The pathology of chronic erosive dermatopathy in Murray cod, *Maccullochella peelii peelii* (Mitchell)

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Abstract

Chronic erosive dermatopathy (CED) is a disease of intensively farmed Murray cod in Australia that has been reported in association with the use of groundwater (mechanically extracted from shallow boreholes) supplies. CED results in focal ulceration of the skin overlying sensory canals of the head and flanks. Trials were conducted at an affected fish farm to study the development of the condition, both in Murray cod and in goldfish, and also to assess the reported recovery of lesions when affected fish were transferred to river water. Grossly, lesions began after 2–3 weeks with degeneration of tissue at the periphery of pores communicating with the sensory canals. Widening of these pores along the axis of the canals resulted from a loss of tissue covering the canal. Histopathologically, hyperplasia of the canal epithelial lining was seen after 3 weeks in borehole water and subsequent necrosis and sloughing of this tissue resulted in the loss of the canal roof. Canal regeneration occurred when fish were transferred from borehole water into river water. The lack of lesions in other organs and the pattern of lesion development support exposure to waterborne factors as the most likely aetiology.

Keywords: dermatopathy, heavy metal, lateral line, Murray cod, pathology, sensory canal.

Introduction

Expansion of intensive freshwater aquaculture in the Australian state of Victoria is restricted largely by the availability of suitable water resources. Consequently high-value warm water species have been cultured in temperature-controlled re-circulation systems, commonly supplied by relatively abundant sources of groundwater. Of the few species suited to the elevated stocking densities required to make these systems economically viable, the Murray cod, *Maccullochella peelii peelii* (Mitchell), is currently the most popular. As production of this species has intensified, several new diseases have emerged, one of which, known as chronic erosive dermatopathy (CED), has so far been recorded only in sites supplied by groundwater (unpublished data). Further evidence of this association with groundwater is the observed resolution of lesions when affected fish are transferred into river water (S. Noble, personal communication).

The full extent of CED within the Murray cod industry is as yet unknown but it has been recorded in at least two sites in Victoria (unpublished data) and is suspected at two more sites in neighbouring New South Wales (M. Landos, personal communication). Affected sites are reported to suffer not only increased mortality and reduced growth rates (B. Ingram, personal communication) but also a reduction in marketability, due to the severe disfigurement of affected fish.

This paper describes the pathological development of the condition and the changes that occurred in lesions when affected fish were transferred to river water.

Materials and methods

Two trials were run at a groundwater-supplied Murray cod farm located close to the Murray River at Rutherglen, Victoria, Australia.

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Development trial

Forty, 6-week-old Murray cod fingerlings, free from external lesions, were obtained from a supplier with no history of CED and maintained in groundwater within the normal, limited recirculation system of the site. Twenty young goldfish, *Carassius auratus* (L.), (age unknown) were obtained from a local retailer and also maintained in groundwater, again within the normal production system, providing a second species for comparison. Water for both these trials formed part of the farm's regular supply and was drawn from a 5-m deep borehole. Before use the water was exposed to low-level ozonation, heated to between 21 and 23 °C and degassed.

Recovery trial

Forty, 15-month-old Murray cod, exhibiting moderate gross signs of CED, were taken from the normal grow-out stock at the site and maintained in water drawn directly from the Murray River. Before use, this water was prophylactically treated with formalin (150 ppm) to reduce parasite loading and then aerated for 3 days. Complete water exchanges were carried out every 72 h.

Production fish

Two sets of samples were taken from the farm's on-growing stock that had been in the production system for 2 or 12 months to evaluate the progression of disease after longer periods in ground water.

Husbandry

Fish were kept in 1200 L black plastic tanks within a darkened and insulated shed. Light levels and feeding regimes were maintained as for normal production. Functioning biofilters were present in the recovery trial and production fish tanks but not in the development trial tanks. Husbandry conditions were maintained as uniform as possible for all three trial populations.

