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POTENTIAL PATHOGENS OF WRASSE (FAMILY: LABRIDAE) FROM SCOTTISH COASTAL WATERS

A thesis presented for the degree of

Doctor of Philosophy to the University of Stirling

by

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DECLARATION

I herby declare that this thesis is wholly my own work and that all sources of information are dully acknowledged. This thesis has neither been submitted nor accepted for another degree.

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ABSTRACT

The use of wrasse (Pisces: Labridae) as cleaner fish to combat infections with the parasitic copepods *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* (Nordmann) (sea-lice) in the culture of *Salmo salar* L. (Atlantic salmon) is now common. Infections with these parasites has caused considerable losses in the industry since its formative years. The use of the wrasse species *Ctenolabrus rupestris* (L.) (goldsinny), *Centrolabrus exoletus* (L.) (rockcook), *Symphodus melops* (= *Crenilabrus melops*) (L.) (corkwing) and *Labrus mixtus* L. (cuckoo) as cleaner fish was first suggested in 1988. The use of these species in the industry is now widespread in Scotland, Ireland and Norway. The fish used are normally caught from the wild before being stocked with *S. salar* smolts during their first year at sea. The fish are routinely collected from waters close to the farm sites to be stocked. As most of the *S. salar* sea production sites in Scotland are located on the west coast of the country, the wrasse to be used in these sites are normally collected from these waters. The movement of wild fish into farm pens presents a risk of disease transfer from wrasse to *S. salar* and vice versa.

Prior to their use as cleaner fish, these four species of wrasse had received little attention as subjects of scientific study. As a result, there was very little information available in the literature regarding their diseases. The present study was undertaken to investigate the potential pathogens present in wild populations in Scottish coastal waters, and, in particular, which of these pathogens, if any, could be transmitted to the *S. salar*. The study also investigated the susceptibility of wrasse to the two major viral diseases of *S. salar* to which they would be exposed in pens.

In order to fully assess the pathogenicity of the potential disease agents under farm conditions, it was first necessary to establish the normal morphology of the wrasse species. Hence, a study of the morphological features of wrasse, with particular emphasis on those features important in the health of the fish was undertaken. Wrasse

were shown to differ in many aspects from salmonids but shared many morphological features with other perciforme fish. Major differences from salmonids were evident in the skin, fins, pancreas, intestine, gonads and heart. There were also aspects of their morphology which differed from other perciforme fish, notably the structure of the heart. These features were regarded to be adaptations to the specific demands of their feeding strategies and habitats. This study was the first of its kind undertaken for wrasse and showed some early contraindications for the use of wrasse in culture; most notable was the marked lipid accumulation in, and resultant degeneration of, the liver resulting from the consumption of high energy *S. salar* feeds.

Once the normal morphological features were established, it was possible to examine the disease status of wrasse. Wild fish were sampled from three different locations on the west coast of Scotland. These sites were all geographically distinct and were all used as sources of wrasse for the *S. salar* farming industry. Samples of wrasse were also obtained from farm sites supplied with wrasse from these wild sites, and an additional number of other geographically distinct farm locations. As a comparison wrasse were also obtained from a wrasse captive breeding facility and another captive location unrelated to the *S. salar* industry, a public aquarium. The fish from all of these sampling sites were examined fully for the presence of parasites, bacteria and, in some cases, viruses. Histological examination was also carried out on all of the fish studied. A total of 24 new parasite host records, and two tentative ones, were recorded from the four wrasse species studied. These new parasite records included protozoa, digeneans, nematodes and crustacea. Parasite infections were found to vary in prevalence, abundance and intensity in respect to the geographical characteristics of sampling sites and also the length of time spent in *S. salar* pens. It was concluded that the separation of wrasse from their natural diet and habitat influenced the degree of parasitism. None of the parasites found to infect wrasse were observed to cause any significant pathology in their hosts other than localised tissue responses.

The possibility of transfer of wrasse parasites to *S. salar* was also investigated experimentally in a series of infections in which parasites dissected from wrasse were introduced to *S. salar* smolts by means of a novel gavage method. None of the parasites used established in the *S. salar,* indicating that there is little risk of transfaunation of parasites between wrasse and *S. salar*. However, this aspect requires further work due to the low number of parasites available and the subsequent low numbers of *S. salar* infected.

Bacterial isolates were obtained from wrasse held in *S. salar* pens but were not found in any of the fish collected from the wild. Most of the bacterial strains isolated would normally be considered as opportunistic pathogens of fish. It was concluded that the relatively high levels of stress, both environmental and physical, that wrasse are subjected to under farm conditions were instrumental in the number of bacterial infections seen in wrasse. Only one pathogenic bacterial infection was seen in any of the fish sampled. This was an isolate of *Aeromonas salmonicida*, the agent known to cause the disease furunculosis, isolated from a wrasse obtained from one of the farm samples. Other authors have reported that this bacterium has already caused substantial losses of wrasse under farm conditions. It was concluded that *Aeromonas salmonicida* will prove to be a major pathogen of wrasse held in *S. salar* pens. No viruses were isolated from any of the wrasse studied.

The susceptibility of wrasse to the most significant pathogens of *S. salar* under farm conditions was also subjected to investigation. In addition to sea-lice infection, the industry lists Infectious Pancreatic Necrosis (IPN) and Pancreas Disease (PD) as of primary importance for further research. Both of these diseases cause substantial losses in the industry.

The susceptibility of wrasse to both of these disease conditions was investigated by means of experimental infections. In the case of IPN wrasse were infected by bathing

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with two different infective doses, a low dose which would be expected to induce the disease in *S. salar* parr and a second dose substantially higher than the first. The *C. rupestris* used were found to be susceptible to IPN. The wrasse developed some of the pathological characteristics typical of the disease in *S. salar*, however, other pathological signs were peculiar to wrasse. The recovery rate from the disease seen in wrasse was far more rapid than that recorded from *S. salar*. Shedding of the virus in the faeces of infected *C. rupestris* was also demonstrated. This study has illustrated for the first time the susceptibility of wrasse to IPN and that they can shed the virus in their faeces. This suggests that infected wrasse could be a source of continual reinfection in an affected sea site.

Experimental infections of *C. rupestris* with PD followed a standard protocol for the reproduction of the disease in *S. salar*. Infection was by means of intraperitoneal injection with putatively infective material obtained from *S. salar* affected with PD. Two infection doses were used, the lowest dose used had been proven to be effective in inducing the disease in *S. salar* parr while the second dose, ten times higher than the first, had been shown to be effective in reproducing PD in *S. salar* smolts. The *C. rupestris* infected did not develop any of the typical signs of the disease seen in *S. salar*. It was, therefore, concluded that wrasse were not susceptible to PD.

The implications of the findings of the study on the continual use of wrasse in the production of *S. salar* is discussed and suggestions for further research are made.

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CHAPTER 1: GENERAL INTRODUCTION

1.1: Salmon Farming: A Historical Perspective

The farming of *Salmo salar* (L.) (Atlantic salmon) is still a relatively young industry in Scotland, only really becoming successful in the 1980s. According to the Scottish Fish Farms Annual Production Survey, 1994, published by the Scottish Office, Agriculture and Fisheries Department, the total number of people employed by the industry in sea production of *S. salar* has increased from 550 in 1985 to 1,226 in 1994. Over the same period total *S. salar* production has increased from approximately 7,000 tonnes to 64,066 tonnes. It is also predicted that the total production will continue to rise but at a slower rate than in the late 1980's. This improved efficiency is partly due to the advent of improved technology and management techniques during both the freshwater and marine stages of production of *S. salar*. Other advances are being made such as the production of S½ and S1½ smolts. This allows companies to target particular times of the year to harvest fish, when the market best suits their product.

As is the case in any new industry, there are still numerous problems inherent in the production of the fish. In the early years of the industry a large percentage of the possible harvest of fish was lost due to disease. The development of vaccines to diseases such as furunculosis and the implementation of the Diseases of Fish Acts, 1939 & 1983, which made the occurrence of certain diseases notifiable, has resulted in a far higher percentage of the parr produced reaching harvestable size and thereby adding to the improved efficiency of the industry. This is reflected in the steady increase in the total percentage of fish recovered from sea sites in recent years, this recovery is currently around 80% of all fish put to sea (Scottish Fish Farms Annual Production Survey 1994).

There is now a three phase approach to combating diseases within the industry. The first phase is legislation, in the form of the Diseases of Fish Acts, which restricts the spreading of diseases away from the point of origin or first reported occurrence. The

second stage is effective husbandry techniques which prevent disease outbreaks in the first instance and also minimise the effect of any outbreaks that do occur. The third and final phase is the use of chemotheraputants to combat the direct effects of diseases and also vaccines to lessen the impact of diseases. It is preferable that the use of chemotheraputants is avoided due to the problems of steadily rising resistance to drugs and also the withdrawal period related to some of the drugs used. However, when they are used they should be administered correctly in a way that significantly reduces the impact of a disease outbreak.

The disease problems which are now causing economically significant losses were, in most cases, considered to be minor problems during the early growth stages of the industry. In 1993 the industry listed its main diseases of concern as;

1. Sea-lice infestation
2. Infectious Pancreatic Necrosis (IPN)
3. Pancreas Disease. (PD)

(Scottish Salmon Growers Association, SSGA)

Both IPN and PD, which are considerations of this study, were of relatively minor concern in the early years of production and it is only now that other diseases are well controlled, that their significance has increased.

The most serious disease of concern to the industry today is parasitic infestation with copepods of the genus Caligidae and, in particular, the species *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann (sea-lice). They were a major problem in the early years when treatments were unsatisfactory but continue to be a major problem because of the build up of parasite populations with the expansion of the industry. These infections are now also relatively well controlled but are still the subject of considerable research as limitations are imposed on the use of chemical and drug treatments.

1.2: The Problems Related to Sea-lice Infestations

With the recognition that sea-lice infections are the main disease problem affecting the industry, much attention has focused on investigating control of the problem. All aspects of lice are currently under investigation including lice biology, prevention of infection and methods for controlling established infections.

1.2.1: Lice Biology and Pathogenicity

There are over 400 species of caligids world wide but only a small percentage of these occur as pests in aquaculture systems. It is estimated that as much as 10% of potential harvestable fish are lost due to caligid infestations world wide (Boxshall & Defaye 1993).

Sea-lice are ectoparasites which feed on the mucus and epithelium of the host fish, utilising a feeding tube which bears a serrated bar to rasp at the surface of the epithelium (Kabata 1979). Damage to the host caused by sea-lice in this way can be severe. There is a tendency for lice to accumulate on the dorsal surface of the fish and erosion of the epithelium on the dorsum, particularly the head, can lead to damage of underlying tissues, with the cranial bones being exposed in severe cases (Jónsdóttir, Bron, Wootten and Turnbull 1992). The loss of osmoregulation resulting from this damage can lead to mortality and may be combined with secondary infections.

The infective stages of sea-lice are water-borne and have a natural reservoir of infection in wild fish. It is therefore very difficult to avoid infections in penned fish. For this reason it has become necessary to employ control measures to minimise infections and therefore damage to, and losses of, *S. salar*.

1.2.2 Lice Control Methods

There are several approaches to the problem of controlling sea lice and the methods used can be split into the following categories:

1. Chemical control;
    - a) Organophosphates.
    - b) Hydrogen peroxide.
    - c) Ivermectin.
    - d) Pyrethroids.
2. Physical;
    - a) Light traps.
    - b) Pumping and filtration.
3. Management;
    - a) Fallowing.
    - b) Single year classes.
    - c) Reduced stocking densities.
4. Biological;
    - a) Vaccines.
    - b) Hyperparasitism.
    - c) Cleaner fish.

1.3: Chemical Treatments

These were reviewed by Roth, Richards & Sommerville (1993a) and Sommerville (1995).

1.3.1: Organophosphates

Until recently the main methods in use in Scotland and Norway to control sea-lice were organophosphates. In Scotland dichlorvos (previously marketed as Nuvan and now as Aquaguard, Ciba Geigy) is used. Organophosphates are applied as a bath

treatment and are used to bathe whole pens of *S. salar*. Aquaguard is the only organophosphate licensed in the UK for use on *S. salar* farms. However, other organophosphates may be given under veterinary prescription or are being used under animal test certificates for research trials such as azamethiphos, marketed as Salmosan by Ciba Geigy, which is currently under trial in Norway. Organophosphates act by inhibiting acetylcholinesterase (AChE) activity resulting in an accumulation of the neurotransmitter acetylcholine, death follows a tetanus like condition (World Health Organisation 1986). Although all stages of lice posses AChE, chalimus stages are not affected by dichlorvos (Walday & Fonnum 1989). The use of such chemotheraputants is expensive and complicated with consideration having to be given to the volume of the pens, water temperature and current systems on the site. Consideration has also to be paid to the lasting damage which can be caused by the prolonged use of such chemicals. The possibility of bioaccumulation of dichlorvos is thought to be slight due to the short half life of the chemical in sea water, however, there is evidence of resistance to dichlorvos in sea lice in some areas resulting in increased numbers of treatments (Jones, Sommerville & Wootten 1992 and Sommerville 1995).

This resistance and the dangers of relying on only one effective treatment makes the development of an effective alternative treatment imperative.

Other organophosphates and related compounds are currently in differing stages of research, some being in the final stages of consideration for the granting of a UK licence. Sommerville (1995) summarises the present situation with regard to these chemicals.

1.3.2: Hydrogen Peroxide

Because of the problems associated with the traditional use of organophosphates, alternatives are now being investigated. One of these alternative chemicals is hydrogen

peroxide, which is now being used commercially on a trial basis. Paramove and Salartect are both treatments consisting of hydrogen peroxide, neither of which have received full licence yet. Hydrogen peroxide would appear to be a more suitable compound than organophosphates for the treatment of lice, as it breaks down quickly to form oxygen and water leaving no harmful residues in the environment. The break down of hydrogen peroxide causes bubbles of oxygen to form. The method by which hydrogen peroxide kills lice is not yet known. However, during and after a treatment, the lice are seen to float to the surface and are unresponsive to external stimuli (Thomassen Pers. Com.). There have been reports of up to 80% mortality of lice with the remainder being removed (Sommerville Pers. Com.). If the mechanisms involved in the death of lice are physical, due to tissue disruption by the formation of bubbles of oxygen, then there should be little possibility of lice becoming resistant to hydrogen peroxide. This compound also has the added advantage of being partially effective against chalimus stages (Thomassen 1993). One disadvantage is the narrow safety margin since prolonged exposures, greater than 20 minutes, causes irreversible gill damage (Sommerville Pers. Com.). Despite this, hydrogen peroxide appears to be a very promising alternative treatment for sea lice but involves a very high cost when compared to treating with Aquaguard.

1.3.3: Ivermectin

The pesticide Ivermectin is the best known oral treatment effective against sea-lice. At the present time it is not licensed for general use in Britain and can only be used as a Prescription only Medicine (POM). That is to say it can only be prescribed by a veterinarian when there are no other alternatives available, for example when a lice population proves resistant to all other treatments (Sommerville 1995). Ivermectin is administered orally with the food and takes several days for the flesh concentration to reach a level high enough to affect lice. There is also a long withdrawal period required between treatment and harvest, preventing the treatment of fish close to harvest.

A large amount of the administered drug passes out, unaltered, in the faeces of the treated fish (Höy, Horstberg & Nafstad 1992) leading to accumulation of ivermectin in the sediments, waste food, and invertebrate life around fish pens. It has been recommended that ivermectin should only be used in extreme cases, if at all (Costello 1993). Indeed the manufacturers have opposed any further development of the drug as a treatment for sea lice (Brewer 1991). There are, however, relatives of this family of chemicals, for example avermectins, which may prove useful for future development (Sommerville Pers. Com.)

1.3.4: Pyrethroids

Pyrethroids (synthetic pyrethrins), which are widely used insecticides, have also been investigated as possible lice treatments. In particular, resmethrin and lambda-cyhalothrin. Pyrethrins are more widely studied in Norway and comparisons between the efficacy of pyrethrins in comparison to dichlorvos made by Boxaspen (1994) illustrated that both compounds had a similar efficacy against sea lice. The use of pyrethrins was reviewed by Roth, Richards & Sommerville (1993a & b). These compounds would appear to be lethal to adult lice and partially effective against chalimus stages. The safety margin for the use of some pyrethroids is very narrow with fish mortalities occurring within a small concentration difference from effective treatment doses. Pyrethroids are also know to be toxic to aquatic life, particularly fish, and their wide spread use could lead to many secondary problems (Roth *et al.* 1993b). Pyrethroids must still be considered as possible alternative chemotheraputants although further research is needed before pyrethroids could be tested on a commercial scale.

1.4: Physical Controls

The main purpose of physical methods is to attempt to interrupt the transmission of the parasites by trapping the infective free-swimming larval stages, or to physically remove the lice from the fish.

1.4.1: Light Traps

Huse, Bjordal, Ferno & Furevik (1990) attempted to reduce lice infections by means of shading pens and placing light traps beside pens to trap the photopositive larval stages. They were unsuccessful. Currently a prototype light trap is being tested at certain Scottish farm sites, but the results as yet are unknown.

1.4.2: Pumping and Filtration

Sites which use a pump ashore system, where fish are grown in tanks on shore, would not appear to suffer from high numbers of lice (McVicar Pers. Com.). These sites are supplied by water pumped from either deep below the surface of the sea, or from bore holes sunk deep enough into a porous rock layer to tap the sea water table. It is possible that the pressure changes experienced by the infective copepodid stages of the lice, as they pass through the large capacity pumps required by these operations, disrupts their ability to attach to their hosts. Another possibility is that the number of infective copepodids deep in the water column is significantly lower than those found on the surface, which would result in a reduced number of infective stages entering the ongrowing tanks. This method would not be effective in sea pen culture due to the pens being open to the sea. Similarly the filtration of sea water to remove the infective stages in sea pens is impracticable (Costello 1993). A different approach to the use of pumps is the removal of lice directly from the fish. Innovac Europe Ltd. have began marketing a combined fish grader and lice remover. The machine utilises a Silkstream live fish transfer pump and a spray bar which administers a lice removing agent to the surface of the fish as they pass through the grader. Lice that are removed from the fish are caught in a filter and disposed of safely. In early trials, the lice killing agent used was Pysal 25, a pyrethroid. The machine performed well when tested on a Sutherland fish farm. The development of this system was probably prompted by the fact that in desperation some farms began pumping fish in normal transfer pumps from one cage to another to reduce

lice burdens and avoid the withdrawal period that would have applied if chemicals had been used.

1.5: Management Strategies

1.5.1: Fallowing

The implementation of a fallow period, between harvesting fish from a site and the introduction of a new year class, effectively delays the onset of lice infections in the new year class. Bron, Sommerville, Wootten & Rae (1993a) postulated that this delay was due to the reduction of farm-generated lice at a given site. New infections were most probably the result of cross infection from wild fish. To be fully effective a whole loch system should be fallowed simultaneously and, in most cases, this will require collaboration between several companies. This approach was used successfully in Loch Sunart on the west coast of Scotland where the whole loch was fallowed between February 1991 and April 1991. This involved three companies and proved to be effective (Grant & Treasurer 1993). According to the Scottish Fish Farms Annual Production Survey 1994 the number of sea sites employing a fallow period in 1994 was 358, or approximately 97% of the operational sea sites.

1.5.2: Single Year Classes

If fish of the same year class are the only fish kept on a site, and all the fish are stocked and harvested at the same time, then the risk of transmitting diseases between age classes is eliminated. Also the management of any disease outbreaks is greatly simplified. If a fallow period is implemented before the stocking of single year class sites then the period before first treatment for lice can be significantly increased (Bron *et al.* 1993a & b).

1.5.3: Lowered Stocking Densities

If the density of fish in pens is reduced then stress levels in the fish are reduced, giving better growth characteristics and also improved ability to withstand infections. This leads to an improvement in the general health of the fish. Lower stocking densities also simplify the treatment of disease outbreaks.

In reality all three of these management strategies would be initiated for maximum success (Bron *et al.* 1993a).

1.6: Biological Controls

1.6.1: Vaccines

Work to develop an effective vaccine against sea lice has also been underway for some time. In heavy lice infestations severe damage can be caused to the epithelium of the fish resulting in exposure of the underlying dermis and ingestion of blood by the lice. Raynard, Munro, King, Bruno, Bricknell, Vahanakki, Sommerville, Petrie, Vivers, Andrade-Salas, Melvin, Amezega, Labus, Coull, Reilly, Mulcahy, O'Donoghue & O'Connell (1994) stated that there has been no evidence that fish acquire an immunity against subsequent lice attacks, therefore, if an antigen was to be effective it would have to be a hidden antigen. The most probable source for these antigens is the intestine of lice. Fish injected by the authors with whole louse extract developed an antibody response to several antigens but did not acquire immunity to lice attack. It was presumed that the complexity of the whole louse extract resulted in antigenic competition and that individual antigens would need to be tested. Several antigens have been purified from lice which illicit antibody responses when injected into mammals (Jenkins, Grayson, Hone, Wrathmell, Gilpen & Munn 1993). These and other antigens developed from various lice tissues by Raynard *et al.* (1994) were subsequently tested histochemically using monoclonal antibodies on sectioned lice (Andrade-Salas, Sommerville, Wootten, Turnbull, Melvin, Amezega & Labus 1993). *S. salar* were then immunised with selected clones and their antibody response monitored using ELISA techniques. These fish were

then challenged with lice. The first few potentially effective antigens are currently under test (Raynard *et al.* 1994).

1.6.2: Hyperparasitism of Lice

Several epizoonts are commonly found on sea lice. The monogenean trematode *Udonella caligorum* Johnston is found on the dorsal surface of lice. The presence of these worms may have a detrimental effect on the lice, however, this has not been established. No obvious detrimental effects to *S. salar* have been observed resulting from the presence of the worms (Minchin & Jackson 1993). Several ciliated protozoans are also seen on the surface of lice. Gresty & Warren (1993) reported the ciliate *Epistylis* Ehrenberg from the carapace fringe and ventral surface of *L. salmonis* removed from *Onchorhynchus keta* Walbaum (Japanese Chum salmon) and attached ciliates of the genera *Trochiloides* Kahl and *Licnophora* Claparéde have been removed from sea lice from *S. salar* from Scottish farms. It is not known if these ciliates have detrimental effects on lice or *S. salar*.

The lack of information on parasites of sea-lice reflects the lack of investigation of the subject. No commercial trials have arisen from this research.

The serious problems with all the above methods led to evaluation of alternative lice control methods.

1.6.3: Cleaner Fish

One of the methods considered was the use of cleaner fish to reduce the lice burden on infected *S. salar*. Symbiotic relationships of this type are widely reported from the wild (Limbaugh 1961). Fish of the genus Labridae are commonly seen as cleaners in tropical waters and their potential as cleaner fish in the production of *S. salar* was investigated initially by Bjordal (1988).

1.7: The Potential of Cleaner Fish as Biological Controls of Fish External Parasites.

1.7.1: Cleaning Behaviour

Symbiotic cleaning relationships have been known throughout the animal kingdom for many years. These relationships are not limited to aquatic animals as several examples can be seen in terrestrial species also. The relationships between the tick bird and the rhinoceros, the Egyptian plover and the crocodile and also the egret and cattle are well known. However, many of the best documented examples are from the marine environment. Most of these records have arisen from the spreading popularity of SCUBA-Diving. These techniques have allowed biologists to observe aquatic animals in their natural environment with the minimum of interference. The range of animals displaying cleaning behaviour is diffuse, with examples being seen in many different groups of teleosts and crustaceans. Limbaugh (1961) presented a review of cleaning behaviour detailing many interesting examples.

Most of the examples seen in crustaceans involve shrimps in tropical waters. For example *Periclimenes pedersoni* Chace (the Pederson shrimp) from the Bahamas sets up cleaning stations in quiet water. The shrimps are always found in association with the anemone *Bartholomea annulata* Leseur. When fish pass by, the shrimp enters a typical pattern of behaviour involving antennae whipping and body swaying to attract the fishes attention. A fish will then approach the shrimp and present areas of its body to be cleaned. If there are any wounds on the fish, these areas will be presented first. The shrimp moves onto the fish and moves rapidly over its surface removing any external parasites and cleaning infected areas. The host fish remains still with it's fins erect and opercula flared, allowing the shrimp to enter the gill chamber and even to exit through the mouth. Fish can often be seen congregating around these cleaning stations waiting to be cleaned.

The relationships between teleost cleaners and their hosts is very similar to that of crustacean cleaners and their hosts. The cleaner fish establish a cleaning station which is visited by fish wishing to be cleaned. The cleaner fish then adopts a typical display to attract potential host fish which, when they approach the cleaning station, adopt a typical posture with fins erect and opercula flared. Generally their bodies will be orientated in a particular fashion also, for example, they may point their bodies upwards towards the surface.

The majority of examples of teleost cleaners involve fish of the genus Labridae (wrasse) in tropical waters where the number of species of wrasse is considerable. Perhaps the most well known of these cleaner wrasse is *Oxyjulis californica* (Gunther) (señorita) from the Californian coast. This is a small brown coloured wrasse with a black spot on the caudal peduncle which is known to clean many different species of host fish.

The species involved in cleaning in these waters are often evolved for the purpose with long pointed snouts to allow the removal of parasites and they are also very brightly coloured in order to stand out from their backgrounds. This is also the case for cleaner shrimps in tropical waters. Cleaners in tropical waters tend to be solitary or in pairs. In contrast to this, cleaners in temperate waters tend to be less colourful and live in shoals which follow host fish, surrounding them and removing parasites. Also, in temperate waters, it is often only the juvenile stages of wrasse that act as cleaners and even at this stage do not rely totally on cleaning as a feeding strategy. Tropical species come closer to being wholly dependent on cleaning as their feeding strategy.

Eibl-Eibesfeldt (1955), considering the significance of coloration in cleaning species, developed the "Guild Mark" theory. He observed that cleaners from widely different zoo-geographical regions evolved very similar coloration. He suggested that this coloration may serve as a guild mark to allow host fishes to recognise cleaner

species. The best known examples are found in the genus Labridae, where all the fish concerned bear longitudinal black stripes.

1.7.2: Cleaning Behaviour in British Wrasse

Observations of cleaning behaviour in some of the European wrasse species had been made as early as 1973 in a marine aquarium (Potts 1973). Two species of labrids *Symphodus melops* (L.) (corkwing) and *Ctenolabrus rupestris* (L.) (goldsinny) were observed to remove the praniza larvae of the isopod *Gnathia maxillaris* (Montagu) from the skin of other fish species in the aquarium. There appeared to be variation in the relationships between the cleaner wrasse and the host fish being cleaned. Some species of host fish, *Pagellus centrodonus* (Cuvier)(pink bream), *Spondiliosoma canthan* (L.) (black bream) and *Labrus bergylta* Ascanius (ballan) would adopt a typical invitation posture when in the presence of the actively cleaning wrasse. This posture consisted of a "head down" position with the unpaired fins erect. Other species of host fish, *Scomber scombrus* L. (mackerel) and *Pleuronectes platessa* L. (plaice), did not actively take part in the cleaning activity and the wrasse would remove parasites opportunistically from the host fish as they passed by. Parasites were also recovered from the stomachs of wild caught specimens of *S. melops* suggesting that cleaning does occur in the wild. Potts (1973) also reports observing *Centrolabrus exoletus* (L.) (rockcook) cleaning specimens of *L. bergylta* in the wild where the specimen of *L. bergylta* had adopted the typical invitation posture described above. Specimens of *L. bergylta* were also observed to adopt the invitation posture while being cleaned by *C. rupestris* specimens in Swedish coastal waters (Hilldén 1981). Hilldén (1983) postulated that species displaying cleaning behaviour evolved from substrate feeders that were opportunistic in their feeding habits. These species may also have selected food items by size, as discussed in chapter 2.4, as is the case for *C. rupestris*. It is also suggested that the ability to take food items quickly would be important in the development of cleaning behaviour (Hilldén 1978a).

The colouration of both *C. rupestris* and *C. exoletus* with dark marks on the caudal peduncle would appear to add weight to the "Guild Mark" theory as their coloration is close to that of *O. californica*. The coloration of non-breeding *C. melops* would also agree in part with this theory (Hilldén 1978a).

The invitation posture, described above, has never been observed in the case of *S. salar* stocked in pens with wrasse. Any lice removed are taken with short, opportunistic movements of the wrasse (Treasurer 1994a). The relationship between wrasse and *S. salar* is therefore best described as being facultative cleaning.

1.7.3: Use of Wrasse for Parasite Control

1.7.3.1 Practical Use: An Historical Perspective

Bjordal (1988 & 1990) first suggested that wrasse could be used to control lice numbers on *S. salar* farms. This suggestion followed a series of small and then full scale trials in Norway. The small scale trials involved stocking 220 *S. salar* smolts in small sized sea pens with a combination of wrasse species and stocking ratios, any wrasse that died being replaced to maintain the stocking ratio. The results of these small scale trials were favourable with the experimental pens needing fewer de-lousing treatments than a control group with no wrasse. *C. rupestris* and *C. exoletus* proved to be slightly more efficient cleaners than *L. mixtus*. The commercial scale trials that followed these experiments were equally successful. These involved the use of three adjacent commercial *S. salar* pens stocked with 20,000, 26,000 and 30,000 smolts respectively. The middle pen was also stocked with 500 *C. rupestris* and 100 *C. exoletus*, giving a wrasse to *S. salar* ratio of 1:43. During the 7 week period of the experiment only a few smolts in the experimental pen were observed with low level lice infections while the control groups received a total of four de-lousing treatments between the two pens. The following year (1989) a number of fish farms in Norway and one in Shetland began to use wrasse to control lice infections. The data from 20 of the Norwegian farms collated

by Bjordal (1991a) suggested that *C. rupestris*, *C. exoletus*, *S. melops* and female and juvenile *Labrus mixtus* L. (Cuckoo) were potential candidates for use as cleaner fish. The next most abundant of the Northern European labrids, *L. bergylta*, did not display any cleaning ability. Its size and aggressive nature also made it unsuitable for use. The initial stocking density suggested by Bjordal was 1 wrasse to 50 *S. salar* smolts (Bjordal 1988, 1990, 1991a,b, 1992).

After the initial, successful trials in Norway, commercial trials were undertaken by the Shetland Salmon Farmers Association. The trials in Shetland were successful, with a mixture of *C. rupestris* and *C. exoletus* being used at a ratio of 1 wrasse to 50 *S. salar* (Anon 1991). A mixture of species was thought to be best to avoid any intraspecific aggression as reported by Bjordal (1991a).

The use of wrasse as biological controls increased sharply in Scotland, Shetland, Norway and Ireland after these early successes (Costello & Bjordal 1990, Costello & Donnelly 1990, Treasurer 1991 a, b, Darwall, Costello & Lysaght 1991).

1.7.3.2: Current Use

The most widely used wrasse species is *C. rupestris* due to its abundance around the coasts of Scotland, Shetland, Norway and Ireland with both *C. exoletus* and *S. melops* being used according to their availability (Costello 1993).

It has been suggested that *C. exoletus* is the most active cleaning species (Costello 1991), although the use of this species is limited by its relative rarity.

Wrasse generally inhabit the sub-littoral regions of rocky coastlines. A review of the biology of the wrasse species found in northern European waters was carried out by Costello in 1991. Wrasse for use on *S. salar* farms are normally caught using fyke nets

or baited, small mesh, prawn creels fished in 2-15 metres of water, although some local variations in fishing methods exist (Costello 1993, Treasurer 1994b). The fish are then stocked with *S. salar* at ratios of 1:50-150 (Costello 1993). It would appear that very few companies screen the wrasse for parasites or other diseases before stocking them into pens.

This lack of screening is surprising considering that high numbers of wrasse losses have been reported in the early years of their use (Darwall *et al.* 1991). The possible causes of these losses are escape of fish due to too large a mesh size being used or holes in the nets and also deaths due to "gilling", predation by piscivorous birds or disease.

The numbers of wrasse losses due to escape or "gilling" can be reduced by the use of small mesh size nets. The mesh size generally used is 12mm. Indeed, it has been suggested that there should be a minimum length, of around 100mm for wrasse caught for stocking of fish farms (Treasurer 1991a). In addition to reducing the number of escapees and death due to "gilling" it also aids in the conservation of the wrasse species by allowing smaller fish to remain in the wild. In one trial carried out by a major fish farming company, 8.6% of the recorded wrasse mortalities could be attributed to "gilling" (Treasurer 1993a). The use of smaller mesh sizes may cause an increase in net fouling requiring more labour to keep the nets clean (West 1991). However, it has been reported that wrasse removed from *S. salar* pens have been found to have net fouling organisms such as *Mytilus edulis* L. (Edible mussel) in addition to lice fragments in their stomachs (Treasurer 1993b). They may also then be regarded as an aid to keeping nets clean although it is not known if an over abundance of fouling organisms would decrease the cleaning ability of the wrasse (Bjordal 1991a). The provision of hides, constructed from sectioned small bore plastic piping, for the wrasse in the pens may aid the retention of wrasse as they would have a refuge in which to shelter (Treasurer 1991a). In addition

to this, if there are observed to be increased mortalities in the winter when wrasse are reported to enter a torpid state due to cold temperatures, wrasse should be removed to suitable holding facilities during periods of low temperature (Darwall *et al.* 1991).

Despite the numerous winter mortalities, the successful cleaning of lice from fish has been observed for the duration of a two year growing cycle with wrasse stocked in the first year (Thorburn 1991, Bjordal 1992). Darwall *et al.* (1991) reported the successful cleaning of brood stock fish of 6.5 kg, in this case, however, the *S. salar* were in a non-feeding phase and in similar trials, with feeding *S. salar*, the wrasse were reported to be predated by *S. salar*. It is generally accepted that the use of wrasse as a biological control of lice is not a complete solution to the problem of lice infestation as they are most successful in the first sea year of *S. salar*. Their use must form a part of a combined management strategy utilising the best control methods depending on the many factors involved in a particular situation (Sommerville 1995).

1.8: Project Aims

It was recognised that there was a need for the health of wrasse in their natural environment to be studied to establish what pathogens could be imported into the farm environment with wild caught wrasse. However, for this survey to be effective the normal morphology of wrasse must first be defined. It was also important to assess the risk of transferring infections from wrasse to *S. salar* and the risk of transfer of *S. salar* pathogens to wrasse. This would allow the appraisal of what disease factors may play major roles in the survival of wrasse under farm conditions.

1.8.1: Wrasse Biology, Morphology and Histology

The first aim of the present study was to describe some relevant aspects of the biology of the four wrasse species currently being used in the industry and also the range of normal morphological features of organs that can be important in disease diagnosis in

these wrasse species. This would enable fish diagnosticians to effectively monitor the health of wrasse in *S. salar* pens or to assess wrasse health prior to stocking in pens. For this process to be successful it is necessary to understand certain aspects of wrasse biology and the range of normal features seen in the fish. This knowledge is also essential before considering the abnormal features caused by diseases due to parasites, bacteria or viruses. At the start of this study there was no knowledge concerning the biology, morphology or histology of wrasse species.

1.8.2: The Natural Infections of Wrasse

The second aim of the present study was to assess what infections occur naturally in wrasse. This would allow the evaluation of which pathogens may be important in wrasse mortalities in the wild. In addition to this, when considering the importation of wrasse into the controlled environment of a *S. salar* farm it is essential to consider which diseases and parasites are present in the fish in the wild in order to avoid disease outbreaks. The knowledge gained from the wild wrasse survey would allow the evaluation of these risks. This information is also important in determining what possible disease problems could arise in wrasse culture. Wrasse are occasionally stocked in *S. salar* farms located in a different area from that in which they were caught. This practice may increase as local stocks decline. It was therefore necessary to extend this part of the study to include different geographical areas for comparison. The practice of moving wrasse between different geographical locations would allow the transportation of pathogens between different areas, possibly introducing new infections. International codes of practice, such as the ICES code of practice on introductions and transfers of marine organisms, are established to prevent these situations occurring. The movement of wrasse from Norway to Scotland has already been prevented by legislation due of the risks of Norwegian *S. salar* diseases such as Viral Haemorrhagic Septicaemia (VHS) and Infectious Haematopoietic Necrosis (IHN) being imported. This restriction in movement

was possible due to the fact that Norway is not within an EEC "free zone" giving Norway a lower disease status when compared to Scotland.

Once the information regarding the naturally occurring infections of wrasse was available it would be possible to fulfil the third aim of this project which was to assess the effect of time in *S. salar* pens on the infections of wrasse. The farm environment is very different from the natural environment of wrasse. The isolation of the wrasse from their natural food sources and habitats may significantly affect the pathogens which affect them. Pathogens which do not cause problems to the wrasse in the wild may be favoured by the change in conditions in the farm pens causing a risk to the health of wrasse.

The fourth aim of this study was to assess the risk to *S. salar* from pathogens imported with wild caught wrasse. The fulfilment of this aim was dependent on the completion of the second and third aims of the study. The information gained from these studies would allow the assessment of risk to *S. salar* from the pathogens found to affect wild wrasse and also the ones which were important to the health of wrasse after a period of time in captivity.

1.8.3: The Susceptibility of Wrasse to *Salmo salar* Pathogens

The fifth and final aim of this project was to assess the risk to wrasse of *S. salar* pathogens. The cost effectiveness of wrasse as cleaner fish depends, at least partly, on their health and how long they survive in *S. salar* pens. An important factor acting upon this is the susceptibility of wrasse to common *S. salar* pathogens. It was, therefore, necessary to determine whether or not wrasse could become infected with pathogens of *S. salar* and act as carriers for these diseases. The importance of this information is two fold;

a) Are these pathogens a risk to wrasse?

b) Is there a risk to *S. salar* from the transfer of these diseases with wrasse?

There is particular risk when considering the transportation of wrasse between totally different geographical areas, such as transferring fish from Ireland or Norway to Scotland.

In addition to sea-lice infections the two disease conditions that the industry regards as most in need of research are Infectious Pancreatic Necrosis (IPN) and Pancreas Disease (PD). The susceptibility of wrasse to these diseases, therefore, needed to be investigated with regards to the above criteria.

CHAPTER 2: WRASSE BIOLOGY AND MORPHOLOGY

2.1 Introduction

The first aim of this part of the project was to describe the biological and morphological characteristics peculiar to the species of wrasse used in the farming of *S. salar*, with particular reference to those features which are important in assessing the health of the wrasse.

There have been no works published which give information on the normal morphological features of the wrasse species important to the *S. salar* farming industry. If fish diagnosticians are to effectively monitor the health of wrasse in *S. salar* pens then this information is essential as a comparison for abnormal features caused by parasites, bacteria or viruses.

What little information has been published mainly deals with distantly related wrasse species from waters other than those used by the *S. salar* industry. Texts such as the common field guides to coastal fish species give general descriptions of the species involved. The following is abstracted from one such guide, Webb, Walwork & Elgood (1981):

Wrasse are perciform fish of the family Labridae with the following common features:

1. Wrasse have a single, elongate dorsal fin with spiny rays anteriorly.
2. Typically the pelvic fins are located anteriorly beneath the pectorals.
3. The anal fin usually has three spiny rays anteriorly.
4. The jaws are protrusible with teeth on the jaws and pharynx.
5. Wrasse have deep bodies which are compressed and have striking colouration.

2.1.1 Description of the Species Used in *Salmo salar* Farming

Only the four species used in the farming of *S. salar* are considered here. These species are *C. rupestris*, *C. exoletus*, *L. mixtus* and *S. melops*. There is a very limited amount of information available on the labrids of British waters. The only texts that deal with them in any depth are the general texts of Webb, Wallwork & Elgood (1981) and Wheeler (1969 & 1978). Considering the emergent interest in the group due to their recently acquired economic importance and also their importance in the ecosystem of the intertidal zone, a more detailed study of the group is warranted. Sayer, Treasurer & Costello (1995) have begun researching aspects of the biology of labrids which could prove in the long term to be very interesting, for example, the ability of at least some of the labrid species involved to enter a state of partial "hibernation" in low water temperatures, a behavioural feature which may be unique amongst fish.

2.1.1.1 *Ctenolabrus rupestris* L. (goldsinny)

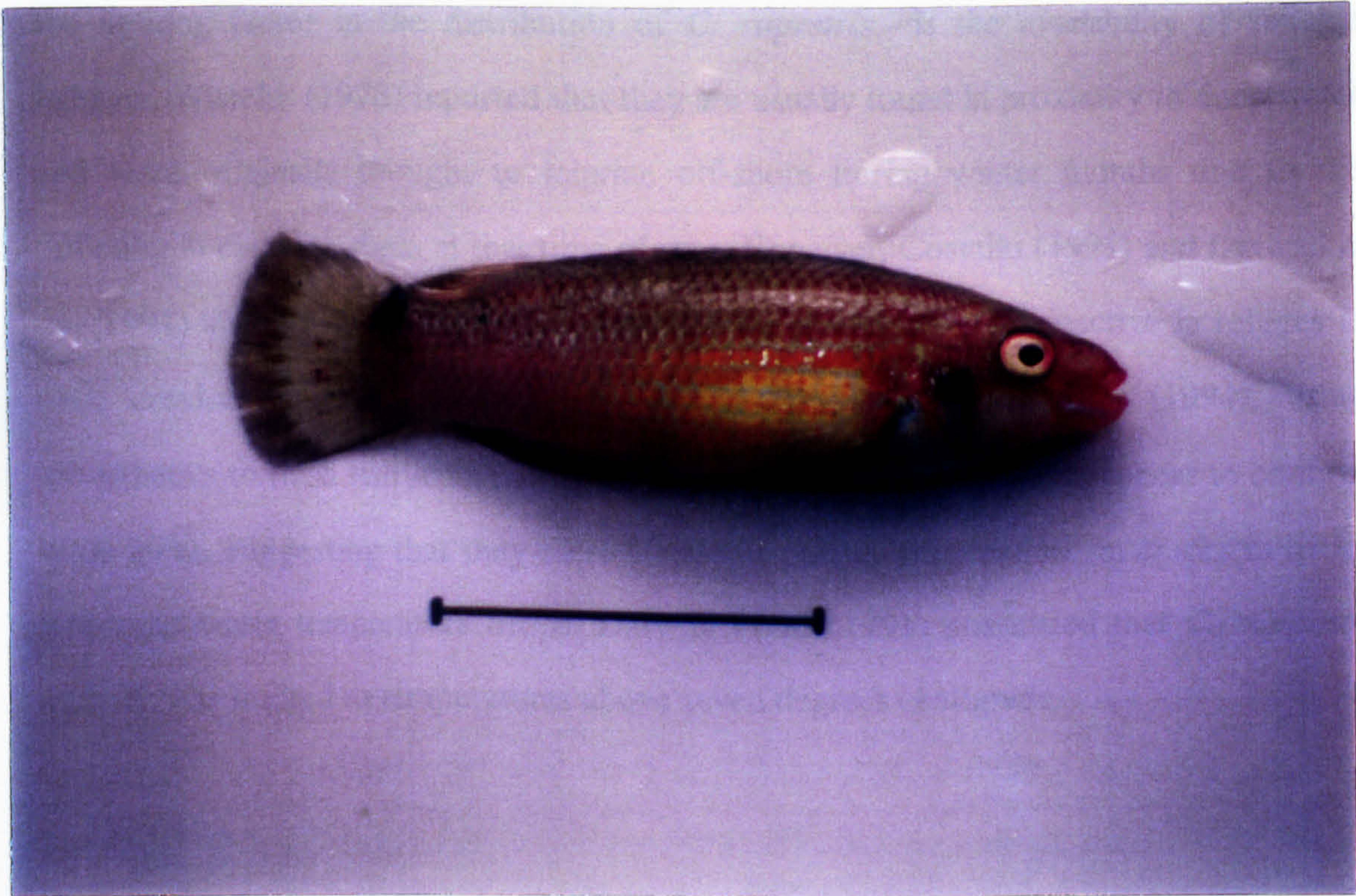
Appearance

This species, illustrated in Fig 2.1, has a slender body with a pointed head and a small mouth. The scales are large and number between 35 and 39 along the length of the lateral line. The pre-operculum is finely serrated on the distal edge but not ventrally. The anal fin typically has seven to eight branched rays. The teeth are found in the front of the jaw and are large, curved and in two rows. Adults can be up to 18 cm in length but are usually around 12cm (Wheeler 1978).

Colouration

C. rupestris is normally brown or reddish-orange in colour, with dusky spots on the dorsal fin membrane between the first and fifth spines and on top of the tail in front of the caudal fin (Wheeler 1978). Adults in breeding condition normally have a golden colour on their flanks and abdomen (Pers. Obs.).

Fig 2.1: An adult *Ctenolabrus rupestris*, scale bar = 5cm.



Distribution

C. rupestris is the most common small wrasse in north European waters, but they are only abundant locally despite the wide distribution. They live close to algal covered rocks in 11 to 50 metres of water, among eel-grass beds and in extreme low-water tidal pools of less than one metre in depth. Sayer, Gibson & Atkinson (1993) postulated that the limiting factor in the distribution of *C. rupestris* is the availability of suitable habitats. Wheeler (1978) reported that they are usually found in proximity to deep water and were originally thought to migrate off-shore during winter months due to the difficulty in catching them at that time of year. However, Costello (1991) and Darwall *et al.* (1991) suggest that during winter months *C. rupestris* remain inactive in refuges in rock crevices and recent work by Sayer, Cameron & Wilkinson (1994), using anaesthetics to flush fish from amongst rocks in winter months, would appear to confirm these ideas, suggesting that they enter a state of partial "hibernation" in cracks in rocks when the water temperature drops. Darwall *et al.* (1991) postulated that *C. rupestris* activity was limited to temperatures above seven degrees centigrade.

Feeding

The most important food item in the diet of *C. rupestris* is crustaceans but they will eat almost anything which is easily captured and abundant amongst the algal habitat. The teeth are adapted for tearing food items off algae (Hilldén 1978a). Unpublished work by Sayer (Pers. Com.) on the stomach contents of wild caught *C. rupestris* would appear to agree with this, as a very wide range of prey items was found in the intestines of the animals examined. These prey items varied from gastropod and lamelibranch molluscs to amphipod, copepod and decapod crustacea. They also included fragments of echinoderms, a full range of polychaetes, teleosts [*Lepadogaster candollei* Risso (Connemara clingfish)] and even some insect larvae.

Reproduction

Males are highly territorial, defending against other males, non-gravid females, young and *C. exoletus*. A single male may spawn with several females over a breeding period, spawning having been seen to take place with up to three different females a day. The females then stay near the territory of the males with whom they spawn and may become territorial themselves after spawning. Spawning occurs within a few days of the fish being seen in shallow water, around mid May, and continues for a period of about one month. Territories are generally around 1.4 m^2 and the literature would suggest that only some males return to the same territory year after year. Once released by the female some eggs sink while others float. It was originally postulated that these two apparently different types of eggs represented two different reproductive strategies. The eggs that floated would be a dispersal phase to permit the colonisation of new areas, while the eggs that sank would repopulate the immediate area (Hilldén 1981). However, Sjolanders, Larson & Engstrom (1972) reported that *C. rupestris* only produce pelagic eggs. Preliminary breeding trials, using *C. rupestris* brood stock caught from the wild, indicated that any eggs that sink were non-viable and, therefore, there is only one breeding strategy, pelagic eggs (Stone 1995). The production of pelagic eggs by *C. rupestris* is unique amongst the labrids. All other species produce demersal eggs. The presence of territoriality and pairwise spawning is inconsistent with this type of reproduction. Sjolanders *et al.* (1972) postulated that *C. rupestris* originally produced demersal eggs similar to those of other labrids before the switch was made to pelagic ones. This may explain the presence of territorial behaviour patterns seen in the species at spawning despite the use od pelagic eggs which do not require to be deffended after being fertilised.

Some males may act as accessory males, they behave and look like females to sneak matings. These males are never chased away by the dominant male. Sham matings occur between the dominant male and a female where no eggs or sperm are released.

This is thought to counter the accessory male's tactics.(Hilldén 1981).

Cleaning behaviour

In the wild there may be a low percentage of this species exhibiting cleaning behaviour. *C. rupestris* are known to establish cleaning stations which are visited by other fish which adopt a cleaning posture typical of that described in chapter 1.7.1. Only *L. bergylta* have been observed as clients in the wild (Potts 1973). *C. rupestris* do not rely totally on cleaning as their only feeding strategy (Hilldén 1983).

2.1.1.2 *Centrolabrus exoletus* L. (rockcook)

Appearance

This species, illustrated in Fig 2.2, is also a deep-bodied, small wrasse with a small mouth and thick lips. The teeth are small and in a single row on the jaw, the anal fin has four to six stout spines and the pre-operculum is serrated. Wheeler (1978) reported that adults can be up to 15 cm in length but are more commonly slightly smaller than this.

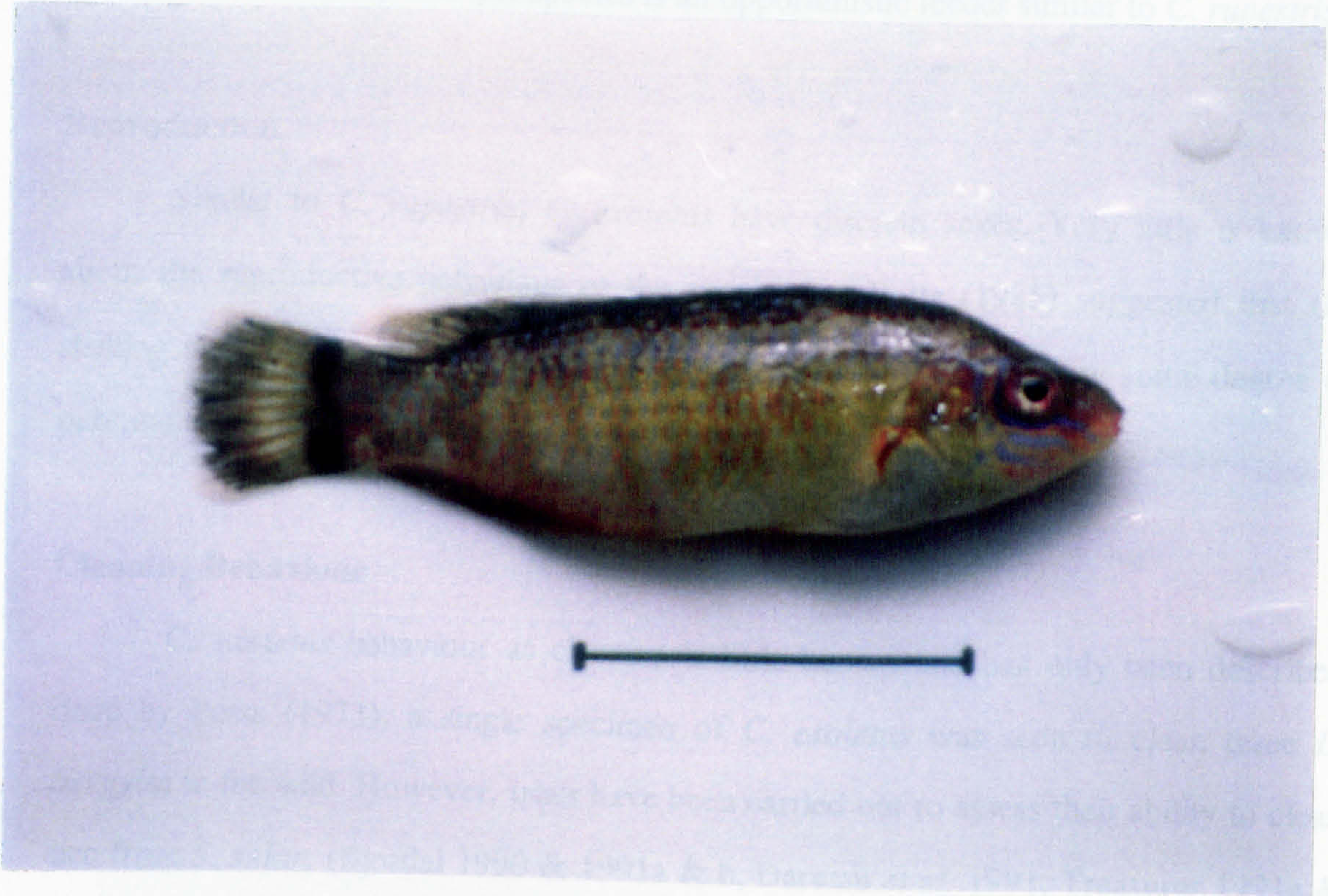
Colouration

C. exoletus generally has a greeny-brown or reddish back and is lighter on the flanks, with a yellowish tinge on the abdomen. The male has blue spots on the vertical fins and on the side of the head; the tail has a dark crescent shaped mark with a light band at the front and the edge of the caudal fin, (Wheeler 1978). Colouration is the main distinguishing feature between this species and *C. rupestris*.

Distribution

Little is known about the general behaviour of *C. exoletus*. However, Wheeler (1978) reported that they are found in localised populations in two to 25 m of water

Fig 2.2: An adult *Centrolabrus exoletus*, scale bar = 5cm.



amongst algae covered rocks and eel-grass beds. The young can be found in shallower water and may occasionally be caught near the extreme low tide level.

Feeding

They eat small crustaceans and are frequently found in prawn traps where they may be scavenging the bait, or feeding on amphipods attracted to the bait (Wheeler 1978). It is most likely that this species is an opportunistic feeder similar to *C. rupestris*.

Reproduction

Similar to *C. rupestris*, *C. exoletus* have discrete sexes. Very little is known about the reproductive behaviour of the species but Potts (1985) suggested that the striking sexual dichromism in the species indicates that it would show some degree of parental care for the demersal eggs.

Cleaning Behaviour

C. exoletus behaviour as cleaners is little known and has only been described once by Potts (1973), a single specimen of *C. exoletus* was seen to clean three *L. bergylta* in the wild. However, trials have been carried out to assess their ability to clean lice from *S. salar*, (Bjordal 1990 & 1991a & b, Darwall *et al.* 1991, Treasurer 1991a & b, & 1993a & b and West 1991). All of these trials indicated that *C. exoletus* acted as an efficient cleaner of lice in a farm environment.

2.1.1.3 *Labrus mixtus* L. (cuckoo)

There is less information available for this species than the others in the study, the only text that deals with the species in any detail is Wheeler (1978).

Fig 2.3: An adult female *Labrus mixtus*, scale bar = 5cm.

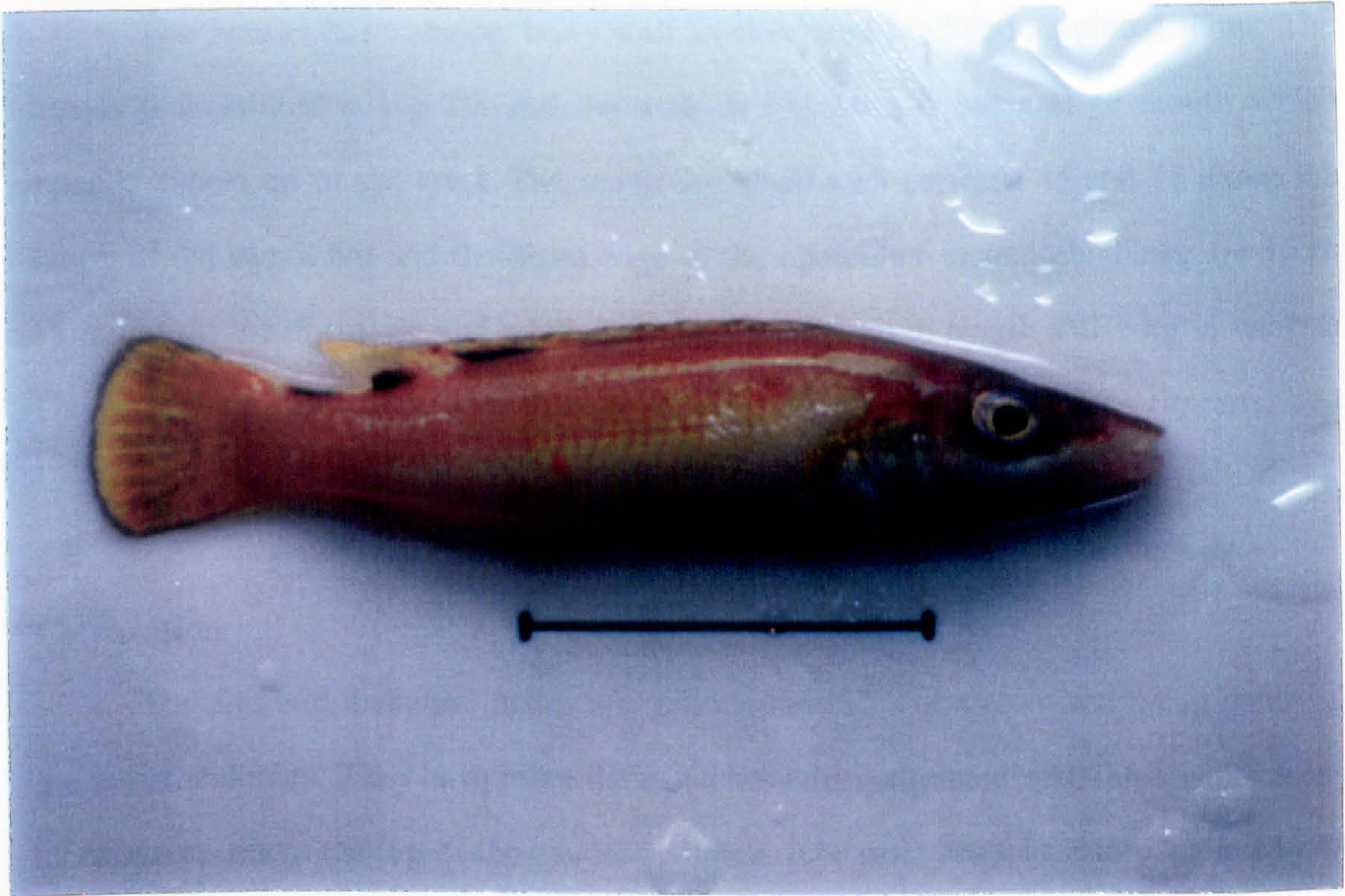
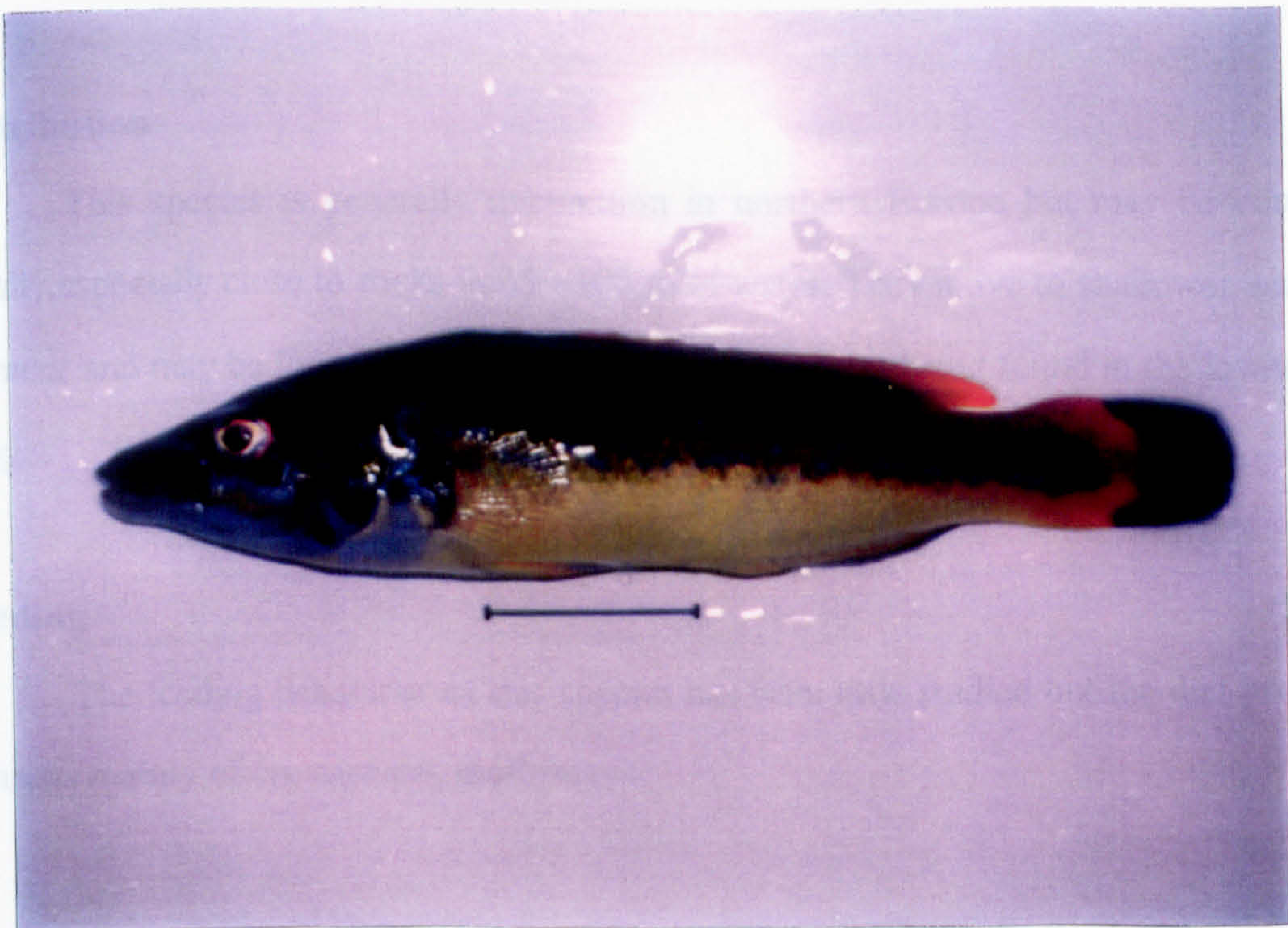


Fig 2.4: An adult male *Labrus mixtus*, scale bar = 5cm.



Appearance

This species has a slender body with an elongated head and a pointed snout. The female is illustrated in Fig 2.3 and the male in Fig 2.4, It has a large mouth which extends almost up to eye level. The scales are small with between 45 and 48 along the length of the lateral line and the distal edge of the operculum is smooth. There are 16 to 18 spines on the dorsal fin and adults can be up to 35 cm in length and 1 kg in weight. The general body shape and size of *L. mixtus* distinguishes it from both *C. rupestris* and *C. exoletus*.

Colouration

Females and immature males are yellow, reddish - orange or red being paler on the lower abdomen. They have three dark patches which alternate with three white areas along the length of the top of the caudal peduncle. The male has a brilliant blue head and blue streaks on the flanks across yellow or orange. During the spawning season the male has a white patch on the top of his head.

Distribution

This species is generally uncommon in northern Europe but may be common locally especially close to rocks in 35 - 108 m of water. They move to shallower water in summer and may be found in 10 m of water, with juveniles being found in the lower tidal fringe.

Feeding

The feeding behaviour of this species has been little studied but the diet probably consists mainly of crustaceans, molluscs etc.

Reproduction

Potts (1985) reports that *L. mixtus* is hermaphrodite. All individuals start life as females and it is only the most dominant individual that becomes male.

The male builds a nest before courting the female who then lays eggs into the nest. Wilson (1958) and Potts (1985) describe these nests as being shallow depressions in coarse substrates close to sublittoral reefs. Growth of the young is slow but they may live to seventeen years of age.

Cleaning Behaviour

Bjordal (1990) indicated that female *L. mixtus* have a limited ability to clean lice from *S. salar*. This finding was corroborated by Darwall *et al.* (1991). However, due to their relative rarity, it is unlikely that this species will ever be important in the commercial use of cleaner fish.

2.1.1.4 *Symphodus (=Crenilabrus) melops* L. (corkwing)

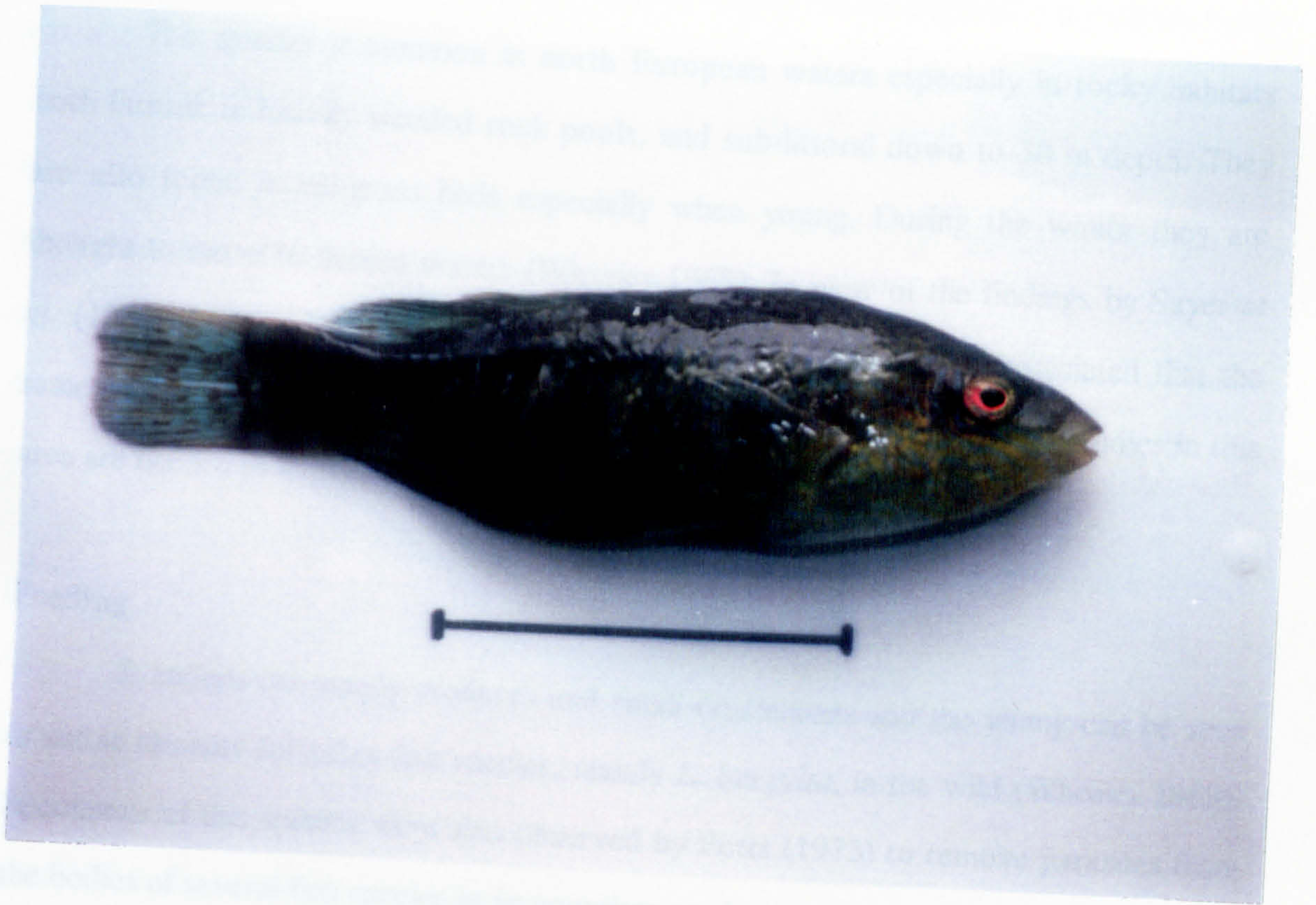
Appearance

This is a deep bodied wrasse with large scales, the species is illustrated in Fig 2.5. There are normally 32 to 36 scales along the lateral line; these scales extend to the head and cheeks where there are several rows. There are three spines on the anal fin which has eight to ten branched rays. The pre-operculum is serrated on the distal and lower edges and the pelvic fins are long, reaching almost to the anus. The teeth are in single series. Adults can be up to 25 cm long but are more usually 15 cm in length (Wheeler 1969).

Colouration

The colour of *S. melops* varies with the habitat. They are usually green to greeny - brown and sometimes reddish. There is a dusky comma shaped mark behind the eye and a pupil sized spot on the tail before the fin, on or below the lateral line. The males

Fig 2.5: An adult *Symphodus melops*, scale bar = 5cm.



have bluish lines on the lower sides of the head and lower abdomen. (Wheeler 1969).

Distribution

This species is common in north European waters especially in rocky habitats both littoral; in heavily weeded rock pools, and sub-littoral down to 30 m depth. They are also found in eel-grass beds especially when young. During the winter they are thought to move to deeper waters (Wheeler 1969). In view of the findings by Sayer *et al.* (1993), in regard to the hibernation of *C. rupestris*, it could be postulated that the same behaviour might be found in other labrids such as *S. melops*. Further studies in this area are needed to confirm this suggestion.

Feeding

S. melops eat mainly molluscs and small crustaceans and the young can be seen to act as cleaners for other fish species, mainly *L. bergylta*, in the wild (Wheeler 1969). Specimens of this species were also observed by Potts (1973) to remove parasites from the bodies of several fish species in an aquarium environment.

Reproduction

They nest among algae in the summer with the male guarding the nest. Potts (1985) describes the nests as predominantly occupying north facing rock crevices and being constructed of several types of algae. The type of algae seems dependent on algal availability. The use of algae was suggested by Potts (1973) to protect the eggs from desiccation, temperature extremes and prevailing turbulence of the sea. However, as the nests are never exposed to the air it is difficult to see how the eggs could be subject to dry conditions or temperature extremes. Potts also suggests that building the nest along a crevice enables the male to spawn with several females and also allows protection of the brood from fertilisation by intruding males. Wheeler (1969) reported that the fry are planktonic and by autumn they drift inshore.

2.1.2: Previous Descriptions of Morphological Features of Wrasse

The fine structure of wrasse has been very poorly described with the literature limited to one paper by Verigina & Kobegenov (1988) describing a histological study of the digestive tract of four species of the family *Symphodus (Crenilabrus)*, which are closely related to the species in the present study, and also a labrid of the family *Cheilo*, which is not as closely related. Four out of five of the species studied fed on a variety of items, mainly molluscs, the fifth species being predominantly herbivorous. The authors examined the intestines of the five different species and drew comparisons based on the diet and structure of the intestines of the different species.

Two papers have been published on the ultrastructure of the reproductive organs of labrids. Bentivegna & Benedetto (1989) studied seasonal variations in the gonads of *Symphodus (Crenilabrus) ocellatus* (Forsskål), a Mediterranean species closely related to *S. melops*. Unlike *S. melops*, *S. ocellatus* is a protogynous hermaphrodite. The second paper on the structure of the gonads of labrids focused on the testes of the protogynous labrid *Thalassoma dupperrey* (saddleback wrasse) (Hourigan, Nakamura, Nagahama, Yamauchi & Grau 1991).

A further paper dealing with the ultrastructure of labrids was published by Lénke (1991). In this paper the author investigated the structure of the opercular gland in several species of tropical labrids that secrete a protective mucosal cocoon in which they rest at night. None of the species in the present study display this behaviour and are not closely related to the species involved.

Geerlink (1989) compared the fin morphology of two labrids, both of the family *Coris*, to that of a cichlid. The two labrids studied were not closely related to those in the present study, but use the same method of locomotion.

If disease diagnosticians serving *S. salar* farms are to recognise abnormal features in wrasse it is essential that the full range of normal features is known first. It appears that the current level of information available in the literature is not sufficient for this purpose. With this view in mind members of the species of wrasse, important in the farming of *S. salar*, were examined histologically and the normal features of the tissues and organs described. All four species of wrasse used in aquaculture have very similar morphologies, indeed it is extremely difficult to differentiate between the species based on histological sections only. For this reason all four species will be dealt with simultaneously and any variations noted.

2.2: Materials and Methods

2.2.1: Collection of Tissue Samples

Samples of normal tissues were removed from fish used for the parasite survey described in Chapter 3A and also from experimental and control fish from the work described in Chapters 4 and 5. The main species studied was *C. rupestris* with a total of 60 wild caught fish, 37 farm held fish and 352 captive bred fish examined. The second species studied was *C. exoletus* with a total of eight wild caught fish and 25 farm held fish examined. Two specimens of *L. mixtus* and *S. melops* were examined. One wild and one from a farm pen, in the case of *S. melops* and two wild caught specimens in the case of *L. mixtus*.

Fish were killed by severing the spinal column immediately behind the head and samples for histology were removed immediately before fixation.

Tissue samples were removed using a scalpel or a pair of sharp scissors. In the case of the kidney, removal of a sample of kidney only was extremely difficult due to its small size and its very close association with the spinal cord. Due to this difficulty it was

decided that the best method for sampling kidney for histological purposes was to remove a steak from the dissected fish which would contain not only kidney but also muscle, spinal cord and skin. Using this method the intact structure of the kidney could be observed.

Tissues were fixed immediately in 10% buffered formol saline for a minimum of 24 hours before histological processing.

2.2.2: Histological Processing

The procedure used for the processing of histological sections followed the standard methods for the procedure and are included in the Appendix.

Tissues were mounted in wax and sections cut at 5-8μm thickness. All sections were routinely stained in haematoxylin and eosin.

2.3: Results

A stylised drawing of the arrangement of the morphological features of a wrasse is shown in Fig 2.6. The fish shown is a female *C. rupestris*.

2.3.1: Skin

The scales on the flanks of wrasse are large and strong giving the skin a very tough property. Due to this tough nature and large scale size it is very difficult to obtain good histological sections of the skin of wrasse. In the majority of skin sections obtained there is separation of the laminar regions which is artefactual. Sections do show numerous mucus cells and melanophores can be seen both below the epithelium and also below the dermis. In most sections the subcutaneous layer is quite thin. These features are illustrated in Fig 2.7 which shows the skin of a *C. exoletus*. Within the skin on the head and cheeks of wrasse numerous small pores can be seen which are believed to have

Fig 2.6: Stylised drawing of a female *C. rupestris* showing the morphological features of a typical wrasse, the left visceral wall has been removed along with the pectoral fin.(E - eye, M - mouth, N - nare, OP - operculum, POP - pre-operculum, DF - dorsal fin, AF - anal fin, PF - pelvic fin, CF - caudal fin, LL - lateral line, L - liver, GB - gall bladder, BD - bile duct, O - ovary, AI - anterior intestine, MI - mid intestine, PI - posterior intestine, S - spleen, B - bladder, U - ureter, SB - swim bladder).

a sensory role. These sensory pits would appear to be extensions of the prominent lateral line. The prominent nares are located dorso-posteriorly in relation to the mouth.

2.3.2: Eyes

The eyes of wrasse are relatively large in comparison to the size of the head and are located slightly anteriorly. The two eyes can be moved independently. Like the rest of the skin the cornea is resistant to abrasion which offers good protection to the eyes. Histologically the eyes of labrids do not differ significantly from those of other fish species.

2.3.3: Opercula

The opercula are heavily armoured and the presence or absence of serrations, and the distribution of those serrations, on the pre-operculum is an important identification feature between species (see the species descriptions above).

2.3.4: Motile Organs

2.3.4.1: Fins

The main locomotory fins in wrasse are the pectoral fins and the posterior section of the dorsal fin, followed by the caudal fin. These fins are flexible with relatively soft fin rays. The other fins of wrasse are supported by rigid fin rays. This is most noticeable in the anterior portion of the dorsal fin where the fin rays are formed into defensive spines which protrude above the upper margin of the fin membrane. The number of fin rays in the dorsal fin is also an important identification feature between species. The pelvic fins are held close to the underside of the abdomen and are supported at their distal margins by a single spiny ray. The anal fin is also equipped with spiny rays. These rays should be noted when handling labrids due to the danger of puncture wounds to the handler. The pectoral fin of a *C. rupestris* is illustrated in Fig 2.8. The epithelium is thicker than that

Fig 2.7: This figure illustrates the skin of a *C. exoletus*. The separation of laminar regions is an artefact of processing. (EP - epithelium, D - dermis, S - scale, ST - subcutaneous tissue, MC - mucus cell, arrow - melanophore), x25 objective.

