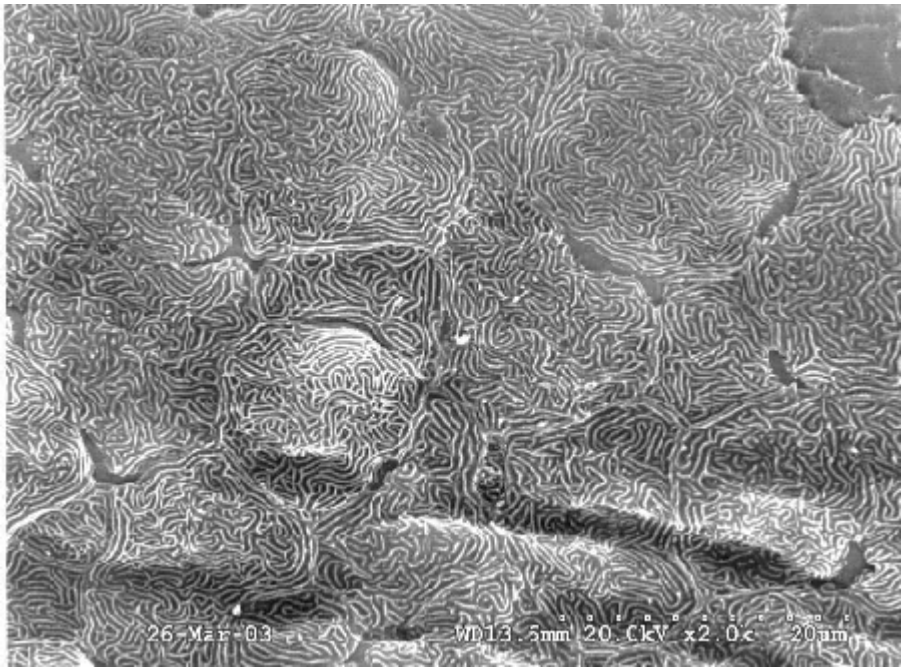


Fig 3.14 **Gills of rainbow trout following exposure to *Actinomycete 12* for 72 hours to show some distortion of the filamental epithelium but the microridging more or less remains intact.**

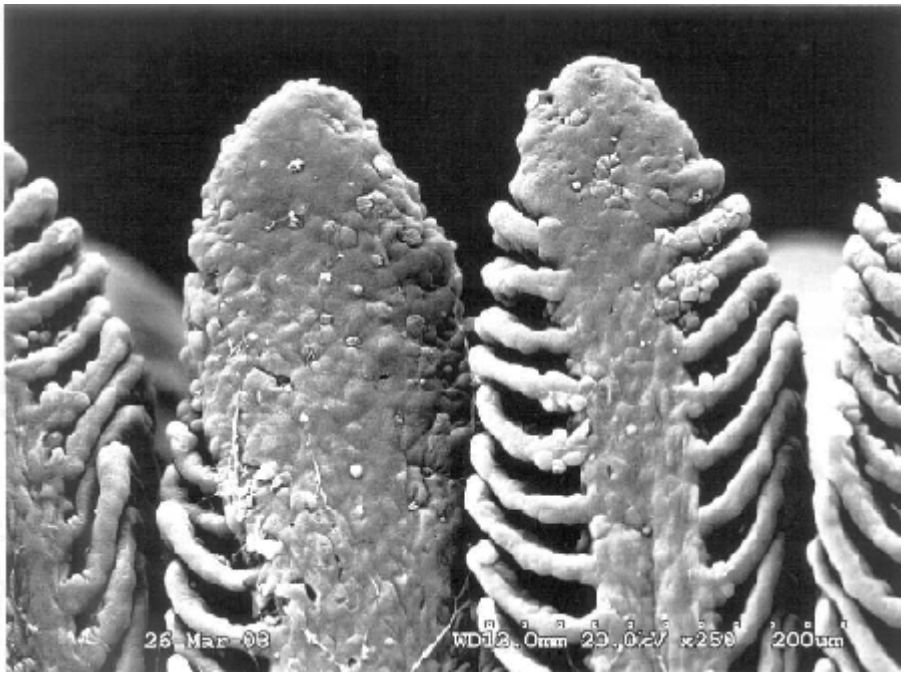


Fig 3.15 **Gills of rainbow trout following exposure to *Actinomycete 12* for 96 hours to show fusion mainly at the tips of secondary lamellae.**

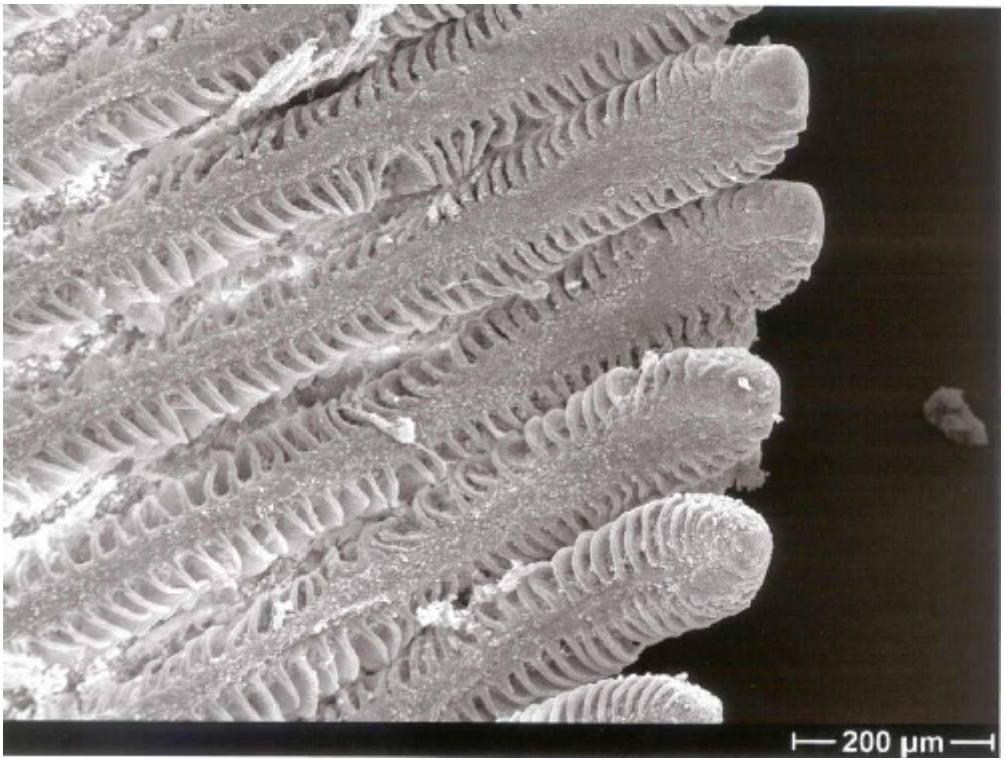


Fig 4.0 **Gills of carp exposed to *Actinomycete* 13 for 24 hours to show collapsing and some fusion at the tips of secondary lamellae; filamental epithelium on primary gill lamellae is also distorted.**

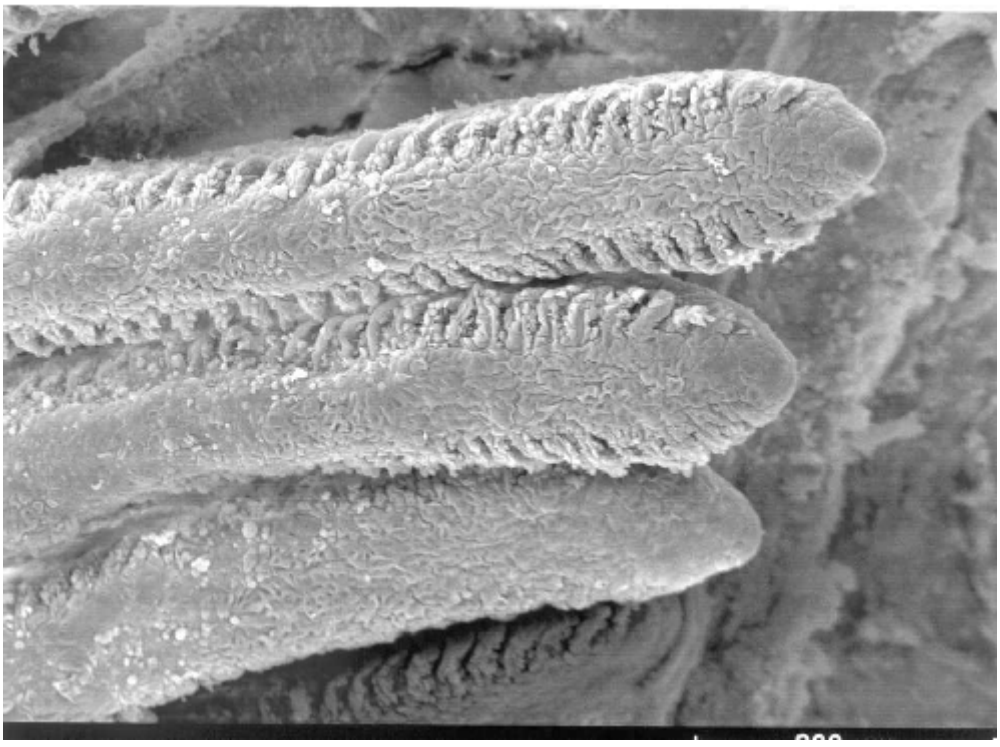


Fig 4.1 **Gills of carp exposed to *Actinomycete* 13 for 48 hours to show hyperplasia leading to show extensive fusion and erosion of secondary lamellae.**

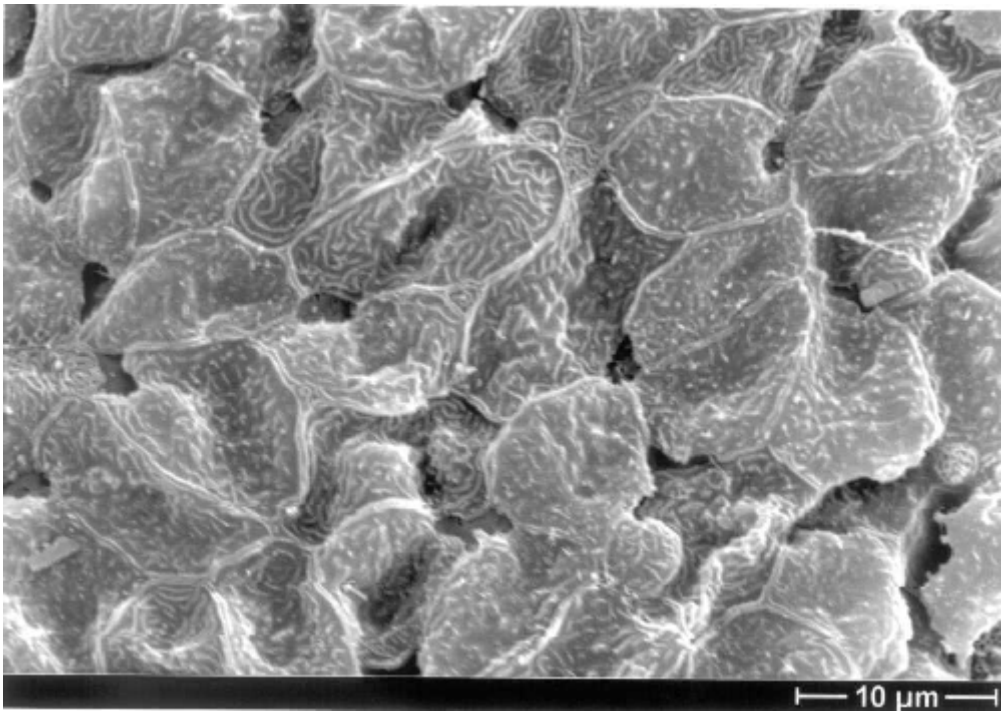


Fig 4.2 **Gills of carp exposed to *Actinomycete* 13 for 48 hours to show distortion and loss of microridging on the filamental epithelium of primary gill lamellae.**

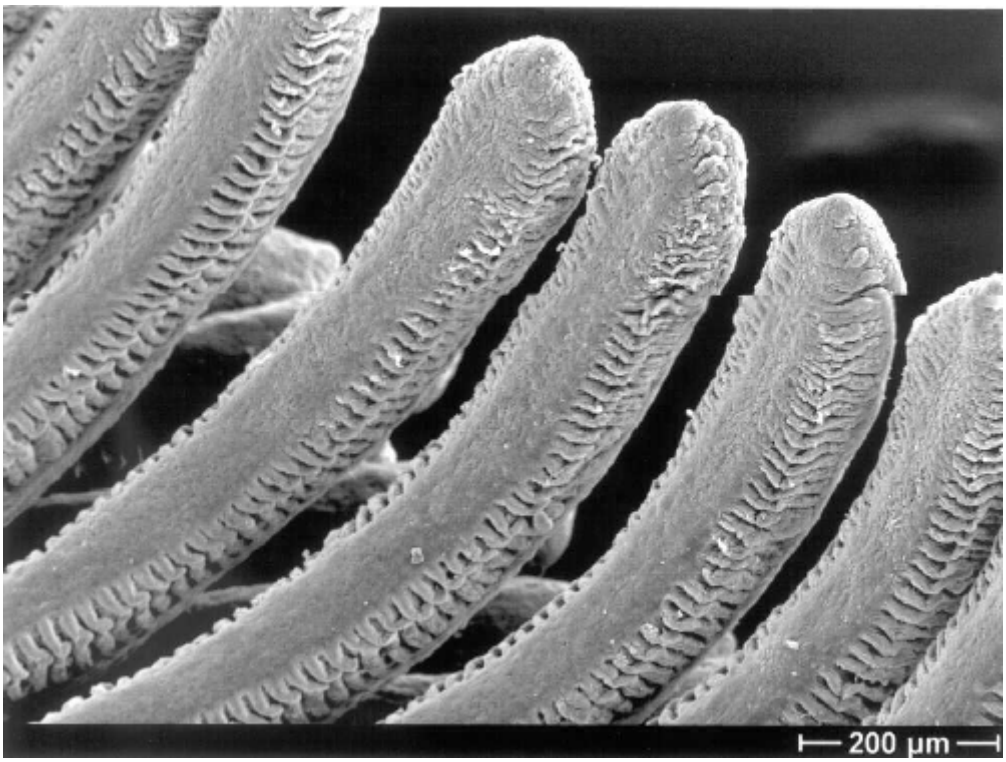


Fig 4.3 **Gills of carp exposed to *Actinomycete* 13 for 96 hours to show hyperplasia leading to fusion and erosion of secondary lamellae.**

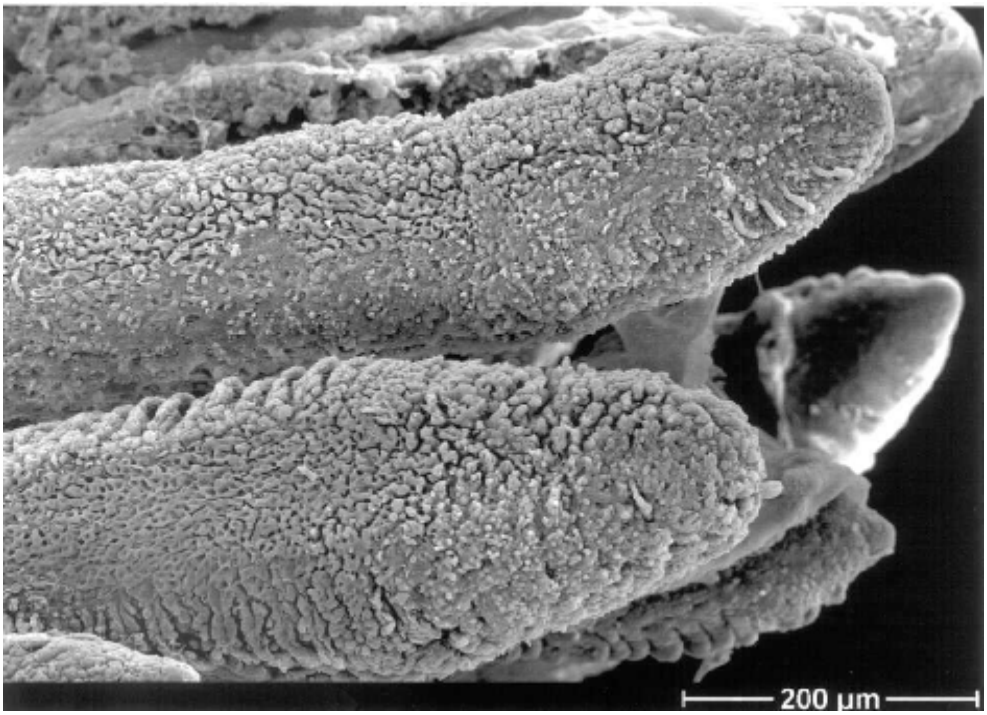


Fig 4.4 **Gills of carp exposed to *Actinomycete* 13 for 96 hours to show hyperplasia leading to extensive fusion and erosion of the secondary lamellae together with a high degree of distortion and necrosis of the filamental epithelium of primary lamellae.**

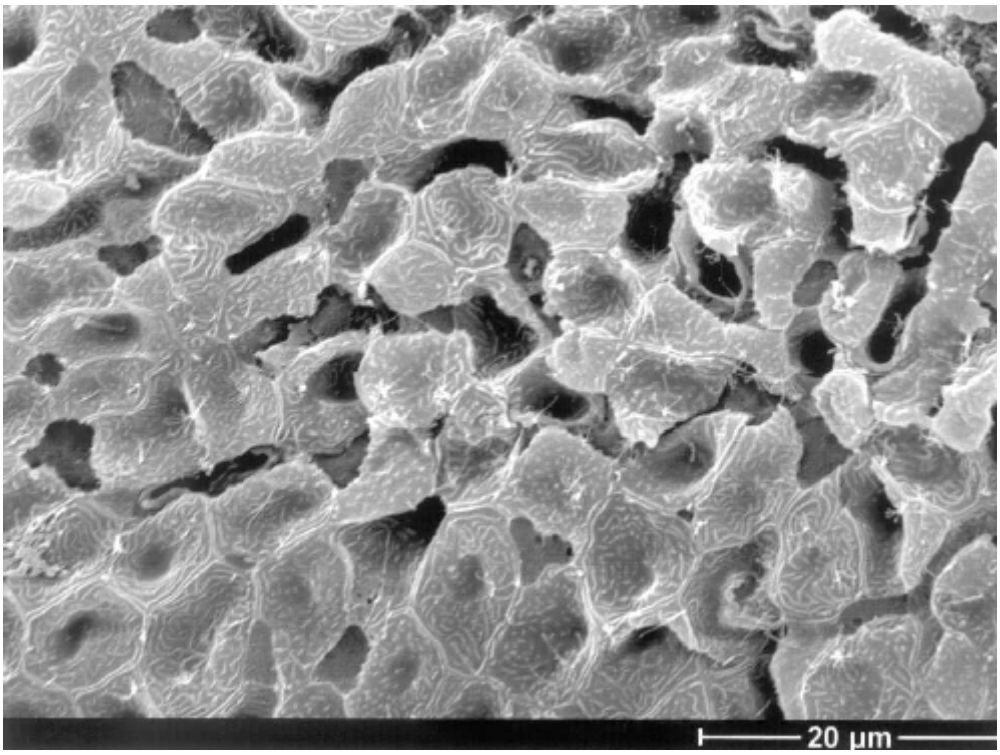


Fig 4.5 **Gills of carp exposed to *Actinomycete* 13 for 96 hours to show a high degree of distortion and loss of microridging on the filamental epithelium of a primary lamella.**

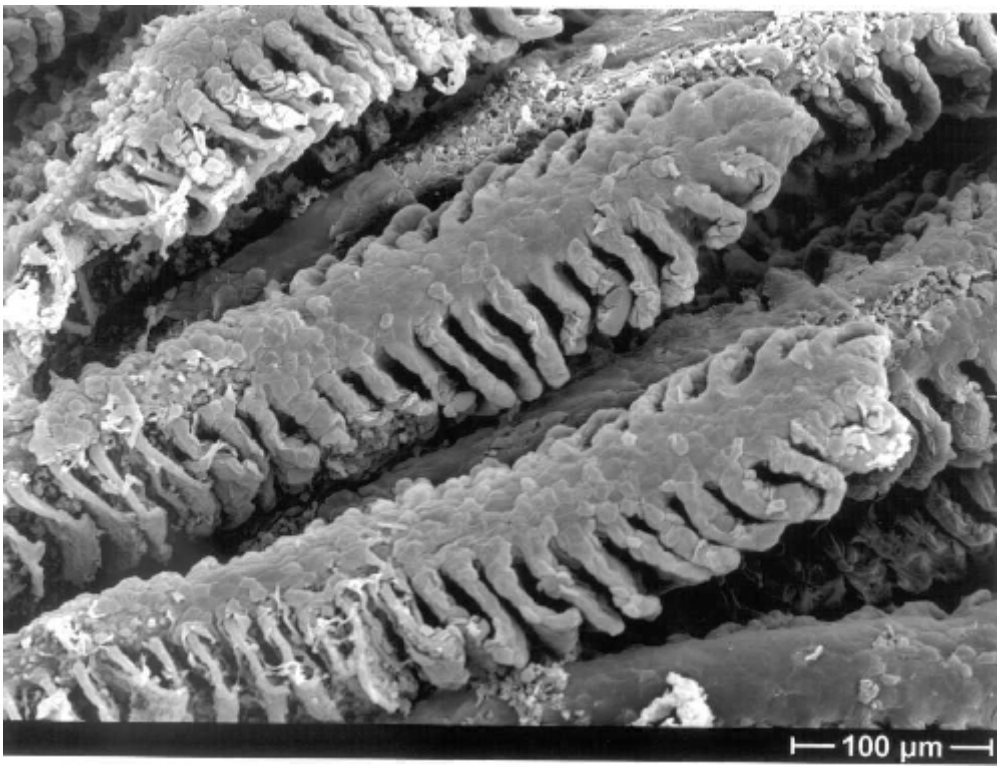


Fig 4.6 **Gills of roach exposed to *Actinomycete* 13 for 24 hours to show collapsing, hyperplasia leading to some fusion and erosion of secondary lamellae.**