Sampling and pathology

Development trials commenced in April 2003 and five sets of samples were obtained at weekly intervals. Goldfish were sampled at 2 and 4 weeks. The recovery trial commenced in February 2003 and fish were sampled at weekly intervals for the first 4 weeks and then at 6, 8 and 10 weeks. In both trials week 0 sample groups formed the healthy and diseased baseline samples. Production fish were sampled in April 2003. Sample groups consisted of three specimens collected via the netting of free-swimming fish. These were killed by anaesthetic overdose using MS222. The abdomen of all specimens was opened and an operculum removed. Specimens were then placed in 10% neutral-buffered formalin for 2 days before being removed and wrapped in formalin-soaked swabs, prior to being airmailed to the Institute of Aquaculture, University of Stirling.

Upon receipt in Scotland all fish were inspected and any gross lesions recorded before internal organs were sampled from two fish in each group. In the recovery trial specimens, tissue samples included brain, oesophagus, semicircular canals, heart, liver, gut and pancreas, spleen, caudal kidney and the eyes and skin. Tissue samples containing the trunk sensory canals were obtained, in all fish, at five evenly spaced points between the caudal operculum and caudal peduncle. The development trial followed the recovery trial and only those organs in which pathology had been previously detected were sampled.

Skin samples were demineralized for 48 h (5:1 formalin:acetic acid) before processing. The remaining tissues were processed directly. Processing and embedding to paraffin wax were carried out routinely and after trimming, skin blocks were further decalcified (CellPath; CellPath PLC, Newton, UK) for 1 h. Sections were taken at 5 μm and following drying at 60 °C the majority were routinely stained with haematoxylin and eosin (H & E). A limited number were stained with periodic acid-Schiff (PAS) to highlight mucus-producing cells.

Results

The most dramatic and consistent gross lesions appeared as progressive erosion and ulceration of skin overlying the sensory canals. Ulceration of the membrane between the fin rays was also noted. In order to appreciate the development of the sensory canal lesions it was necessary to consider normal canal morphology.

Healthy Murray cod possessed a series of bilaterally symmetrical sensory canals on the head and a single, bilateral, sensory lateral line canal extending the entire length of the trunk. The exact structure of
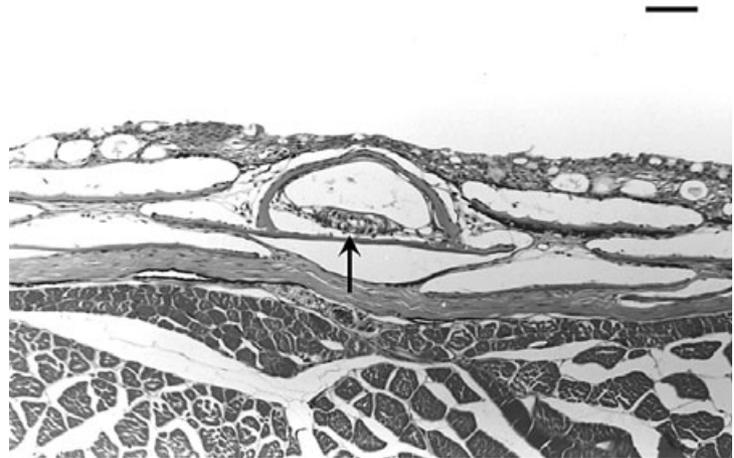
Figure 1 Cross-section of lateral line canal with neuromast (↑), in a healthy Murray cod (H&E, bar = 100 μm).

the lateral line canal has not been described in this species but it appeared to be a discontinuous arrangement of chambers each opening to the outside via a single pore. This was supported by the absence of canal structures in some cross-sections of healthy controls. As in other species, the two- to three-cell thick epithelial lining of the canal contained large numbers of mucous cells and regularly spaced neuromasts (Fig. 1) connected by an epithelial cord, four to five cells wide. By contrast, lateral line canals in goldfish were continuous, with a single pore on the posterior aspect of each scale (Bailey 1937), and with a lining three to seven cells thick (Fig. 2).

In addition to mucous cells, healthy Murray cod epidermis contained some PAS-positive cells whose cytoplasm contained a thread-like matrix and occasional visible nuclei. These far less abundant

cells were probably club cells, although this was not confirmed. Normal epidermis and superficial dermis also possessed multifocal accumulations of mononuclear cells, presumably lymphocytes, predominantly in the vicinity of blood vessels.