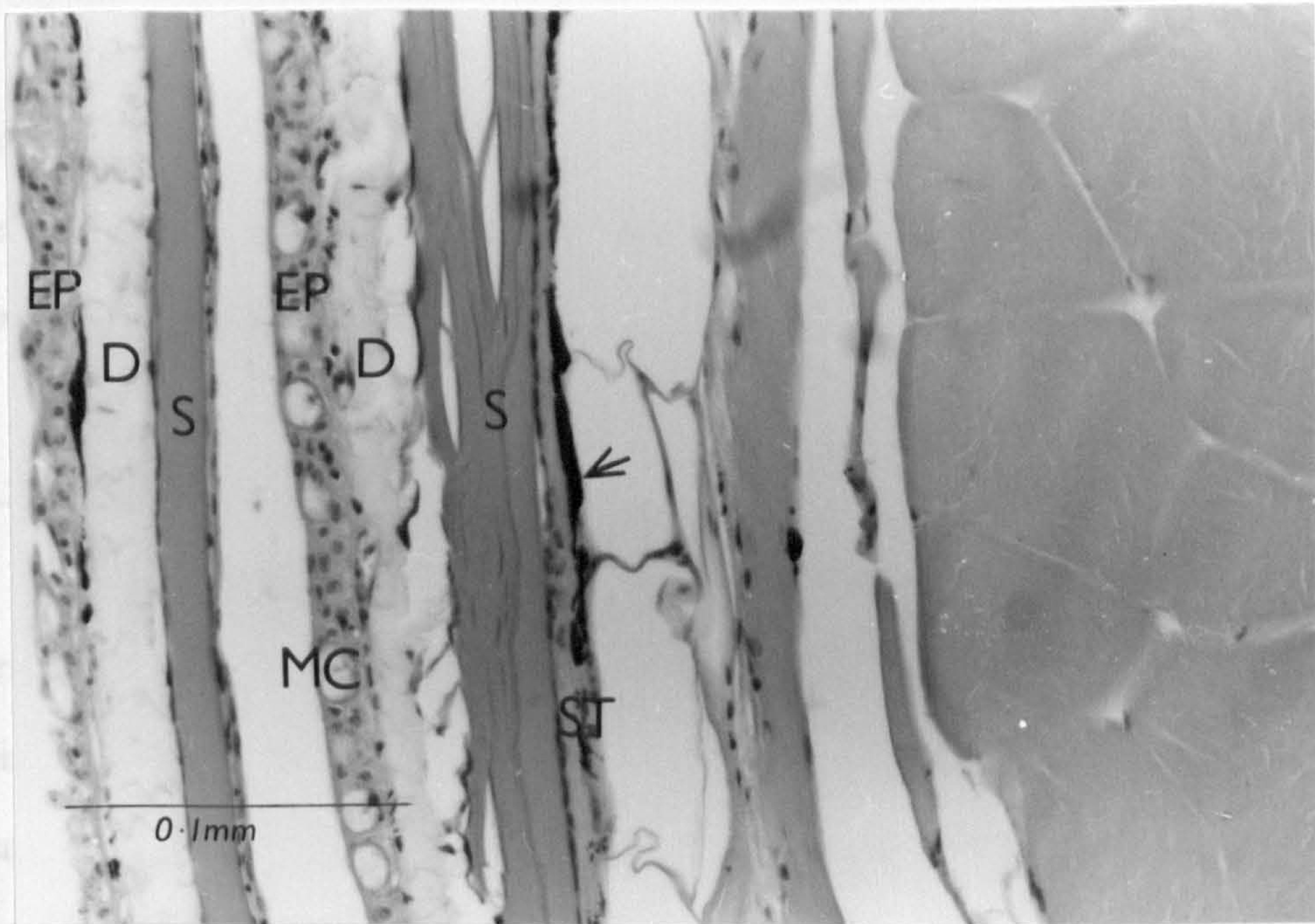
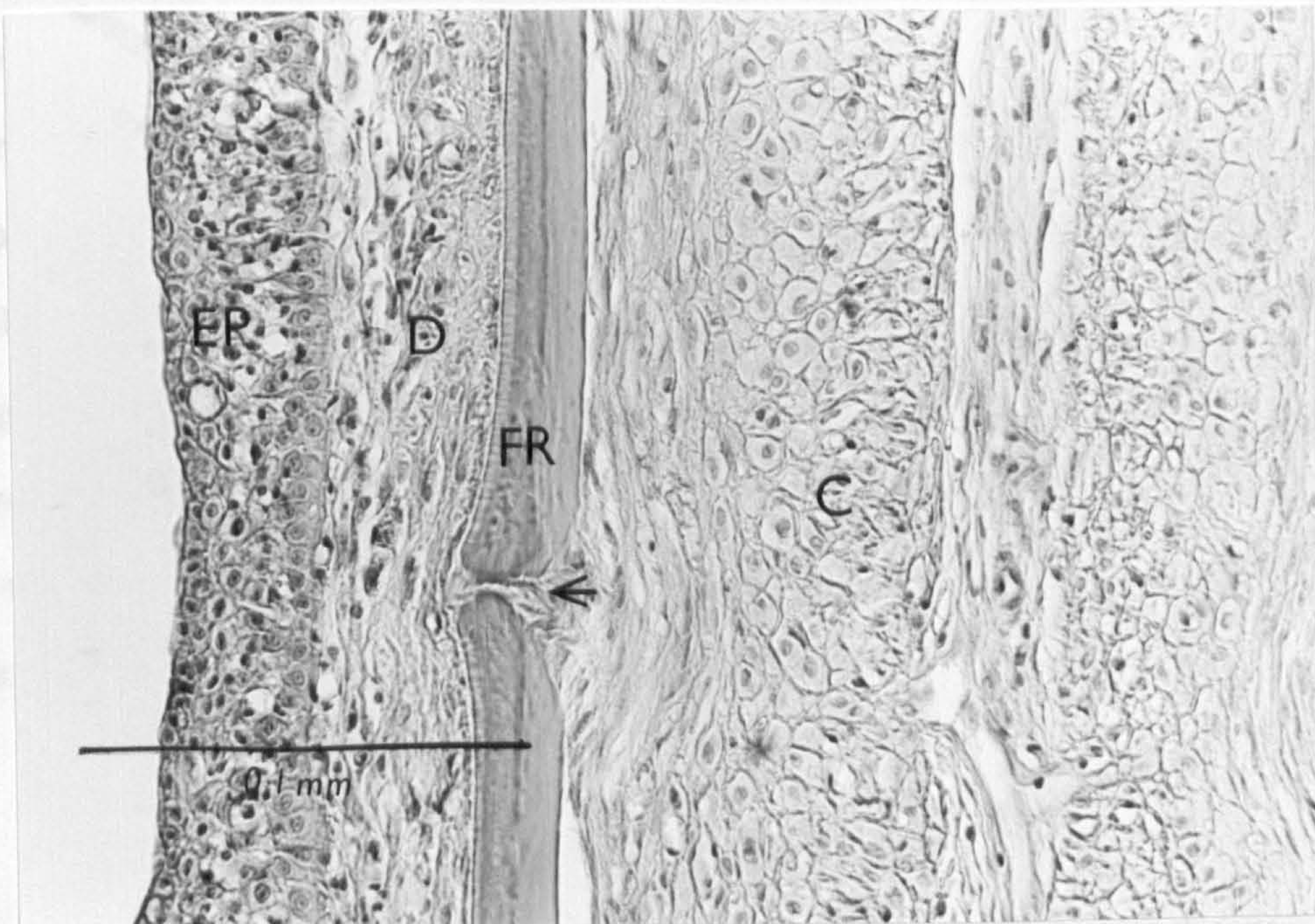


Fig 2.8: Section through the pectoral fin of a *C. rupestris*, x40 objective (EP - epithelium, D - dermis, FR - fin ray, arrow - fin ray junction, ST - subcutaneous tissue, C - cartilage).



seen in the skin section illustrated in Fig 2.7, as is the dermis. The flexible fin ray can clearly be seen, as can the cartilaginous support in the centre of the fin.

In general the fin membranes are nearly transparent except for the dorsal and caudal fins which are opaque. A light pink coloration can often be seen around the base of the pectoral fins and the posterior region of the dorsal fin. This coloration is due to the concentration of red muscle fibres in these regions.

2.3.4.2: Musculature

The flesh of all four species is firm and pale in colour. As mentioned above the majority of red muscle is located around the base of the locomotory fins. The remainder is white muscle. The myotome structure is quite compact with very little fat deposition in the junctions between muscle fibres and myotomes, this is illustrated in Fig 2.9, taken from a *S. melops*.

2.3.5: Gills

The gills are arranged in the classical design; with four gill bars, the posterior gill bar being firmly attached to the posterior wall of the opercular chamber. A pseudobranch is present, being seen as a dark red organ embedded in the tissue of the inner surface of the opperculum. The gill bars are strong and give considerable support to the primary lamellae, as illustrated in Fig 2.10 which shows the cartilage in the centre of the gill bar along with a block of muscle located at the proximal end of the gill bar. The base of the gill rakers can be seen at the bottom of the section. It can also be seen from this figure that the epithelium of the gill rakers is similar to that of the pectoral fins in terms of thickness.

In histological section numerous chloride cells and mucus cells can be seen at the base of the secondary lamellae, illustrated in Fig 2.11. There are no significant

Fig 2.9: The muscle arrangement in a *S. melops*, x10 objective (MF - muscle fibre, arrow - muscle fibre junction, MJ - myotome junction).

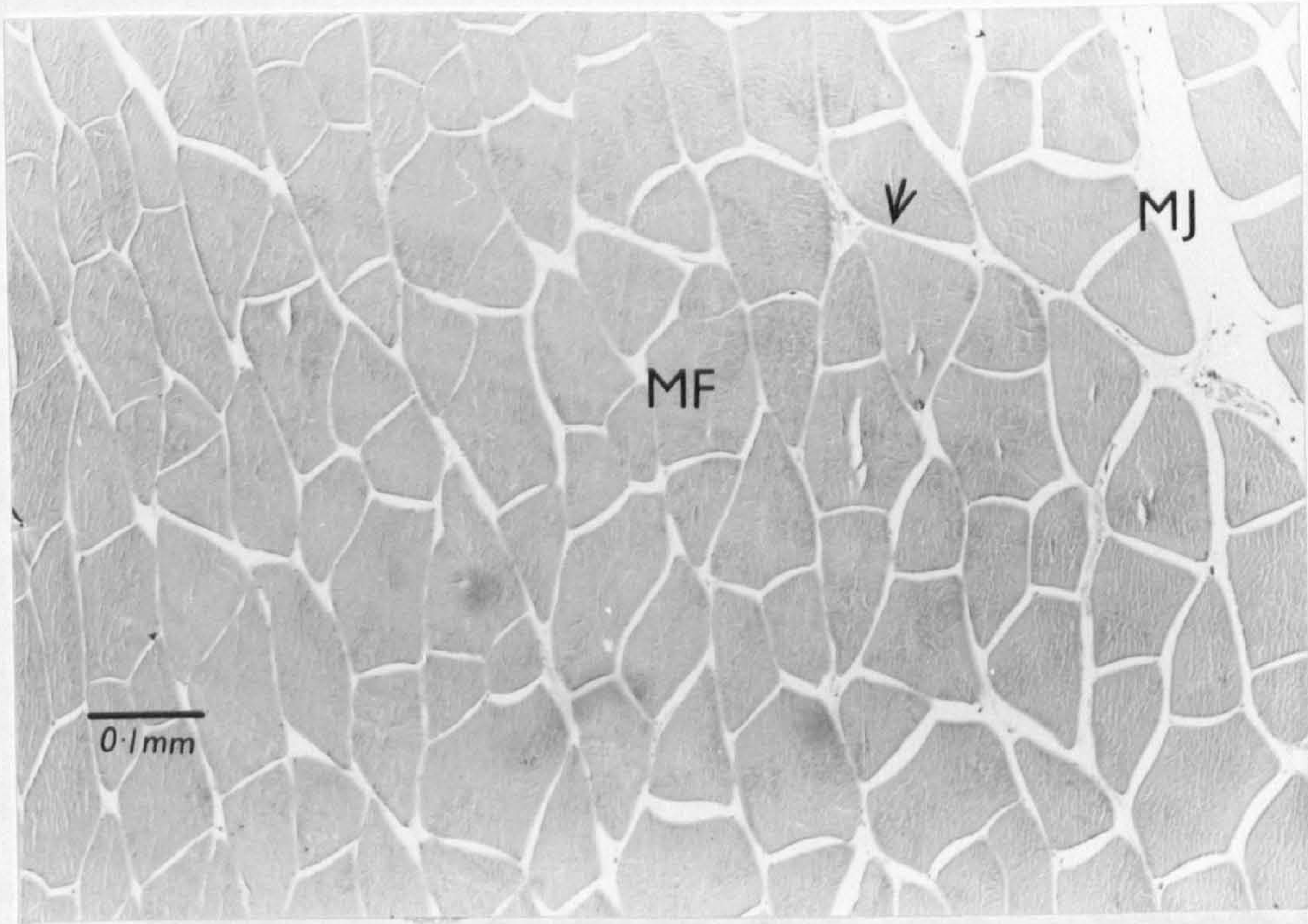


Fig 2.10: The gill bar of a *C. rupestris*, the bases of the primary lamellae can be seen at the top of the frame and the bases of the gill rakers at the bottom. The section was taken close to the proximal end of the gill bar and the muscle block which joins to the cartilage of the gill bar can be seen (PL - primary lamella, SL - secondary lamella, GR - gill raker, M - muscle, EP - epidermis, C - cartilage), x10 objective.

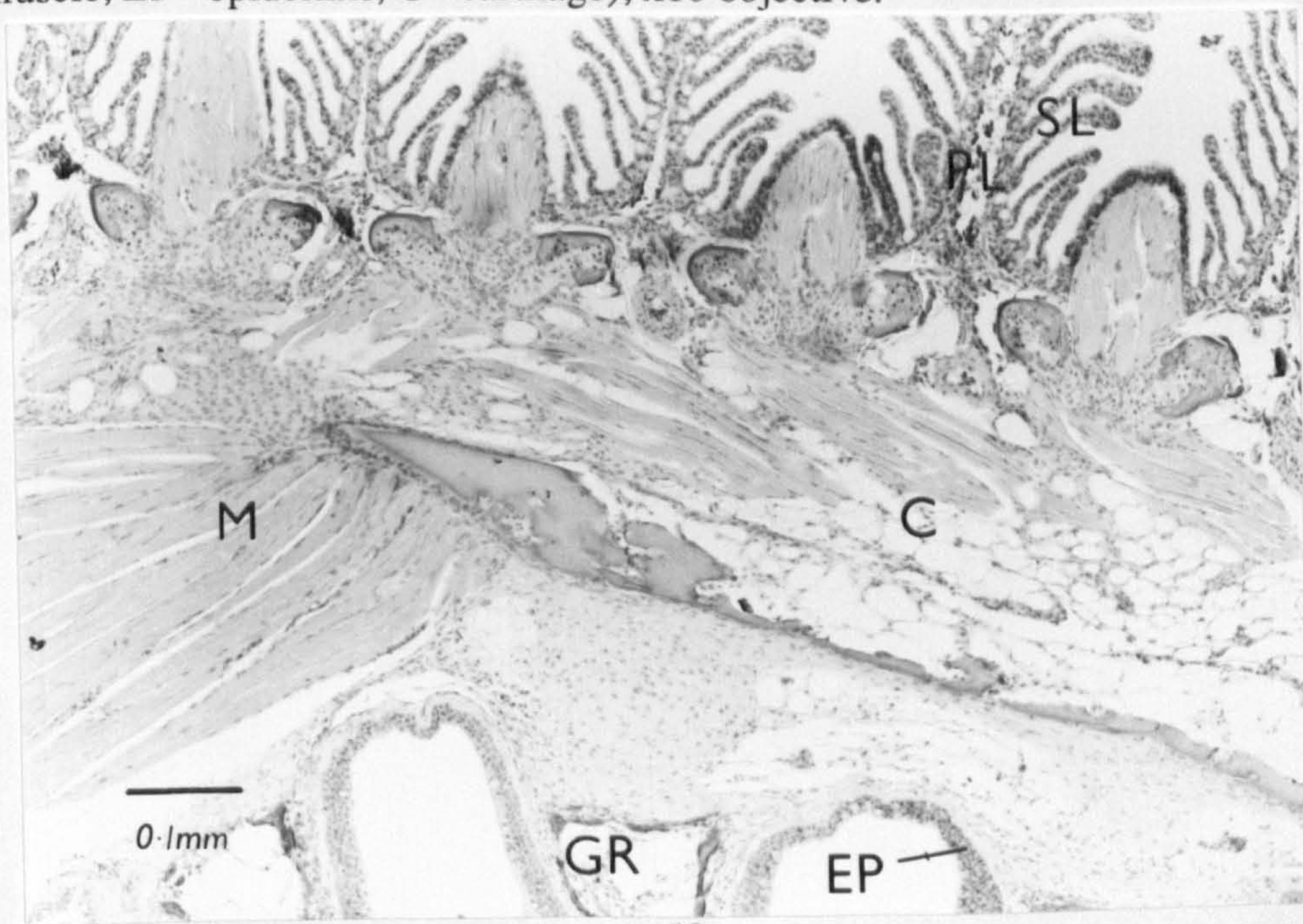
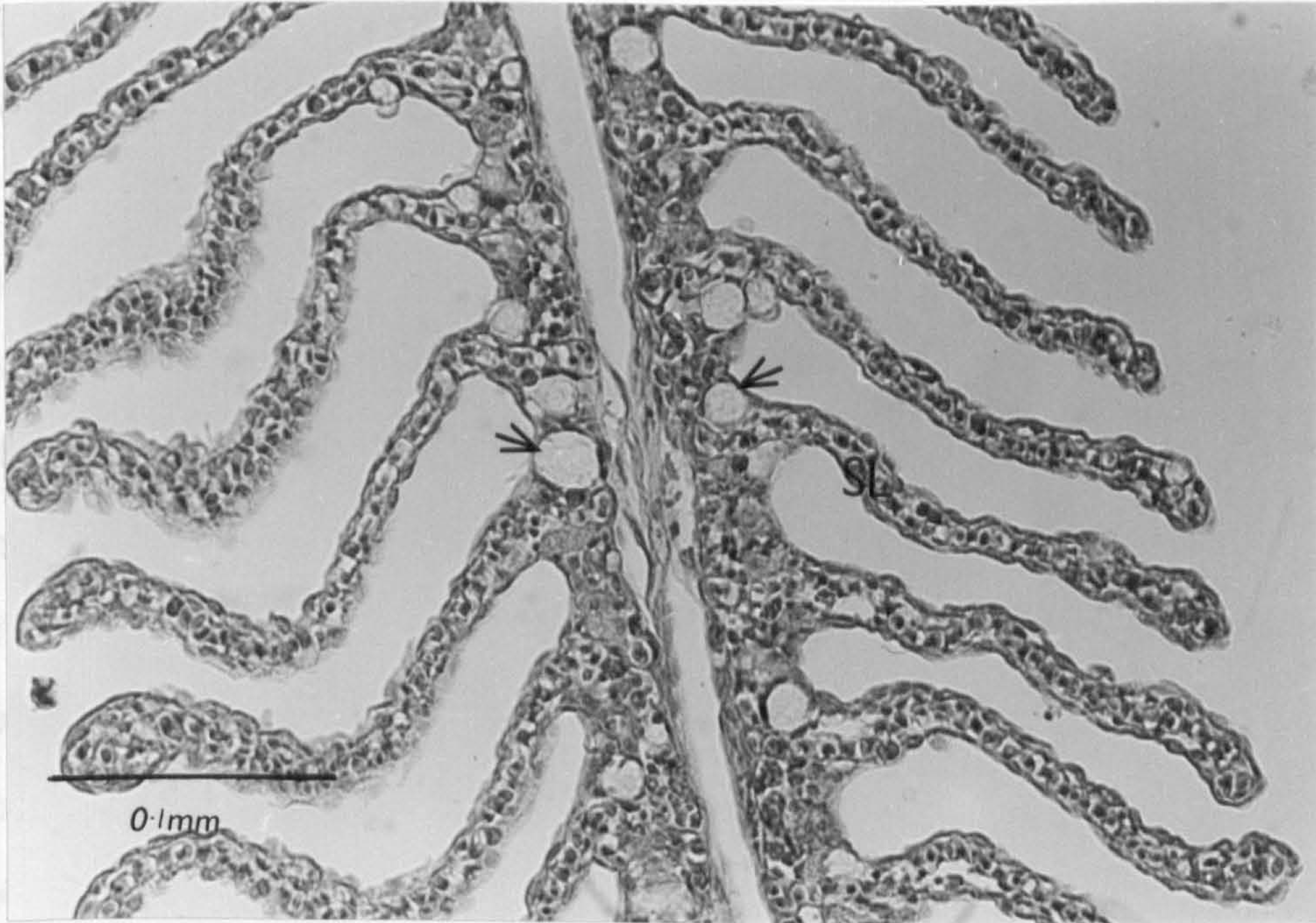


Fig 2.11: A section of the primary lamella of a *C. exoletus* showing the chloride cells located at the base of some of the secondary lamellae, x25 objective (SL - secondary lamella, arrows - chloride cells).



differences between the gills of labrids when compared to other wholly marine teleosts. As with other fish species, the gills of a healthy fish are dark pink to red in colour.

2.3.6: Vascular System

2.3.6.1: Heart

The atrium is a very delicate sac-like organ which will, in most cases, be damaged during dissection making its identification difficult. The ventricle in all four wrasse species is a thin walled organ with a spongy appearance when viewed histologically, due to the highly convoluted nature of the ventricle walls. There is no obvious differentiation throughout the ventricle. The convoluted nature of the ventricle muscle fibres is illustrated in Fig 2.12 which shows the heart of a *C. rupestris*. The serous membrane surrounding the ventricle is very thin often only being a single cell in thickness. This feature is illustrated in Fig 2.13, this section being from a *L. mixtus*. The endocardium is not visible in most sections. The bulbous arteriosus is a more muscular organ but this development is less than would be seen in salmonid hearts. The normal heart is light pink in colour.

2.3.6.2: Spleen

The spleen is dark red in colour and is commonly attached loosely to the posterior intestine although its exact location on the intestine can vary. The serous membrane is very thin, perhaps only one cell thick. Little differentiation can be seen histologically within the spleen. Melanomacrophage centres are often evident as well as accumulations of haemocidirin granules. These features are not arranged around any common pattern. These features are illustrated in Fig 2.14 from the spleen of a *C. rupestris*.

Fig 2.12: The convoluted nature of the ventricle of the heart from a *C. rupestris*. Red blood cells can be seen in the centre of the ventricle. While the serous membrane around the heart is visible the endocardium is not, x10 objective (RBC - red blood cells, MF - muscle fibres, SM - serous membrane).

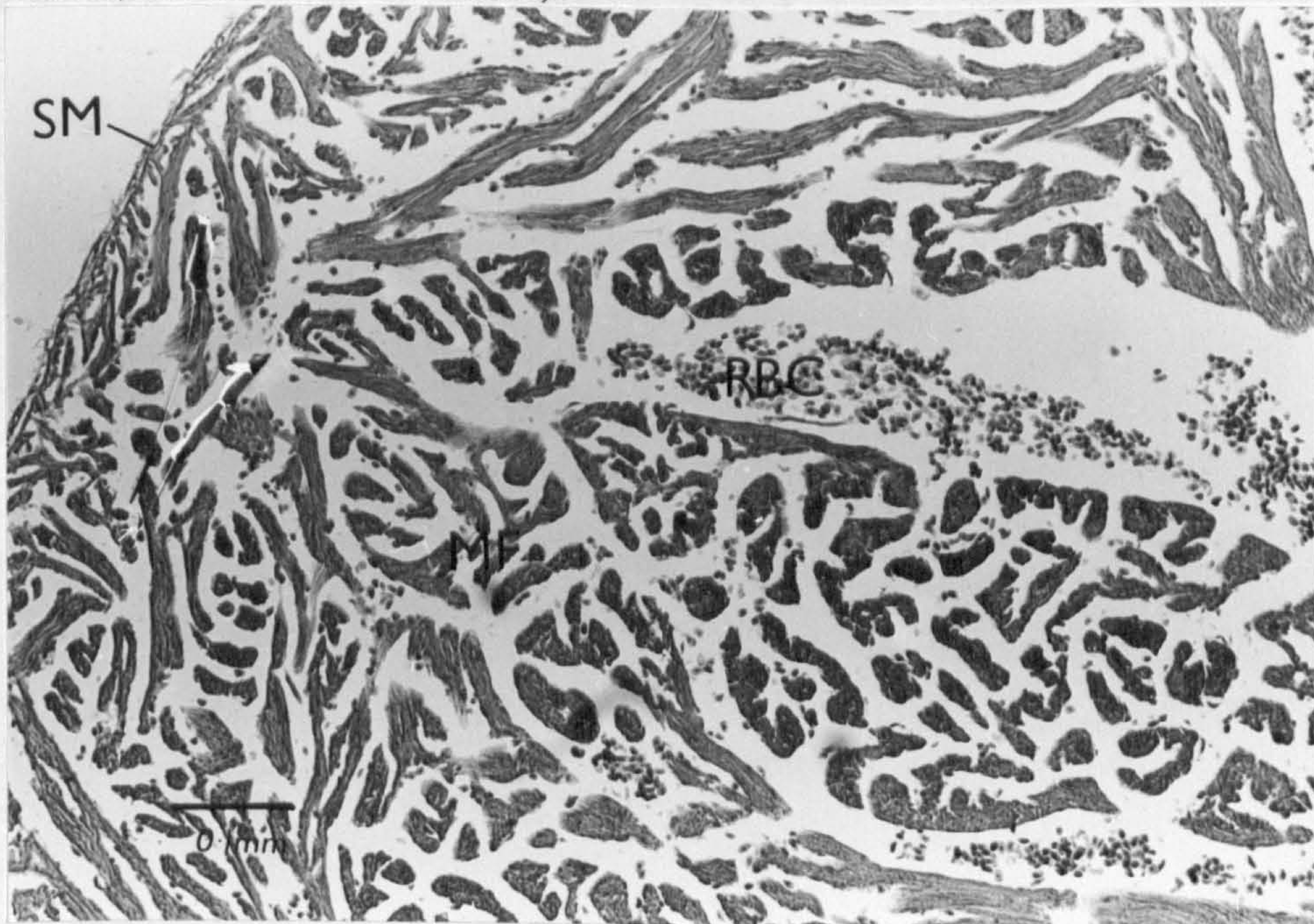


Fig 2.13: Higher powered, x40 objective, view of the serous membrane of the ventricle of a *L. mixtus*. The single cell thickness of the membrane can be clearly seen (MF - muscle fibres, SM - serous membrane).

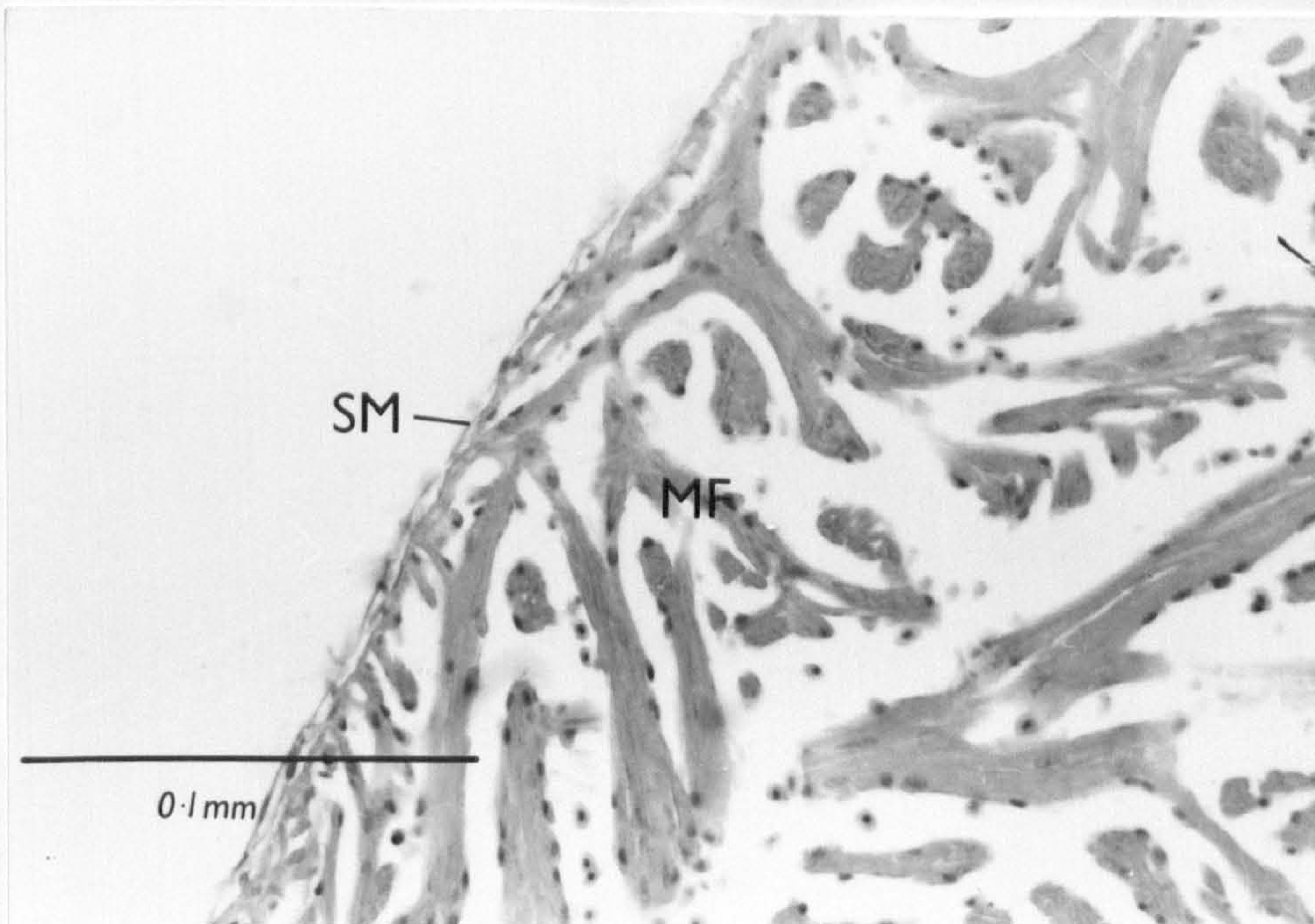
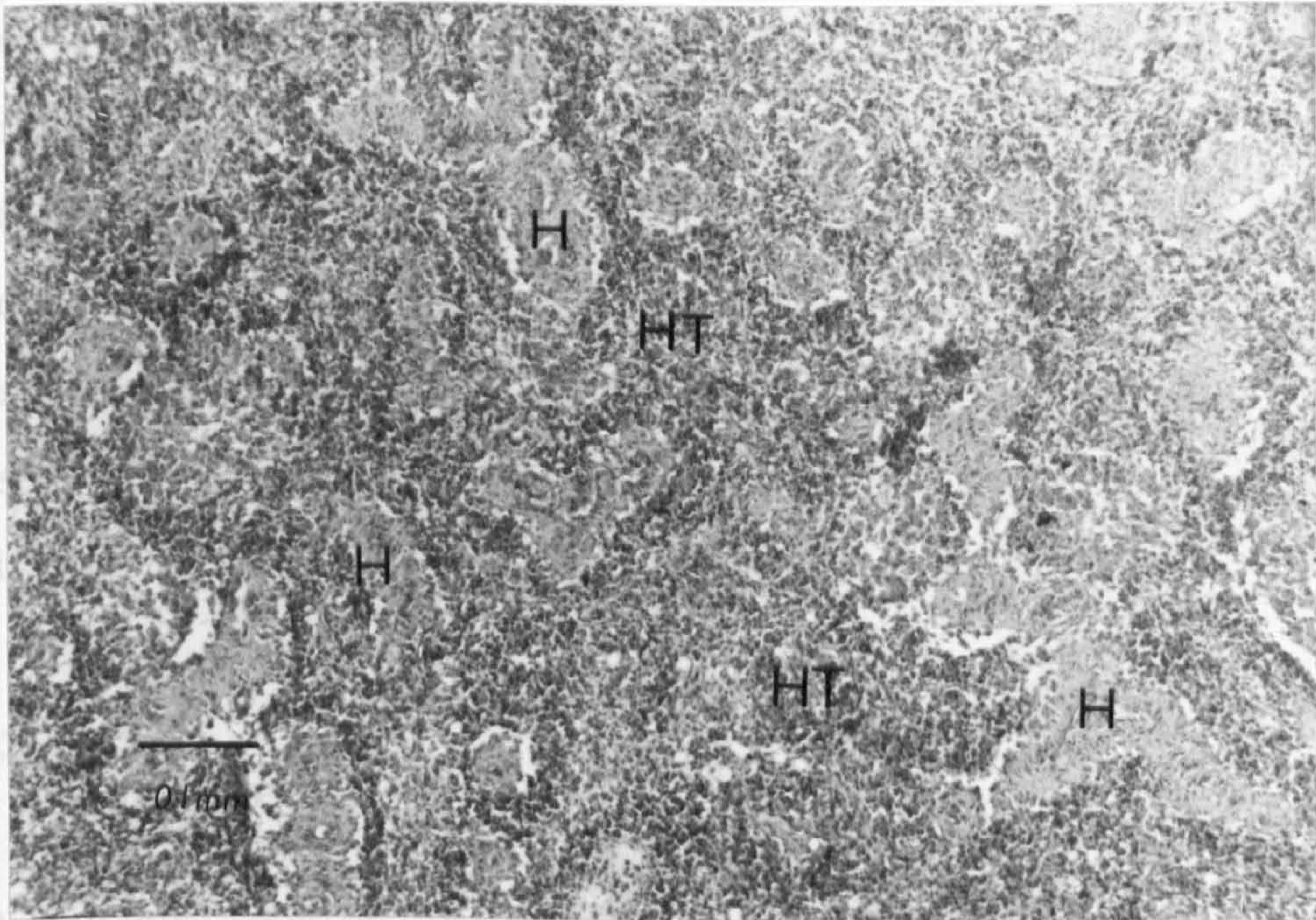


Fig 2.14: A view of the spleen of a *C. rupestris* taken with a x10 objective. Accumulations of hemocidirin can be seen as the slightly lighter areas (HT - hematopoietic tissue, H - hemosiderin).



2.3.7: Digestive System

2.3.7.1: Mouth

The jaws of wrasse are protrusible allowing the fish to remove even firmly attached organisms from the substrate. The jaws are strong with either a single or double row of sharp, curved teeth. Teeth can also be found on the top of the pharynx. A hardened plate on the bottom of the pharynx forms a grinding plate which is used in conjunction with the pharyngeal teeth to crush molluscs and crustaceans which compose the majority of the diet. Bogachik (1969) described how labrids eject the crushed shells of their prey from the mouth. This results in very little inert material entering the intestine which would otherwise reduce the digestive efficiency of the intestine.

2.3.7.2: Alimentary Tract

A short, muscular oesophagus merges with the anterior intestine. For the purposes of description, the anterior intestine is considered to begin at the point where the bile duct enters the intestine. There is no true stomach only a slight widening of the intestine prior to it narrowing again as it doubles back at the posterior margin of the liver. This fold may be regarded as the margin between the anterior and mid intestines. A further fold marks the junction of the mid and posterior intestines which is also characterised by another gradual narrowing of the intestine. The posterior intestine then runs directly to the anus where it merges with the urinary duct to form the vent.

Morphologically there are few features which distinguish the different regions of the intestine in the four species of wrasse. There are normally a greater number of goblet cells in the mid section of the intestine compared to the anterior and posterior sections. This difference is not always easily distinguished. Figs 2.15 to 2.17 show sections from the intestine of a *C. rupestris*. Fig 2.15 is from the anterior intestine, Fig 2.16 from the mid section and 2.17 from the posterior section. The other difference that can be noted in the different regions is the degree of convolution in the intestinal villi. While this

Fig 2.15: Anterior intestine from a *C. rupestris*, x10 objective. Mucus cells are clearly visible in the intestinal epithelium (IW - intestinal wall, IE - intesinal epithelium, arrows - mucus cells).

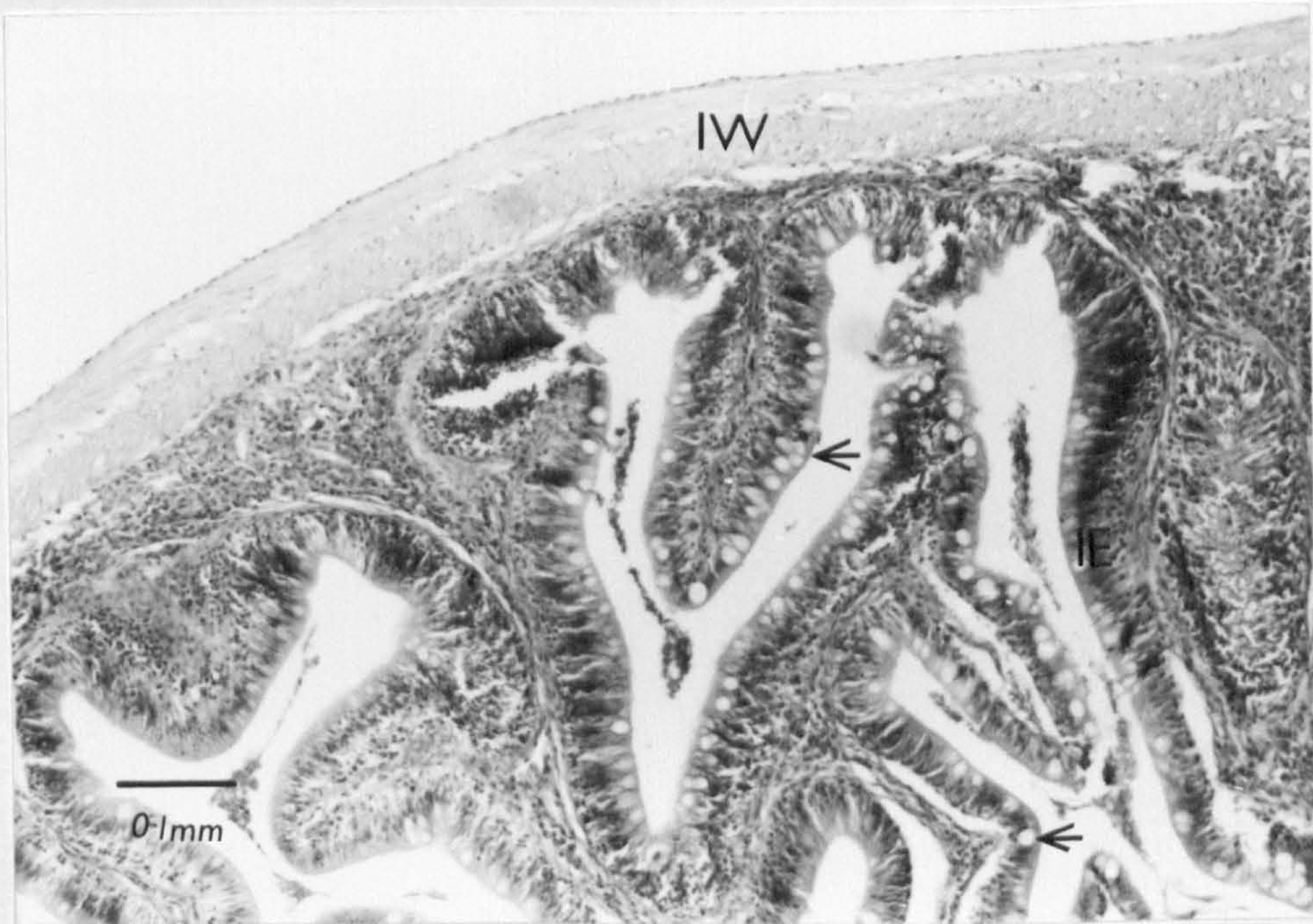


Fig 2.16: Mid intestine from the same *C. rupestris*, x6.3 objective. Again the mucus cells are clearly visible, there is a greater number of mucus cells in the mid intestine than in the anterior or posterior portions of the intestine (IW - intestinal wall, IE - intestinal epithelium, arrows - mucus cells).

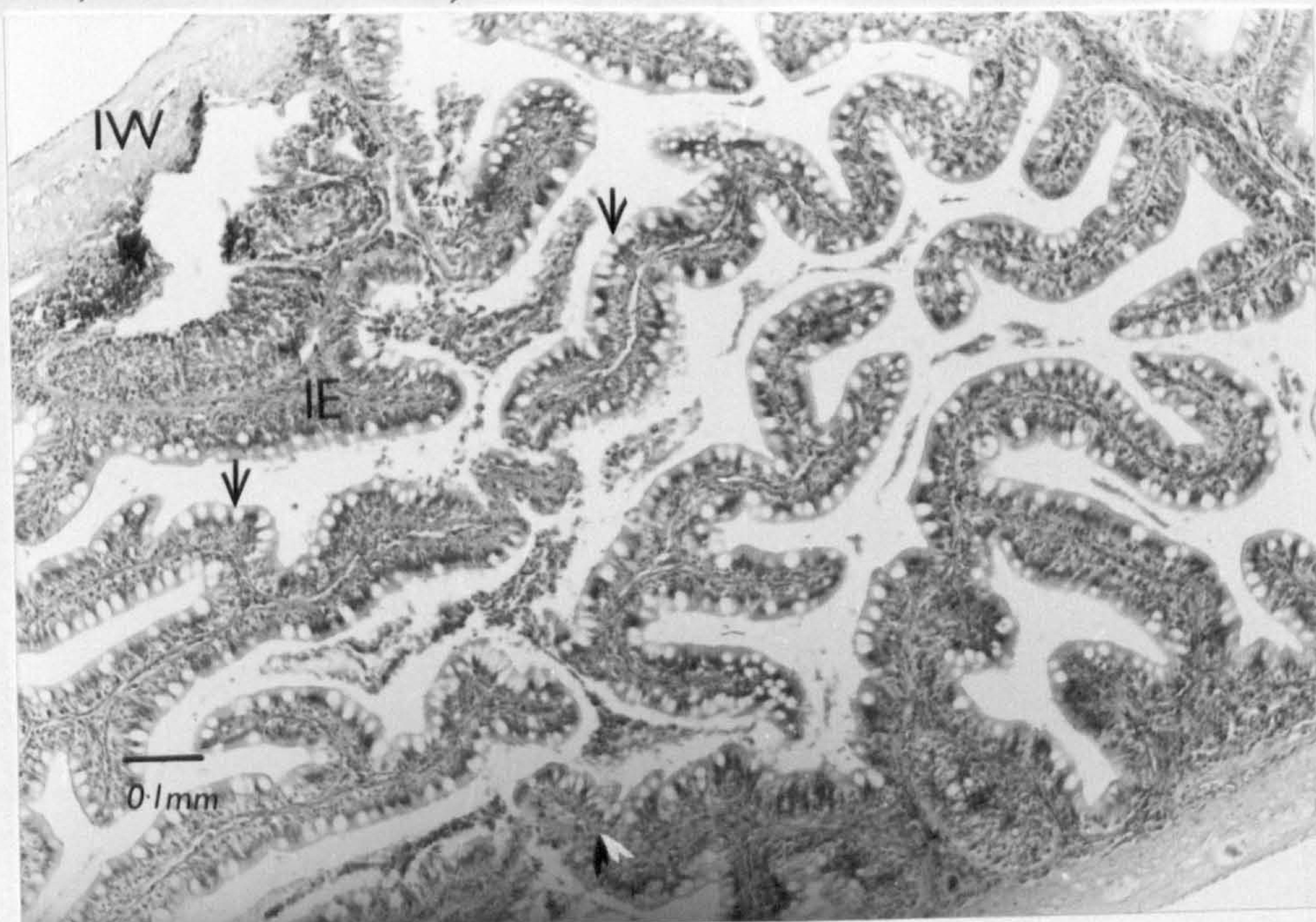
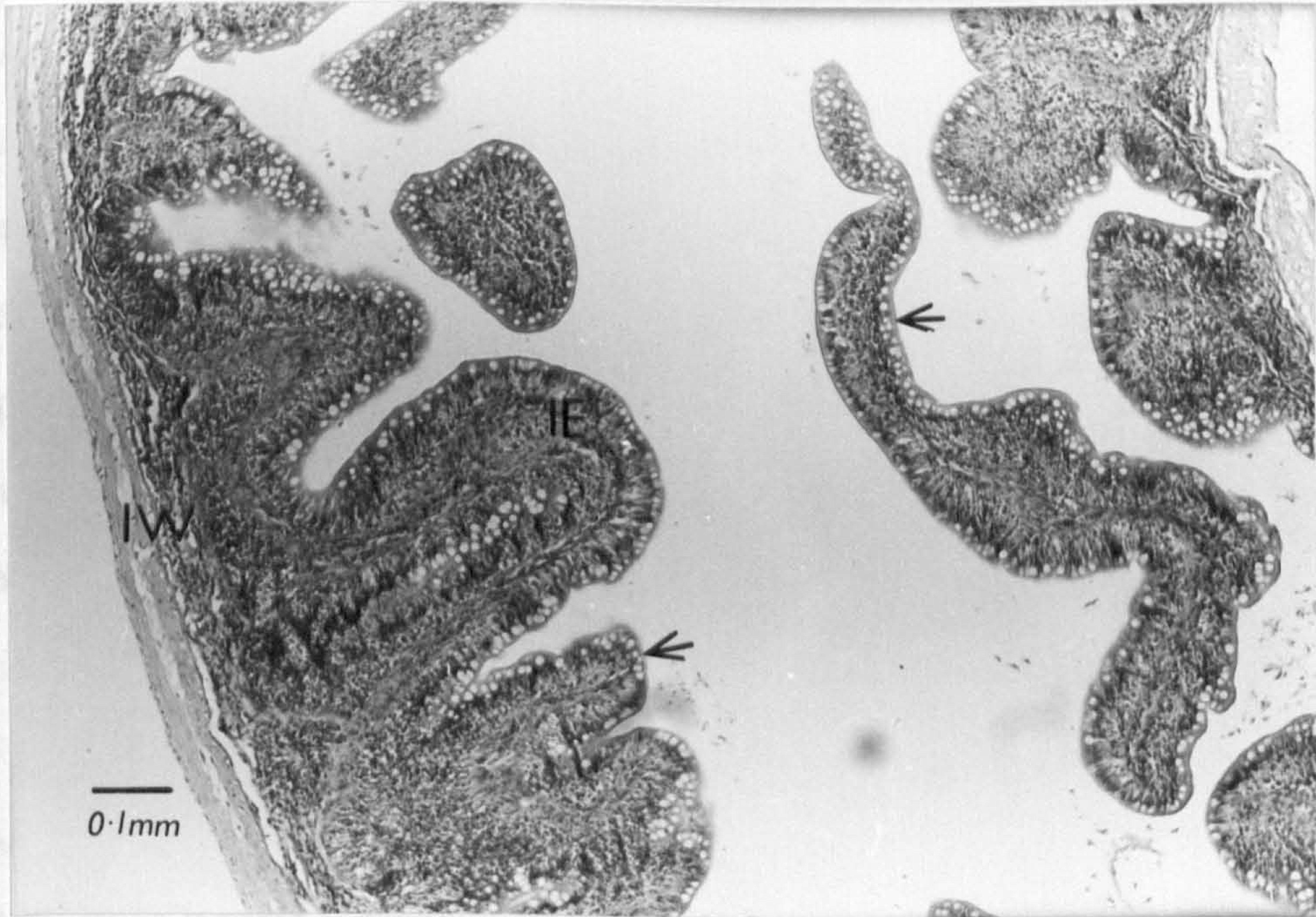


Fig 2.17: Posterior intestine of the same *C. rupestris*, x6.3 objective. Fewer mucus cells are seen in this area of the intestine than in the mid intestine, in addition to this the intestinal villi are less convoluted (IW - intestinal wall, IE - intestinal epithelium, arrows - mucus cells).



factor is common in the anterior and mid intestines the villi are less convoluted in the posterior intestine.

The intestinal wall is uniform in thickness throughout the length of the intestine. The serous membrane of the intestine is more substantial than that of the other organs already described, but is still only a few cells thick. The tella subserosa is thin and indistinct. The wall of the intestine is muscular with both transverse and longitudinal muscle layers being obvious within the muscularis. There would appear to be no stratum compactum in wrasse with the submucosa lying directly above the muscularis. There are often eosinophylic granular cells within the submucosa and the presence of low numbers of these cells may be regarded as normal. The cells of the mucosal layer of the four species studied in the present work are compact with a microvillus border which can only be seen under high magnification. These features are illustrated in Fig 2.18 which shows a section from the mid intestine of a *C. exoletus*. A rectal valve is present in the caudal part of the intestine which is composed of highly convoluted epithelial folds with muscular cords at their bases.

2.3.7.3: Liver

The liver of wrasse is a bi-lobed structure which varies from a light fawn to a mid brown in colour depending on the diet of the wrasse. The most obvious feature of the liver in histological sections is the presence of strands of pancreatic tissue which pass through the liver in discrete ducts, although depending on the area sectioned these strands are not always apparent.

The appearance of the hepatocytes varied considerably depending on diet. In fish recently caught from the wild the liver was compact with well defined cells and very few lipid droplets, Fig 2.19. In fish which had been held in *S. salar* pens the livers had a more "vacuolated" appearance due to the presence of high numbers of lipid droplets in the

Fig 2.18: The structure of the intestinal wall from a *C. exoletus*. The various layers of the imtestinal wall can be seen, x25 objective (SMe serous membrane, M - muscularis, SM - sub mucosa, LP - lamina propria, EP - epithelium, arrows - EGC's).

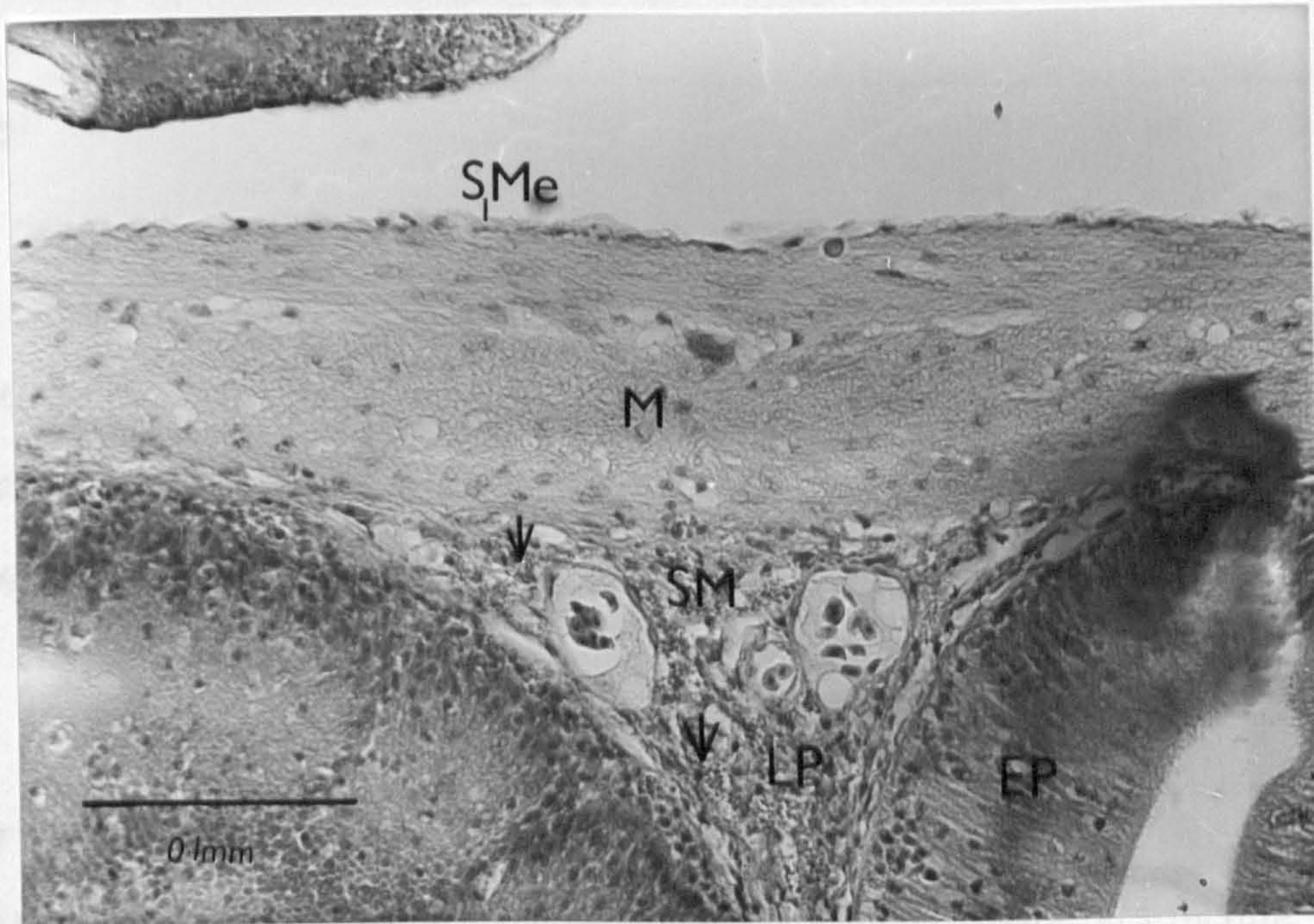
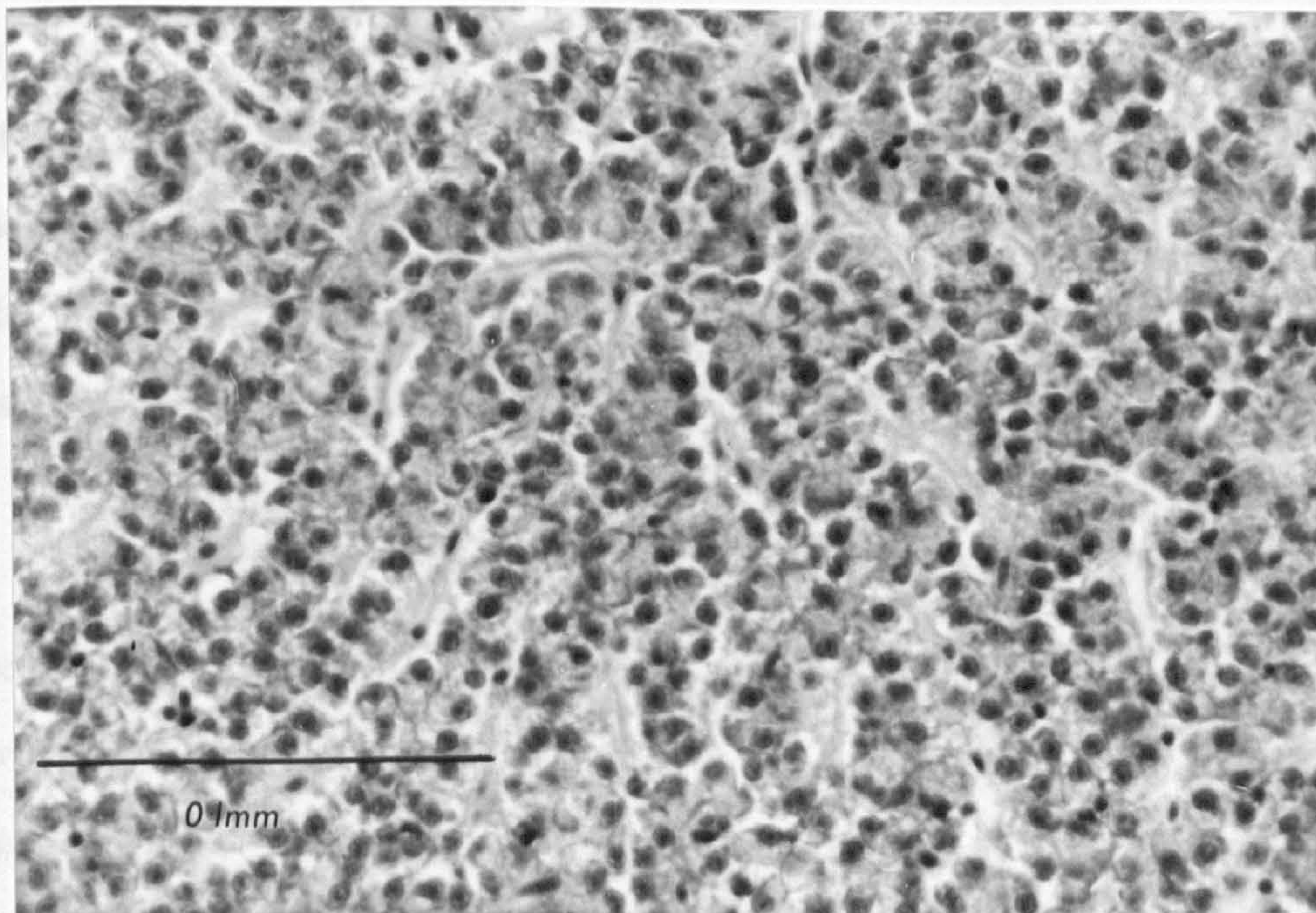


Fig 2.19: The compact hepatocyte structure of the liver from a wild caught *C. exoletus*, x40 objective.



hepatocytes. Fig 2.20 shows a liver from a *C. rupestris* with some lipid accumulation in the hepatocytes while Fig 2.21 shows the liver from a *C. rupestris* with chronic lipid accumulation.

2.3.7.4: Pancreas

The pancreas of wrasse is very diffuse with acinar cells being found throughout the liver (hepatic pancreas) illustrated in Fig 2.22, adipose tissue (fat pancreas) illustrated in Fig 2.23 and associated with the outside of the intestine (intestinal pancreas) illustrated in Fig 2.24. Figures 2.22.-2.24 were all taken from sections of a *C.rupestris*.

The hepatic pancreas is closely associated with blood vessels passing through the liver as most strands of pancreas would appear to have a blood vessel in their centre. The pancreatic tissue found in the adipose tissues is more variable in structure. It can be seen as small collections of acinar cells amongst the adipose cells (Fig 2.23 shows adipose pancreas from a *C. exoletus*) or as large congregations of acinar cells (Fig 2.25 shows adipose pancreas from a *L. mixtus*). The pancreatic tissue associated with the intestine more closely resembles that seen in the liver being organised into discrete strands, illustrated in Fig 2.24 from a *C. rupestris*. Often pancreatic ducts can be seen leading from this associated pancreas into the submucosa of the intestine, one of these ducts is shown in Fig 2.26 from a *C. rupestris*.

The endocrine pancreas is relatively difficult to locate as it would appear to be present in only a few areas in each fish. The location of these areas does not seem to follow any particular pattern, however, once located these areas can be seen to contain large amounts of endocrine tissue, this is illustrated in Fig 2.27.

Fig 2.20: The hepatocyte structure of the liver from a *C. rupestris* from a farm location with lipid vacuoles in the hepatocytes, x40 objective (arrows - lipid vacuoles).

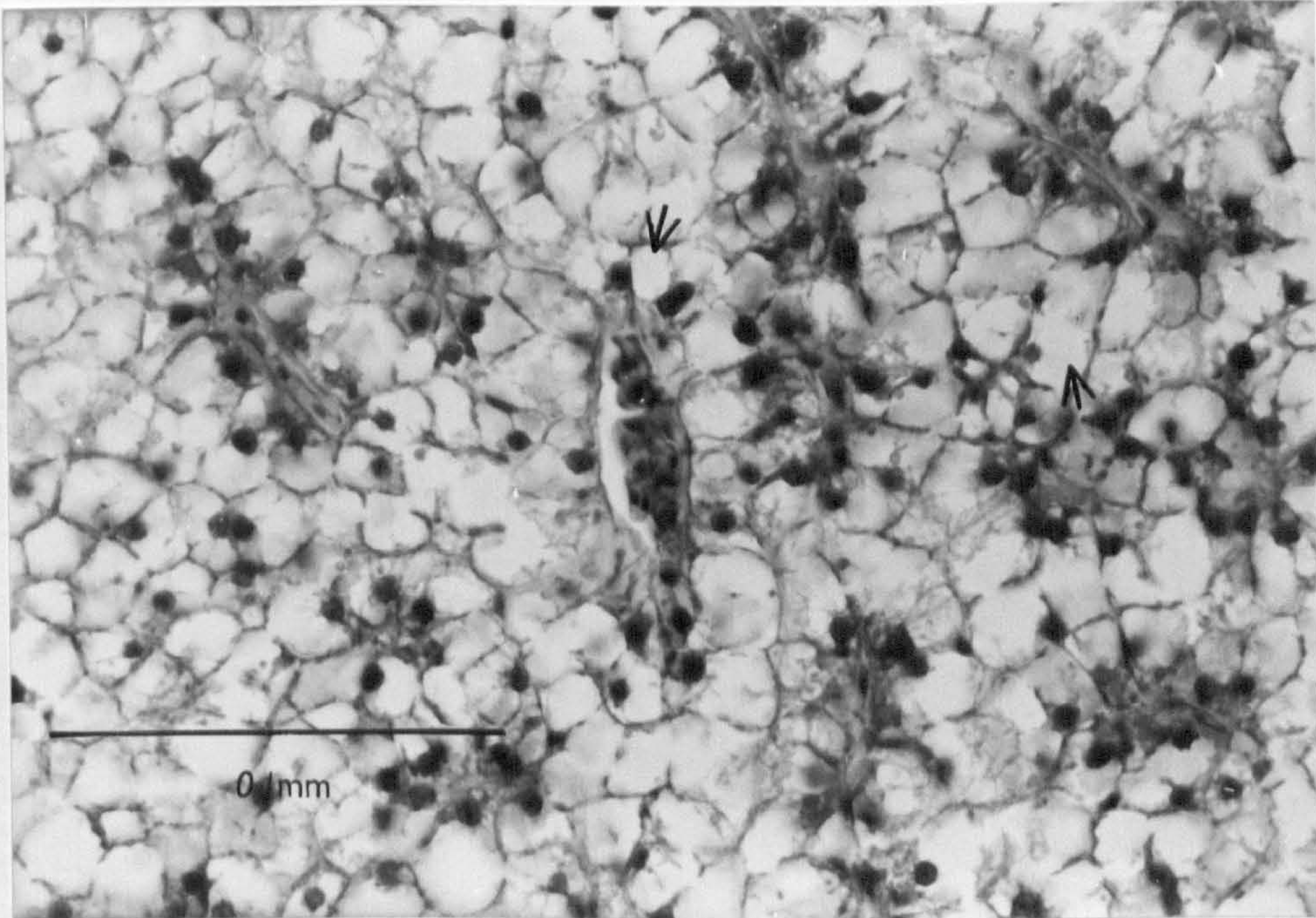


Fig 2.21: The hepatocyte structure of the liver of a *C. rupestris* with a large amount of lipid vacuoles in the hepatocytes, x40 objective (arrows - lipid vacuoles).

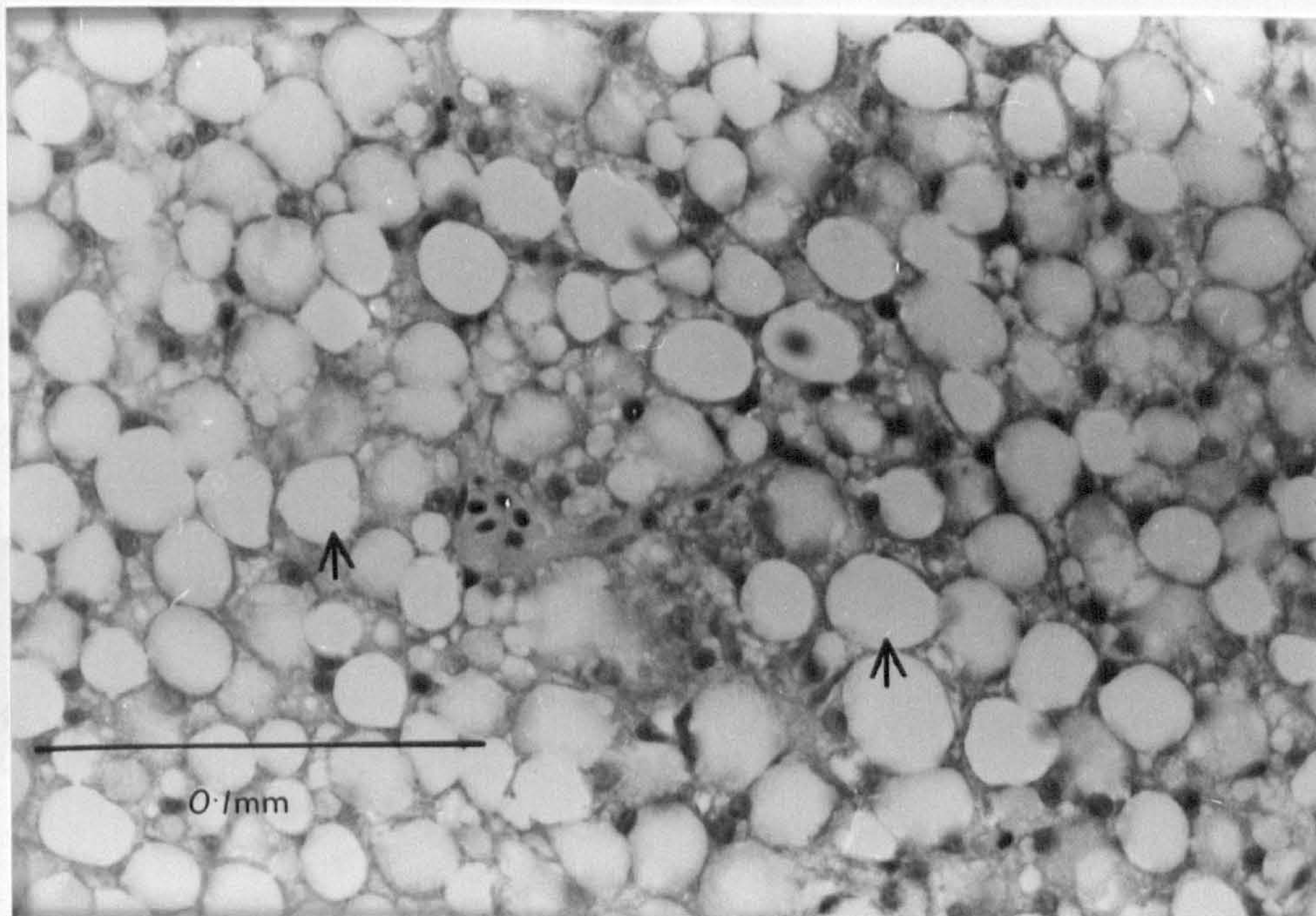


Fig 2.22: Hepatopancreatic tissue in the liver of a *C. rupestris*, x25 objective (A - acinar tissue, B - blood vessel, H - hepatocytes)

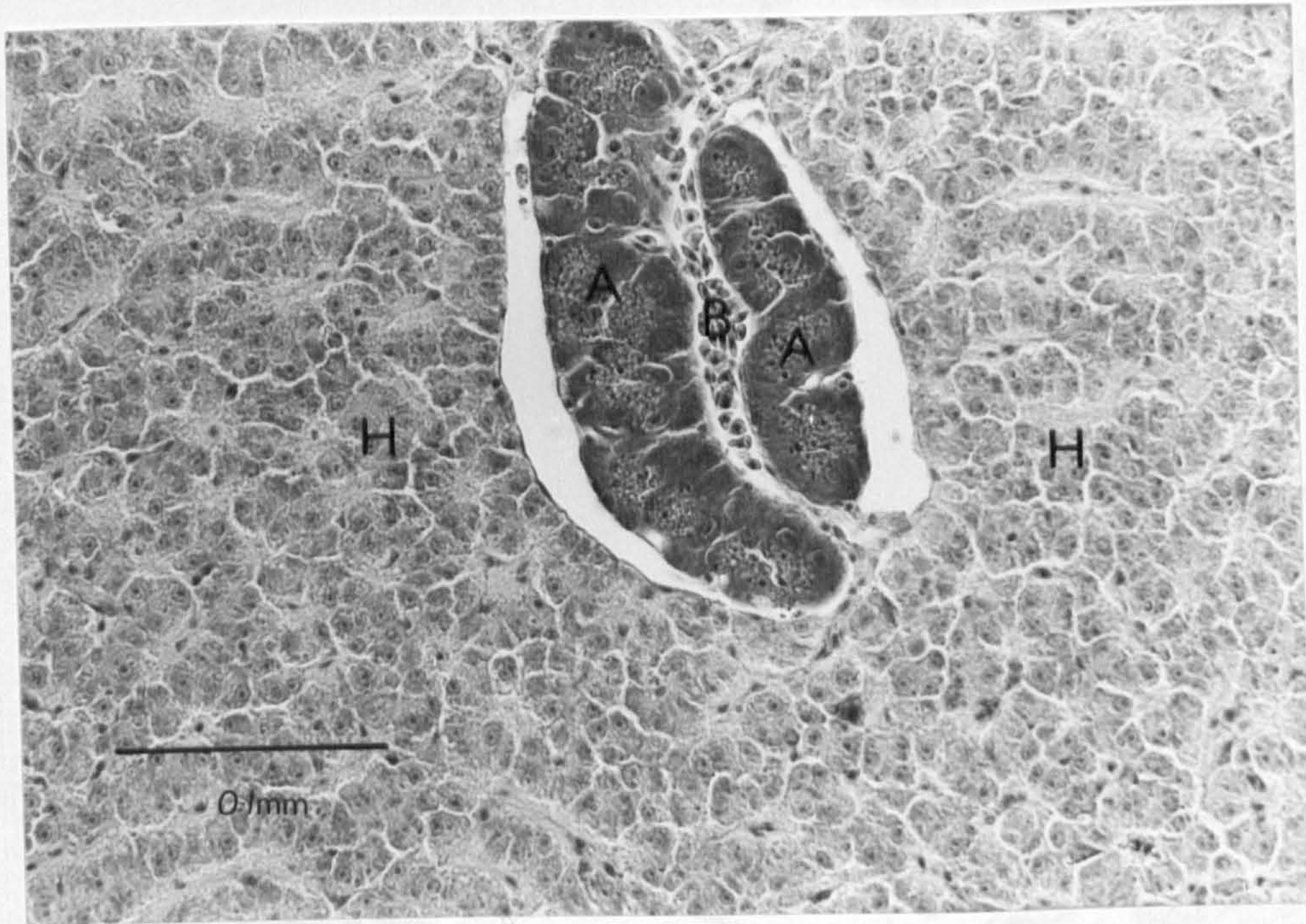


Fig 2.23: Adipose pancreas from a *C. rupestris*, x10 objective (A - acinar tissue, arrows - adipose cells with lipid vacuoles, I - intestine).

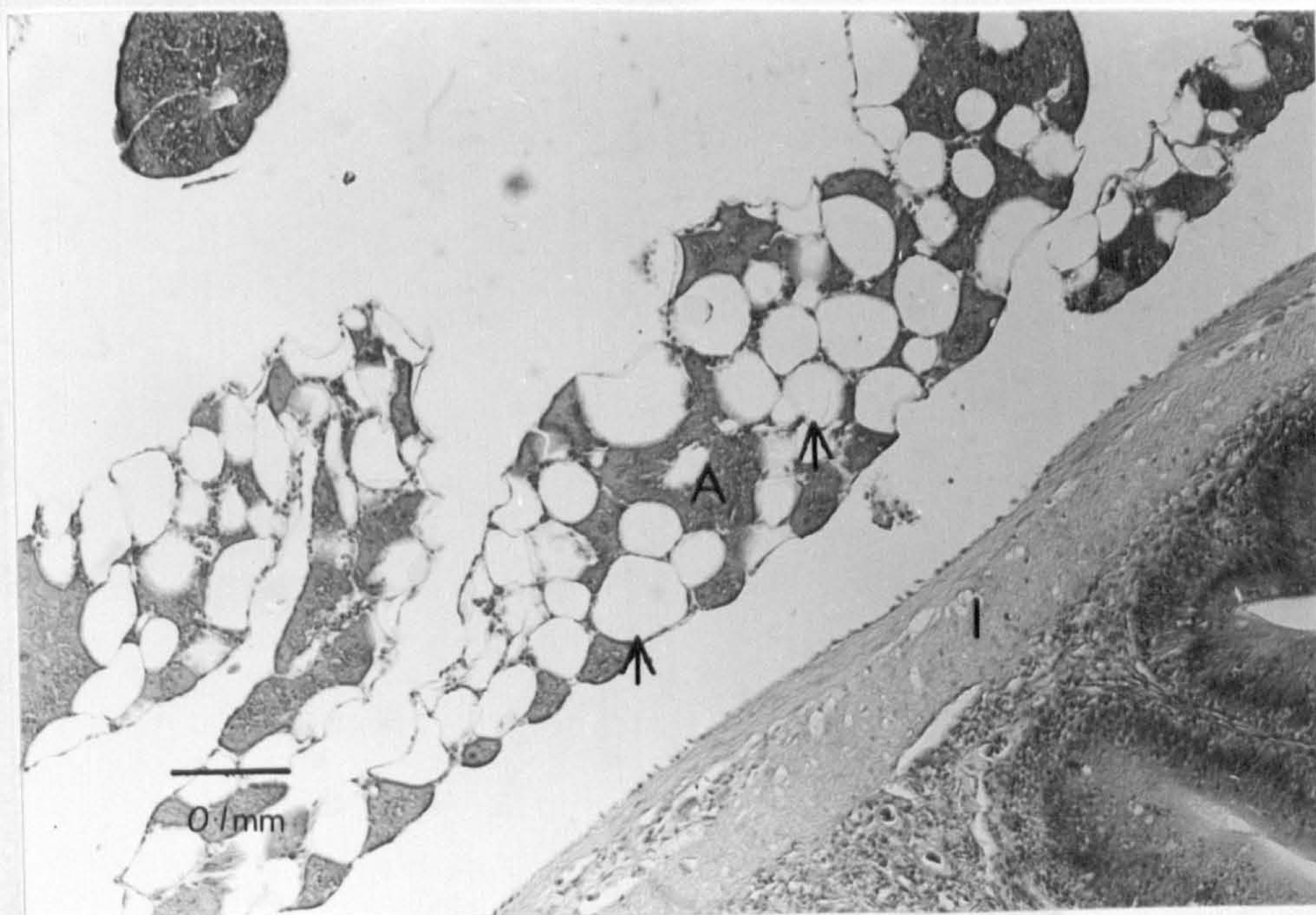


Fig 2.24: Intestinal pancreatic tissue of a *C. rupestris* associated with a pancreatic duct, x6.3 objective (A - acinar tissue, D - pancreatic duct, I - intestine).

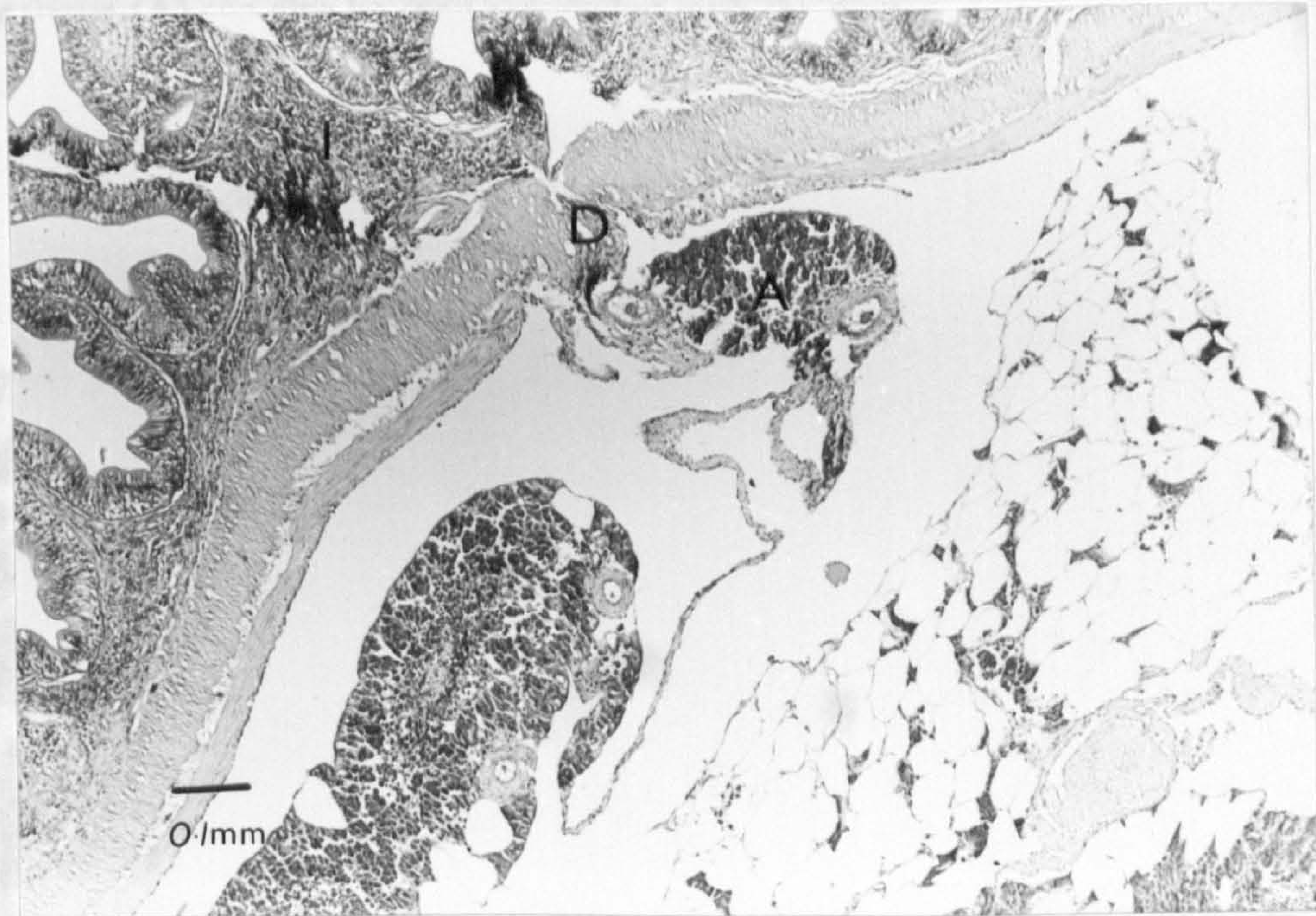


Fig 2.25: Adipose pancreatic tissue from a *L. mixtus*, x6.3 objective. An accumulation of acinar tissue (A) can be seen interspersed with adipose cells (arrows).