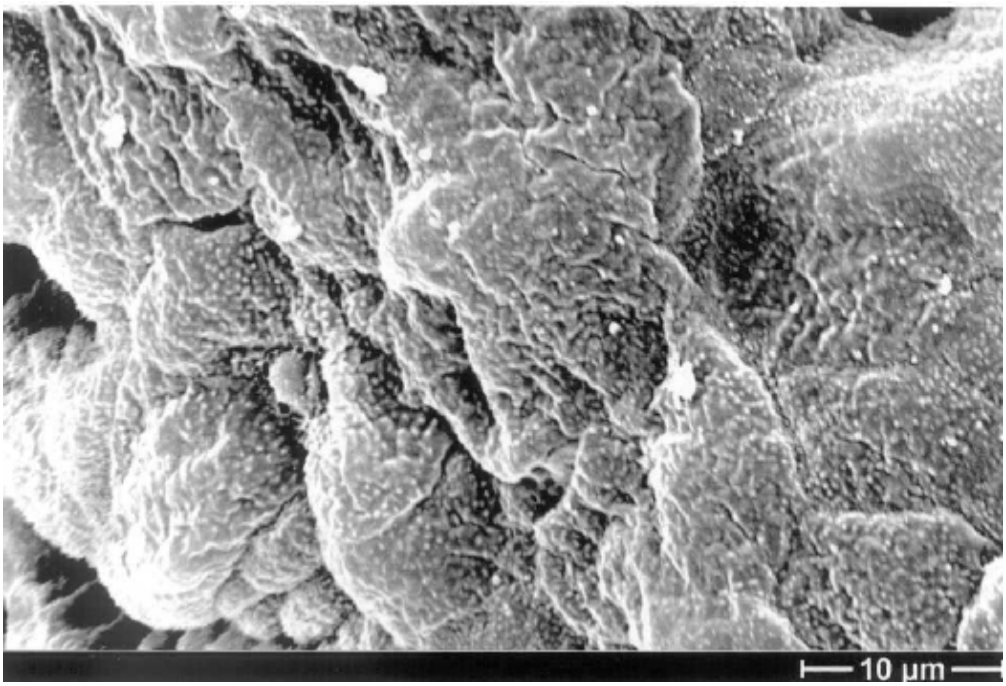


Fig 4.7 **Gills of roach exposed to *Actinomycete* 13 for 24 hours to show a moderate loss of microridging on the filamental epithelium of a primary lamella.**

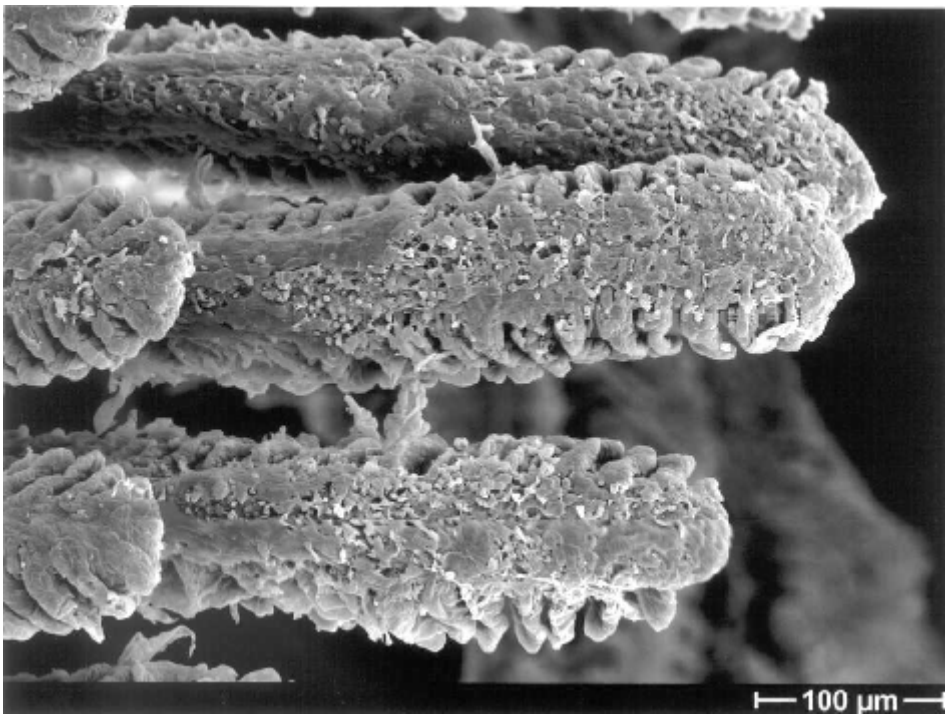


Fig 4.8 **Gills of roach exposed to *Actinomycete* 13 for 72 hours to show hyperplasia leading to extensive fusion, erosion and necrosis of secondary lamellae and filamental epithelia of primary lamellae.**

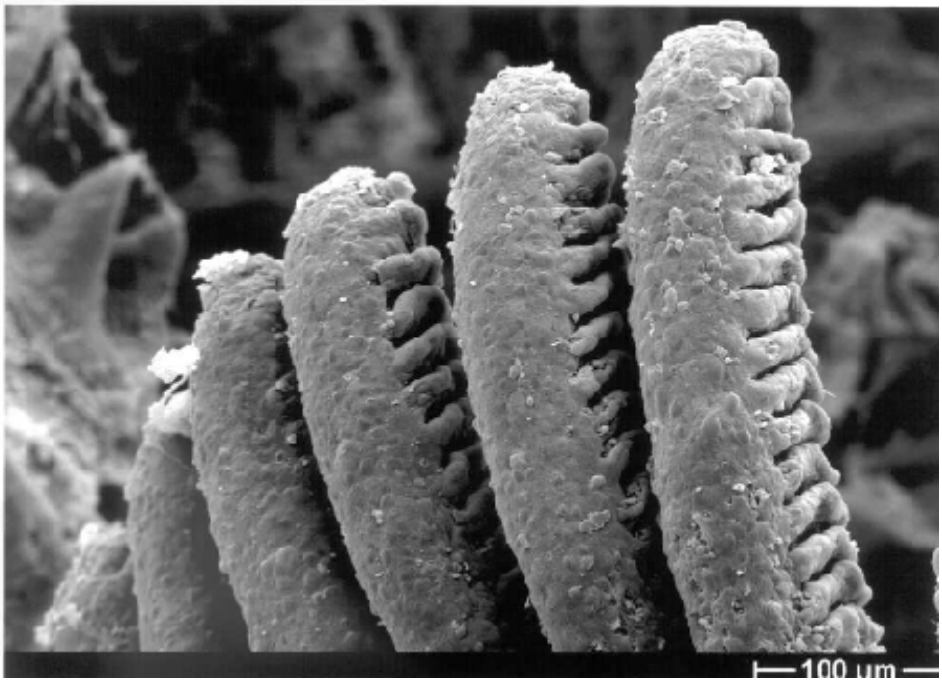


Fig 4.9 **Gills of roach exposed to *Actinomycete* 13 for 72 hours to show hyperplasia of secondary lamellae and filamental epithelia of primary lamellae.**

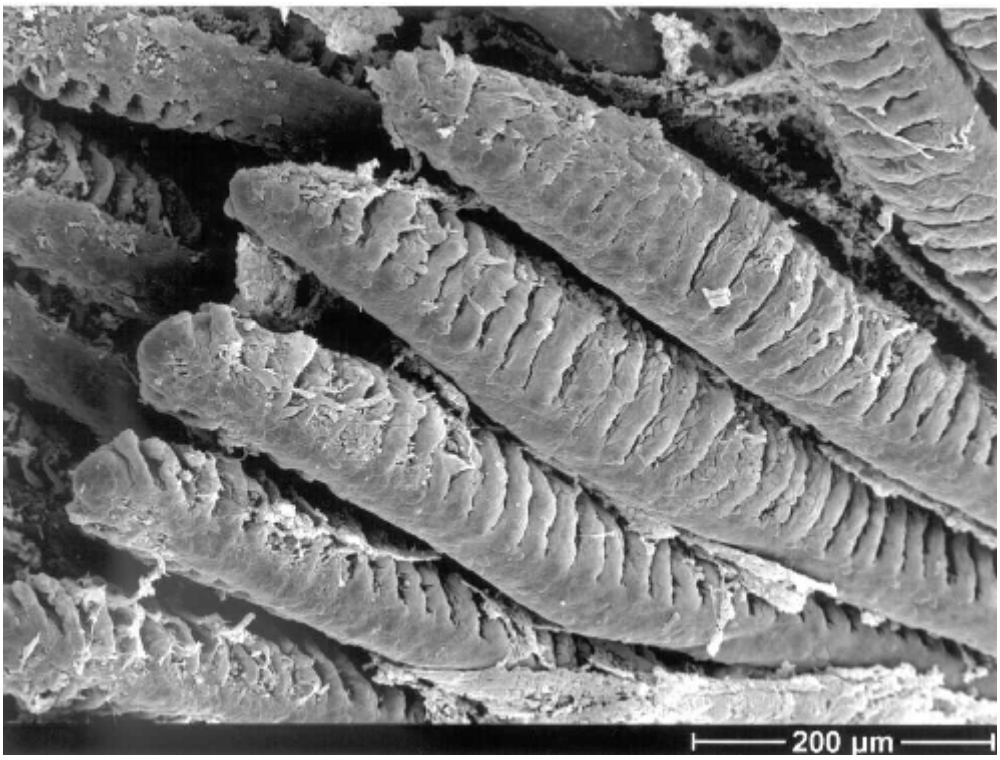


Fig 4.10 **Gills of roach exposed to *Actinomycete* 13 for 96 hours to show hyperplasia leading to extensive fusion and erosion of secondary lamellae.**

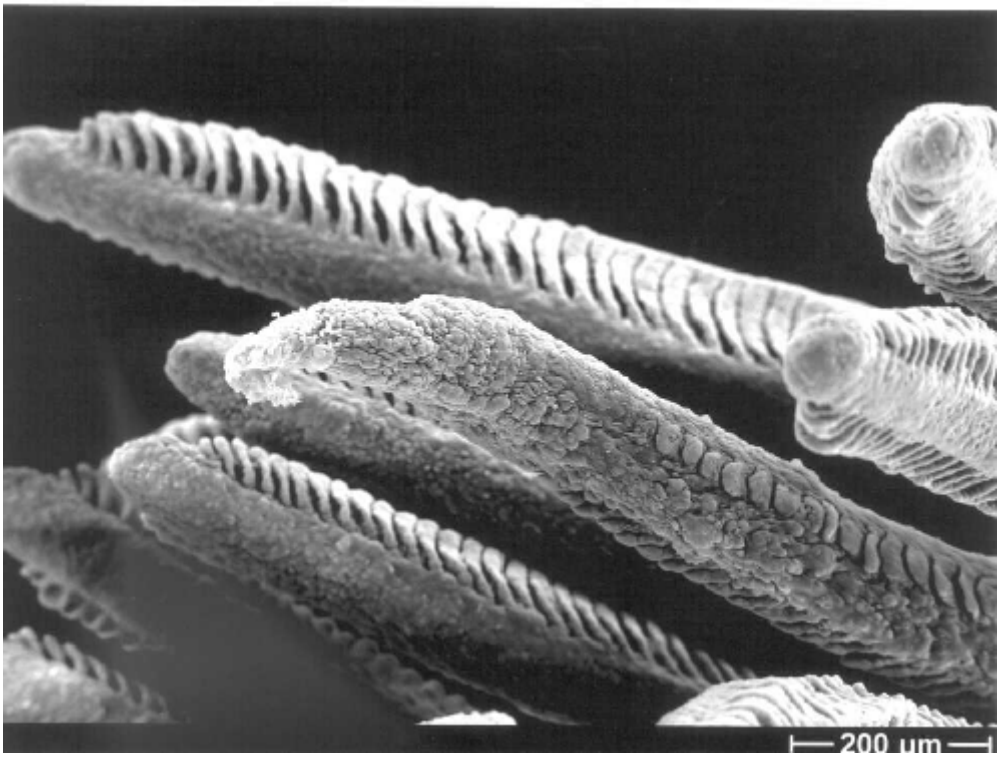


Fig 4.11 **Gills of rainbow trout exposed to *Actinomycete* 13 for 24 hours to show collapsing and fusion especially at the tips of secondary lamellae.**

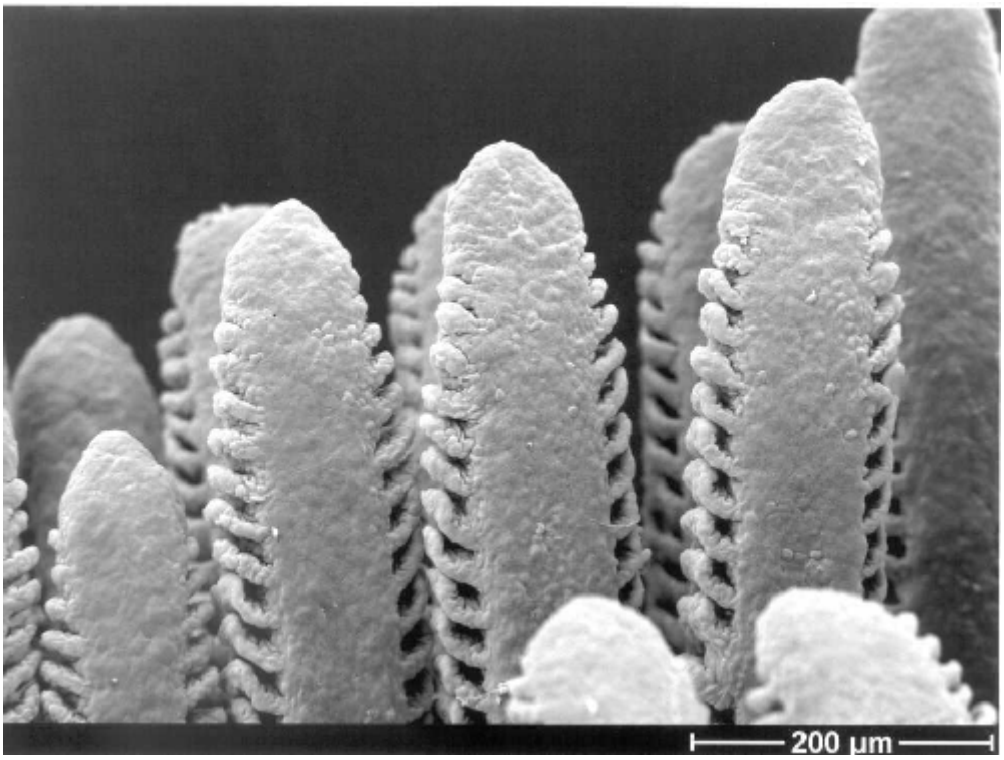


Fig 4.12 **Gills of rainbow trout exposed to *Actinomycete* 13 for 24 hours to show hyperplasia leading to fusion of secondary lamellae especially at the tips.**

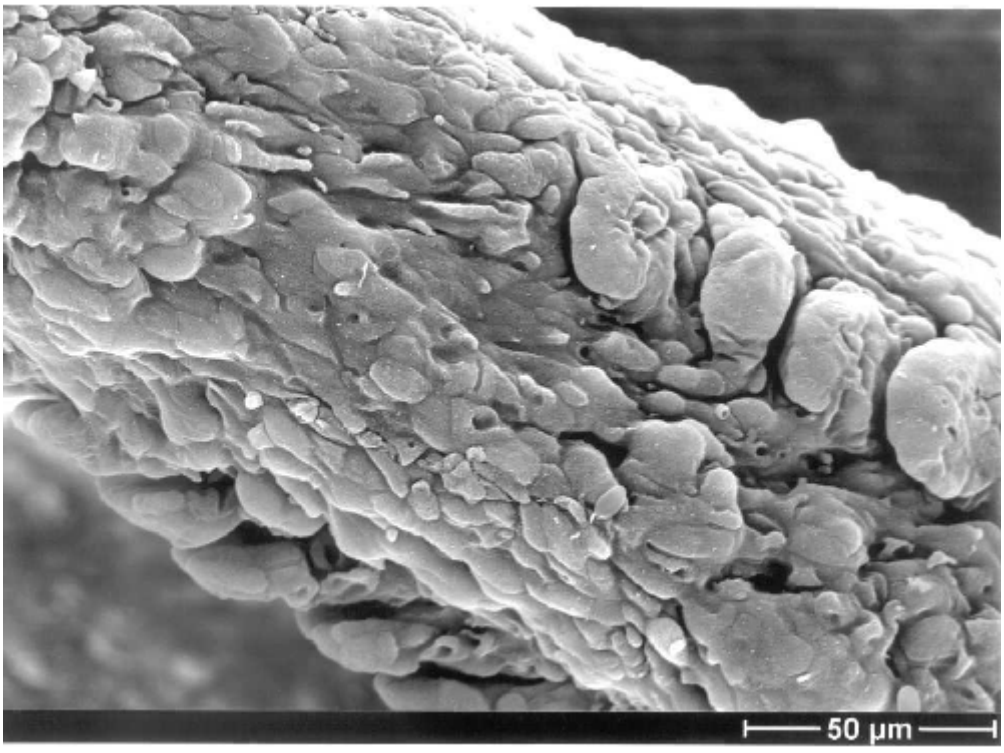


Fig 4.13 **Gills of rainbow trout exposed to *Actinomycete* 13 for 24 hours to show a high degree of distortion on the filamental epithelium of a primary lamella.**

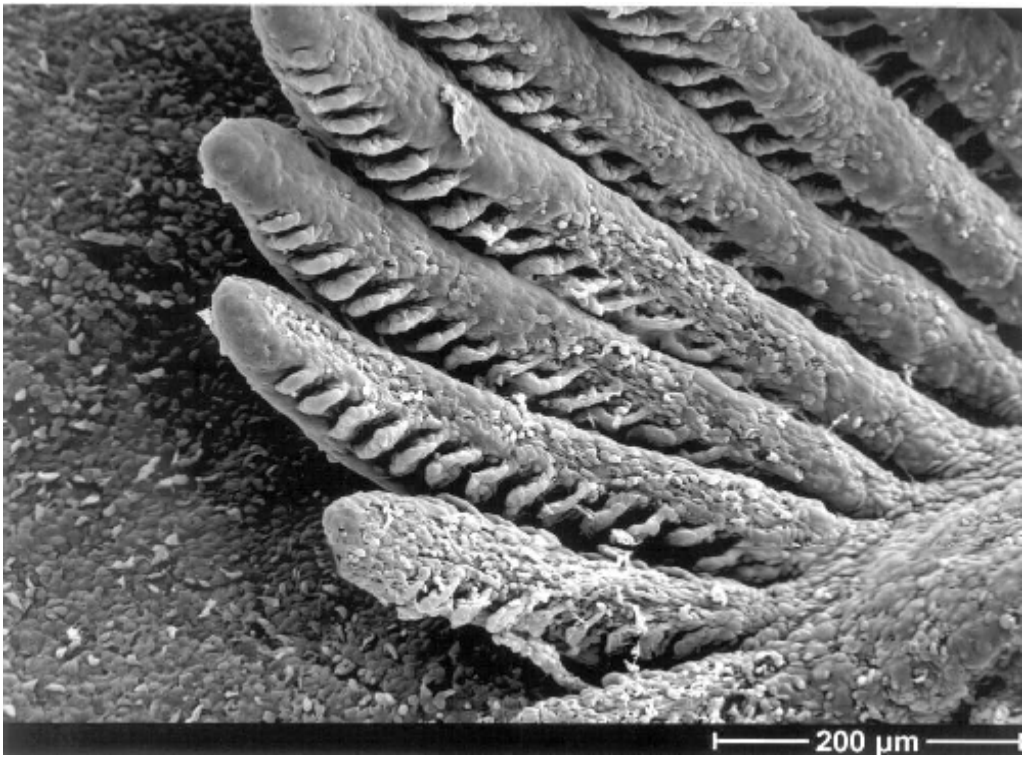


Fig 4.14 **Gills of rainbow trout exposed to *Actinomycete* 13 for 24 hours to show erosion of secondary lamellae and distortion/erosion of filamental epithelia of primary lamellae.**

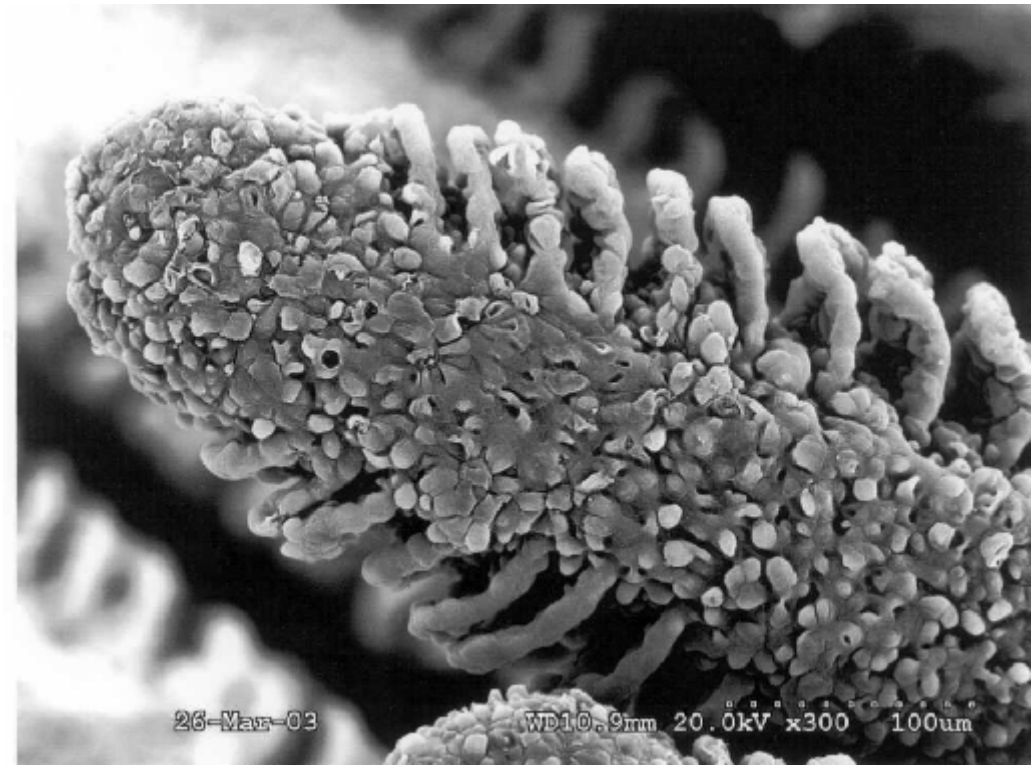


Fig 4.15 **Gills of rainbow trout exposed to *Actinomycete* 13 for 48 hours to show fusion of the tip of a secondary lamella and a high degree of distortion on the filamental epithelium of a primary lamella.**