CED lesion development

Gross lesion development in the Murray cod followed a pattern similar to that described by Trott (1999). Approximately 2–3 weeks after exposure to groundwater, the pores that communicate with the sensory canals of the head and trunk started to enlarge, surrounded by narrow haloes of dermal pallor, the result of necrosis and waterlogging of the canal roof. Lesion development was generally bilaterally symmetrical. As the condition progressed, the pores elongated along the axis of the
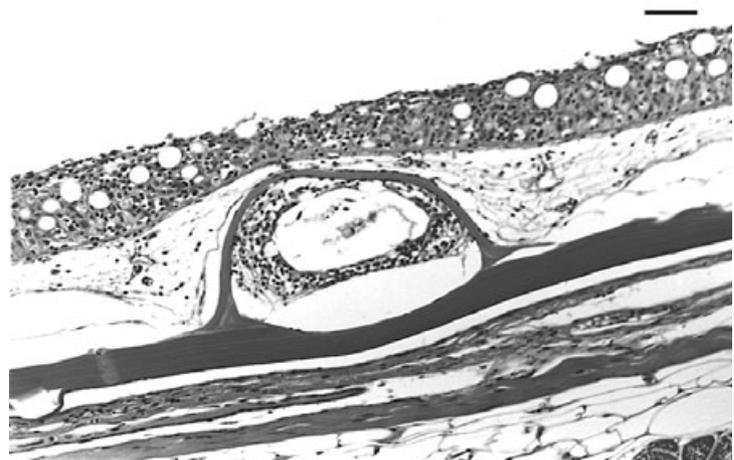

Figure 2 Cross-section of lateral line canal in a healthy goldfish (H&E, bar = 100 μm).

Figure 3 Gross view of head sensory canals and flank lateral line in a CED-affected Murray cod.

underlying canal, and at around 4–5 weeks, began to coalesce, exposing the bed of the canal. In the later stages of development, as seen in the 2- and 12-month production fish, the majority of the sensory canal beds were exposed, and on the head, ulceration of superficial tissue started to extend into surrounding areas (Fig. 3). Even in severe cases, however, small regions of apparently healthy skin were seen covering the canals.

In goldfish, after 2 weeks in groundwater, the normally pronounced canal outline was not visible in the thoracic region of some goldfish. After 4 weeks, cranial enlargement of the pores was seen, while those in caudal trunk regions remained normal.

Erosion of fins in Murray cod was noted grossly after approximately 2 months in groundwater and began at the distal fin tips, with erosion of the thin fin

membranes between rays (Fig. 4). By 12 months all fins were ragged, with only finger-like rays remaining. No fin lesions were seen in any of the goldfish.

Histopathology

In Murray cod the first changes were noted 3 weeks after exposure to groundwater as marked hyperplasia of the epithelium in all affected canals in the 3- and 4-week sample groups. This was frequently severe enough to occlude the canal lumen (Fig. 5). Necrosis of this hyperplastic tissue with an accompanying inflammatory response was present in several sections. This process involved both the underlying and overlying scale that formed the boundaries of the canal but seldom extended beyond this into the surrounding tissues (Fig. 6). By 5 weeks the tissue overlaying the canals was often completely necrotic and had sloughed in several sections (Fig. 7), although interestingly canal neuromasts in these sections showed no evidence of degeneration even when the bed of the canal was fully exposed. The same changes were generally apparent in goldfish, although hyperplasia appeared after only 2 weeks (Fig. 8) and continued with necrosis in the 4-week group. No loss of tissue covering the canals was observed within the trial period in goldfish. The lateral line nerve appeared normal in both species in all sections in which it was present.

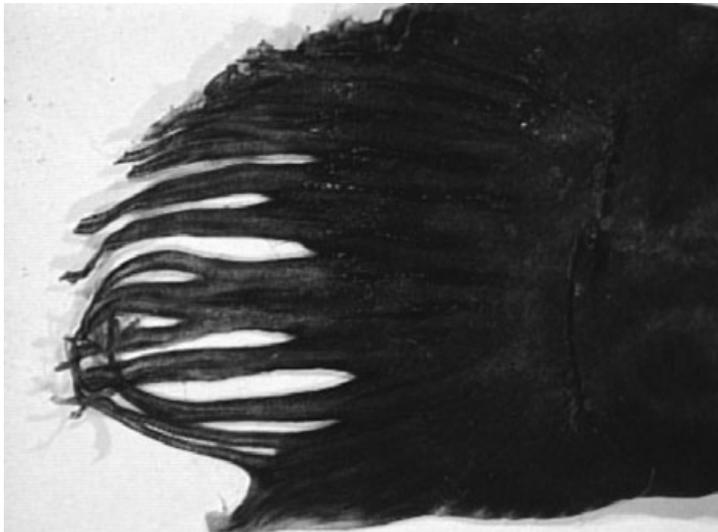Minimal pathological changes were noted in the skin away from the sensory canals although a