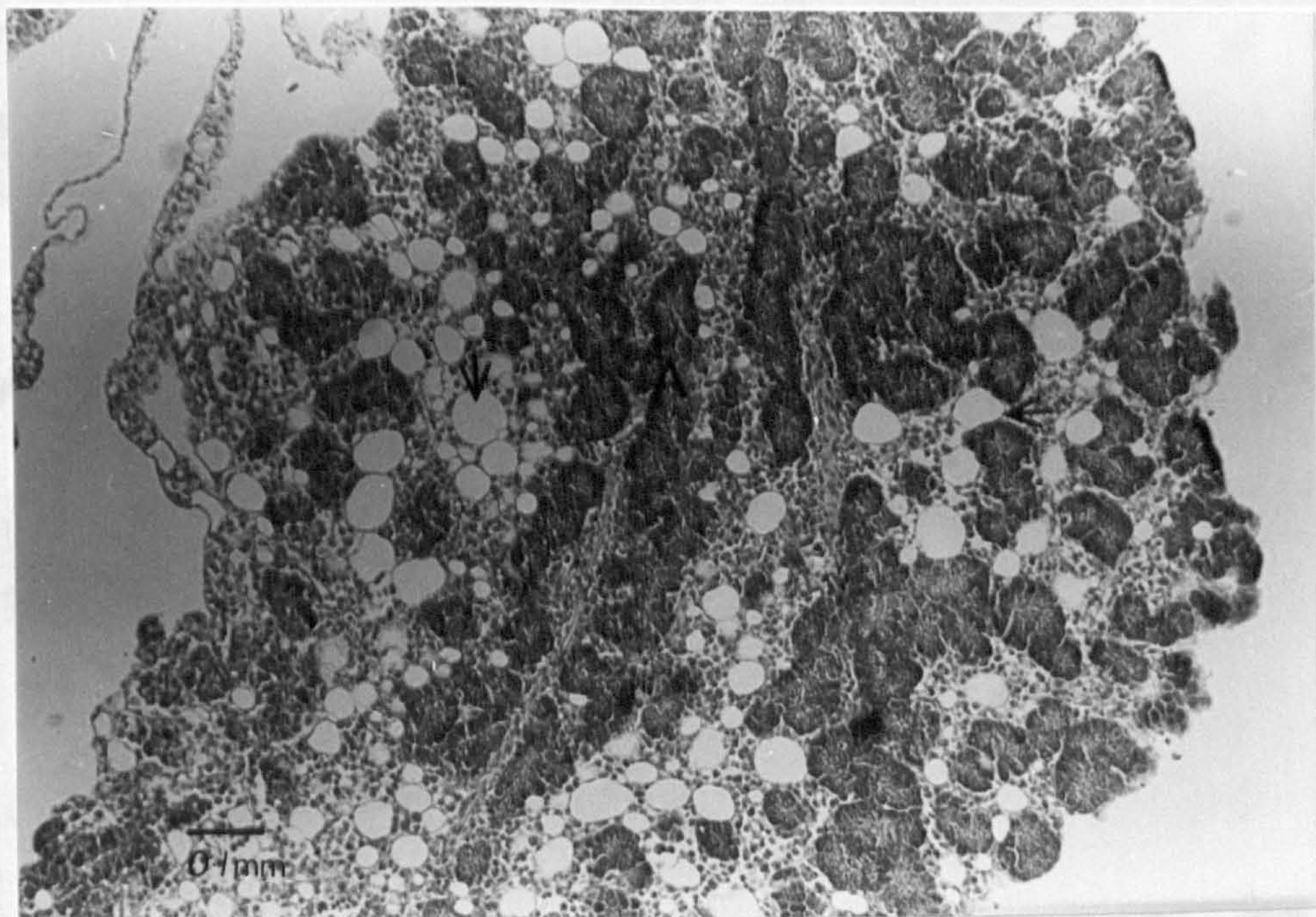


Fig 2.26: A pancreatic duct (PD) of a *C. rupestris* leading into the muscularis (M) of the intestine, the duct passes through to the submucosa of the intestine, x40 objective. Acinar tissue (A) can also be seen associated with the duct.

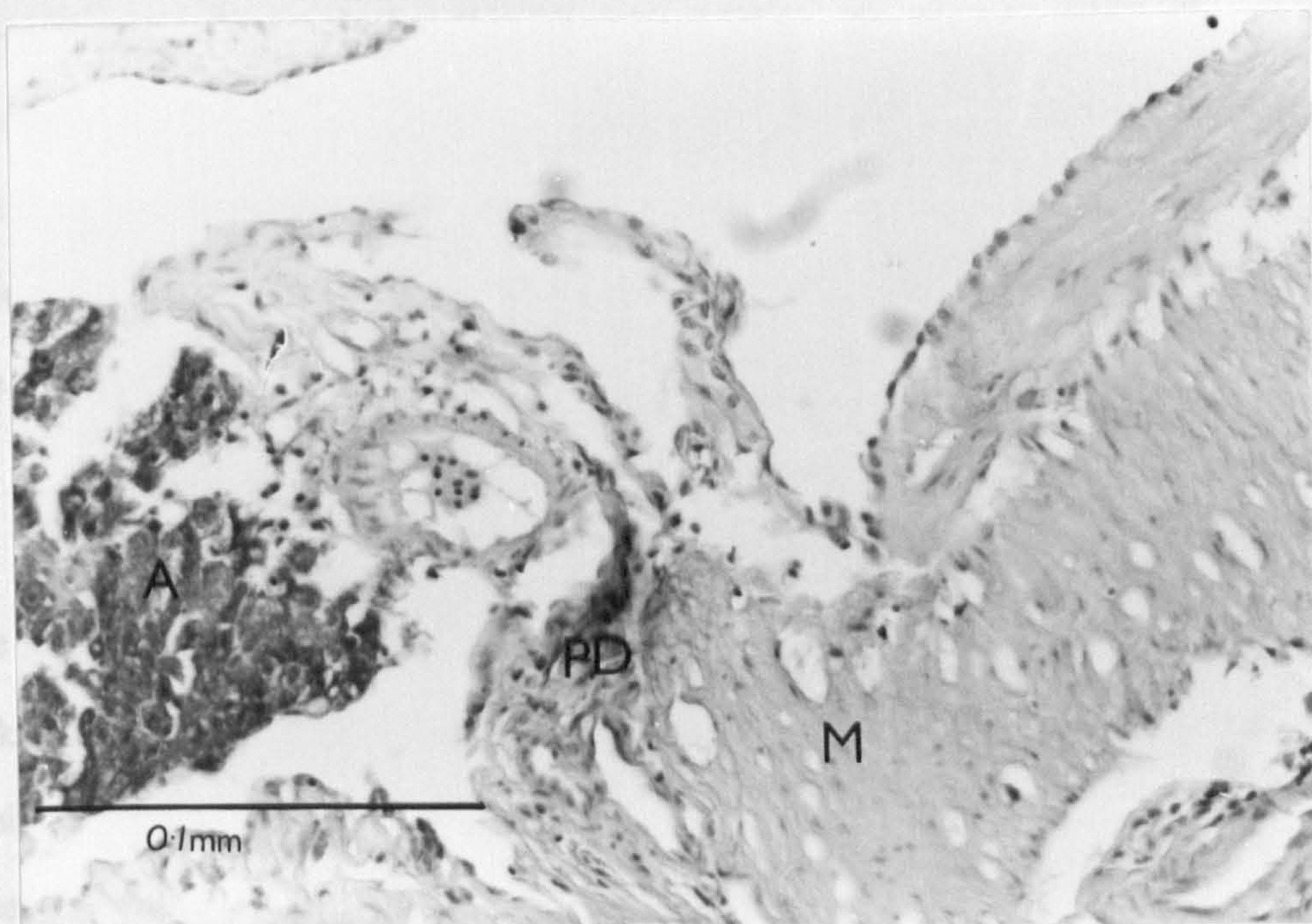
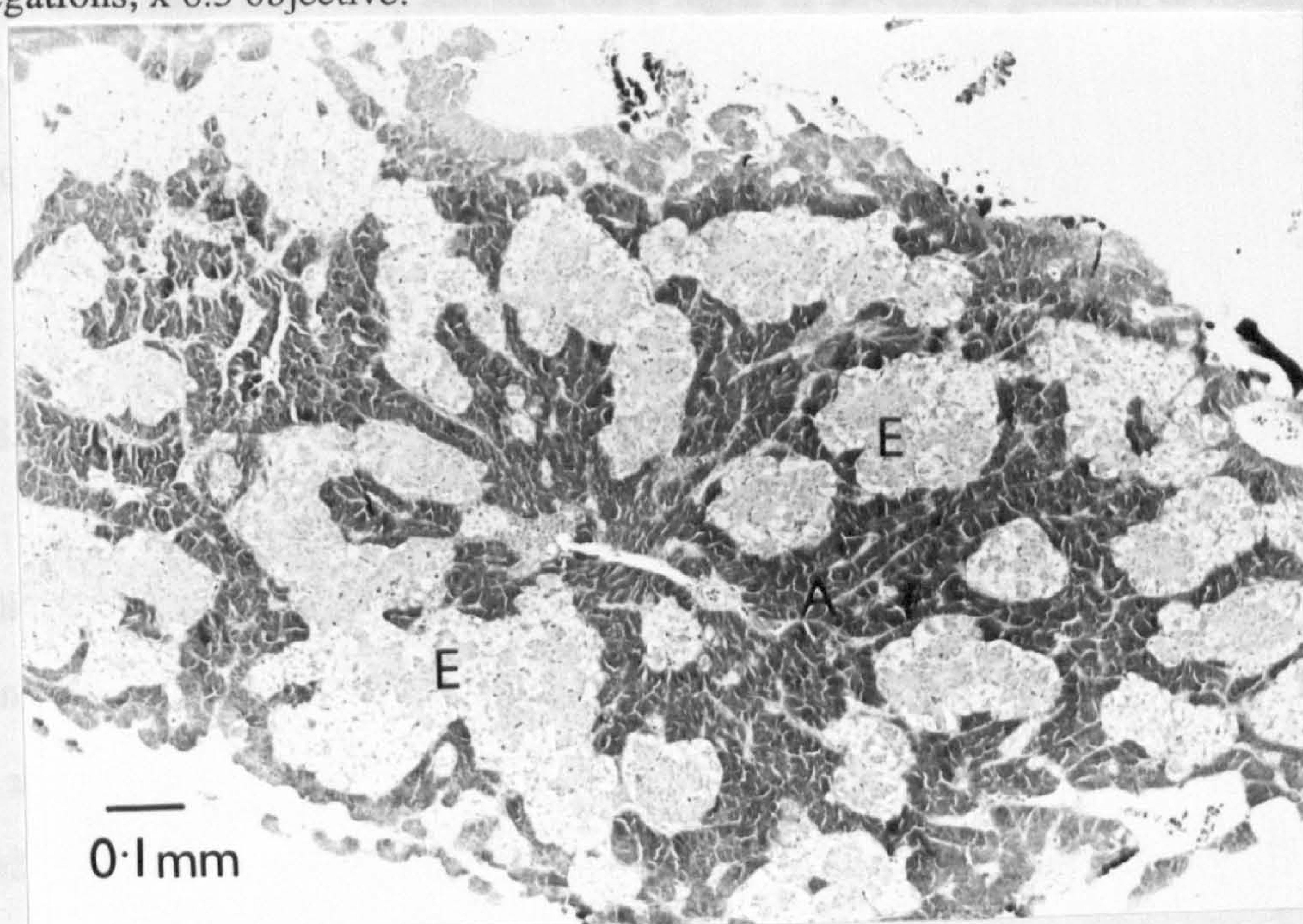


Fig 2.27: Endocrine pancreas (E) surrounded with exocrine pancreas or acinar tissue (A) from *C. rupestris* illustrating that endocrine pancreas is found in localised aggregations, x 6.3 objective.



2.3.7.5: Kidney

When seen in sections collected as described in section 2.2 of this chapter the kidney is seen to be triangular in shape bordered by a very thin serous membrane and closely associated with the peritoneum. The kidney of a *C. rupestris* is illustrated in Fig 2.28 which shows the kidney with the vena cava in its centre. A nerve ganglion can be seen between the kidney and the peritoneum, the separation of the kidney from the peritoneum being an artefact of processing. The base of a vertebra can be seen on the left edge of the frame.

No corpuscles of Stannius were observed in the wrasse kidneys, but, this may have depended on the sections of the kidney taken. The only other difference noted in the kidney when compared to other wholly marine fish species was the presence of large numbers of EGC's in the intertubular spaces. This may be considered as normal, despite it being an unusual feature in other fish species, unless accompanied by parasitic infection.

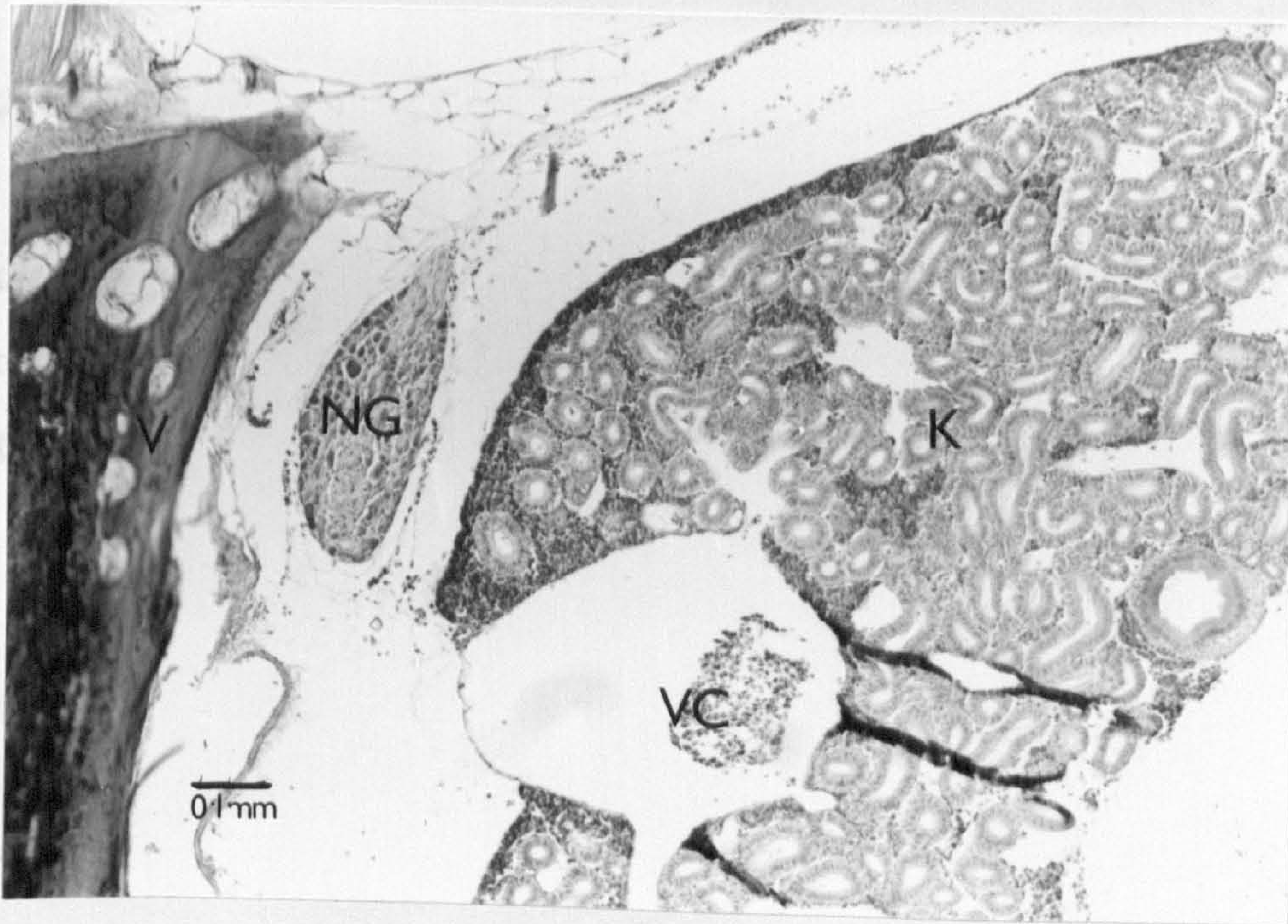
2.3.8: Reproductive Organs

Even relatively small fish can show signs of advanced gonadal development. In two year old fish, of approximately five grams in weight that were bred from wild caught brood stock, ova in advanced stages of maturity could be seen in the ovaries. Similarly, fully formed sperm can be seen in small numbers in male fish of the same age.

2.3.8.1: Ovaries

The ovaries in wrasse consist of two sac like organs originating at the vent. In small fish these organs are still obvious as small orange coloured structures. In larger fish during the reproductive season (late June till late August approximately), these organs can account for half of the volume of the visceral cavity giving the abdomen of mature female fish a translucent quality. The ovaries of wrasse are asynchronic i.e. all stages of

Fig 2.28: A low powered, x6.3 objective, view of the kidney (K) of a *C. exoletus*. The vena cava (VC) can be seen running through the middle of the kidney. A nerve ganglion (NG) can be seen between the kidney and the peritoneum. The separation of the kidney from the peritoneum is and artefact of the processing. The base of a vertebra (V) can be on the left of the frame.



development of ova can be seen at any one time, the asynchronic ovary of a *L. mixtus* is shown in Fig 2.29. Fully mature ova that are not shed are reabsorbed by the female, Fig 2.30 shows a mature ova in the ovary of a *L. mixtus* degenerating and being reabsorbed. The serous membrane of the ovary is slightly thicker than that of other organs of wrasse, as shown in Fig 2.31.

2.3.8.2: Testis

The testes appear glandular in histological section. Mature sperm accumulate in fluid filled spaces in the testes. These spaces are presumably linked to the epididimus. In mature fish these mature sperm appear as masses of darkly blue pigmented dots in section due to their basophilic nature. The testis of a *C. exoletus* is shown in Fig 2.32.

The details of the ultrastructure of the testis of the labrid *T. duperrey* were discussed by Hourigan *et al.* (1991).

2.4 Discussion

The morphology and histology of the family Labridae is very different from salmonids, with which fish farm site diagnosticians are most familiar. Extrapolation cannot, therefore, be made from normal *S. salar* morphology. A more suitable comparison could be made with other perciform marine fish. However, the majority of histological texts refer almost exclusively to salmonid fishes.

The skin of wrasse is similar to the majority of teleosts, but, the size and thickness of the scales may differ from other fish species. These relatively large scales give the skin a very robust property which is perhaps an adaptation to living in the relatively turbulent area of the sublittoral coast line. A delicate skin would be very easily damaged when living in rock crevices. The thick skin also makes wrasse relatively

Fig 2. 29: Asynchronic ovary of a *L. mixtus* showing the wide range of ova development seen in the ovaries of wrasse, x6.3 objective. Newly forming ova (arrows) can be seen as well as mature ova (M) and also degenerating ones (D).

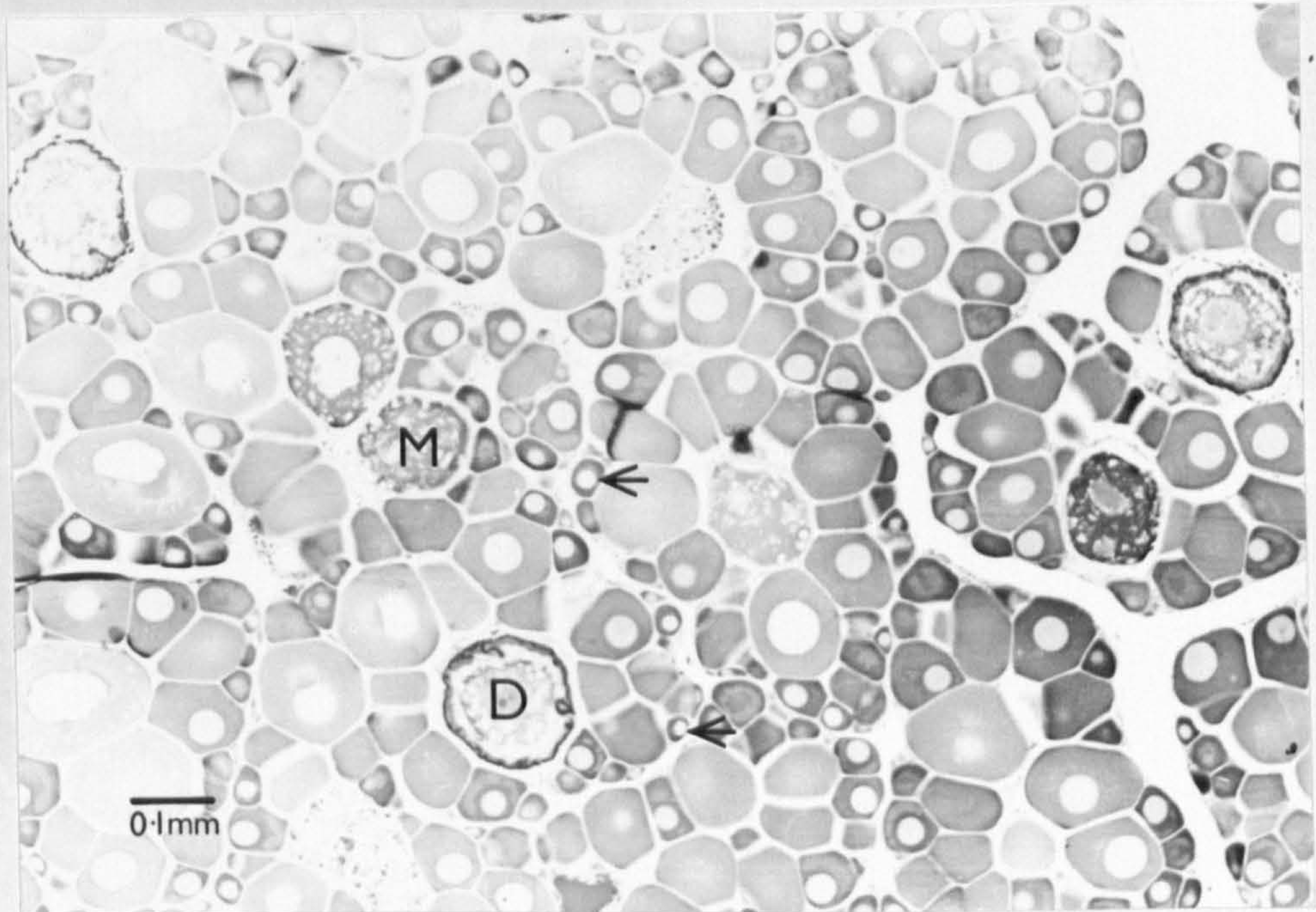


Fig 2.30: A degenerating ova (D) being reabsorbed by the *L. mixtus*, newly forming ova (N) can be seen very close by, x40 objective.

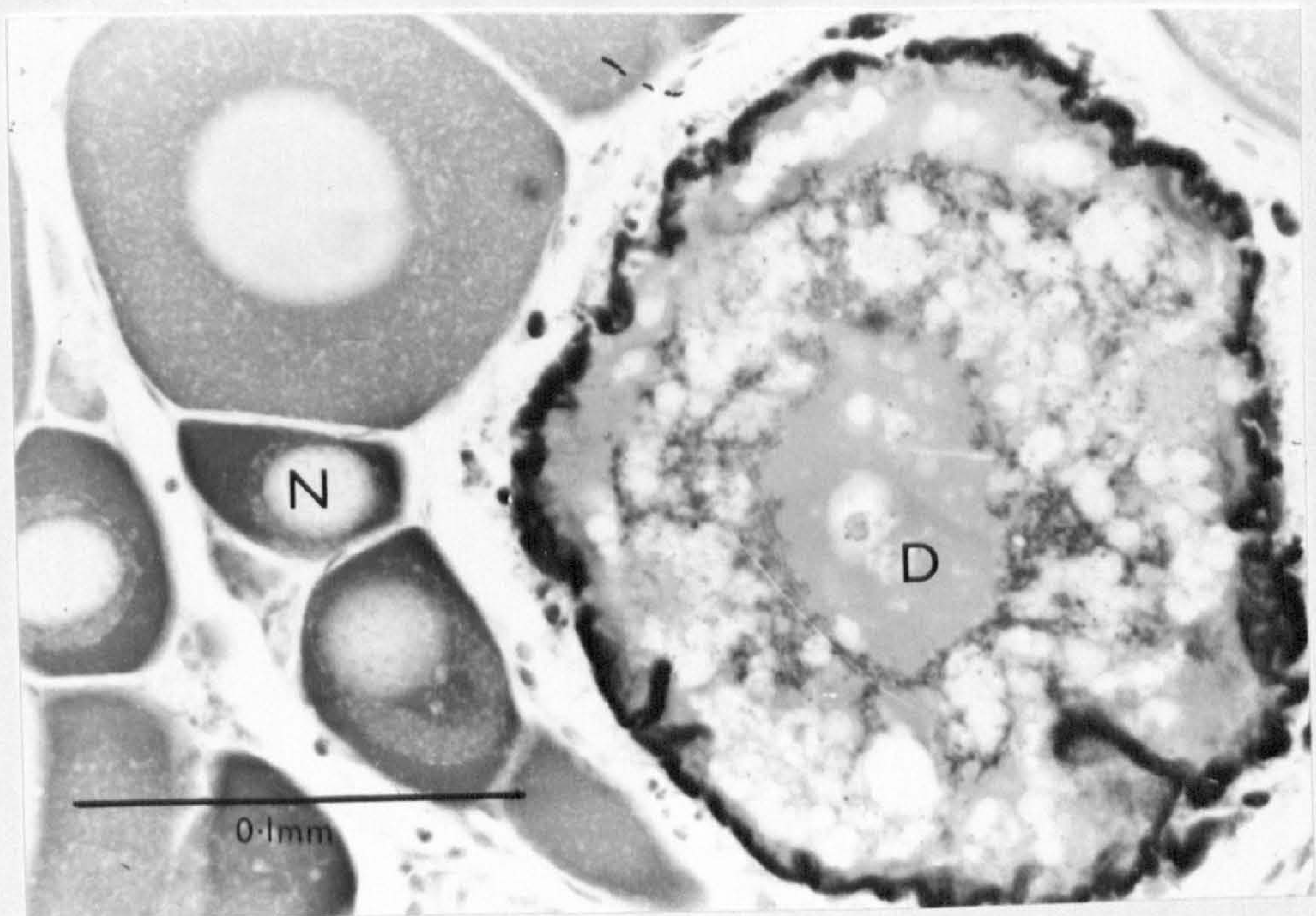


Fig 2.31: The serous membrane (arrow) surrounding the ovary of a *L. mixtus*, x40 objective. It can be seen that the serous membrane is several cells thick in areas.

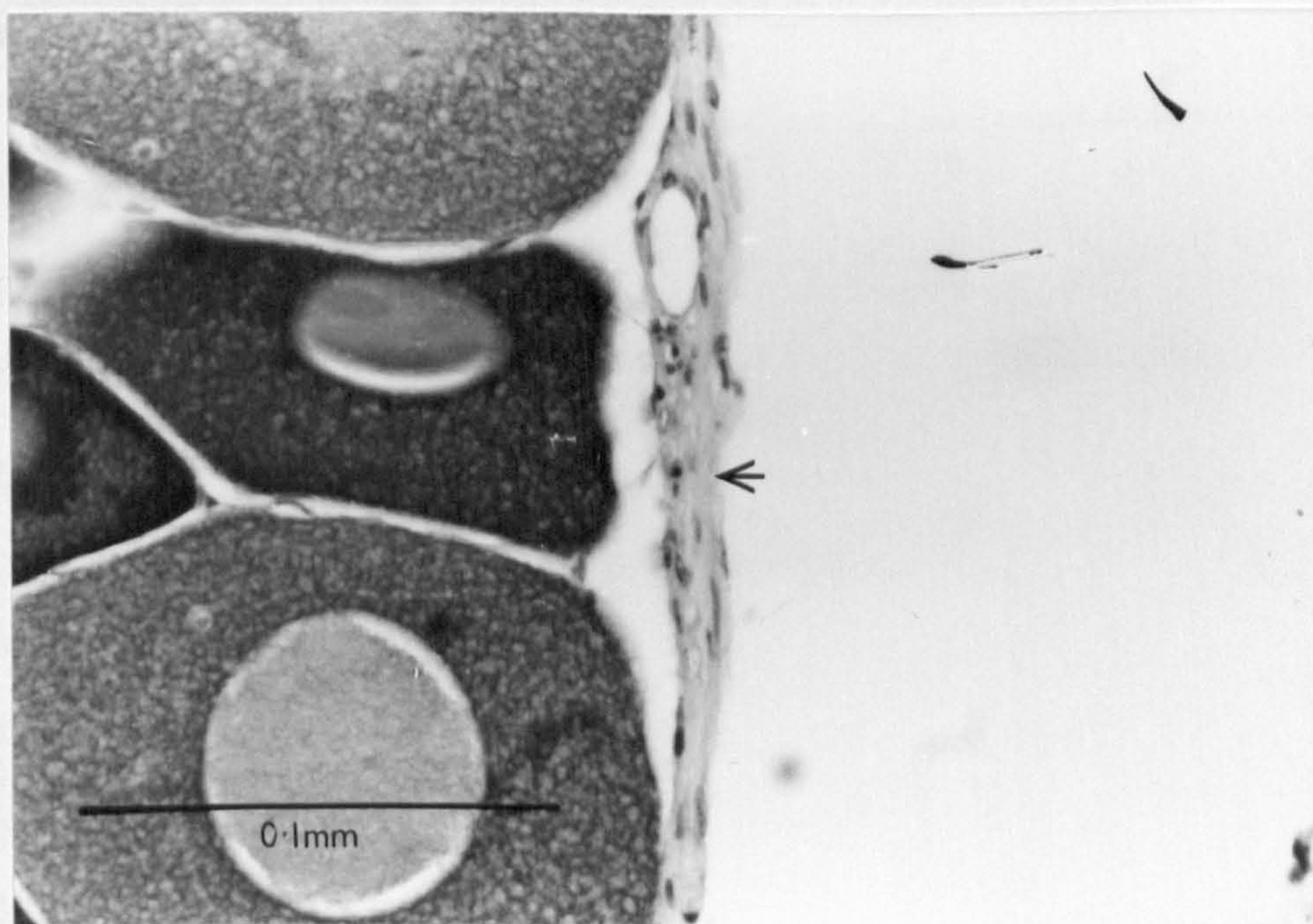
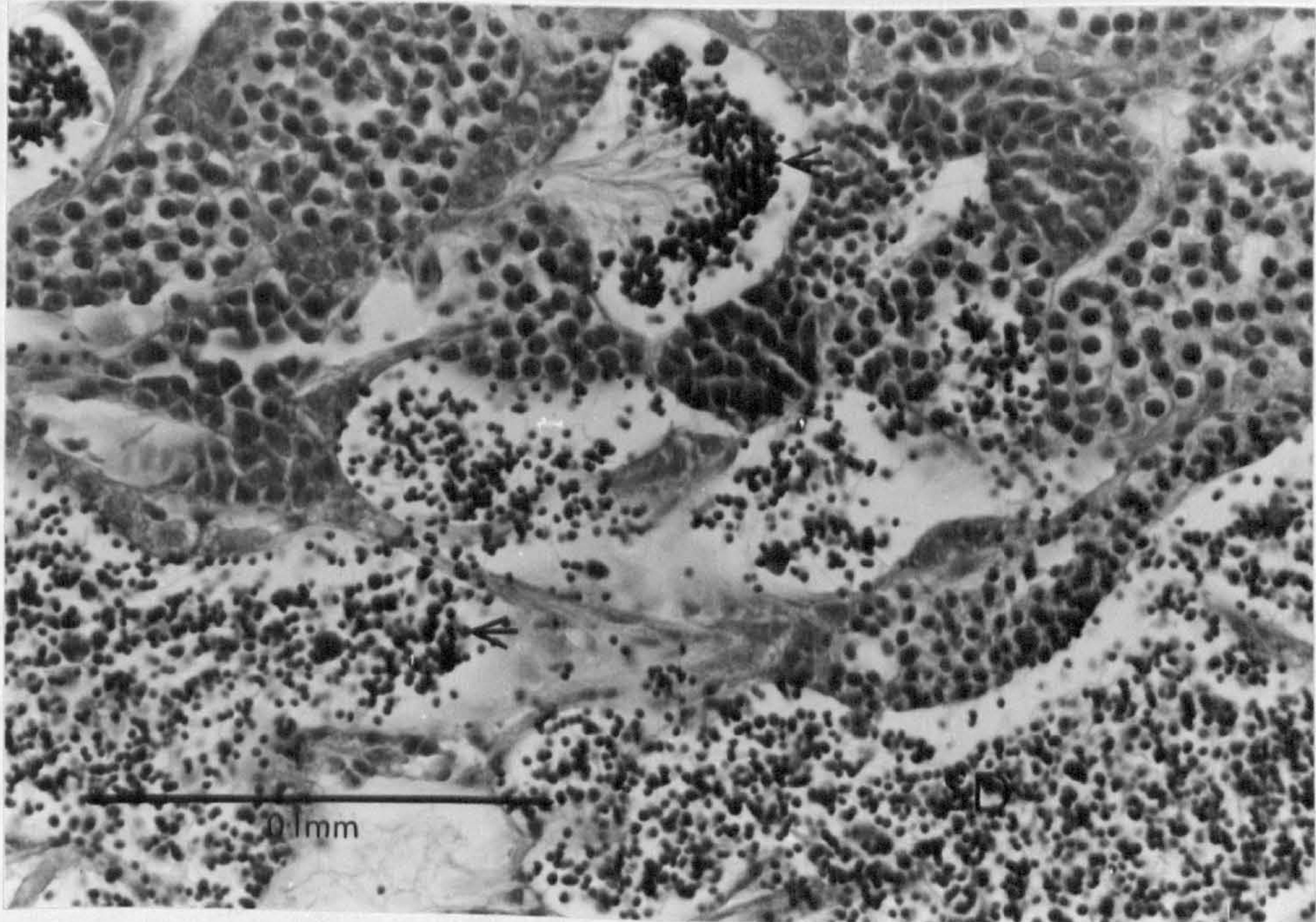


Fig 2.32: A relatively high powered, x40 objective, view of the testes of a *C. rupestris*. The basophilic nuclei of the sperm (arrows) can clearly be seen accumulating in the sperm duct (SD) at the base of the frame.



resistant to handling and net damage. This resistance to damage must also help limit the uptake of water borne disease agents.

Adaptations by labrids allowing them to use their pectoral fins as the main means of locomotion have driven several morphological changes which differentiate them further from salmonids. The use of the pectoral fins for sustained swimming has necessitated the accumulation of red muscle fibres around the base of these fins. In salmonids the main method of locomotion is by using the caudal fin, for this reason the red muscle fibres in salmonids is concentrated in a thin band along the length of the lateral line. The use of the pectorals in labrids also allows them to be very highly manoeuvrable, being able to swim in any direction and able to swim in and out of rock crevices. This manoeuvrability combined with the relatively dextrous mouth parts of labrids allows them to pick small items of food from a range of surfaces. This ability makes them ideally suited to the role of cleaner fish. Hilldén (1983 & 1978a) postulated that cleaning behaviour could most easily evolve in species that were opportunistic substrate feeders with the ability to take food items quickly. The labrids in the current study all fulfil these criteria.

The high degree of manoeuvrability of the fins has also allowed them to be used for display (Potts 1974) and has enabled a more comprehensive range of social behaviours to be evolved by the labrids compared to the salmonids such as complex nest building (Potts 1985). The dexterity required for this behaviour would not be possible without the ability to move in any direction very accurately.

The thin walled nature of the heart of labrids, with a very thin serous membrane surrounding the organ and thin cardiac muscle fibres, would be regarded as being abnormal if seen in salmonids. This feature is most probably an adaptation to the way of life of the labrid fishes. They do not undertake prolonged periods of high energy

activities requiring an increased blood pressure and flow rate, the heart does not, therefore, have to be very muscular. The very thin serous membrane of the heart would allow it to expand to a greater degree than a similar sized heart with a thick elastic serous membrane as is seen in salmonids. This would have the effect of giving a large capacity for blood when the heart is fully distended. The small amount of cardiac muscle in the ventricle would, however, result in a relatively low velocity of the blood leaving the heart and, therefore, a low blood pressure in the circulatory system as a whole. Perhaps this is an adaptation to the partial "hibernation" that some labrids would appear to enter during periods of low temperature which Sayer *et al.* (1993) described. The high heart volume would allow the heart rate to drop while still maintaining a reasonably good flow rate of oxygenated blood. Measurement of the cardaic pressure and pulse rate of wrasse at different ambient temperatures could provide interesting results.

The most striking feature of the intestine of labrids is the absence of a stomach. Verigina & Kobegenov (1988) studied the following five species of wrasse from the Black sea; (*Symphodus roissall* (=*Crenilabrus*) *quinquelineatus* Risso (quail wrasse), *S.* (*C.*) *tinca* L. (eye-spot wrasse), *S.* (*C.*) *cinereus* (=*C. griseus*) (Bonnaterre) (gray wrasse) and *S.* (*C.*) *ocellatus* (Forsskål) (green sea parrot). They concluded that the absence of a stomach in labrids could be a result of the neutralisation of gastric juice by the calcium carbonate in mollusc shells which would make the stomach unnecessary. However, Bogachik (1969) describes how labrids eject the crushed shells of their prey from their mouths before the food is ingested; with the shells being crushed by the substantial pharyngeal teeth. This results in very little inert material entering the intestine, thereby, preventing the majority of the calcium carbonate from the shells of their prey entering the intestine. These two hypotheses would appear to contradict each other. A detailed examination of the enzyme activity and pH range within the intestine of wrasse is needed to clarify this situation.

In the work by Verigina & Kobegenov (1988) the wrasse species which ate mostly molluscs had folded intestines while the one that was herbivorous had fewer folds. Salmonids are also predatory and will take a large range of prey items. They have no pharyngeal teeth so their stomachs have to be very muscular to crush their prey items. They also have far longer intestines than a comparatively sized labrid and intestinal caeca are also present. These additional features may be needed to compensate for the higher level of inert material entering the intestinal tract in salmonids when compared to wrasse. The short intestines seen in wrasse may be made possible by the low amounts of inert material that enters the intestine due to the rejection of the crushed shells of their prey that takes place before digestion. Another important factor in the digestive efficiency of fishes is the degree of convolution of the intestinal villi (Verigina & Kobegenov 1988). Wrasse have a high degree of convolution in the intestinal villi which will increase the surface area and thus the efficiency of the intestine allowing for a shorter intestine. Perhaps examination of the intestinal efficiency of wrasse would clarify this point.

The liver of labrids would appear to vary considerably depending upon the diet of the fish. In wild caught specimens the liver is very compact when compared to that of farm held specimens which had access to the high energy pelleted feeds fed to the *S. salar*. This would suggest that the liver plays a major role in the storing of excess lipids in wrasse. The very low amount of inter-myotomal fat deposition and relatively low amounts of abdominal adipose tissue would also support this hypothesis. In the light of this, the diet of wrasse held in *S. salar* pens should be given careful consideration if the population of cleaner fish is to be kept healthy. Excess amounts of lipid in the livers of labrids will almost certainly impair the function of the organ. The major area of deposition of excess lipids in salmonids is the adipose tissue of the abdomen, so that the function of the liver is not impaired by high levels of lipid in the diet, or certainly to a lesser degree. However, as reported by Roberts & Shepherd (1986), *S. salar* fed with feeds with the wrong combination of lipids, or rancid lipids, can be affected by lipid

degeneration of the liver. This indicates that the type of lipid and its composition is very important in the way the lipid is utilised and stored.

The distribution of the pancreatic tissue of wrasse is very different from that seen in salmonids. In salmonids the pancreatic tissue is limited to the adipose and connective tissue which lies between the intestinal caeca. In contrast to this, labrid pancreas is very diffuse being found in the liver, adipose tissue and also in strands attached to the intestine. This arrangement is typical of that seen in perciform fish. The presence of relatively large amounts of endocrine pancreas in small discrete areas suggests a localisation of function within the pancreas to a degree not seen in salmonids.

The kidney of wrasse is typical of that seen in other fish, but, the relatively high numbers of EGC's present may be regarded as abnormal in other fish species. This finding was seen too many times in wrasse to be considered coincidental.

No differences were detected between the ovarian structure of the *L. mixtus* examined and the other three wrasse species. This is perhaps unexpected as *L. mixtus* was reported to be a protogynous hermaphrodite by Potts (1985) while the other three species remain the same sex throughout their life span. However, as *L. mixtus* begin life as functional females before changing to functional males the ovaries may not be significantly different from those of other species. Unfortunately it is only the testes of Initial phase and Terminal phase males of *T. dupperrey*, another protogynous wrasse species, which have been previously studied (Hourigan *et al.* 1991). The authors made no comment on the transition between female and male in this species or the structure of the ovary in the species.

The presence of mature eggs and sperm in small captive bred fish is interesting. However, whether wild fish of the same age and size would be sexually mature is

uncertain. Fish of this size would not be expected to compete well with fully grown fish for matings although males of a small size do act as accessory and satellite males and sneak matings with females being courted by larger males (Potts 1985, Hilldén 1981). This does not explain the presence of mature eggs in females of the same age. There could be no possible advantage to a female of producing eggs which have to be reabsorbed into the body due to a lack of suitable suitors. This, however, may not be the case, the fact that a male will spawn with several females during the breeding season suggesting that even small females will most likely have a reasonable chance of a successful mating. The advanced state of maturity seen in these young fish could also be related to a high energy diet that the young wrasse were fed. This could have increased the rate of maturation in the fish. Care should therefore be taken when extrapolating from this data. The asynchronous nature of egg development in labrids is typical of that seen in multiple spawners. In salmonids, where egg and sperm development is synchronous, it is far better to delay maturity until there is a far higher possibility of a successful mating.

Wrasse are ideally suited to life amongst the kelp and rocks of the sublittoral region, but, they may not be as well suited to the conditions found in *S. salar* pens. For example, their natural behaviour of seeking shelter may lead to a high number of wrasse becoming trapped in the nets of pens contributing to the number of deaths. The apparent storage of excess lipid in the liver of wrasse may also contribute to poor health, while the apparent "hibernation" state that wrasse enter during periods of cold weather may result in them being more open to attack from *S. salar* or other predators, it will also result in the wrasse being more susceptible to stress. Both of these factors will contribute to the mortalities of wrasse in pens.

CHAPTER 3: SECTION A; SURVEY OF DISEASES OF WILD AND FARM HELD WRASSE

3A.1 Introduction

3A.1.1 Wrasse Diseases: Current Knowledge

There is very little information in the literature relating to the diseases of wrasse. This is mainly due to the fact that prior to their use in the farming of *S. salar* they were poorly studied. What little literature there is available mainly deals with wrasse from outside British waters.

From the four wrasse species which are the subject of this study there have only been 15 species of parasite from 13 different genera reported. These parasites are summarised in Table 3A.1. These reports are not spread evenly over the four wrasse species involved in this study. Ten parasite species have been reported from *S. melops* while only one species has been reported from *C. exoletus*. Similarly there are few reports of bacterial isolates recovered from wrasse. The only work on the subject relates to the isolation of typical strains of the bacterium *Aeromonas salmonicida* from wrasse held in *S. salar* pens (Treasurer & Laidler 1994) and the isolation of atypical strains of *A. salmonicida* from wild caught wrasse from Irish coastal waters (Frerichs, Millar & McManus 1992). There have been no reports as yet of viral isolates from wrasse, either in the wild or under farm conditions.

It would be expected that small inshore fish species such as wrasse would host more parasites and diseases than already reported. Their feeding strategies, as described in the relevant sections of chapter 2, involve foraging for a wide variety of food items from many different invertebrate groups in the shallow waters of the intertidal zone of

Wrasse species	Parasite species	Location of infection	Location of report	Author
Ctenolabrus rupestris	**Copepoda**			
	Hatschekia cluthae (Scott)	gills	Britain	Kabata 1979
	Hatschekia pygmaea Scott & Scott	gills	West Scotland	Kabata 1979
	Trematoda			
	Cryptocotyle lingua (Creplin)	not reported	Norway	Costello 1991
	Proctoeces maculatus (Looss)	intestine	Mediterranean	Prevot 1965
	Protozoa			
	Trichodina sp.	not reported	Norway	Costello 1991
	Coccidia			
	Eimeria banyulensis Lom & Dykova	intestinal epithelium	Mediterranean	Daoudi 1987
Centrolabrus exoletus	**Copepoda**			
	Leposphilus labrei Hesse	cephalic canals (head)	not reported	Quignard 1968
Symphodus melops	**Copepoda**			
	Leposphilus labrei Hesse	lateral line canal	not reported	Quignard 1968
	Hatschekia pygmaea Scott & Scott	gills	Britain	Scott & Scott 1913, Leigh-Sharp 1933.,
	Isopoda			
	Nerocila bivittata (Risso)	fins	Mediterranean	Trilles 1961, 1964, 1968
	Anilocra frontalis Edward	not reported	Mediterranean	Legrand 1952, Trilles 1968, 1965, 1977
	Gnathia maxillaris Scott (larvae)	not reported	aquaria	Wilson 1958, Potts 1973
	Trematoda			
	Cryptocotyle lingua (Creplin)	not reported	Ireland	Costello 1991
	Proctoeces maculatus (Looss)	intestine	Mediterranean	Prevot 1965
	Helicometra fasciata Rudolphi	intestine	Mediterranean	Rudolphi 1819, Papoutsoglou 1976
	Nematoda			
	Cucullanus micropapillatus Törnquist	intestine	Mediterranean	Petter & Radujkovic 1986
	Microsporean			
	Ichthyosporidium giganteum (Thélohan)	connective tissues	France & Holland	Möller & Anders 1986
	Myxosporean			
	Sphaerospora divergens Thélohan	kidney	Norway	Auerbach 1912
Labrus mixtus	**Copepoda**			
	Caligus labracis Scott	not reported	Britain	Kabata 1979
	Hatschekia labracis (van Beneden)	gills	Britain	Kabata 1979
	Trematoda			
	Helicometra fasciata Rudolphi	intestine	Adriatic	Ergens 1960, Sey 1970
	Zoogonus rubellus (Olsson)	intestine	Britain	Bray & Gibson 1986

Table 3A.1: Summary of reported parasites from the four European wrasse species under study.

the shore. Many parasites use small invertebrates as intermediate hosts. For example, some nematodes, cestodes and acanthocephalans utilise zooplanktonic crustacea such as copepods and ostracods as their intermediate hosts (Svendsen 1990 & Cheng 1973). These invertebrate groups constitute a major part of the diet of wrasse and this would facilitate the infection of wrasse with several different parasite groups. In addition to the risk of infection from their diet, wrasse are also open to infection by parasites which have free swimming infective stages. For example, many digenean parasites utilise gastropod molluscs as intermediate hosts. The infective cercariae of the parasites are released from the snail hosts directly into the water. There are many species of mollusc which live in the same intertidal regions as the wrasse, leaving the wrasse open to infection.

3A.1.2: Recorded Parasites of British Wrasse

Only 25% of the parasite records relating to the four wrasse species in the present study are from British coastal waters. The others vary in location from Norway to Ireland and the Mediterranean.

3A.1.2.1: *Ctenolabrus rupestris*

There are six parasites recorded from *C. rupestris*. Costello (1991) reported that protozoans of the genus *Trichodina* have been seen on *C. rupestris* in Norwegian waters. These parasites are considered to be ectocommensals and in many situations there is no associated pathology. However, under farm conditions they may cause severe infection and may be lethal to fry and fingerlings. Trichodinids have a direct life-cycle and, under suitable conditions, their numbers can increase dramatically in a short period

of time. Trichodinids are one of the most ubiquitous parasites of fish, they are common in both fresh and salt water and can infect the intestine as well as the epithelium of the gills or skin.

Kabata (1979) reviewed reports of the copepods *Hatschekia cluthae* (Scott) and *Hatschekia pygmaea* Scott & Scott from *C. rupestris* and noted that all previous records of *H. cluthae* have been from *C. rupestris* in British waters, the exact location of the record is not given. He also gives his own record of the parasite from *L. bergylta* in Scottish waters. He also gives the only record of the second copepod known to infect *C. rupestris*, *H. pygmea*. This record was from the west coast of Scotland. He reported that infections with either of these species of parasite are very rare and they are normally in low abundance. This information would suggest that both *H. cluthae* and *H. pygmaea* are very family specific, affecting only wrasse. Both of these parasites have a direct life-cycle with no intermediate hosts and are found attached to the gills of their hosts.

There have been reports of two digenean parasite species affecting *C. rupestris*. Costello (1991) reported that the metacercariae of *Cryptocotyle lingua* (Creplin), a member of the family Heterophyidae, have been seen on *C. rupestris* from both Norwegian and Irish coastal waters. This parasite uses the gastropod molluscs *Littorina littorea* (L.) and *Littorina saxatilis* (Olivi) as intermediate hosts (Zavras & James 1979 and Davis & Farley 1973). These molluscs can be found in large numbers on littoral regions of the Scottish coastline. *C. lingua* has been reported from many different fish species from many different groups including the salmonid *Salvelinus alpinus* L. (Arctic charr) in Norwegian waters (Kristoffersen 1988 & 1991), the gadoids *Merlangius*

merlangus (L.) (whiting), *Trisopterus luscus* (L.) (pouting), the flat fish *Pleuronectes platessa* L. (plaice) and *Platichthys flesus* (L.) (flounder) from the Medway estuary in England (van den Broek 1979) and the clupeid *Clupea harengus* L. (herring) in American waters (Sindermann & Farrin 1962). These are only selected host records to illustrate the wide range of fish species affected by the parasite. The final hosts for *C. lingua* are sea birds but it has been reported from mammals, including man (Babbott, Frye & Gordon 1961). The wide range of both intermediate and final hosts for *C. lingua* reflects the broad specificity of the parasite. It has been demonstrated that penetration of the epithelium of juvenile fish by large numbers of cercariae of *C. lingua* may cause mortalities (Sindermann & Rosenfield 1954, Steele 1966, MacKenzie 1968 and Mackenzie 1971). Once the infection is established the metacercariae of *C. lingua* are long lived and cause little damage to their host. The most noticeable effect of *C. lingua* is the presence of "Black spots" in inshore marine fish. These pigment spots are due to the accumulation of melanocytes around the area of infection with the digenean (Sindermann & Farrin 1962). The pathology associated with the presence of *C. lingua* in fish is reviewed by McQueen, Mackenzie, Roberts & Young (1973).

The other digenean parasite recorded from *C. rupestris* is *Proctoeces maculatus* (Looss). Prevot (1965) reported adults of this species of digenean from *C. rupestris* in the Mediterranean but gave no further information regarding location of infection or any pathology involved. This digenean has a complex life-cycle which is given in detail by Bray (1983). The life-cycle involves bivalve molluscs hosting both adult, metacercarial and sporocyst stages, gastropod molluscs hosting metacercarial and adult stages, cephalopod molluscs hosting metacercarial stages, echinoderms also hosting

metacercarial stages and annelid worms hosting both adult and metacercarial stages as well as sparid and labrid fish hosting adult stages.

The only other parasite described from *C. rupestris* is the coccidian *Eimeria banyulensis* Lom & Dykova which was reported by Daoudi (1987) from the Mediterranean coast of France. These coccidians infected the intestinal epithelium of their hosts and they are known to cause extensive pathology, no information on this was noted by the authors.

3A.1.2.2: *Centrolabrus exoletus*

There is only a single report of a parasite from *C. exoletus* by Quignard (1968) who reported that the copepod parasite *Leposphilus labrei* Hesse produced swelling in the tissues immediately above the eyes in this species of wrasse. These swellings were caused by the tissue reactions to the presence of the parasite in the cephalic canals of the head. The location of capture of the infected fish was not given but it is presumed that they were sampled from the Mediterranean area. *L. labrei* is a copepod of the family Philichthyidae which employs a direct life-cycle. The marked pathology it causes seems to be localised in the area of infection. The philichthyids are unique amongst copepod parasites in that they live completely enclosed within their hosts tissues with only a single duct linking them to the external environment. This duct marks their entry point into their host (Kabata 1979).

3A.1.2.3: *Symphodus melops*

There are more parasite records relating to *S. melops* than to any of the other three wrasse species involved in this study. Ten parasite species, each from different genera, have been reported.

Two species of copepod parasites have been recorded from *S. melops*. Quignard (1968) reported *L. labrei* in this host as well as in *C. exoletus*, however, in *S. melops* the parasite can only be found in the lateral line canal and not in the cephalic canals of the head as seen in *C. exoletus*. The presence of the parasite is normally indicated by a small swelling along the lateral line. Donnelly & Reynolds (1994) reported a 30.7% prevalence of infection with *L. labrei* in *S. melops* which were captured in Irish waters to be used as cleaner fish. They also noted that male *S. melops* had significantly higher numbers of parasites than females or juveniles and suggested that this difference was related to the size of the fish as prevalence increased above a host length of 100mm. They also found that there were significantly greater numbers of infections involving the left side of the fish when compared to infections of the right side and suggested that this may have been due to asymmetric swimming motions in fish !

The other copepodid parasite species reported from *S. melops* is *H. pygmaea*, Kabata (1979) could only find two reports of this parasite, one was from the Solway Firth and one from Plymouth, in both of these cases the host was *Crenilabrus melops* (=*S. melops*) he also gave his own report of the parasite from *C. rupestris*, as described

in section 3A.1.2.1. *H. pygmea* has since been described from *S. melops* in British waters by Scott & Scott (1913) and Leigh-Sharp (1933).

Three parasitic isopod species have been recorded from *S. melops*. *Nerocila bivittata* (Risso) and *Anilocra frontalis* Edwards were both reported from *S. melops* in the Mediterranean by Legrand (1952) and Trilles (1968, 1965 & 1977). The authors remarked that both of these species would appear to show a preference for wrasse as their hosts but that they are not solely found on wrasse. Wilson (1958) and Potts (1973) both reported that specimens of *S. melops* held in aquaria became infected with the praniza larvae of the isopod *Gnathia maxillaris* (Montagu). The adults of this isopod live in the substrate and it is only the larval stage that is parasitic.

Three species of digenean parasites have been recorded from *S. melops*. Costello (1991) reported that the metacercariae of *C. lingua* was seen to infect *S. melops* in Ireland but the location or severity of infection was not reported. Prevot (1965) reported *P. maculatus* and Rudolphi (1819) & Pappoutsoglou (1976) reported *Helicometra fasciata* Rudolphi from the Mediterranean. The infections were in the intestine, but, no information is given regarding any pathology invloved. As the parasites were recorded as adults it may be assumed that they were recovered from the intestine of the fish.

The single nematode to be recorded from any of the wrasse species under study was reported to infect *S. melops* in the Mediterranean by Petter & Radujkovic (1986). The species involved was *Cucullanus micropapillatus* Törnquist. It was recovered from

the intestine of six wrasse species including *S. melops*, the other five wrasse species involved are not found in British waters. Four out of five of these other hosts were also of the family *Symphodus* with the fifth species belonging to the family *Labrus*. No other hosts for this parasite were reported. This would appear to indicate a specificity for wrasse species as final hosts.

Of the endoparasitic sporozoans, the microsporean parasite *Ichthyosporidium giganteum* (Thélohan) has been reported by Möller & Anders (1986) as infecting the connective tissues of *S. melops* and the myxosporean, *Sphaerospora divergens* Thélohan, was reported from the kidney of *S. melops* in Norwegian waters by Auerbach (1912), however, no details of the infection were given.

3A.1.2.4: *Labrus mixtus*

The fourth wrasse species involved in this study, *L. mixtus*, has a record of three parasite species of three different genera. Kabata (1979) reported that *L. mixtus* from British waters were found to be infected with the copepod parasites *Caligus labracis* Scott, found on the skin, and with *Hatschekia labracis* (van Beneden), found on the gills. He reported that infections with *C. labracis* are limited almost exclusively to *L. bergylta* and *L. mixtus* from British waters, thus indicating a high degree of family specificity. The same can be said for the copepod *H. labracis*. Infections would appear to be limited to the gills of *L. mixtus* and *L. bergylta* in British coastal waters and those of adjacent European coasts. He observed high intensities of infection on *L. mixtus* with

up to 267 specimens being reported from a single host. Both of these parasites utilise a direct life-cycle.

As Table 3A.1 shows, most of the reports of parasites in wrasse are not from British waters, indeed, prior to this study it was not known if some of the parasites involved would be present in the Scottish coastal waters. It is therefore important to establish which parasites affect British species and most especially which parasites are prevalent in wrasse from the areas fished to provide wrasse for stocking in *S. salar* farms. It is also important to understand what happens to these parasites when wrasse are stocked in farm pens. Some parasites may find the conditions more favourable than others due to the different conditions. The wrasse may also acquire new parasites in the new environment.

Parasites which utilise a direct life-cycle may be acquired directly, by wrasse, from the *S. salar* in pens while those with indirect life-cycles infecting wrasse may use *S. salar* as a final host. The risk of transfer of parasites from wrasse to *S. salar* and also from *S. salar* to wrasse must be considered. Darwall *et al.* (1991) reported that large *S. salar* were seen attacking wrasse in pens. If this is the case there is a risk of transfaunation of parasites from wrasse to *S. salar*. It is unlikely that any of the parasites so far reported from wrasse could cause any health problems to *S. salar* in a culture situation. The only parasite reported from both wrasse and *S. salar* is *C. lingua*. This parasite is regarded as being of economic significance to salmonid culture (Roberts & Shepherd 1986), but, there is no risk of transfaunation of *C. lingua* from wrasse to *S. salar*.

The majority of the wrasse used to stock Scottish farms are caught from the wild on the west coast of Scotland as the wrasse species involved are most common on this part of the coastline. Very little is known regarding the reproductive capabilities of these species, in particular their ability to recover from heavy fishing pressure. Wrasse would appear to fulfil an important role in the ecology of the intertidal zone of the shore. They are important predators on small invertebrates and are alos imoortant items of prey to animals such as seals, congers and some piscivorous birds. Due to this important role, the removal of large numbers of wrasse from a population may affect the balance of parasite populations in the food web of the intertidal zone. It was decided to concentrate the sampling effort in the west coast of Scotland because of the lack of information relating to the diseases of wrasse in this area and because of their recently acquired economic significance.

3A.2: Materials and Methods

3A.2.1: Wild Caught Fish

3A.2.1.1: Selection of Sampling Sites.

Representative areas for sampling were chosen on the basis of location, geography, proximity to *S. salar* sites using wrasse and ease of boat access for sampling. Three locations on the Scottish west coast were chosen. The locations of these sites are indicated by the symbols A, B and C on Figure 3A.1. The three sites varied considerably:

Fig 3A.1: Location of the sample sites on the west coast of Scotland, wild, farm, captive and incidental samples are shown, figure not to scale.

Site A was located within a long sea loch which ran approximately east-west. The shallow mouth of the sea loch resulted in very low amounts of tidal flushing with significant flushing only taking place during spring tides. The effects of weather were minimal due to the protection gained from surrounding mountains. Rough seas were only experienced during times of strong westerly winds. The sample site itself was centred around a small group of islands in the middle of the loch. The loch has been extensively used for the farming of *S. salar* for many years and several different companies still operate sites within the loch. There is also a resident population of Grey Seals in the loch. In addition to the use of the loch for commercial *S. salar* culture it is also used for recreational fishing. All of the farms in the loch were fallowed for three months in 1991 in an attempt to control sealice numbers.

Site B was located within a relatively short sea loch which ran approximately southwest to northeast. There was relatively good tidal flushing but the loch was well protected from the influence of weather by surrounding mountains, strong south westerly winds being the only conditions which produced rough seas. As with Site A, the sample site was centred around a small group of islands in the centre of the loch. Site B has also been extensively used for the farming of *S. salar* for many years, but, there are relatively fewer farm sites located within the loch when compared to Site A.

Site C was exposed and open to strong tidal flushing from the west. Almost any wind direction from northerly, though westerly, to southerly would produce rough seas. The sampling site was around a small group of offshore islands. Similar to the other two

sites there is a strong historical connection with *S. salar* farming in the area. There are several farm sites in operation in the area surrounding the sampling site.

3.2.1.2: Collection of Fish

The wrasse were caught using small mesh size prawn creels, as shown in Figure 3A.2 during the period 11.07.92 to 18.09.92. The creels were baited with the bivalve molluscs *Patella vulgata* L. and *Patella aspera* Lamarck (common limpets), *Mytilus edulis* Linné (edible mussel) and the crabs *Portunus puber* Leach (velvet crab) and *Carcinus maenas* L.(shore crab) before being laid down in two to five meters of water as close to the shore as possible. The creel lines were left for 45 to 60 minutes before being retrieved. Any wrasse caught were transferred to a large plastic bag filled with local sea water on board the boat. Once ashore, the fish were transferred to fresh sea water before the bags were oxygenated and sealed for transport. Care was taken not to let the bags overheat. The number of fish caught was small due to several factors: the limited number of creels available for fishing, the time consuming nature of the fishing method and constraints of the weather on the number of sampling trips undertaken. However, the fish that were obtained were in perfect condition and the exact location of capture was known as well as the time in captivity.

3A.2.2 Farm Held Fish

3A.2.2.1: Selection of Sampling Sites and Collection of Fish

Five separate farm sites were sampled from the west coast of Scotland. These sites were numbered 1-5 and their locations are shown in Figure 3A.1. Wrasse held in farm pens were found to frequent the bottom of the nets including the dead sock, it was

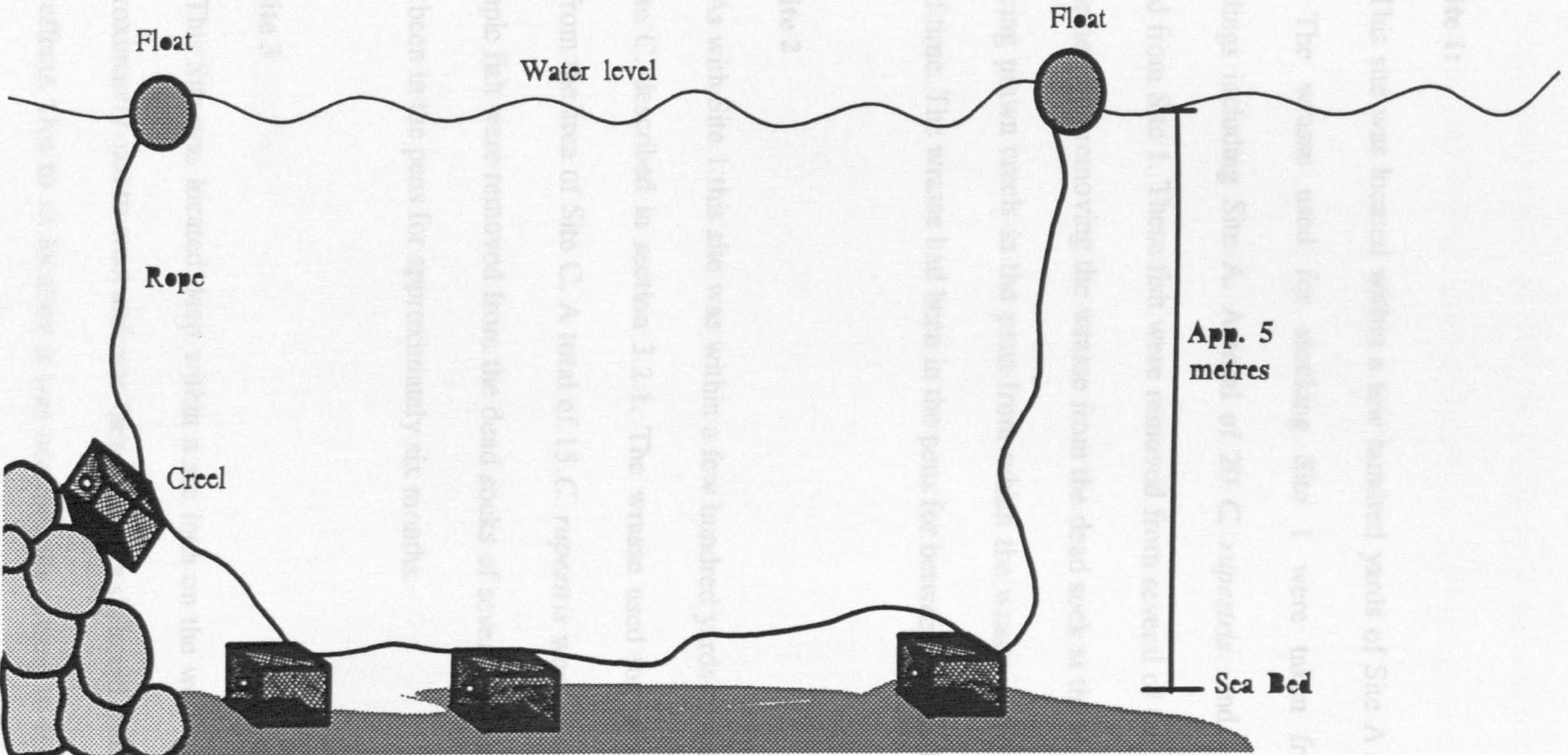


Fig 3A.2: Wrasse capture apparatus, not to scale.

found that shallowing the nets to gain access to the dead sock was a convenient method of collecting wrasse from the pens.

Farm Site 1:

This site was located within a few hundred yards of Site A described in section 3A.2.1. The wrasse used for stocking Site 1 were taken from the immediate surroundings including Site A. A total of 20 *C. rupestris* and 14 *C. exoletus* were examined from Site 1. These fish were removed from several of the pens on the farm by lifting the nets and removing the wrasse from the dead sock at the bottom of the nets and also placing prawn creels in the pens from which the wrasse could be removed after a period of time. The wrasse had been in the pens for between four and nine months.

Farm Site 2

As with Site 1 this site was within a few hundred yards of one of the wild sample sites, Site C, described in section 3.2.1. The wrasse used for stocking the farm were caught from the area of Site C. A total of 15 *C. rupestris* were examined from this site. The sample fish were removed from the dead socks of several different pens on the farm and had been in the pens for approximately six months.

Farm Site 3

This Site was located deep within a sea loch on the west of Scotland. The loch ran approximately east to west and was therefore very sheltered from any severe weather or tidal effects. Due to its location it was occasionally subject to reduced salinity due to fresh water run-off from the surrounding land. This was more of a problem during the

winter months. The wrasse used to stock the farm were obtained from a variety of locations, one of which was Site B, described in section 3.2.1. A sample of nine *C. rupestris* were examined from this site, they had been in the pens for approximately eight months. The sample fish were removed from the pens by lifting the dead socks.

Farm Site 4

Site 4 was also located on the west of Scotland. Although the farm was coastal it was reasonably sheltered by the Island of Mull to the west and by Loch Linnhe to the north. The fish used to stock the site were originally caught on the south coast of Mull and stocked in the farm pens approximately eight months before being sampled. They were removed from the dead socks of several different pens on the farm.

Farm Site 5

Site 5 was located on the eastern shore of the island of Skye on the west coast of Scotland. It was in a sheltered location but was subjected to quite strong tidal action. The wrasse used to stock the farm were caught from the immediate surroundings of the farm approximately one year before being sampled. A total of eight *C. exoletus* and two *C. rupestris* were sampled.

3A.2.3: Captive Fish

In addition to these farm held samples of wrasse, two samples were obtained from other locations where these wrasse were held captive.

Captive Site I

A sample of 12 *C. rupestris* were obtained from the wrasse breeding facilities at Hunterston. These wrasse had been caught in the Firth of Clyde approximately six months before being sampled as broodstock and were surplus to requirements. During their time in the facilities they had received one oxytetracycline treatment for a suspected bacterial infection and four formalin bath treatments for suspected protozoan infections. The exact dates and dosages used for these treatments were not known.

Captive Site II

A sample of five *C. rupestris* and one *C. exoletus* were obtained from a public aquarium on the west coast of Scotland. The fish were originally supplied by Farm Site 3 and had been held in various display tanks for approximately six months before being sampled. The fish were originally caught off the south coast of Mull.

3A.2.4: Incidental Samples

Nematodes removed from the intestine of three *C. rupestris,* obtained from the Oban area on the west coast of Scotland, were supplied fixed in 80% ethyl alcohol by Dr. M. Sayer, Dunstaffanage Marine Laboratory. The fish were part of a population study being carried out by Dr. Sayer. The nematodes were cleared in beechwood creosote for identification.

Parasites dissected from *C. rupestris* and *S. melops* from the coast around the Isle of Cumbrae were supplied by Dr. M. Sayer. The fish were part of the above study being carried out by Dr. Sayer. The samples were from 13 *C. rupestris* and 46 *S. melops*.

The parasites were received fixed in 80% ethyl alcohol. The parasites were cleared in beechwood creosote for identification in the case of the nematodes and in the case of acathocephalans, examined grossly using a dissecting microscope.

3A.2.5: Fish Maintenance

Fish from the different sample locations were transported live to the laboratory where they were kept in static aquaria with internal filtration (Fluval internal filters with mesh insert) and held at a constant temperature of 12°C prior to examination for bacteria and parasites. Fish from different localities were held separately in sea water from the location of capture. Each sample of fish was stocked into clean tanks which had been washed, sterilised and thoroughly dried, including the filters and media, beforehand. This ensured that no cross-infection between samples could take place. Fish were examined as soon after capture as possible, however, due to the length of time needed to examine individual fish thoroughly, some of the fish in the samples were held for a maximum of 14 days prior to examination. The fish were offered grade 1 *S. salar* pelleted food but only some of the fish fed during this time.

3A.2.6: Post Mortem Procedures

The fish were killed by severing the spinal column just behind the head and a full examination was carried out on each fish immediately post mortem as follows:-

1) After the spinal column was severed, blood was drawn out of the blood vessel within the spinal column. A drop of this blood was placed on a slide and allowed to clot before being covered with a cover slip and placed in a covered petri dish for later

examination. A second drop of blood was smeared onto a separate slide and allowed to air dry. The slide was then fixed in absolute methanol for thirty seconds and allowed to dry prior to staining with Giemsa.

2) Skin scrapes were taken from each side of the fish. On larger fish scrapes were also taken from the dorsal surface. These scrapes were spread thinly on a glass slide and covered with a coverslip, and were then kept moist in a covered petri dish for later examination.

3) The right operculum was then removed with scissors and the gills removed. These were placed in 10% buffered formalin for fixation for subsequent histological examination.

4) The left operculum was removed and the gill arches were dissected out using small scissors. The gill bars were then separated from the filaments using a scalpel before the filaments were mounted in sea water on a slide. These preparations were then placed in a covered petri dish for later examination.

5) An incision was then made on the ventral surface of the fish from the rear vent to the pericardial cavity using a scalpel and a flap of skin from the left flank was removed and the internal organs teased away from the kidney by blunt dissection.

6) A heat sterilised wire loop was used to remove a small sample of kidney. This was used to inoculate sterile agar plates containing various medias, see section 3.2.8. These plates were then placed in an incubator at 22°C.

7) Samples of liver, intestine, gonad, heart, spleen and kidney were removed and placed in 10% buffered formol saline for fixation. In the case of the kidney samples, steaks were removed from the centre of the fish to include muscle skin and kidney tissue in the same sample.

3A.2.7: Parasitological Examination

The skin and gills of the fish were examined grossly for any obvious parasites or lesions. The skin scrapes, gill squashes and blood clots prepared earlier were then examined for any protozoan or metazoan parasites using an SM-LUX compound microscope. The skin and gill preparations were scanned at x250 magnification and the blood clot at x400 magnification.

The internal organs of the fish were then removed, placed in fish physiological saline and examined grossly for parasites using an Olympus binocular microscope at x10 and x40 magnification. The liver and spleen were teased apart using forceps and the intestine opened along its length using scissors. Squashes were then made of the tissues and these examined for parasites using the compound microscope at x100 to x400 magnification.

Any digenean metacercariae found in the visceral cavity were removed. Some of these were dissected from their capsules using tweezers prior to being placed in Berland's fluid (9 parts Glacial Acetic Acid and 1 part formalin) for five to ten minutes for fixation and relaxation. They were then placed in 80% ethyl alcohol for storage. The remaining metacercariae were placed directly into 80% ethyl alcohol. The digenean metacercaria *C. lingua* was not dissected from the fish, but the location and numbers of these parasites was noted.

Any adult digenean and nematode parasites found were placed in Berland's fluid for 5-10 minutes to relax and fix the specimen before they were transferred to 80% ethyl

alcohol for storage (Baylis 1922, Berland 1982). Digenean parasites were then identified with the aid of standard texts (Bray & Gibson 1982 & 1986, Nicoll, 1910, 1913a, 1913b,1916,)

Monogenean parasites were fixed and stored in 80% ethyl alcohol.

Digeneans and monogeneans were stained using Mayer's Paracarmine after the procedures detailed in the British Museum (Natural History), Department of Zoology (Parasitic Worms Division), information sheets. These are only available from the British Museum.

Larval acanthocephalans and larval cestodes were placed in distilled water and stored at 4°C for 4-12 hours to evert the proboscis before being transferred to 80% ethyl alcohol for fixation and storage. Acanthocephalans and cestodes were identified using Yamaguti (1959)

Nematode parasites were fixed and stored in 80% ethyl alcohol and were identified using descriptions in the literature (Berland 1961, Yoshinaga, Ogawa & Wakabayashi 1987 and Cannon 1977)). Acanthocephalans and nematodes were cleared with beechwood creosote to aid identification.

Crustacean parasites were placed directly into 80% ethyl alcohol for fixation and storage and were identified using Kabata (1979).

The site of all parasites was carefully noted and care was taken to label parasites from individual fish and to count the total numbers recovered. In the case of protozoa on the skin and gills, an arbitrary six point scale was used to indicate the level of infection. If no protozoa were present a score of zero was given, and if the infection was very high then a score of five was given. Infections between these two levels were given appropriate values. Due to the ability of many protozoan parasites to multiply quickly in tank conditions it was felt more important to record the presence and relative severity of infections rather than the total number of parasites present.

Data are presented as prevalence, [defined as the number of individuals of a host species infected with a parasite divided by the number of hosts examined multiplied by 100 to give a percentage], abundance, [defined as the total number of a parasite species divided by the total number of host species (infected and uninfected) in the sample, and range, [defined as the least number of parasites noted to the greatest number noted in the host species in the sample] (Margolis, Esch, Holmes, Kuris & Shad 1982).

3A.2.8: Bacteriology

Sterile loops were used to remove smears from the kidney and any lesions observed on all the fish in all three sampling site groups. These smears were streaked onto agar plates containing the following medias :-

A. Tryptone Soya Agar

B. Tryptone Soya Agar with Sodium Chloride

C. Mueller Hinton media

The formula and methods of preparation for these media are detailed in Appendix. These three medias were chosen so that as large a range of bacteria could be detected as possible without the use of many different media types.

These plates were then incubated at 22°C for 3 to 4 days. If any significant growth was observed on the plates then standard identification techniques were employed to type the bacteria. Details of the techniques used are given in the Appendix. If no growth was detected then the plates were incubated at 22°C for a further two weeks. If there was still no growth present then the plates were recorded as zero.

3A.2.9: Virology

3A.2.9.1: Wild Samples

A sample of 10 *C. rupestris* from Site A was tested for the presence of viruses by the Marine Laboratory in Aberdeen. The 10 fish were split into two pools of five. The fish were killed by severing the spinal column immediately behind the head. The spleen, kidney and intestine of each fish was then placed in Hank's Balanced Salt Solution. The two pools of tissues were then sent to the Marine Laboratory where they were tested on CHSE cells for the presence of viruses. Details of the techniques used are given in the materials and methods section of Chapter 4.

3A.2.9.2: Farm Samples

In addition to sampling for bacteria and parasites, the fish from Site 2 were also sampled for the presence of viruses. The techniques used were the same as described in section 3A.2.9.1.

3A.3: RESULTS

3A.3.1: Wild Caught Fish

A total of 25 fish consisting of 19 *C. rupestris*, six *C. exoletus* and one *L. mixtus* were examined from Site A. Nineteen were examined from Site B, 18 *C. rupestris* and one *S. melops*, and twenty from Site C, 19 *C. rupestris* and one *L. mixtus*.

Twelve species of parasites were found in *C. rupestris*, seven species in *C. exoletus*, seven in *L. mixtus* and three in the *S. melops*.

Most of the parasites had a single preferred site in the body although some were found in more than one location. For example metacercariae of the digenean *C. lingua* were found in the skin, fins and gills and the acanthocephalan *Corynosoma* sp. was seen throughout the viscera.

3A.3.1.1 *Ctenolabrus rupestris*

The average weights and lengths of the fish together with the ranges are shown in Table 3A.2. Table 3A.3 contains the data for prevalence, mean abundance and range for the parasites recovered from *C. rupestris* from the three sample sites.

As shown in Table 3A.3 the prevalence of *Trichodina* spp. infections was similar in both Site A and C with approximately one third of the fish infected, however, in the sample from Site B high prevalence of infection was noted with approximately two thirds of the fish infected. Trichodinids were only seen on the gills of *C. rupestris* and never on the skin.

TABLE 3A.2: Length and weight data for *C. rupestris* from the three sample sites.