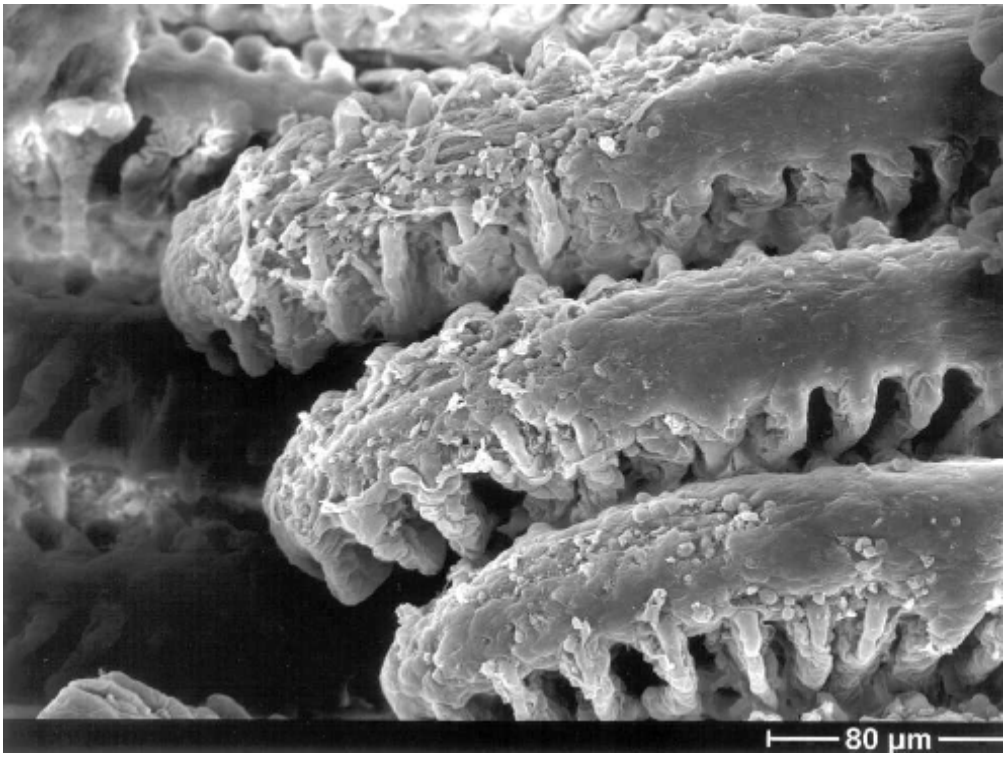


Fig 4.16 **Gills of rainbow trout exposed to *Actinomycete* 13 for 96 hours to show erosion and necrosis of secondary lamellae and also on the filamental epithelium of primary lamellae.**

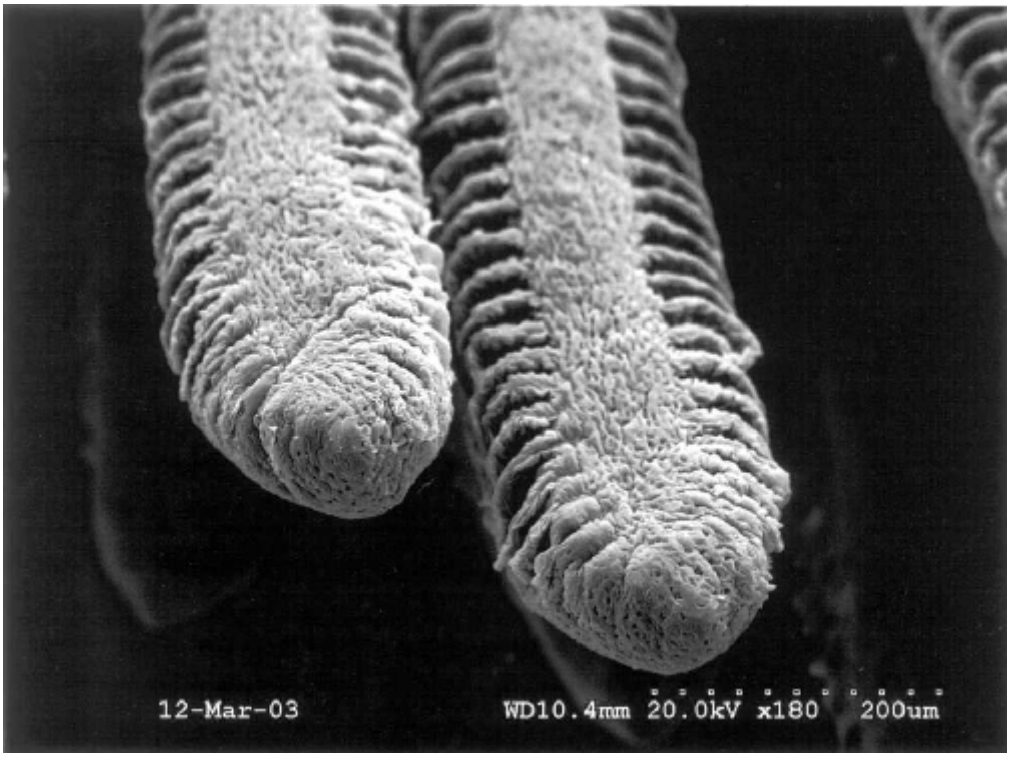


Fig 5.0 **Gills of carp exposed to *Actinomycete* 15 for 24 hours to show some fusion and distortion of secondary lamellae near the tips.**

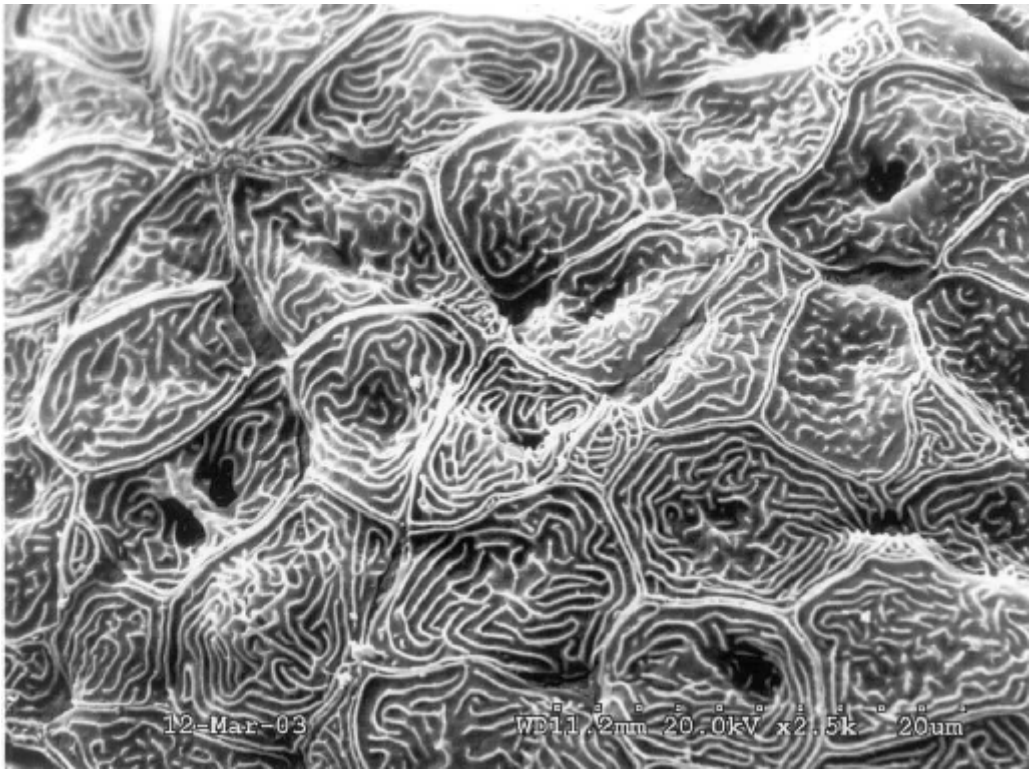


Fig 5.1 **Gills of carp exposed to *Actinomycete* 15 for 24 hours to show a low degree of distortion and little loss of microridging on the filamental epithelium of a primary lamella.**

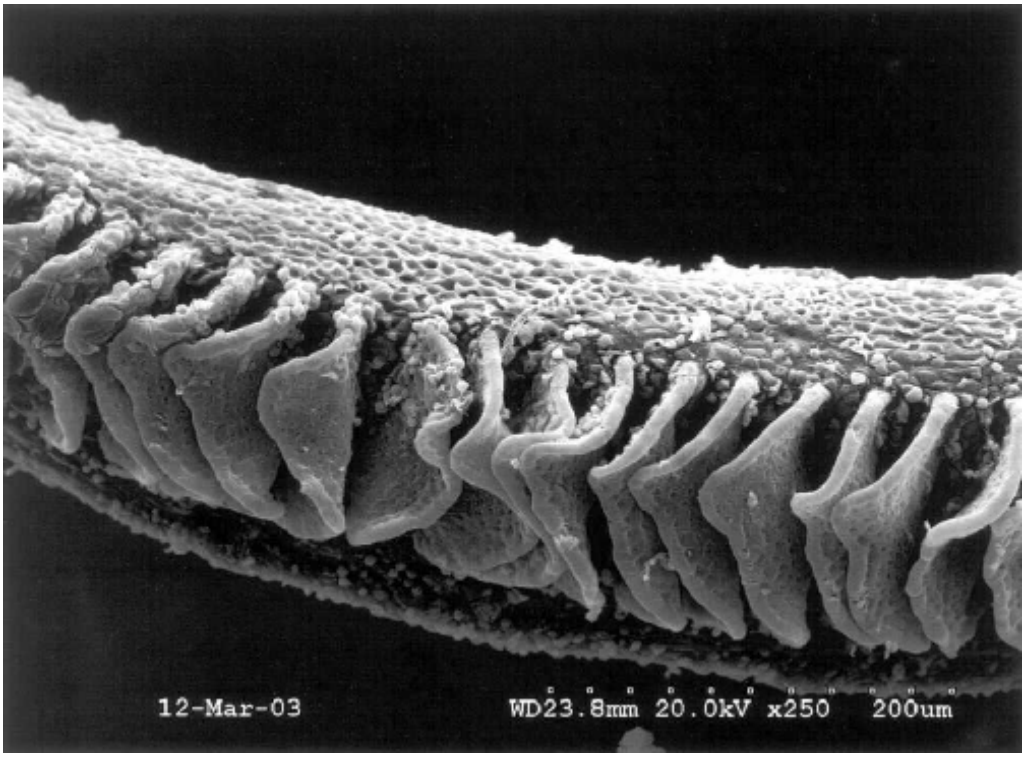


Fig 5.2 **Gills of carp exposed to *Actinomycete 1*5 for 48 hours to show collapsing of secondary lamellae and a moderate degree of distortion on the filamental epithelium of a primary lamella.**

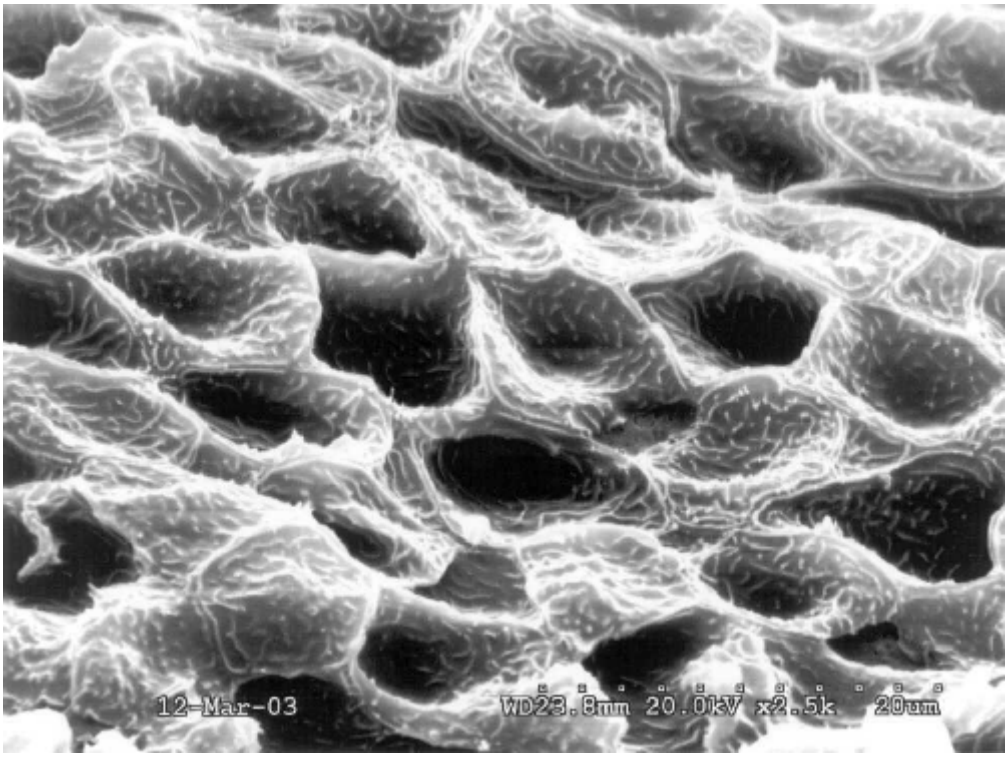


Fig 5.3 **Gills of carp exposed to *Actinomycete* 15 for 48 hours to show a moderate degree of distortion and some loss of microridging on the filamental epithelium of a primary lamella.**

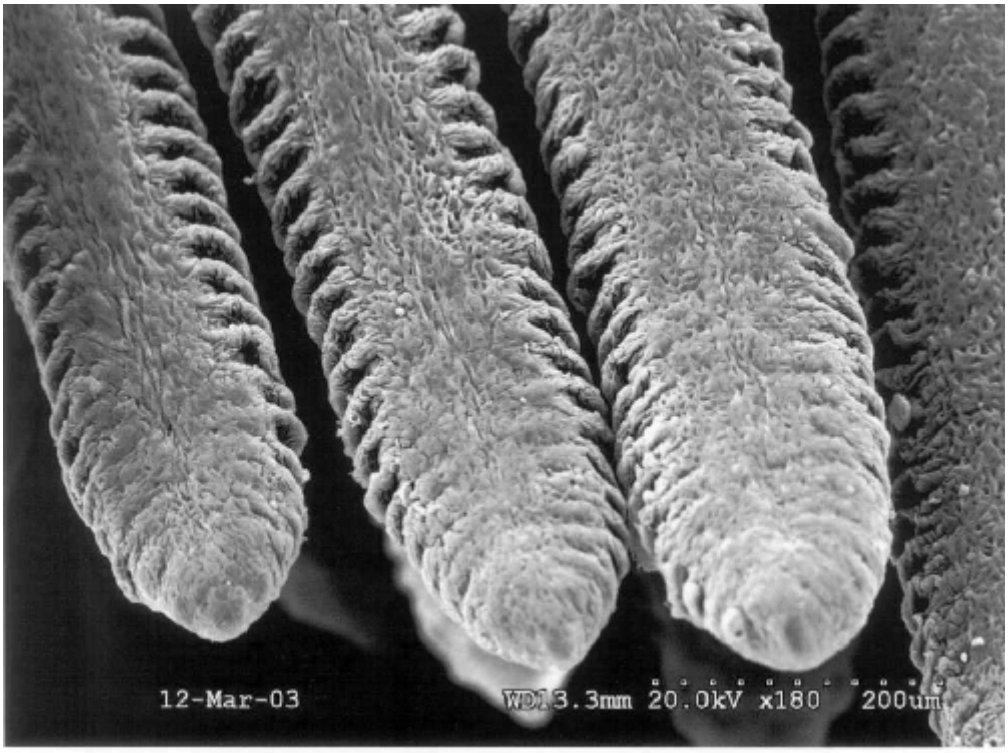


Fig 5.4 **Gills of carp exposed to *Actinomycete* 15 for 48 hours to show hyperplasia leading to fusion of secondary lamellae especially at the tips.**

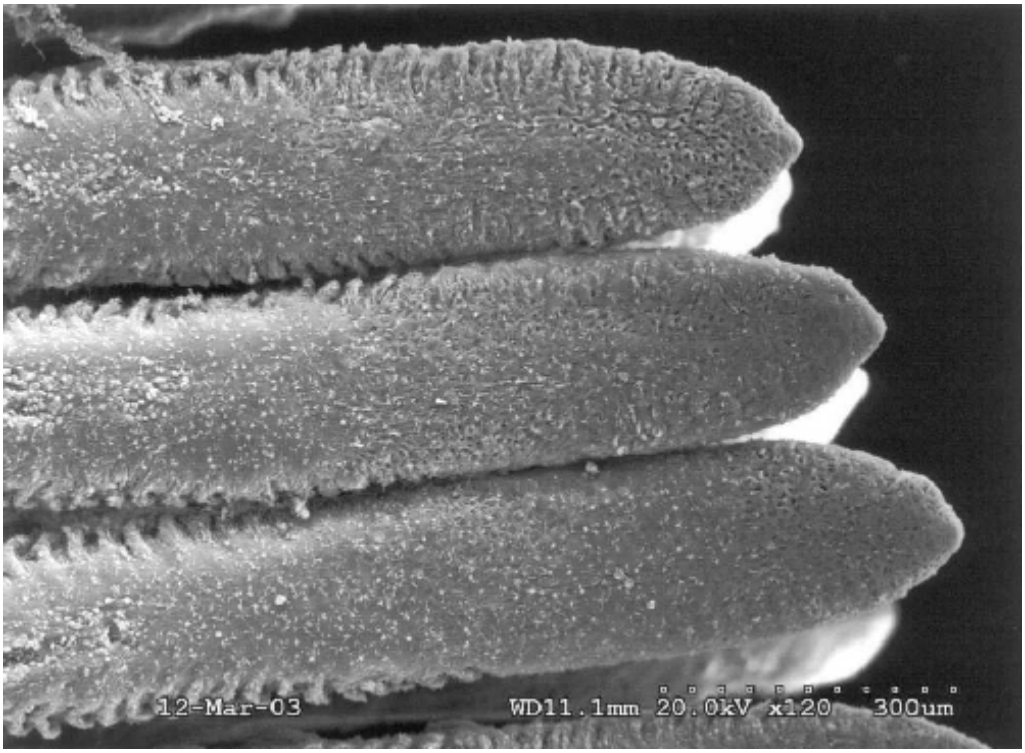


Fig 5.5 **Gills of carp exposed to *Actinomycete* 15 for 96 hours to show more extensive hyperplasia leading to fusion of secondary lamellae.**

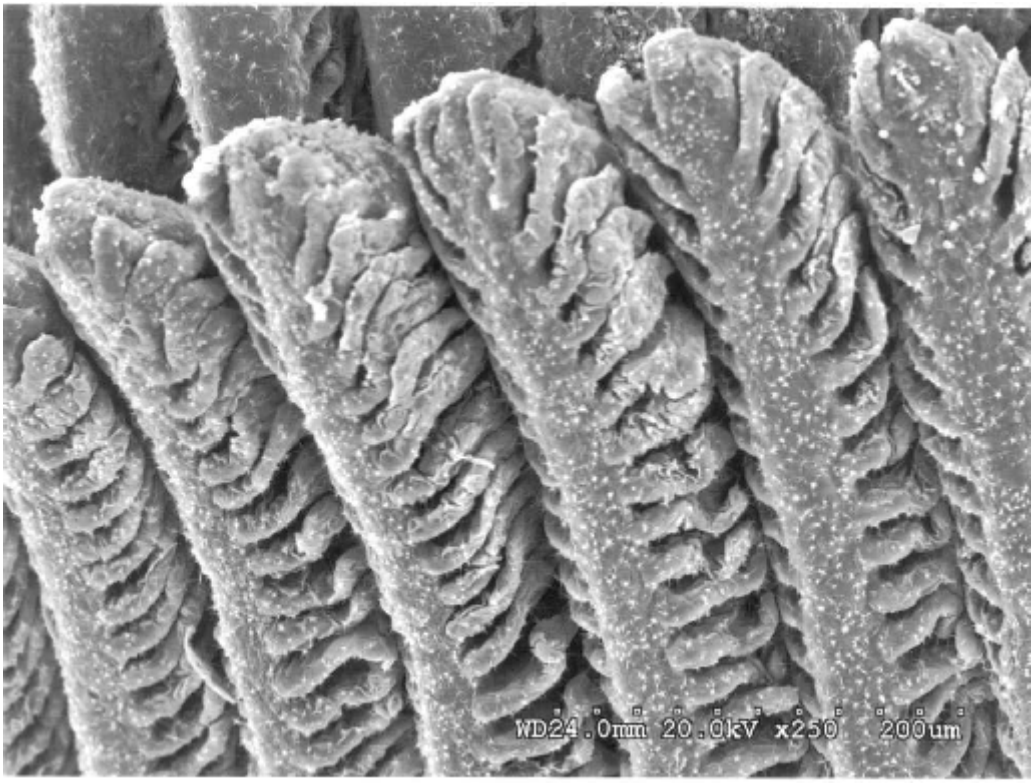


Fig 5.6 **Gills of roach exposed to *Actinomycete* 15 for 24 hours to show collapsing of secondary lamellae but no fusion.**