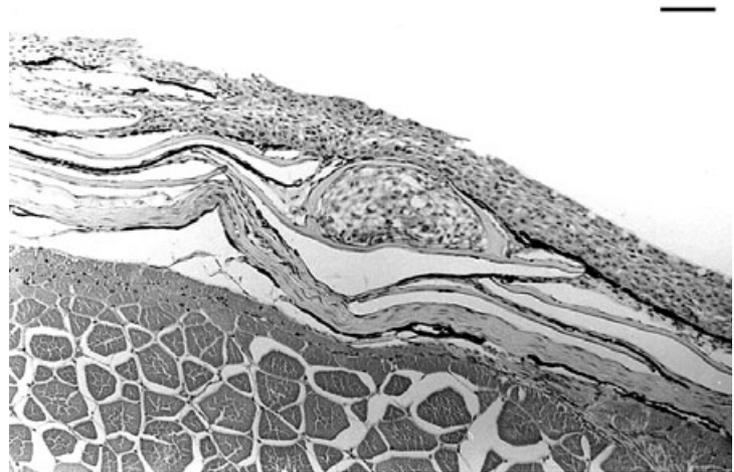**Figure 4** Fin erosion in a CED-affected Murray cod.

Figure 5 Cross-section of lateral line canal occluded by hyperplasia in a Murray cod after 3 weeks in the development trial (H&E, bar = 100 μm).
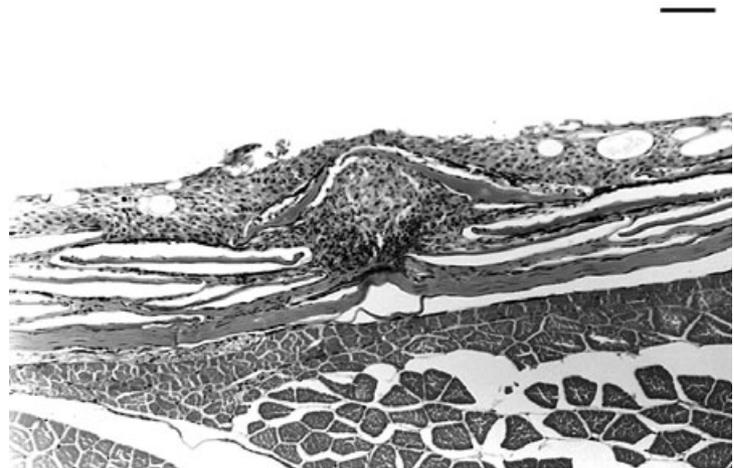

Figure 6 Cross-section of lateral line canal occluded with hyperplastic tissue and exhibiting necrosis of this tissue extending to the underlying scale in a Murray cod after 4 weeks in the development trial (H&E, bar = 50 μm).
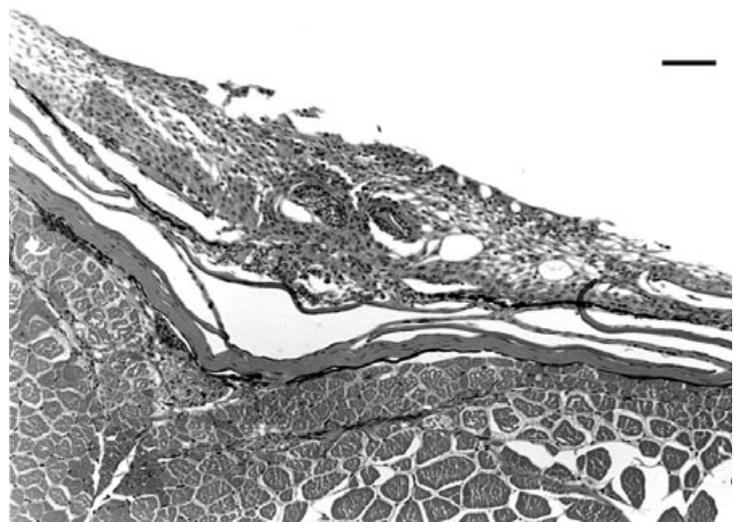