Location	Mean Weight (g)	Weight Range (g)	Mean Length (mm)	Length Range (mm)
Site A N=18	24.64	5.05 - 50.14	99.6	63 - 132
Site B N=18	20.66	8.86 - 44.10	97.9	74 - 127
Site C N=19	28.61	14.82 - 41.79	107.0	87 - 122

Parasite species (and developmental stage)	Site of infection	Site A, N=18			Site B, N=18			Site C, N=19		
		P	A	R	P	A	R	P	A	R
Protozoans										
Trichodina spp.	Skin & Gills	27.8	3	1-5	66.7	4	2-5	31.6	3	2-5
Monogenea										
Microcotyle donavini Van Beneden & Hesse (adult)	Gills	5.6	0.1	0-1		0			0	
Digenea										
Cryptocotyle lingua (Creplin) (metacercaria)	Skin & Gills	77.8	37.7	0-166	94.4	89.8	0-348	94.7	155.1	0-661
Lecithochirium rufoviride (Rudolphi) Lühe (metacercaria)	Connective tissue	72.2	2.4	0-9	27.8	0.4	0-3	10.5	0.2	0-3
Macvicaria alacris Loosse (adult)	Intestine	66.7	4.3	0-18	77.8	3.1	0-8	10.5	0.3	0-3
Nematoda										
Contracaecum osculatum Rudolphi (L3 larvae)	Connective tissue (Liver)	61.1	2.9	0-16	94.4	8.3	0-23	94.7	18.0	0-96
Hysterothylacium aduncum Rudolphi (L3 larvae)	Connective tissue	44.4	4.0	0-42	72.2	3.1	0-8	89.5	9.3	0-53
Anisakis simplex Rudolphi (L3 larvae)	Connective tissue	5.6	0.9	0-16	66.7	1.1	0-5	52.6	3.8	0-18
Acanthocephala										
Corynosoma sp. (acanthella)	Connective tissue	16.7	0.3	0-3	44.4	1.7	0-8	10.5	0.2	0-2
Crustacea										
Caligus centrodonti Baird (pre-adult)	Skin	16.7	0.2	0-1	27.8	0.4	0-3	15.8	0.3	0-2
Hatschekia cluthae (Scott) (adult)	Skin	5.6	0.2	0-1	5.6	0.1	0-1	5.3	0.1	0-1

TABLE 3A.3: Parasite prevalence, mean abundance and range data for *C. rupestris* from the three sample sites.
P= Prevalence, A= Abundance, R= Range of infection.

A single specimen of the monogenean *Microcotyle donavini* Van Beneden & Hesse was found in the survey on the gills of a single *C. rupestris* from Site A (Fig 3A.3).

The metacercaria of *C. lingua* was the most common of the three digenean species seen in *C. rupestris* achieving almost 100% prevalence in both the Site B and Site C samples. The mean abundance of infection was also high, but, the level of infection in different individuals varied greatly, ranging from 2-661, for the Site C sample.

The prevalence data for *Lecithochirium rufoviride* (Rudolphi) Lühe (Fig 3A.4), the only other metacercaria found, was more variable than *C. lingua*. Site A fish had the highest infection rate at 72.2% whereas in Site C it was 19.5%. However, the abundance of infection in all the sites was low at less than 2.5 parasites per fish.

Adults of *Macvicaria alacris* (= *Lebouria alacris*, =*Plagioporous alacer*) Looss (Fig 3A.5) was also common in the samples, more so in the Site A and Site B samples, where prevalence was high and mean abundance moderate. The highest number of these parasites was seen in a fish from the Site A sample which harboured 18 individuals in the intestine. Both mean abundance and prevalence for *M. alacris* was far lower in the Site C sample than in the other two sites.

Three species of nematodes were found in the fish, *Contracaecum osculatum* Rudolphi, *Hysterothylacium aduncum* Rudolphi and *Anisakis simplex* Rudolphi, were

Key to abbreviations for figure 3A.3 :

M - mouth.

GA - genital atrium.

IC - intestinal caecum.

T - testes.

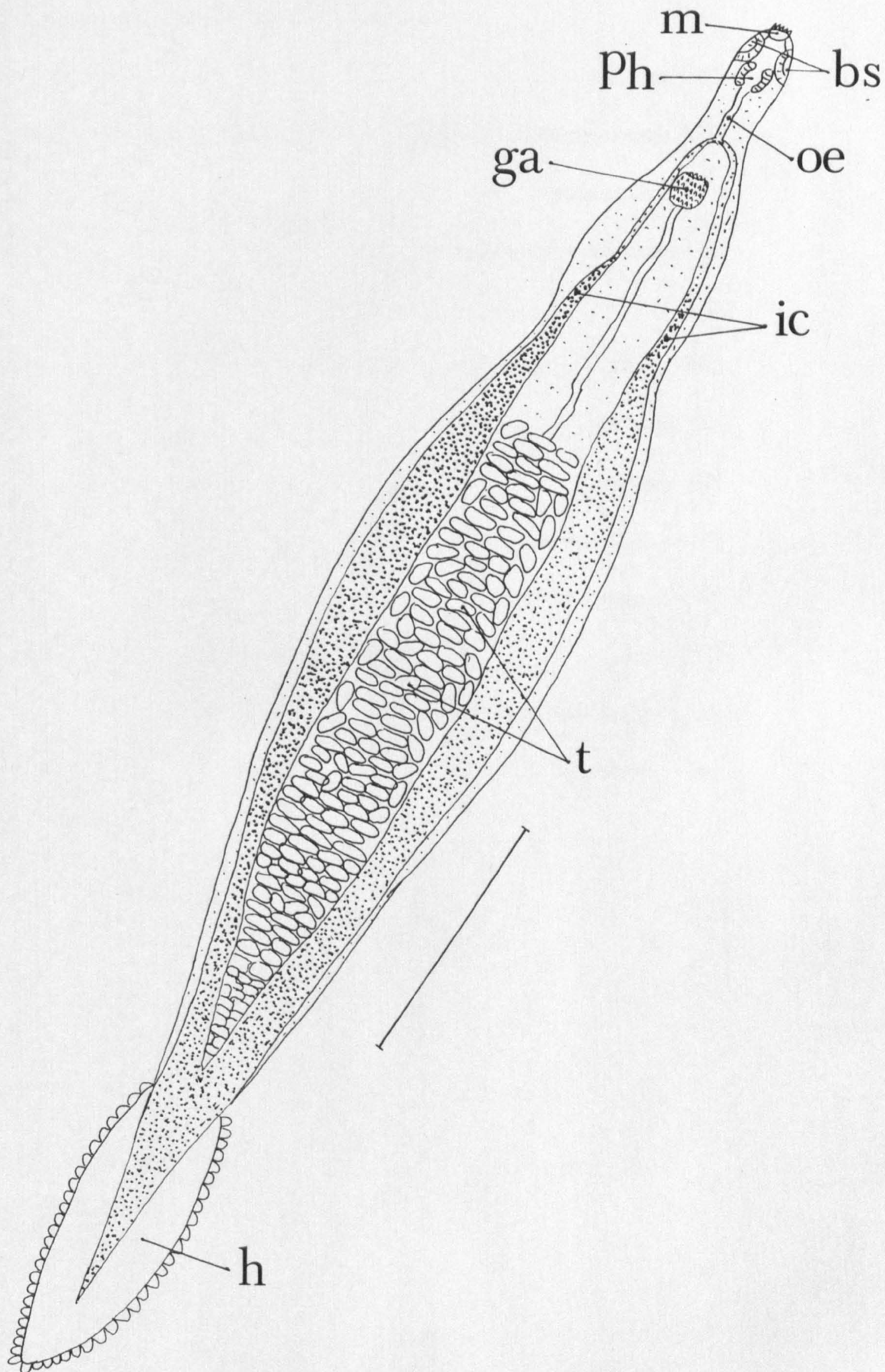
Ph - pharynx.

BS - buccal sucker.

H - haptor.

OE - oesophagus.

Fig 3A.3: *M. donavini*, partly after van Beneden & Hesse (1864). scale bar = 1mm.



Key to abreviations for Figures 3A.4 & 3A.5.

OS - oral sucker.

VS - ventral sucker.

CP - cirrus pouch.

ID - intenstinal diverticulum.

O - ovary.

OV - ovum.

T1, T2 - testes.

PH - pharynx.

GA - genatil atrium.

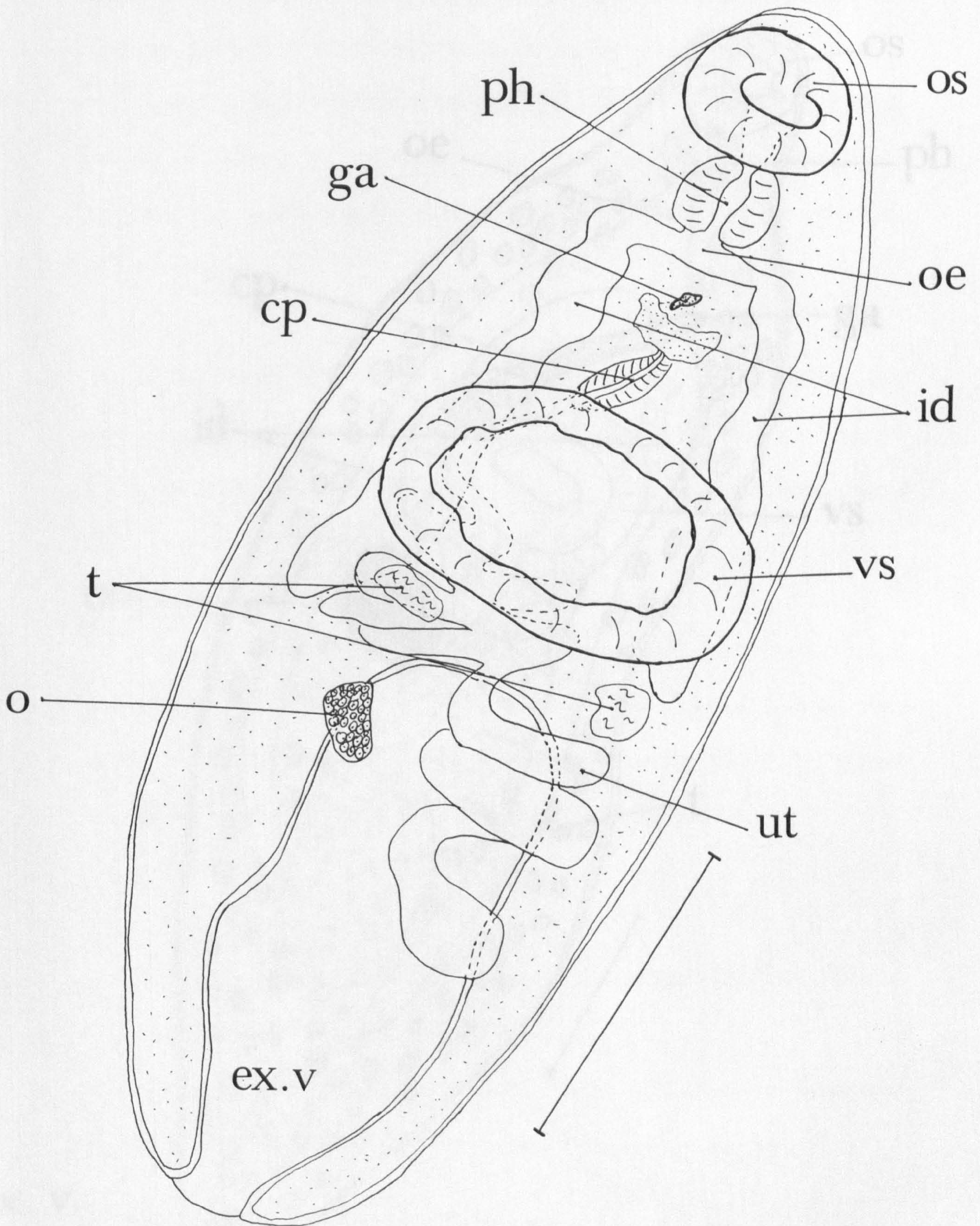
EV - excretory vesicle.

UT - uterus.

SR - seminal recepticle.

OE - oesophagus.

Fig 3A.4: Metacercaria of *L. rufoviride*, composite drawing from specimens stained with Mayers Paracarmine. Scale bar = 200μm.



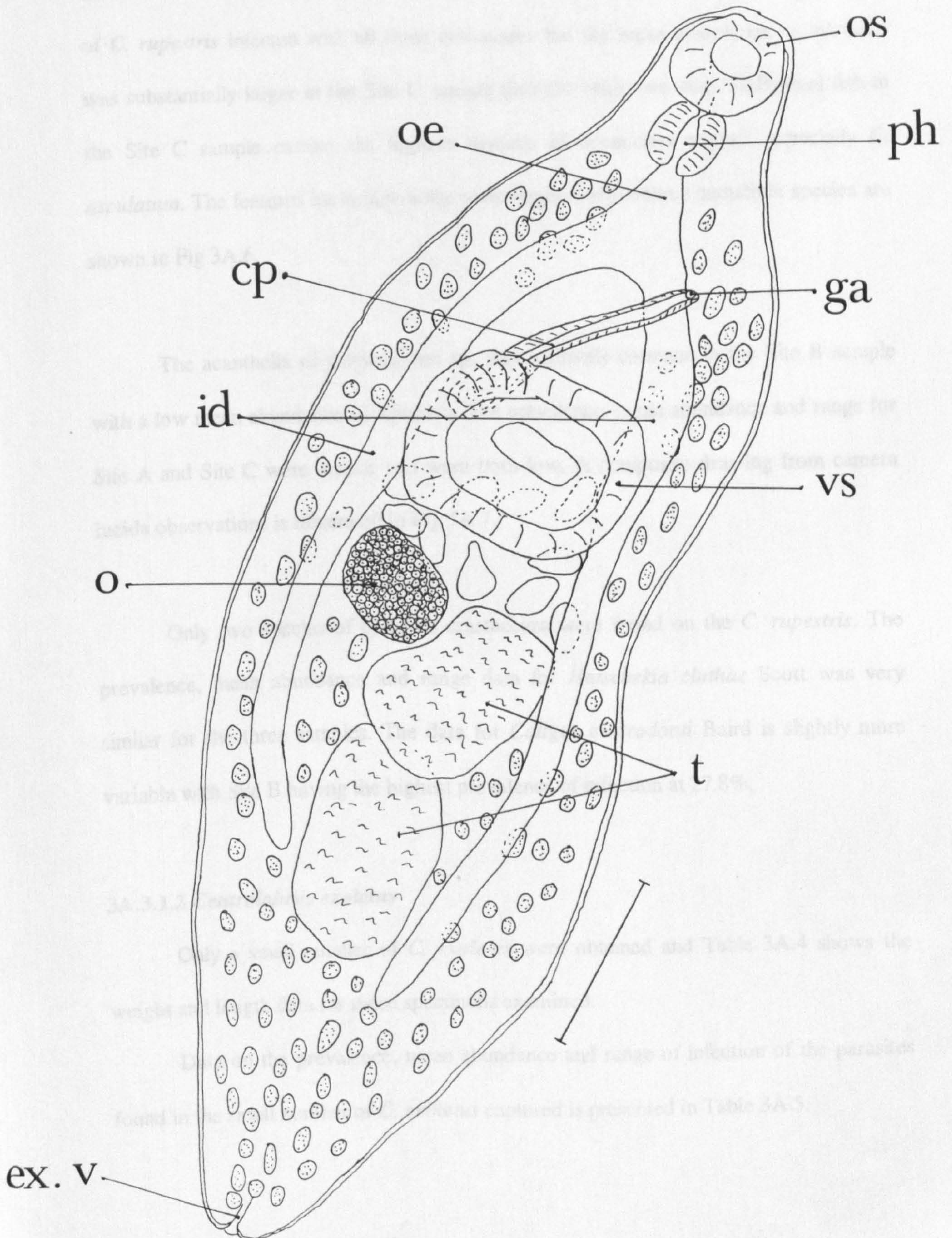


Fig 3A.5: Adult *M. alacris*, some details from Gibson & Bray (1982). Scale bar = 200μm.

common in all three of the sites sampled, with the exception of *A. simplex* in the sample from Site A which had a prevalence of only 5.6%. Site B and Site C had similar numbers of *C. rupestris* infected with all three nematodes but the mean abundance of infection was substantially larger in the Site C sample than the other two sites. Individual fish in the Site C sample carried the highest burdens of nematodes overall, especially *C. osculatum*. The features important in the identification of the three nematode species are shown in Fig 3A.6.

The acanthella of *Corynosoma* sp. was relatively common in the Site B sample with a low mean abundance of infection. The prevalence, mean abundance and range for Site A and Site C were similar and were both low. A composite drawing from camera lucida observations is illustrated in Fig 3A.7.

Only two species of parasitic crustaceans were found on the *C. rupestris*. The prevalence, mean abundance and range data for *Hatschekia cluthae* Scott was very similar for the three samples. The data for *Caligus centrodonti* Baird is slightly more variable with Site B having the highest prevalence of infection at 27.8%.

3A.3.1.2 *Centrolabrus exoletus*

Only a small number of *C. exoletus* were obtained and Table 3A.4 shows the weight and length data for those specimens examined.

Data on the prevalence, mean abundance and range of infection of the parasites found in the small number of *C. exoletus* captured is presented in Table 3A.5.

Key to abbreviations for figures 3A.6 & 3A.7

Nematodes (Fig 3A.6)

- BT - boring tooth.
- NR - nerve ring.
- EP - excretory pore.
- ED - excretory duct.
- EC - excretory canal.
- AN - anus.
- R - rectum.
- INT - intestine.
- IC - intestinal ceacum.
- VA - ventricular appendix.
- PV - preventriculum.
- V - ventriculum.
- OE - oesophagus.
- M - mucron.

Acanthocephala (Fig 3A.7)

- PB - proboscis.
- PR - proboscis receptacle.
- T1, T2 - testes.
- CG - cement gland.
- CR - cement reservoir.
- NM - nerve mass.

Fig 3A.6: Identification features of the three nematode species involved in the present study. A - *C. osculatum*, B - *H. aduncum* and & C - *A. simplex*. After Smith & Wootten (1984 a & b), scale bars = 200µm.

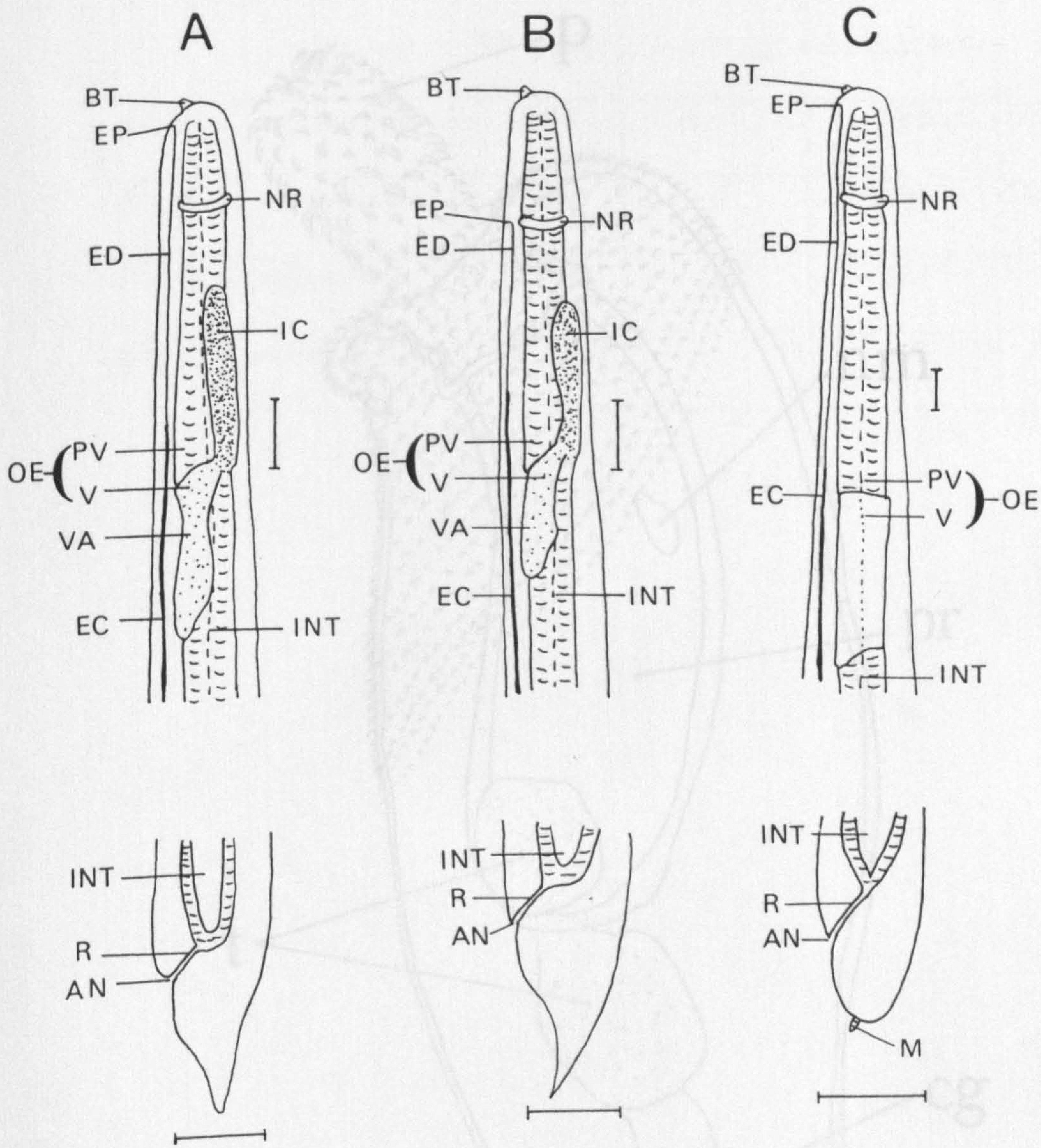


Fig 3A.7: Acanthella of *Corynosoma* sp.. Composite drawing from camera lucida observations, scale bar = 200μm.

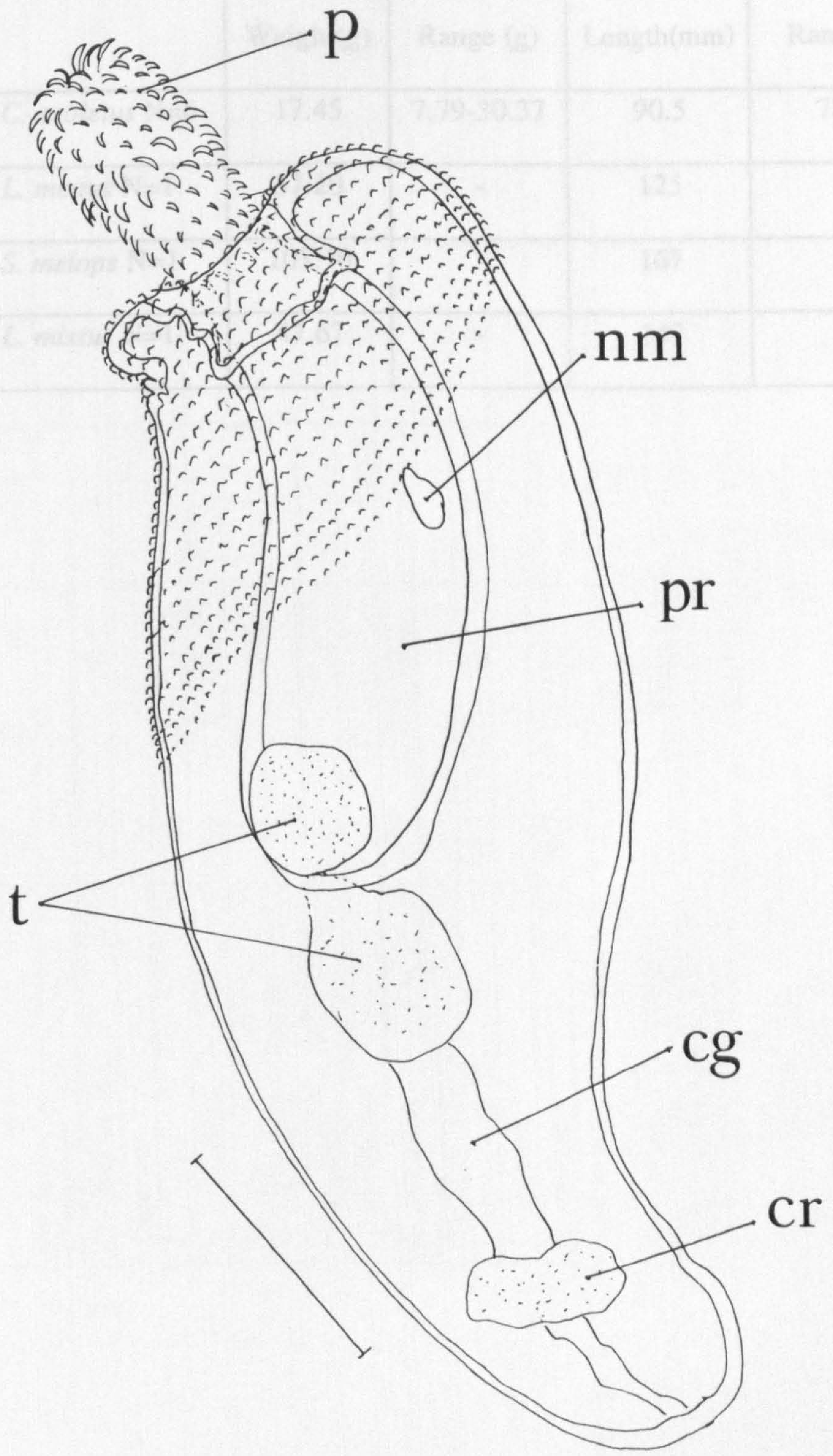


Table 3A.4: Weight and length data for *C. exoletus*, *L. mixtus* and *S. melops* for Sites A, B & C.

Site No. & species	Mean Weight(g)	Weight Range (g)	Mean Length(mm)	Length Range(mm)
Site A, *C. exoletus* N=6	17.45	7.79-30.37	90.5	72-110
Site A, *L. mixtus* N=1	37.20	-	125	-
Site B, *S. melops* N=1	101.70	-	167	-
Site C, *L. mixtus* N=1	47.67	-	147	-

Parasite species	*C. exoletus* Site A N=6			*L. mixtus* Site A N=1	*L. mixtus* Site C N=1	*S. melops* Site B N=1
	P	A	R	Total	Total	Total
Protozoa						
Trichodina spp.	16.7	1	0-1	0	4 (Skin)	4 (Skin and Gills)
Digenea						
C. lingua	100	17	3-42	3	5	15
L. rufoviride	33.3	0.3	0-1	0	0	0
M. alacris	100	7.7	1-23	1	13	0
H. pulchella		0		8	3	0
Nematoda						
C. osculatum		0		1	0	0
H. aduncum	16.7	0.2	0-1	0	2	0
Acanthocephala						
Corynosoma sp.	16.7	0.2	0-1	0	0	0
Crustacea						
H. cluthae	16.7	0.2	0-1	1	0	0

Table 3A.5: Parasite prevalence, abundance and range data for *C. exoletus* and total parasite numbers for *L. mixtus* and *S. melops* from sample Sites A, B & C.

P= Prevalence, A= Abundance and R= Range of infection.

The prevalence of infection with *Trichodina* spp. in *C. exoletus* was low as with the *C. rupestris*.

Also similar to the *C. rupestris*, there was a high prevalence of *C. lingua* (100%), however, the mean abundance and range was far lower than in *C. rupestris*. *M. alacris* also reached a prevalence of 100%, compared to a maximum of approximately 78% in *C. rupestris*, with the abundance and range also relatively high with one fish harbouring twenty three individuals. One third of the fish were also infected with *L. rufoviride* but the mean abundance and range of infection of this parasite were very low.

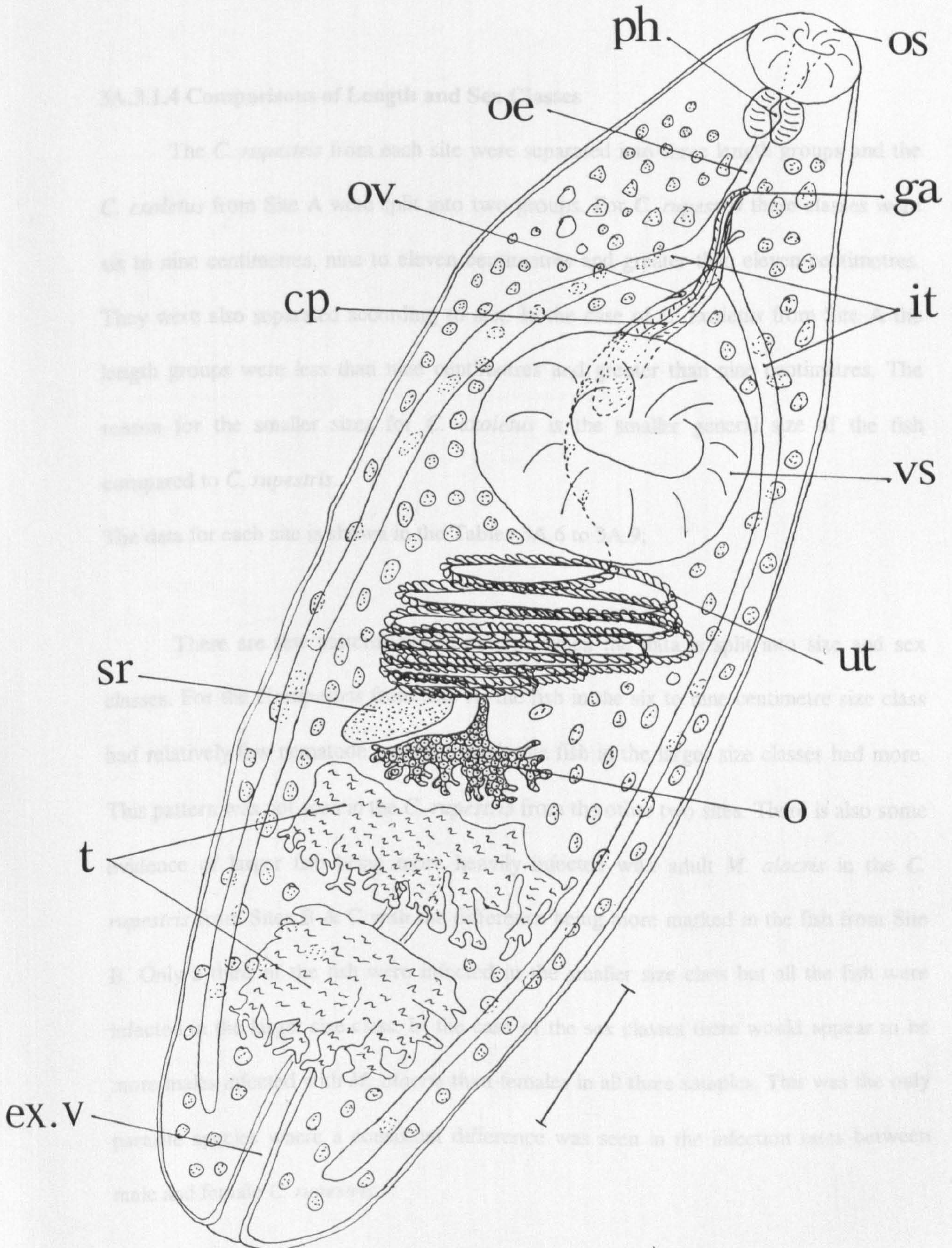
The only nematode species found in the *C. exoletus* was *H. aduncum* and the number of fish infected was low. Numbers were also generally low for the *Corynosoma* sp. and *Hatschekia cluthae* (Scott).

3A.3.1.3: *Labrus mixtus* and *Symphodus melops*

The data for weight and length for these two species is shown in Table 3A.4. Very few of these species were caught. The data for total number of parasites found in the *L. mixtus* and *S. melops* is included in Table 3A.5.

The two *L. mixtus* examined harboured most of the parasites already seen in the other species of wrasse, but, in addition, they both contained specimens of the adult digenean *Helicometra pulchella* Rud. which was not seen in any of the other species of wrasse, Fig 3A.7 illustrates this species of digenean. The trichodinids seen on the Site C fish were only seen on the skin. As was the case with *C. rupestris* and *C. exoletus*.

Fig 3A.8: Adult *H. pulchella*, adapted from Nicoll (1910), scale bar = 200μm. Abreviations as for Figs 3A.5 & 6.



The *S. melops* examined harboured few parasites with trichodinids being seen on both the skin and the gills of the fish.

3A.3.1.4 Comparisons of Length and Sex Classes

The *C. rupestris* from each site were separated into three length groups and the *C. exoletus* from Site A were split into two groups. For *C. rupestris* these classes were six to nine centimetres, nine to eleven centimetres and greater than eleven centimetres. They were also separated according to sex. In the case of *C. exoletus* from Site A the length groups were less than nine centimetres and greater than nine centimetres. The reason for the smaller sizes for *C. exoletus* is the smaller general size of the fish compared to *C. rupestris*.

The data for each site is shown in the Tables 3A.6 to 3A.9;

There are few patterns which emerge when the data is split into size and sex classes. For the *C. rupestris* from Site A, the fish in the six to nine centimetre size class had relatively few nematode parasites while the fish in the larger size classes had more. This pattern was not seen in the *C. rupestris* from the other two sites. There is also some evidence of larger fish being more heavily infected with adult *M. alacris* in the *C. rupestris* from Sites B & C with the difference being more marked in the fish from Site B. Only a third of the fish were infected in the smaller size class but all the fish were infected in the larger size class. In the case of the sex classes there would appear to be more males infected with *M. alacris* than females in all three samples. This was the only parasite species where a consistent difference was seen in the infection rates between male and female *C. rupestris*.

Parasite species	6-9 cm N=5			9-11cm N=7			11+ cm N=6			Males N=10			Females N=8		
	P	A	R	P	A	R	P	A	R	P	A	R	P	A	R
Protozoans															
Trichodina sp.	40	1	0-4	28.6	1	0-5	30	1	0-3	30	1	0-5	25	0.5	0-4
Monogenea															
M. donavini		0			0		17	0.2	0-1	10	0.1	0-1		0	
Digenea															
L. rufoviride	66.7	0.7	0-1	85.7	2.6	0-5	83.3	2.7	0-9	90	2	0-5	62.5	2.2	0-9
M. alacris	60	2.6	2-23	85.7	5.6	0-18	50	4.3	0-10	90	6.1	0-18	37.5	2.9	0-14
H. pulchella	0				0			0			0			0	
C. lingua	100	22	2-71	85.7	67.8	0-166	50	22	0-199	90	67.8	0-166	62.5	8.9	0-33
Nematoda															
C. osculatum	20	0.2	0-1	57.1	2.3	0-9	100	6	1-16	60	2.5	0-16	50	3.2	0-10
H. aduncum		0		57.1	6.4	0-42	66.7	4.5	0-19	40	2.2	0-19	62.5	6.4	0-42
A. simplex		0			0		16.7	2.7	0-16	10	1.6	0-16		0	
Acanthocephala															
Corynosoma sp.	20	0.2	0-1	14.3	0.1	0-1	16.7	0.5	0-3	10	0.1	0-1	25	0.5	0-1
Crustacea															
C. centrodonti		0		28.6	0.3	0-1	33.3	0.3	0-1	10	0.1	0-1	25	0.2	0-1
H. cluthae	20	0.2	0-1		0			0		20	0.2	0-1	12.5	0.1	0-1

Table 3A.6: The parasites according to three different size classes and both sex classes of *C. rupestris* from Site A.

P= Prevalence, A= Abundance, R= Range of infection.

Parasite species	6-9 cm N=6			9-11cm N=9			11+ cm N=3			Males N=6			Females N=12		
	P	A	R	P	A	R	P	A	R	P	A	R	P	A	R
Protozoans															
Trichodina	83.3	4	0-5	55.6	3	0-5	66.7	1	0-4	66.7	4	0-5	66.7	3	0-5
Digenea															
L. rufoviride	33.3	0.8	0-3	33	0.3	0-1		0		33.3	0.5	0-2	25	0.4	0-3
M. alacris	33.3	0.8	0-2	88.9	3.3	0-8	100	3.7	1-8	100	3.2	1-8	66.7	2.2	0-8
H. pulchella	0				0			0			0		0		
C. lingua	100	66.3	19-348	88.9	57.4	0-133	100	28.7	6-66	100	90	42-193	91.7	83.6	0-348
Nematoda															
C. osculatum	100	9.7	3-16	88.9	7	0-23	33.3	0.3	0-1	100	5.7	1-16	91.7	9.5	0-23
H. aduncum	83.3	3.3	0-8	55.6	2.2	0-7		0		66.7	2	0-5	75	3.6	0-23
A. simplex	66.7	1.7	0-5	55.6	0.8	0-3	100	1	0-1	66.7	1	0-3	66.7	1.2	0-5
Acanthocephala															
Corynosoma sp.	16.7	0.3	0-2	55.6	1.9	0-6		0		33.3	0.8	0-4	50	2.2	0-8
Crustacea															
C. centrodonti		0		33.3	0.6	0-3	33.3	1	0-1		0		33.3	0.5	0-3
H. cluthae		0		11.1	0.1	0-1		0			0		8.3	0.1	0-1

Table 3A.7: Parasites from three different size classes and both sex classes of *C. rupestris* from Site B.

P= Prevalence, A= Abundance, R= Range of infection.

Parasite species	6-9 cm N=1	9-11cm N=10			11+ cm N=8			Males N=11			Females N=8		
	Total	P	A	R	P	A	R	P	A	R	P	A	R
Protozoans													
Trichodina sp.	0	40	1	0-5	12.5	1	0-5	36.4	1	0-5		0	
Digenea													
L. rufoviride	0	10	0.3	0-3	12.5	0.1	0-1	9.1	0.1	0-1	12.5	0.4	0-3
M. alacris	0	10	0.2	0-2	12.5	0.4	0-3	18.2	0.4	0-3		0	
H. pulchella	0		0			0		0				0	
C. lingua	45	100	104.5	2-400	87.5	232.1	0-661	90.9	137.6	0-400	100	179.1	5-661
Nematoda													
C. osculatum	23	100	21.4	2-96	87.5	13	0-47	100	21.8	2-96	87.5	12.6	0-37
H. aduncum	7	80	10.3	0-53	100	67	3-20	90.9	12.5	0-53	87.5	4.9	0-12
A. simplex	6	50	4.7	0-18	50	3.8	0-14	45.4	4.3	0-18	62.5	3.2	0-13
Acanthocephala													
Corynosoma sp.	2	10	0.2	0-2	12.5	0.1	0-1		0		12.5	0.2	0-2
Crustacea													
C. centrodonti	0	20	0.3	0-2	25	0.2	0-2	9.1	0.2	0-2		0	
H. cluthae	0	10	0.1	0-1		0		9.1	0.1	0-1		0	

Table 3A.8: Parasites from three different size classes and both sex classes of *C. rupestris* from Site C.

P= Prevalence, A= Abundance, R= Range of infection.

Parasite species	<9 cm N=2			>9 cm N=3		
	P	**A**	**R**	**P**	**A**	**R**
Protozoans						
Trichodina sp.	33.3	0.33	0-1		0	
Digenea						
L. rufoviride		0		66.7	0.7	0-1
M. alacris	100	2.33	1-4	100	13	2-23
H. pulchella		0			0	
C. lingua	100	3	1-5	100	46.5	18-42
Nematoda						
H. aduncum		0		33	0.3	0-1
Acanthocephala						
Corynosoma sp.	33.3	0.33	0-1		0	
Crustacea						
H. cluthae		0		33	0.3	0-1

Table 3A.9: The parasites of two different size classes of *C. exoletus* from Site A

P= Prevalence, A= Abundance and R= Range of infection.

The different size classes of *C. exoletus* from Site A revealed that larger fish were infected with *L. rufoviride*, *H. aduncum* and *H. cluthae* more often than smaller fish. No other differences were present.

Consideration was given to combining the data for the fish from all three wild samples giving a larger data set to investigate size and sex differences. This would involve the testing of significant differences for parasite infections between sites before the data could be confidently combined. The sample sizes from the three sites were considered too small to provide reliable statistical analysis.

3A.3.1.5: Bacteriology

No bacteria were isolated from any of the fish from wild sample Sites A, B or C.

3A.3.1.6: Virology

The fish from Site A tested for the presence of virus all proved to be negative.

3A.3.2 Farm Held Wrasse

The average weights and lengths of the fish from all five farm sites, together with the ranges of these measurements are shown in Table 3A.10. The prevalence, abundance and ranges for the parasites from the fish from Sites 1, 2, 3, and 5 are shown in Table 3A.11.

Table 3A.10: Mean and range of weights and lengths for fish from Sites 1 to 5.

Site No. & species	**Mean weight (g)**	**Weight range (g)**	**Mean length (mm)**	**Length range (mm)**
Site 1, *C. exoletus* N= 14	25.75	13.14-43.32	100	86-122
Site 1, *C. rupestris* N= 2	31.12	23.64-38.80	112.5	105-120
Site 2,*C. rupestris* N= 15	37.03	21.0-80.80	114.3	97-143
Site 3, *C. rupestris* N= 9	28.68	22.07-50.47	102.2	98-132
Site 4, *C. rupestris* N= 10	26.5	17.1-44	108.0	99-123
Site 4, *C. exoletus* N= 2	14.25	11.6-16.9	90.5	84-97
Site 5, *C. rupestris* N= 2	32.65	22.4-42.9	113.5	103-124
Site 5, *C. exoletus* N= 8	27.58	14.4-44.4	105.0	88-124

Parasite species	Farm Site 1 N=14 *C. exoletus*			Farm Site 1 N=2 *C. rupestris*			Farm Site 2 N=15 *C. rupestris*			Farm Site 3 N=9 *C. rupestris*			Farm Site 4 *C. rupestris* N=10			Farm Site 4 *C. exoletus* N=2			Farm Site 5 *C. rupestris* N=2			Farm Site 5 *C. exoletus* N=8		
	P	A	R	P	A	R	P	A	R	P	A	R	P	A	R	P	A	R	P	A	R	P	A	R
Protozoa																								
Trichodinids	50	2	0-4	50	2.5	0-5	50	2	0-4		0		100	2.7	1-5	100	3	3-3	50	2	0-4	62.5	1.5	0-4
Ciliates & Flagellates		0			0			0		12.5	1	0-1		0			0			0			0	
Digenea																								
C. lingua	100	99.2	5-315	100	5	1-9	6.7	0.1	0-1		0		60	50.8	0-300	100	71.5	49-94	100	68.5	48-89	100	38	8-123
L. rufoviride	21.4	1	0-11	50	0.5	0-1	13.3	0.3	0-2	62.5	10.4	0-34	90	10.9	0-37	100	13.5	1-26		0		12.5	1.4	0-11
M. alacris	50	1.9	0-11	50	2.5	0-5	20	0.8	0-6	25	0.25	0-1	30	2.3	0-21	100	18	11-25		0		62.5	3.8	0-14
Z. rubellus	7.1	0.1	0-1		0			0			0			0			0			0			0	
Nematoda																								
A. simplex		0			0		60	1.3	0-5	12.5	0.1	0-1	60	1	0-3		0			0		12.5	0.1	0-1
H. aduncum		0		50	2	0-4	93.3	5	0-16	87.6	1.6	0-5	50	1.9	0-11	50	0.5	0-1		0		12.5	0.1	0-1
C. osculatum	42.9	0.6	0-4	100	1	1	93.3	11.4	0-26	62.5	1.6	0-5	70	4.8	0-29	50	3	0-6	50	0.5	0-1	37.5	0.6	0-3
Acanthocephala																								
Corynosoma sp.	14.3	0.3	0-3	50	0.5	0-1	6.7	0.1	0-2	12.5	0.1	0-1	30	0.3	0-1		0		100	4.5	1-8	25	0.2	0-1
Crustacea																								
L. labrei		0			0			0		10.1	0.1	0-1		0			0			0			0	
Cestoda																								
N. lingualis		0			0		6.7	0.1	0-1	12.5	0.1	0-1		0			0			0			0	

Table 3A.11: Parasite prevalence, abundance and ranges for the fish from Sites 1 to 5.

P= Prevalence, A= Abundance, R= Range of infection.

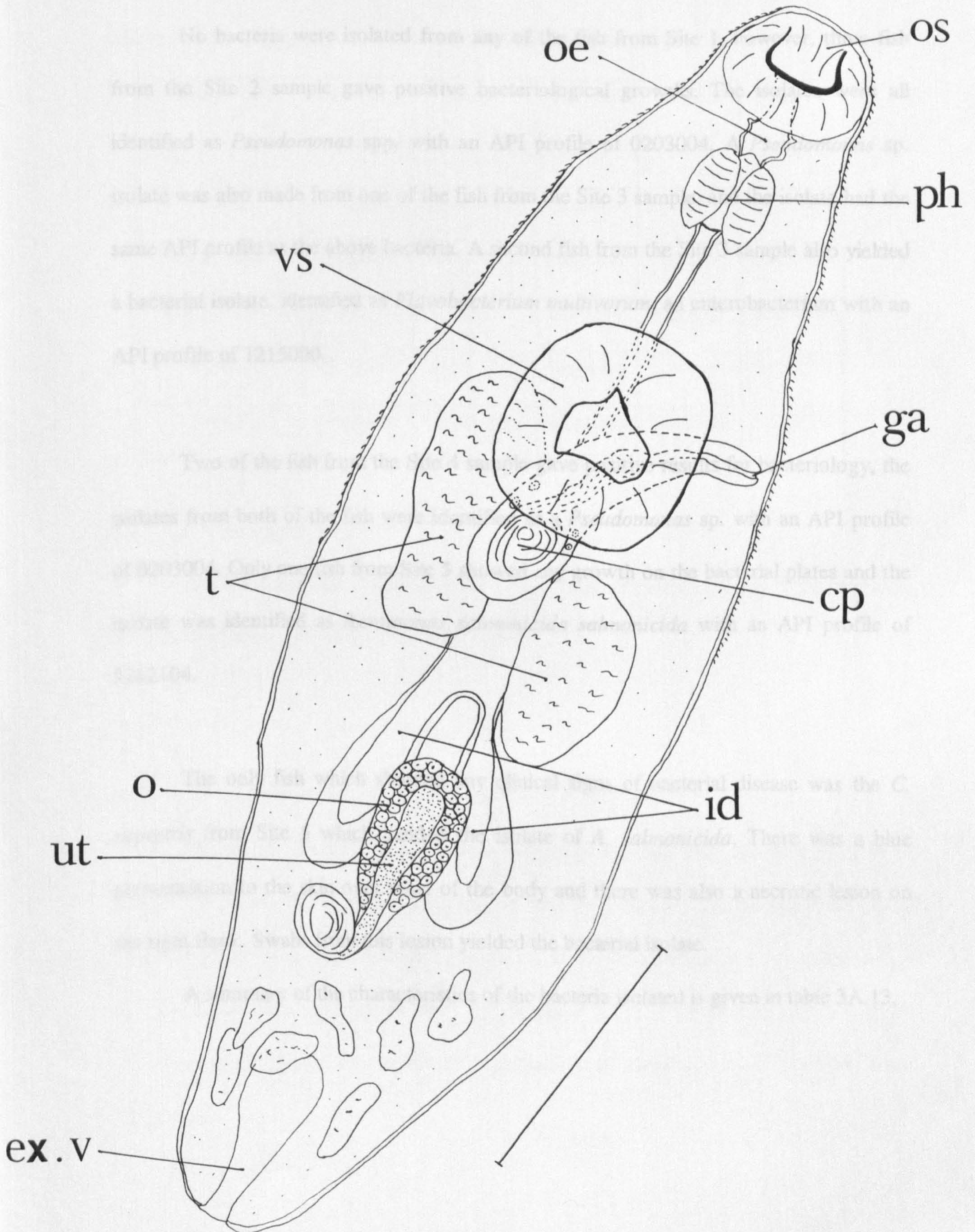
Trichodinids were seen on the fish from all farm sites except Site 3. Prevalence ranged from 50% to 100%, however, abundance was generally low. In the *C. rupestris* from Site 3 there was mixed infection with ciliates and flagellates on one of the fish.

Metacercariae of *C. lingua* were seen in all the samples except for the *C. rupestris* from Sites 1 & 3. Prevalence was high as was abundance. Similarly *L rufoviride* was seen in all sites except the *C. rupestris* from Site 5. The third most common digenean seen in the samples was the adult form of *M. alacris*. This parasite was seen in all groups of fish with the exception of the *C. rupestris* from Site 5. A single specimen of the adult form of *Zoogonus rubellus* (Olsson) was recovered from the intestine of a *C. exoletus* in the Site 1 sample, this species is illustrated in Fig 3A.9. L3 larvae of the nematode *C. osculatum* were seen in all samples of fish. Abundance of infection was particularly high in the *C. rupestris* from Site 1. The prevalence and abundance for the other two nematode species varied though all of the sites in both fish species.

Acanthella of *Corynosoma* sp. were seen in most fish except the *C. exoletus* from Site 4. The prevalence in the samples ranged from zero to 100%, but, the abundance of infection was generally low.

The cestode *N. lingualis* was seen in two of the fish, one *C. rupestris* from Site 2 and one *C. rupestris* from Site 3.

Fig 3A.9: Adult Z. *rubellus*, after Bray & Gibson (1986), scale bar = 200μm.



3A.3.2.2: Bacteriology Testing

No bacteria were isolated from any of the fish from Site 1, however, three fish from the Site 2 sample gave positive bacteriological growths. The isolates were all identified as *Pseudomonas* spp. with an API profile of 0203004. A *Pseudomonas* sp. isolate was also made from one of the fish from the Site 3 sample, and the isolate had the same API profile as the above bacteria. A second fish from the Site 3 sample also yielded a bacterial isolate, identified as *Flavobacterium multivorum*, an enterobacterium with an API profile of 1215000.

Two of the fish from the Site 4 sample gave positive results for bacteriology, the isolates from both of the fish were identified as a *Pseudomonas* sp. with an API profile of 0203004. Only one fish from Site 5 showed any growth on the bacterial plates and the isolate was identified as *Aeromonas salmonicida salmonicida* with an API profile of 5262104.

The only fish which showed any clinical signs of bacterial disease was the *C. rupestris* from Site 5 which yielded the isolate of *A. salmonicida*. There was a blue pigmentation to the skin over most of the body and there was also a necrotic lesion on the right flank. Swabs from this lesion yielded the bacterial isolate.

A summary of the characteristics of the bacteria isolated is given in table 3A.13.

Table 3A.13: Characteristics of bacterial isolates from farm samples.

Sample Site	Motility (+/-)	Shape	Gram (+/-)	Oxidase (+/-)	API Profile
Site 2	Negative	Med. Rod	Negative	Possitive	0203004
Site 3, Fish 1	Negative	Short Rod	Negative	Possitive	0203004
Site 3, Fish 2	Negative	Cocci	Negative	Negative	1215000
Site 4	Negative	Med. Rod	Negative	Possitive	0203004
Site 5	Negative	Short Rod	Negative	Possitive	5262104

3A.3.2.3: Virological Testing

The fish from Site 2 tested for the presence of viruses all proved to be negative. No fish from any of the other sites were tested.

3A.3.3: Comparisons Between Wild and Farm Held Fish

The wrasse from Farm site 1 were originally obtained from the vicinity of Wild Site A. Therefore, these two samples can be directly compared to investigate the effect of the length of time spent in a farm pen on the level of parasitism. Fig 3A.10 illustrates the differences in prevalence between the parasites of *C. exoletus* from Sites A & 1. While Fig 3A.11 shows the differences in prevalence of parasites from *C. rupestris* from these sites.

Similarly, the wrasse stocked in Farm site C were obtained from Wild site 2, these are also compared. The parasite prevalence of *C. rupestris* from Sites C & 2 are compared in Fig 3A.12. Farm site 3 obtained wrasse from a variety of wild locations one of these was that used in Wild site B. Therefore, the parasites of *C. rupestris* from Sites B & 3 are compared in Fig 3A.13.

The most obvious differences in parasite prevalence between the *C. exoletus* from Wild Site A and Farm Site 1 is the increase in the numbers of trichodinids affecting the fish and the decrease in the numbers of *M. alacris*. The numbers of trichodinids on the farm held fish were three times higher than those on the wild fish while the number of adult *M. alacris* is half that of the numbers seen in the wild caught fish. These patterns were also seen in the *C. rupestris* from the same sites. In addition to this, the numbers of *C. lingua* and *C. osculatum* increased in the farm held fish when compared to *the C. rupestris* from the wild while the numbers of *L. rufoviride* decreased. In Both *C. rupestris* and *C. exoletus*, no crustacean parasites were seen on the farm held fish.

Fig 3A.10 Comparative prevalence of parasites of *C.exoletus* from Wild Site A and Farm Site 1

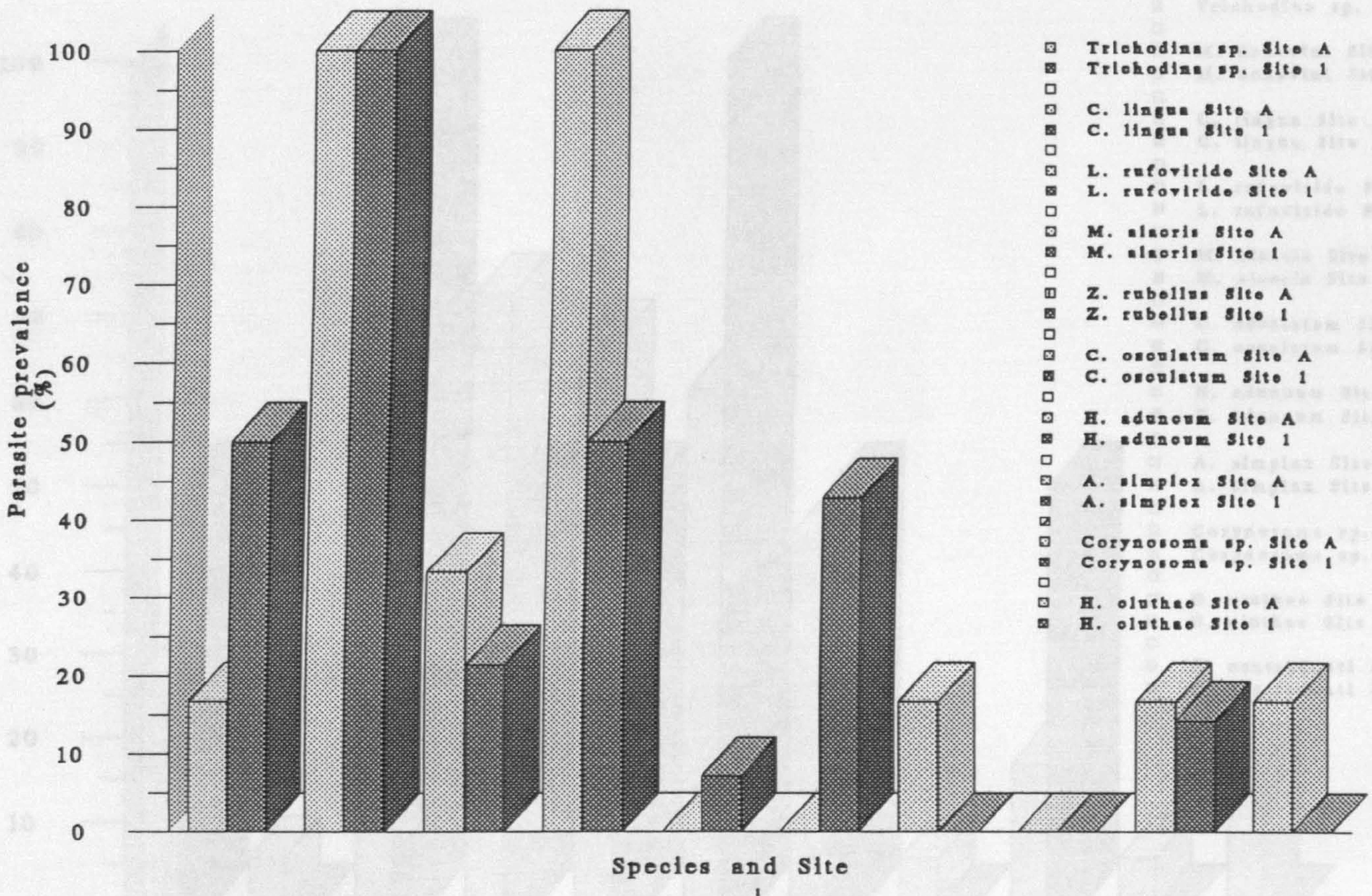


Fig 3A.11: Comparative prevalence of parasites of C. rupestris from Wild Site A and Farm Site 1.

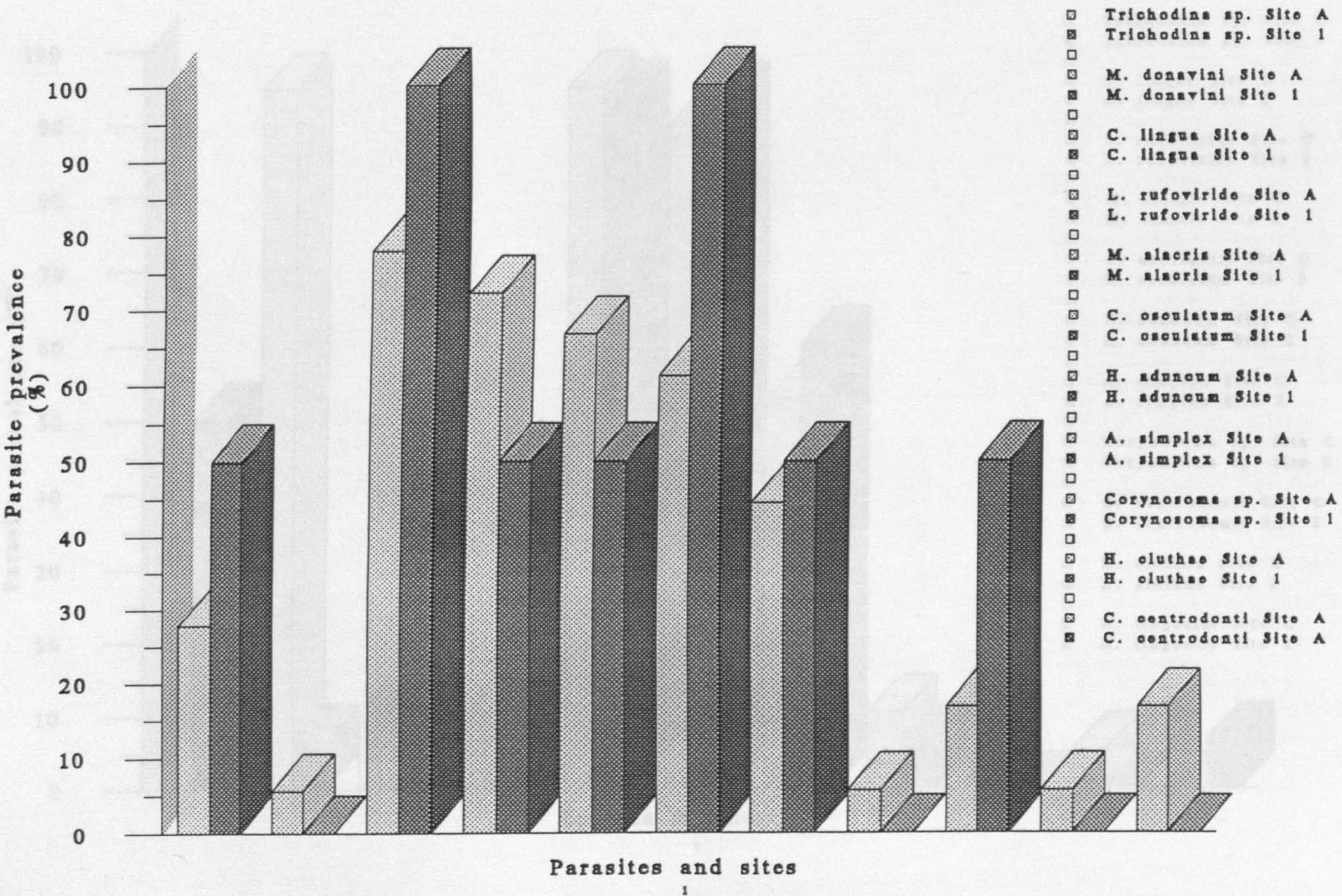


Fig 3A.12: Comparative prevalence of parasites of *C. rupestris* from Wild Site C and Farm Site 2.

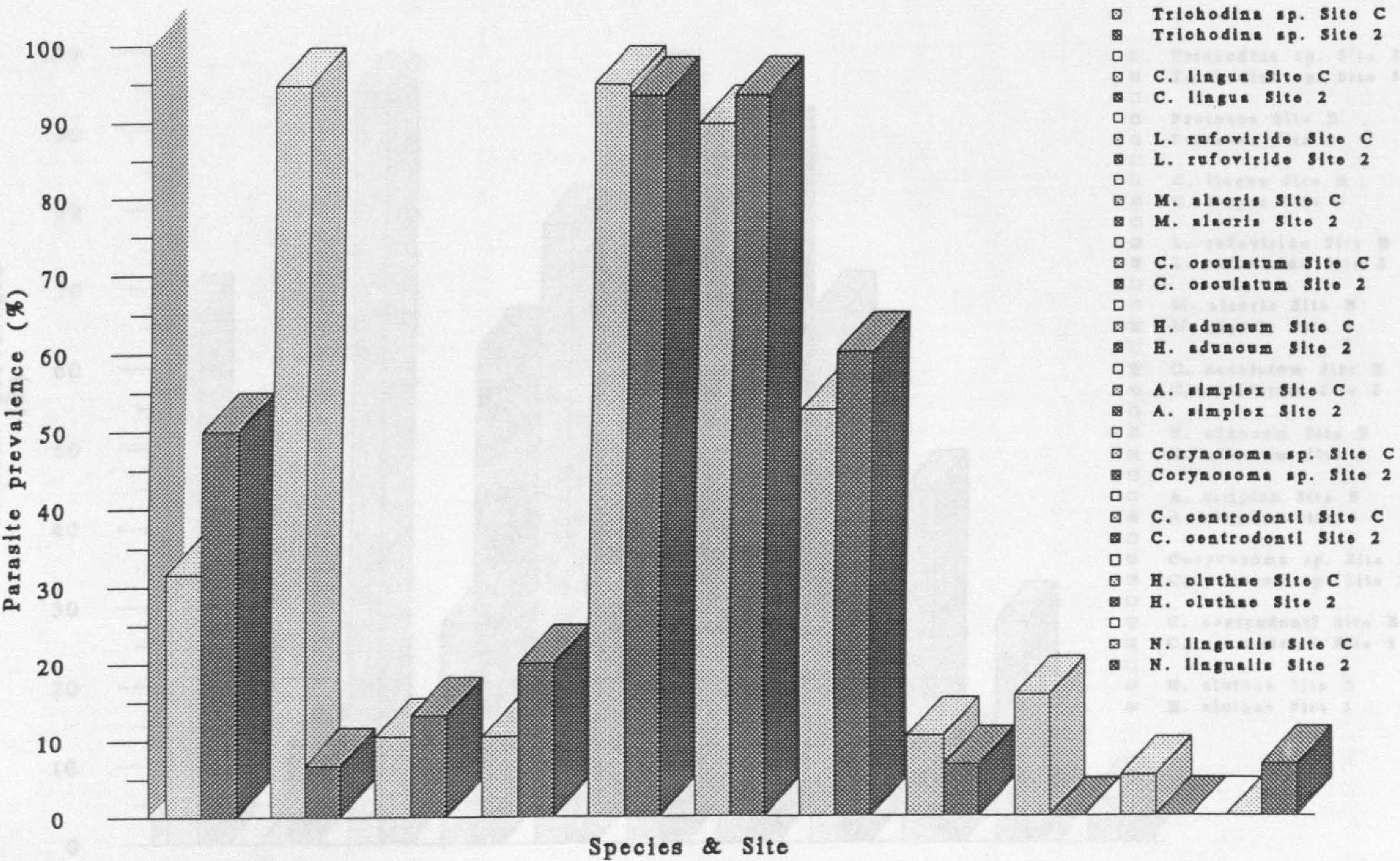
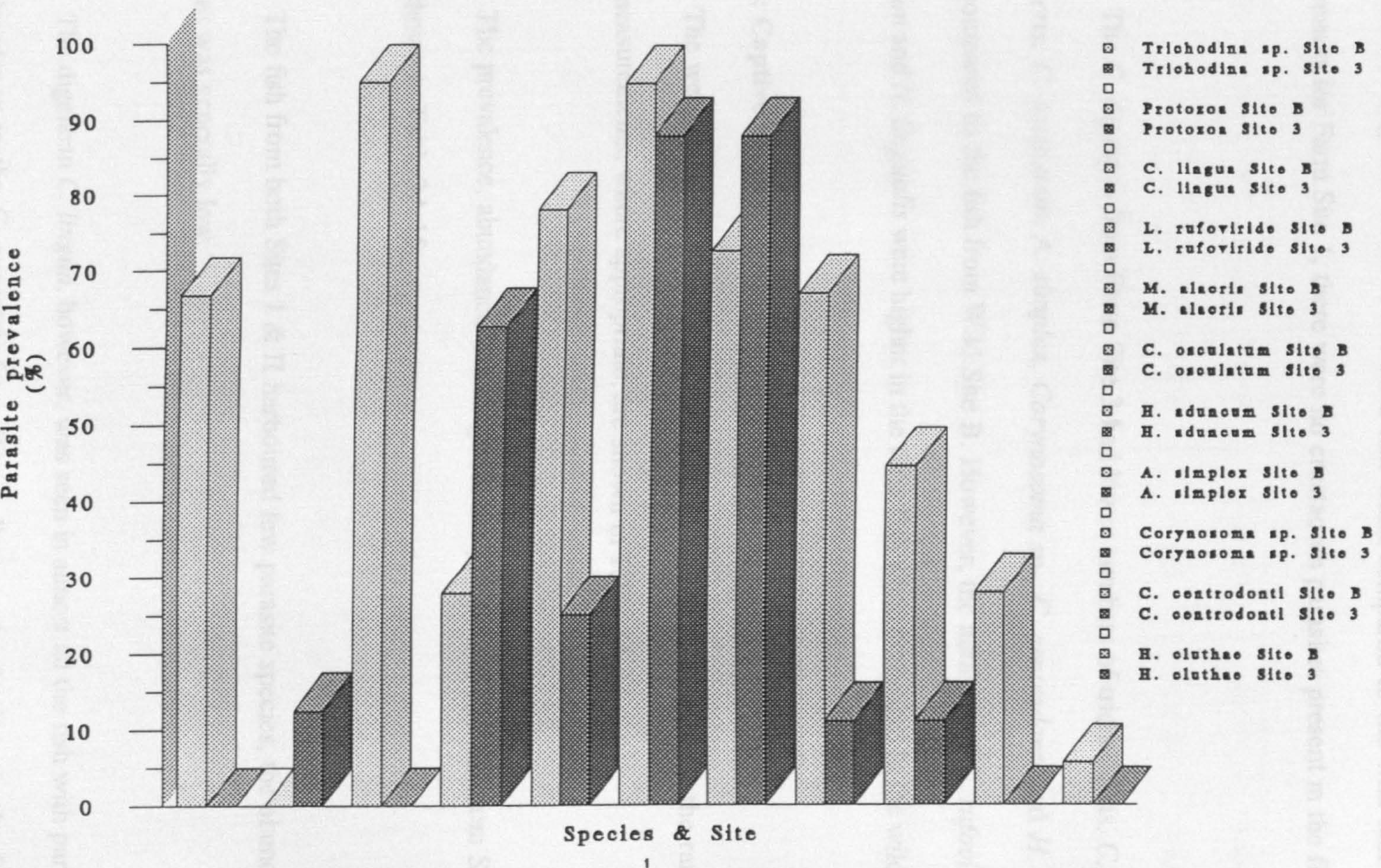


Fig 3A.13: Comparative prevalence of parasites of *C. rupestris* from Wild Site B and Farm Site 3.



The only major differences in parasite prevalence seen between the *C. rupestris* from Wild Site C and Farm Site 2 were the increase in trichodinid numbers and the decrease in *C. lingua* numbers in the farm fish when compared to the wild fish. Similar to the results for Farm Site 1, there were no crustacean parasites present in the fish from Site 2.

The *C. rupestris* from Farm Site 3 had lower numbers of trichodinids, *C. lingua*, *M. alacris*, *C. osculatum*, *A. simplex*, *Corynosoma* sp., *C. centrodonti* and *H. cluthae* when compared to the fish from Wild Site B. However, the numbers of *L. rufoviride*, *H. aduncum* and *N. lingualis* were higher in the farm fish when compared to the wild fish.

3A.3.4: Captive Wrasse

The weights and lengths for the fish from Sites I & II, along with the ranges for these measurements, where appropriate, are shown in Table 3A.14.

The prevalence, abundance and range for the parasites of the fish from Sites I & II are shown in Table 3A.15.

The fish from both Sites I & II harboured few parasite species, the abundance of parasites was generally low.

The digenean *C. lingua*, however, was seen in almost all the fish with particularly high abundance in the *C. rupestris* from Site I. Protozoal infections in the fish were common but their abundance was very low with a mixed ciliate and flagellate infection

Table 3A.14: Mean and range of weights and lengths for fish samples from captive Sites I & II.

Site No. & Species	**Mean weight (g)**	**Weight range (g)**	**Mean length (mm)**	**Length range (mm)**
Site I, *C. rupestris* N= 12	37.03	21.0-80.80	114.3	97-143
Site II, *C. rupestris* N= 5	10.66	4.88-24.25	81.6	70-110
Site II, *C. exoletus* N= 1	n/a	20.33	n/a	99

Parasite species	Site I N=12 *C. rupestris*			Site II N=5 *C. rupestris*			Site II N=1 *C. exoletus*
	P	**A**	**R**	**P**	**A**	**R**	**Total**
Protozoa							
Trichodina sp.		0		60	0.4	0-1	1
Ciliates & flagellates	50	0.6	0-2		0		0
Digenea							
C. lingua	91.7	228	0-940	100	31	20-54	27
L. rufoviride	0			20	0.8	0-4	5
M. alacris	16.7	0.2	0-1		0		5
Nematoda							
C. osculatum	8.3	0.1	0-1	20	0.2	0-1	0

Table 3A.15: Prevalence, abundance and range of parasites from samples I & II.

P= Prevalence, A= Abundance, R= range of infection.

observed on the skin and gills of half of the fish from the Site I sample. Although a relatively high proportion of the fish were infected, the abundance of infection was low. The nematode *C. osculatum* was seen in both samples but again at a low prevalence.

3A.3.4.2: Bacteriology Testing

No bacterial isolates were obtained from any of the fish in samples I & II.

3A.3.5: Incidental Samples

Due to poor fixation of many of the samples, identification of the parasites was difficult. However, the nematodes from the three fish from Oban were all identified as adult *H. aduncum*. One of the fish harboured 7 adult *H. aduncum* while the other two fish harboured one each. The parasites identified from the samples supplied by Dr. Sayer from the waters around Cumbrae are shown in Table 3A.16.

A relatively high percentage of samples was lost due to poor fixation or the storage tubes drying out before being received. Twenty three percent of the *C. rupestris* samples and 15 percent of the *S. melops* samples were rendered unidentifiable due to these reasons. It was found to be impossible to identify the acanthocephalans from the samples due to the fact that they were all larval stages and the proboscis had not been everted prior to fixation. The need for proper fixation and storage of specimens is well illustrated by this exercise where much potentially valuable data was lost.

Table 3A.16: Prevalence, abundance and range of parasites from the Cumbrae samples.

Parasite Species	***C. rupestris* N=13**			***S. melops* N=46**		
	P	**A**	**R**	**P**	**A**	**R**
Adult *H. aduncum*	8	0.08	0-1	2	0.04	0-1
L3 *H. aduncum*	23	0.23	0-1	9	0.13	0-3
L3 *C. osculatum*	54	0.60	0-2	63	1.4	0-8
Acanthocephala		0		28	0.9	0-10

P= Prevalence, A= Abundance, R= Range of infection.

3A.3.6: Histopathology

3A.3.6.1: Parasites

In general the pathology associated with the parasites identified in the study was localised in the immediate area surrounding the parasite.

There was no pathology associated with trichodinid or protozoal infections in any of the fish examined in histological section. Even in fish where there was heavy infection with these parasites (Fig 3A.14), there was not even slight hyperplasia (Fig 3A.15).

No specific pathology was seen on the gills of wrasse infected with copepod parasites, but, localised gill hyperplasia, and in some cases total fusion of gill lamellae due to chronic hyperplasia, was seen in some fish (Fig 3A.16). No parasites were seen in these sections, however, this type of damage is typical of that caused by copepod parasites of the family *Hatschekia*. The method of attachment in this group is by means of two large hooked appendages which cause localised tissue damage. There was some limited evidence of atrophy of the secondary lamellae which would also be expected if a large copepodid parasite had been present on the gill.

The host reaction to the presence of established *C. lingua* metacercariae was very limited in most cases. Metacercariae were seen not only in the skin (Fig 3A.17), but deeply embedded in the gills. The gill infections were seen both within the primary lamella (Fig 3A.18) and deep within the gill bar (Fig 3A.19). Infections with *C. lingua* were also seen deep within the musculature in some fish.

Fig 3A.14: This figure illustrates a heavy infection with *Trichodina* sp. on the gills of a *C. rupestris*. Multiple trichodinids (arrowed) can be seen between the secondary gill lamellae (x25 objective).

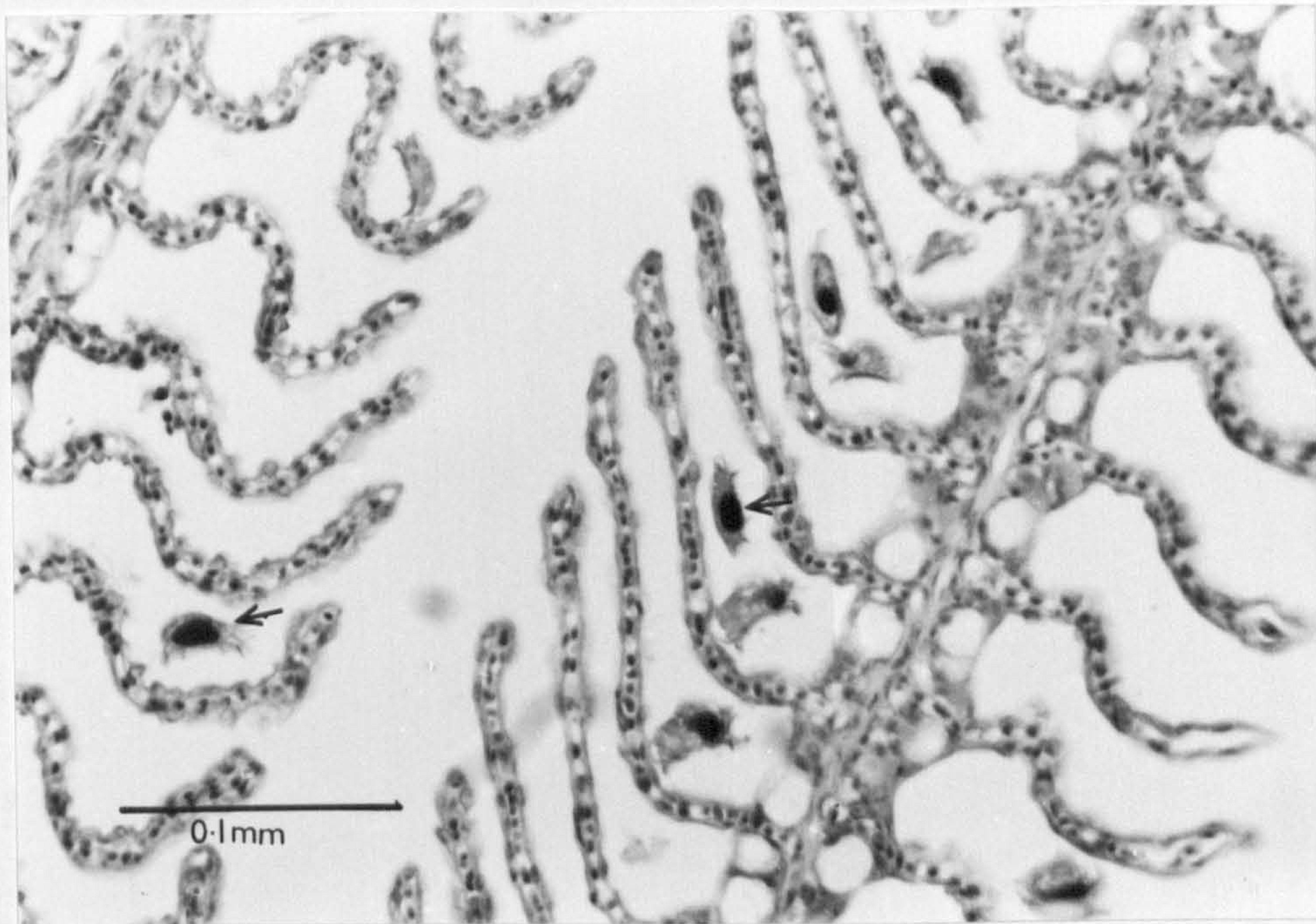


Fig 3A.15: This figure shows a magnified view of a heavy trichodinid infection on the gills of a *C. rupestris*. No inflammatory response or hyperplasia can be seen associated with the presence of the parasites (x40 objective).