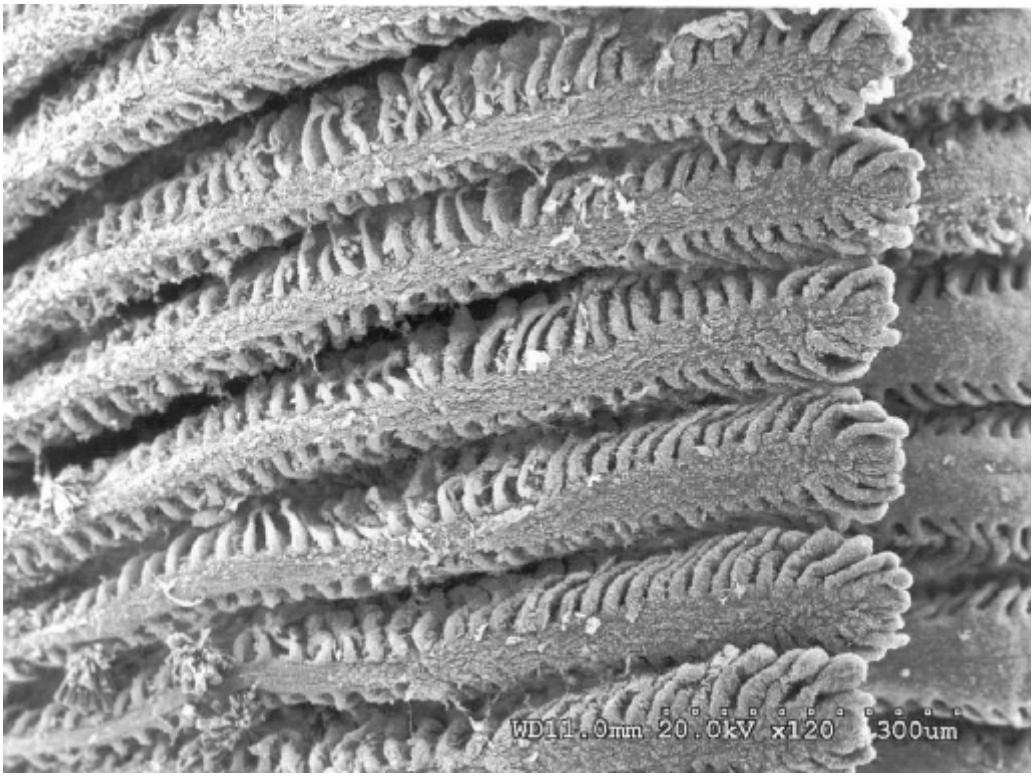


Fig 5.7 **Gills of roach exposed to *Actinomycete* 15 for 72 hours to show collapsing of secondary lamellae and a moderate degree of distortion to the filamental epithelium of primary lamellae.**

Fig 5.8 **Gills of roach exposed to *Actinomycete* 15 for 96 hours to show some fusion of secondary lamellae.**

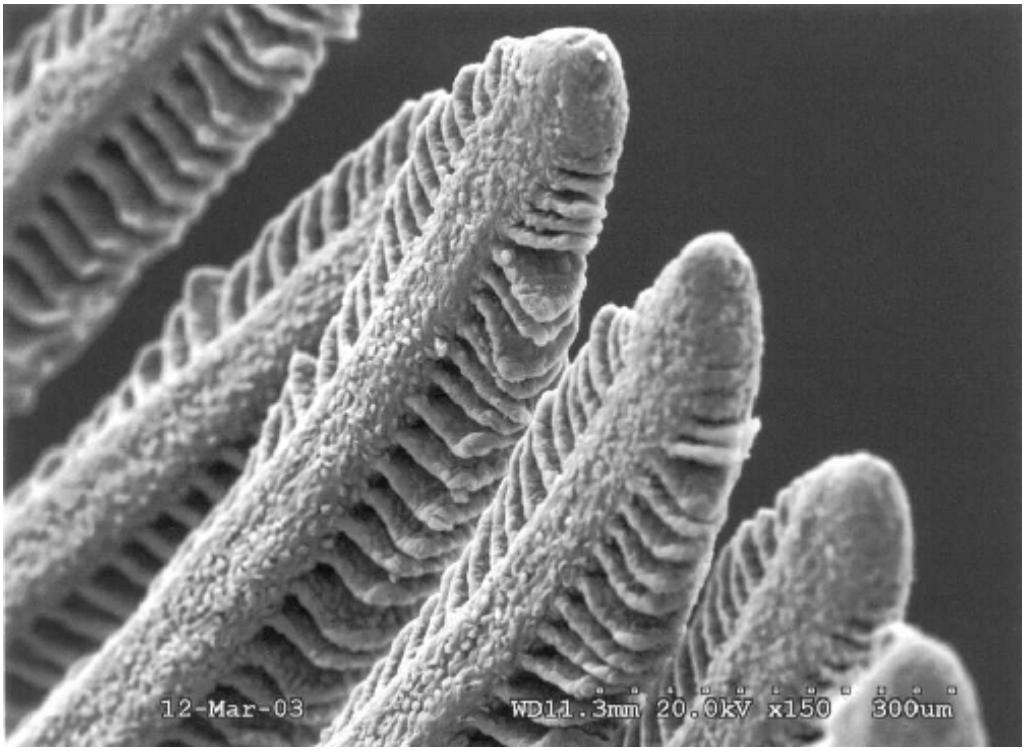


Fig 5.9 **Gills of rainbow trout exposed to *Actinomycete 1*5 for 24 hours to show collapsing and fusion near the tips of secondary lamellae; the filamental epithelia on primary lamellae are also moderately distorted.**

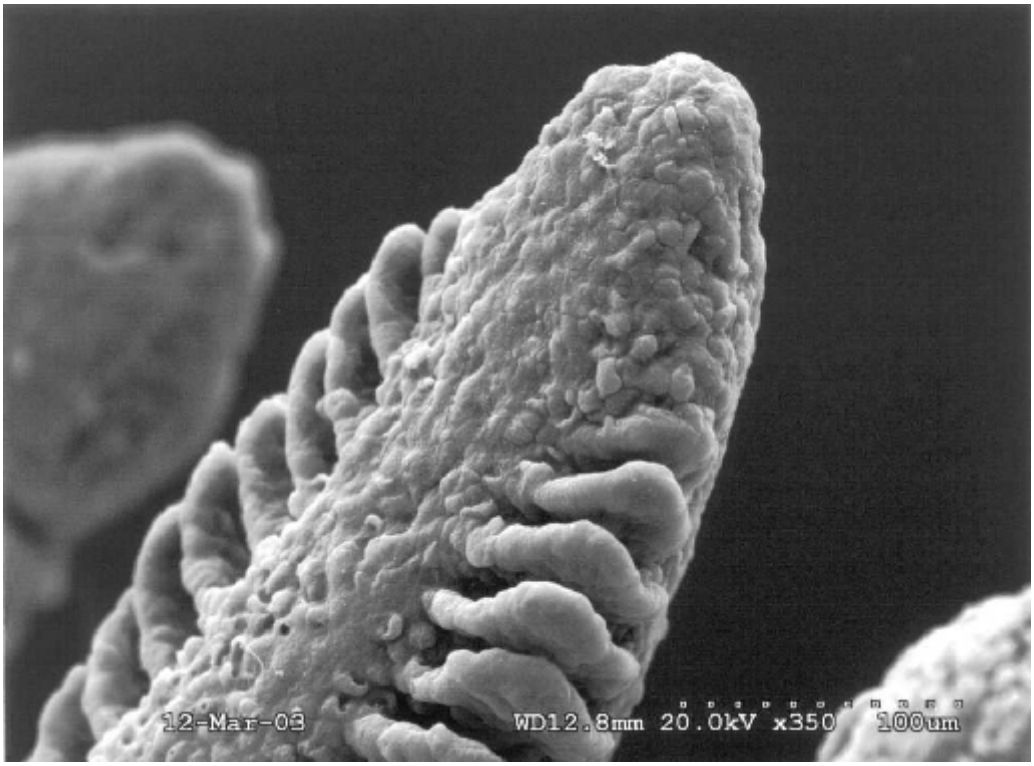


Fig 5.10 **Gills of rainbow trout exposed to *Actinomycete* 15 for 24 hours to show fusion near the tip of a secondary lamellae plus distortion on the filamental epithelium of the primary lamella.**

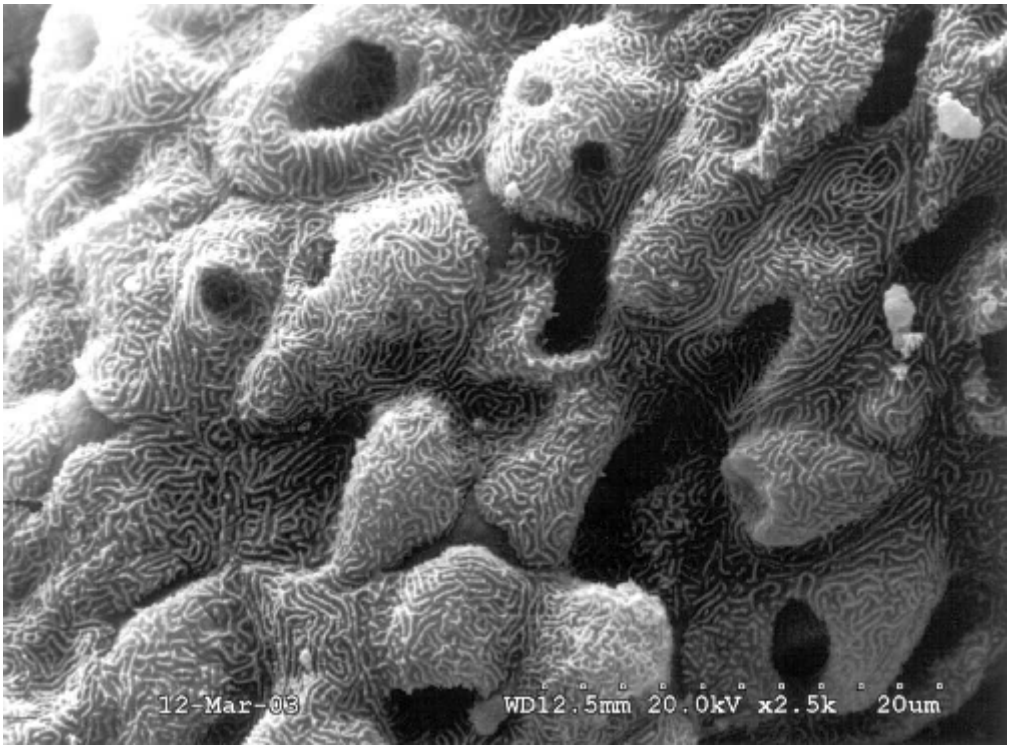


Fig 5.11 **Gills of rainbow trout exposed to *Actinomycete* 15 for 24 hours to show distortion on the filamental epithelium of a primary lamella but the microridging remains intact.**

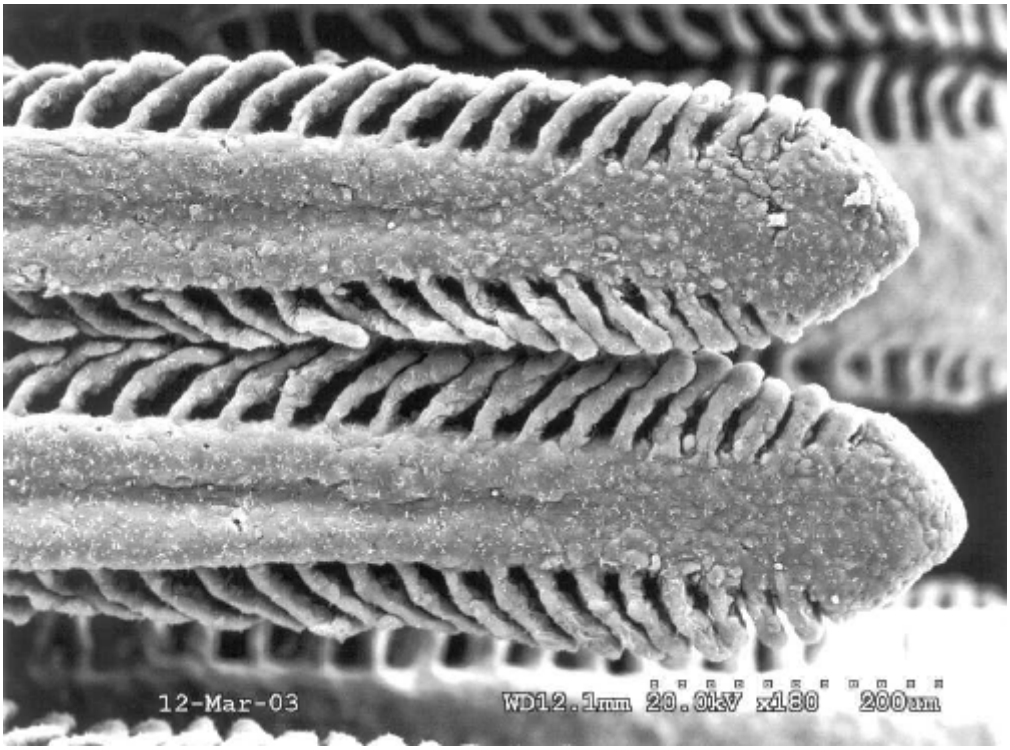


Fig 5.12 **Gills of rainbow trout exposed to *Actinomycete* 15 for 72 hours to show hyperplasia leading to fusion of secondary lamellae.**

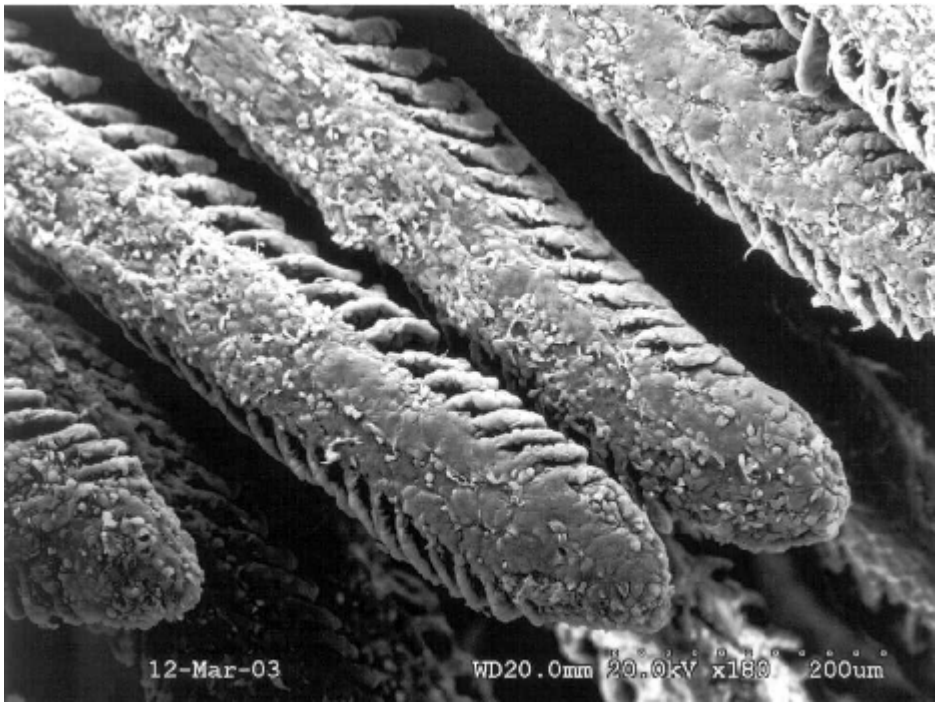


Fig 5.13 **Gills of rainbow trout exposed to *Actinomycete 1*5 for 96 hours to show hyperplasia leading to more extensive fusion and erosion of secondary lamellae and distortion/erosion of filamental epithelia on the primary lamellae.**

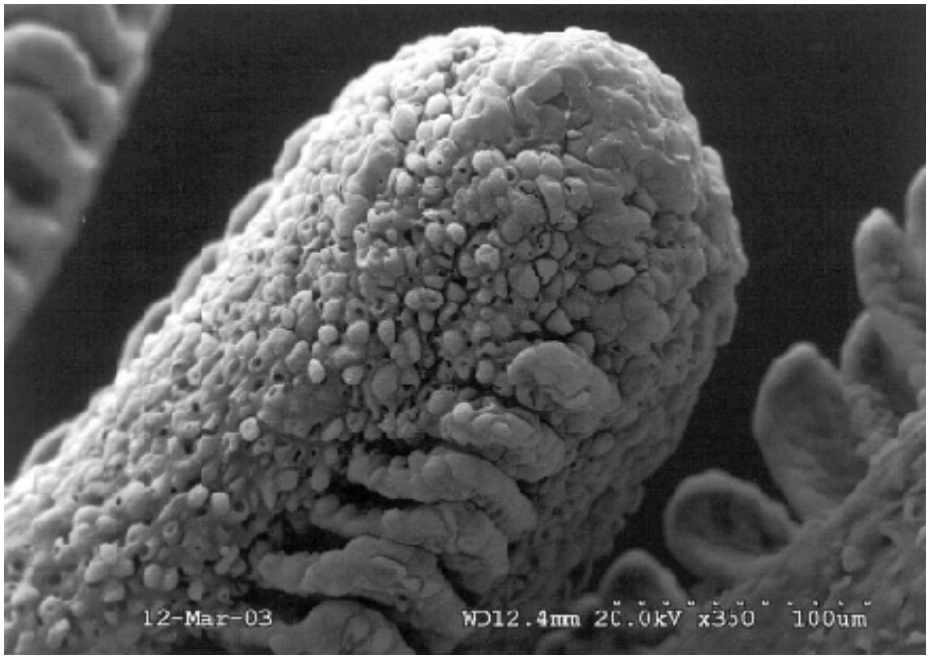


Fig 5.14 **Gills of rainbow trout exposed to *Actinomycete* 15 for 96 hours to show a high degree of distortion on the filamental epithelium near the tip of a secondary lamella.**

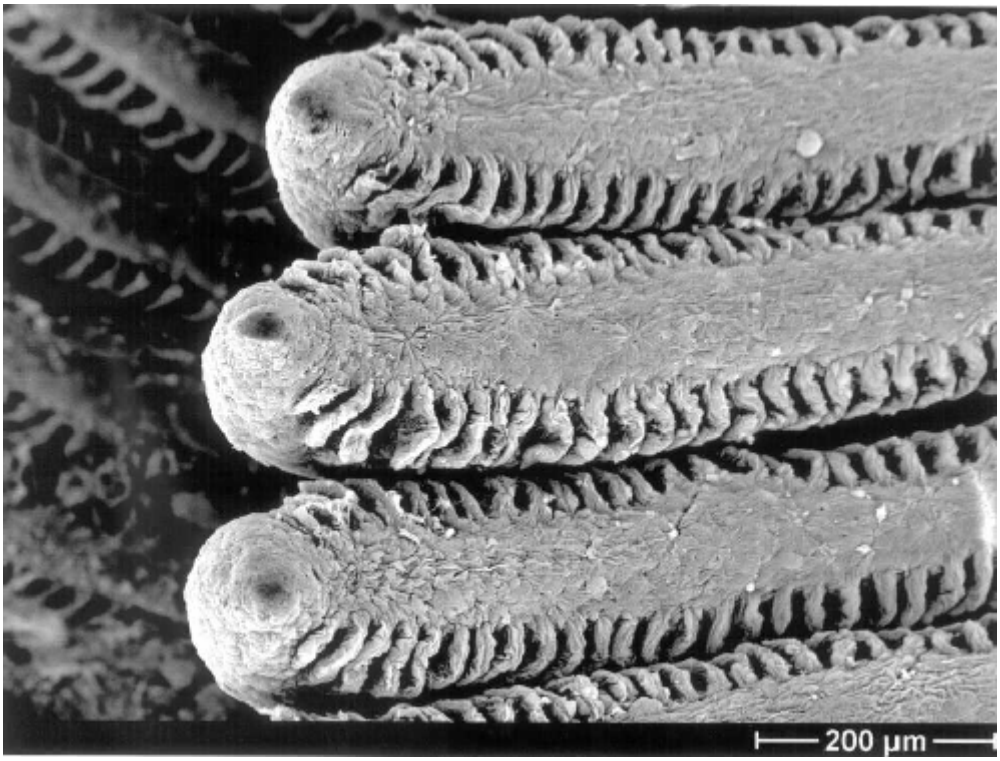


Fig 6.0 **Gills of carp exposed to *Actinomycete* 17 for 24 hours to show collapsing and hyperplasia of the secondary lamellae with a moderate degree of distortion to the filamental epithelium of primary lamellae.**

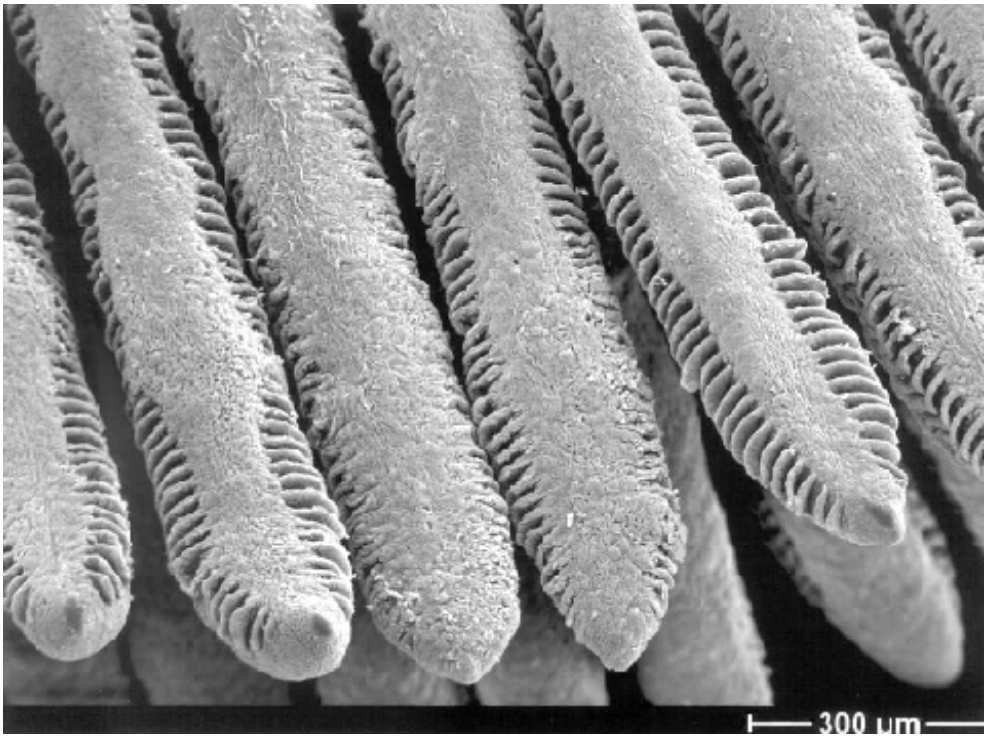


Fig 6.1 **Gills of carp exposed to *Actinomycete* 17 for 48 hours to show hyperplasia leading to fusion of secondary lamellae and a moderate degree of distortion to the filamental epithelium of primary lamellae.**