Figure 7 Cross-section of hyperplastic and necrotic lateral line canal with sloughing of superficial tissue in a Murray cod after 5 weeks in the development trial (H&E, bar = 100 μm).
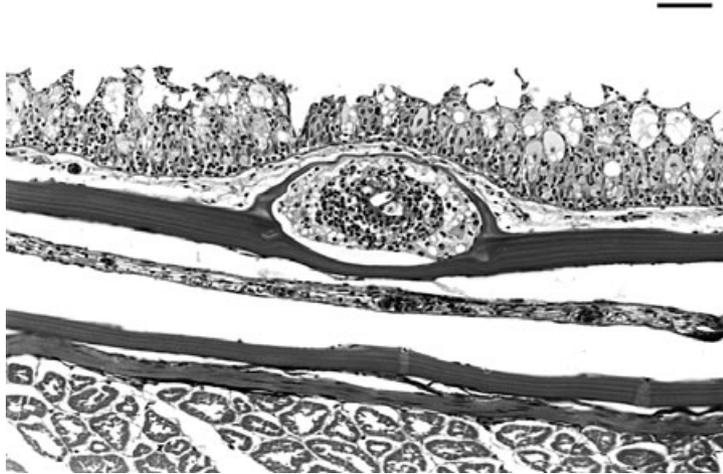
Figure 8 Cross-section of lateral line canal containing hyperplastic and necrotic epithelium in a goldfish after 2 weeks in the development trial (H&E, bar = 50 μm).
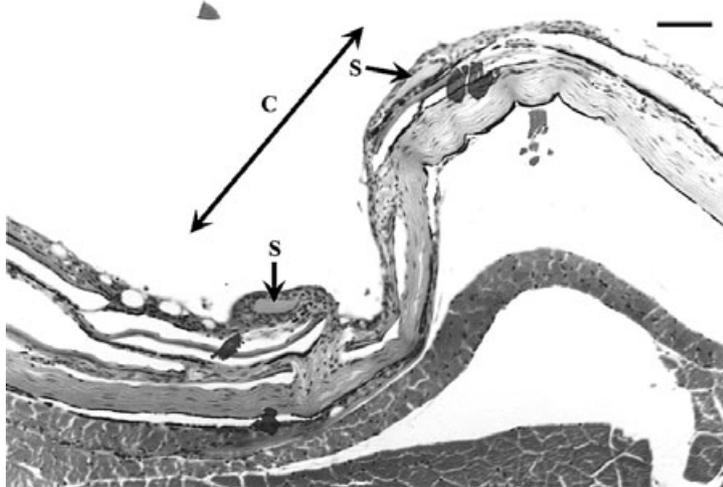
Figure 9 Cross-section of lateral line canal (C) showing absence of normal canal architecture and presence of scale fragments (S) in a Murray cod after 4 weeks in the development trial (H&E, bar = 50 μm).

generalized epidermitis, with inflammatory cell infiltration and necrosis, was apparent in 4- and 5-week development trial Murray cod and 4-week goldfish samples. Superficial neuromasts and taste buds appeared healthy in all Murray cod samples from the development trial. These structures, however, were vacuolated and degenerate in 2-week goldfish samples.