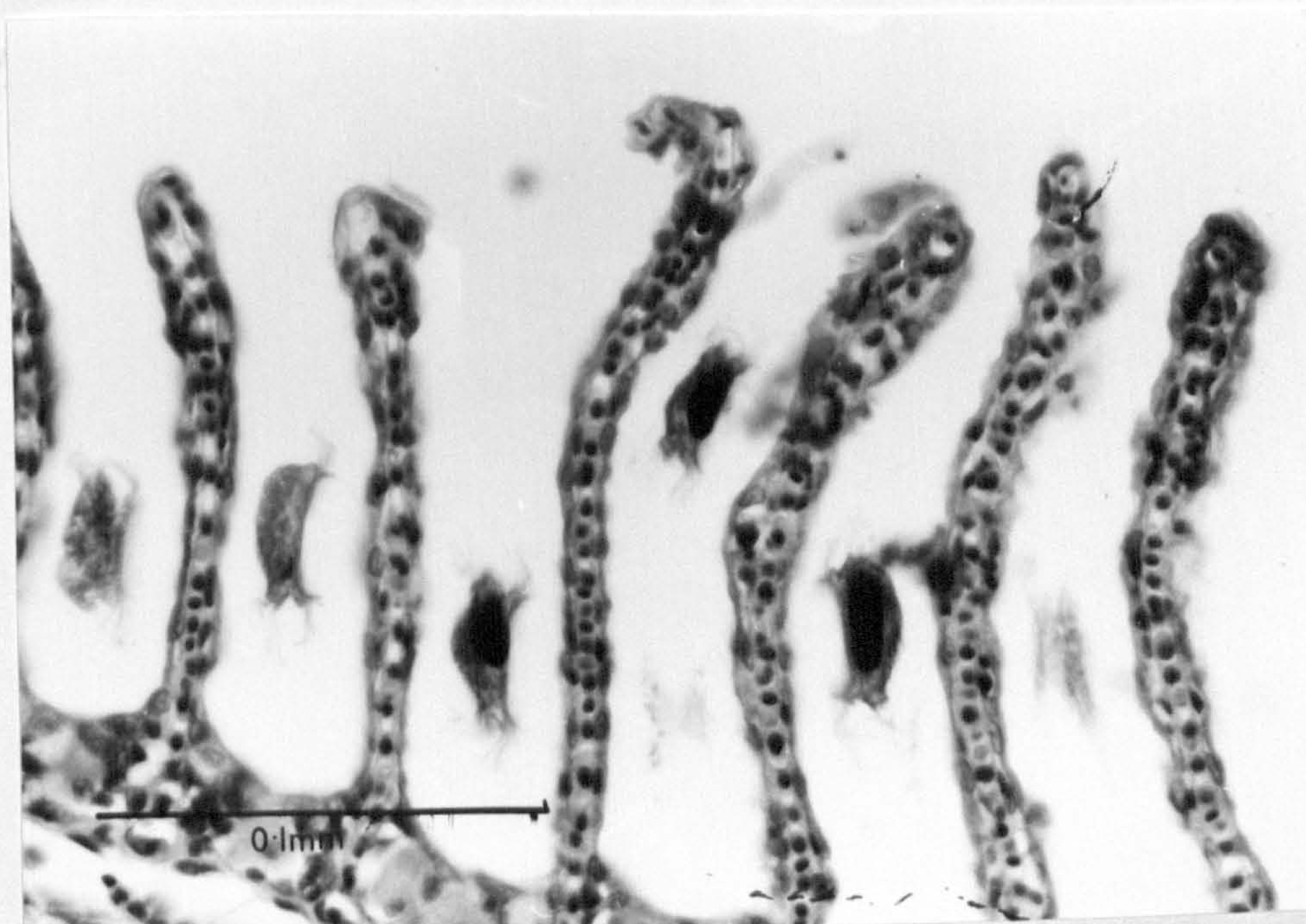


Fig 3A.16: This figure illustrates chronic localised hyperplasia of the secondary gill lamellae in a *C. exoletus*. Very little remains of the original gill structure in the area affected (HP) while surrounding lamella are normal (N) (x25 objective).

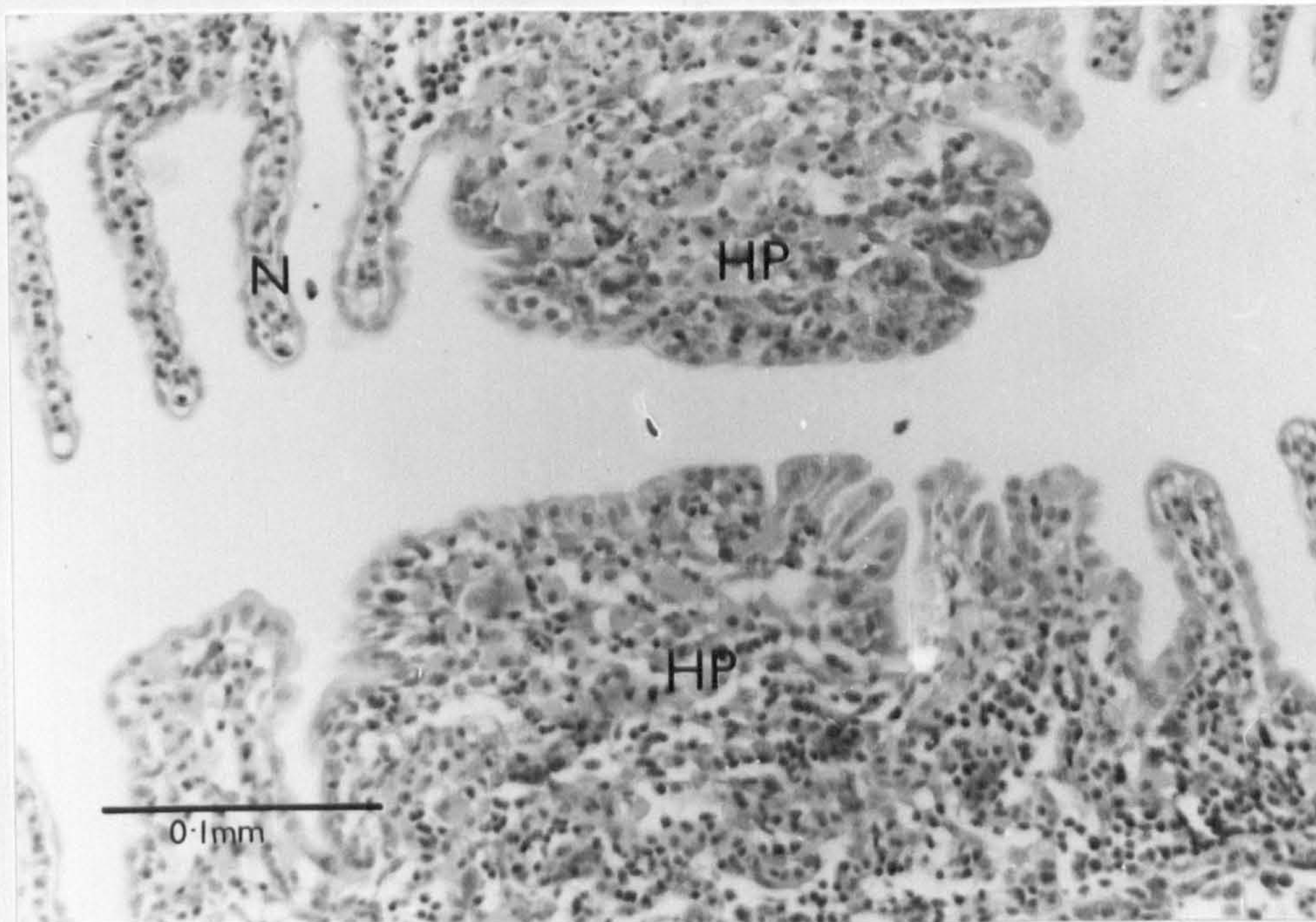


Fig 3A.17: An established infection with a metacercaria of *C. lingua* (DG) lying directly below the epithelium (EP) of a *C. exoletus* on top of a scale (S). There is little evidence of a tissue response to the digenean with only a limited number of melanocytes present (arrowed) (x40 objective).

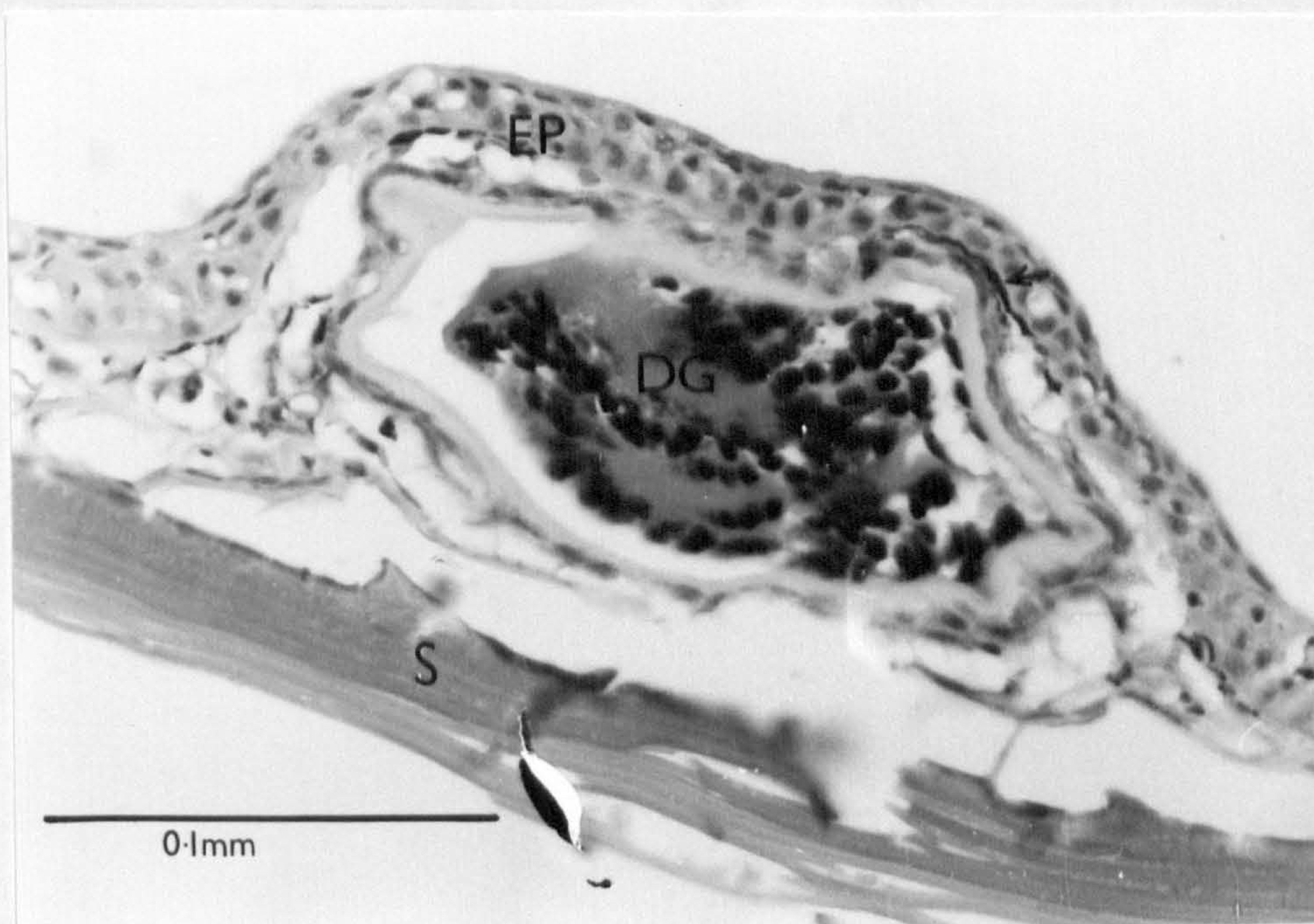


Fig 3A.18: In this figure a *C. lingua* (DG) has established in the primary lamella of a *C. rupestris*. The parasite is surrounded by a thin, bilaminate host capsule. Despite considerable localised tissue disruption the secondary lamellae are undamaged (x25 objective).

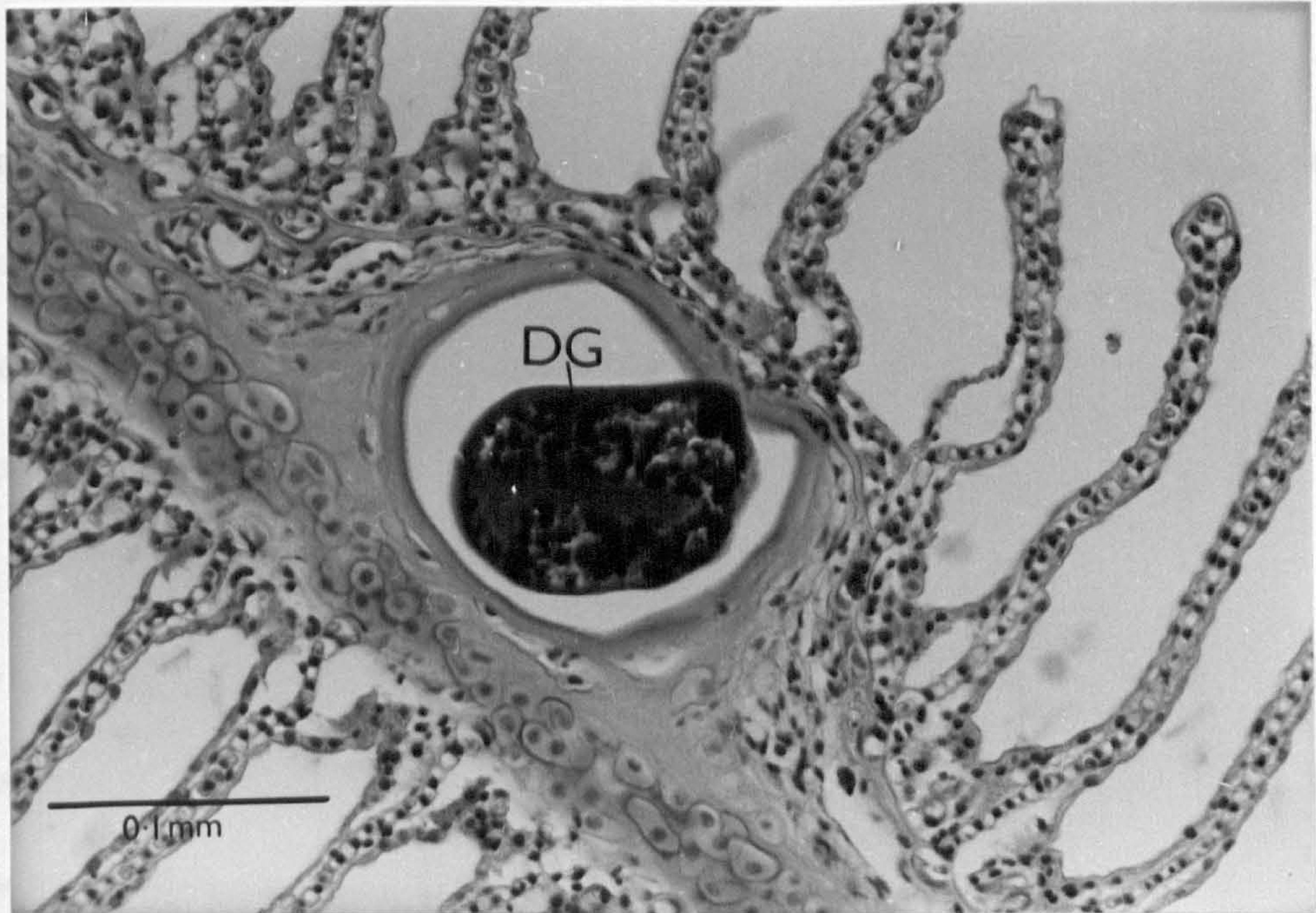


Fig 3A.19: In this case a *C. lingua* (DG) established deep in the supportive cartilage at the tip of a gill bar in a *C. rupestris*. The host capsule (C) is considerably thicker in this specimen and there are numerous melanocytes (arrowed) surrounding the parasite (x25 objective).

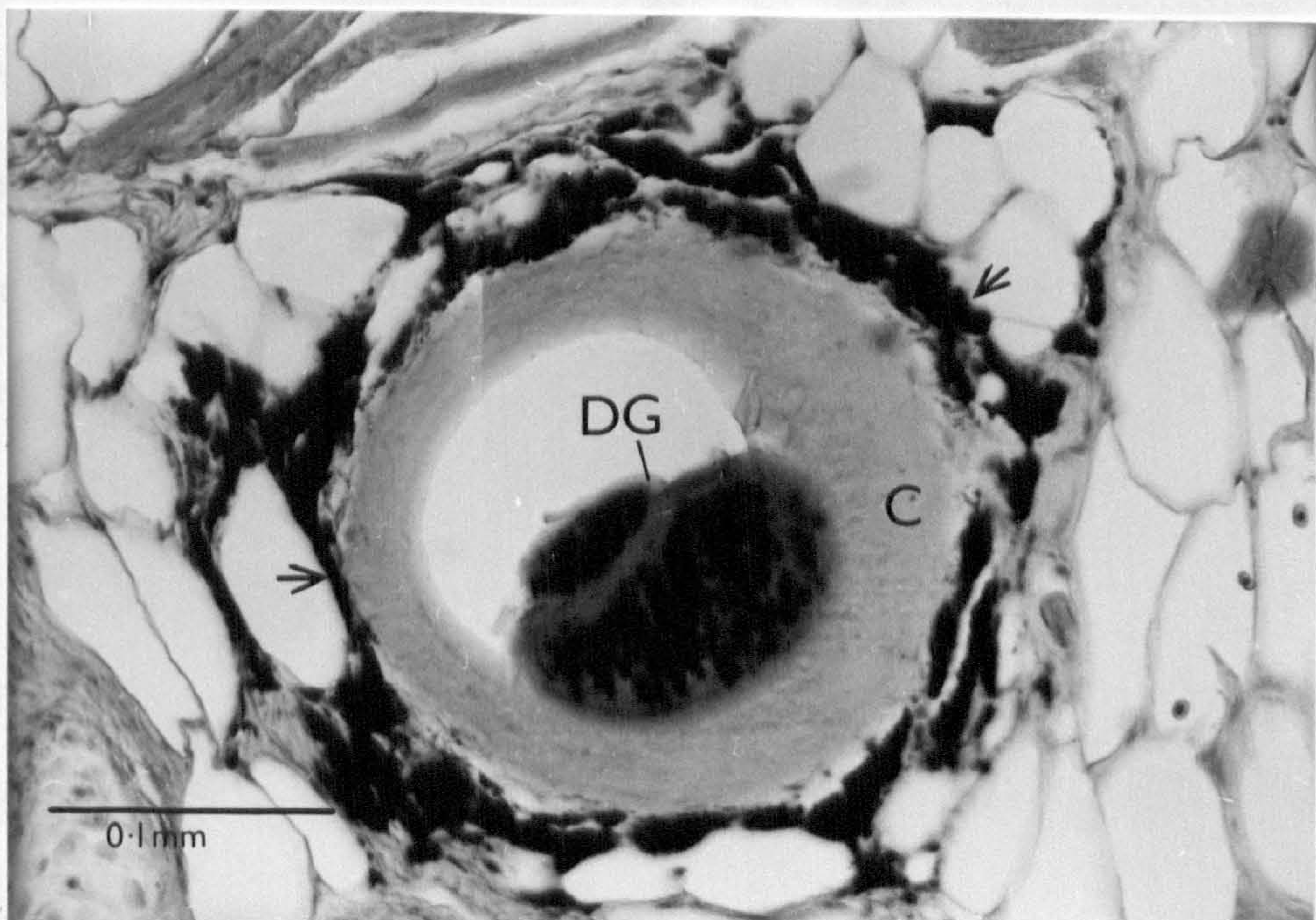


Fig 3A.20 illustrates a single *C. lingua* cyst within the musculature of a small *C. rupestris*, five grams in weight. The degree of melanisation was considerably variable with some cysts attracting no melanocytes while others were surrounded with many.

In most cases the pathology associated with infections was limited to a bilaminar cyst of host origin. The degree of inflammatory response varied slightly but was always quite limited. Some separation and disassociation of the epithelium with formation of vesicles can be seen, but, this is an artefact of processing.

No metacercarial *L. rufoviride* were observed in histological section despite these parasites being present in reasonably large numbers in some fish. This was most likely to be due to the fact that they were found only in the connective tissue of the abdominal cavity and were easily dislodged during dissection. This would result in the majority of the parasites becoming disassociated from the tissues during histological processing.

The adults of *M. alacris* observed in the intestine of wrasse were seen to be very closely associated with the villi of the intestine (Fig 3A.21). As with most adult digeneans, the attachment of these parasites is by the muscular ventral sucker. Localised tissue damage can be seen with the invasion of the immediate area with EGC's (Fig 3A.22).

The histopathology observed in the presence of nematode parasites varied depending on the organ affected. In the case of nematodes infecting the liver, the **Fig**

3A.20: A well established *C. lingua* (DG) cyst can be seen deep within the musculature (M) of a small *C. rupestris*, approximately 5g in weight. There is a thickened host capsule (C) with some associated melanocytes (arrowed) (x10 objective).

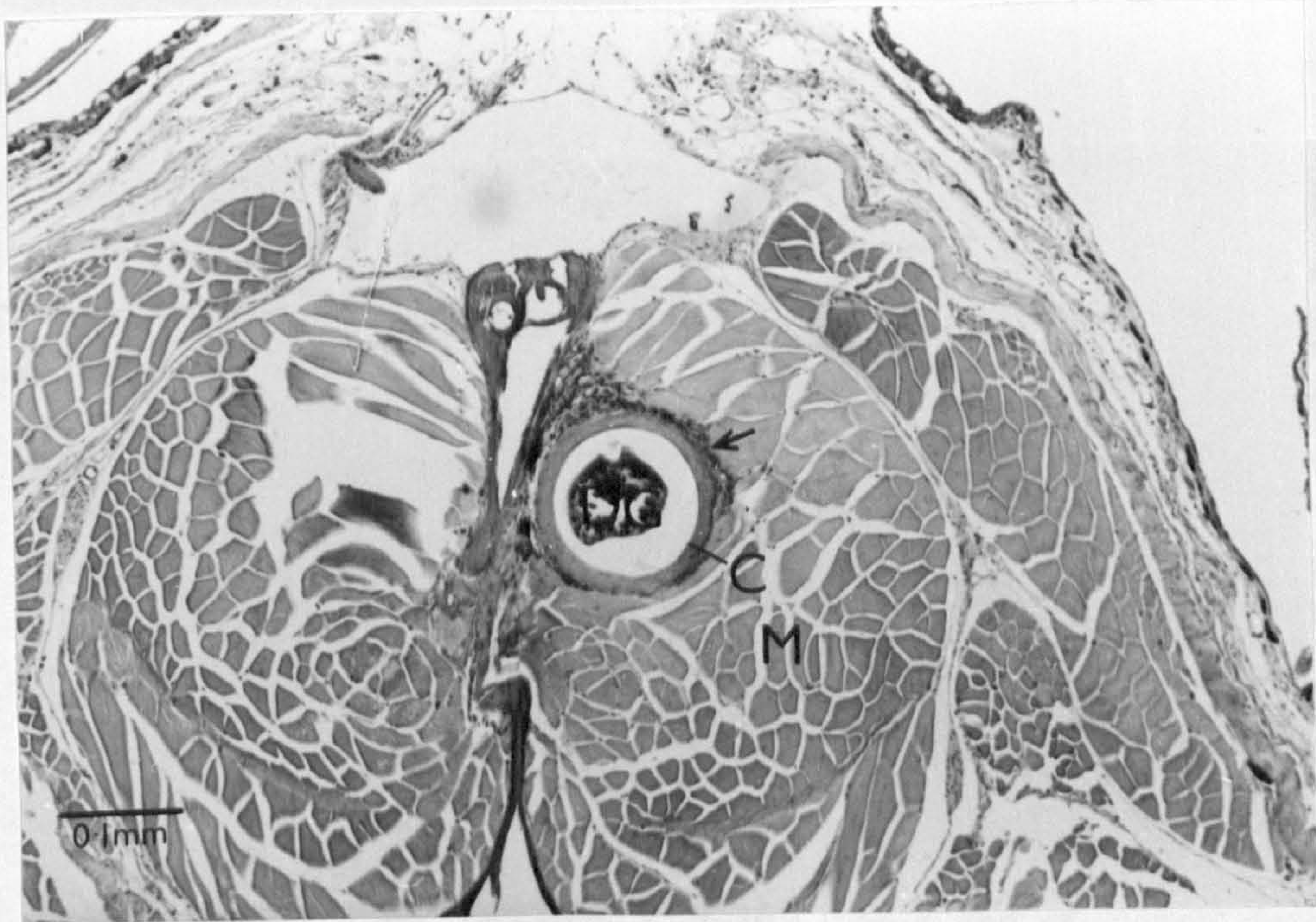


Fig 3A.21: Two adult *M. alacris* digeneans (DG) located in the intestine of a *C. rupestris*. The digeneans are closely associated with the intestinal villi with one attached to a fold in the intestine by means of the ventral sucker (x10 objective).

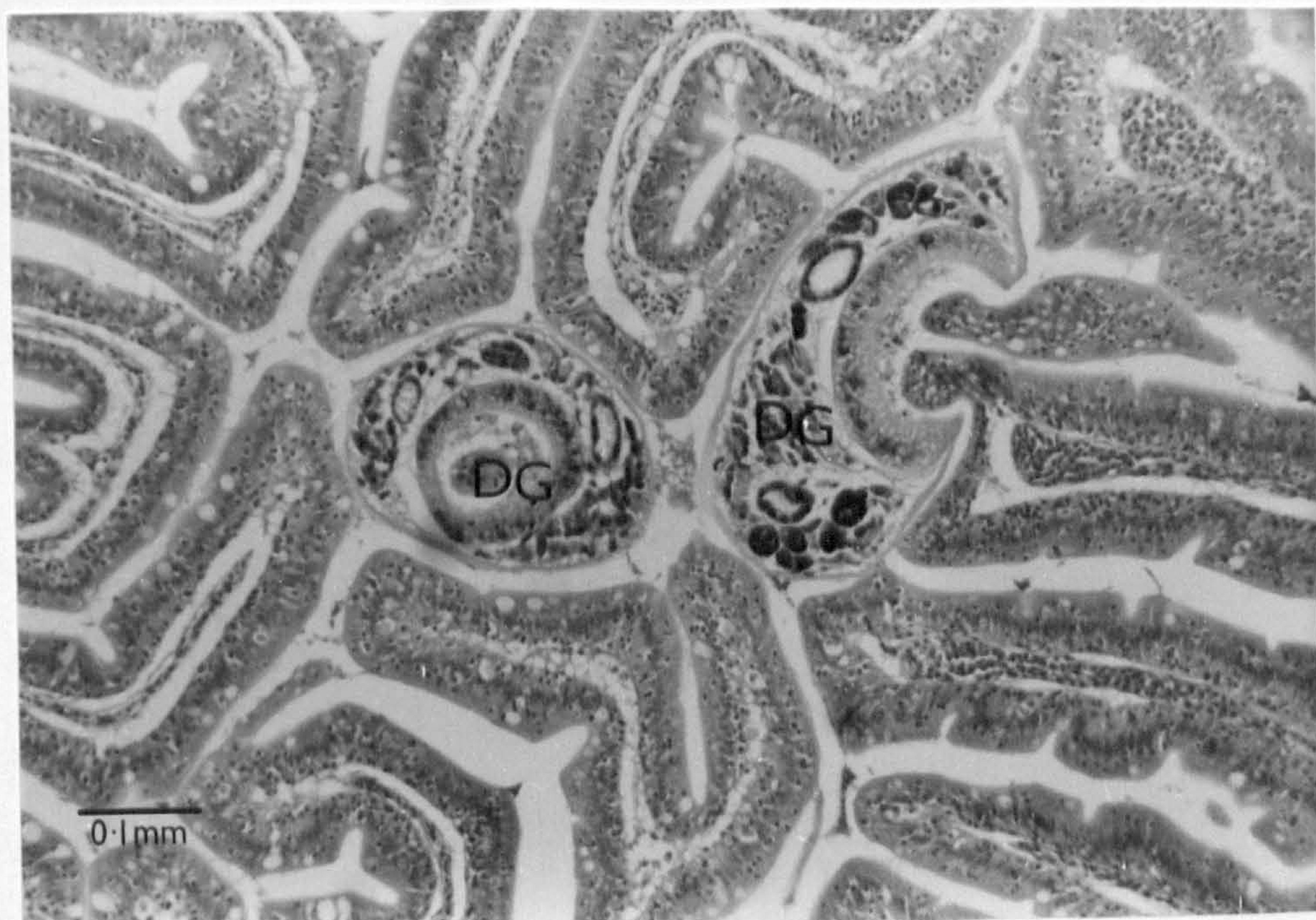
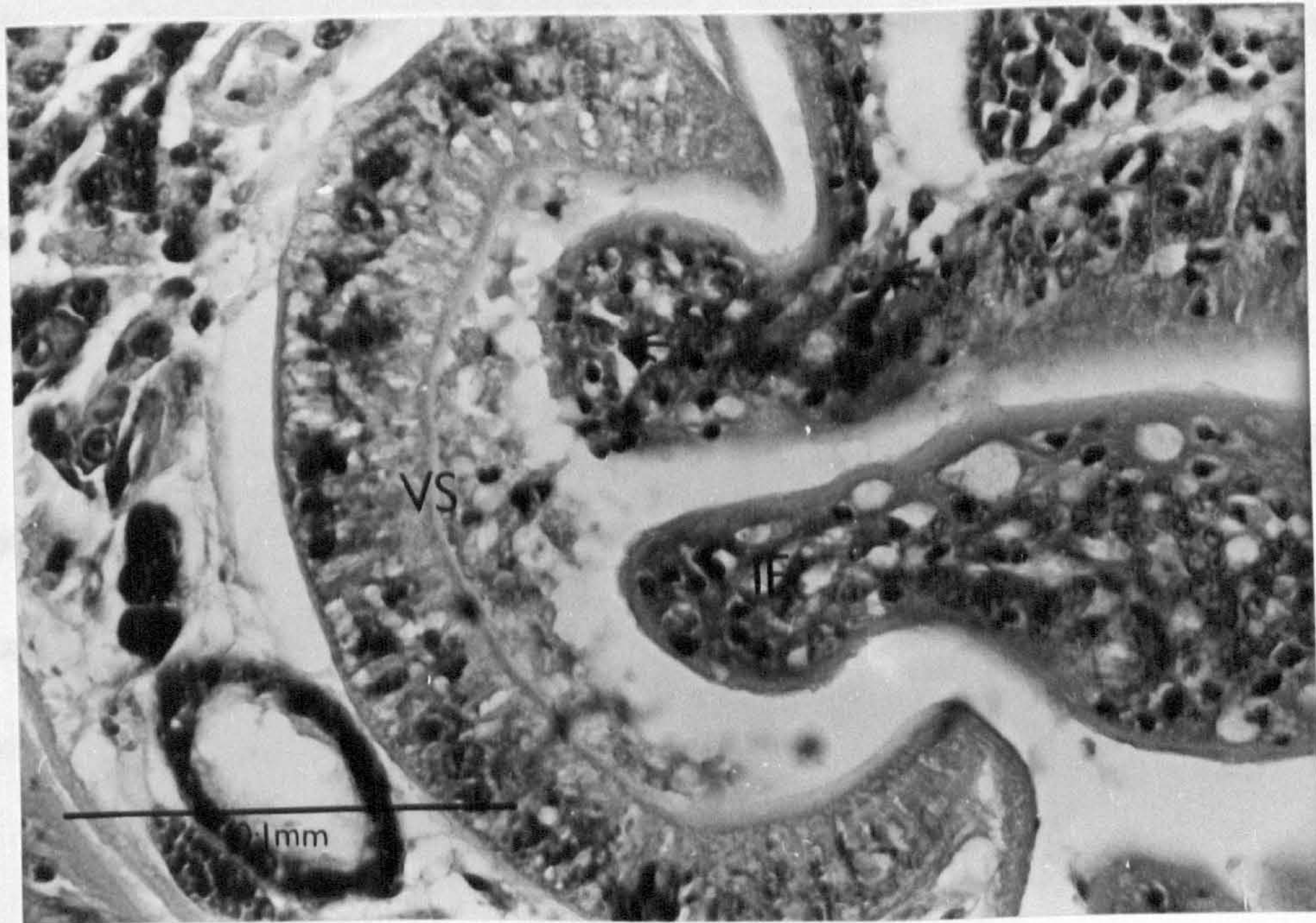


Fig 3A.22: Higher powered view (x40 objective) of the attachment site of an adult *M. alacris* in the intestine of a *C. rupestris*. The ventral sucker (VS) can be seen gripping the intestinal epithelium (IE) causing localised tissue disruption. Eosinophilic granular cells (EGCs) (arrowed) can be seen in the damaged area.



parasites could be seen surrounded by hepatocytes with very little host response associated with them (Fig 3A.23). In most cases the nematodes were enclosed in a thin capsule of host origin (Fig 3A.24). However, in some cases there was a substantially greater tissue response associated with encapsulated nematodes, these nematodes were normally necrotic and were surrounded by a thick, fibrotic capsule of host origin (Fig 3A.25). It is difficult to say whether this capsule results in the death of the nematode or is a response to the release of toxins liberated by the decaying nematode.

There was also more variation in the histopathology associated with nematodes present in the connective tissues. Very often the nematodes were found in close association with blood vessels. In these cases large amounts of blood products could be seen within the parasite capsule (Fig 3A.26).

Histological examination of the *L. mixtus* from Farm Site A revealed a heavy coccidian infection affecting the intestinal epithelium, this being the only fish in the survey found to be infected with coccidians. Unfortunately, the presence of the parasites was only recognised during the histological examination, not at the time of parasitological examination. Therefore, no identification could be made of the species of coccidian involved. There was little tissue disruption despite the large numbers of parasite oocysts located in the intestinal epithelium (Fig 3A.27). This disruption was limited to the immediate area of the cysts, this localised tissue disruption is illustrated in Fig 3A.28. An increased number of EGC's were observed in the intestinal epithelium of the infected fish indicating an inflammatory response to the infection.

Fig 3A.23: A nematode, most probably *C. osculatum* due to its proximity to the liver, surrounded by a thin host capsule and hepatocytes in the liver of a *C. rupestris* (x10 objective).

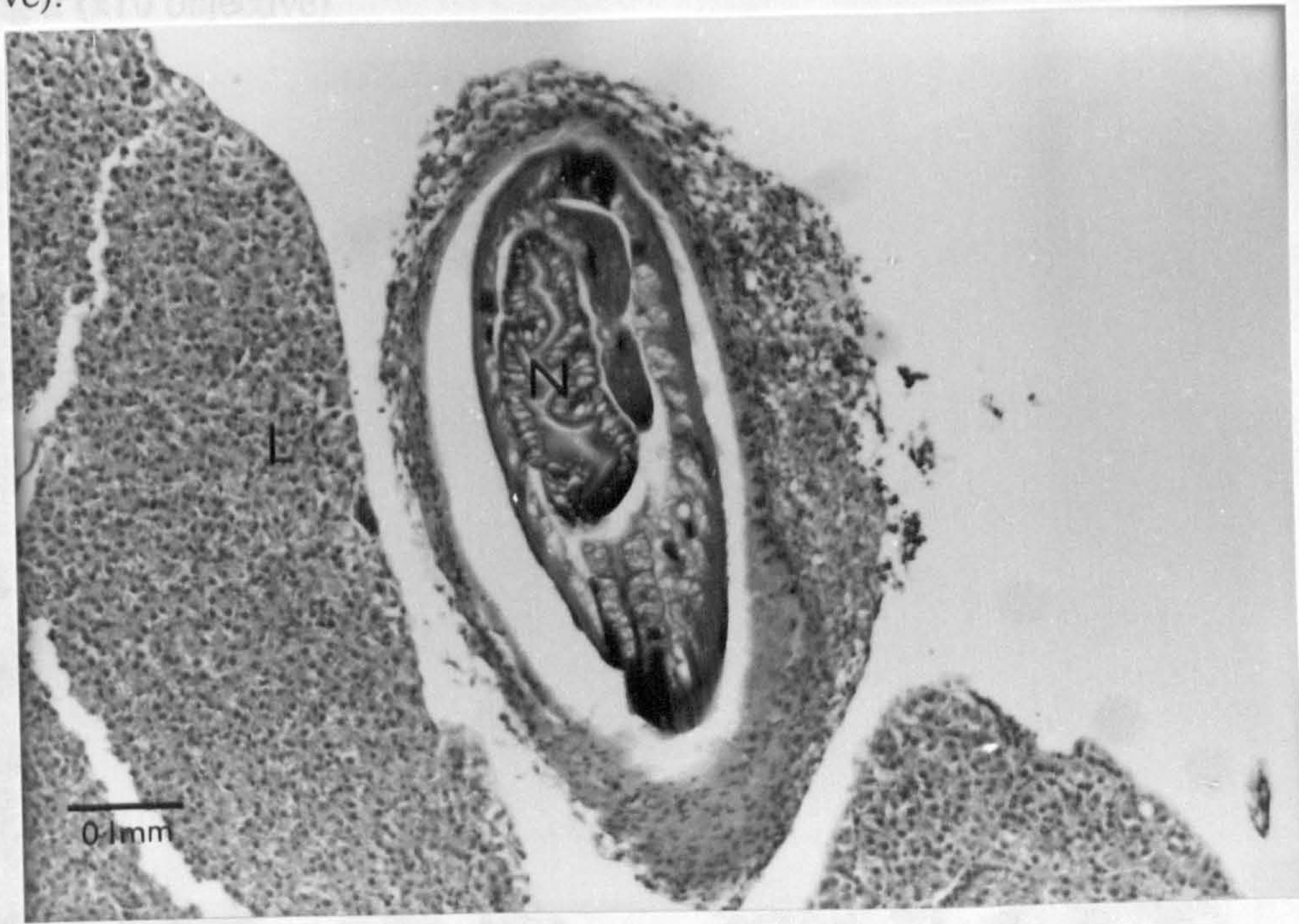


Fig 3A.24: Higher powered view (x40 objective) of the host capsule surrounding a nematode situated within liver tissue of a *C. rupestris*. The wall of the capsule (C) can be seen to be composed of host fibrocytes and is distinct in composition from the nematode tegument (NT). There are a few EGC's present (arrowed) but the inflammatory response is very limited.

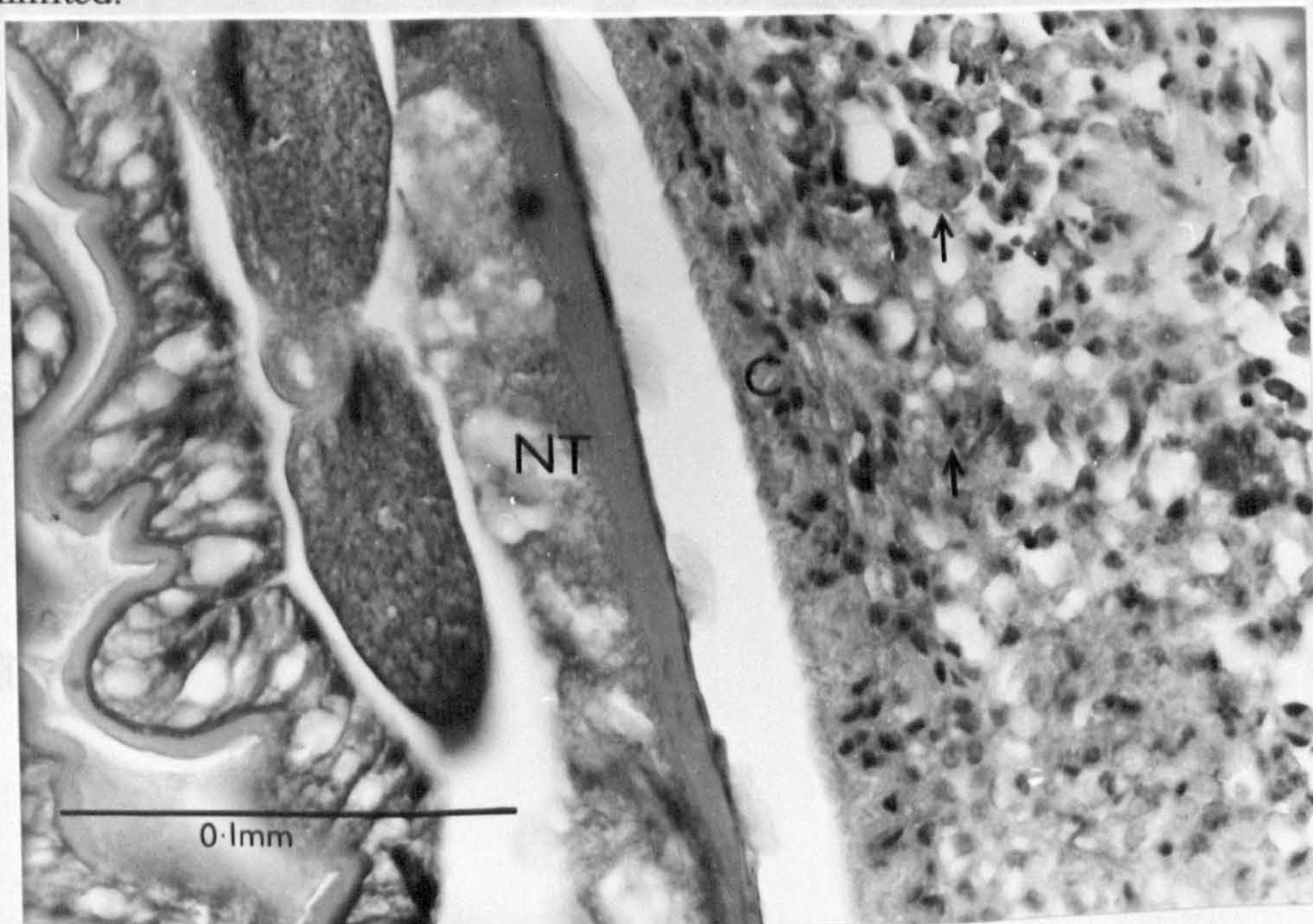


Fig 3A.25: A necrotic nematode (N) can be seen in association with pancreatic tissue (P) in a *L. mixtus*. The host capsule (C) is thick with considerable fibrotic tissue (FT) surrounding it (x10 objective).

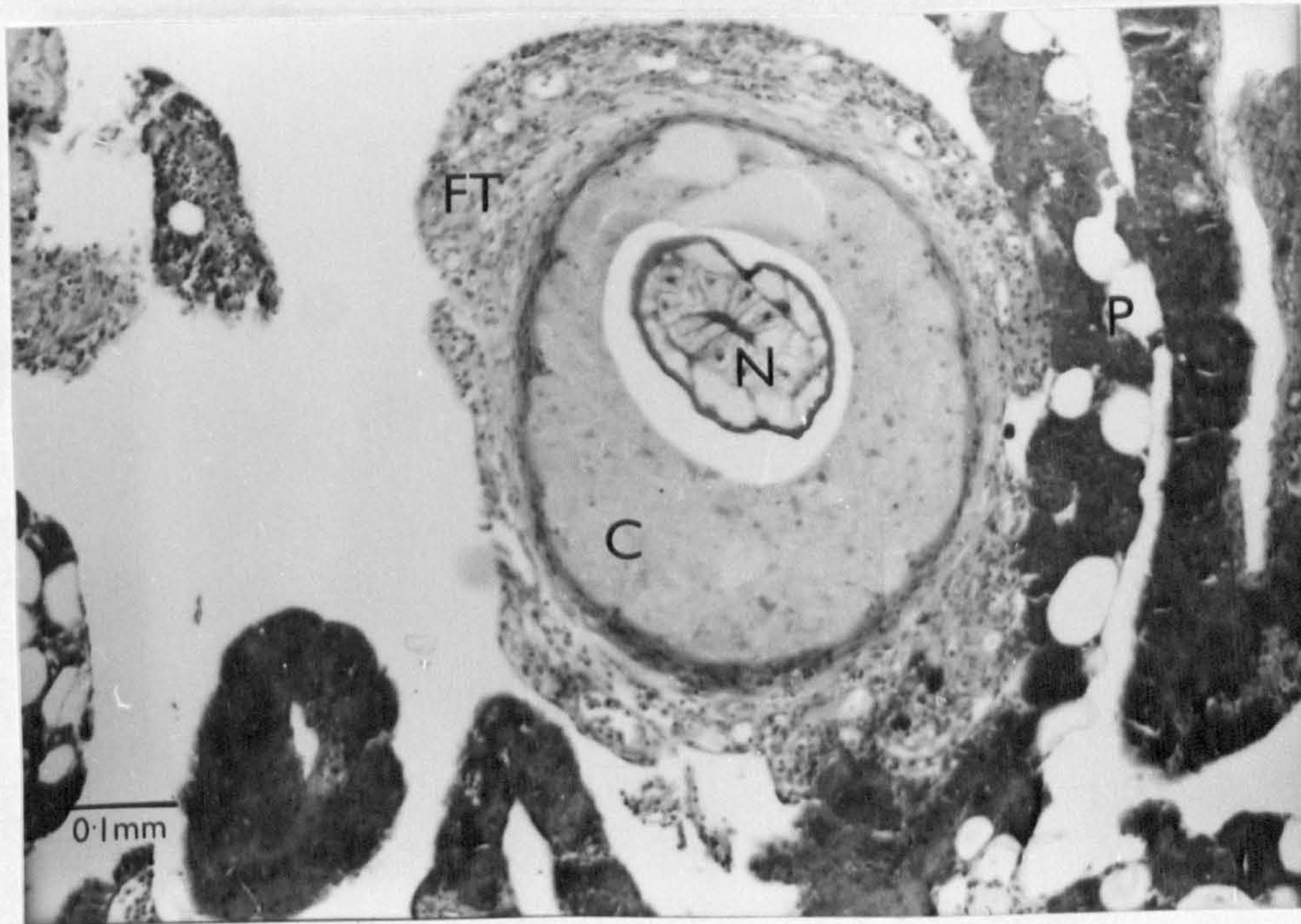


Fig 3A.26: Several nematodes (arrowed) are seen in section within the connective tissue of a *C. rupestris*. The capsule of one of the nematodes can be seen to be filled with blood products (BP) (x6.3 objective).

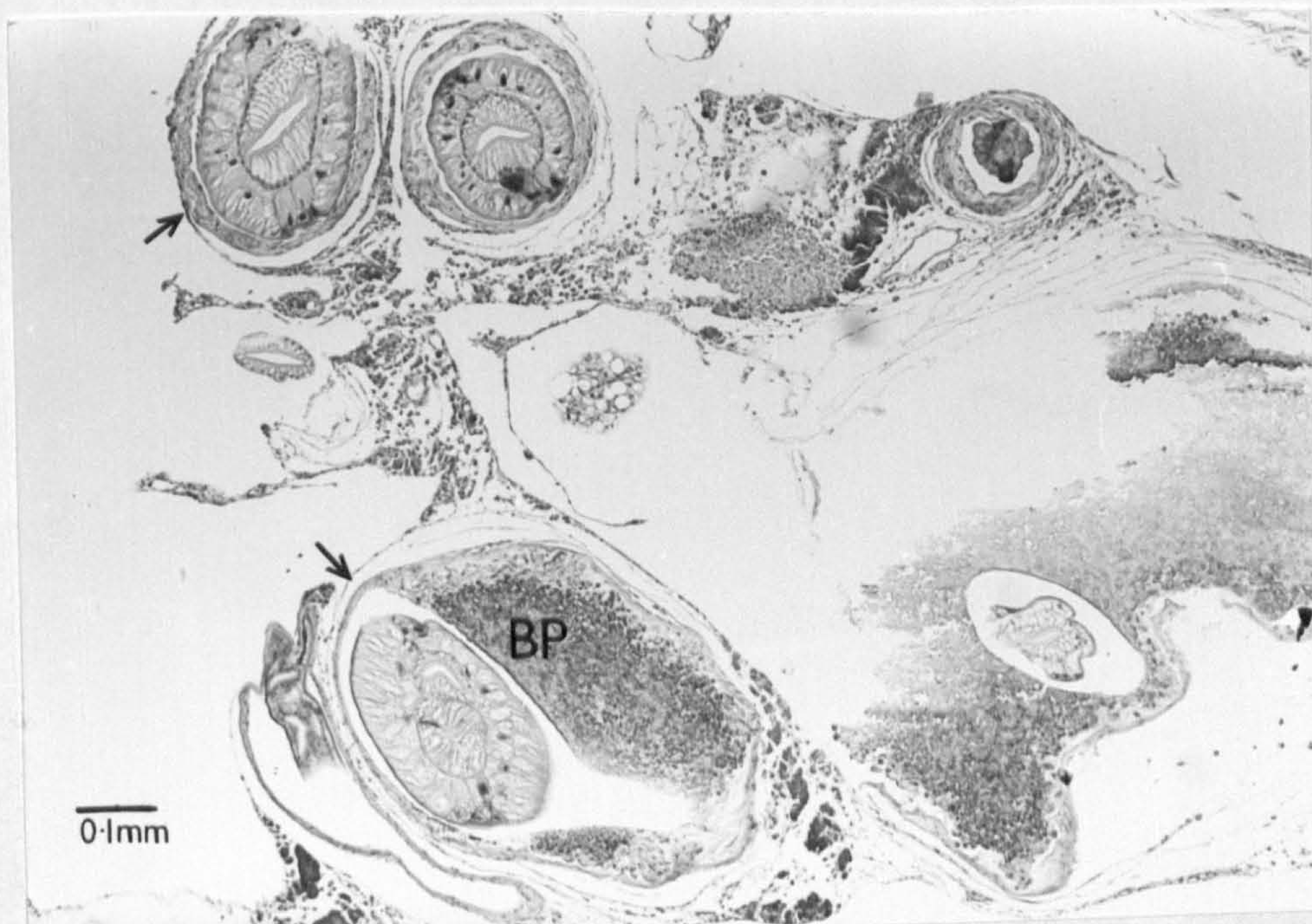


Fig 3A. 27: A low powered view (x6.3 objective) of the intestine of a *L. mixtus* infected with an unknown coccidian. Numerous oocysts can be seen (some arrowed) located within the epithelial layer of the intestine.

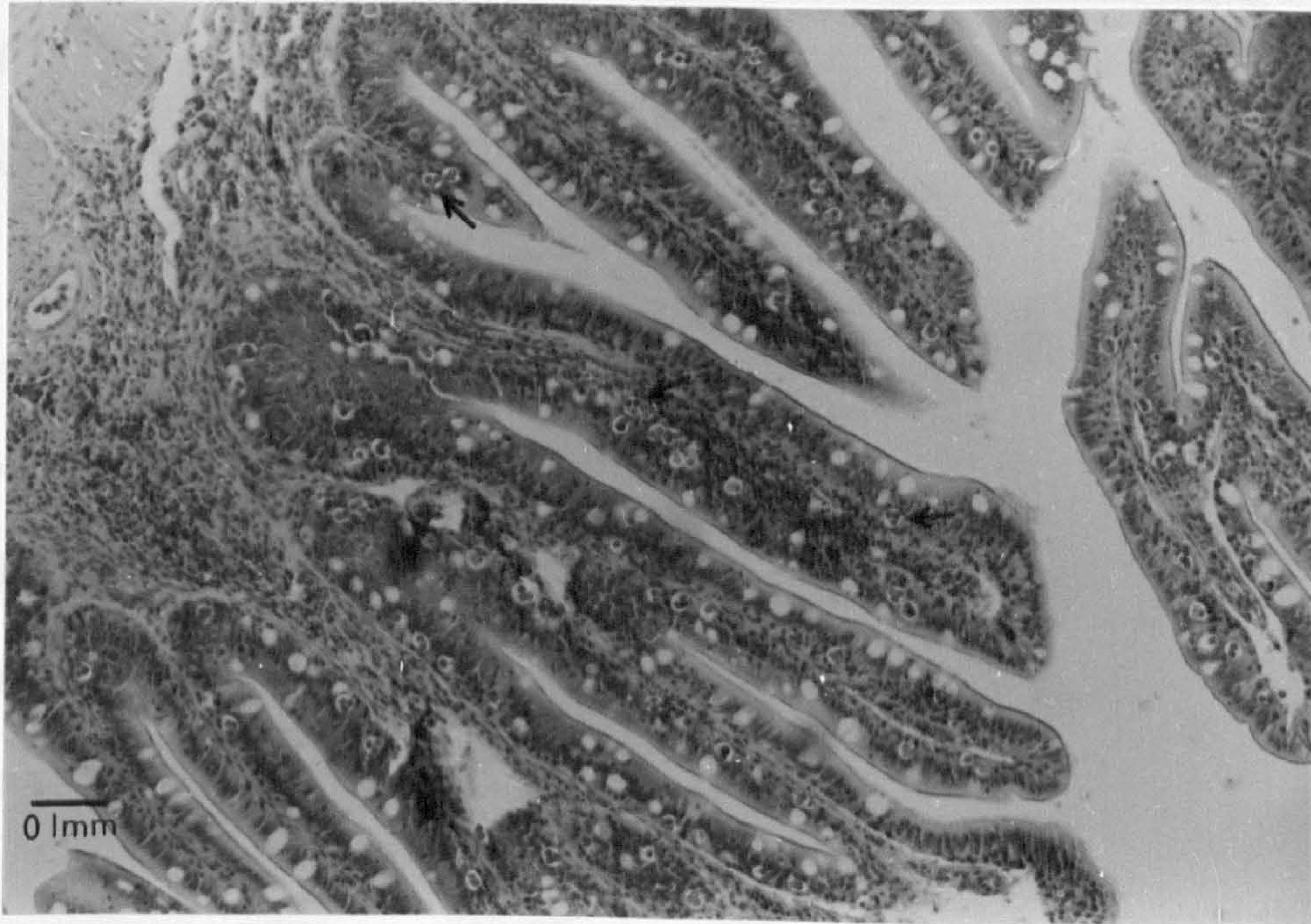
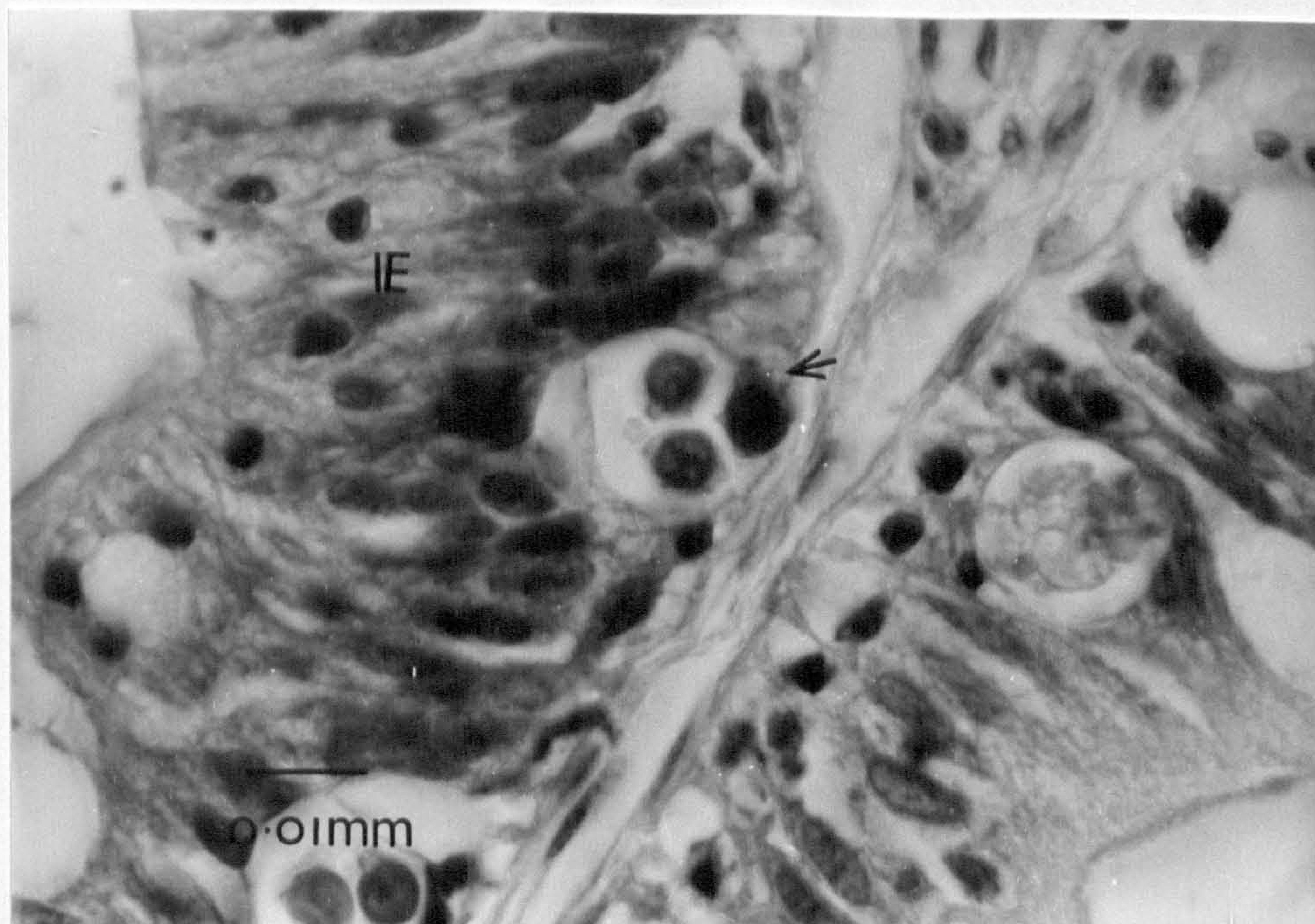


Fig 3A.28: A higher view of a coccidian oocyst (arrowed) located within the intestinal epithelium (IE) of a *C. rupestris*. Three spores are located within each oocyst. Little tissue disruption can be seen in the area of infection.



3A.3.6.2: Bacteriology

Pathology associated with bacterial infection in wrasse was observed only in the *C. rupestris* from Captive Site I. Bacterial colonies were observed in the gills of one of the fish where there was considerable tissue disruption in the area of the bacterial colony (Fig 3A.29).

These colonies were typical of those seen in *Aeromonas salmonicida salmonicida* infections in *S. salar*. Bacterial necrosis was also observed in sections of a skin lesion from one of the fish. These lesions caused severe muscular necrosis associated with an invasive bacterial infection (Fig 3A.30 & 3A.31). Staining with Gram Humberston, see Appendix, indicated that the bacteria were gram positive (Fig 3A.32).

Fig 3A.29: An extensive bacterial colony (BC) in the primary lamella of a C. rupestris from Captive Site I. Considerable tissue disruption can be seen with a significant inflammatory response (x10 objective).

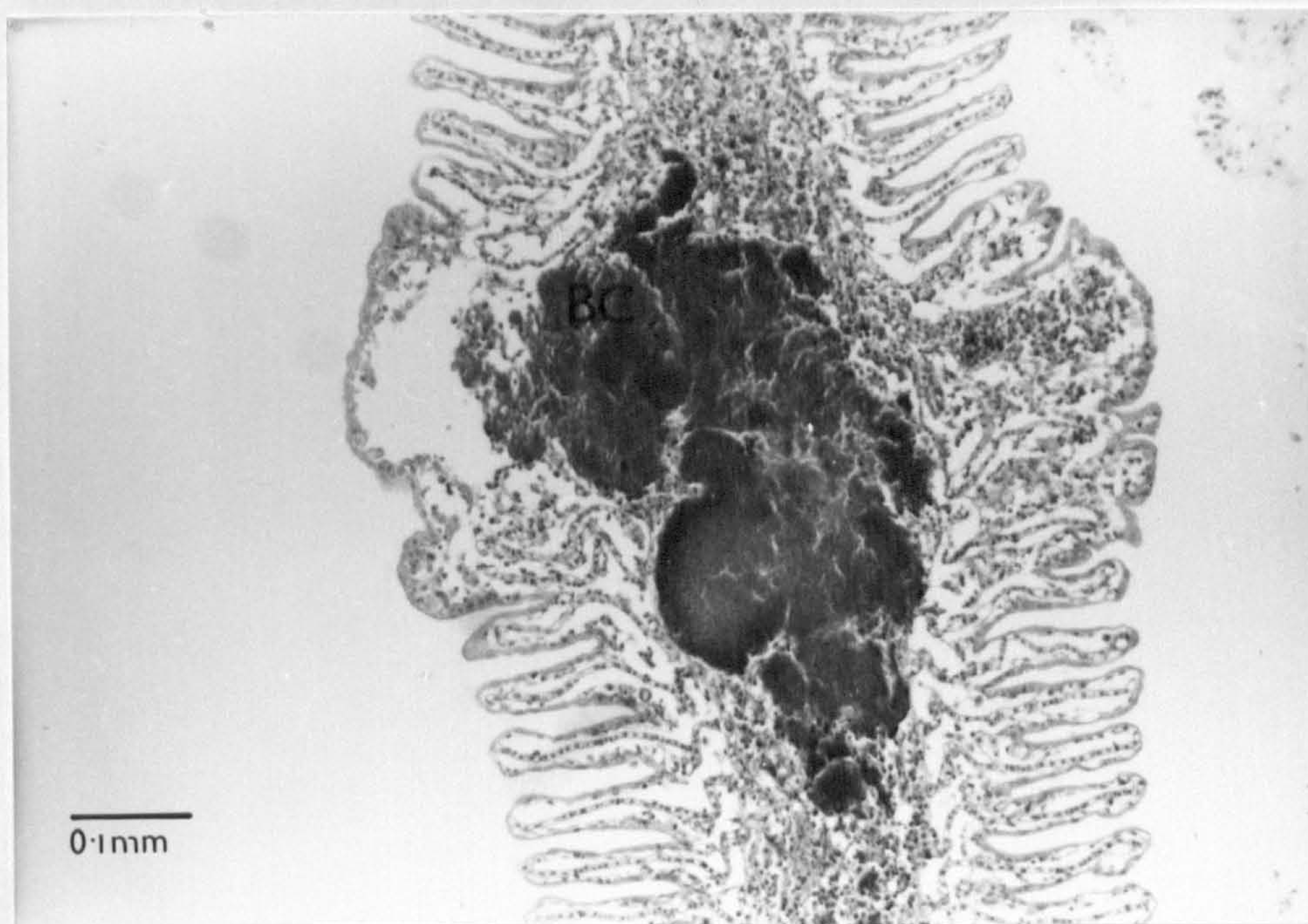


Fig. 3A.30: Invasive bacterial lesion in the musculature of a *C. rupestris* from Captive Site I most probably caused by *Aeromonas salmonicida salmonicida*. Severe muscular necrosis can be seen which penetrates deep into the musculature (x6.3 objective).

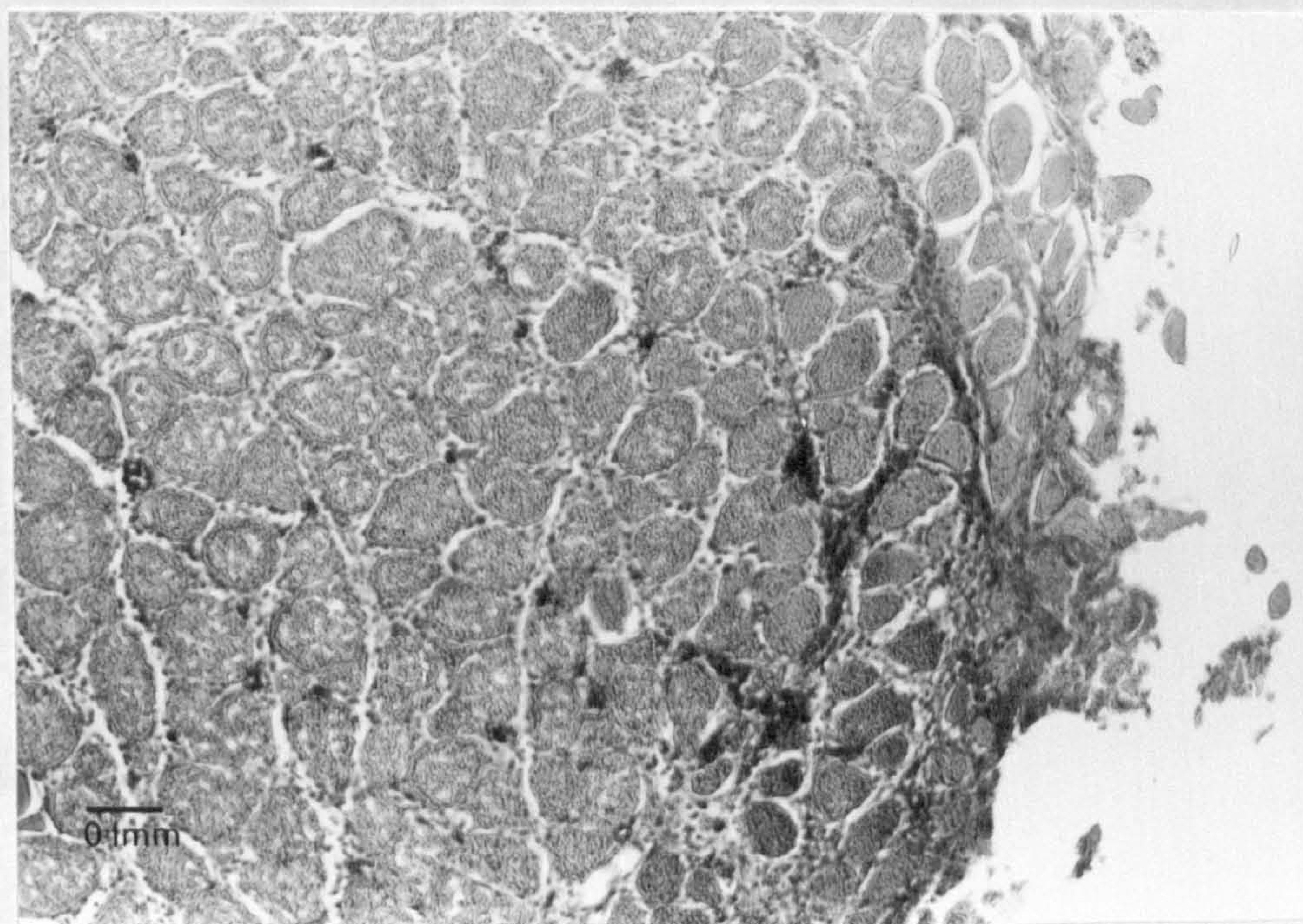


Fig 3A.31: Severe bacterial necrosis in muscle of a C. rupestris from Captive Site I. Red blood cells and leukocytes (arrowed) can be clearly seen in the junctions between muscle fibres.

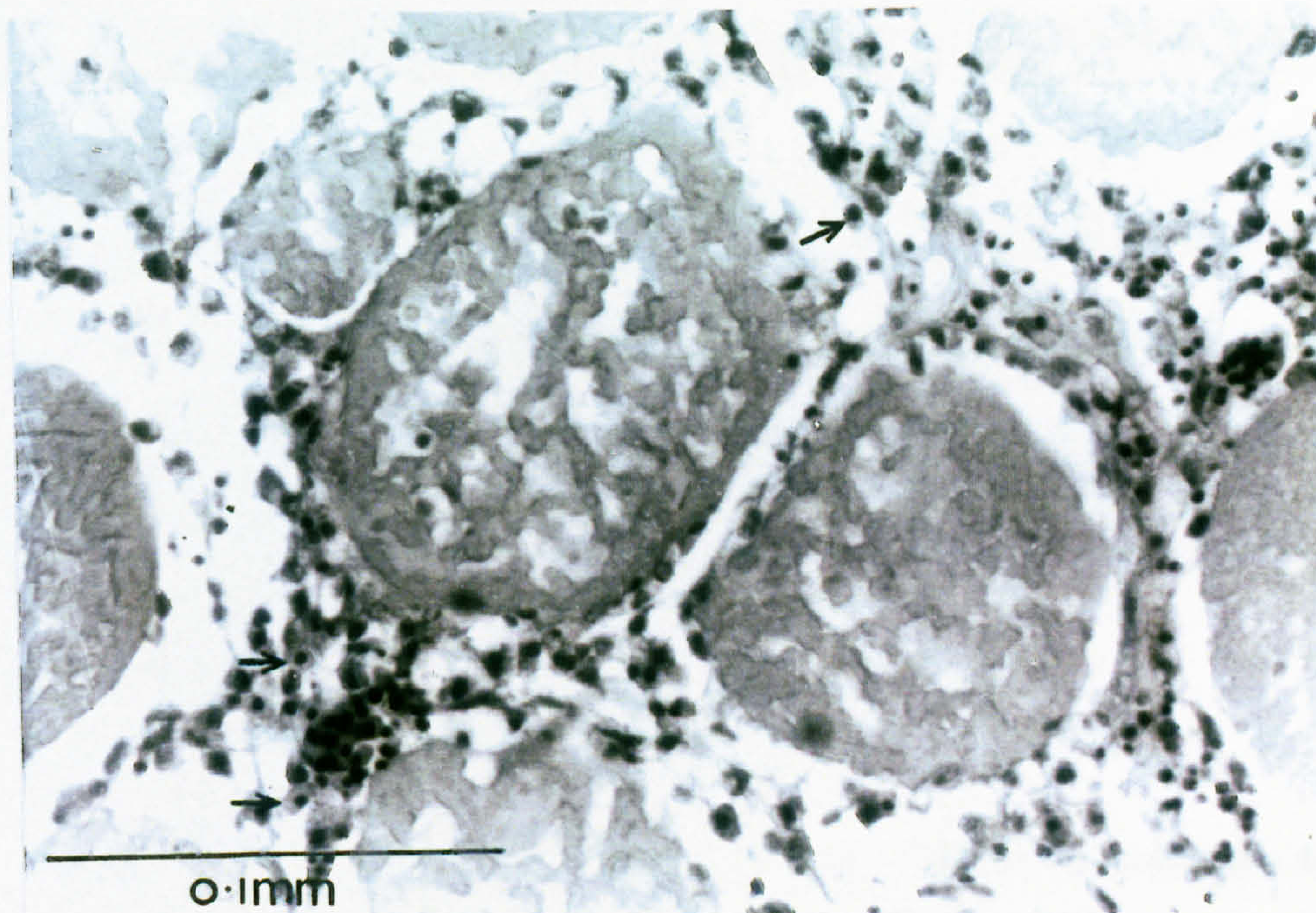
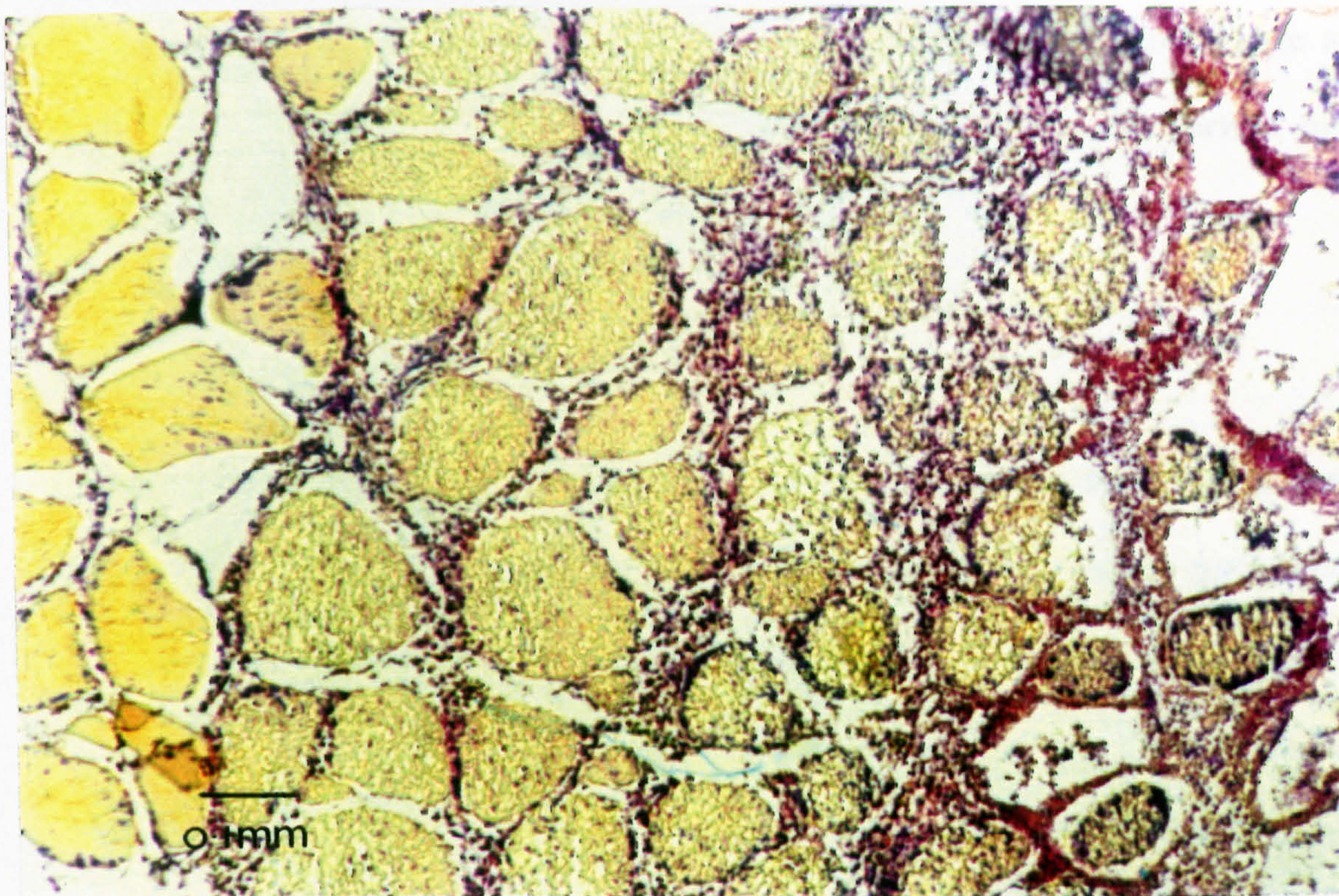


Fig 3A. 32: Gram Humberston stain of bacterial necrosis in muscle of *C. rupestris* showing that the bacteria present are gram positive (x10 objective).



3A.4 Discussion

Sample sizes from all sites were small. In the wild samples this was due to the difficulty involved in fishing for the wrasse. This also affected the species composition of the samples. Larger sample sizes could have been obtained if the wrasse were purchased from the commercial fishermen supplying *S. salar* farms, but, it was decided that fishing for the wrasse would be preferable to buying fish due to the difficulty involved in ascertaining the exact location of capture of commercially caught fish. There was also the risk that fish from different locations had been mixed after capture resulting in a possibility of parasite transfer.

In the case of farm held fish it was very difficult to persuade the farm management to part with more than a few wrasse due to their own difficulty in obtaining the fish and the relatively high price that they had paid for them. At the time wild caught wrasse were being sold for around £2.50 each. Once stocked in pens wrasse have a limited period of usefulness due to mortalities and losses; any wrasse that survive are very important in keeping numbers of lice low in pens.

3A.4.1: New Host Records

A survey of the available literature showed that the results from the disease survey represents a total of 24 new host parasite records. Of the twelve species of parasites collected from *C. rupestris* in this study seven are new host records. These are the digenean *L. rufoviride*, the monogenean *M. donavini*, the nematodes *C. osculatum*, *H. aduncum* and *A. simplex*, the cestode *N. lingualis* and the copepod *C. centrodonti*. In the case of *C. exoletus*, all of the nine positive parasite species identifications resulting

from this study are new host records, these are *Trichodina* sp., the digeneans *C. lingua*, *L. rufoviride*, *M. alacris* and *Z. rubellus*, the nematodes *H. aduncum*, *C. osculatum* and *A. simplex* and the copepod *H. cluthae*.

The acanthocephalan species recorded from both *C. rupestris* and *C. exoletus* could not be determined with confidence as it was only present in larval form. It has been tentatively identified as *Corynosoma strumosum* (Rudolphi). If this is the case this is also a new host record for both wrasse species.

Similarly all of the seven parasites recorded from *L. mixtus* in this study are new host records. These are *Trichodina* sp., *C. lingua*, *M. alacris*, *H. pulchella*, *C. osculatum*, *H. aduncum* and *H. cluthae*. Of the three parasites recorded from *S. melops* in the present study two are new host records, these are *Trichodina* sp. and *C. centrodonti*.

Although no species of *Trichodina* have been recorded from *C. exoletus*, *L. mixtus* or *S. melops* previously their presence on these fish is not surprising given the wide spread distribution of the genus. Trichodinids are one of the most widely reported groups of external protozoans of fish in both fresh and salt water. Members of the genus *Trichodina* have been reported from *C. rupestris* in Norway (Costello 1991) and in Scotland by Treasurer (1991a). At low levels trichodinids do not normally cause pathological changes in their host. It is possible under stressful conditions, such as holding after capture and stocking in farm cages, that the trichodinid numbers on the wrasse may increase resulting in pathological effects. Due to the direct life-cycle of

trichodinids and their ability to quickly multiply, they are potentially dangerous in holding facilities. This is especially so if water quality is poor or stress levels are high. Hilldén (1981) reported that in the wild the territories of *C. rupestris* were approximately 1.4m^2. The wrasse in farm pens are likely to be stocked in higher numbers than this as wrasse in *S. salar* pens are normally found to inhabit the dead sock of pens in relatively large numbers (Pers. Obs.). Under these circumstances, host to host transfer of parasites with direct life-cycles, such as *Trichodina* sp., is liable to be greater than that seen in the wild. This could result in higher numbers of infection with these parasites than would be seen in their natural habitat. These factors, linked with poor water quality, could cause increased infections with trichodinids. Under these circumstances it is likely that they may cause significant pathology, notably hyperplasia of the gills. However, in the present study no such pathology was seen. The high levels of infection recorded in some of the fish sampled could be a reflection of a very rapid increase in trichodinid numbers due to the stress induced by transport and also the holding conditions of the fish. If this was the case, then pathology related to the infections would not always be evident as it can take several days of high numbers of trichodinids to induce hyperplasia in the gills of fish (Sommerville Pers. Com.).