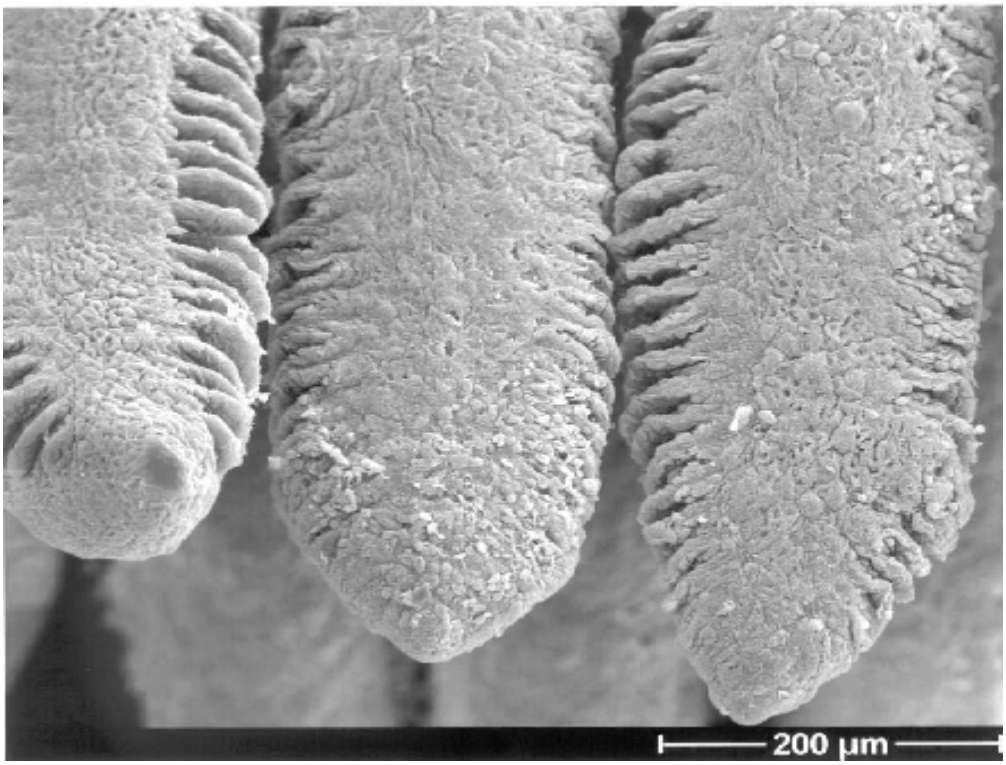


Fig 6.2 **Gills of carp exposed to *Actinomycete* 17 for 48 hours to show hyperplasia leading to fusion of secondary lamellae and a moderate degree of distortion to the filamental epithelium of primary lamellae.**

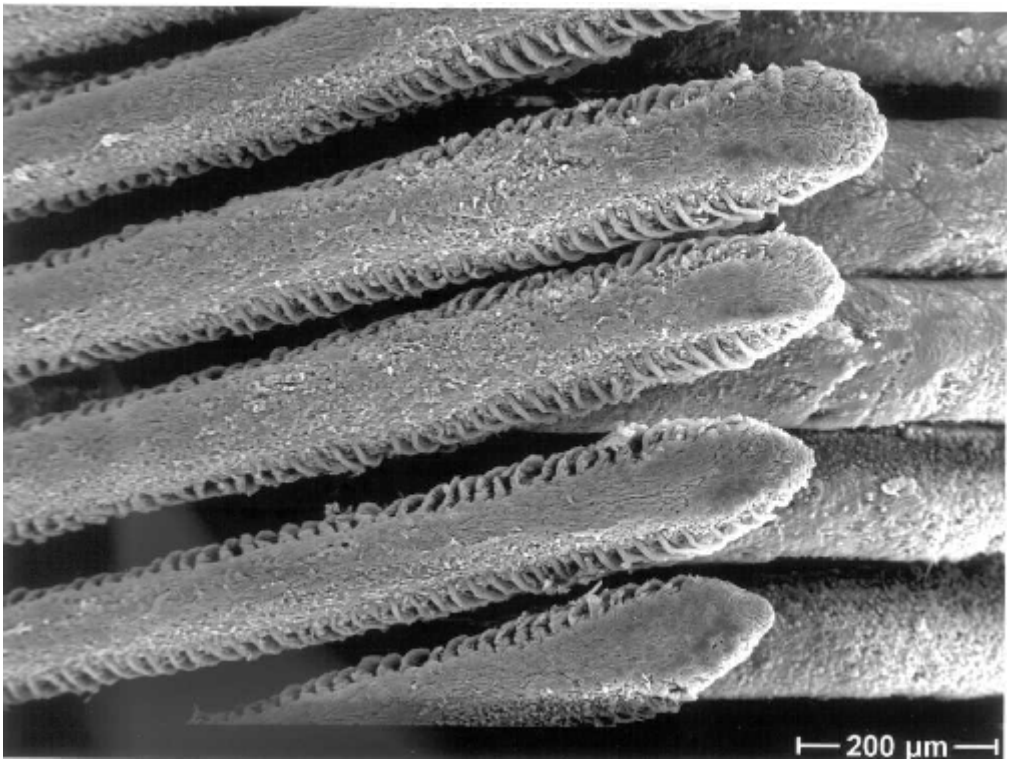


Fig 6.3 **Gills of carp exposed to *Actinomycete* 17 for 72 hours to show hyperplasia leading to fusion of secondary lamellae and a moderate degree of distortion to the filamental epithelium of primary lamellae.**

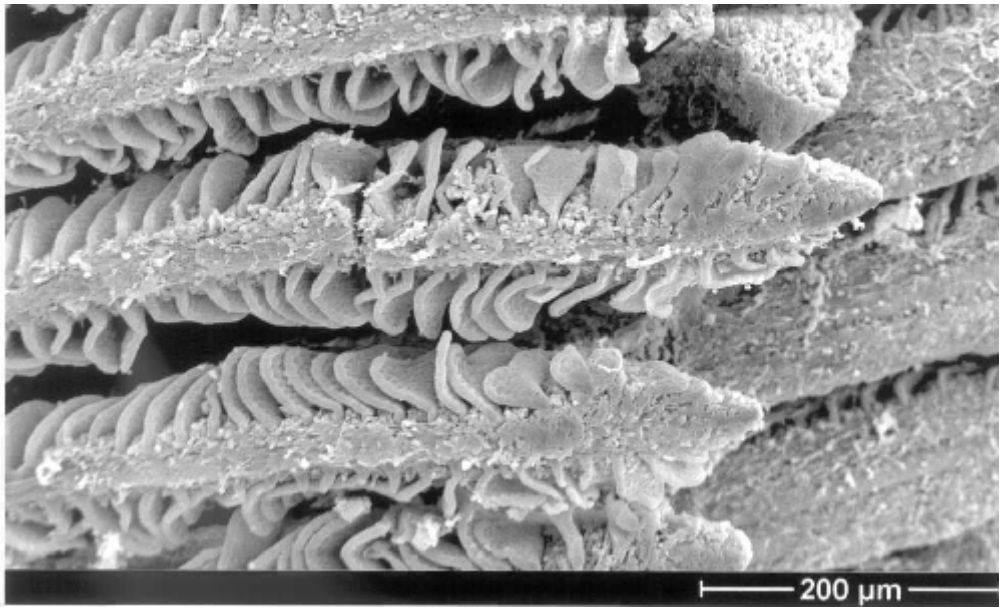


Fig 6.4 **Gills of carp exposed to *Actinomycete* 17 for 96 hours to show collapsing, fusion and necrosis of secondary lamellae together with a moderate degree of distortion to the filamental epithelium of primary lamellae.**

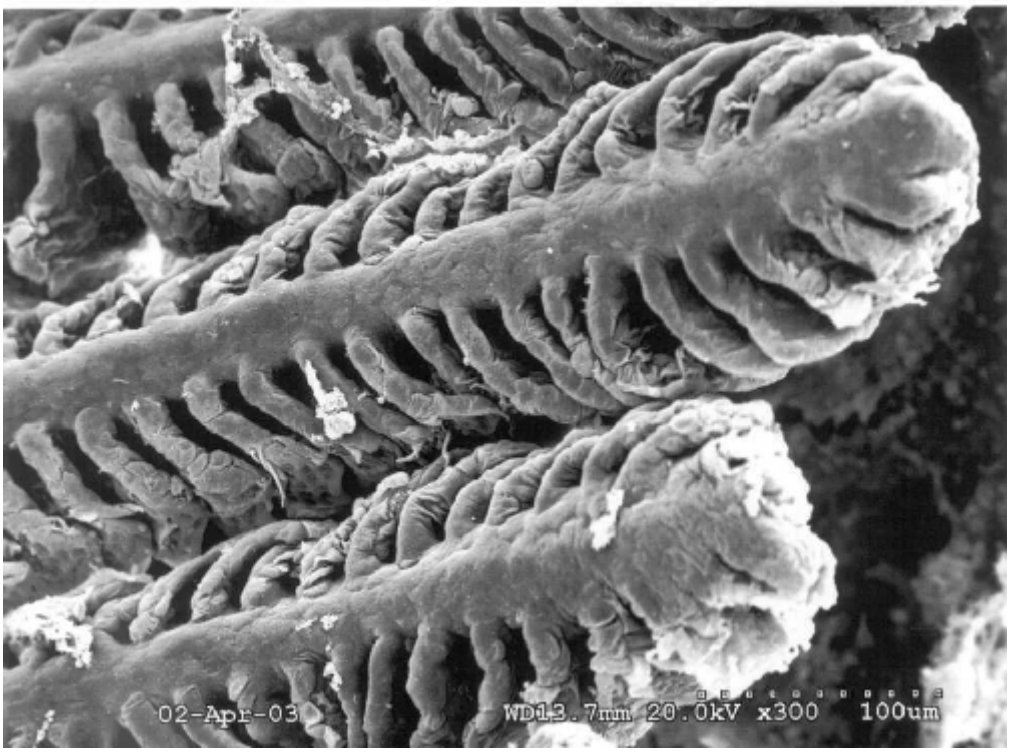


Fig 6.5 **Gills of roach exposed to *Actinomycete* 17 for 72 hours to show collapsing hyperplasia and some fusion and erosion of secondary lamellae.**

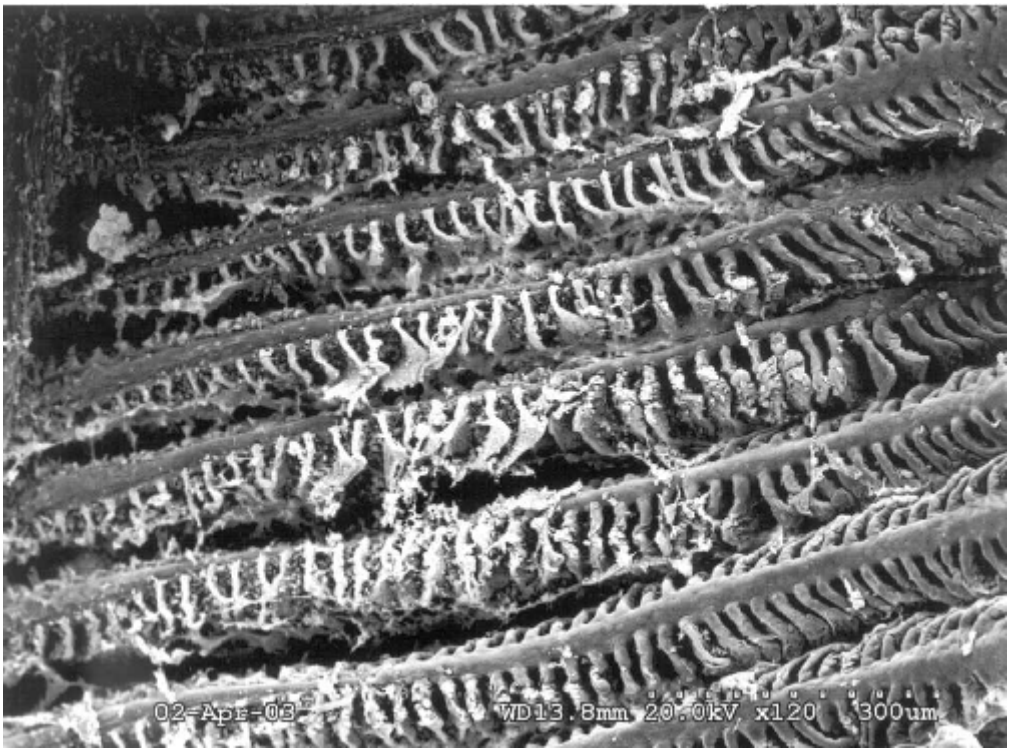


Fig 6.6 **Gills of roach exposed to *Actinomycete* 17 for 72 hours to show collapsing and erosion of secondary lamellae and a high degree of distortion/erosion on the filamental epithelia on primary lamellae.**

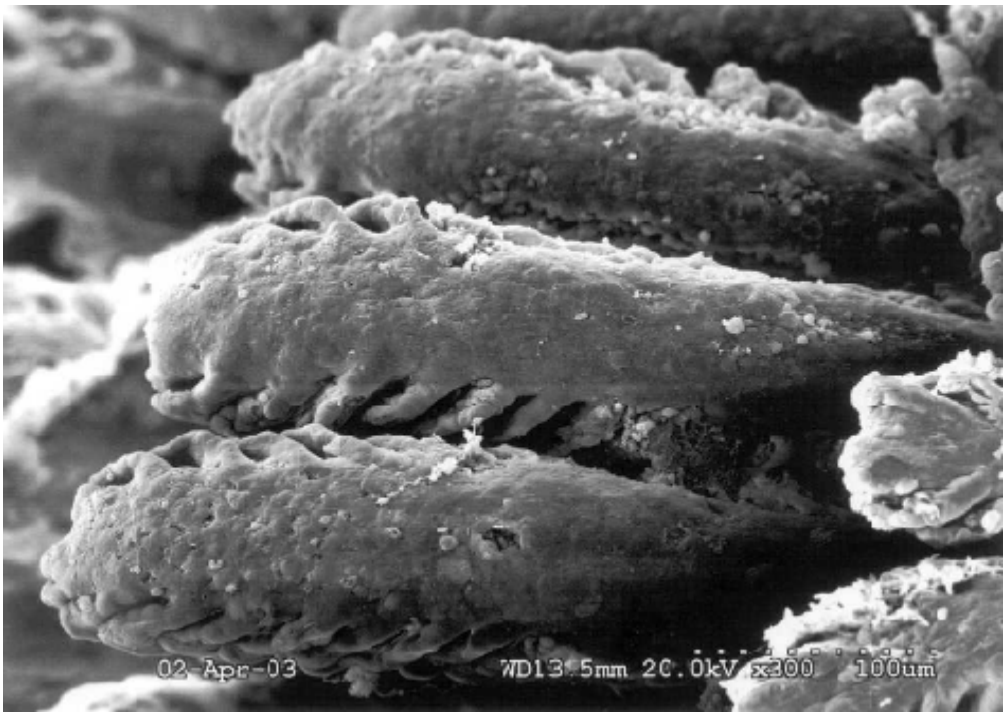


Fig 6.7 **Gills of roach exposed to *Actinomycete* 17 for 96 hours to show hyperplasia leading to fusion of secondary lamellae and a high degree of distortion/erosion on the filamental epithelia of primary lamellae.**

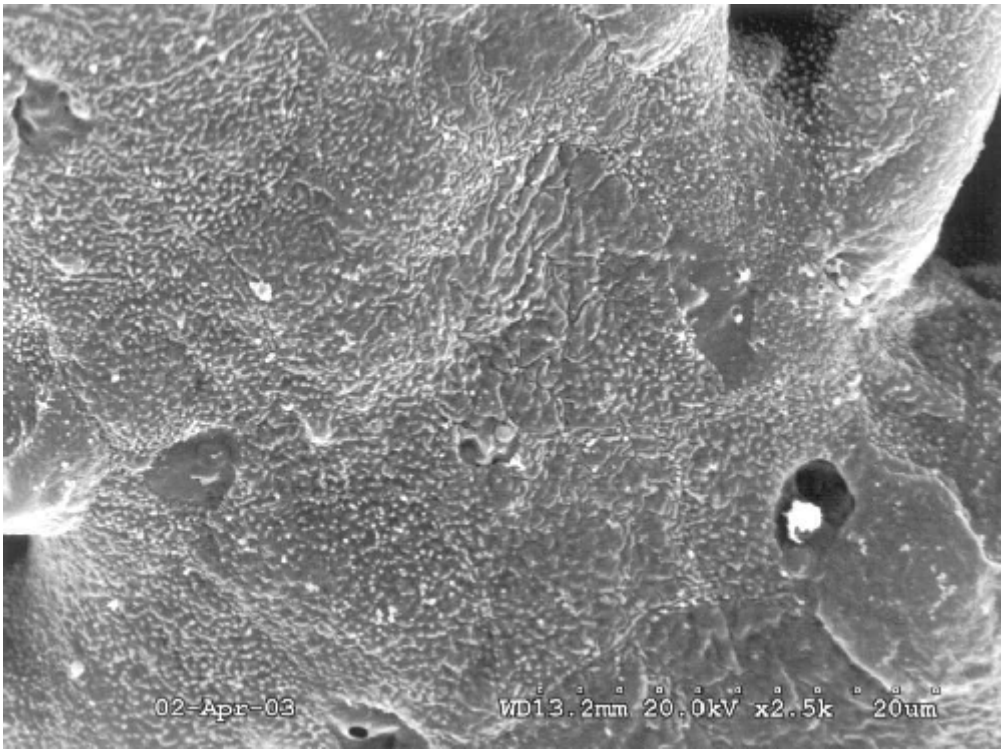


Fig 6.8 **Gills of roach exposed to *Actinomycete* 17 for 96 hours to show a moderate degree of loss of microridging on the filamental epithelium of a primary lamella.**

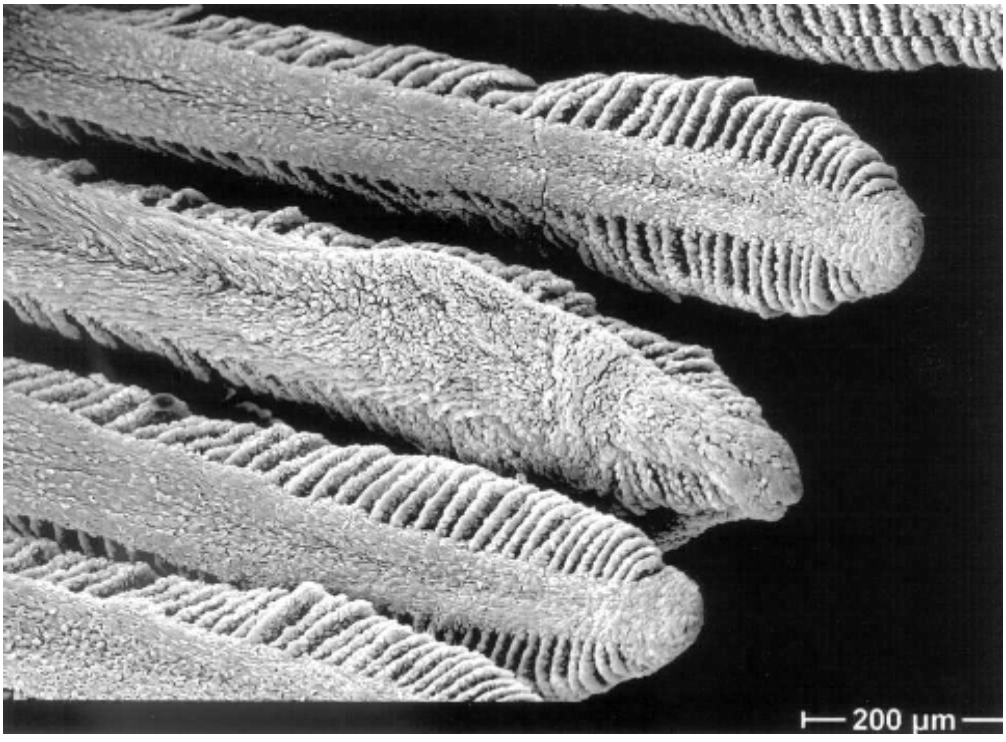


Fig 6.9 **Gills of rainbow trout exposed to *Actinomycete* 17 for 24 hours to show collapsing, hyperplasia leading to some fusion near the tips of secondary lamellae; the filamental epithelium on some primary lamellae shows a high degree of distortion.**

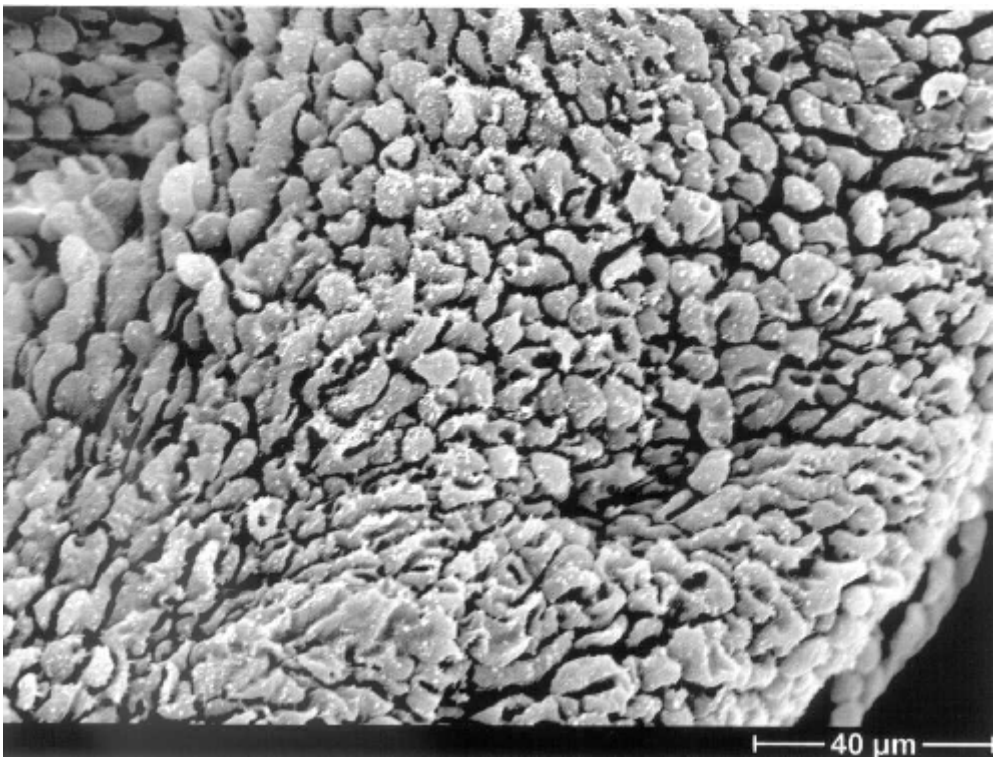


Fig 6.10 **Gills of rainbow trout exposed to *Actinomycete* 17 for 24 hours to show a high degree of distortion on the filamental epithelium of a primary lamella.**