CED lesion recovery

In canal sections in which the overlying tissue had sloughed, normal canal architecture was lost and replaced by an elongated depression, lined with thin secretory epithelium containing a distinct central epithelial cord, with or without peripheral

scale fragments (Fig. 9). This appearance was considered to represent the very first stage of lesion recovery and was seen grossly as short, shallow and often pale channels in the region of the trunk sensory canal. Large, pale, active osteoblasts were commonly seen surrounding the scale fragments in these sections, and in subsequent sections crests formed at the edges of the thin canal epidermis (two to three cells thick compared with eight to nine cells thick in the surrounding epidermis). These crests comprised enlarging scale fragments, and in some cases, hyperplastic dermal fibroblasts. Consequently, the canal region acquired a more excavated appearance as the tissue crests of the sidewalls began to extend over the defect (Fig. 10). Closer examination of the enlarged tips of these
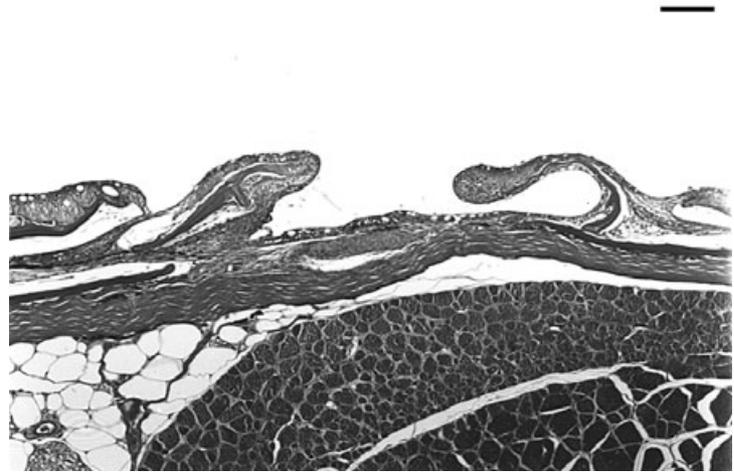
Figure 10 Cross-section of regenerating lateral line canal in a Murray cod after 2 months in the production system (H&E, bar = 100 μm).
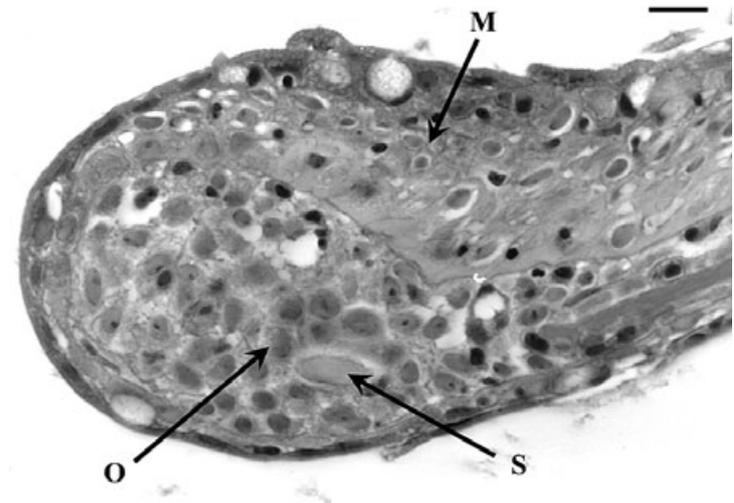

Figure 11 Crest of regenerating tissue at periphery of lateral line canal with scale fragments (S), active osteoblasts (O) and malpighian cells (M) (H&E, bar = 10 μm).

tissue crests revealed hyperplasia of active osteoblasts and malpighian cells (Fig. 11). Eventually, the regenerating dermal and epidermal tissue joined over the canal to fully enclose the lumen (Fig. 12). Early reformed canals were distinguishable from healthy canals by the presence of large active osteoblasts lining the scale of the newly fused canal roof. It is interesting to note that regeneration of parts of the canal was seen not only in the recovery trial but also to a limited degree in all the later stages of the development trial as well as in production fish. Furthermore, as with CED lesion development, there was much individual variation regarding rate and extent of recovery. However, by 8–10 weeks into the recovery trial, gross appearance and the number of fully or nearly formed

canal histological sections was sufficient to suggest that the majority of specimens were mostly structurally recovered.