The genus *Microcotyle* was established by van Beneden & Hesse (1864) with a description of *M. donavini* from the gills of the wrasse *Labrus donavini*, this species being now regarded as the type species for the genus (Williams 1991). This parasite has also been recorded from the gills of the *L. bergylta* (Radjukovic and Euzet 1989) and therefore would appear to be a parasite of labrids. Not only is this finding a host record but it is the first record of this parasite in British waters.

The copepod *H. cluthae* is described from *C. exoletus* and *S. melops* for the first time. This species was listed by Kabata (1979) as occurring solely on *L. bergylta* and *C. rupestris* in British waters. This record of the parasite from two of the other wrasse species from British waters is not surprising considering the very close relationship of all three species of wrasse. The record of *C. centrodonti* from *C. rupestris* and *L. mixtus* adds two species to the list of hosts recorded for this copepod. Kabata (1979) reported that most of the previous reports of this parasite have been from perciform fish, mainly labrids such as *L. bergylta*, but there have also been reports from species such as *Sebastes marinus* (L.) (golden redfish).

The final hosts of *L. rufoviride* are *C. conger*, and *Anguilla anguilla* L., (European eel). The adult has been described from the intestine of both these species of fish by several authors, as documented by Gibson and Bray (1986). These records range from Plymouth to Aberdeen and the North and West of Scotland. The metacercaria of *L. rufoviride* has been described by Gibson & Bray (1986) from *G. morhua*, *Blennius pholis* L.(shanny) and *Psetta maxima* L. (turbot). The first intermediate host for *L. rufoviride* is thought to be the benthic gastropod mollusc *Gibbula cineraria* (L.) (topshell). The cercaria has only been described once from a naturally infected snail by Pelseneer (1906) although Koie (1990) did succeed in experimentally infecting *G. cineraria* by exposing them to embryonated eggs of *L. rufoviride*. As the cercaria of *L. rufoviride* are free swimming after release from the snail host, it is not unusual to find the metacercaria in inshore fish species.

Similarly, the presence of *C. lingua* infections in *L. mixtus* and *C. exoletus* would be expected due to the low specificity of the parasite in inshore fish populations. it is perhaps surprising that *S. melops* was not discovered to be infected with the species. This is likely to be a result of the small sample size for this wrasse species rather than it not being susceptible to infection with *C. lingua*.

The record of adults of the digenean *M. alacris* from *L. mixtus* adds to the evidence that this parasite is family specific to labrids.

The specimens of the adult digenean *H. pulchella* recorded from *L. mixtus* from both Wild Sites A & C would suggest a degree of species specificity of this parasite for *L. mixtus* due to the geographical differences seen between the two sites. Nicoll (1910) described *H. pulchella* from the intestine of *L. bergylta* and *C. conger* from the Firth of Clyde but also noted that he had found the species in many different host species, mainly from the south coast of England. Therefore, *H. pulchella* may not be as species specific as the data from the present study suggests. A digenean of the same family, *H. fasciata*, was reported by Rudolphi (1819) & Papoutsoglou (1976) from *S. melops* from Mediterranean waters and by Ergens (1960) & Sey (1970) from the Adriatic *in L. mixtus*. The two species of digeneans are quite similar in appearance but can be distinguished on the basis of body shape and testes structure.

A single specimen of the adult digenean *Z. rubellus* was recovered from the intestine of a *C. exoletus* from Farm Site 1. Bray & Gibson (1986) gave their own records of this digenean from the intestines of *Anarhichas lupus* L. (wolf fish), *L.*

bergylta, *Labrus bimaculatus* (=L. mixtus), *Limanda limanda* L. (common dab), *P. flesus* and *P. platessa*. They also reviewed reports of the parasite from *Blennius pholis* and *Trachurus trachurus* L. (horse mackerel or scad). It would appear that *Z. rubellus* has a low degree of species specificity. They concluded that the second intermediate hosts for *Z. rubellus* are likely to be echinoderms and nereis worms. However, considering the wide diversity of final hosts for *Z. rubellus* it would be surprising if the intermediate host was limited to only two groups of invertebrates. Bjordal (1991b) reported that stomach content analysis of *C. rupestris* and *C. exoletus* from *S. salar* pens revealed that in addition to lice they had been feeding on crustaceans, polychaetes, mussels and tunicates. It is possible, thereby, that wrasse, browsing on such invertebrates in farm pens, could be inadvertently preying on intermediate hosts of parasites such as *Z. rubellus*.

The first intermediate host of the three nematode species recorded are thought to be species such as ctenophores, chaetognaths, polychaetes and crustaceans such as euphausiids and mysids (Smith, Elarife, Wootten, Pike &Burt, 1990, Svendsen, 1990). Most of these species are zooplanktonic. Wrasse must be preying on these creatures in order to acquire nematode infections due to the indirect nature of the life-cycles of nematodes. As indicated in section 2.1.1.1, *C. rupestris* feed on a large variety of foods, therefore it feasible that these mainly planktonic animals could comprise part of their diet. A second route of infection for *A. simplex* L3 and *H. aduncum* adults is from other fish where wrasse would be acting as a second intermediate or paratenic host to the parasite in the case of *A. simplex* and as a final host in the case of *H. aduncum* (Smith

1974 and Wootten & Smith, 1975). Wrasse are not known to be piscivorous to any great extent so this method of infection will be limited.

Wootten (1978) described the L3 larvae of *C. osculatum* from *M. merlangus*, *Melanogramus aeglefinus* L. (Haddock) and *Trisopterus minutus* L. (Poor Cod) from the Moray Firth. Harford Williams (1959) also reported this species from *Alosa fallax* Lacepéde (Twaite Shad) from the South Minch, *Conger conger* L., (conger eel) and *Gadus virens* (*Pollachius virens* L.) (Saithe) from the west of Ireland and *Gadus callarius* (*Gadus morhua callarius* L.) (Baltic Cod) and *Scomber scombrus* L. (Mackerel) from north of Ireland. In addition to these records, Khalil (1969) reported *C. osculatum* from *Clupea harengus* L. (Herring) from all around the coast of Britain. Adult forms of *C. osculatum* have been reported from *Halichoerus grypus* Fabricius (Grey Seal) from the Shetland Isles and from the western Scottish islands (Young 1972).

The L3 larvae of *A. simplex* are also well documented. Wootten (1978) reported this species from *Trisopterus esmarkii* Nilsson (Norway Pout) and *Gadus morhua* L. (Cod) in the North Sea and from *T. minutus* from the Moray Firth. Wootten & Waddell (1977) also described *A. simplex* as being widespread in both *G. morhua* and *M. merlangus* in Scottish waters while at the same time stating that the abundance of the two species differed considerably between different geographical locations, for example *A. simplex* is scarce in cod from the South Minch but abundant in cod from offshore southern North Sea. No explanation was given for these differences.

Young (1972) documented *A. simplex* L3 larvae and adults from various aquatic mammals from around the Scottish coast. These mammals included *H. grypus*, *Balaenoptera acutorostrata* Lacepéde (Minke Whale), *Balaenoptera physalis* L. (Fin Whale), *Physeter catodon* L. (Sperm Whale), *Globicephala melaena* Traill (Pilot Whale) and *Phocaena phocaena* L. (Harbour Porpoise).

Although reports of *A. simplex* and *C. osculatum* from wrasse have not been seen before, their occurrence in these fish is not surprising due to the large number of fish species reported to be infected by this parasite. It is, however, unusual to see *A. simplex* in inshore species. Jones (1994) reviewing the literature relating to the distribution of common ascarid nematodes concluded that both of the above nematode species would be found in areas where there was the greatest number of suitable hosts. For *A. simplex* this is offshore waters whereas for *C. osculatum* this is in coastal waters.

There would appear to be few records of *H. aduncum* from British waters but the L2 larvae of this species are common in zooplankton in Norwegian waters (Svendsen, 1990) while Øresland (1986) also reported the presence of L2 larvae of *H. aduncum* in chaetognath *Sagitta setosa* Mueller from the English channel. In addition to these records, Smith (1983) reported the L2 larvae of *H. aduncum* from euphausiids from locations to the east and west of Scotland. L3 larvae of *H. aduncum* in fish were reported to be abundant in small gadoids in both offshore and inshore waters, whereas L3 larvae of *C. osculatum* were localised in distribution around the Scottish coast possibly reflecting the distribution of the seal final host (Wootten 1978). Jones (1994) recorded L3 larvae of this species from *G. morhua*, *M. aegelfinus* and *Micromesistius*

poutassou (Risso) (blue whiting) from waters off the east coast of Scotland and from *Myoxocephalus scorpius* L. (bull rout) from coastal waters on the east coast of Scotland. Berland (1961) considered the distribution of *H. aduncum* to be circumpolar in the northern hemisphere where it infects fish in both temperate and cold waters.

The larval cestode, *N. lingualis*, recorded from *C. rupestris* from Farm Sites 2 & 3 represent new host records for this parasite species. Yamaguti (1959) reviewed reports of *N. lingualis* from many different hosts including teleosts and elasmobranchs from many different genera. The larval stage of the parasite would appear to be equally widely distributed illustrating the low degree of specificity of this parasite.

Probably the most significant of these host records is *A. simplex* as this is the nematode associated with the disease Anisikiasis in humans. If, as some reports suggest (Darwall *et al.* 1991), *S. salar* on occasion attack and consume moribund wrasse in cages, there may be a potential for *S. salar* to act as second intermediate hosts for *A. simplex*. This would provide a possible infection path to humans. It is fortunate, therefore, that the present stringent processing and screening techniques for *S. salar* fillets are in place. Most of the nematodes will be killed by the cooking of fish at high temperatures but not by low temperature treatments. There is a modern preference for dishes incorporating either lightly cooked or raw fish originating from countries such as Scandinavia, Holland and Japan. These dishes include pickled herring or rollmops, shashimi and sushie. All of these dishes are likely to contain living nematodes. In all of the cultures from which these dishes originate there is a social history of anisakiasis. Kagei & Isogaki (1992) detailed a case study where a 58 year old Japanese woman

suffered severe intestinal pains after eating a dish of sashimi. A total of 56 larval *A. simplex* were removed from her stomach using biopsy techniques. In a previous study carried out in France, 21 cases of human anisakiasis were reported in a 33 month period (Hubert, Bacou & Belveze 1989). Eight fish species were identified to be the sources of some of the infections and in two cases *S. salar* was involved. The authors identified the consumption of pickled or raw fish as a major source of infection with *A. simplex*. It is essential, therefore, that the risk of infected fish reaching the public is minimised. Currently *S. salar* is rarely eaten raw as the traditional methods involved in its preparation mostly involve cooking. The only methods currently in use for the preparation of *S. salar* which could leave the public open to infection are the cold smoking of fillets and the consumption of *S. salar* as "gravad lax". The first method is hazardous as the core temperature of the fillet does not reach a level sufficiently high enough to kill the nematodes. The flesh of the fish must reach at least 60°C for a period of time greater than one minute to ensure the death of the nematodes (Margolis 1977). The larvae of anisakines are also very tolerant of freezing. They will survive the temperatures used in most domestic freezers (-5°C to -10°C) and must be stored at -35°C for at least 24 hours before all nematodes are killed (Deardroff & Throm 1988). The only freezing method which uses temperatures this low is the commercial blast freezing of fish. The second method requires the fillets of *S. salar* to be lightly pickled and sliced very thinly before they are eaten, the risks from this method are obvious.

It is important to note that *A. simplex* has never been reported from farmed *S. salar* in Scotland and processing techniques are designed to ensure that there is no chance of human infection. Regular checks are made and on the basis of these, export

sales, for example to Japan, are certified as *Anisakis* free (McVicar Pers. Com.). The possibility of the transfer of nematodes between wrasse and *S. salar* is investigated in Chapter 3B.

3A.4.2 Wild Fish

3A.4.2.1 Comparisons Between *Ctenolabrus rupestris* and *Centrolabrus exoletus*

3A.4.2.1.1 External Parasites of Wild Caught Fish

It is not known whether the species of *Trichodina* on the wrasse could pose a health risk for stock *S. salar*. In the present study trichodinid numbers actually fell with length of time in the holding tanks in the laboratory. This is not to say that where wrasse are held in holding facilities prior to stocking in cages the numbers will not increase. In the laboratory holding tanks, fish were held in very high quality conditions to reduce stress as much as possible, but this may not be the case on farms.

The prevalence of trichodinid infection in both Sites A and C was very similar. It could be that these levels reflect the level of infection after one week in static holding tanks rather than the level of infection in the wild. The level of infection in the Site B sample, however, was double that for the other two samples despite identical handling techniques being employed. This would perhaps suggest that the level of intensification of infection in captivity is not always constant. However, it is considered that in this study the level of infection with trichodinids was most likely to be a reflection of the original level of infection in the wild rather than an accurate indication of this infection. Both the samples from Sites A and C were captured in June 1992 and the sample from

Site B was captured in August of the same year. The differences in infection may be a reflection of the increased sea temperature in August compared to June.

In both *C. rupestris* and *C. exoletus* the trichodinids only infected the gills and not the epithelium of the body surface. The level of infection for *C. exoletus* was approximately half that for *C. rupestris* from the same site. This may suggest a difference in the habitats between the two fish or a different species of trichodinid infecting the two wrasse species.

The crustacean *C. centrodonti* was most abundant in the *C. rupestris* from the Site B sample, but, the mean abundance and range of infection was very similar to the other two samples. These differences would not appear be significant. This parasite was not found on the *C. exoletus* from Site A despite the presence of the parasite in the area. It has previously been recorded from *C. exoletus* by Bron & Treasurer (1991), therefore it may reflect the small sample size of *C. exoletus* from Site A.

The data for *H. cluthae* infections of *C. rupestris* is remarkably similar for all three areas with *H. cluthae* infections of *C. exoletus* in the Site A sample slightly more numerous than those of *C. rupestris* from the same sample. The uniform distribution of this parasite on *C. rupestris* from the three different localities suggests that this parasite is widely distributed around the coast.

3A.4.2.1.2 Internal Parasites

By far the most common digenean in both the *C. rupestris* and *C. exoletus* was the metacercaria of *C. lingua*. This is a very common parasite of inshore marine fish

being visible as small black spots, (approximately 1mm in diameter), on the skin and gills of the host. The final hosts for this parasite are piscivorous birds and it is generally regarded as causing no harm to the adult fish host (McQueen, MacKenzie, Roberts & Young 1973). The histopathology seen associated with this parasite in wrasse would appear to agree with these findings. However, individual fish of adult size, around 11cm, were seen to be harbouring up to six hundred *C. lingua* on the skin and gills (Site C). All of the *C. lingua* observed in the wrasse species involved in this study were well established specimens, therefore the damage caused by the cercariae penetrating the fish was not observed. Thulin (1986) stated that *C. lingua* cercaria may kill young fish and alter the behaviour of others, while metacercariae may blind fish if they establish within the cornea. Presumably the disruption to the epithelium caused by large numbers of cercariae simultaneously penetrating the host causes osmoregulatory difficulties. Sindermann & Farrin (1962) reported that cercarial release from the snail host is seasonal being influenced by temperature, salinity and state of the tide with more cercariae released during high tides and increased temperatures. The authors described that on the northern coast of New England, USA cercarial release from the littorinid host took place predominantly during the months May to November. This coincided with the annual peak in sea water temperatures. This seasonality of release would result in increased numbers of cercariae in the water in the and summer months in Scottish waters when wrasse are found predominantly in shallow coastal waters and would lead to occasionally high levels of infection. Unlike other fish species, such as gadoids, wrasse spend most of their lives in shallow coastal waters. This will allow them to accumulate *C. lingua* infections throughout their life. Other fish species are only susceptible to infection during the time as juveniles where they use the tidal zone as a habitat.

Infections with *C. lingua* were common in both Sites B & C reflecting the even distribution of the snail host and final host in most areas of the coast. The fish from Site A, both *C. rupestris* and *C. exoletus*, had lower prevalence and abundance of infection than the fish from the other two sites, this was reflected also in lower ranges of infection. This is perhaps surprising given the very sheltered nature of the site with low tidal flushing. These factors would be expected to increase the length of time that the cercariae were concentrated in the water column before being dispersed. Further work is needed to investigate the distribution of littorinid snails and final bird hosts in the area of Site A. The levels of populations of these organisms may be lower in Site A than in the other two sites giving rise to the lower infection rates with *C. lingua* in the area.

The metacercariae of the digenean *L. rufoviride* were present in both *C. rupestris* and *C. exoletus* being twice as abundant in *C. rupestris* as in *C. exoletus*. This parasite did not cause significant pathology in any of the fish examined, however, all the specimens recovered were well established and, as with *C. lingua*, it is possible that the penetrating cercariae would produce more significant pathology during their migratory stage. The lack of evidence of any chronic pathology in the wrasse species investigated suggests that any such pathology is short term. It is not known whether *S. salar* can act as a final host for this parasite. Unfortunately there is a lack of literature on this subject as *L. rufoviride* has received little attention as a subject of study. The difference in prevalence between *C. exoletus* and *C. rupestris* may be due to a difference in the feeding behaviour of the two species. Both species of wrasse are found in the same habitat on the coastline and a degree of niche separation would be expected to prevent the two species from coming into direct competition with each other.

The most common nematode was *C. osculatum* in all three samples of *C. rupestris*. The infections were greatest in the Site C sample. The fish in this sample harboured the greatest number of nematodes for all three wrasse species. A possible explanation for this is that the diet of *C. rupestris* in the Site C area was significantly different from *C. rupestris* in other areas enabling them to pick up more nematodes from the first intermediate host. The first intermediate host is assumed to be a zooplanktonic species (Smith *et al.* 1990, Svendsen 1990). This high prevalence could be due to a superabundance of the first intermediate host of the nematode in the Site C area or that the fish are being very selective. A third possible explanation for the difference is that there is a higher number of infective stages of the nematodes in the Site C area compared to the other two sites, which may be related to the two month's difference in the samples from Sites A and B compared to Site C. It could also be the case that the greater exposure to the open sea seen at Site C results in a higher number of the planktonic hosts of the infective stages being present allowing the wrasse to build up higher infections of the nematodes. Unfortunately there is no data on the abundance of infective stages of *C. osculatum* at different times of the year.

The *C. exoletus* obtained from Site A harboured only one species of nematode, this was *H. aduncum*. The other two species (*C. osculatum* and *A. simplex*) were present in the Site A area as they were found in *C. rupestris*. This would, again, point to possible differences in feeding behaviour between the two species. The greatest number of *C. osculatum* and *A. simplex* were seen in the larger specimens of *C. rupestris*. This could be a result of accumulation of the parasites throughout the life of the fish. It could also result from the parasites using larger intermediate hosts than those of *H. aduncum*. If

this was the case then it is only the larger fish that would be able to prey on them. This would also explain the absence of C. *osculatum* and *A. simplex* in the smaller *C. exoletus* when compared to *C. rupestris*. The number of final hosts of nematodes may also affect the level of infection seen in wrasse. Corbet & Harris (1991) identify breeding populations of *Phoca vitulina* L. (common seal) in the vicinity of Sites A & B while there are none in the immediate area of Site C. This species of seal are known to be concentrated around the breeding grounds even out of the breeding season. There are also breeding sites of *H. grypus* (grey seal) closer to Sites A & B than Site C, however, this species of seal is only found in the breeding area during pupping. At other times they congregate in other areas. Both species of seal are known to be opportunistic in their feeding, preying mainly on small teleosts, and they are also known to travel large distances between haul out areas and feeding grounds. The specific foraging behaviour of seals on the west coast of Scotland is not known, therefore, it is impossible to say from the information available if foraging seals are more common in one of the sampling areas than another. Both of these aspects need further investigation in order to establish the reasons for the differences in nematode prevalence in the different sampling areas.

Acanthella of *Corynosoma* sp. were more abundant in *C. rupestris* from Site B than in the fish from the other two areas. The data for the acanthocephalans in the *C. exoletus* were almost identical to the data for the *C. rupestris* from Site A. This is surprising as Site A and Site B are quite close geographically. Although the full life cycle of corynosomid acanthocephalans is not known, the first intermediate host is thought to be planktonic micro-crustaceans such as ostracods (Yamaguti 1959). The final hosts are marine mammals and aquatic birds. It is possible that there is a large number of these

first intermediate hosts or final hosts in the Site B area compared to the other two sites producing the higher infection rate.

From the small sample studied it would appear that *C. exoletus* are less parasitised than *C. rupestris* from the same area. The differences between the two species of wrasse are most probably due to differences in the diet of the two species. This may be related to the smaller size of *C. exoletus* when compared to *C. rupestris* of equivalent age, resulting in *C. exoletus* preying on smaller food items than *C. rupestris*.

3A.4.2.1.3 Comparisons of Length and Sex Classes

There would not appear to be any common pattern running through the size class results for the three sites for either *C. exoletus* or *C. rupestris*. For sites A and C there is some evidence for accumulation of nematodes with increasing fish length in *C. rupestris* but this is not the case in the site B fish. The same can be said for *M. alacris* in sites B and C compared to site A. There are no clear patterns of differences between the sexes in either *C. rupestris* or *C. exoletus*. The sample sizes in the different size and sex groups were very small due to the small sample sizes in the original groups

3A.4.2.2 Parasites of *L. mixtus* and *S. melops*.

Very few conclusions can be drawn from the small number of fish examined. However, with the exception of *C. lingua* all of the parasites recorded from these two wrasse species are new host records.

Trichodinids were found on the skin and not the gills of *L. mixtus* but on both surfaces for *S. melops*, this would indicate that it is most probably a different species of

trichodinid which infects the two different species of wrasse. In the case of *S. melops* there could be two different species of trichodinids, one infecting the gills and the other infecting the skin.

The digenean parasite *H. pulchella* was found in *L. mixtus* in areas where it was not found in the other species of wrasse. This parasite has been recorded from other wrasse species such as *L. bergylta* and *C. rupestris* at Plymouth and also from *S. melops*, but not from British waters (Nicoll 1916). Therefore, it is not clear why *H. pulchella* was not found in the other species. However, a far larger sample size is needed before any firm conclusions can be drawn regarding these two wrasse species.

3A.4.2.3: Farm Held Fish

3A.4.2.3.1: Comparison With Wild Caught Fish

The mean weights and lengths of the fish from the farm pens were greater than that of the fish from the wild and showed less variation. A possible reason for this is the direct and passive selection of larger fish for the use as cleaner fish. The use of 12mm mesh nets on *S. salar* sites will result in any small wrasse escaping through the mesh. To combat this some farmers only buy fish over a minimum size. Treasurer (1991a) recommended that only wrasse larger than a total length of 100mm should be used to stock farm sites to avoid the problem of loss of fish. The selection of this larger size class will have the added benefit of only removing the older fish from a population. Hilldén (1978b) illustrated that *C. rupestris* of 100mm in length in Norwegian waters were a minimum of three years old. During the present study *C. rupestris* of two years of age

were sexually mature. This would allow wrasse one year to reproduce before they were caught for use in *S. salar* farms. This may help to sustain wrasse stocks.

In general the fish sampled from the farm sites harboured fewer parasites than those from the wild. However, the fish used to stock Farm Site 1 were collected from the area of Wild Site A, these fish had been in the farm pens for between four and nine months before sampling. The levels of trichodinds on both *C. rupestris* and *C. exoletus* from the Farm Site were higher than in the fish from Wild Site A. This difference was also seen in the fish from Farm Site 2 when compared to the fish from Wild Site C, which was used to source fish for stocking in the farm around six months prior to sampling. This increase could be due to a reduction in water quality in the immediate vicinity of the farm. This pattern was not seen when the fish from Farm Site 3 were compared to those from Wild Site B. The reduced salinity occasionally experienced at farm Site 3 could have resulted in lower numbers of protozoa.

There was also a general trend in these three pairs of directly comparable samples for a reduction in the number of *M. alacris*. Adult digeneans normally have a limited life span in the intestine of the fish host, so after the length of time the fish spent in the pens it would be expected that the number of these parasites would reduce, providing that the fish did not have access to their natural food sources. None of the wrasse sampled had been held in *S. salar* pens for more than eight months, assuming that no re-infection occurred during their time in pens, the life span of adult *M. alacris* must exceed this period. As reported earlier, Bjordal (1991b) recorded many different invertebrates from

the intestines of wrasse in *S. salar* pens indicating that they is a possibility that natural infection with parasites can still take place.

The most significant reduction in all of the farm samples was in the number of external metazoan parasites. There was only a single specimen recovered from any of the farm fish. This was the single *L. labrei* recovered from the gills of a *C. rupestris* in Site 3. Costello (1991) suggested that the low number of external parasites on wrasse was a result of intraspecific cleaning. If this was the case the wild and farm fish would be expected to have similar numbers of external parasites. This activity may play a role in reducing the numbers of these parasites in the wild. However, the very low numbers seen in the farm fish may be a result of delousing treatments carried out in the pens in which the wrasse were held. These treatments are likely to affect the number of crustacean parasites recovered from the farm held fish. The only crustacean recovered was a single specimen of *L. labrei* seen on a *C. rupestris* from Farm Site 3. The fact that this parasite lives embedded within the dermis of the host may protect it from delousing treatments. The wrasse had spent eight months in the farm pens prior to being sampled which may exceed the natural life of these parasites, the larvae of which may not survive in the farm conditions to re-infect the fish.

While the prevalence of infection with trichodinids was high in Sites 4 & 5 the intensities were low and evidence of pathogenic effects was absent. This suggests that protozoal infections will not prove to be a hazard to the health of wrasse in *S. salar* pens.

Infections with the digenean *C lingua* were similar in both wild caught and farm held fish. The level of infection of this parasite is seasonal, depending upon the intermediate host, periwinkles. This will not be affected by the wrasse being in *S. salar* pens as opposed to being in the wild. The only factor which could affect this is the distance from an infected population of snails. In the wild, wrasse live in amongst the kelp, close to the littoral habitat of the snails, whereas, in pens, they are elevated from the sea bed and generally a few hundred meters from the shore. This could reduce the abundance of infection as the tides and currents would disperse the cercariae of *C. lingua* once released from the snails. However, farmed *S. salar* are often seen to be infected with *C. lingua* metacercaria, hence, the holding of fish in pens offshore is not necessarily a barrier to infection with *C. lingua*. The level of infection is likely to be highly dependent on factors such as; proximity to a rocky shore line, depth of water below the pens and current systems around the pens.

As would be expected, the levels of infection with the metacercaria of *L rufoviride* was similar in both farm held fish and wild caught fish. The metacercariae of *L. rufoviride* are long lived in the fish intermediate host and the infections observed in the farm held fish were probably acquired prior to capture. It may also be that, although the fish are held in farm conditions, they may still become infected by the free swimming cercaria of this digenean parasite. However, as the molluscan host of the parasite, *G. cineraria*, is most common on kelp in shallow water (pers. obs.), it is difficult to see how large numbers of cercaria could infect fish held in pens in deeper water. Fish held in shallow pens close to the shore will be more susceptible to infection.

Nematode infections were still common in the fish examined from farm sites, but, in general the abundance of infection was lower than that seen in the wild caught fish. This could reflect a reduction in the number of parasites harboured by each fish due to the death of the parasites or being killed by the hosts defence mechanisms. Despite the fact that wrasse do browse on fouling organisms in cages, and are, thereby, foraging in a natural manner, the level of available planktonic prey items, which also forms part of their natural diet in the wild, may be reduced due to the physical barrier of the nets around the pens. These planktonic organisms, for example euphausiids, are considered to be the intermediate hosts for the nematode species recorded from wrasse in this study. This would result in a reduction of infective stages entering the fish. In contrast to this, the numbers of acanthocephala seen in the farm held fish when compared to those in wild caught fish were not significantly different. As the intermediate hosts of this parasite is also planktonic, the wrasse in pens would appear to be able to consume at least a limited number of these items. The diet of wrasse in cages requires more work in order to clarify the sources of infection of these parasites.

3A.4.2.4: Captive Fish

There were few parasites found on the captive held fish from either of the two samples. The formalin bath treatments that the fish had received may have influenced the number of external parasites but would not have affected the internal ones. The presence of the metacercaria of *C. lingua* in the fish from both sites and *L. rufoviride* from the fish from site I most probably is the result of infections acquired before capture. The presence of the adult digenean *M. alacris* in the intestine of *C. exoletus* from site I and the *C. rupestris* from Site II suggests that the fish still had access to a fairly normal diet

while in captivity. The fish from the sample from Site I were fed on fresh *M. edulis* (mussels) and other similar items collected from the surrounding area. These food items could have been the source of continuing infections with *M. alacris*. However, due to the lack of information relating to the life-cycle of *M. alacris* it is difficult to speculate on the source of these infections. The lack of infections with nematodes, with the exception of *C. osculatum* is unusual in light of the findings from the farm sites. In the case of the fish from Site I the fish sampled were small and there may not have been sufficient time for the accumulation of infection with nematodes that would be expected in older fish. The fish from Site II were captured from the Firth of Clyde, and the number of infective stages of the nematodes may have been low in this area. The presence of ciliates, flagellates and trichodinids on the fish from both samples may be an indication of poor water quality in the captive situations.

Comparing the fish from Captive Site II to the fish from Farm Site 3, where they were collected from, reveals very poor correlation between the two samples. The average weights and lengths for the *C. rupestris* from Captive Site II were far lower than those from Farm Site 3. This could indicate that the fish supplied to the aquarium were deemed too small to be stocked into pens and were, therefore, surplus to the requirements of the farm. Farm Site 3 used several different sources for the wrasse it used to stock the pens, so the ones given to the aquarium could easily have been caught from different location from those sampled from the farm itself. This would explain the poor correlation in the parasite infections between the two samples.

A.4.2.5: Incidental Samples

It is difficult to draw any firm conclusions from this data, the fixation was relatively poor and there was no certainty that all of the parasites from the fish had been removed.

The presence of adult forms of *H. aduncum* in *C. rupestris* from the Oban area and *C. rupestris* and *S. melops* from the Isle of Cumbrae indicates that wrasse can act as final hosts for *H. aduncum* though adults were never found in any of the fish sampled. The mechanism of infection involved must include the consumption of prey infected with L3 stages of the parasite. Smith (1983) reported that euphausiids were found to be infected with L3 stages of *H. aduncum* in inshore waters on the west coast of Scotland. These infections were rare but could be locally abundant giving rise to localised infections with adult nematodes if the fish had been feeding on these infected euphausiids. This suggests local abundance of these infected euphausiids in both the Cumbrae area and the Oban area.

3A.4.2.6: Bacteria

The only isolates obtained from the fish sampled were from fish held in farm pens and this would suggest that endemic and acquired bacterial infections may be of major significance in the use of wrasse in aquaculture. The majority of the bacteria isolated were of an opportunistic nature. *Pseudomonas* infections are often seen as secondary infections or they only occur after stress. *Aeromonas salmonicida*, however, is a major pathogen of farmed *S. salar* which is currently well controlled in *S. salar* by the use of vaccines. Treasurer & Laidler (1994) have already reported major losses of wrasse in

pens due to *Aeromonas salmonicida salmonicida* infections. Research into the use of vaccines in wrasse is perhaps warranted.

The bacterial colonies observed in the gills of fish from captive Site I were typical of those seen in *S. salar* infected with *Aeromonas salmonicida salmonicida.* Also the muscle necrosis caused by a gram positive bacterium in the same fish is typical of that seen in *S. salar*, although, the lesion was more invasive than would be expected in *S. salar*. Although no bacteria were isolated from these fish on the plates the strain present could have been a slow growing one which was not picked up by the techniques employed. It is also possible that the infection was present only on the surface of the gills and was not systemic. It is, therefore, not possible to say if *Aeromonas salmonicida salmonicida* was responsible for the lesions.

From these results there would appear to be little health risk to *S. salar* in pens stocked with wild caught wrasse. However, the possibility of transfaunation of wrasse parasites to *S. salar* requires further investigation.

There is little detailed information on diets available for the wrasse species from the present study. However, due to the similarity in parasite species infecting the four different wrasse species it is reasonable to assume that their diets are quite similar. The parasites which utilise direct life-cycles such as the protozoans and copepods will not be affected by the feeding behaviour of their hosts.

The isolation of wrasse from their natural diet would appear to be very important in the reduction of parasite infections. Fish held in captive situations in tanks or aquaria had the lowest infections with parasites when compared to those of farm held fish which were able to browse on fouling organisms and zooplankton.

3A.4.2.7: Histopathology Associated With Disease in Wrasse

In general the histopathology associated with the presence of parasites in wrasse was very limited. The majority of the parasites were seen to be encapsulated in thin walled capsules of host origin. This would suggest that the parasites found in wrasse are well adapted to parasitising these species. If the wrasse had been aberrant hosts to these parasites the tissue reactions seen would have been more severe. None of the parasites recorded in this survey could be considered to pose significant health risks to wrasse. The only exceptions to this would be if the parasites were present in large numbers. In these cases the cumulative effects of the numerous parasites would cause significant pathology.

The pathology seen associated with infection with the bacterium *Aeromonas salmonicida* was more severe than any other pathogen seen in wrasse. This finding concurs with the opinion of Treasurer & Cox (1991) who stated that *Aeromonas salmonicida* will prove to be a major pathogen of wrasse in *S. salar* culture.

The present study has increased the knowledge of the level of parasitism seen in the four native species of wrasse used in *S. salar* culture. Despite numerous new parasite host records, few of the parasites could be considered truly pathogenic to their host. No

substantial pathology was observed in association with any of the parasites. Similarly, few of the parasites can be considered as potentially harmful to wrasse or *S. salar* under farm conditions. Some of the parasite species recorded utilise fish as final hosts, so it may be possible that these parasites could transfer to *S. salar*. The possibility of this transfaunation is examined in Chapter 3B.

CHAPTER 3, SECTION B: PARASITE CROSS INFECTION EXPERIMENTS

3B.1: Introduction

The possibility of parasite transfer between wrasse and *S. salar* is an important consideration when examining the interaction of the two species in a farm situation. Darwall *et al.* (1991) reported that under certain circumstances, such as starvation of *S. salar* prior to harvesting, *S. salar* may attack and consume wrasse in the pens. This would provide a route by which the parasites affecting wrasse could enter *S. salar* and possibly utilise them as final or paratenic hosts. From the survey of diseases carried out in Chapter 3A, several parasites were identified as being potential parasites of *S. salar*. The two nematode species *H. aduncum* and *C. osculatum* were both very common in wrasse species from most of the sample sites. Adult *H. aduncum* have been observed by Wootten in the intestine of *S. salar* (Wootten Pers. Comm.), the L3 larvae of this parasite were also recorded from wild caught *S. salar* by Holst, Nilsen, Hodneland & Nylund (1993). The L3 larvae of *C. osculatum* was also found in such high numbers in some of the wrasse sampled that its role as a possible parasite of *S. salar* required investigation. The digenean *L. rufoviride* utilises fish as its final host; *C. conger* and *A. anguilla*, as does *N. lingualis*. A variety of hosts have been reported for *N. lingualis* which include fish from the following groups; Pleuronectidae, Scombridae, Rajidae and Scorpionidae, suggesting that *N. lingualis* has a low degree of species specificity at the plerocercoid stages. The acanthocephalan *Corynosoma* sp., (if the species used was *C. stromosum* as suspected) utilises mammals and birds from many different genera as final hosts as does the juvenile stage, which have been reported from the following families; *Cottus*, *Coregonus*, *Clupea*, *Sciaena*, *Osmerus*, *Salvelinus*, *Anguilla*, *Perca*, *Lucioperca*, *Pleuronectes*, *Platichthys* and *Rhombus*. This demonstrates a very low degree of species specificity.

In order to investigate the possibility that these parasites could be transferred to *S. salar* in the artificial habitat created by the farm cages, a series of experiments was conducted in which these parasites were introduced to *S. salar* smolts.

3B.2: Materials and Methods

3B.2.1: Experimental Design

Only a small number of viable parasites were available at any one time and, therefore, the experimental groups were neccessarily small.

3B.2.1.1: Experiment 1

Three experimental groups of five *S. salar* smolts, approximately 50g in weight, were used consisting of a control group and two test groups. Test group 1 were infected with L3 larvae of *C. osculatum* and Test group 2 were infected with L3 larvae of *H. aduncum*.

The fish in the two test groups were infected with five nematodes each by gavage while the Control group was gavaged with physiological saline. The gavage technique is described in section 3B.2.4.

Sampling of fish was carried out at 36 hours, four days, nine days and twelve days post infection with one fish being removed from each group at these times.

3B.2.1.2: Experiment 2

Experiment 2 was carried out subsequent to Experiment 1, as more material became available. An attempt was made to clarify and expand the results obtained in Experiment 1. Five experimental groups of *S. salar* smolts were used, a Control group and four test groups. The Control group consisted of four *S. salar* smolts which were

each gavaged with saline and the food paste as in Experiment 1. Test group 1 consisted of four *S. salar* smolts which were infected with six L3 larvae of *H. aduncum* and Test group 2 also consisted of four *S. salar* smolts which were each infected with ten *L. rufoviride* metacercariae. Test group 3, contained two *S. salar* smolts which were each infected with three *Corynosoma* sp. acanthella, thought to be *C. stromosum*, while Test group 4 also contained two *S. salar* smolts which were each infected with one *N. lingualis*.

Test groups 3 and 4 were sampled at 24 hours and four days post infection, while the Control group and Test groups 1 and 2 were also sampled at eight and 12 days post infection. One fish was removed from each group at each of these times. Fish were sampled at 24 hours post infection in order to investigate the possible early establishment of parasites.

The experimental design is summarised in Table 3B.1

Experiment 1 (stock of 15 *S. salar*)				**Experiment 2 (stock of 16 *S. salar*)**					
Sampling interval	Control (5 fish)	Test 1 (5 fish) *C. osculatum*	Test 2 (5 fish) *H. aduncum*	**Sampling interval**	Control (4 fish)	Test 1 (4 fish) *H. aduncum*	Test 2 (4 fish) *L. rufoviride*	Test 3 (2 fish) *Corynosoma* sp.	Test 4 (2 fish) *N. lingualis*
36 hours	1	1	1	24 hours	1	1	1	1	1
4 days	1	1	1	4 days	1	1	1	1	1
9 days	1	1	1	8 days	1	1	1	0	0
12 days 1	1	1	1	12 days	1	1	1	0	0
12 days 2	1	1	1						

Table 3B.1: Experimental design for Experiments 1 & 2 showing the sampling intervals for each experiment and the number of fish removed from the groups at these times.

3B.2.2: Source of Parasites

Parasites were dissected from wild caught *C. rupestris* obtained from Site C for Experiment 1 and Site A for Experiment 2, Sites C and A are described in Chapter 3A.2.1.1. The parasites were then identified to species level before being stored live in physiological saline at 4°C. The time of storage varied as it took several days to dissect all the wrasse to obtain the parasites. Some of the parasites were in physiological saline for five days, approximately 80% of the saline was changed daily. Only the most active parasites were used for the infections.

Site C was chosen as a source of fish for Experiment 1 due the high nematode burden exhibited by fish from this site during the survey of diseases described in Chapter 3A.. However, the number of parasites recovered was fewer than expected so Site A was used as a source of parasites for Experiment 2 due to it relatively easier accessibility.

3B.2.3: Source of *Salmo salar*

The experimental fish were S1 *S. salar* smolts obtained from Howietoun fish farm before transfer to sea water to ensure they had not been exposed to the risk of infection with any of the parasite species used in the experiment. The *S. salar* were then transferred to full strength sea water, 35 parts per thousand salinity, prepared from Instant Ocean sea salts. Two weeks were allowed for acclimatisation. The *S. salar* were kept at a constant 12°C, with a light regime of 12L:12D, for the duration of the experiment.

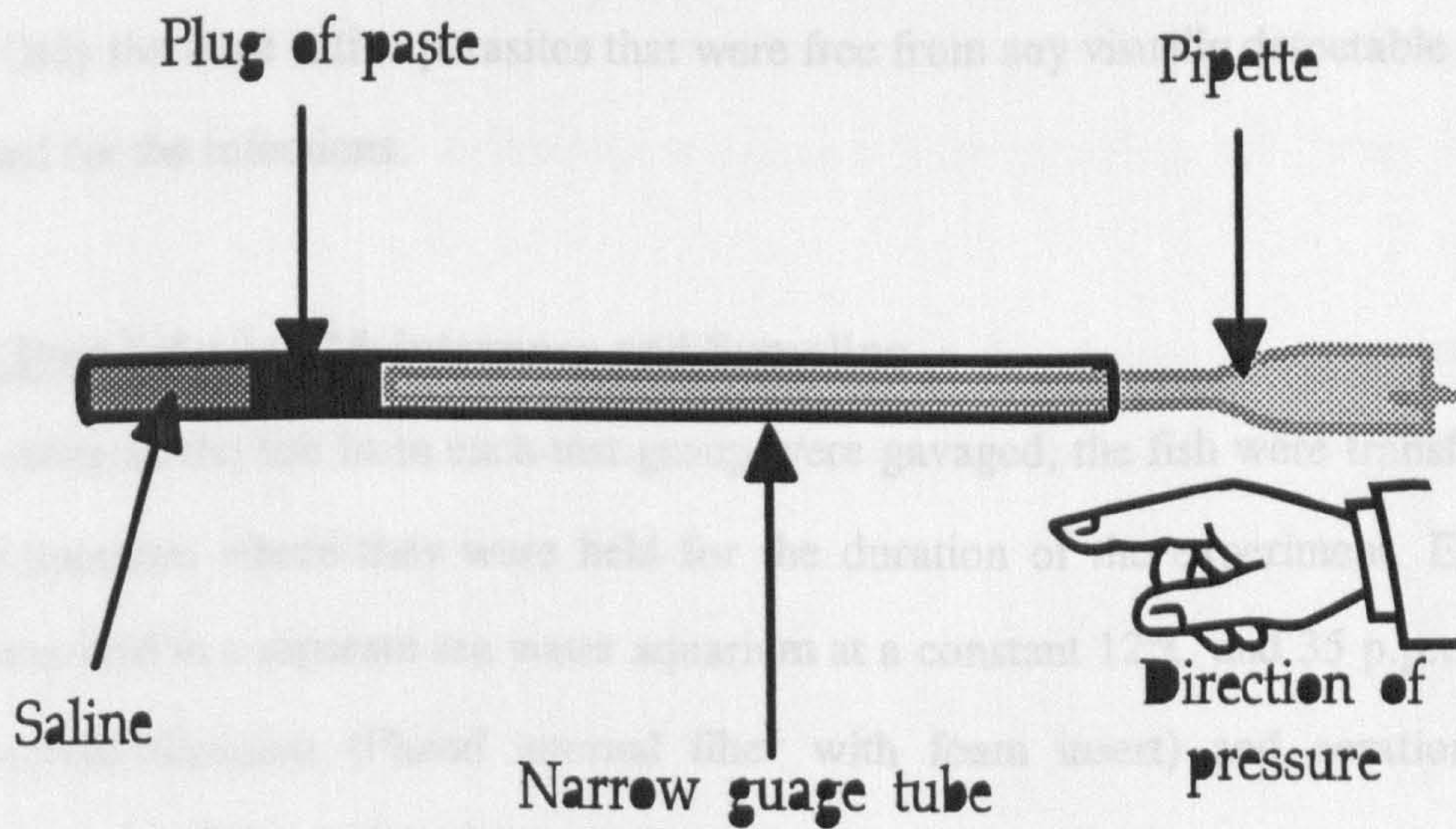
3B.2.4: Method of Infection

A novel method of gavage was used to infect the fish, as illustrated in Fig 3B.1. A thick paste was made using grade one *S. salar* pellets mixed with a small amount of physiological saline. This paste was used to make small pellets which were used to plug

a short length of narrow gauge plastic tube. The plug of paste was then pushed down the tube using a long glass pipette. A distance of approximately one centimetre was left between the tip of the tube and the plug of paste. This space was filled with physiological saline. For the test groups, the parasites to be used for the infections could be placed in this small volume of saline in the tip of the tube. The tube was then inserted in the mouth and oesophagus of an anaesthetised *S. salar* until the tip was located in the stomach of the fish. A long length pipette was then passed down the tube to force the plug of paste out of the tube, thereby delivering the parasites and a very small volume of saline and fish food directly into the fish's stomach. In the case of the Control group, the same method was followed except for the absence of parasites in the delivery tube.

The fish for each experiment were removed randomly from a stock tank and placed in a small aquarium which was used as a holding tank. Individual fish were then placed in a tank containing five millilitres of ten percent Benzocaine for every eight litres of sea water, until they were sufficiently anaesthetised for easy handling. The fish were then placed on a wet paper towel and gavaged using the method illustrated in Fig 3B.2. After gavage the fish were marked using a Panjet marker with each test group of fish being marked with a different, easily identifiable combination of marks. They were then placed in a second small aquarium for recovery, where they were closely observed for approximately ten minutes to ensure that no regurgitation of parasites took place. The

Figure 3B.1: The apparatus used to infect the *S. Salar* in both Experiment 1 & 2, not to scale.



fish from each test group were then placed in a separate tank where they remained for the duration of the experiment. Each tank held only one test group of fish. In each experiment the Control group was gavaged first. All test groups were infected individually with careful cleaning of the apparatus between groups to ensure no accidental infections took place.

Only the most active parasites that were free from any visually detectable damage were used for the infections.

3B.2.5: Post Infection Maintenance and Sampling

After all the fish from each test group were gavaged, the fish were transferred to a large aquarium where they were held for the duration of the experiment. Each test group was held in a separate sea water aquarium at a constant 12°C and 35 p.p.t. salinity with internal filtration (Fluval internal filter with foam insert) and aeration. After transferring the fish to their large tanks, the water from the recovery tank and the holding tank was filtered through a 60μm mesh twice. This mesh was then examined under a binocular dissecting microscope for the presence of regurgitated parasites.

A single fish was removed at random from each group at the intervals described in section 3B.4.2 above and examined for the presence of parasites in the viscera and musculature. In the case of Experiment 1, two samples were taken on day 12 post infection.

At the end of the experiment the water from the experimental tanks was filtered through a 60μm mesh. The filters from the tanks were carefully washed and the washings were filtered. The mesh was then examined using a binocular dissecting microscope for the presence of parasites.

3B.3: Results

3B.3.1: Experiment 1:

Two nematodes were recovered post infection. These were L3 larvae of *C. osculatum* recovered from a fish in Test group 1, 36 hours post infection. The nematodes were recovered from the intestine immediately posterior to the pyloric caeca. No nematodes were recovered from the samples at three days post infection, nine days post infection or 12 days post infection. Due to the negative results of these later samples, the experiment was terminated at day 12 and the remaining fish sampled.

No further parasites were recovered after 36 hours and no nematodes were recovered from the filtered water from the tanks used for infection, or from those used for holding the fish for the duration of the experiment.

3B.3.2: Experiment 2:

At 24 hours post infection five L3 larvae of *H. aduncum* were recovered from the fish sampled from Test group 1. Three of these nematodes were located in the pyloric caeca area of the intestine and two from the rectal area of the intestine. A single *H. aduncum* was also recovered from the posterior intestine of the fish sampled at four days post infection from the same group. No parasites were recovered from the fish sampled at eight or 12 days post infection and no parasites were recovered from any of the fish in the other groups, or from the filtered tank waters or filter medias.

The results of both experiments are summarised in Table 3B.2.

Experiment 1

Sampling interval	Test group 1 (No. of *C. osculatum* recovered)	Test group 2 (No. of *H. aduncum* recovered)
36 hours	2	0
4 days	0	0
9 days	0	0
12 days 1	0	0
12 days 2	0	0

Experiment 2

Sampling interval	Test group 1 (No. of *H. aduncum* recovered)	Test group 2 (No. of *L. rufoviride* recovered)	Test group 3 (No. of *Corynosoma* sp. recovered)	Test group 4 (No. of *N. lingualis* recovered)
24 hours	5	0	0	0
4 days	1	0	0	0
8 days	0	0		
12 days	0	0		

Table 3B.2: Summary of the results for Experiments 1 & 2 showing the number of parasites recovered from the sampled fish at each sampling interval.

3B.4: Discussion

The method of gavage devised for the experiments worked very well. No parasites were regurgitated immediately after gavage or at any other time during the course of the experiments.

Adult *H. aduncum* have been observed in the intestine of *S. salar* (Wootten Pers. Com.) suggesting that L3 larvae of *H. aduncum* are able to remain in the intestine of the experimental fish, moult to L4 and then develop into adult stages using *S. salar* as a final host. This was not observed in Experiment 1. A possible explanation for this is that the nematodes were held for too long a period at 4°C before being introduced into the *S. salar*. This may have prevented them from being active enough in the intestine of the fish to establish and remain in the intestine. It is also possible that they may have been damaged during dissection from the wrasse preventing them from being viable. However, as only the most active nematodes free from any visually detectable damage were used, the number of non-viable worms introduced into the test fish should have been minimal. As reported by Holst *et al.* (1993) adults of *H. aduncum* have been reported from wild caught *S. salar*, so they can act as final hosts for this parasite. However, the results of this study suggest that the method of infection in these cases is likely to be the consumption of planktonic crustacea infected with L2 stages of *H. aduncum* rather than the consumption of fish species already infected with L3 larvae of *H. aduncum*. In an attempt to clarify these results a second group of fish was infected with *H. aduncum* in the second experiment.

The five *H. aduncum* recovered from the fish sampled 24 hours post gavage in Experiment 2 represent an 83% recovery of the parasites introduced into the fish, while the single nematode recovered four days post gavage represents a 17% recovery. All of the nematodes recovered were L3 larvae. This would suggest that the nematodes did not establish in the *S. salar*. The fact that one of the nematodes survived in the intestine of

the experimental *S. salar* for four days post infection suggests that the nematodes were viable when introduced into the *S. salar*.

The findings for *C. osculatum* concur with the findings of Smith *et al.* (1990). These workers attempted to infect *Oncorhynchus mykiss* Richardson (rainbow trout) with *C. osculatum* removed from *Halichoerus grypus* (grey seal). It was found that larval *C. osculatum* survived only for a maximum of two days in the intestine of rainbow trout after oral introduction, however, newly hatched *C. osculatum* introduced directly into the body cavity by intraperitoneal injection did survive and developed into L3 larvae. These authors suggested that L3 *C. osculatum* do not possess the necessary mechanisms to allow penetration of the gut wall and re-encystment in a paratenic host.

The fact that no *L. rufoviride* established in the intestine of the *S. salar* is surprising as the final host for this parasite is either *C. conger* or *A. anguilla*, therefore, they are capable of using fish as their final host. However, both of these eels have morphologies radically different from that of *S. salar*, suggesting that *S. salar* is not a suitable final host for this digenean.

The failure to establish by both *Corynosoma* sp. and *N. lingualis* may have been a reflection of the small number of parasites used to infect the experimental fish. Considering the large range of fish species utilised by *N. lingualis* as hosts, including some salmonids, it would be expected that this parasite would utilise *S. salar* as a final host. More work is needed using larger numbers of parasites to investigate this possibility further. In contrast to this, *Corynosoma* sp. utilises mammals and birds as final hosts. It would not, therefore, be expected to establish in the intestine of *S. salar*, however, the large range of fish intermediate hosts reported for the parasite might suggest the possibility of it utilising *S. salar* as a paratenic host. This did not occur

during the above two experiments, again further work is required using larger numbers of parasites to investigate this possibility.

Due to the low prevalence and intensity of infection with *N. lingualis* and *Corynosoma* sp. observed in wrasse it would be very difficult to obtain large numbers of viable parasites from wrasse species in British waters to carry out large scale trials making further work on the subject very difficult. In general, in nature the number of parasites which successfully establish in a host would rarely approach 100% of those which enter the host. For this reason it is necessary to use a large infective dose in infection experiments. Unfortunately large numbers of the parasites were not available in this series of experiments . Further work with larger numbers of parasites is needed to establish, for certain, that the parasites of *C. rupestris* cannot use *S. salar* as either a final or intermediate host, however, the results obtained in the above experiments would suggest that this is the case.

The nematode *A. simplex* was also found in the wrasse species examined in Chapter 3A. This species was generally present in lower numbers than either *H. aduncum* or *C. osculatum*, and was not present at all in the wrasse used as a source of parasites for the above experiments. *A. simplex* has been linked with severe gastrointestinal diseases in humans after the consumption of raw or partially cooked fish infected with L3 larvae of this nematode, therefore there is more of a health risk to humans associated with the presence of *A. simplex* than with any other parasite species recorded from wrasse. Experimental cross infections should, therefore, be carried out using *A. simplex*. However, due to the relative scarcity of this species in wrasse, it may be necessary to obtain suitable numbers of these nematodes from other fish species. One possible source of L3 *A. simplex* is *Clupea harengus* L. (herring) which are known to be infected with large numbers of these parasites (Davey 1972, McGladdery 1986, Smith & Wootten 1984a, van Banning & Becker 1978). *C. harengus* are readily available from

the wild and from fish mongers depending upon the time of year and would, therefore, be a far better source of parasites than wild caught wrasse. This method could also be applied to the other two nematode species shown to infect wrasse. This method does not, however, investigate what effect the internal environment of wrasse has on the ability of nematodes to re-infect a paratenic host. The nematodes may be rendered unable to re-infect a paratenic host by the conditions within the wrasse host.

It would appear that the parasites used in this series of experiments are unable to transfer from wrasse to *S. salar*. As the parastite species chosen were the ones regarded as being the most likely to be able to transfer to *S. salar* on the basis of their life-cycles, it is unlikely that any of the other parasites recorded from wrasse will be capable of transfaunation either. In order to ensure that this is the case, further experiments would have to be carried out using larger infection doses and larger sample sizes.

CHAPTER 4: INFECTIOUS PANCREATIC NECROSIS VIRUS: EXPERIMENTAL INFECTION OF *Ctenolabrus rupestris* .

4.1: Introduction

The disease known as Infectious Pancreatic Necrosis (IPN) was first described in 1955 from *Salvelinus fontinalis* (Mitchill) (brook trout) in America by the authors Wood, Sniesko & Yasutake (1955), however, a disease with similar features was described by M^c^Gonigle in 1941 and termed acute catarrhal enteritis. Wolf, Sneiszko, Dunbar & Pyle (1960) first determined the viral origins of the disease by describing cell degeneration in cell cultures inoculated with bacteria free material from an unstated trout species which had died due to IPN infection. The authors also reported that the disease was already causing severe mortalities in *S. fontinalis*, *O. mykiss* and *Salmo trutta* L. (brown trout) in fresh water in America. The authors Besse & Kinkelin (1965) described the disease in continental Europe where it affected *O. mykiss* in fresh water and the disease was first described in the United Kingdom, also from *O. mykiss* in fresh water, by Ball, Munro, Ellis, Elson, Hodgkiss & McFarlane (1971). Sano also reported the disease from *O. mykiss* in Japan in 1976. It appeared that mortalities due to IPN were mainly confined to fry and fingerling stages of salmonids (Wolf *et al.* 1960), however, the virus has been isolated from adult salmonids where there were none of the typical signs of disease, for example Wolf & Pettijohn (1970) reported the isolation of IPNV from *Onchorhynchus kisutch* (Walbaum) (Coho salmon) in the USA, Ljungberg & Jorgensen (1972) reported the virus from *Salvelinus alpinus* (Arctic char) in Sweden and Ahne (1980) reported the virus from *Hucho hucho* (Danube salmon) in Germany. By the late

1970's the disease had been reported in 10 European countries, North America and Japan and was thus more widespread than any other known viral fish disease (Hill 1978).

Carrier fish infected with IPNV are known to shed the virus in their faeces and there is evidence that vertical transmission via the eggs and seminal fluid of infected salmonids occurs (Bullock, Rucker, Amend, Wolf & Stuckey 1976 and Ahne 1983). This will result in the continual shedding of virus by fish after they are moved to sea with the accompanying risk of infection to fish in the immediate viscinity. IPNV continues to be a major problem in the culture of salmonids in Britain, indeed a meeting of the Co-ordination of Fisheries Research and Development (CFRD) working group on fish diseases in aquaculture in 1993 listed IPN as it's second highest priority for research after sea lice infestation. This working group is comprised of interested parties involved in research in aquaculture, including industry and university scientists, policy makers and representatives of the industry.

4.1.1: Characteristics of the Virus

The IPN virus is the most comprehensively classified fish virus. Hill (1978) provides a detailed account of the characteristics of the virus. It is a non-enveloped icosahedral virus with a diameter of 59nm. Each capsid is composed of 180 sub-units shared by 92 pentagonal and hexagonal capsomers. There are several serotypes of IPNV with European serotypes being significantly different from those found in North America. For many years the VR-299 strain, isolated in North America, was used as a reference strain in infection experiments, however, several other strains were isolated in North America which proved to have different properties to those of VR-299

(McMichael, Fryer & Pilcher 1975). European strains of IPNV include N1 strain which was isolated from *S. salar* fry in Norway (Christie, Håverstein, Djupvik, Ness & Endresen (1988)) and Ab and Sp strains isolated from *O. mykiss* in Denmark (Jørgensen & Kehlet 1971). Details of the IPNV Sp. serotype used in this study are discussed in section 4.1.4.

4.1.2: Non Salmonid Hosts

Although IPN is generally regarded as being a disease affecting fresh water stages of salmonids, there have also been numerous reports from non salmonid species of fish in both fresh and salt water, along with reports from non-fish species. Adair & Ferguson (1981) described the isolation of IPNV from a single *Carassius auratus* (L.) (goldfish) imported from the far east, a *Symphodus discus* (Henckel) (discus fish) donated by a tropical fish keeper and an *Abramis brama* (L.) (bream) recovered from a slow moving English river. The isolation of IPNV would appear to have been incidental and none of the fish displayed any pathological signs of IPNV. The isolates from the three fish were neutralised by antiserum to IPNV, strain Ab. Antiserum for strains Sp or VR-299 were ineffective. Munro, Liversidge & Elson (1976) isolated IPNV from *Lampetra fluviatilis* L. (river lamprey), *Perca fluviatilis* L. (perch) and *Phoxinus phoxinus* L. (minnow) from Loch Awe in Scotland. In a three year survey of the fish from Loch Awe with the aim of assessing the extent to which wild fish were infected with IPNV, the authors reported that the virus was present in 0.9% of the population of *L. fluviatilis*, 2.5% of the population of *P. fluviatilis* and 0.2% of the population of *P. phoxinus* present in the loch. It was concluded that the sources of infection within the Loch Awe system were the numerous salmonid farms in the loch.

The virus has also been reported from *Esox lucius* L. (pike) in Germany (Ahne 1978) in this case the isolate was neutralised by normal trout serum and was not pathogenic to young trout fry or young pike, the isolation would appear to have been incidental. Sonstegard & McDermott (1972) described the isolation of IPNV from *Catastomus commersoni* Lacepéde (white sucker) from Canada, the fish sampled were taken from the waste water raceways of a fresh water *O. mykiss* farm. Hudson, Bucke & Forrest (1981) isolated IPNV Ab serotype from farmed *Anguilla anguilla* L. (European eel) in the UK, the fish had shown no external lesions or signs of disease and were sampled as part of a routine health monitoring programme.

Although all the above records have been from fresh water species, IPNV has also been found in wild caught marine fish, for example, *Limanda limanda* L.(common dab) (Diamant, Smail, McFarlane & Thomson 1988). As no pathology was reported from the fish sampled it is likely that they were carriers showing no signs of disease. The N1 serotype of IPN virus was isolated from both *Psetta maxima* (turbot) and *Hippoglossus hippoglossus* L. (halibut) from Norwegian waters (Mortensen, Hjeltnes, Rødseth, Krogsrud & Christie 1990).

The virus has also been isolated from marine molluscs *Meretrix lusoria* Roeding in Japan (Lo, Hong, Huang & Wang 1988 and Lo, Lin, Liu, Wang & Kou 1990). Perhaps more significantly, it has been isolated from *Pecten maximus* (L.) (scallop) and *Mytilus edulis* L. (mussel) collected from the vicinity of a *S. salar* farm in Norway (Mortensen *et al.* 1990). The authors suspected that the virus was present as result of accumulation of particulate matter during filter feeding, a process which would facilitate

the spread of virus through the normal predator prey pathways. This was demonstrated by isolating IPNV from the prawns *Pandalus borealis* Krøyer and *Palaemon elegans* L. that had been feeding on dead *P. maximus* infected with IPNV (Mortensen 1993).

In addition to these records IPNV has been isolated from the faeces of several birds from the vicinity of fresh water salmonid hatchery sites in America. The bird species involved were all native American species and largely piscivorous (McAllister & Owen 1992). Also, Peters & Neukirch (1986) demonstrated that *Ardea cinerea*, (heron) was capable of shedding IPNV in its faeces for up to 13 days after being fed on infected fish. Similar experiments by Eskildsen & Vestergård Jørgensen (1973) and Sonstegard & McDermott (1972) demonstrated that IPNV can be shed in the faeces of gulls, chickens, owls and small predatory mammals. It must, therefore, be assumed that the spread of IPNV by predatory birds and mammals is also possible in Britain.

The ability of the virus to thrive in both salt and fresh water is demonstrated by the number of hosts and habitats from which it has been isolated and also survival studies on the virus in water of varying salinity. Toranzo, Barja, Lemos & Hetrick (1983) demonstrated that the North American VR-299 serotype of IPNV can survive for up to 35 days in untreated estuarine water of 11.5 ppt salinity.

4.1.3: IPNV in Salmonids

IPNV is a notifiable disease in Scotland and restriction orders have been imposed on the movement of fish and eggs in some cases to prevent the spread of the disease (Knott & Munro 1986). Fish which survive initial infection with the virus are likely to be

carriers for the disease and shed virus in their faeces, eggs and seminal fluids.(Frantsi & Savan 1971, Bullock *et al.* 1976 and Ahne 1983). The duration of virus shedding can be highly species specific (Wolf, Quimby, Carlson & Bullock 1968, Billi & Wolf 1969 and Frantsi & Savan 1971). The certification of brood stock as disease free requires that both fish and eggs are tested for the presence of the virus resulting in considerable cost to the producer.

The precise cellular site of replication of the virus is not known, however, there would appear to be at least some replication within leukocytes (MacDonald & Moore 1982 and Knott & Munro 1986). The authors speculated that the use of leukocytes as a cellular replication site would possibly render the carrier fish more susceptible to infections with other diseases. Knott and Munro (1986) reported that IPNV could only be recovered from fish once the leukocytes had been stimulated with a mitogen (phytohemagglutinin). This method would enable the virus to remain in a resting state until it's host becomes immunologically challenged before reactivating. The authors Tate, Kodama & Izawa (1990) verified that the induction of a carrier state in *O. mykiss* resulted in immunosupression.

The clinical nature of the disease in salmonids was summarised by Hill (1978). Out breaks are normally characterised by sudden, progressive increases in mortalities mostly affecting the fastest growing individuals. Total mortalities during an outbreak vary greatly but may reach 90% or more in the most severe cases. The authors reported that visceral titres of virus peak at 10^7-10^9 infective particles per gram of tissue and large amounts of virus are secreted in the faeces. The number of viral particles in

hatchery water during an outbreak may be as high as $10^5 TCID_{50}$ per millilitre (Desautels & MacKelvie 1975). Under these conditions horizontal transmission takes place readily. There is a marked resistance to the disease with increasing host age. The clinical signs of IPNV include darkening of pigmentation, distention of the abdomen and a corkscrewing swimming motion in the most severely infected fish. There may also be some exopthalmia. Acute catarrhal enteritis is also normally present along with petechial haemorrhages affecting the anterior viscera. The liver and spleen are normally enlarged and pale.

The authors McKnight & Roberts (1976) give a comprehensive description of the histopathology associated with the naturally occurring disease. They demonstrated that the histopathology associated with the disease mainly affects the pancreas. Severe necrosis of the acinar cells of the pancreas can be seen along with nuclear pyknosis, karyorrhexis and occasional intracytoplasmic inclusions. The pyloric caeca and intestinal lumen show acute enteritis with sloughing of epithelial cells.

4.1.4: IPNV in Scotland

IPNV has a widespread distribution in farmed *S. salar* at sea sites in Scotland (Munro & Smail 1992), it is therefore likely that wrasse being used on these sites will be exposed to IPNV. Smail, Greirson & Munro (1986) reported that only two strains of IPNV are known from Scottish waters these are the Sp and SS strains. The Sp strain has been associated with commercial losses of first feeding fry of *O. mykiss* in Scotland (Ball, Munro, Ellis, Elson, Hodgkiss & McFarlane 1971) and was demonstrated to be virulent to *S. salar* sacfry by Smail *et al.* (1986). Chronic persistence of the Sp strain has

also been shown in older fish (Knott & Munro 1986). This strain was also shown to be pathogenic to *O. mykiss* fry by Dorson & Torchy (1981). The Sp serotype was first isolated from *O. mykiss* in Norway.

No studies to date have examined the susceptibility of wrasse to IPNV. This study was designed to investigate the susceptibility of *C. rupestris* to IPNV Shetland strain (a subtype of Sp serotype first isolated from fish from the Shetland Isles) (Pryde, Melvin & Munro 1993). The Sp serotype was used due to its links with fish mortalities in Scotland and its prevalence in fish from Scottish waters. Unfortunately, the level of exposure and the concentration of virus in the water within infected pens is not known, therefore, the infection doses used in the experiments were derived from previous unpublished work by Smail on *S. salar*.

4.2: Materials and Methods

A group of 190 *C. rupestris* of approximately five grams in weight were used for the experiments and were held in one metre diameter flow-through tanks. These fish had been reared in captivity from wild broodstock and were, therefore, of a known age, two years, and had a known disease history. As far as was known the fish had not been exposed to IPNV. However, to clarify this, 20 of the fish were killed three weeks prior to the start of the experiment and a pool of internal organs from all 20 fish was checked for IPNV. This check was carried out by standard virological techniques using Chinook Salmon Embryo (CHSE) cells, the tests proved to be negative. Two experiments were carried out to investigate the response of *C. rupestris* to different challenge levels of

IPNV. Experiment 1 was carried out in the temperature range of 12-13°C. The second experiment was carried out at 7-8°C using an increased dose, approximately six times that of experiment 1, and a bathing time five times greater than that of experiment 1, in order to provide a stronger challenge.

4.2.1: Virus Growth

4.2.1.1: Experiment 1

The virus isolate to be used in the experimental infection was identified to be within the IPNV-Sp serotype by a serum neutralisation test using rabbit anti-IPNV antiserum, this was carried out by, Dr. D.A. Smail at the Marine Laboratory, four weeks before the growing of the virus. This isolate of IPNV-Sp (Shetland strain), at pass number five, was then grown on CHSE-214 cells as follows: Five Roux bottles of sub-confluent cell monolayers were infected at a low level with virus, an average of 0.01 plaque forming units (pfu) per cell was used. The virus was then recovered from the infected cell monolayers nine days post infection. The culture medium was clarified by low speed centrifugation and the virus precipitated by means of polyethylene glycol (PEG 6000) using the method of Dixon & Hill (1983). The virus stock solution to be used in the bathing experiments was prepared by resuspending the PEG precipitate in 11ml Hanks Balanced Salt Solution (HBSS). Aliquotes of this stock solution were then titrated on CHSE cells using an agar overlay to prevent dehydration of the cells during incubation. The cell monolayers were then fixed 48 hours post infection with formol saline (eight percent) and stained with a crystal violet solution. Any plaques present were then obvious as clear areas within the blue staining cell monolayers, the plaques were then counted to allow the calculation of the virus titre in the stock solution, this titre was

8.2×10^8 pfu/ml. Ten millilitres of the virus stock solution was then combined with 20 litres of sea water to produce the bathing solution, the viral titre of the final bathing solution was 4.1×10^5 pfu/ml.

4.2.1.2: Experiment 2

A recent isolate of IPNV-Sp. (Shetland strain), at passes nine and ten, was grown in five Roux bottles of CHSE-214 cells as above. Because the yield of virus was low at pass nine, the PEG precipitates (as described above) from the two passes were pooled and the cell pellet of pass ten extracted with Arklone (ICI Ltd.), to recover more of the virus, by the method of Dixon & Hill (1983). The virus stock solutions from the PEG precipitates of pass nine and ten were combined with the stock viral solution obtained from the extraction of the pellet from pass ten, this resulted in a total stock solution volume of 24ml. In the absence of CHSE cells in good condition, the total viral stock solution was then titrated on CHH-1 (Chum Salmon Heart) cells and this gave a titration value of $1.0 \times 10^{9.0}$ pfu/ml. Titres have previously been shown to be comparable on CHSE and CHH-1 cell lines (Lannan, Winton & Fryer 1984). The total viral stock solution was then diluted in ten litres of sea water to produce a uniform solution, the ten litres of sea water was then further split to give two bathing solutions of five litres, the virus titre of which was calculated to be 2.4×10^6 pfu/ml.

4.2.2: Experimental Infection with Virus

4.2.2.1: Experiment 1

A sample of 120 fish from the original captive bred stock was randomly split into three groups of 40 fish. Two groups (Replicates 1 & 2) were used as replicates and each

received a bath challenge with IPNV-Sp (Shetland strain) in 20 litres of sea water at a concentration of 4.1×10^5 pfu/ml for one hour. The third group was used as a control and received a bath challenge in 20 litres of sea water with two millilitres of HBSS for one hour.

The challenge tanks were aerated for ten minutes in every 30. Bathing was carried out in isolated tanks well separated from the experimental holding facilities to avoid air-borne viral particles contaminating the holding tanks, the fish were then transferred back into the holding tanks with sterile nets being used at all stages.

Post infection, ten fish from each group were sampled at one week intervals as follows: A sample of all organs was removed and fixed in eight percent buffered formol saline for histological processing. The remaining viscera, along with a sample of kidney, were prepared for virological processing as described in section 4.2.3.

4.2.2.2: Experiment 2

The remaining 50 fish from the same captive bred stock as that used for Experiment 1 were used for this experiment. The fish were randomly split into three groups. Two experimental groups of 20 fish and one Control group of ten fish. Two groups (Replicates 1 & 2) received a bath challenge with IPNV-Sp. (Shetland strain) in five litres of sea water at a concentration of 2.4×10^6 pfu/ml for five hours. The Control group received a bath challenge in five litres of sea water with ten millilitres of HBSS for five hours. The tank systems and protocol was the same as that used in Experiment 1.

The sampling protocol for Experiment 2 was as follows: Five fish per week were sampled from the experimental groups, two fish were removed from the Control group each week except for week three, when three control fish were sampled, and the fourth sample was taken at five weeks post infection. From week two onwards, in addition to the sampling of fish tissue, the faeces from each experimental sample were pooled and the virus particle concentration determined. The faecal matter was collected by squeezing the dissected intestine with sterile forceps to eject the contents into a sterile Bijoux bottle. The faecal matter from all five fish in the sample was pooled and prepared for virological processing as described in section 4.2.4.

4.2.3: Examination of Experimental Fish for Virus

Samples of kidney and viscera were weighed to ± 0.001g and homogenised using a Stomacher 80 (Seward Medical) in 99 volumes of HBSS to give a 100 times dilution of the tissue. The homogenate was clarified by sedimentation at 300G for 15 minutes at 4°C. The supernatant was then passed through a low-protein binding Millipore filter (HV-0.45µm) and 0.2ml of the filtrate was used to inoculate a well on a 24 well plate containing CHSE cells in Experiment 1 and CHH-1 cells in Experiment 2. Six serial, ten times dilutions, from 10^2 times to 10^7 times, were made by cross well transfer of inoculum into Eagle's Minimal Essential Maintenance Medium (EMEMM). The cells were incubated at 15°C for one hour to allow for absorption of the virus by the cells. The inoculum was then removed and replaced with agar in EMEMM. Plates were incubated for 48 hours at 15°C and fixed and stained as above. The plaques were counted and the tissue titre of virus expressed as pfu per gram (pfu/g), in relation to the tissue dilution and the inoculum volume.

4.2.4: Examination of Faeces for Virus

The pooled faeces samples were weighed as above. They were then diluted with 19 volumes of HBSS to give a dilution of 20 times. The diluted samples were then shaken vigorously before being clarified as above. The supernatant was then passed through a low-protein binding Millipore filter (GV-0.22μm) and 0.5ml of filtrate was used to inoculate a well on a 24 well plate containing CHH-1 cells. A further five serial, ten times dilutions were made by cross well transfer into EMEMM. The plates were then incubated, fixed, stained and the faecal virus titres expressed as above.

4.2.5: Examination of Control Fish for Virus

Samples of viscera were weighed, homogenised and clarified as above, with the exception that samples were diluted with 49 volumes of HBSS to give a dilution of 50 times. Filtrates of the supernatant were made as before and CHSE cells (Experiment 1) or CHH-1 cells (Experiment 2) in 24 well plates inoculated using 0.2ml of filtrate. A further five times dilution in the inoculum was made by in-well transfer. Two wells were inoculated for each fish in the control group.

4.2.6: Histological Processing

Samples for histology were processed using standard wax impregnation and embedding techniques as described in Appendix 1. A total of 340 slides prepared from the tissues of 170 fish were examined during the course of the two experiments.

4.3: Results

4.3.1: Experiment 1

The titres from the control fish showed no virus to be present in any of the four weekly samples. The number of fish found to be infected with IPNV from each weekly sample for both replicates is shown in Table 4.1. The titres recorded from the individuals infected and the average for the ten fish are also shown in Table 4.1. The plaques recorded were typical of IPNV, the input virus, with respect to both their size (1-2mm diameter at 48 hours) and their rate of development (Smail Pers. Com.).

For Replicate 1, two fish out of ten were infected one week post infection. Two weeks post infection, there were three out of ten fish infected, week three post infection only showed two fish out of ten fish infected with IPNV and by week four no plaques were recorded from any of the test fish.

Replicate 2 showed the same basic pattern with ten percent of fish infected at week one, two out of ten fish infected at two weeks post infection and no infection detectable by weeks three and four. The results are shown in Table 4.1 and Figure 4.1.

Histological examination of pancreas, posterior intestine, kidney and liver of all the fish was carried out. There was no sign of the typical histopathological changes in the pancreas associated with IPNV infection in salmonids, even in the fish that produced a positive titre for the virus. Fig 4.2 shows pancreas from a fish from Replicate 1 of Experiment 1, one week post infection. The acinar cells show no sign of pathology associated with IPNV infection. Fig 4.3 shows the hepatic pancreas of a fish from

Table 4.1: Individual and mean fish titres of IPNV for Replicates 1 & 2 for Experiment 1.

Week	No. of fish infected	Replicate 1 Fish Titres (pfu/g)		No. of fish infected	Replicate 2 Fish Titres (pfu/g)	
		Individual Titres	Mean of 10		Individual Titres	Mean of 10
1	2/10	5.55×10^2 1.17×10^3	1.72×10^2	1/10	3.33×10^3	3.33×10^2
2	3/10	1.67×10^3 5.55×10^2 1.11×10^3	3.33×10^2	2/10	5.55×10^2 5.55×10^2	1.11×10^2
3	2/10	5.55×10^2 5.55×10^2	1.11×10^2	0/10	0	0
4	0/10	0	0	0/10	0	0

The value 5.55×10^2 pfu/g represents the lowest detectable titre by this methodology.

Figure 4.1: Tissue titres of IPNV for Replicates 1 & 2 for Experiment 1 (values are sample means and y-error bars represent Standard Error).