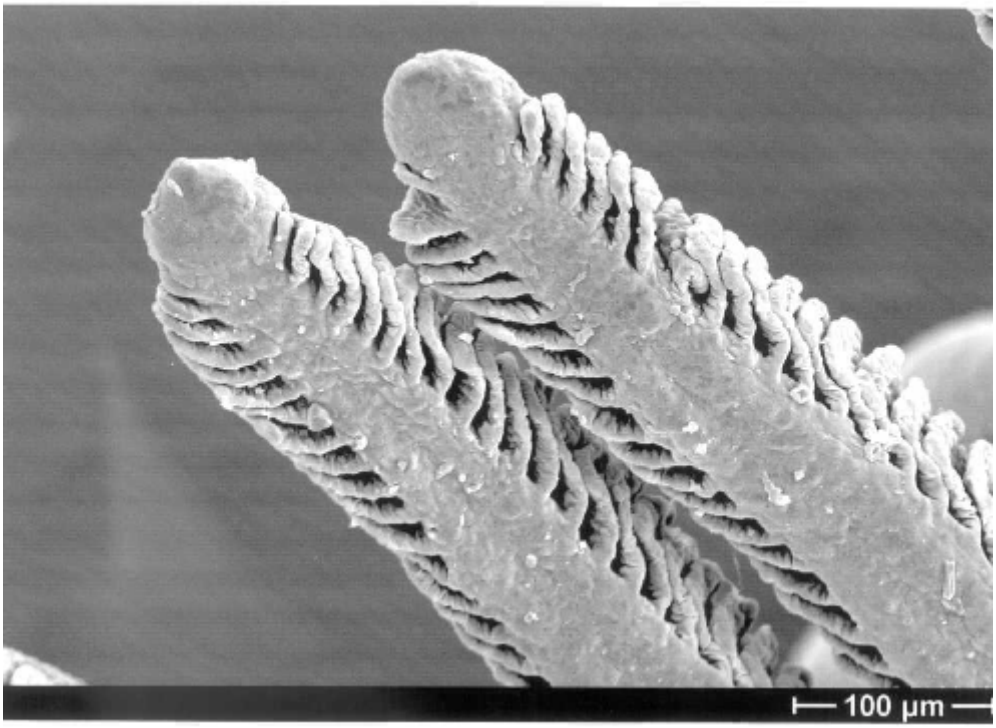


Fig 6.11 **Gills of rainbow trout exposed to *Actinomycete* 17 for 72 hours to show collapsing and hyperplasia of secondary lamellae.**

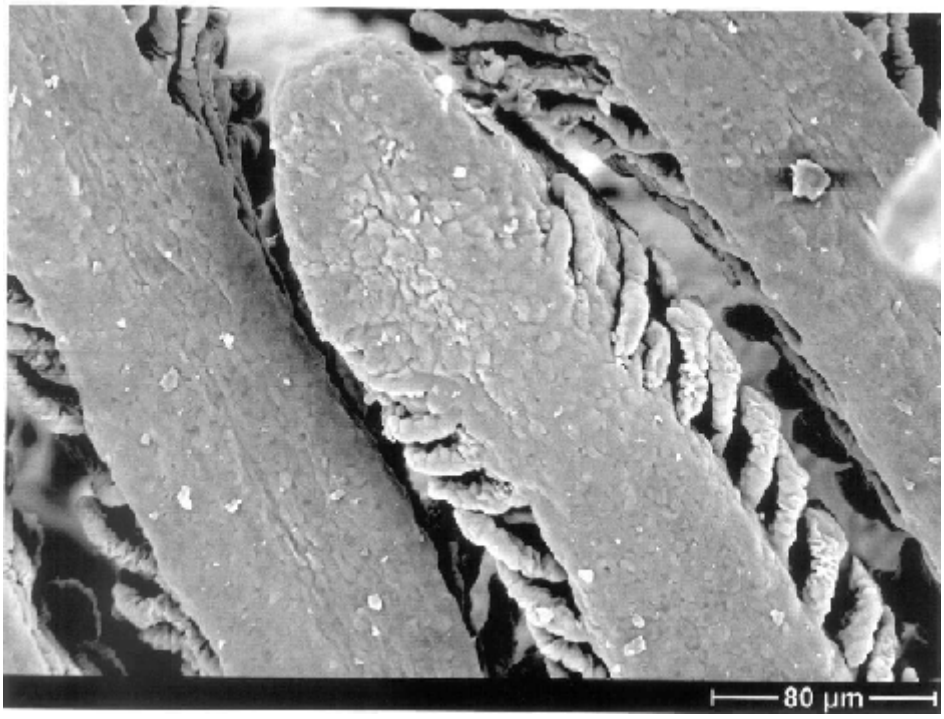


Fig 6.12 **Gills of rainbow trout exposed to *Actinomycete* 17 for 96 hours to show hyperplasia leading to fusion and erosion of secondary lamellae.**

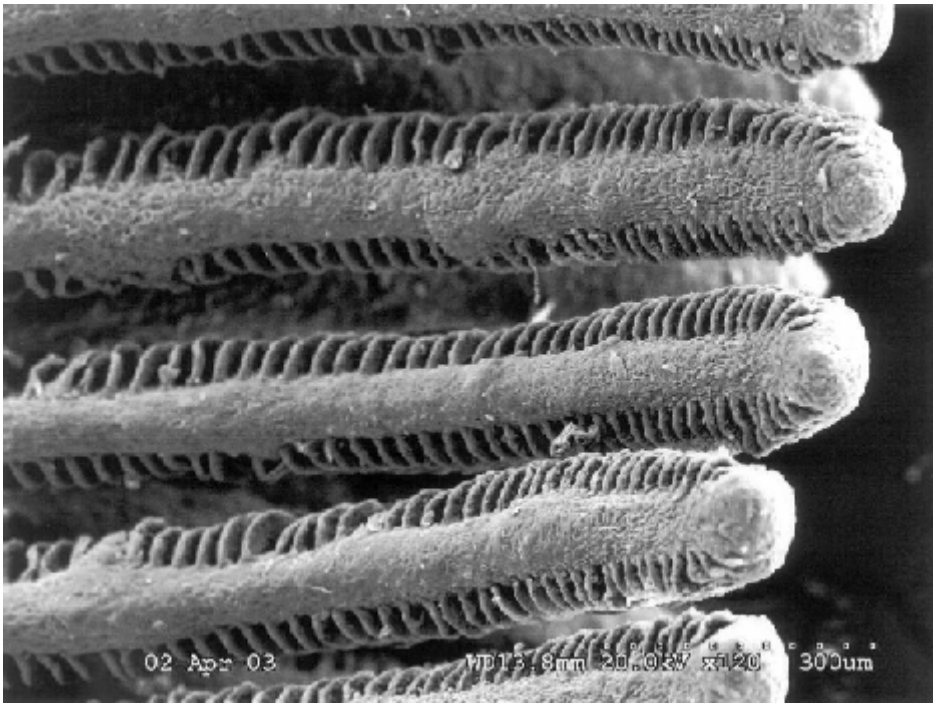


Fig 7.0 **Gills of carp exposed to *Oscillatoria* sp for 24 hours to show some collapsing and hyperplasia of secondary lamellae together with a moderate degree of distortion and swelling on the filamental epithelium of primary lamellae.**

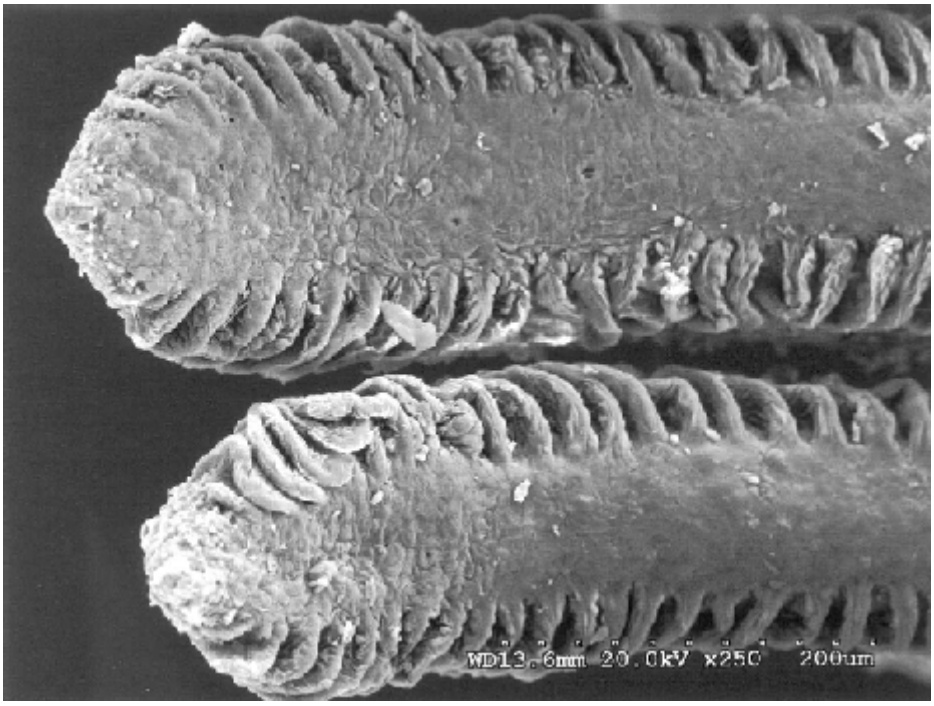


Fig 7.1 **Gills of carp exposed to *Oscillatoria* sp for 48 hours to show collapsing and hyperplasia leading to fusion of secondary lamellae near the tips.**

Fig 7.2 **Gills of carp exposed to *Oscillatoria* sp for 48 hours to show a moderate degree of distortion on the filamental epithelium of a primary lamella together with a loss of microridging.**

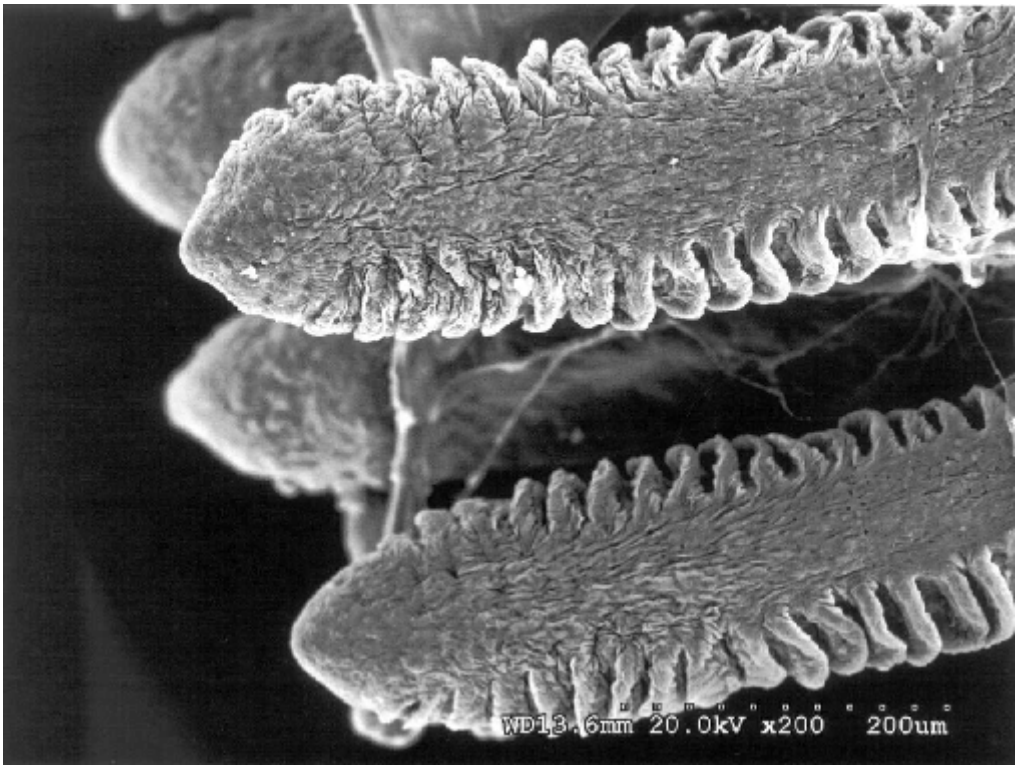


Fig 7.3 **Gills of carp exposed to *Oscillatoria* sp for 72 hours to show collapsing and hyperplasia leading to fusion of secondary lamellae.**

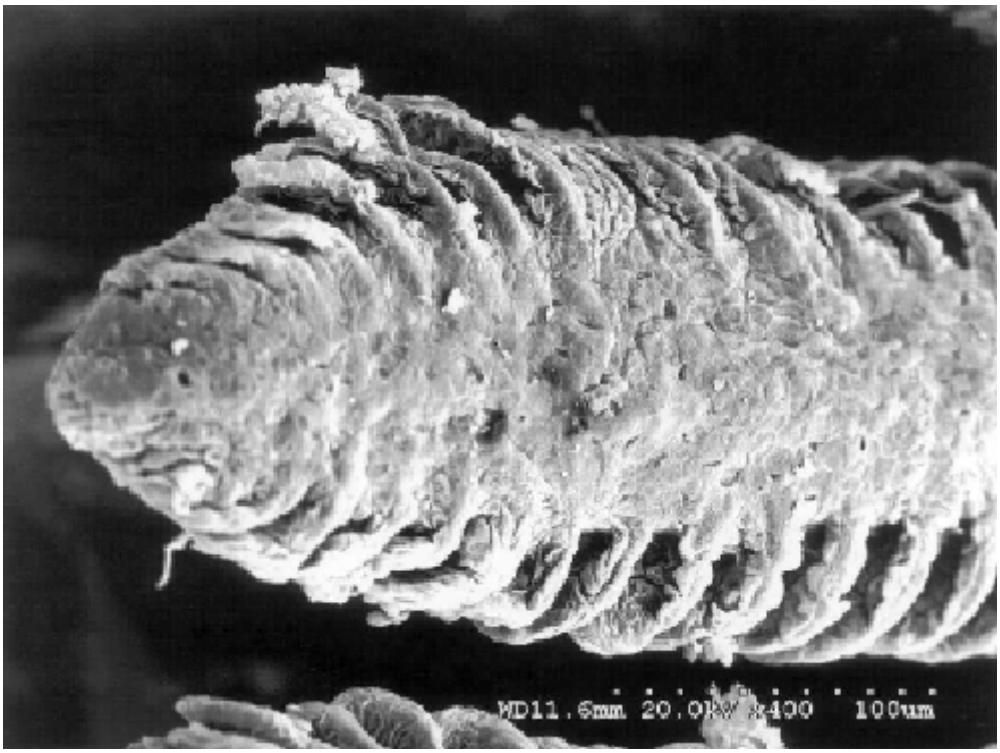


Fig 7.4 **Gills of carp exposed to *Oscillatoria* sp for 96 hours to show more extensive hyperplasia, fusion and erosion of the secondary lamellae.**

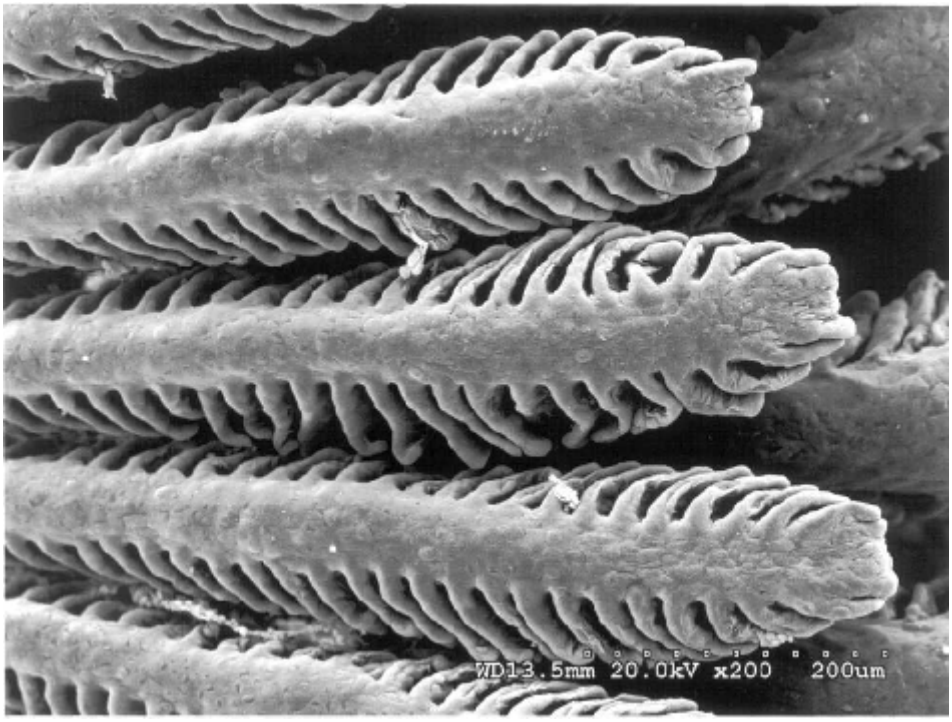


Fig 7.5 **Gills of roach exposed to *Oscillatoria* sp for 24 hours to show collapsing and hyperplasia of secondary lamellae but no evidence of fusion.**

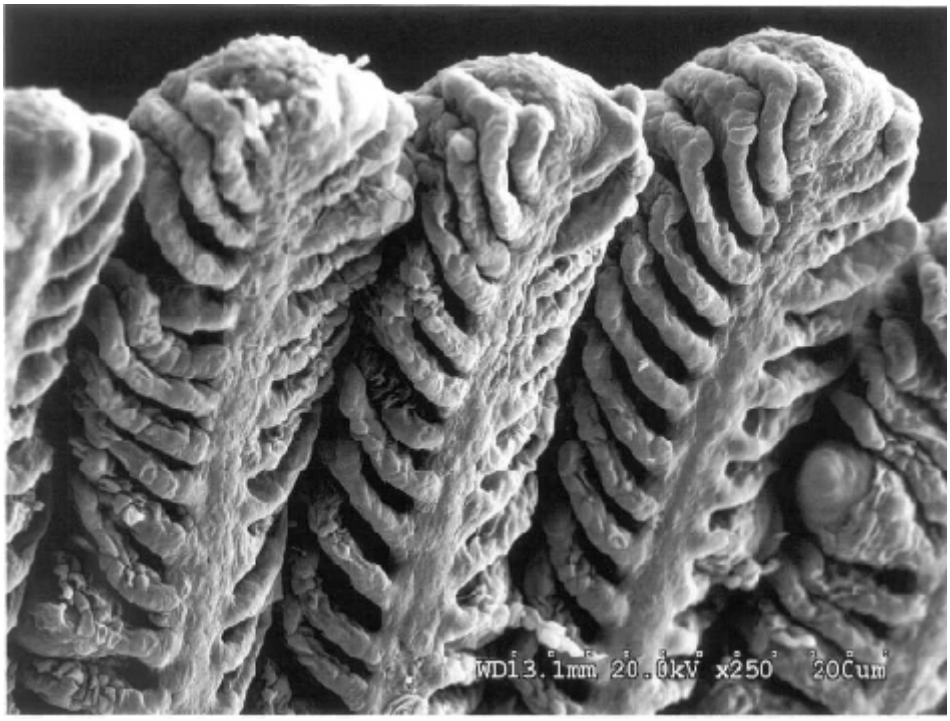


Fig 7.6 **Gills of roach exposed to *Oscillatoria* sp for 72 hours to show collapsing, hyperplasia together with some erosion of secondary lamellae.**

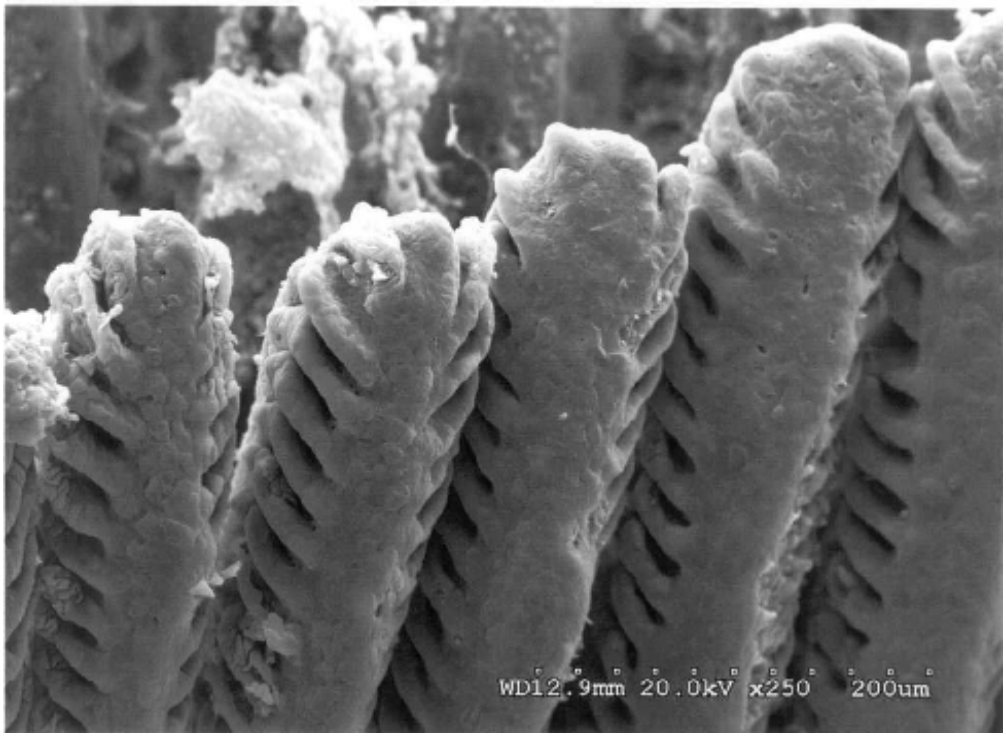


Fig 7.7 **Gills of roach exposed to *Oscillatoria* sp for 96 hours to show increasing hyperplasia leading to fusion/erosion of secondary lamellae.**