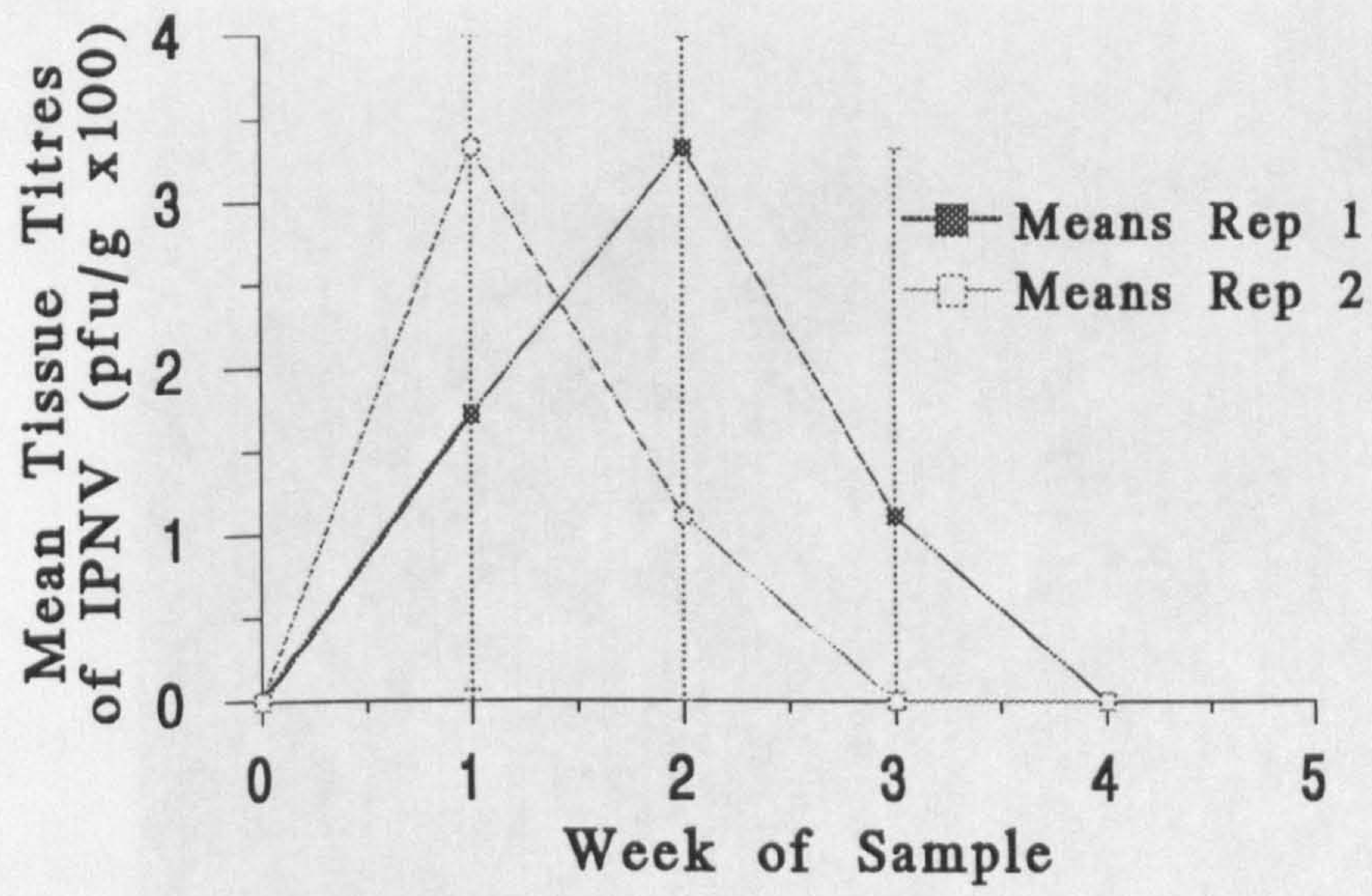


Fig 4.2: Normal exocrine pancreas in a fish from Replicate 1 one week post infection showing normal acinar cells (A), x25 objective.

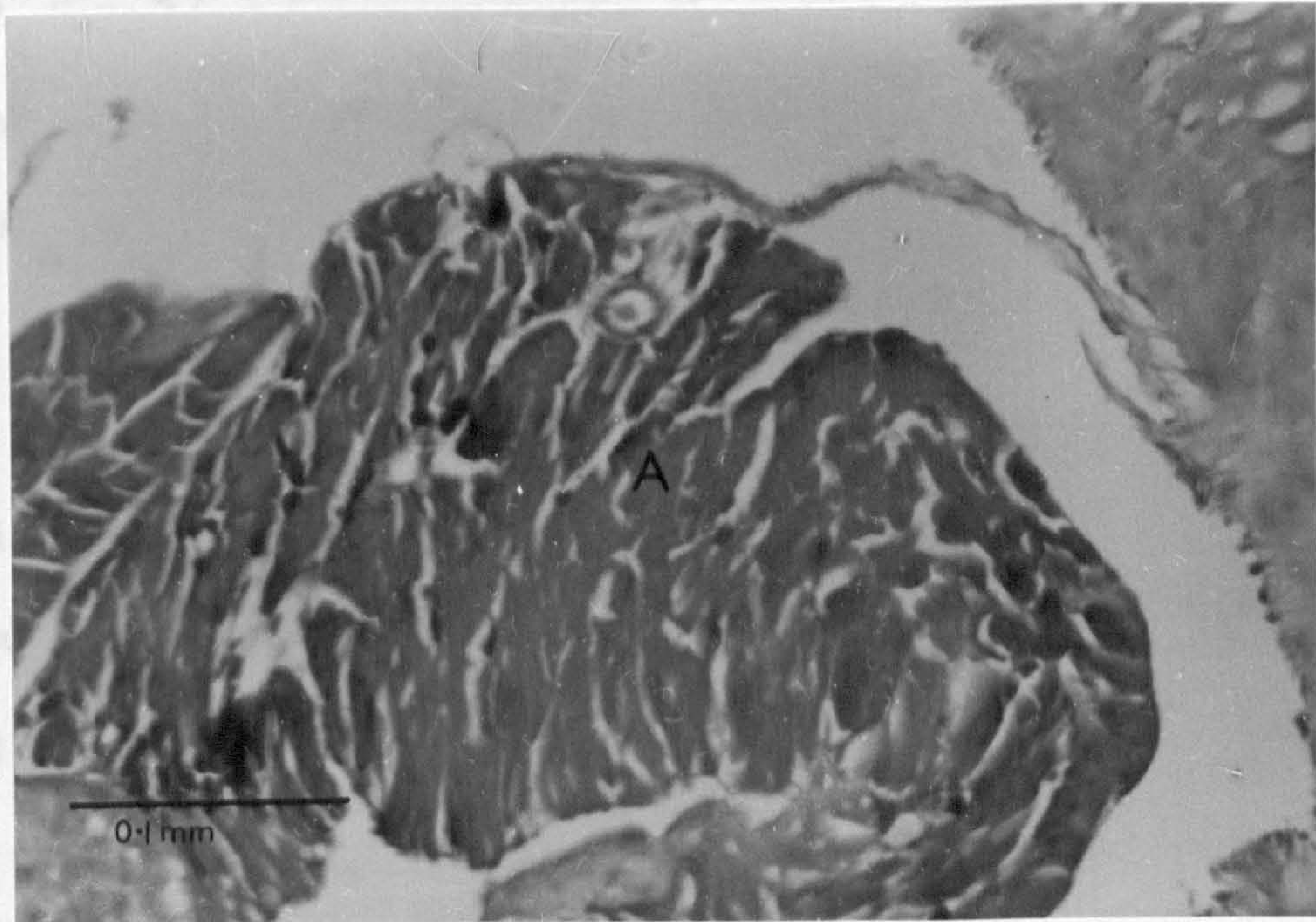
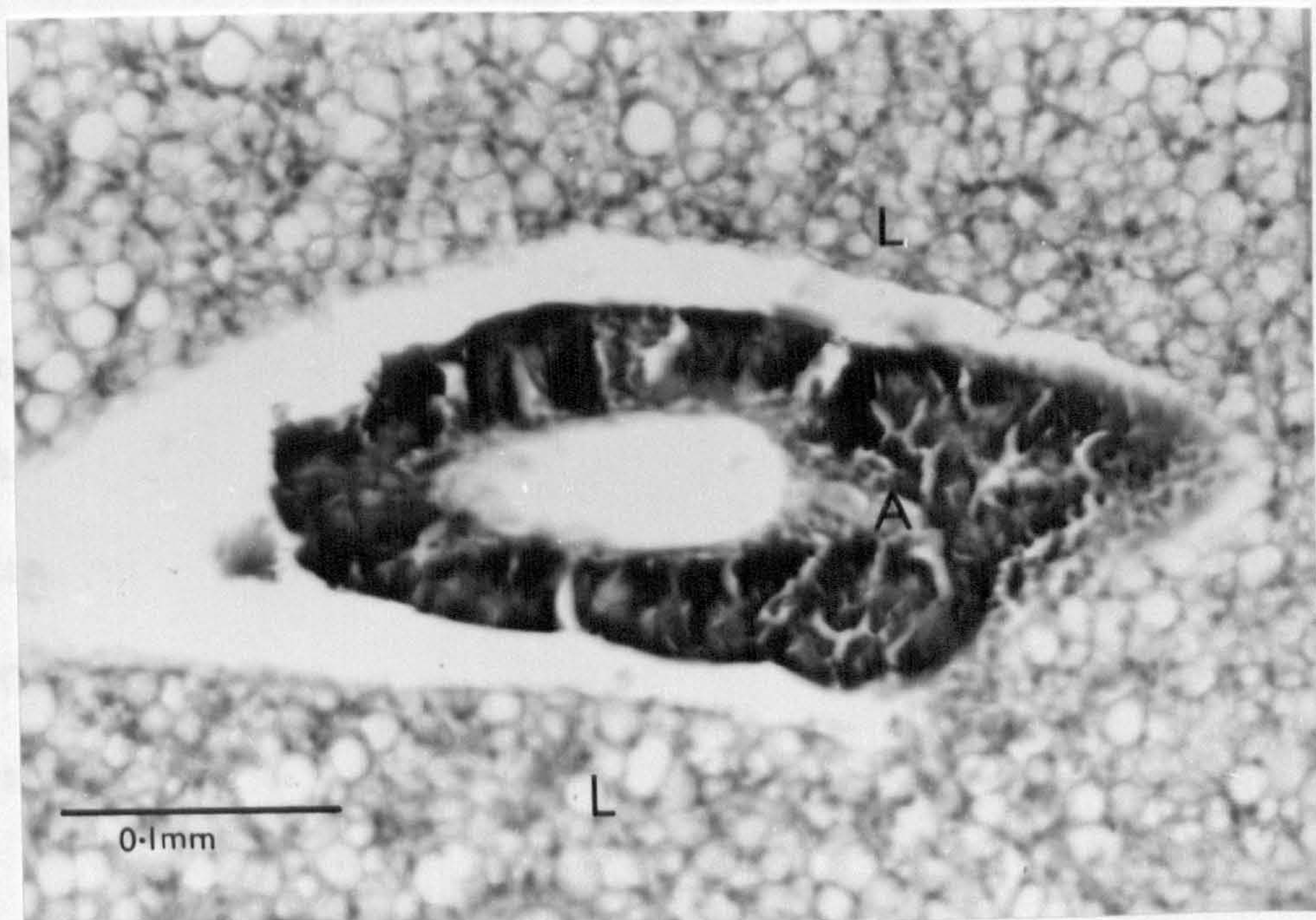


Fig 4.3: Liver pancreas from infected fish from Replicate 1 two weeks post infection showing a normal strand of acinar tissue (A) passing through the liver (L), x25 objective.



Replicate 2 of Experiment 1, two weeks post infection, again there is no sign of any pathology which could be associated with infection with IPNV. The intestine of a fish from Replicate 2 from Experiment 1 two weeks post infection is shown in Fig 4.4. The intestinal epithelium is entirely within normal limits and shows no sign of the enteritis which is associated with IPNV infection in salmonids.

All the other tissues were within the normal ranges as described in Chapter 2.1.1.1. No abnormalities in pigmentation, feeding response or swimming patterns were observed in any of the fish for the duration of the experiment. There were no mortalities during the course of the experiment.

4.3.2: Experiment 2

As in Experiment 1, the control fish titres showed no virus to be present in any of the samples. The titres recorded for the experimental fish are shown in Table 4.2 and the average titres for the fish from Replicates 1 & 2 are displayed in Fig 4.5.

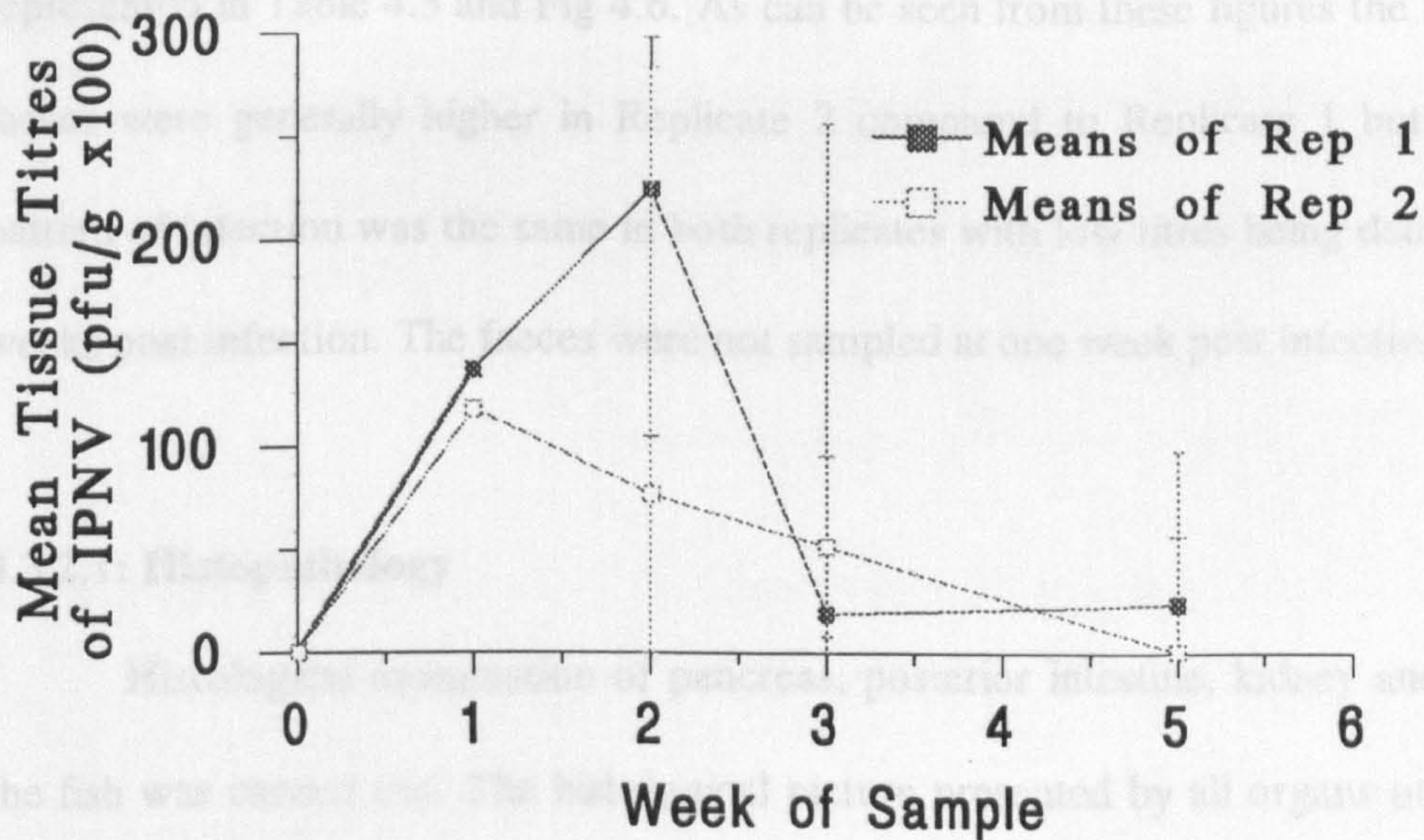
One week post infection three out of the five fish for Replicate 1 and four out of five fish for Replicate 2 were infected with IPNV. By week two a further four out of five fish for Replicate 1 and all five of the fish for replicate 2 infected. The results for week three showed a decrease with only two out of five fish (Replicate 1) and three out of five fish (Replicate 2) infected. On average the titres for Replicate 1 were higher than those for Replicate 2. By week five in Replicate 2 titres had dropped to a level that was only just detectable.

Fig 4.4: Normal intestine (N) from a fish from Replicate 2 two weeks post infection.

Table 4.2: Individual and mean titres of IPNV for Replicates 1 & 2 for Experiment 2.

Week	Replicate 1 Fish Titres (pfu/g)		Replicate 2 Fish Titres (pfu/g)	
	Individual Titres	Mean of Five	Individual Titres	Mean of Five
1	1.56×10^4	1.37×10^4	1.67×10^3	1.18×10^4
	3.05×10^4		3.33×10^3	
	2.22×10^4		5.33×10^4	
	0.00		5.55×10^2	
	0.00		0.00	
2	5.55×10^2	2.26×10^4	2.22×10^3	7.22×10^3
	2.78×10^4		1.22×10^5	
	6.56×10^4		6.33×10^4	
	1.89×10^4		6.50×10^4	
	0.00		7.22×10^3	
3	1.11×10^3	1.89×10^3	2.44×10^4	5.19×10^3
	8.33×10^3		1.17×10^4	
	0.00		0.00	
	0.00		0.00	
	0.00		0.00	
5	6.67×10^3	2.44×10^3	5.55×10^2	1.11×10^2
	5.55×10^3		0.00	
	0.00		0.00	
	0.00		0.00	
	0.00		0.00	

Fig 4.5: Tissue titres of IPNV for Replicates 1 & 2 for Experiment 2 (values are sample means and y-error bars represent Standard Error).



Virus was present in the faeces of both replicate groups with the highest titre being recorded at two weeks post infection. The titres for the faecal samples are represented in Table 4.3 and Fig 4.6. As can be seen from these figures the titres for the faeces were generally higher in Replicate 2 compared to Replicate 1 but the general pattern of infection was the same in both replicates with low titres being detected by five weeks post infection. The faeces were not sampled at one week post infection

4.3.2.1: Histopathology

Histological examination of pancreas, posterior intestine, kidney and liver of all the fish was carried out. The histological picture presented by all organs other than the pancreas was found to be within the normal range, as described in Chapter 2.1.1.1, in all of the fish. In both the replicate groups necrosis of the pancreas was seen which was similar to the histopathology seen in *S. salar*. There was marked necrosis observed one week post infection in four out of the five fish from Replicate 1 and all five fish from Replicate 2. Focal areas of pancreatic necrosis were observed in the exocrine pancreas. This necrosis was first visible as cell shrinkage due to loss of cytoplasm, this is illustrated in Fig 4.7 taken from a fish from Replicate 2 of Experiment 2, one week post infection. The endocrine pancreas was seen to be necrotic in a single fish (Fig 4.8), again this necrosis was present as cytoplasmic loss. Up to 70% of the exocrine pancreas was observed to be necrotic in some fish.

By two weeks post infection the number of fish displaying necrosis had dropped to two out of five fish for Replicate 1 and four out of five fish for Replicate 2. Fig 4.9

Table 4.3: Titres of faecal sample pools (pfu/g) for Replicates 1 & 2 for Experiment 2.

Week	Replicate 1	Replicate 2
2	1.20×10^4	1.03×10^4
3	8.89×10^2	8.89×10^2
5	1.78×10^3	4.44×10^2

Figure 4.6: Faecal titres of IPNV for Experiment 2 (Values are individual titres)

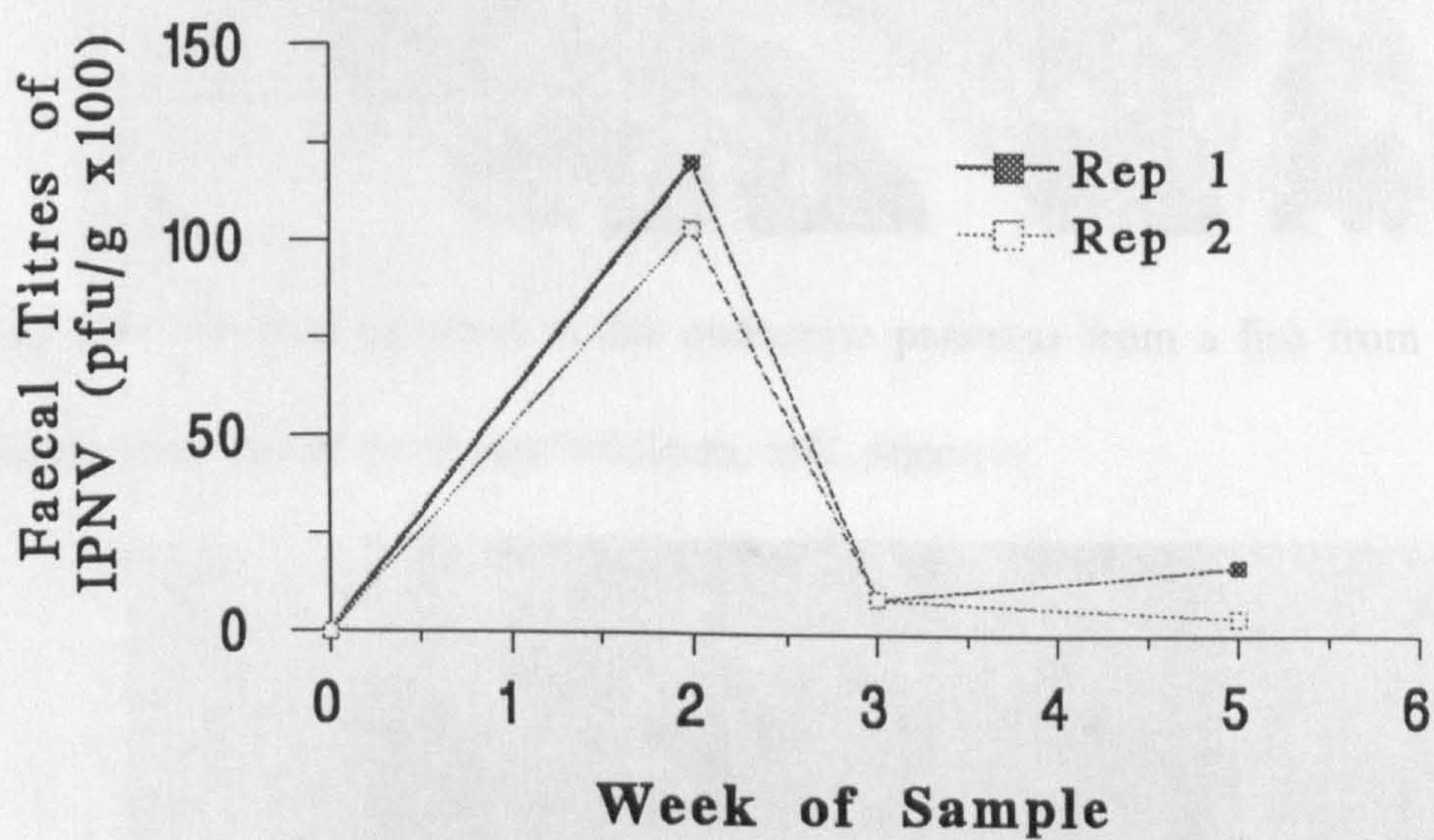


Fig 4.7: Early signs of necrosis characterised by cell shrinkage (arrows) due to cytoplasmic loss, x40 objective. Section from fish from Replicate 1 of Experiment 2, one week post infection.

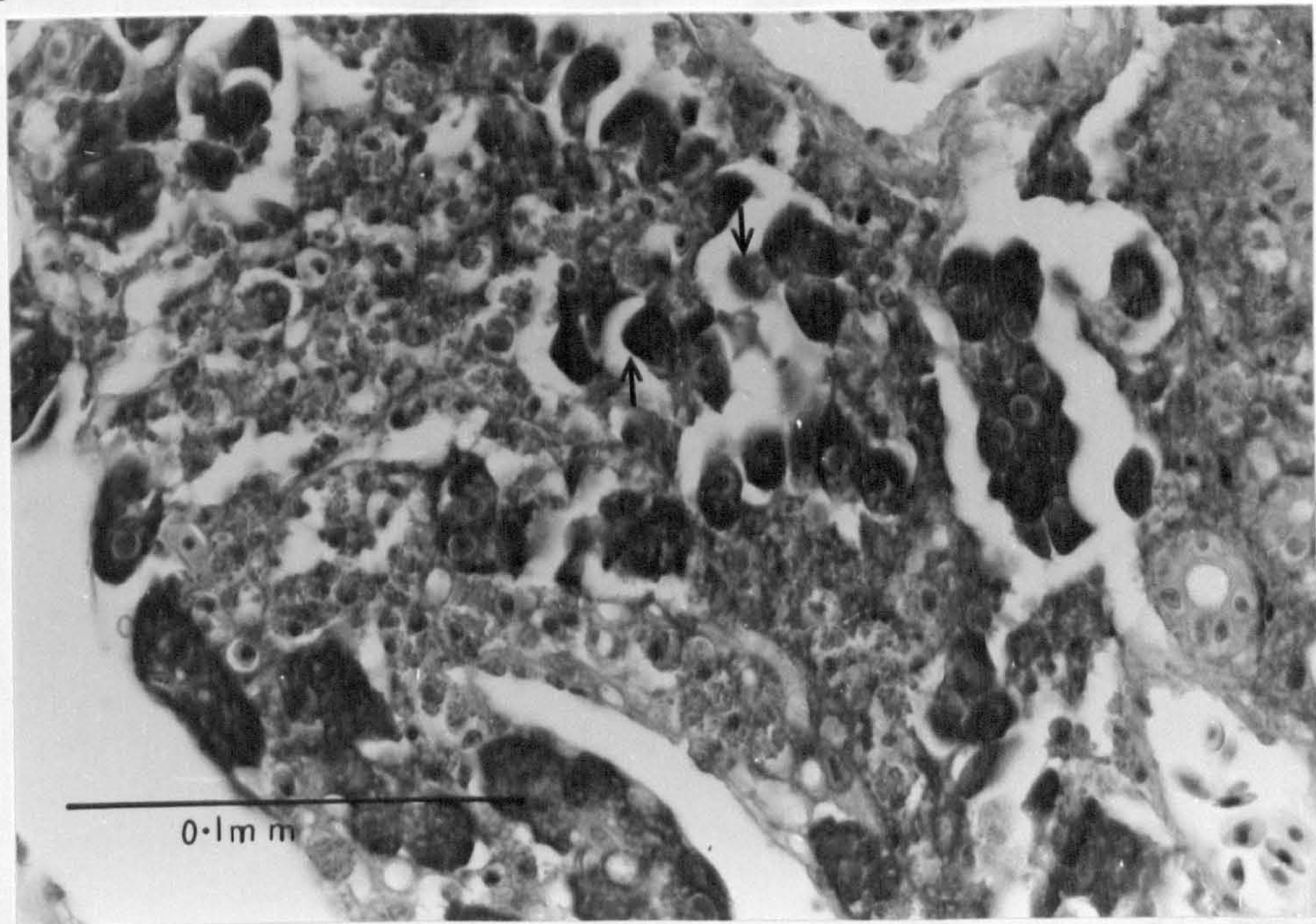


Fig 4.8: Necrosis (arrows) in the endocrine pancreas from a fish from Replicate 1 of Experiment 1, one week post infection, x40 objective.

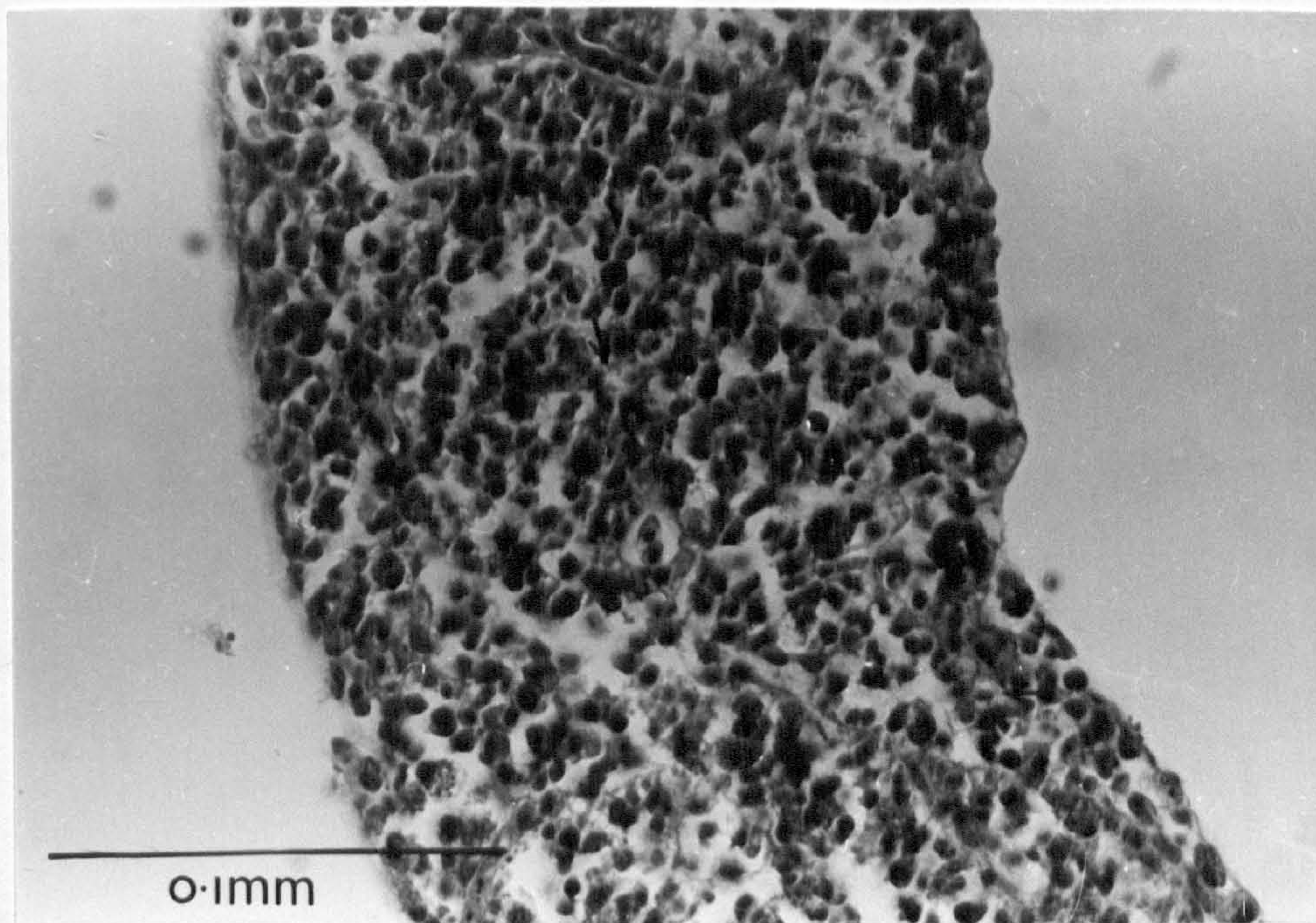
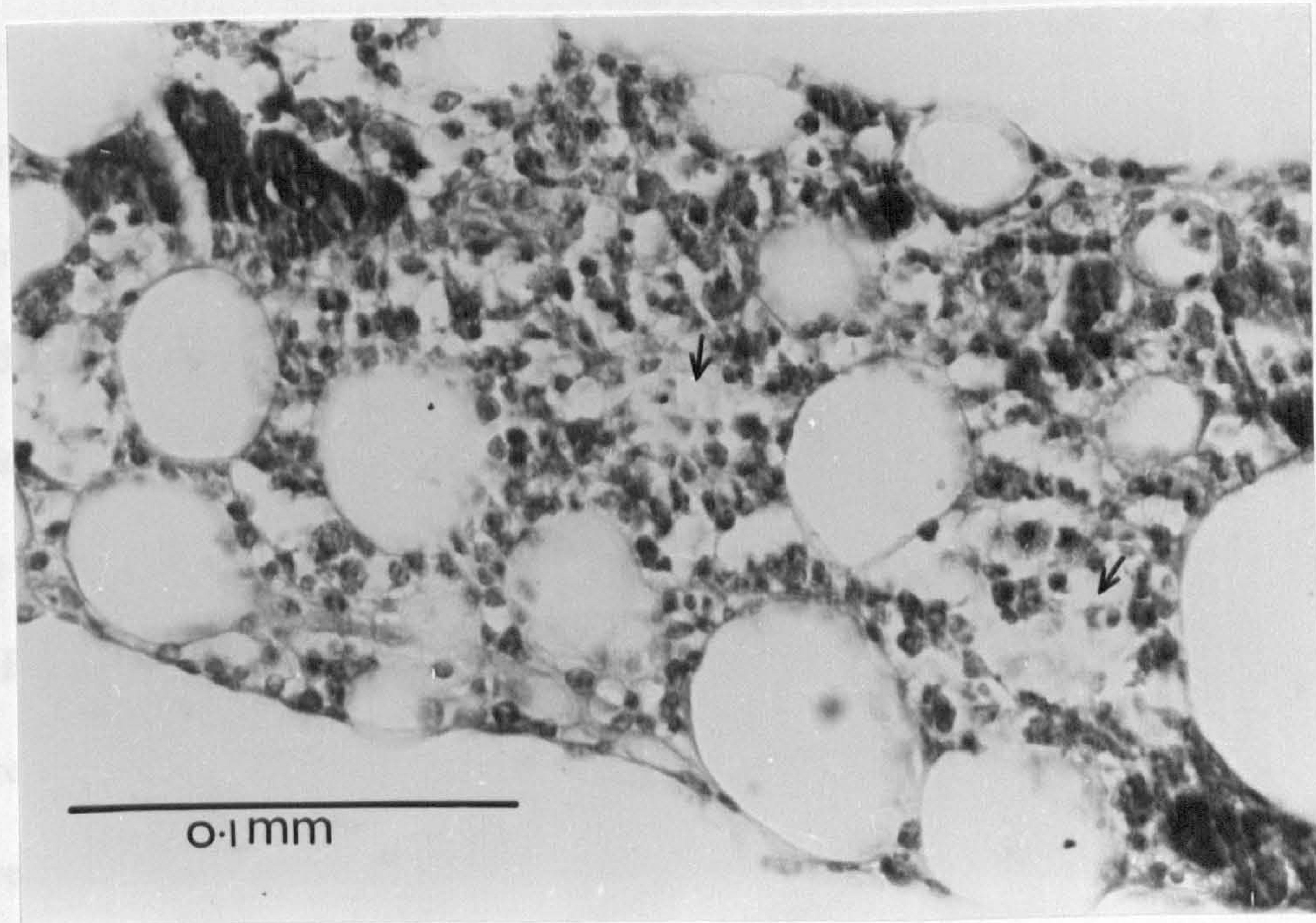


Fig 4.9: Severe pancreatic necrosis from a fish from Replicate 2 of Experiment 2. two weeks post infection. Area of complete acinar tissue loss can be seen (arrows), x40 objective.



shows severe necrosis of the acinar cells of a fish from Replicate 2 of Experiment 2, two weeks post infection. Total destruction of the acinar tissue can be seen while some acinar cells remain unaffected. This necrosis was most evident in the adipose tissues where the only remnants of pancreas to be seen was the presence of ghost cells. These represent the areas within the adipose tissue and supporting connective tissue which used to be occupied by acinar cells before the cells degenerated. Fig 4.10 shows ghost cells within the adipose tissue of a fish from Replicate 1 of Experiment 2, two weeks post infection. Within the areas of severe necrosis liberated zymogen granules could be seen due to the break down of acinar cells, these zymogen granules are illustrated in Fig 4.11 from a fish from Replicate 2 of Experiment 2, two weeks post infection. In most of the fish, areas of apparently normal pancreas could be seen, often very close to severely affected areas of pancreas, Fig 4.12 illustrates this in a fish from Replicate 2 of Experiment 2, one week post infection. No further active necrosis was seen after this time in either replicate. From three weeks post infection onwards there was a steady increase in the number of fish showing signs of recovery. Fig 4.13 shows the pancreas from a fish from Replicate 2 of Experiment 2, three weeks post infection. Fibrocytes can be seen in the area that would have been filled with acinar cells prior to the infection, normal acinar cells are also visible. This fibrosis was most obvious in a fish from Replicate 2 of Experiment 2, five weeks post infection. As shown in Fig 4.14, an area of marked fibrosis is present closely associated with the serous membrane of the intestine. This could have been a pancreatic duct which has fibrosed after loss of the acinar tissue.

The most striking feature was the fact that the pancreatic tissue within the liver was almost completely unaffected by necrosis. As can be seen in Fig 4.15 the pancreatic

Fig 4.10: Ghost cells (arrows) from the adipose tissue of a fish from Replicate 1 of Experiment 2, two weeks post infection, x40 objective.

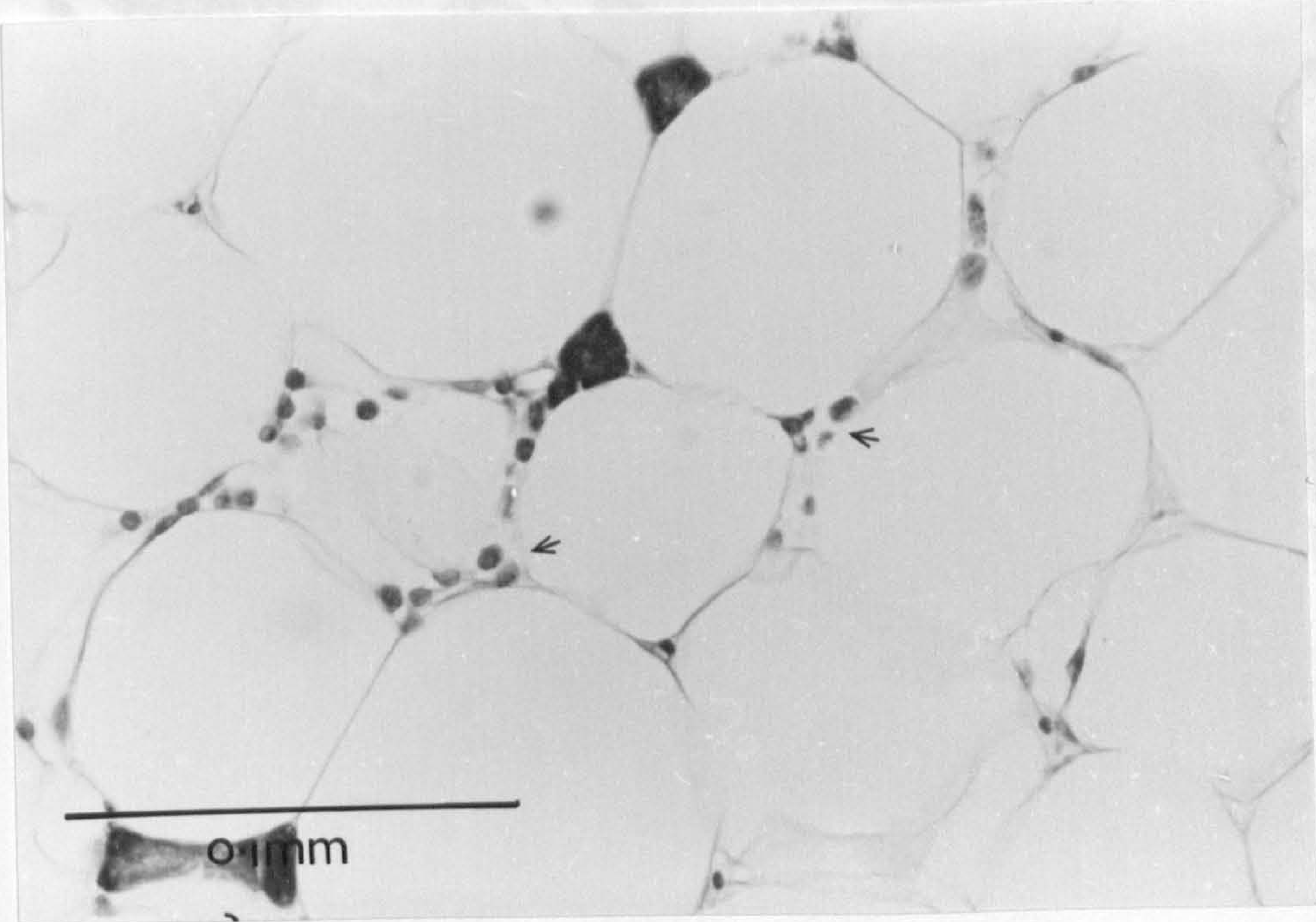


Fig 4.11: Released zymogen granules (arrows) within a necrotic lesion in the pancreas of a fish from Replicate 2 of Experiment 2, two week post infection, x100 objective.

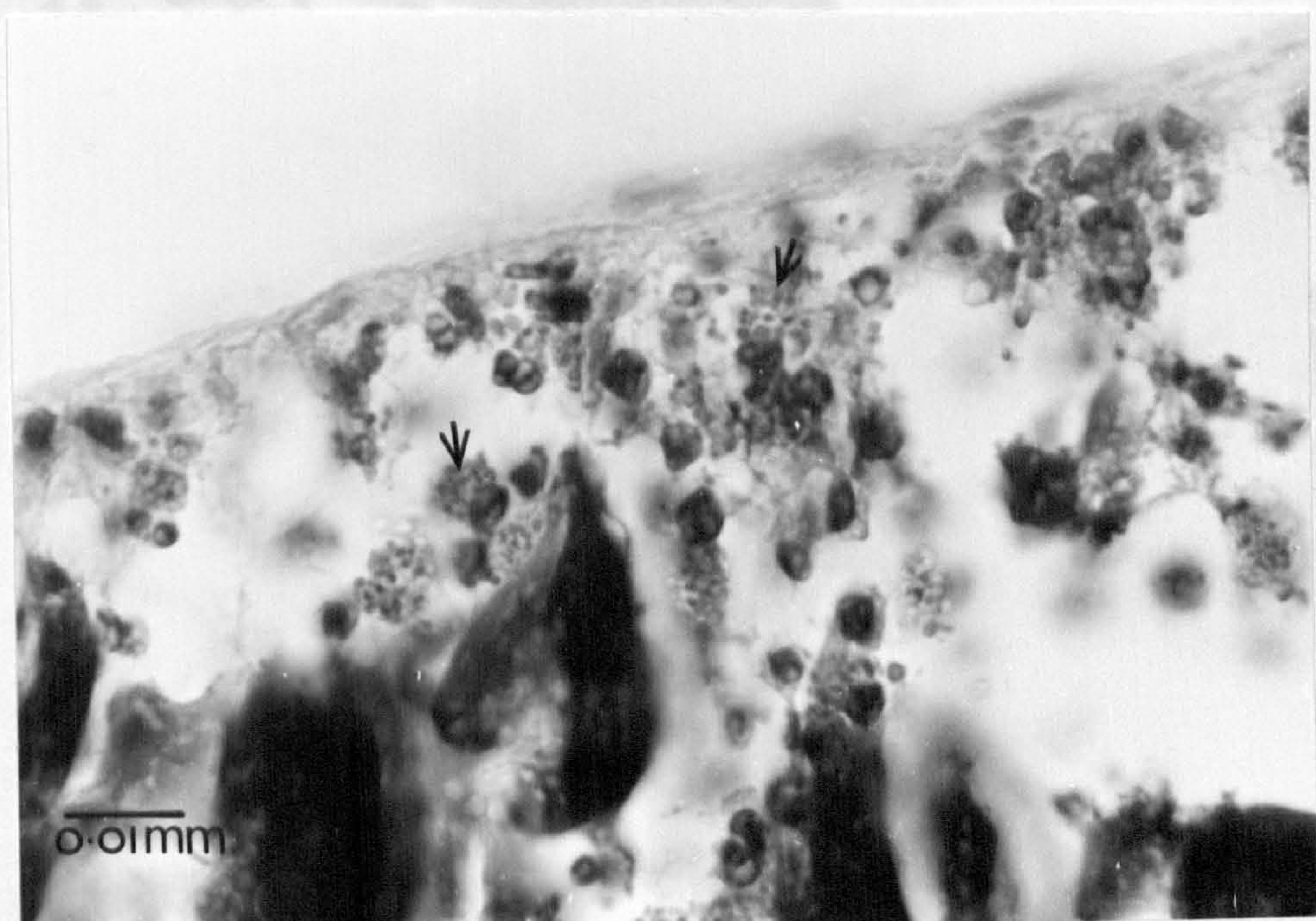


Fig 4.12: Areas of normal pancreas (N) beside necrotic lesions (L) in a fish from Replicate 2 of Experiment 2, one week post infection, x6.3 objective.

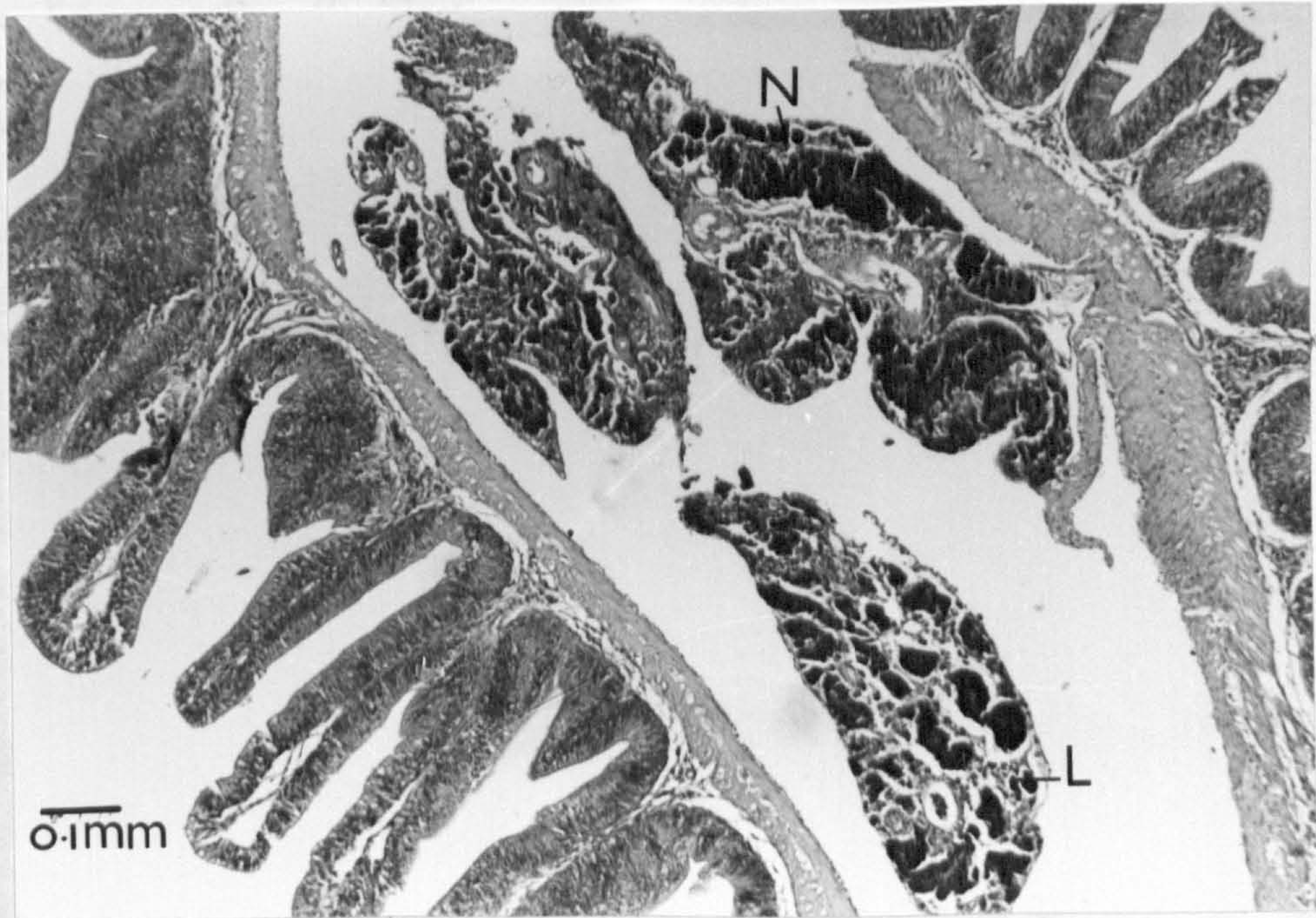


Fig 4.13: Early signs of recovery in a fish from Replicate 2 of Experiment 2. three weeks post infection. The recovery is characterised by the presence of fibrocytes (arrows), x40 objective.

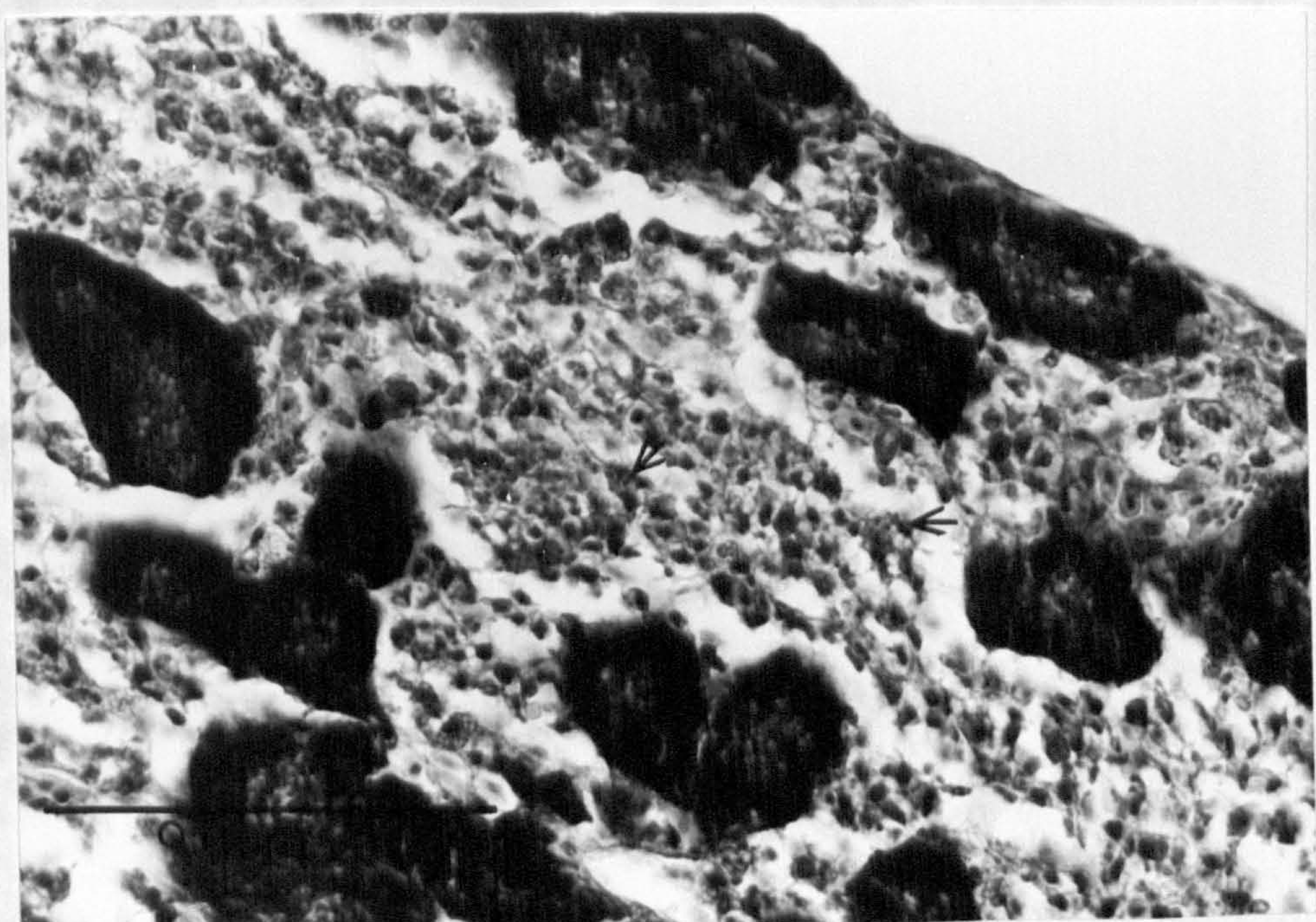


Fig 4.14: Severe fibrosis (F) in pancreatic tissue closely associated with the serous membrane and muscularis (M) of the intestine. The basophilic areas (arrows) are dissociated acinar cells, x40 objective.

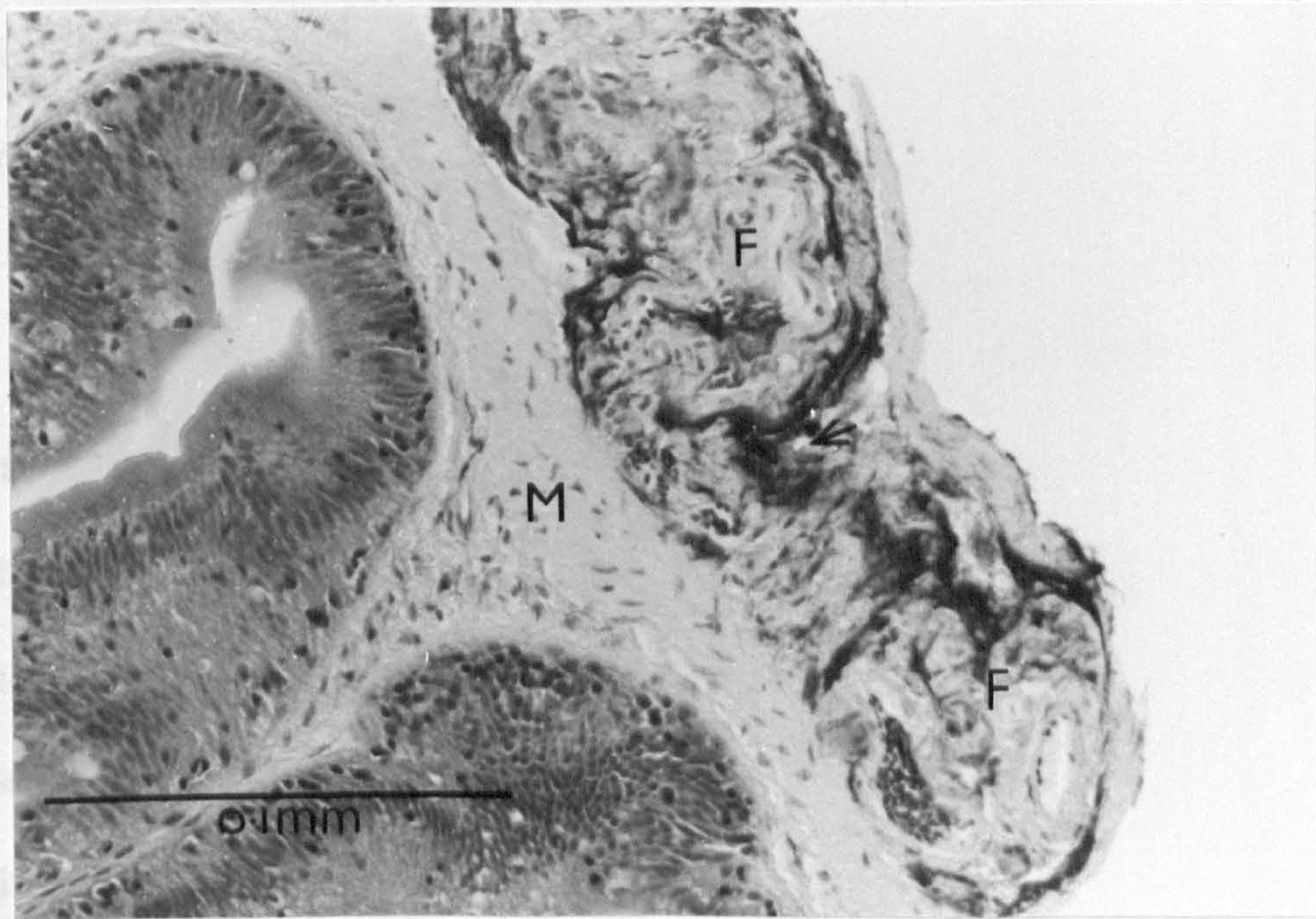
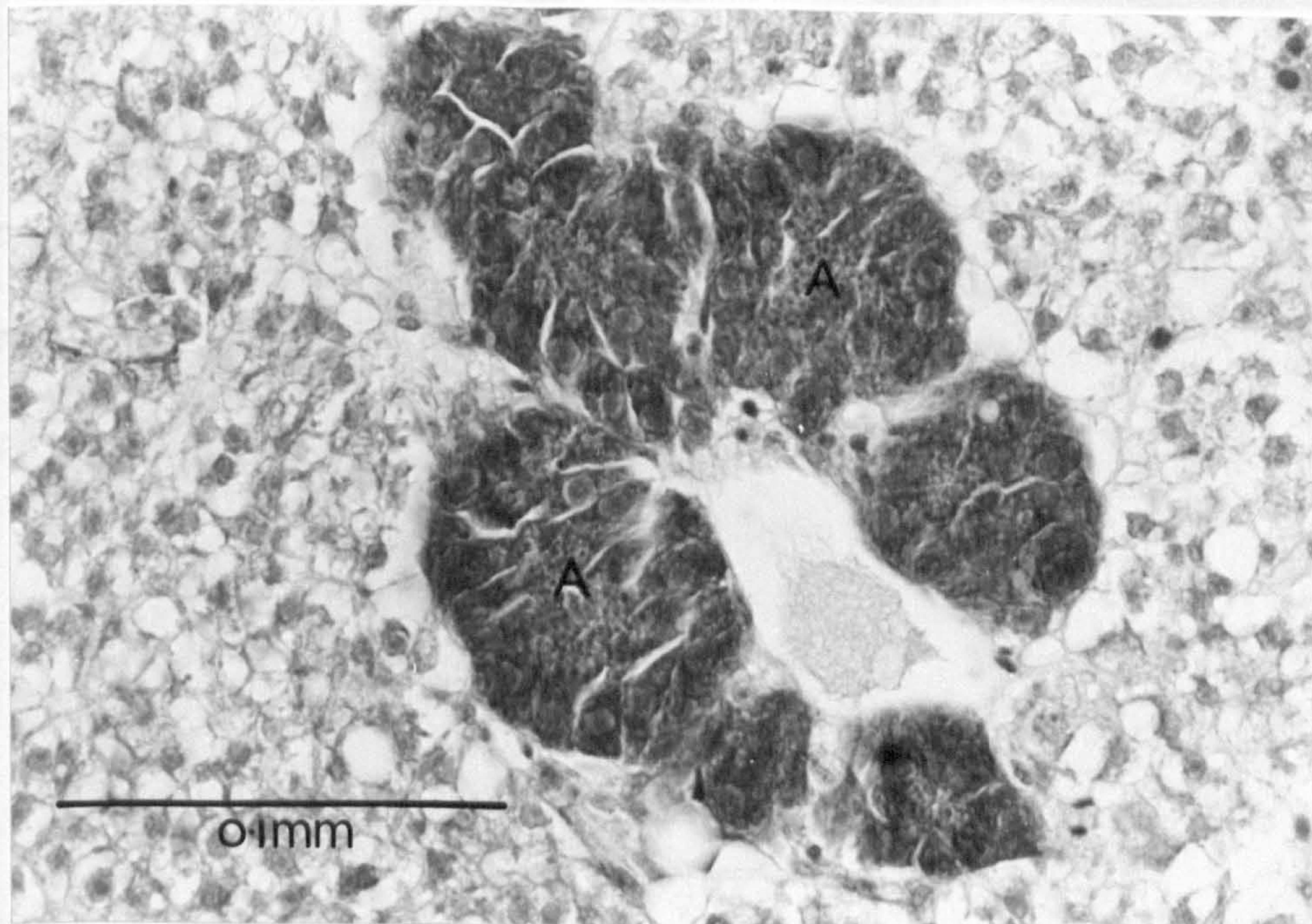


Fig 4.15: Hepatic pancreatic tissue in a fish from Replicate 2 of Experiment 2, two weeks post infection. The acinar cells (A) are normal, x40 objective.



strands passing directly through the liver appears normal, the section is from a fish from Replicate 2 of Experiment 2, two weeks post infection. However, the pancreatic strands passing close to the edge of the liver were seen to be affected by necrosis on the outer edge of the liver. This is illustrated in Fig 4.16 taken from a fish from Replicate 1 of Experiment 2, two weeks post infection.

Only one area of intestinal necrosis was observed in any of the 40 fish examined from the two replicates. This was in a fish from Replicate 2 of Experiment 2, one week post infection. The area of necrosis was very small and was limited to an area of the epithelium very close to the point of entry of a pancreatic duct into the submucosa. The section is shown if Fig 4.17. There was no evidence of enteritis in any of the fish examined and food particles were seen in the lumen of the intestine from three weeks post infection onwards. Fig 4.18 shows food particles in the intestinal lumen of a fish from Replicate 1 of Experiment 2, three weeks post infection.

Some darkening of pigmentation was seen in the most heavily infected fish but no abnormalities were seen in swimming behaviour. The rate of feeding was poor during the first two weeks of Experiment 2, but returned to normal by week three post infection. There were no mortalities during the course of the experiment.

Fig 4.16: Necrotic acinar cells (NE) within a pancreatic strand close to the edge of the liver (L) of a fish from Replicate 2 of Experiment 2, two weeks post infection, x40 objective.

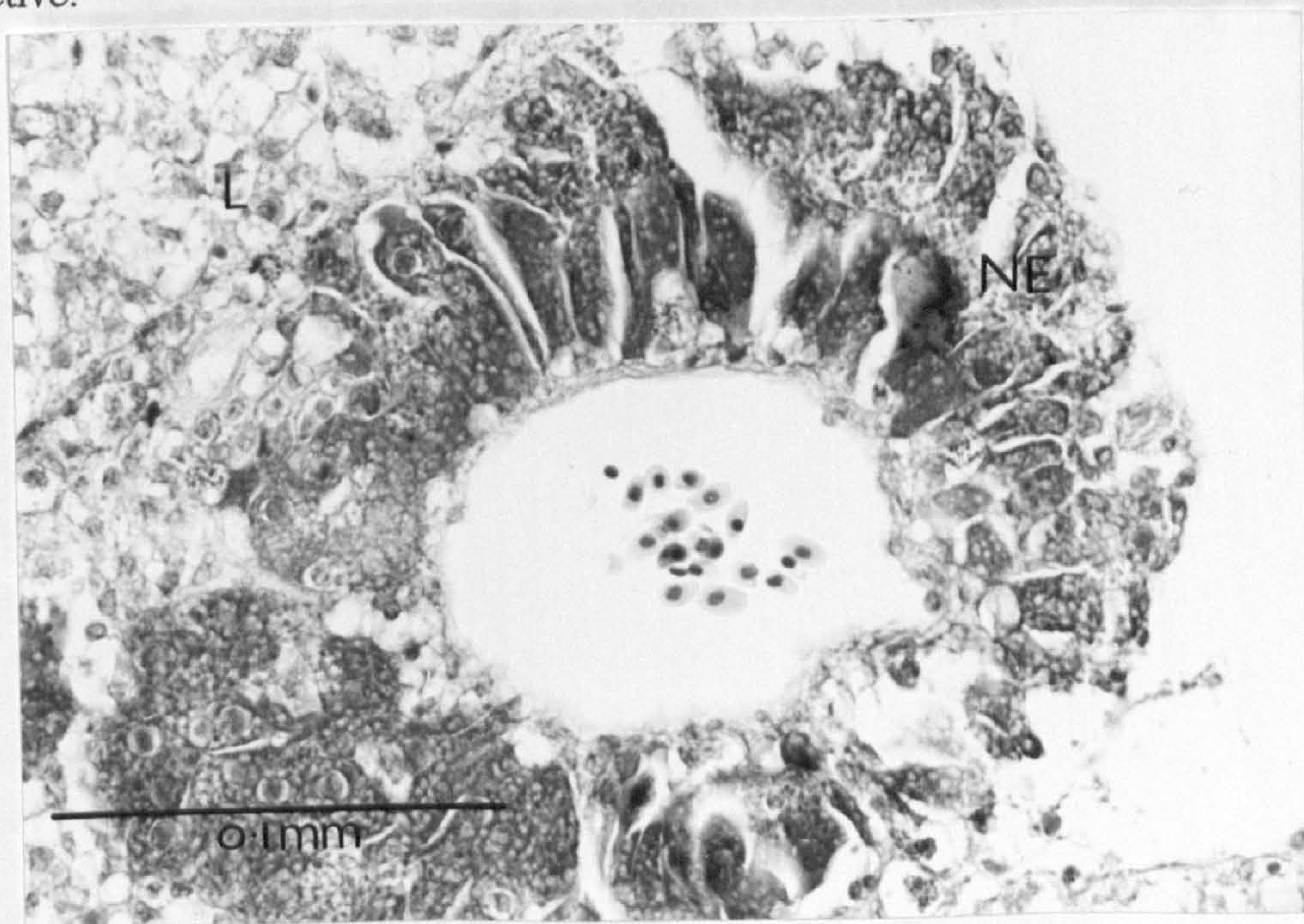


Fig 4.17: Necrosis of the intestinal epithelium (NE) in a fish from Replicate 2 of Experiment 2, one week post infection. The area of necrosis was located close to the entry point of a pancreatic duct, x40 objective.

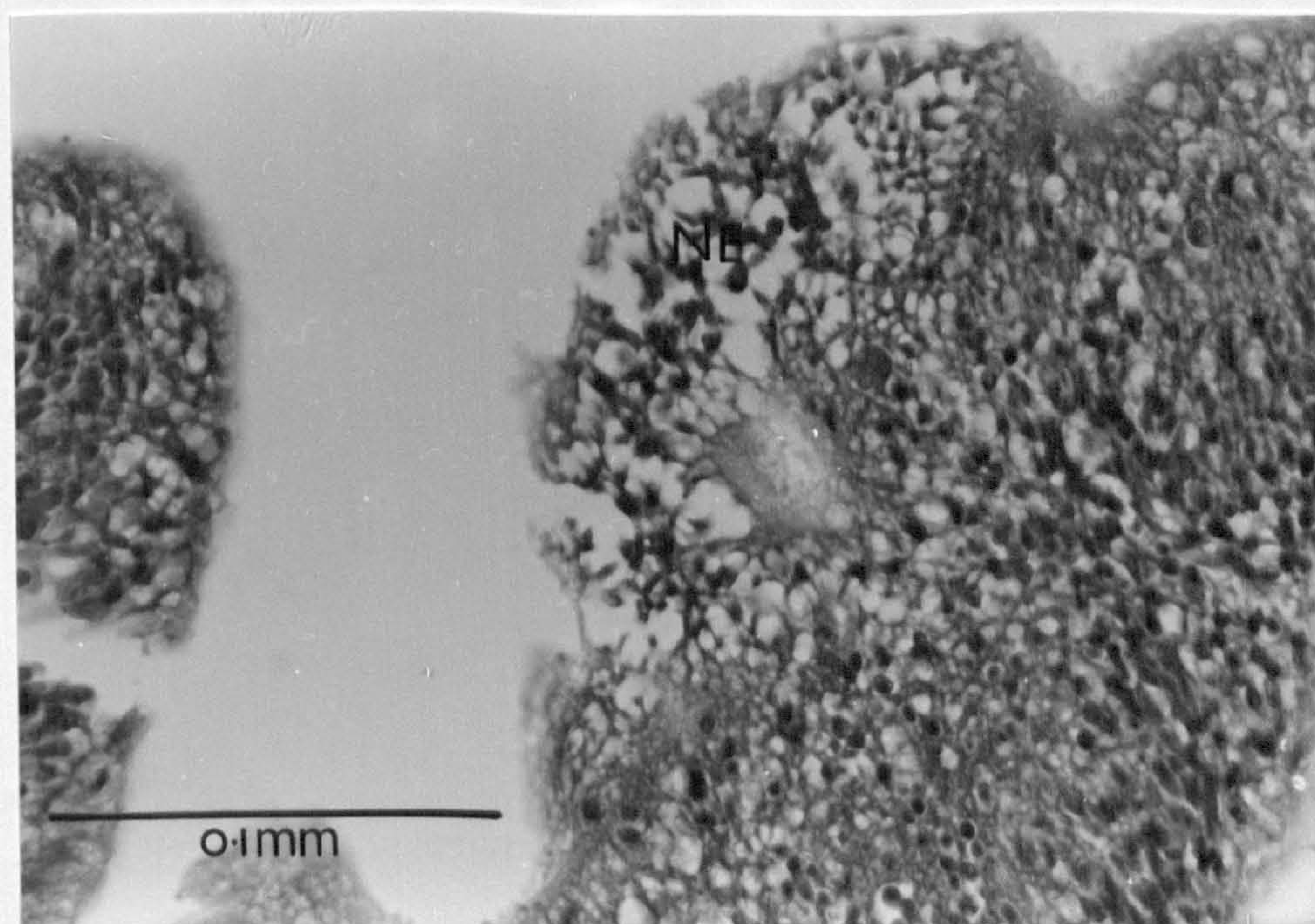
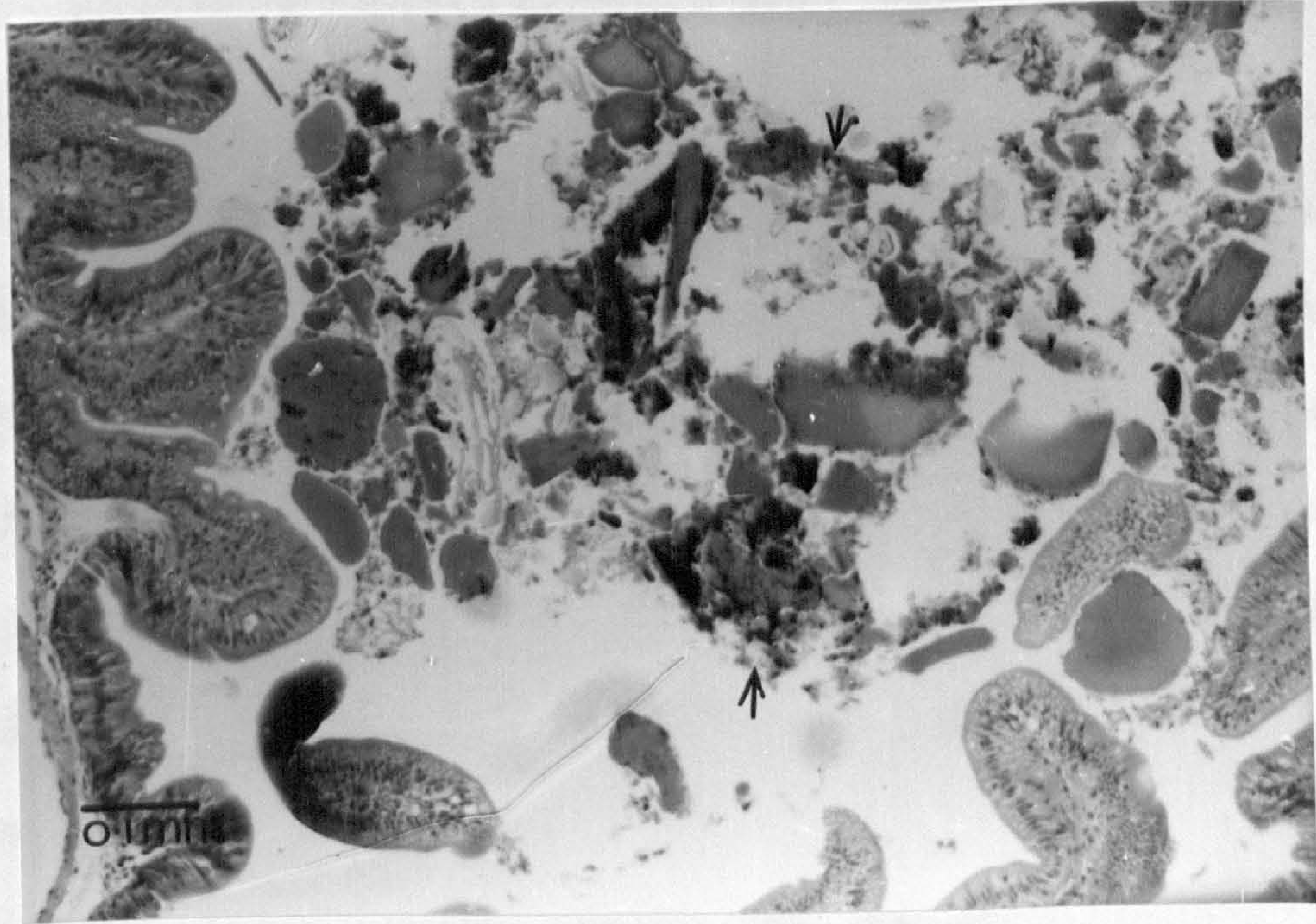


Fig 4.18: Food particles (arrows) in the intestinal lumen of a fish from Replicate 1 of Experiment 2, three weeks post infection, x10 objective.



4.4: Discussion

The challenge dose used in Experiment 1 produced a maximum prevalence of infection two weeks post infection (Replicate 1), with three out of ten fish being infected, and zero prevalence by four weeks post infection. The results of Experiment 1 suggested that *C. rupestris* are susceptible to IPNV, however, the prevalence of infection achieved was low in both replicates. There was a degree of heterogeneity displayed in the fish regarding the rate of uptake of virus. Some fish displayed infections far higher than others in the same replicate. These differences resulted in the skewing of the data giving peaks of infection at different weeks post infection for the two different replicates. This skewing is also due in part to the low sample sizes used. Further studies were needed with a higher challenge level.

McAllister & Owens (1986) suggested a standard bathing time of five hours at a concentration of 10^5 pfu/ml for infection of *Salvelinus fontinalis* (Mitchell) (brook trout) with IPNV. It was decided to follow this protocol for the second experiment using a higher challenge dose to produce a stronger challenge in the wrasse compared to Experiment 1. The results obtained did indeed show that *C. rupestris* are susceptible to IPNV with detectable tissue titres being obtained throughout the five week duration of the experiment. This was reflected in the higher prevalence of infection of wrasse which reached 100% for Replicate 2 at two weeks post infection.

The replication of the virus in the host produced a pathological response which was reversible. Recovery from the virus was rapid suggesting some degree of resistance

to the virus was developing. The clinical and histopathological picture varied considerably from those seen in salmonids. Only some of the fish exhibited darkening in pigmentation and none displayed the characteristic spiral swimming motion of severely affected salmonids Also feeding was resumed quickly after infection, with food being seen in the intestines of infected fish three weeks after infection. However, the wrasse used were older than *S. salar* fry or parr of comparable size normally infected with IPNV and, therefore, no direct comparison can be made between clinical signs in the two species. The necrosis of pancreatic tissue was typical of that seen in salmonids. The first signs of necrosis was a rounding of the cells and loss of cytoplasm this was accompanied in some cases with pyknotic nuclei. The acinar cells then quickly broke down and ghost cells could be seen within the adipose tissue. Necrotic lesions could be seen where the entirety of the acinar tissue was lost. There was also one area of necrosis observed in the endocrine tissue of one fish from the first weeks sample post infection, such necrosis of endocrine pancreas is not seen in *S. salar* infected with the virus. Also, in contrast to *S. salar,* there was no enteritis seen in any of the wrasse infected. Only one very small area of intestinal necrosis was observed. This was in an area where a pancreatic duct entered the intestine. This would indicate that the only tissue affected by IPNV in wrasse is the pancreas and any virus liberated in the faeces originates from the breakdown of pancreas resulting in infective particles being flushed through the pancreatic ducts into the lumen of the intestine.

The presence of the virus in the faeces demonstrates that wrasse infected by bath challenge with IPNV, while showing little clinical signs of infection, excrete the virus in their faeces. This would suggest that wrasse infected in *S. salar* pens with IPNV are a

potential source of re-infection for the *S. salar*. Smail *et al.* (1986) reported that the Sp serotype of IPNV could be detected in variable amounts in *S. salar* up to two and a half years after infection at the sac-fry stage. This suggests that virus excretion in carrier fish may also occur for a protracted period of time post infection. It is reasonable to assume, therefore, that wrasse which are stocked in pens affected with IPNV will also act as carriers for the disease, shedding virus for a considerable amount of time after infection.

Wrasse are not cultured commonly and can only be obtained in large numbers form the wild. Fish from the wild are less suitable for experimental work due to their unknown disease history. Only small numbers of tank bred fish were available and sample sizes were thus necessarily small, giving rise to the large variation seen in the data. A contributory factor to this variation was undoubtedly the use of the bath challenge, resulting in low uptake of virus. Using a bath challenge system offers experimental conditions close to the natural situation, however, there is no way to regulate the amount of virus taken up by individual fish.

It is interesting to note that in Experiment 1, where the temperature was relatively high, the highest tissue titres were seen at one week post infection in Replicate 2, but, in Experiment 2 where temperatures were lower, the maximum titres were seen two weeks post infection. Lapierre, Larrivée & Berthiaume (1986) reported that mortalities due to IPNV in *S. fontinalis* occurred quicker after infection and were greater in number at 15°C than at five degrees centigrade, presumably because virus multiplication and tissue damage is faster at the higher temperature.

In both experiments the appearance of the disease, and in particular the first signs of pathological changes in Experiment 2, occur over a similar time course to that in *S. salar*. For example Swanson & Gillespie (1979) reported that yearling *S. salar* of unstated size, inoculated by intraperitoneal injection with 2.5 x $\log_{10}6.0$ TCID50 IPNV VR-299 showed histopathological changes in the pancreas from three to nine days post infection. These lesions were associated with virus titres in the low titre range of 2-4 $\log_{10}$ TCID50 per 0.2ml pancreas homogenate.

However, the greatest difference between *C. rupestris* and *S. salar* infections is that the lesions in the former were seen to be far more extensive at low virus titres and even involved the endocrine pancreas tissue, which is never seen in salmonids. This finding suggests that *C. rupestris* are more susceptible to IPNV infection, when exposed to levels of infection used in Experiment 2, than *S. salar*. The relatively greater susceptibility of *C. rupestris* is surprising considering that the *C. rupestris* used were over two years old and their immune system would be fully functional. Testing for virus antibodies was not carried out as part of this experiment owing to its short duration.

In *S. salar* of a similar age to the wrasse used it would be expected to see a chronic persistence of infection after an initial onset of pathological signs, which suggests that IPNV does not stimulate a completely effective immune response in young *S. salar*. The quick recovery demonstrated by *C. rupestris* suggests that the immune response is not the only factor affecting recovery from IPNV. Ellis (Pers. Comm.) speculates that the production of specific antibodies to an antigen would take up to six weeks at the temperatures experienced in Experiments 1 and 2. Phagositosis of viral

particles by macrophages may play an important role in the early recovery of *C. rupestris* infected with IPNV.