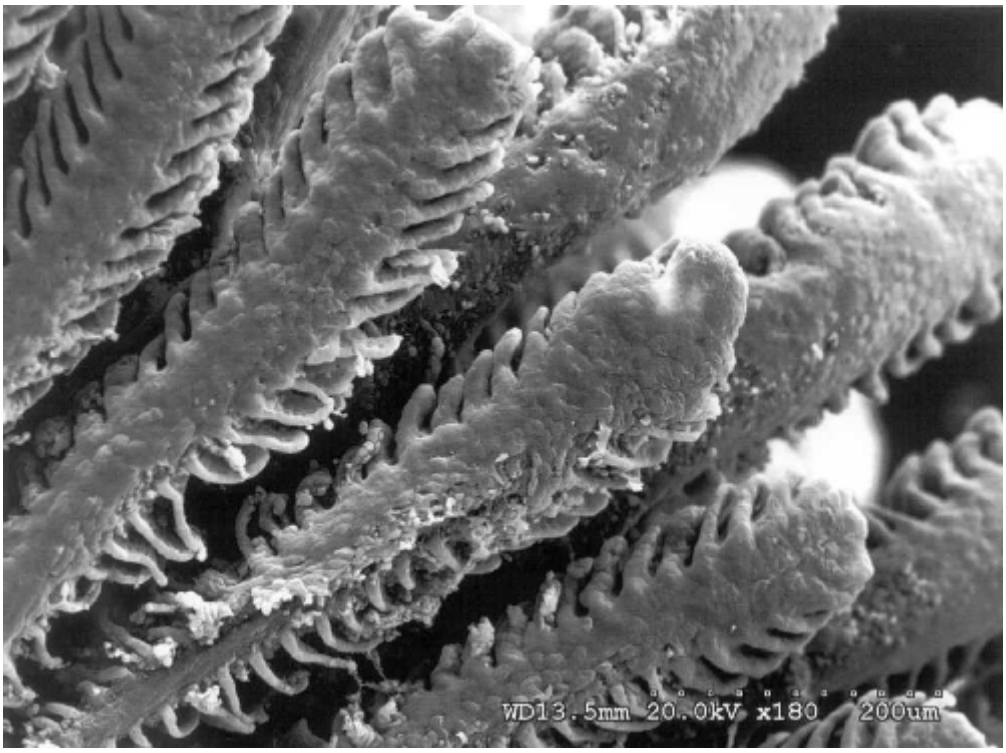


Fig 7.8 **Gills of roach exposed to *Oscillatoria* sp for 96 hours to show a high proportion of fusion of secondary lamellae and a high degree of distortion/erosion of the filamental epithelium of primary lamellae.**

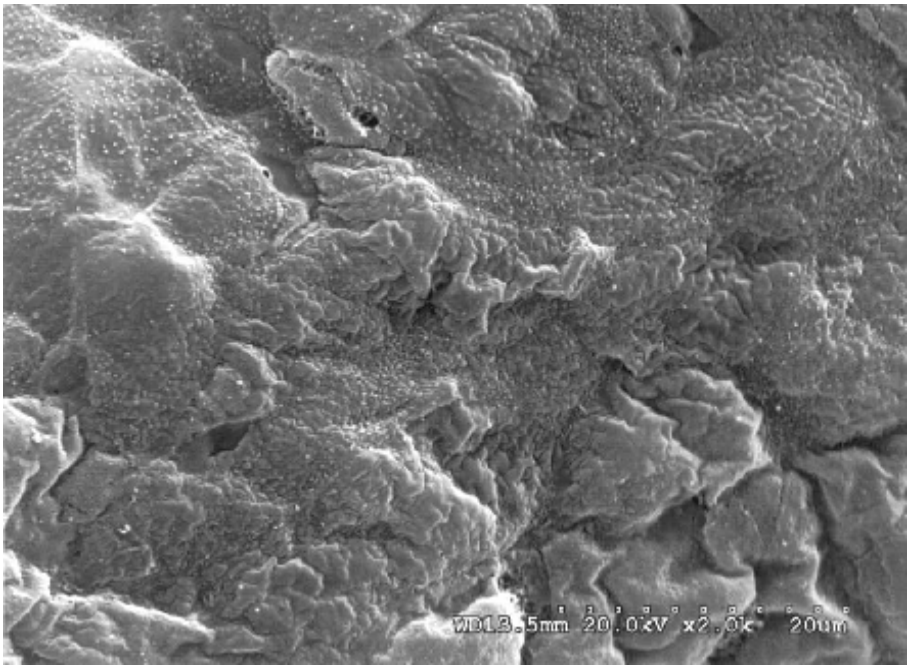


Fig 7.9 **Gills of roach exposed to *Oscillatoria* sp for 96 hours to show the filamental epithelium on a primary lamella with a high degree of loss of microridging.**

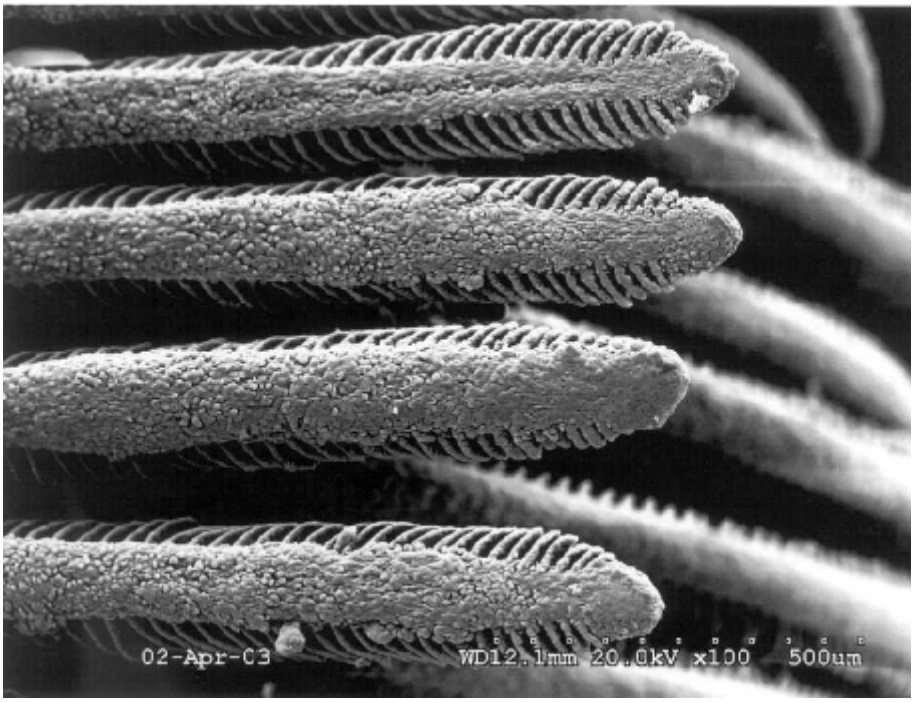


Fig 7.10 **Gills of rainbow trout exposed to *Oscillatoria* sp for 24 hours to show hyperplasia leading to fusion near the tips of secondary lamellae and a moderate degree of distortion on the filamental epithelia of primary lamellae.**

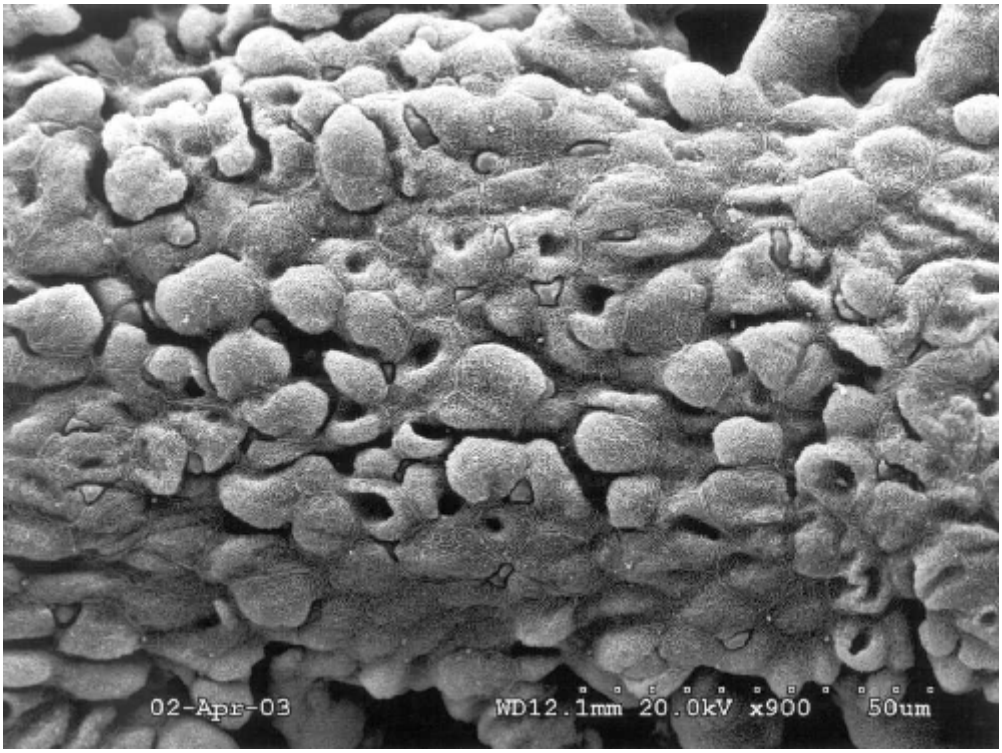


Fig 7.11 **Gills of rainbow trout exposed to *Oscillatoria* sp for 24 hours to show a moderate degree of distortion on the filamental epithelium of a primary lamella.**

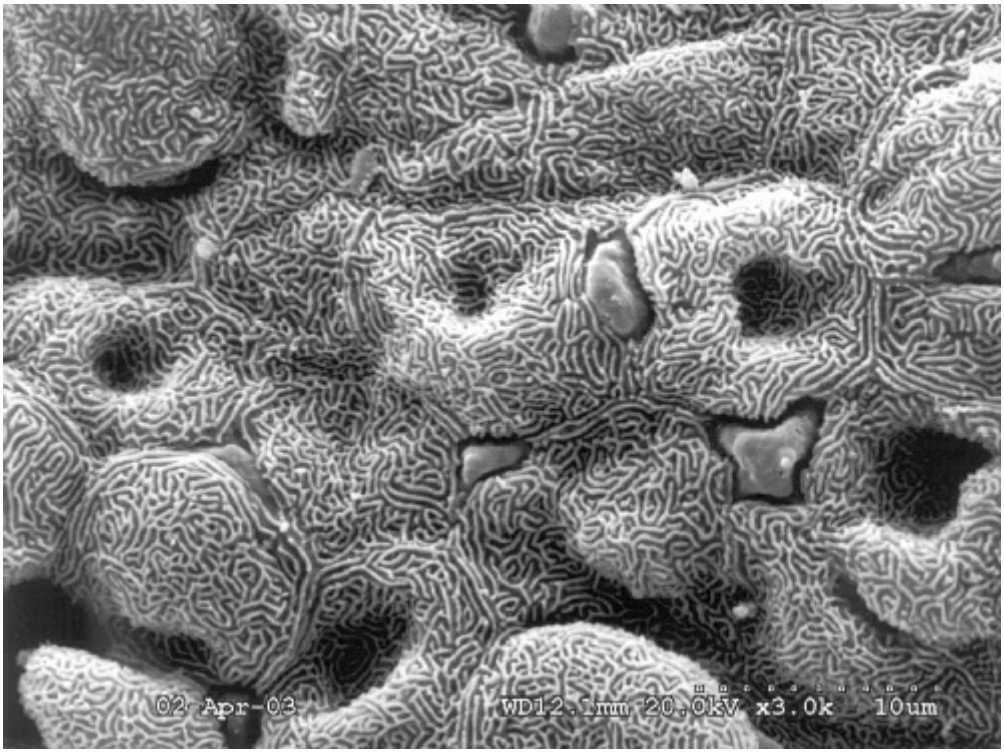


Fig 7.12 **Gills of rainbow trout exposed to *Oscillatoria* sp for 24 hours to show that the majority of the microridging remains intact despite distortion to the filamental epithelium.**

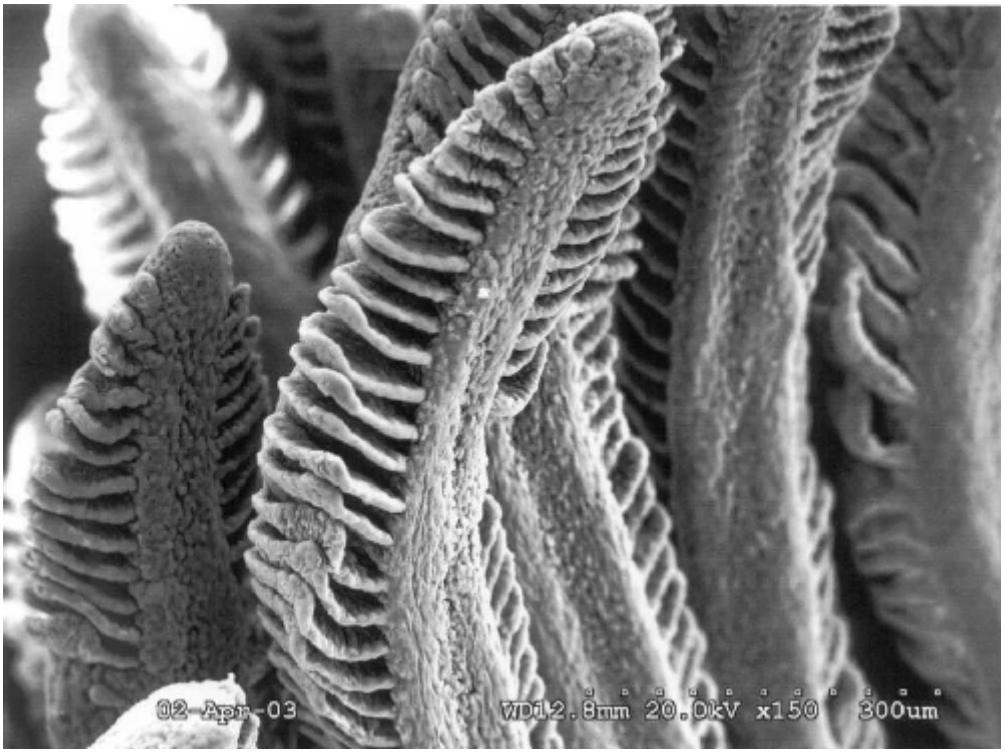


Fig 7.13 **Gills of rainbow trout exposed to *Oscillatoria* sp for 48 hours to show collapsing and some fusion near the tips of secondary lamellae.**

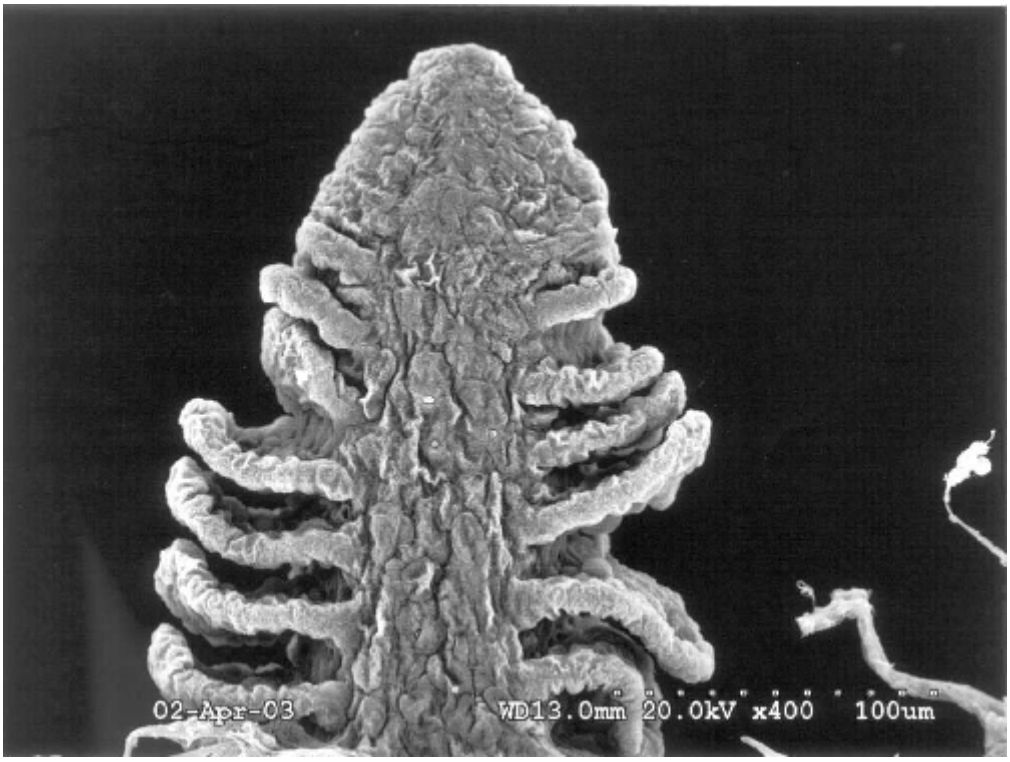


Fig 7.14 **Gills of rainbow trout exposed to *Oscillatoria* sp for 72 hours to show collapsing, hyperplasia and fusion of the secondary lamella near the tip.**

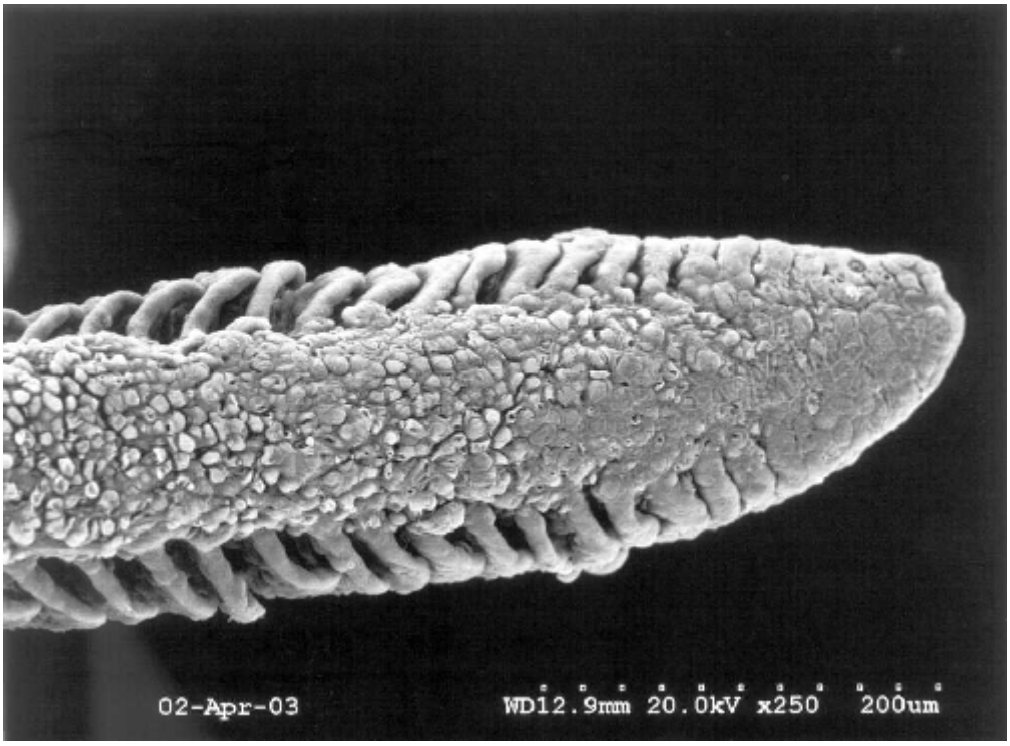


Fig 7.15 **Gills of rainbow trout exposed to *Oscillatoria* sp for 96 hours to show more extensive hyperplasia and fusion of the secondary lamella together with a high degree of distortion on the filamental epithelium.**

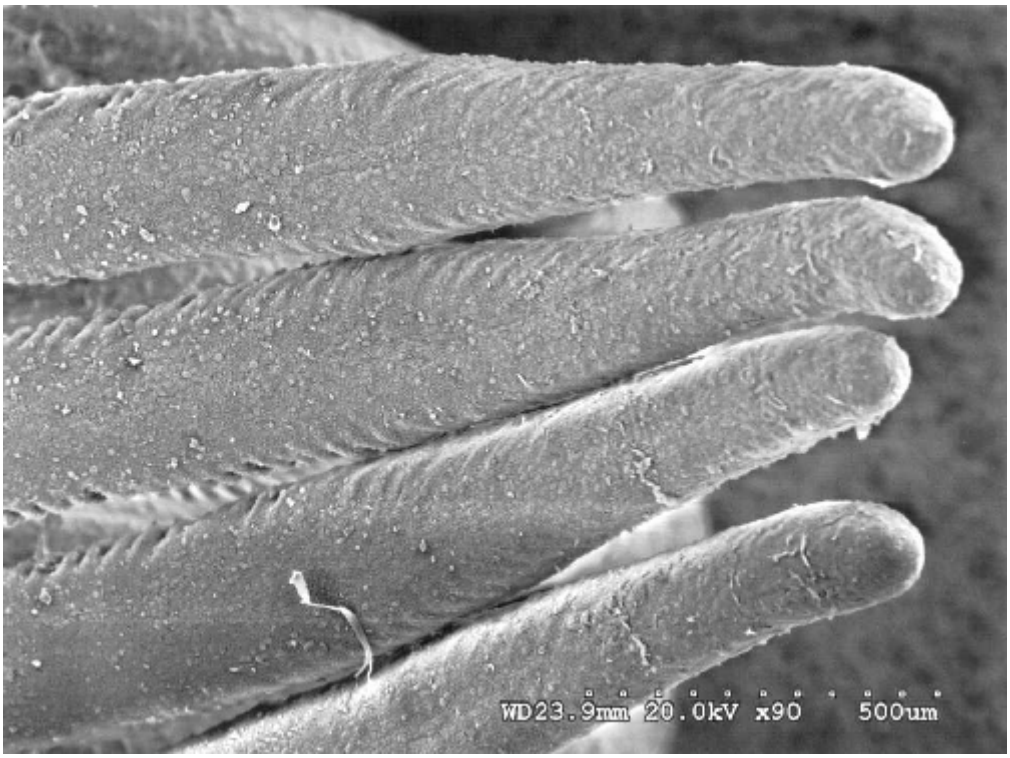


Fig 8.0 **Gills of bream from One House Pond, Stowmarket to show hyperplasia leading to total fusion of secondary lamellae.**