Although some individual fish in both experiments were apparently negative for virus, this does not necessarily mean that there was no virus present in the fish. Due to the constraints of the sampling methods the lowest detectable level of virus is 5.55×10^2 pfu/g. It is possible, therefore, that wrasse may act as carriers for IPNV by harbouring the virus at very low levels without succumbing to the disease. Infection may then be activated during times of chronic stress producing pathology of the pancreas and higher virus titres, for example, in the winter when the water temperature drops. Wrasse are generally known to be susceptible to low water temperatures when held in *S. salar* pens (Darwall *et al.* 1991). During the winter in the wild *C. rupestris* can be found in a state close to "hibernation" in rock crevices (Sayer *et al.* 1993). In *S. salar* pens there are no such refuges for the wrasse to retreat to when the water temperature drops. In addition, there will be constant sources of stress for the wrasse from both net maintenance and from the *S. salar* in the pens. This combination of environmental and physiological stress, possibly combined with lowered salinity due to increased fresh water run-off at this time, is likely to reduce their natural ability to resist pathogens resulting in an increase in susceptibility to any disease agents in the farm environment. This would also provide a method for continual infection of *S. salar* not only with IPNV but any diseases that are affecting the farm at the same time as IPNV.

It cannot be concluded, however, from this experiment that IPNV has a role in wrasse mortality. Proof of combined effects, for example, infection with multiple diseases or under conditions of high stress, will also need experimental studies.

Wrasse are normally caught from the areas surrounding *S. salar* farms before being stocked with post smolt *S. salar* for their first year at sea. There is, thereby, considerable economic pressure to move wrasse between different sites at the end of the first year at sea to be stocked with incoming smolts. The fact that the wrasse were still excreting virus, five weeks post infection, indicates that movement of wrasse from IPN infected *S. salar* sites should not take place as this would facilitate the spread of the disease.

Although the experiment only investigated the susceptibility of *C. rupestris* to IPNV the morphology of all four wrasse species is so similar that it can be assumed that all four species will act in a similar fashion to infections with this virus, however, if a source of guaranteed disease free stock of *C. exoletus*, *S. melops* and *L. mixtus* could be found the above experiments could be repeated to investigate their susceptibility to the virus. In addition to this experiments investigating the possible carrier status of wrasse infected with IPNV could provide interesting results, if an examination of the fish for the presence of antibodies to IPNV was combined with the carrier status experiments this would significantly widen our understanding of the disease in wrasse.

CHAPTER 5: PANCREAS DISEASE

5.1: Introduction

5.1.1: A Historical Perspective of Pancreas Disease

In 1976 a new disease condition was described in *S. salar* which involved the total degeneration of the exocrine pancreas of fish held under farm conditions (Munro, Ellis, McVicar, McLay & Needham 1984). The disease was termed Pancreas Disease (PD) due to the degeneration of exocrine pancreas and subsequent acute emaciation of fish affected with the disease. It was quickly established that these outbreaks were not caused by the other agent of the known disease affecting the pancreas, Infectious Pancreatic Necrosis Virus (IPNV). The two diseases could be differentiated on the basis of their histopathological differences and the fact that PD only occured in salt water where IPN could be seen in both environments (McVicar 1986).

There followed an upsurge in the recognition of PD cases in the early eighties which established the disease as a major cause of loss in the culture of *S. salar*. By 1986 the disease had been reported from 19% of the *S. salar* sea water production sites in Scotland and was continuing to spread (McVicar 1986). Poppe, Rimstad & Hyllseth (1989) reported that outbreaks of the disease were also evident in Norwegian waters, although the number of outbreaks and mortalities associated with the disease were relatively fewer in Norwegian sea production sites than in the Scottish industry. The disease was also prevalent in Irish sea water production sites (Rodger, Murphy, Drinan & Rice 1991).

5.1.2: Clinical and Pathological Signs of Pancreas Disease

Diagnosis of the disease can only be made conclusively from the histopathology induced in the pancreas. The histopathological changes associated with the disease are limited to the total necrosis of the exocrine pancreas in *S. salar*. The sequence of histopathological changes associated with PD were described by Munro, Ellis, McVicar, McLay and Needham (1984) as pre-acute, acute and post-acute (including recovery). These developmental stages were further elucidated by McVicar (1987) with the aid of electron microscope observations. This author described "Increased condensation of the endoplasmic reticulum into sheets and whorls, loss of zymogen, the appearance of cytoplasmic vacuolation and cloudy swellings of cells" as being the only signs seen before the collapse of exocrine cells. The vacuolation seen in the exocrine cells prior to collapse is due to mitochondrial dilation in the cells.

Acute myopathy of skeletal and cardiac musculature has been reported from fish affected with PD in Norway, Scotland and Ireland (Ferguson, Rice & Lynas 1986a, Ferguson, Roberts, Richards, Collins & Rice 1986b, McVicar 1987, Ferguson, Poppe & Speare 1990 and Rodger, Murphy, Drinan & Rice 1991). This myopathy was assumed to be due to dietary deficiency and prolonged physical stress. The myopathy seen involved all areas of the musculature of the affected fish. Poston, Combs & Leibovitz (1976) reported that experimentally induced deficiencies in selenium and vitamin E produced myopathy of the cardiac and skeletal musculature. This myopathy was very similar to that seen in fish affected with PD. Ferguson *et al.* (1986a) reported that the levels of selenium and vitamin E in fish affected with PD was low. The authors concluded that these deficiencies were either the cause of, or result of, PD. However,

work by Bell, McVicar & Cowey (1987), Rodger *et al.* (1991) and McCoy, McLoughlin, Rice & Kennedy (1994) has shown that the levels of selenium and vitamin E defficiency seen in fish affected with PD are due to PD rather than being the cause of the disease.

McVicar (1987) highlighted the problems in diagnosing a disease condition solely on the basis of pancreatic change. For example Solangi & Overstreet (1982) described histopathological changes in the pancreas of two estuarine fishes which resembled the changes seen in PD affected *S. salar*, but which were due to oil contamination. Many conditions produce very similar pathological changes due to the limited number of ways that fish tissues can respond to disease or poisoning. Thus it is very important to review all of the factors involved in an outbreak of PD along with the histopathological picture.

In most cases the first sign of disease is a loss of feeding response and decrease in food conversion ability. Severely affected fish become "eel like" and emaciated. It is at this time that signs of pancreatic necrosis are first seen in histological sections. Recovery from the disease may occur at any time during the course of the disease, but a variable proportion of the severely affected fish never recover. Recovery would appear to be closely related to the amount of stress to which the fish are subjected. Most of the mortalities in an outbreak are attributable to secondary disease problems such as sea-lice, furunculosis or vibriosis (McVicar 1987).

5.1.3: Aetiology of Pancreas Disease

Early research was unable to link the disease with a single cause as it appeared to affect sites at random, however, once affected, PD would re-occur on a site year after year. It was also noted that fish previously exposed to the disease showed signs of increased resistance.

Numerous attempts were made to isolate a disease agent from fish affected in outbreaks, using several cell lines in the tests, but without success. Poppe, Rimstad & Hyllseth (1989) reported seeing cytopathic effects typical of PD in CHSE-214 cell lines induced by homogenates prepared from the midkidney of *S. salar* from a PD infected farm population. These homogenates had been filtered through a 0.45μm membrane (Millex HA). However, filtered homogenates from pyloric caeca, brain and gills showed no CPE in the same cell line. This level of filtration effectively ruled out any disease agent other than a virus. Serum neutralisation tests carried out on the isolated virus identified it as IPNV-sp serotype. It was concluded that IPNV was secondary to PD infection in these fish.

McVicar (1987) presented the results of field and experimental trials which demonstrated that naive *S. salar* could become affected with PD if subjected to effluent water from tanks housing fish diplaying signs of the disease. These results illustrated that the disease could be passed on through water between affected and naive *S. salar* and, therefore, that PD had an infectious primary aetiology. Fish which recover from the disease also display a resistance to reinfection but could pass the disease on to naive fish.

Following this work a series of experiments carried out by Raynard and Houghton (1993) established a standard method for experimental infection of naive *S. salar* with PD. This method involved the injection of 0.45µm filtered homogenated kidney tissue, removed from PD affected *S. salar*, into naive *S. salar*. These injected fish would then develop the characteristic signs of the disease. The experimentally infected fish were then sampled and the kidney homogenised and filtered before being injected into a second batch of naive *S. salar*. If this second experimental group developed the disease the experiment was considered to satisfy the conditions of Koch's postulate. The absence of any indication of vertical transmission is important when establishing the disease free status of naive fish. As reported earlier, there is increased resistance to PD in fish previously exposed to the disease. This method is now accepted by goverment scientists as the standard method for reproduction of PD.

This experimental technique illustrated that the agent responsible for PD was an infectious agent which could be transferred between fish by means of infective tissue. It did not, however, explain the origins of the infective agent in farm conditions.

The fact that outbreaks appear to occur at random, independent of location, age of site or year class of fish present would suggest that the source of infection comes from outwith the affected farm. It has been suggested that this source could well be wild fish passing by the farm and subsequently infecting the fish in the cages. The synchronous nature of outbreaks which very quickly become epidemic in nature would also appear to confirm this suggestion (McVicar 1987).

Very recently a toga-like virus has been isolated from the tissues of PD infected fish by Nelson, McLoughlin, Rowley, Platten & McCormick (1995). During infection trials, fish infected with this isolated virus developed histopathological signs indistinguishable from those seen in PD outbreaks. This indicates that the isolated virus is the causative agent of PD, the authors therefore suggest that the virus be named Salmon Pancreas Disease Virus (SPDV). Fish affected with PD from a Scottish *S. salar* farm were shown to possess antibodies to SPDV in their blood, indicating that SPDV is present in Scottish *S. salar* farms. The authors suggested that it may now be possible to develop rapid detection techniques, for example antigen and nucleic acid detection tests, for the disease. McLoughlin (1995) reports that work is now underway to develop a vaccine against SPDV.

5.1.4: The Continuing Problems Related with Pancreas Disease

Pancreas Disease has continued to be a major cause of loss in the *S. salar* industry and, since control of other major diseases, such as furunculosis and vibriosis, has been acheived by new managment techniques or vaccines, PD, along with sea lice infection and IPN remain the three most serious disease conditions in *S. salar* culture, see Chapter 1.1. Considerable financial losses can be attributed to PD due to reduced growth during outbreaks and the neccessity to cull severely affected fish from a farm population. PD has been reported from all the European countries in which *S. salar* are cultured (Raynard, Houghton and Munro 1992) and the growth in the number of PD cases has closely mirrored the development of the industry (McVicar 1987).

5.1.5: The Aims of the Present Work

As PD has only been described from *S. salar*, and no other fish species have been examined for the typical symptoms of the disease, it would be very difficult to establish which species, if any, are responsible for transmitting the disease to farmed *S. salar*.

The use of wrasse in the farming of *S. salar* for the control of sea lice exposes farmed *S. salar* to common inshore fish species which could be vectors for the agent of PD, and also exposes wrasse to the disease agent of PD potentially being carried by *S. salar*. Wrasse have not been examined for PD due to the difficulty in identifying the disease in any species other than *S. salar*, and it is not known if they are susceptible to the disease. If it could be demonstrated that they are indeed susceptible to PD, the results could be very important to the future use of wrasse in the farming of *S. salar*.

With this view in mind work was undertaken to expose wrasse to PD using the experimental protocol laid out by Raynard and Houghton (1993) This was in an attempt to establish whether or not they are susceptible to the disease and also to investigate the possibilty that wrasse could transfer the disease to *S. salar*.

5.2: Materials and Methods

The experiment was carried out in two stages. The first stage being the injection of naive *C. rupestris* with infective kidney homogenate from PD infected *S. salar*. This first stage of the experiment also involved the cohabitation of the injected *C. rupestris* with naive *S. salar* smolts to check for cross infection between the species via water.

The second stage of the experiment involved the injection of *S. salar* parr with kidney homogenate removed from the *C. rupestris* in stage one. This was to establish whether or not tissues from wrasse injected with infective PD material were in themselves infective for *S. salar*. This stage was carried out to satisfy the requirements of Koch's postulate.

5.2.1: Stage One

5.2.1.1: Experimental Design

A group of 150 captive bred *C. rupestris* were used for the experiment. These fish were all the same age, approximately two and a half years old, and were obtained from Captive Site I, described in Chapter 3A.2.3. The brood stock fish were caught near the Isle of Cumbrae, in the Firth of Clyde, an area with no links with *S. salar* farming. It was, therefore, considered to be highly unlikely that either the brood stock or their offspring had been previously exposed to PD.

A sub-sample of 20 fish were randomly removed from the group and anaethsetised using Benzocaine, at a dose of five milletres of ten percent Benzocaine in eight litres of sea water, and then weighed. These weights are shown in Table 5.1. After they had recovered they were returned to the same holding tanks as the rest of the group. This group of 150 fish were then split randomly into three groups of 50 fish (a Control group and Groups 1 and 2).

Table 5.1 Weights for 20 randomly selected *C. rupestris* taken from a population of 150 fish.

Fish Number	Weight (g)	Fish Number	Weight (g)
1	7.40	11	5.71
2	4.06	12	5.25
3	5.40	13	8.25
4	3.50	14	3.80
5	5.40	15	6.40
6	6.10	16	3.77
7	5.55	17	4.49
8	4.66	18	3.59
9	3.94	19	3.60
10	2.70	20	4.47

Total weight of fish = 98.04g,

Mean weight of fish = 4.92 ±1.40g.

A group of 75 S1 *S. salar* smolts from the marine research facilities of S.O.A.F.D. at Aultbea, a site certified as PD free, were randomly split into three groups of 25 fish. Each group was cohabited with one of the three *C. rupestris* groups.

5.2.1.2: Method of Infection

Two samples of kidney homogenate were supplied by Dr. Houghton of the Marine Laboratory at Aberdeen. One of these samples was from *S. salar* which were known to be free from PD and was used for the controls. The second sample was obtained from *S. salar* experimentally infected with PD. The protein content of these samples had been calculated by use of a Sigma Diagnostics Micro Protein Determination kit, phenol reagent method for biological fluids, Procedure Number 690. The total protein content of each of the two samples was 98000μg/ml.

The Control group of wrasse were injected with kidney homogenate at a dose level of 30μg/g body weight. The body weight was taken to be the mean weight calculated from the subsample. This resulted in a neccessary effective dose of 147μg per fish. This dose was obtained by diluting the one millitre of control sample, with a protein concentration of 98000μg/ml, with 6.5ml of phosphate buffered saline (PBS) (Gibco) to give an effective protein concentration of 147μg/100μl for the stock solution. The 50 *C. rupestris* from Group 1 were then given injections of 100μl of the diluted kidney homogenate, and marked with a Panjet marker before being placed in a flow-through sea water tank with 25 of the naive *S. salar*.

The first treatment group (Group 1) received a 100μl injection of infective kidney homogenate at a concentration of 3μg/g body weight. This was acheived by diluting 100μl of homogenate, with a protein concentration of 98000μg/ml, with 6.5 ml of PBS to give an effective protein concentration of 14.7μg protein/100μl of fluid. Once injected the 50 fish from Group 1 were marked with a Panjet marker and placed in a flow through system with twenty five of the naive *S. salar*.

The second treatment group (Group 2) received an experimental dose of 30μg/g body weight in 100μl of fluid by carrying out the same dilution on the infective kidney sample as described for that of the Control group sample. These 50 fish were also marked after injection and placed in a flow through system with 25 naive *S. salar*.

The experimental dose levels were obtained from the work by Raynard & Houghton (1993). These authors reported that in experiments carried out in fresh water a dose level of 3μg/g body weight in 100μl of kidney homogenate, obtained from PD affected *S. salar*, resulted in a significant number of injected *S. salar* parr displaying the typical pathological signs associated with PD in *S. salar*. Experiments in sea water using dose levels of 30μg/g body weight in 100μl of kidney homogenate, obtained from PD affected *S. salar*, resulted in significant numbers of *S. salar* smolts displaying the same signs. The higher dose level of 30μg/g body weight in 100μl of kidney homogenate were used to inject the Control group fish in order to ensure that any contamination of the control homogenate with the infective agent of PD would be detected.

5.2.1.3: Sampling

Sampling was carried out at seven day intervals post injection for a five week period. Ten *C. rupestris* and five *S. salar* were removed from each group per sample. The fish were killed by a sharp blow to the head and samples of liver, adipose tissue, intestine and kidney were removed from the sampled fish and placed in eight percent buffered formol saline for histological processing. In the case of the *S. salar* samples of intestinal caeca were also removed for histological processing. Histological processing followed the standard techniques detailed in the Appendix The remaining organs from each fish were placed in individual sterile Bijoux bottles and frozen at -70°C for later processing.

The fish from each group were sampled separately starting with the control group. All equipment was sterilised with an iodine based disinfectant between groups to eliminate the risk of cross contamination of samples.

5.2.2: Stage 2

5.2.2.1: Experimental Design

The second stage of the experiment was carried out in fresh water and involved the injection of homogenised tissues, removed from the *C. rupestris* experimentally infected in Stage 1 of the experiment, into naive *S. salar* parr.

A group of 180 one year old *S. salar* parr were obtained from the PD free marine research facilities of S.O.A.F.D. at Aultbea. A sub sample of 20 of these fish were anaethsetised using Benzocaine, at the same dose as before, and weighed. The average

weight for these 20 fish was used in the future calculations of the infective dose. These weights are shown in Table 5.2.

The fish were split randomly into six groups of 30 fish. These fish were then transfered to separate flow through tanks. The groups, thus formed, were for use as tests of Koch's postulate, one group for each of the five weeks of sampling in the first part of the experiment and one Control group.

5.2.2.2: Homogenate Preparation

Control Group

Two of the visceral samples from each of the five weekly samples of ten *C. rupestris* in the Control group of the first stage of the experiment were chosen at random and defrosted. The resulting ten visceral samples were then pooled and one millitre of PBS was added. The pooled sample was then placed in a sterile test tube and homogenised mechanically before being passed through a 0.22μm (Millipore) membrane filter. The filtered homogenate was then stored on ice in a sterile sample tube.

Experimental Groups

The visceral organs from the ten *C. rupestris* sampled at one week post injection in Stage 1, were pooled and homogenised as described above. This procedure was repeated for each of the other four weekly samples. Each sample was dealt with seperately, using a new set of autoclave-sterilised equipment each time. These samples, after filtration, were then stored on ice in sterile epindorph tubes.

Table 5.2 Weights for 20 randomly selected *S. salar* parr from a population of 180 fish.

Fish Number	Weight (g)	Fish Number	Weight(g)
1	43.68	11	24.12
2	38.85	12	33.25
3	42.36	13	31.22
4	39.77	14	41.11
5	42.70	15	46.62
6	35.55	16	31.04
7	27.09	17	29.35
8	31.00	18	37.71
9	26.59	19	25.96
10	23.41	20	32.80

Total weight of fish = 684.18g,

Mean weight of fish = 34.283±7.05g.

The control sample and the five weekly samples were then analysed for protein content by use of a Sigma Diagnostics Micro Protein Determination kit, procedure number 690.

5.2.2.3: Method of Calibration

A calibration curve consisting of the absorbancies at 725nm, obtained using a spectrophotometre, of varying concentrations of an egg albumen protein standard was obtained by following the instructions supplied with the protein determination kit. The absorbancies and concentrations used to construct the calibration curve are shown in Table 5.3, the calibration curve is shown in Fig 5.1.

5.2.2.4: Protein Analysis Protocol

The protocol supplied with the determination kit was followed to obtain absorbancy readings at 725nm, using a spectrophotometre, for each of the samples. These absorbancies were then read against the calibration curve obtained from the protein standard dilutions to obtain the figures for the protein content of the samples. These protein concentrations are shown in Table 5.4.

5.2.2.5: Calculation of the Dose Level for Injection into the Naive *Salmo salar*

Given the number of fish to be injected, the average fish weight of 34.2g, the volume of homogenate (0.8ml) and the protein content of the homogenates (22mg/ml) the highest protein dose possible was 5μg/g body weight. This gave a dose per fish of 171μg protein. The injection volume was to be 100μl, therefore, the stock solution

Table 5.3 Absorbances and protein contents for the protein standard calibration dilutions.

Standard Tube	Absorbance at 725nm	Protein Content (mg/ml)
1	0.000	0
2	0.316	25
3	0.568	50
4	0.843	75
5	0.998	100

Fig 5.1: Calibration curve constructed from the absorbancy values, at 725nm, of different dilutions of egg albumen protein standard.

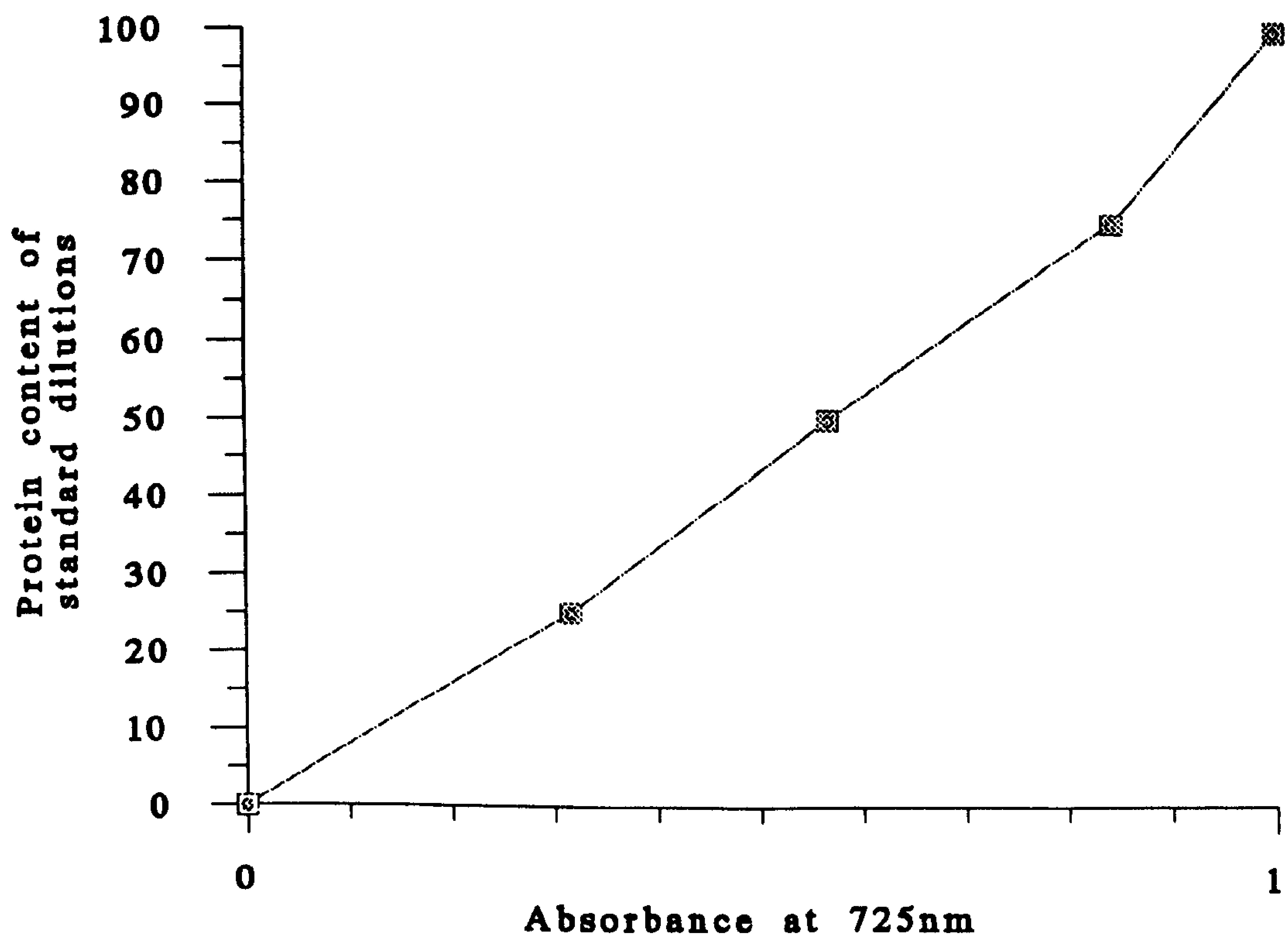


Table 5.4 Absorbances and protein concentrations of test solution dilutions, after the removal of 200μl for protein content analysis there was only 800μl of stock solutions left.

Sample Number	Absorbance at 725nm	Protein content (mg/ml)/(mg/0.8ml)
Control x10 Control x100	1.995 0.385	32/25.6
1 x10 1 x100	1.540 0.274	22/17.6
2 x10 2 x100	1.762 0.275	22/17.6
3 x10 3 x100	1.849 0.296	23/18.4
4 x10 4 x100	1.594 0.274	22/17.6
5 x10 5 x100	1.699 0.269	22/17.6

should contain 1.71mg protein/ml. The dilutions made to obtain this protein concentration are shown in Table 5.5:

5.2.2.6: Injection of fish

The 30 *S. salar* from each group were injected with 100µl of the stock solution corresponding to their group. They were then panjet marked and placed in flow through tanks, with one tank housing one group of fish.

5.2.2.7: Sampling Protocol

At seven day intervals post injection, ten fish from each group of *S. salar* were removed, killed by a sharp blow to the head and samples of pyloric caeca, intestine, liver, kidney and abdominal adipose tissue fixed in 8% formol saline for histological processing.

5.2.2.8: Histological Proccessing

The procedure used for the processing of histological sections followed the standard methods for the procedure as described in the Appendix.

5.3: Results

5.3.1: Stage 1

There was a total of four mortalities of *C .rupestris* during the five week duration of the experiment. All of these mortalities occured in the second week of the experiment. The four mortalities were spread through all three groups of fish and thus

Table 5.5 Column one relates to the sample number and column two relates to the dilution required to produce a protein concentration of 1.71mg protein/ml solution. Column three indicates the amount of PBS added to 0.8ml of stock solution to produce the dilution given in column two.

Sample Number	Dilution required	Amount of PBS (ml)
Control	14.9	11.12
1	10.3	7.44
2	10.3	7.44
3	10.8	7.84
4	10.3	7.44
5	10.3	7.44

death was considered to be due to stress induced during the handling involved in the injection of the fish.

No pathology, which could be considered to be typical of PD in salmonids, was seen in either the experimentally infected *C. rupestris* or the cohabitted *S. salar* during the five weeks of the experiment. The pancreatic tissues in both species remained within the normal levels expected for this tissue when compared to control fish and the descriptions given in Chapter 2. The pancreas from a *C. rupestris* from Group 1 from two weeks post injection is shown in Fig 5.2 illustrating that there was no change in this tissue, the pancreas from a co-habiting *S. salar* from the same group is shown in Fig 5.3 illustrating the same thing. A section of pancreas from a *C. rupestris* from Group 2, two weeks post infection is shown in Fig 5.4, with the same tissue from a *S. salar* from the same sample illustrated in Fig 5.5.

Food was consistently seen in the alimentary tracts of the sampled fish indicating that there was no reduction in feeding activity.

The only pathology which could be regarded as unusual was an increase in melanised areas seen in the adipose tissues of the injected *C. rupestris*. However, these melanised areas were also seen in the control fish. Fig 5.6 illustrates one of these melanotic areas in the connective tissue from a *C. rupestris* from the Control group from two weeks post injection.

Fig 5.2: Normal pancreatic (N) tissue of a *C. rupestris* from Group 1, two weeks post infection, x40 objective.

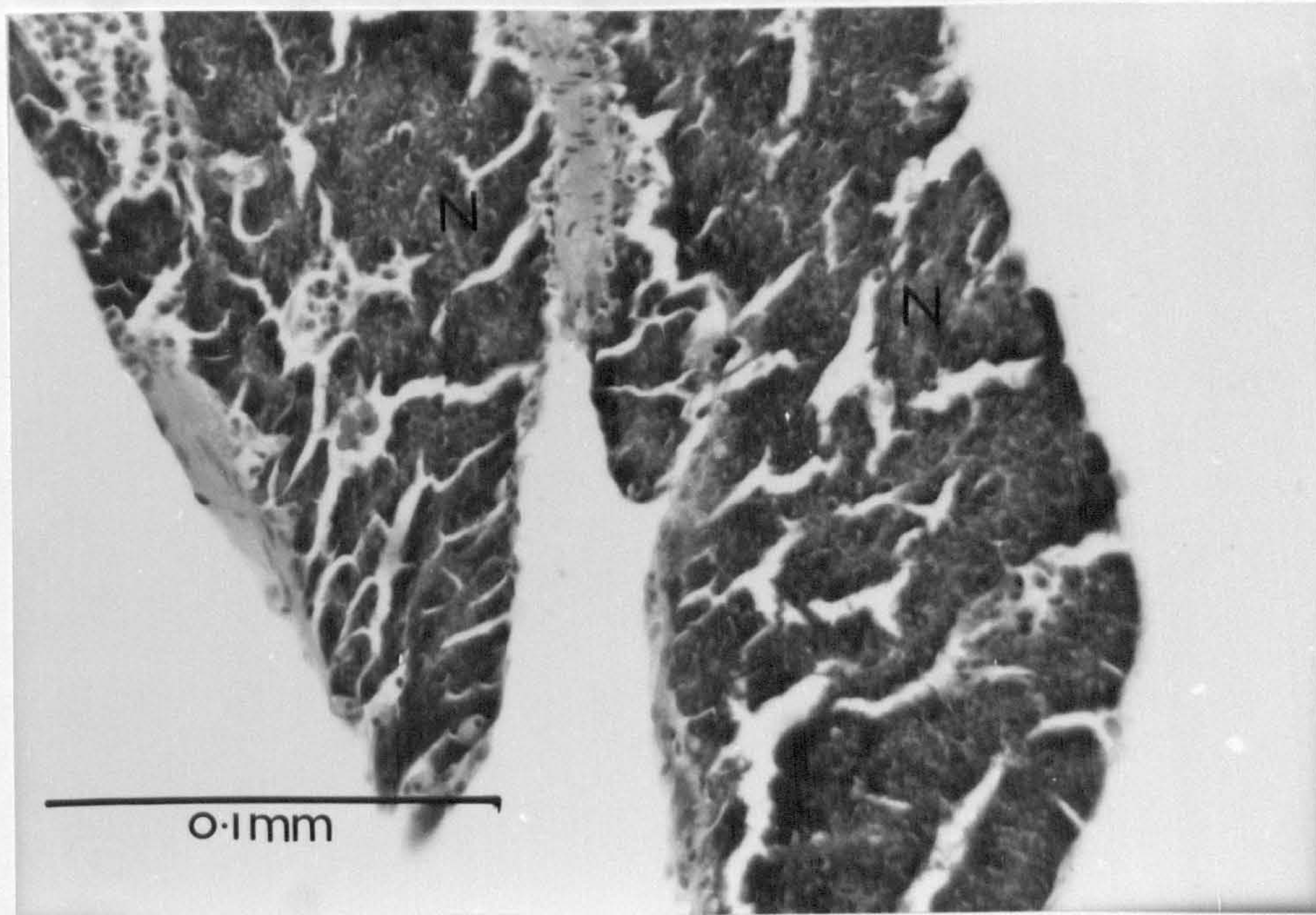


Fig 5.3: Normal pancreatic tissue (N) from a *S. salar* from Group 1, two weeks post infection, x40 objective.

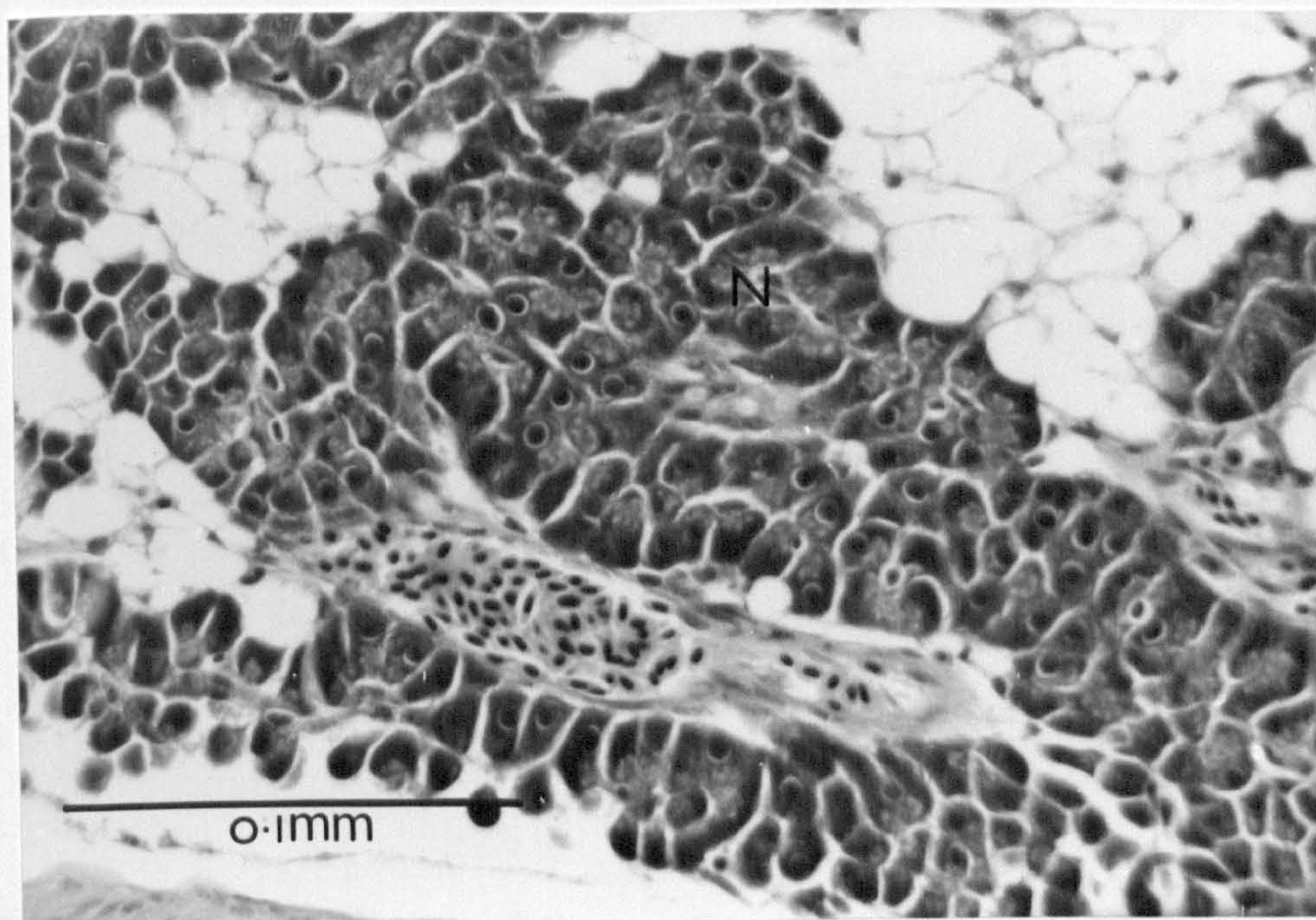


Fig 5.4: Normal pancreatic tissue (N) from a *C. rupestris* from Group 2, two weeks post infection, x40 objective.

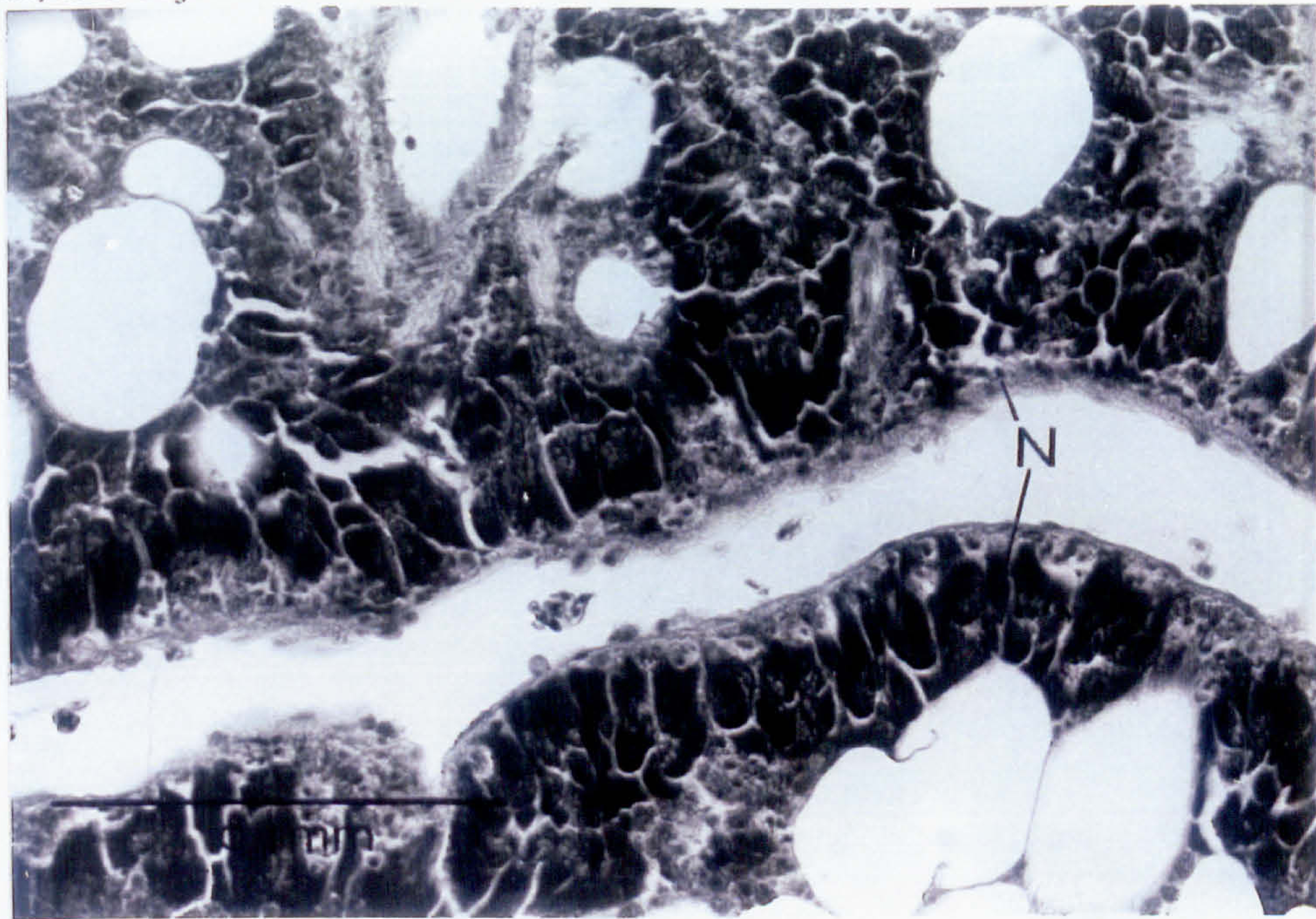


Fig 5.5: Normal pancreatic tissue (N) from a *S. salar* from Group 2, two weeks post infection, x40 objective.

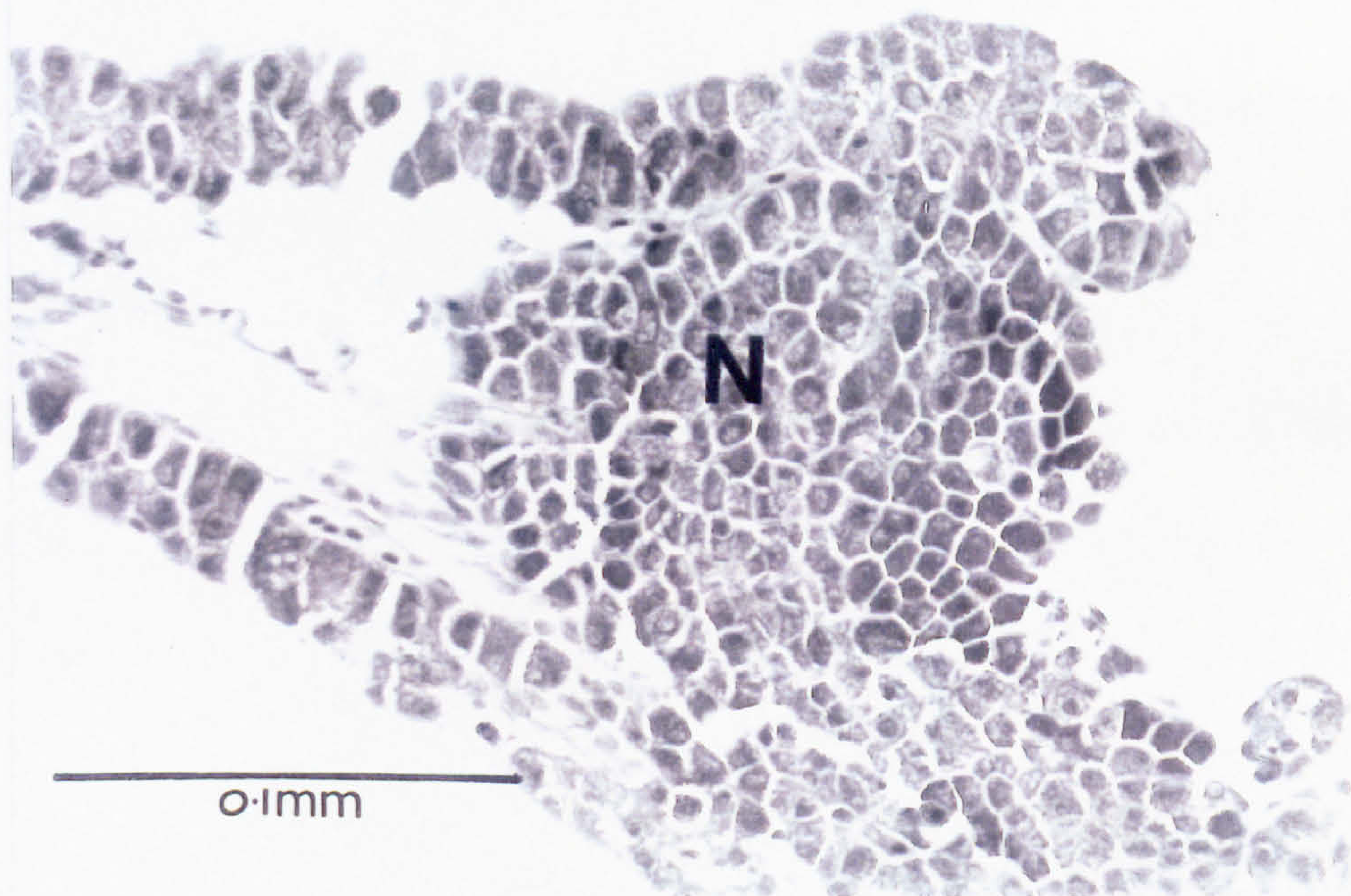
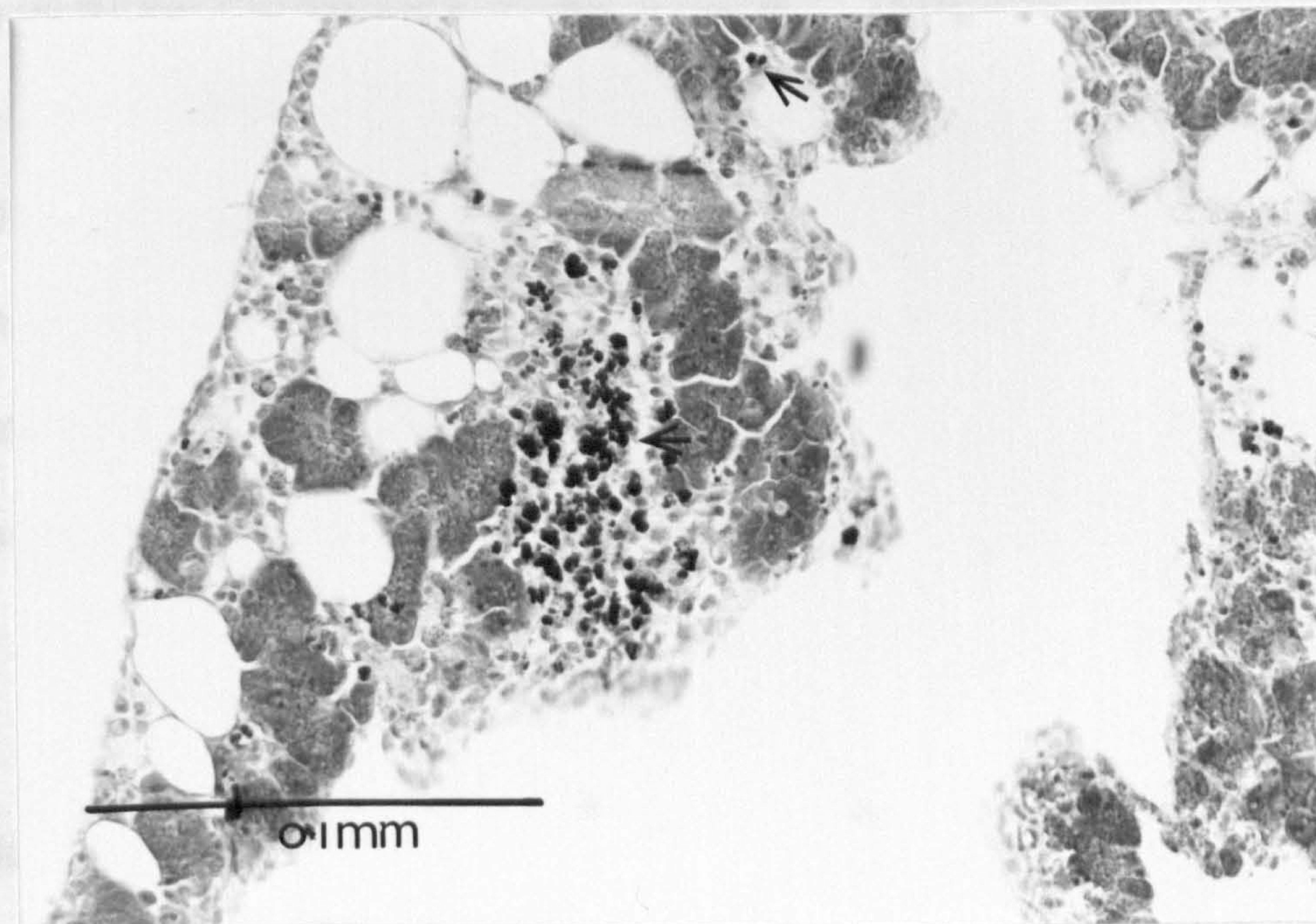


Fig 5.6: Melanotic area (arrows) within the pancreatic and connective tissues of *a C. rupestris* from the Control group, two weeks post infection, x40 objective.



5.3.2: Stage Two

There was no sign of any typical pathology associated with PD in any of the *S. salar* from the three weekly samples taken during the experiment. Food was consistently seen in the intestines of the fish indicating that there was no reduction in feeding activity. Fig 5.7 shows the pancreas of a *S. salar* from Group 3 three weeks post infection illustrating that no changes were seen in this tissue during the duration of the experiment. Fig 5.8 shows food particles in the intestine of a *S. salar* from Group 4, three weeks post infection.

5.4: Discussion

The absence of any abnormal histological signs in any of the sampled *C. rupestris* injected with infective PD kidney homogenate suggests that *C. rupestris* are not susceptible to PD. The dosage rates used were directly equivalent to those which would be expected to induce the disease in *S. salar*. Previous and subsequent experiments with the same pool of kidney homogenate as was used in the first part of this experiment have proven that the homogenate was indeed infective to *S. salar* (Houghton and Raynard Pers. Com.). The methods of preparation of homogenate for injection were identical to that used by Raynard & Houghton in their experiments and there appears to be no doubt that the homogenate was indeed infective when it was injected into the *C. rupestris*.

The presence of melanotic areas in both the experimental and control *C. rupestris* is suspected to be a direct result of the intraperitoneal injections disrupting the tissues rather than a reaction to the material injected. These melanotic areas would appear to be

Fig 5.7: Normal pancreatic tissue (N) from a *S. salar* from Group 3, three weeks post infection, x40 objective.

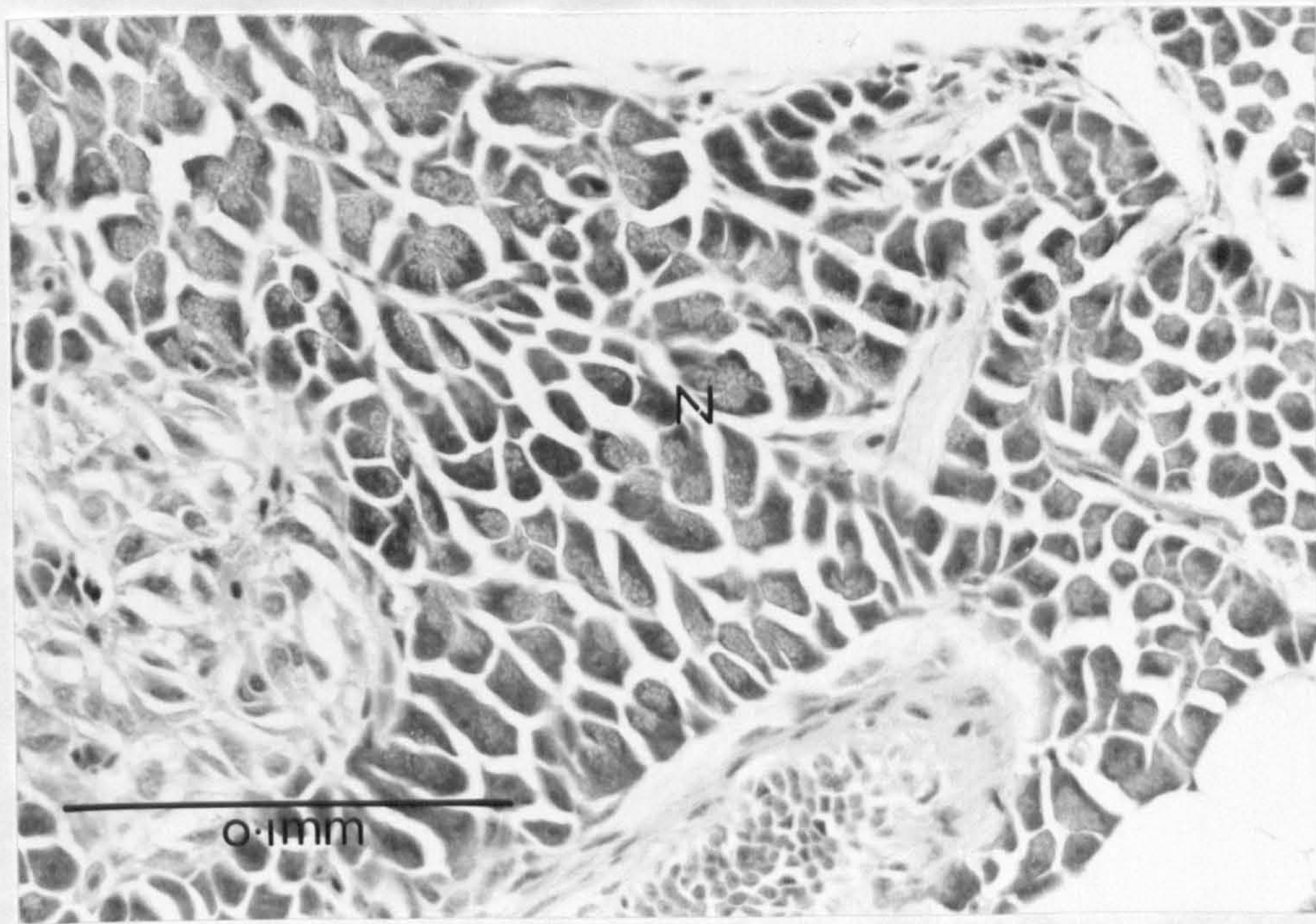
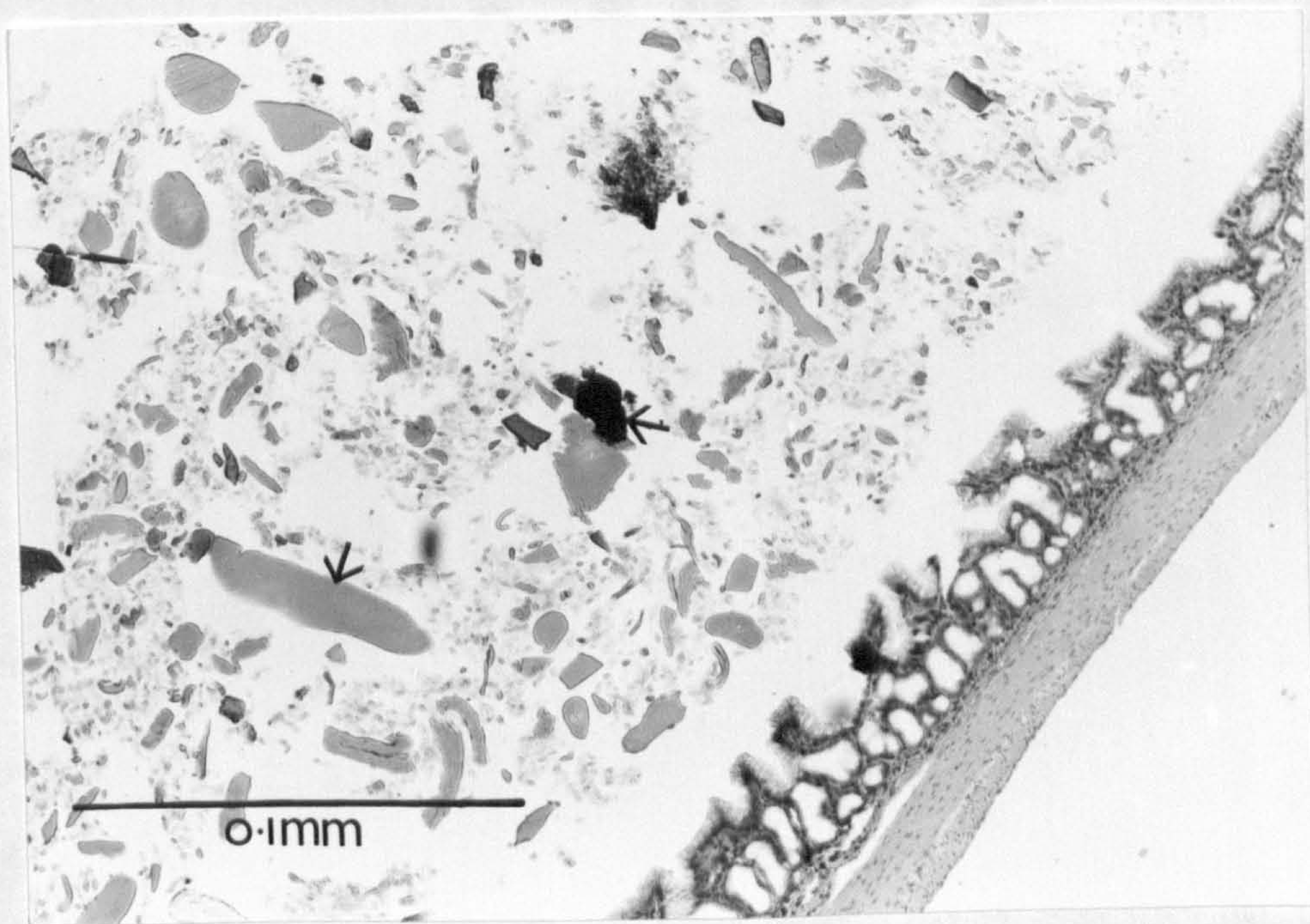


Fig 5.8: Food particles in the intestine of a *S. salar* from Group 4, three weeks post infection, x40 objective.



the result of localised inflammatory response to tissue damage caused by the needle. The fact that these melanotic areas were seen in the control fish as well as the experimental fish would reinforce this hypothesis.

The failure to transfer the disease via water to the cohabited *S. salar* would suggest that the infective material was not simply shed by the *C. rupestris*. Or at least, if it was shed, it was in a state which was not infective for *S. salar*. The presence of localised inflammation at the site of injection as indicated by the areas of melanisation could indicate that the disease agents were destroyed by the immune system of the *C. rupestris*.

The failure to transmit the disease via the homogenised visceral organs of the experimentally injected *C. rupestris* to naive *S. salar* parr would also indicate that wrasse tissues are not affected by the infective agent of PD.

The most likely explanation for this finding is that the mechanisms which allow the infective agent of PD to enter the pancreatic tissues of *S. salar* are not present in *C. rupestris*. These mechanisms are most probably cell based and indicate that PD is very species specific for *S. salar*. However, experiments involving other species of fish would be needed to verify this. With the isolation of a possible viral agent of PD (Nelson *et al.* 1995) more detailed studies on the infection mechanisms involved in PD may become possible. It is likely that PD would affect other species of salmonids, but, as no experiments have been carried out on any other species other than *S. salar* and *C .rupestris* it is not possible to say for certain at this time if this is the case.

From these results it may be concluded that PD will not pose a problem to the use of *C. rupestris* in the farming of *S. salar*. They are neither affected by PD nor do they appear to transmit the disease to uninfected *S. salar*. The results also suggest that *C. rupestris* are not a wild source of the disease. Although this experiment only involved *C. rupestris* and it would be desirable that other wrasse species are tested. *C. exoletus*, *S. melops* and *L. mixtus*, being closely related to *C. rupestris*, would be expected to display very similar reactions to any diseases, suggesting that they are also not susceptible to PD.

CHAPTER 6: SUMMARY AND SUGGESTIONS FOR FURTHER WORK

6.1: Conclusions From the Present Study

A review of the available literature revealed that there is very little known about the four wrasse species currently being used to control sea-lice infestations in *S. salar* culture. Limited information is available regarding the distribution and behaviour of wrasse, but, other aspects of their biology have been very poorly studied. In particular, there is scant information available regarding the diet of wrasse and little relating to their diseases. Similarly the detailed morphology of wrasse had been poorly studied with what little work that has been carried out being of limited relevance to the study of their diseases.

In order to investigate abnormal conditions it is first necessary to understand the normal ones. With this view in mind a detailed study of the normal morphological features relevant to the monitoring of the health of fish was undertaken. This study revealed that wrasse have many features which set them apart from salmonids, which are the most frequent subject of study for health diagnosticians in the industry in the UK. Many of the features described from the four wrasse species studied were common to perciform fish. For example, all four wrasse species were found to be hepatopancreatic. The pancreas in wrasse is a diffuse organ with strands of pancreatic tissue being found throughout the connective tissue of the visceral cavity and the liver. In all salmonids the pancreatic tissue is localised around the intestinal caeca. However, there were also features that were peculiar to wrasse. In common with other labrids studied previously, the four species included in this study were found to lack a well defined stomach. Their intestines were short and unspecialised and even in histological section it was very

difficult to differentiate between the regions. This is likely to be an adaptation to their diet. Though little is known, wrasse are regarded as being opportunistic feeders preying on small invertebrates, but, there is very little knowledge of dietary differences between the different species. Another feature which set wrasse apart from salmonids was the structure of the heart. The ventricular structure found in wrasse was unusual. The serous membrane surrounding the ventricle was very thin, only one to two cells thick, and the ventricle itself was very convoluted in structure with the cardiac muscle being present in thinner fibres than would be seen in other fish such as salmonids. This would result in the heart being able to accommodate a large volume of blood but would also result in relatively low blood pressure. This may be an adaptation to the "hibernation" state which wrasse appear to enter during cold periods. The large volume of the heart would allow the rate of pumping to drop while still maintaining a relatively large flow of blood through the gills. Salmonids have a very muscular heart with a relatively small volume which enables them to pump blood through the gills and the rest of the body at a steady rate maintaining a high blood pressure. The small volume of the heart does not allow for slowing down the rate of beating as this will significantly reduce the rate of blood flow.

The degree of lipid vacuoles in the liver differed between wrasse from different locations. Those from the wild had compact hepatocytes with few lipid vacuoles, while those from farm localities often had large numbers of vacuoles in the hepatocytes. This was thought to be due to the storage of excess lipid in the liver. The source of this lipid was likely to be the high energy pelleted feeds that are fed to *S. salar* under farm conditions since such a conditions is commonly found in the livers of farmed *S. salar* fed such a diet.

The gonadal structure of wrasse also differed significantly from that of salmonids. The presence of asynchronic ovaries in wrasse allows the use of a multiple spawning strategy during the breeding season. Salmonids only spawn once per reproductive cycle and the gonadal development is, therefore, synchronic.

Other aspects of the morphology of wrasse were similar to those of *S. salar* which enables direct comparison, for example the structure and response to excess lipid of hepatocytes was similar in both groups.

Once the range of normal morphological features was established, it was possible to investigate the diseases which affect the four species of wrasse involved in the present study. Although some parasite species had been reported previously from the wrasse being studied, the parasite records were by no means comprehensive. This proved to be the case when a total of 24 new host records were discovered from the four wrasse species. These host records were spread throughout several different parasite genera. Most of the new host records were not unexpected and the absence of previous descriptions can be explained by the lack of interest in wrasse prior to this study. These parasites included *Trichodina* spp. which were recorded from all four wrasse species. They also included *C. lingua* which is a very common parasite of inshore fish species, and proved to be the most common parasite infecting all four wrasse species studied. Other parasite species were recorded from these labrids for the first time but were previously known from other labrids such as *L. bergylta*. The most significant of the new host records was the presence of the L3 stages of anisakine nematodes, *C. osculatum*, *H. aduncum* and *A. simplex* in *C. rupestris* and *C. exoletus*. *L. mixtus* and *S. melops* were

found to be infected with *H. aduncum* and *C. osculatum*, but not with *A. simplex*. These nematodes, in particular *A. simplex*, have been linked to severe parasite infections in humans, where ingestion of partly cooked or raw fish infected with these parasites caused acute gastrointestinal disease in the human host. Every method, no matter how indirect, by which these parasites enter fish destined for human consumption must be considered to be important.

The prevalence, abundance and intensity of parasite infections were seen to vary between different sample locations and, in most cases, these differences could be linked to the different geographical conditions acting on the individual sampling sites. There were other parasites such as the acanthocephalan *Corynosoma* sp. which were remarkably constant in the level of infection between different locations on the west of Scotland. Levels of infection were also seen to vary with the time spent in *S. salar* pens on farms. Infections with *Trichodina* spp. increased, in most cases, with an increased length of time spent in farm pens, but were not observed to cause pathology, while the levels of other parasites generally decreased, for example the adult digenean *M. alacris*. In most cases these decreases were thought to be due to the isolation of the wrasse from their natural diet and hence, the source of re-infection with the parasites. The level of long lived parasites (for example the L3 nematode larvae) was, on average, unaltered with time spent in pens. Conversely, the level of infection with parasitic copepods was drastically reduced in farm samples with no copepod crustaceans being found from any of the fish from farm samples. This was thought to be due to de-lousing treatments carried out on the farms. Even in pens stocked with wrasse the occasional de-lousing treatment may still be required.

The isolation of pathogenic bacterial strains, namely *Aeromonas salmonicida*, from wrasse in culture conditions suggests that infection with such pathogens could be a significant factor in their future use. The isolation of other, opportunistic, bacteria indicates that wrasse in culture conditions are generally quite susceptible to bacterial infections.

Despite testing two separate samples of wrasse, one from a wild location and one from a farm location, no virus was isolated from the wrasse.

Experimental cross infection of *S. salar* smolts with some of the most significant parasites of wrasse resulted in no cross infections taking place. The only parasite which was recovered from the intestine of the *S. salar* experimental hosts was *H. aduncum* but even this species did not establish well. The method used for the infection was very successful with no parasites being regurgitated post infection. However, the number of parasites used were small and ideally a larger dose would have been used if they had been available. These results would indicate that there is little risk of transfaunation of wrasse parasites to *S. salar* but the experiments should be repeated with larger experimental groups infected with more parasites. The nematode species *H. aduncum* and *A. simplex* have been reported previously from *S. salar* and therefore there is a very real risk of the S. salar acting as paratenic hosts to the parasites. It may be that changes in the physiology of the parasites to the internal environment of wrasse makes them unable to re-infect *S. salar*.

Experimental induction of Infectious Pancreatic Necrosis (IPN) in *C. rupestris* demonstrated conclusively for the first time that this species is susceptible to the virus which causes the disease in salmonids. However, the fish used showed a very good recovery ability, with almost total recovery by five weeks post infection. It was impossible to investigate the possibility that these fish were then carriers for the virus due to the low availability of a guaranteed disease free stock fish for experimental purposes. The pathology associated with the disease was shown to vary from that seen in salmonids. In particular, there was no evidence of the widespread enteritis which characterises the disease in salmonids. However, it was demonstrated that wrasse infected with IPNV shed the virus in their faeces. This is a major diagnostic criterion for the identification of carrier fish in other species. In addition, in wrasse the endocrine pancreas was seen to be affected, whereas, this tissue does not suffer from necrosis in salmonids affected by the disease. These results would indicate that while IPN may not play a role in wrasse mortalities under farm conditions, their movement away from sites which are known to suffer from IPN infections should be limited due to the possibility that wrasse may act as carriers for the disease.

In contrast to the success of the IPN infections Pancreas Disease (PD) could not be induced in *C. rupestris*. Using two different experimental doses, both of which would have induced the disease in *S. salar*, the wrasse infected did not develop any of the typical histopathological or gross clinical signs which characterise the disease in *S. salar*. This indicates that wrasse are not susceptible to infection with the virus which causes this disease and that PD will not play a role in wrasse mortalities under farm conditions.

6.2: The Future use of Wrasse

The use of wrasse to control sea lice infestations continues to be a major factor in the management programmes of major *S. salar* producing companies aimed at controlling infections with these parasites. Indeed, the largest *S. salar* producer in Britain, stocked wrasse in all of their smolt sites in 1995 and continue to regard this method of sea lice control as being effective (Treasurer Pers. Com.). It is extremely important that the use of wrasse forms an integral part of a varied control programme to prevent overdependence on any one method. The use of wrasse, however, brings with it a new set of requirements that have to be met by the *S. salar* producer. The small size of wrasse when compared to *S. salar* results in the prolonged use of small mesh sizes for nets. This results in increased labour costs as net fouling becomes more of a problem. Hides must also be provided in the pens to ensure that the wrasse have refuges, which is especially important during periods of low temperature or lowered salinity when the wrasse will suffer severely from stress. Indeed during these times it is probably advisable to remove the wrasse from the *S. salar* pens to a separate holding facility as free from stress as possible to reduce the possibility of infection with opportunistic pathogens. In addition to ensuring that the physical needs of wrasse are met the producer must also ensure that the health requirements of wrasse are met.

6.3: Potential Disease Problems to the Continued use of Wrasse

Wrasse posses radically different morphologies when compared to *S. salar* and these differences must be understood if the wrasse are to be maintained in a healthy

state. The review of the morphology of wrasse presented in chapter 2, which is the first of it's kind, should aid diagnosticians to monitor the health of the wrasse in their charge.

It would appear that, although wrasse are infected with many varied species of parasites in the wild, none of these parasites pose a threat to either the health of wrasse or *S. salar* under culture conditions. The species of parasites affecting wrasse seem to be incapable of transferring to *S. salar* even if wrasse were predated by *S. salar*. The greatest threat to the health of wrasse in culture conditions could be the microbial diseases of *S. salar*. Many of the bacterial conditions which caused heavy mortalities of *S. salar* during the formative years of the industry are now well controlled by the use vaccines and improved management practises. The occurrence of furunculosis is now far less than it was, due to the widespread use of vaccines against the disease. Despite this, large numbers of wrasse would appear to die from the disease under culture conditions (Treasurer & Cox 1991). The infection of wrasse with other, opportunistic bacteria must also be considered. These infections were common in the fish sampled from farm locations but were not seen in fish taken from the wild. Indeed, there has only been one documented bacterial isolation from wild wrasse. These infections are likely to be closely linked to the amount of stress suffered by the wrasse in farm pens and also physical trauma experienced by the wrasse. Net damage causes skin lesions which easily become infected, and the lack of a normal diet for wrasse in pens is likely to result in immunosupression due to poor diet. This would have the result that wrasse are less able to fight infections in pens than they would be in the wild.

One of the diseases which continues to be problematical in the production of *S. salar* is IPN. Outbreaks of the disease can occur in parr in fresh water production units

and adult fish in sea sites. The fish which survive infection become carriers of the virus and continue to shed infective viral particles in their faeces, eggs and seminal fluid. This shedding continues for a considerable time, hence, wrasse stocked with such carrier fish are liable to be exposed to water borne viral particles. Wrasse are susceptible to the virus, they also possess a very good recovery ability when infected with the virus. The role of wrasse as carriers for the disease is still not known due to a lack of fish with which to carry out the work. These results should be taken into consideration when considering the movement of wrasse between farm sites or holding facilities.

Pancreas disease is limited entirely to the sea water production stage of *S. salar* farming. Despite belief that the disease was viral in origin it is only recently that the virus which causes the disease has been identified. The disease is regarded by the industry as being a major concern to the production of *S. salar*. Due to the uncertain nature of the disease it is still not notifiable despite being found in many sea sites in Scotland; many of these sites will also be stocked with wrasse. This situation may change following the report that the virus responsible for the disease has been identified. From the work carried out for the present study it would appear that wrasse are not susceptible to PD and that this disease will not play a major role in the future use of wrasse.

6.4: The Need for Future Research

More work is required regarding the biology of the wrasse species used in the *S. salar* industry. If the present level of fishing for wrasse is sustained it is essential that we understand as much as possible about the population dynamics of wrasse. This may allow the setting of quotas for their capture to prevent overfishing of the stocks. This

information, if combined with parasitological surveys, would also help us to understand the relationships between the parasites affecting wrasse and the size and sex of individual fish.

Information on population dynamics, diet and parasite infections from different localities will aid in the elucidation of the diet of wrasse and how it may vary depending on geographical differences. This may also allow us to speculate on the life-cycles of some of the parasites such as *M. alacris* which have not, as yet, been fully elucidated. This information may also help us to understand how wrasse can acquire infections with nematode parasites which are normally limited to fish from offshore locations.

Investigation of the role of wrasse as prey items for predators is also deserving of further investigation. As one of the more common groups of inshore fish in British waters their role in the ecology of this environment would be better understood if their place in the food web of that environment was better known. This information would also help us to understand the role of wrasse in the life-cycles of some of the parasites recorded from them.

The risk of transfaunation of wrasse parasites to *S. salar* should be further investigated. In an industry where the aesthetic appeal of the final product is extremely important, the risk of any parasite infections must be carefully considered. The difficulty in the present study was to obtain sufficient numbers of parasites from wrasse to carry out cross infection experiments with large numbers of fish. Now that the parasites of wrasse are better understood these parasites could be obtained from other areas such as

from other fish species known to carry the same species of parasite. This method may allow the infection of *S. salar* smolts with significantly higher numbers of parasites.

Further research is also needed to investigate the possibility that wrasse may act as carriers for IPNV. This could be carried out by stress testing fish previously infected by the virus or by testing for the shedding of the virus in the faeces and seminal fluids of fish previously infected with the virus. The nature of the mechanisms responsible for the rapid recovery of wrasse from IPNV could be investigated further. This would require the testing for the production of specific antibodies to the virus. These techniques are now well established for other fish species and their adaptation to wrasse would not be difficult. These techniques could also be used to investigate how wrasse combat infection with the virus responsible for PD. Although no pathology was seen in fish infected with this virus, there may have been some cellular reaction to the presence with the production of antibodies.

It would appear that one of the most significant pathogens of wrasse under farm conditions is the bacterium *Aeromonas salmonicida*. Although some work has already been carried out on infections with this bacterium further research is merited. In particular trials using the commercially available vaccines against *Aeromonas salmonicida* could be carried out to investigate its protective properties for wrasse in farm conditions. This may significantly reduce the mortalities experienced in wrasse in farm pens.

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APPENDIX

Histological processing

1. Samples are allowed to fix in 10% formol saline for a minimum of twenty four hours.

2. Samples are then placed in tissue cassettes before being loaded into an automatic wax embedding machine which takes the tissues through a number of alcohol concentrations to remove any water before impregnating the tissues with paraffin wax. This process takes approximately twenty hours.

3. Tissues are then embedded in small wax blocks, these blocks are chilled on a cold plate to harden the wax.

4. A microtome is then used to trim in the blocks to expose the tissues to be sectioned.

5. The trimmed blocks are then placed in water at room temperature for approximately one hour before again being placed on a cold plate to chill the tissues.

6. The blocks are then sectioned on a microtome with three to four satisfactory 5 μ sections being removed.

7. The sectioned are floated in a water bath at approximately 40°C for a couple of minutes before being floated on to the surface of a clean microscope slide.

8. The slides are marked appropriately using a diamond marked before being dried on a hot plate for a minimum of thirty minutes.

9. After this period the slides are then placed in an oven at 60°C overnight

10. The slides are then stained using Haemotoxylin and Eosin stains by passing the slides through the following solutions for the time indicated:

I. Xylene	5 mins
II. 100% Alcohol	2 mins
III. 100% Alcohol	1 min
IV. 100% Methanol	1 min
V. Running tap water	2 mins
VI. Haemotoxylin	5 mins
VII. Running tap water	until clear
VIII. Scots Tap Water Solution.	1 min
IX. 50% Acid Alcohol	4 quick dips
X. Running tap water	wash well
XI. Eosin	5 mins
XII. Running tap water	Quick wash
XIII.100% Methanol	30 secs
XIV. 100% Alcohol	1 min
XV. 100% Alcohol	2½ min
XVI. Xylene	5 mins

11. From the last xylene change the slides are mounted with a coverslip using pertex as a mountant and sealant.

12. After several hours the slides can be cleaned and examined under the microscope.

Preparation of Medias for Bacteriology

All the medias are prepared in a 500ml autoclavable flask, the following ingredients are required for each media;

Tryptone Soya Agar (TSA) media.

1. 20g TSA.
2. 500ml distilled water.

TSA with salt media.

1. 10g sodium chloride.
2. 20g TSA.
3. 500ml distilled water.

Mueller Hinton media.

1. 0.5g 1-cysteine hydrochloride.
2. 19g Mueller Hinton agar.
3. 500ml distilled water.

Once all the components are added to the flasks they are autoclaved at 120 $lbin^2$ fro 15 minutes to sterilise the mixtures. The flasks are then allowed to cool until they reach a temperature at which they can be comfortably held. They are then poured into

sterile petri dishes in volumes of approximately 25ml before being allowed to cool at room temperature.

Bacteriological Methods

1. Gram Staining

I. Take a clean glass slide and, using a sterile loop, asepticaly place a loop full of sterile saline or distilled water onto the slide.

II. Remove a minute quantity of culture using a sterile loop and emulsify the culture in the liquid on the slide, and spread evenly.

III. Allow the slide to dry naturally.

IV. The dried slide is held in forceps with the film upwards and slowly passed through a Bunsen flame three times to fix the film. When the slide is just too hot to be borne on the back of the hand, fixation is complete.

V. The slide is allowed to cool and placed on the staining rack.

VI. Cover the slide with crystal violet solution and leave it on the slide for approximately one minute.

VII. Hold the slide at a steep slope and wash off the residual stain with iodine, then cover the slide with fresh iodine and leave for approximately one minute.

VIII. Tip off the iodine, holding the slide at a steep slope and pour an alcohol/acetone mixture over the slide from the upper end, so as to cover its whole surface.

IX. Repeat the washing with alcohol/acetone until no more colour runs off the slide freely.

X. Wash the slide with water.

XI. after shaking off the excess water flood the whole slide with a safranin counter stain and leave for approximately two minutes.

XII. Wash the slide thoroughly with water and then dry.

XIII. Carefully clean excess stain off the bottom of the slide.

XIV. Microscopically examine the stained slide under a x40 objective then under oil immersion with x100 objective.

Gram positive organisms - blue/purple cells

Gram negative organisms - red/pink cells

2. Motility test

I. Place soft paraffin or Vaseline on the four corners of a coverslip.

II. Place the coverslip, paraffin up, on the bench.

III. Sterilise a bacteriological loop and allow to cool.

IV. Pick up a loop full of sterile saline and place it on the coverslip. Re-sterilise the loop and pick up a minute amount of growth from an agar plate and gently emulsify the growth in the saline. Remember to sterilise the loop after use.

V. Gently lower a microscope slide onto the paraffin mounds without allowing the slide to touch the drop of culture.

VI. Quickly, but gently, invert the slide so that the drop is hanging from the coverslip.

VII. Carefully place the slide onto the microscope and rack down the x40 objective until it is just touching the coverslip. Take care not to depress the coverslip.

VIII. Rack up the objective slowly until the image is focused.

IX. Examine for evidence of bacterial motility.

X. The shape of the bacteria can also be determined at this point.

3. Oxidase Test

I. Place an "Oxidase strip" into a clean petri dish.

II. Sterilise a platinum wire and allow to cool.

III. Pick up a heavy inoculum of pure growth from a culture plate using the tip of the wire.

IV. Smear the inoculum over the area of filter paper containing the "Oxidase reagent".

V. Re-sterilise the wire.

VI. Observe the smear for up to thirty seconds and note any colour change.

Results.

A deep blue colour developing within thirty seconds indicates oxidation of the reagent and a positive result.

API 20E Microbial Identification Kit

Full instructions for the use of these kits are supplied with the kit as is a summary table of the results to aid in the interpretation of the results.