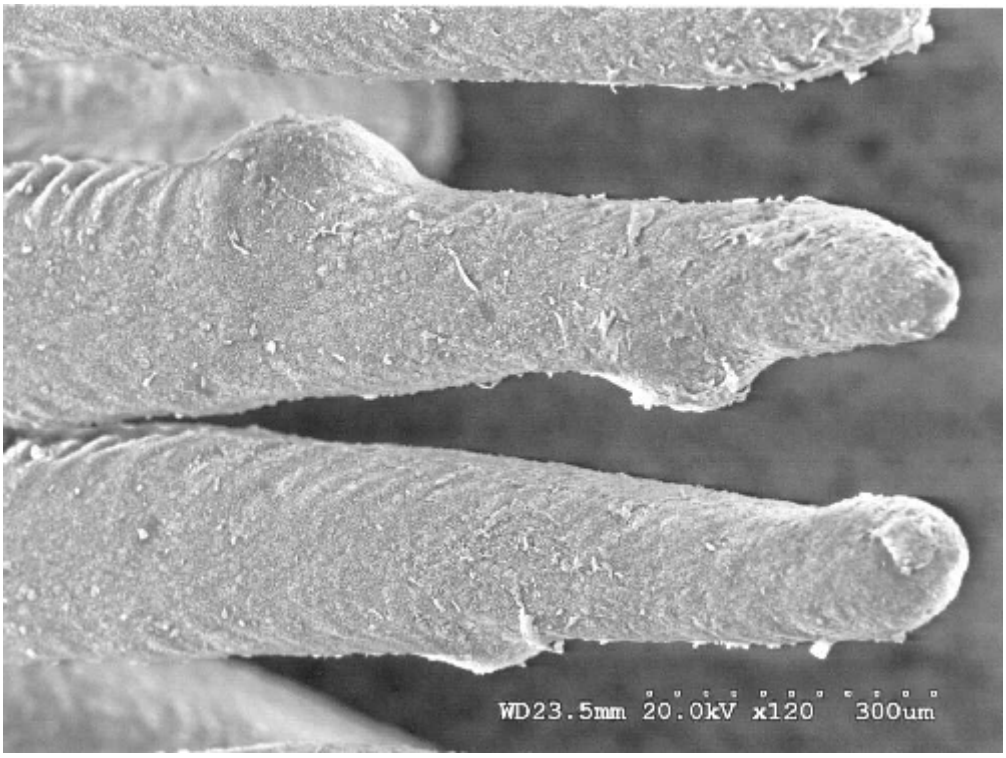


Fig 8.1 **Gills of bream from One House Pond, Stowmarket to show swellings on the secondary lamellae possibly due to a *Myxobolus* cyst.**

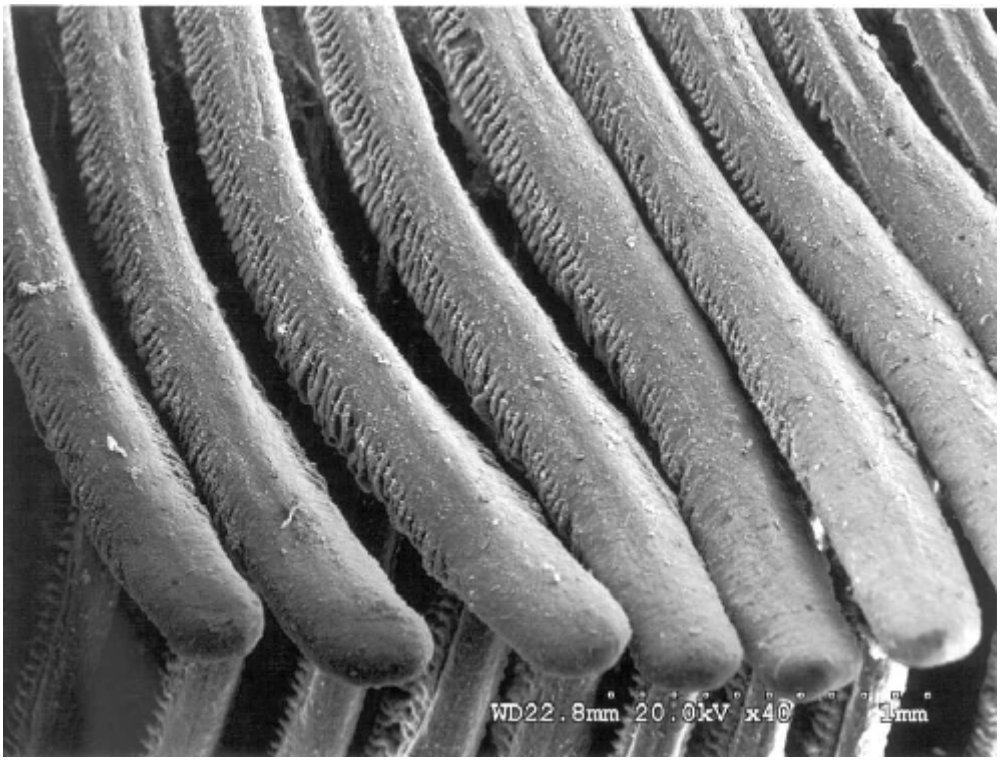


Fig 8.2 **Gills of roach from One House Pond, Stowmarket to show hyperplasia leading to extensive fusion of secondary lamellae but less so than in the gills of bream (Fig 8.0).**

Fig 8.3 **Gills of roach from One House Pond, Stowmarket which show collapsing and extensive fusion of the secondary lamellae.**

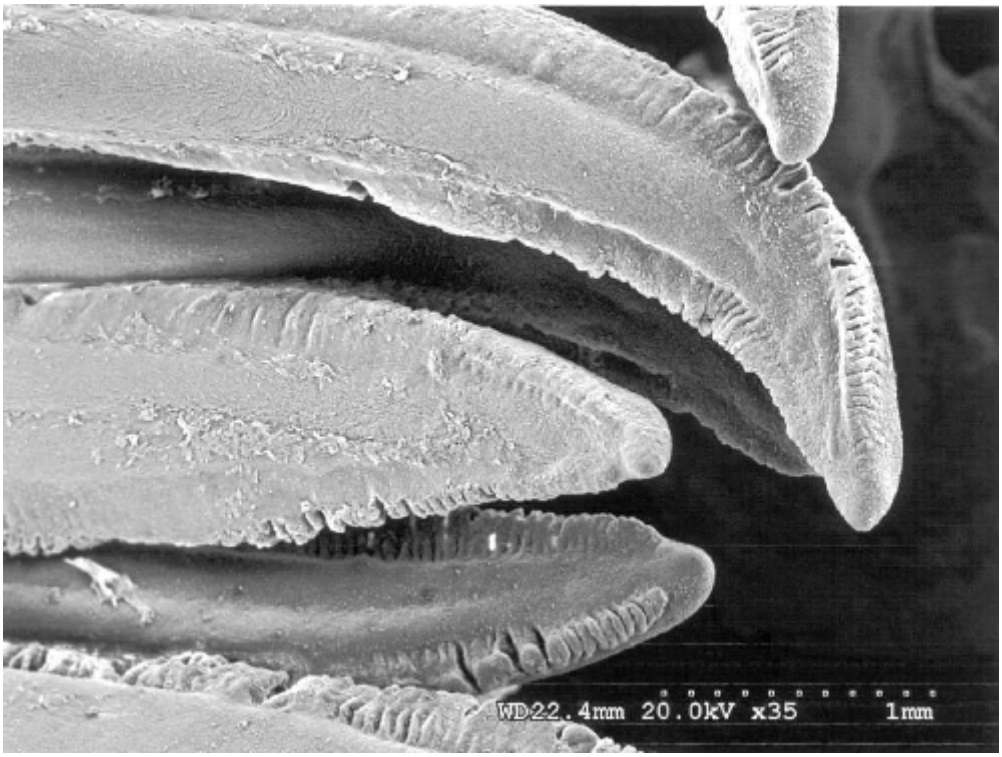


Fig 8.4 **Gills of rainbow trout from Highfield Farm Reservoir, Essex to show collapsing and hyperplasia leading to extensive fusion of the secondary lamellae especially near the tips.**

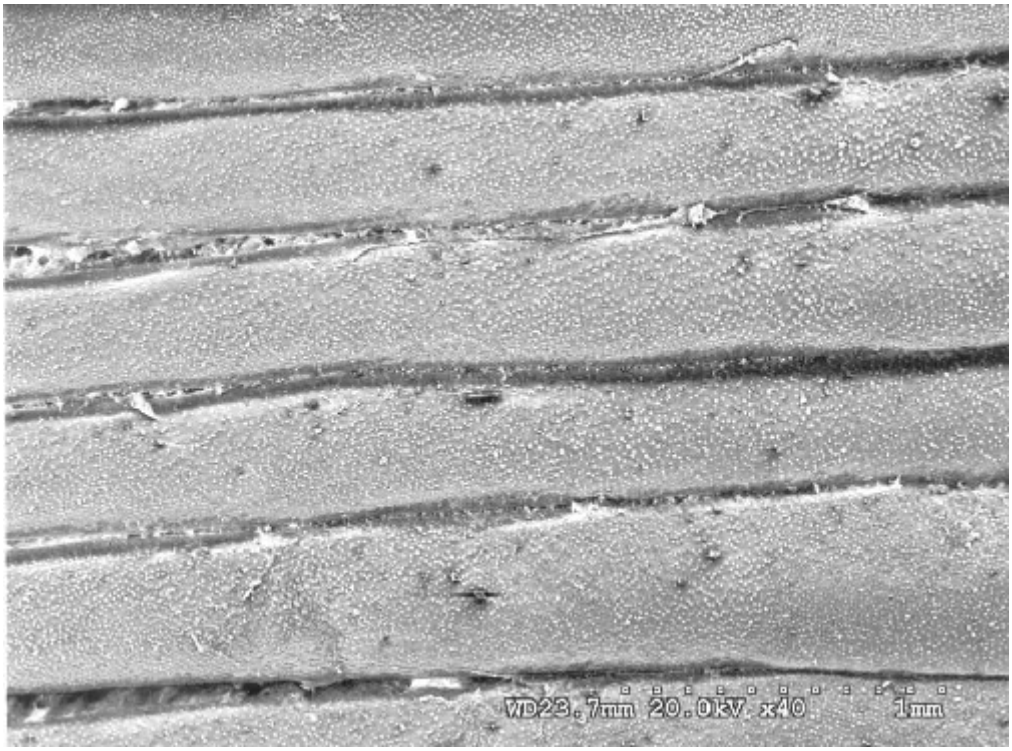


Fig 8.5 **Gills of rainbow trout from Highfield Farm Reservoir, Essex to show hyperplasia leading to total fusion of secondary lamellae along the length of the primary lamellae.**

Fig 8.6 **Gills of rainbow trout from Highfield Farm Reservoir, Essex to show plugs of mucus on the filamental epithelium of a primary lamella.**

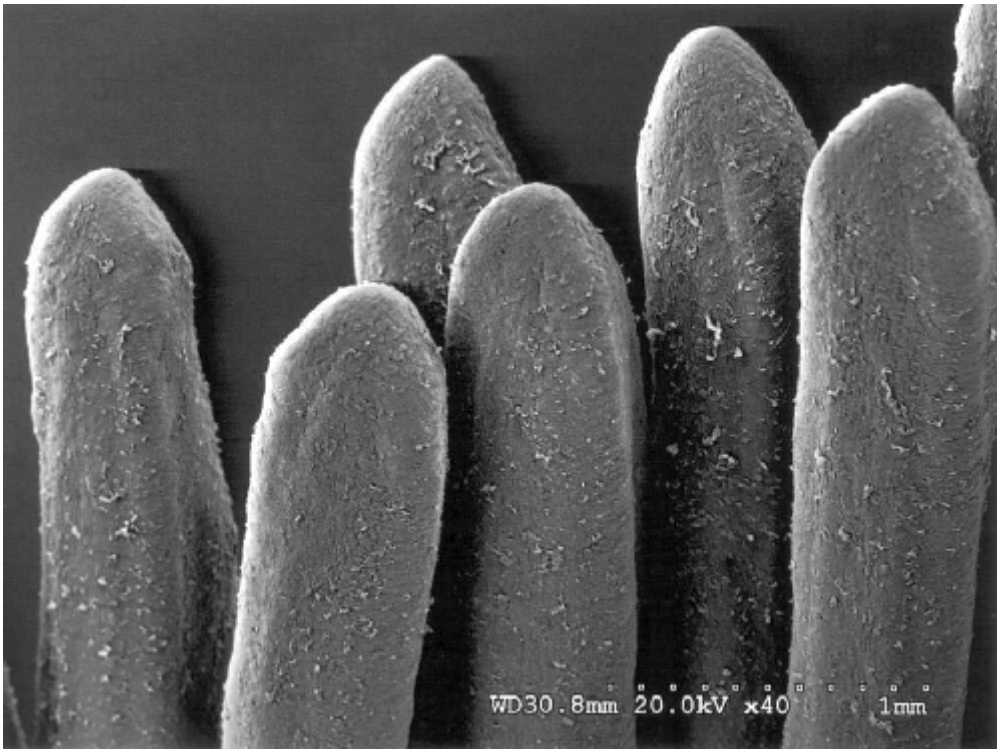


Fig 8.7 **Gills of carp from Bovington No 1 Pit, Hatfield Peverel, Essex to show hyperplasia leading to total fusion of secondary lamellae.**

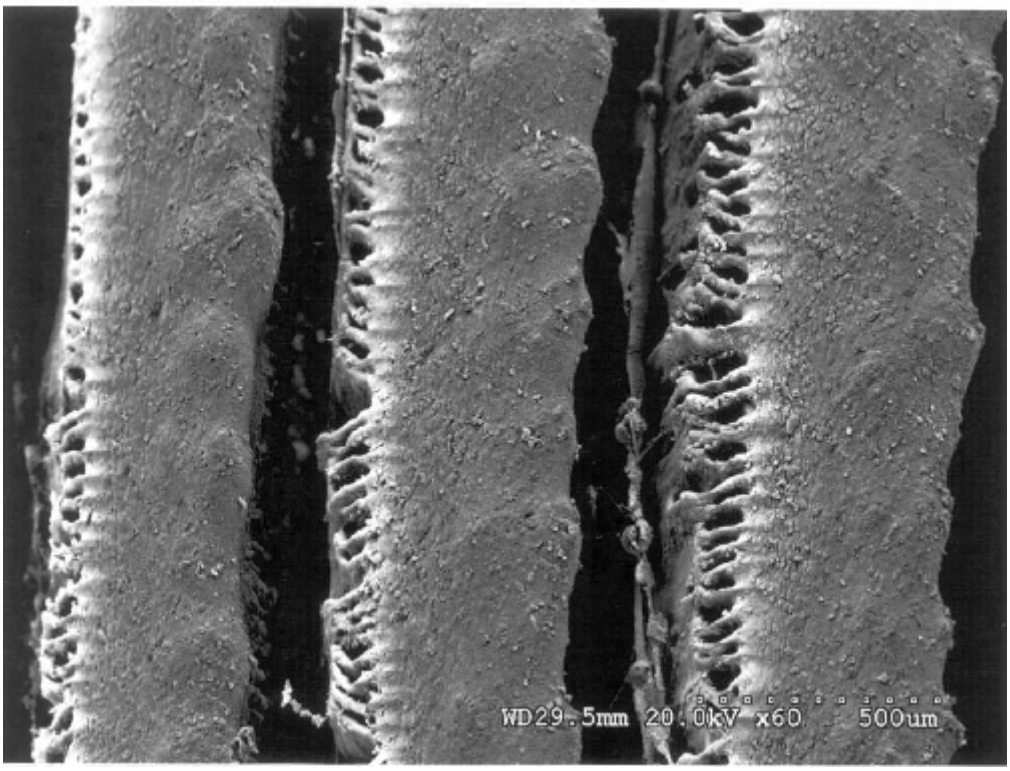


Fig 8.8 **Gills of carp from Bovington No 1 Pit, Hatfield Peverel, Essex to show collapsing of secondary lamellae and telangiectasis (swellings) along the length of the filamental epithelia of primary lamellae.**

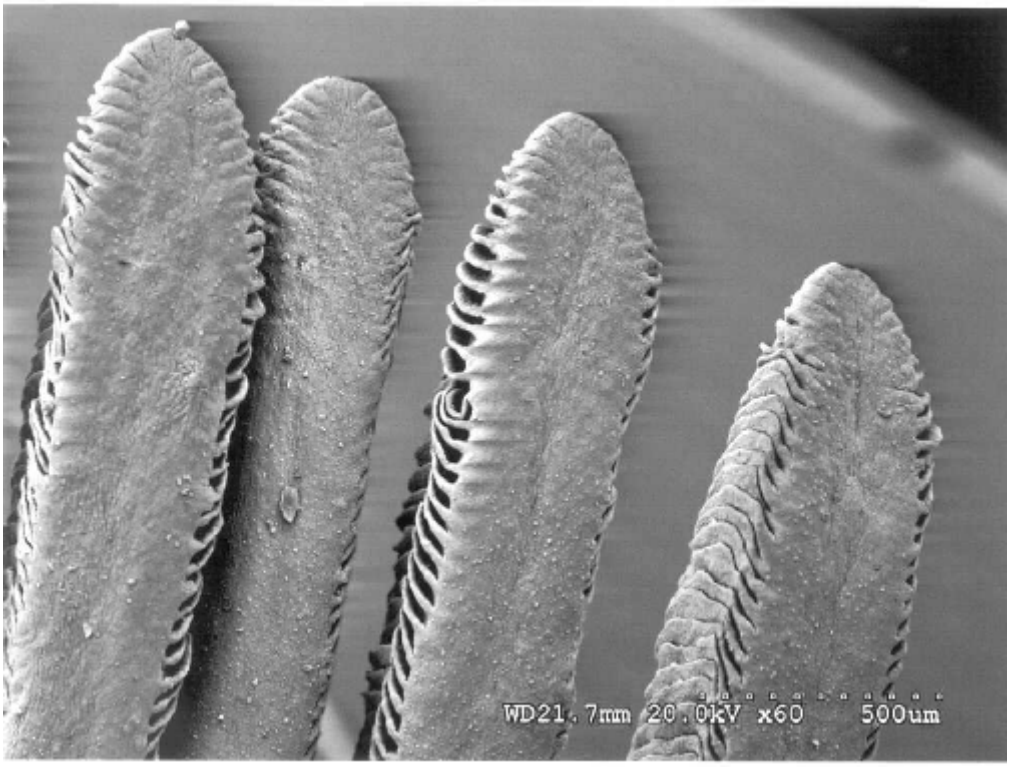


Fig 8.9 **Gills of carp from Cockaynes Pit, Alresford, Essex to show collapsing and hyperplasia leading to fusion of secondary lamellae especially near the tips.**

Fig 8.10 **Gills of carp from Cockaynes Pit, Alresford, Essex to show telangiectasis (swellings) on the filamental epithelia of primary lamellae.**

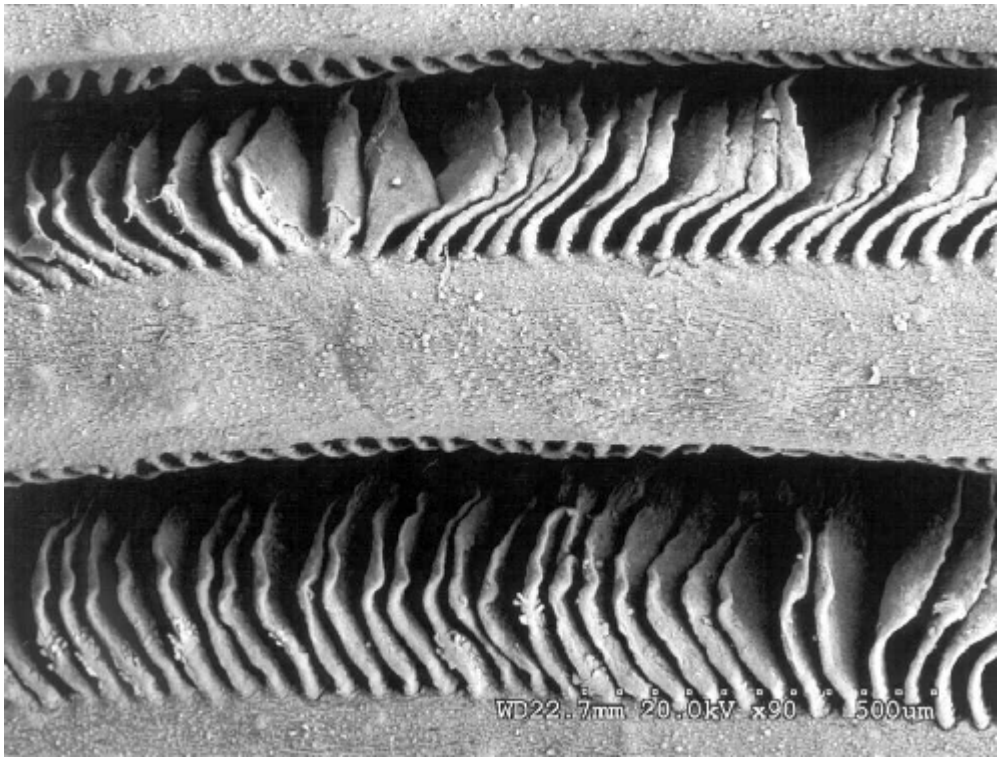


Fig 8.11 **Gills of carp from Cockaynes Pit, Alresford, Essex to show collapsing of secondary lamellae along the length of primary lamellae.**