One of the most striking results of this study was the lack of notable or consistent internal changes associated with CED-affected fish, despite the presence of marked external lesions. There was limited filamental necrosis in the gills of the 3-week developmental trial group. Ocular pathology, in the form of exophthalmia, was detected in a variety of CED-affected Murray cod but not goldfish. This, however, was also apparent in healthy Murray cod and is a common finding in Murray cod farms unaffected by CED at times of poor water quality and thus is not considered a specific component of this condition.
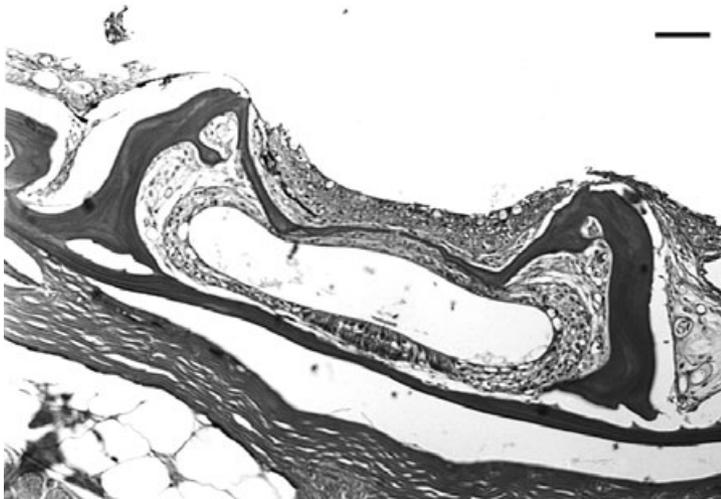
Figure 12 Cross-section of regenerated lateral line canal in a Murray cod after 10 weeks in the recovery trial (H&E, bar = 25 μm).

Discussion

As a result of this study it was possible to confirm that lesions in affected fish resolved when they were transferred to river water. The presence of extensive CED lesions in 2- and 12-month production fish suggests that significant recovery from CED did not occur whilst fish were maintained in groundwater. These fish effectively acted as a control for the gross lesions in the recovery trial. However, small regions of apparently normal lateral line tissue were noted grossly in these fish and limited canal regeneration was seen to occur histologically in damaged canals. From this study it was not possible to determine whether the intact lateral line regions were tissue that was unaffected by the condition or the result of full canal regeneration. Weekly sampling of production fish over an extended period would have provided more information on the regeneration process and its variation with time.

The development of lesions in groundwater and their subsequent resolution in river water suggested that some component of the groundwater in this region was responsible for the development of CED. Control groups of unaffected fish kept in river water alongside the development trial would have been useful to support this hypothesis, unfortunately this small production unit did not have enough free tank space for such a control. The restriction of significant lesions to the epidermis and dermis implied a locally acting agent rather than a systemic one. This was further reinforced by the absence of lesions within the semicircular canals, as these organs have the same basic structure as the sensory canals but are not connected to the external environment in teleosts (Lowenstein 1971) and they remained unaffected even in fish exhibiting severe external lesions.

In seeking a reason for the highly specific distribution of CED lesions it is proposed that some characteristic of the sensory canals facilitated the concentration or retention of a waterborne toxin. Past studies have documented the binding ability of body mucus for heavy metal contaminants (Handy & Eddy 1988). Literature regarding the luminal content of fish lateral line canals is scarce but Patt & Patt (1969) commented that it is filled with gelatinous or semi-gelatinous mucus secreted from goblet cells in the epithelial lining. It is likely that the mucus content of the sensory canals has a slower rate of turnover than elsewhere on the body due to canal structure and hydrodynamics, thereby increasing contact time with any toxin adsorbed by the mucus. The apparent discrepancy between the timings of gross lesion development and histological lesion development suggests that pathology of the lateral line canal does not develop at the same rate along its length, with the tissue around the pores being affected first. These pores provide a portal between the external environment and the mucus contents of the canal. Thus, a toxin diffusion gradient would be likely to form with the highest concentrations nearest the pores resulting in the tissues of these regions undergoing hyperplasia and necrosis first. This being the case the stage of canal hyperplasia and necrosis seen histologically would be dependent on the location of the section relative to the pores.

Furthermore, the mucus-trapping ability of the canal would be expected to be greater the more complete the overlying roof of tissue. In fact, if little or none of this roof tissue were present, mucus could be expected to be sloughed relatively rapidly, reducing the duration of exposure to any adsorbed toxin and allowing early stages of recovery in fish exhibiting even severe gross lesions. A previous study by Bailey (1937) demonstrated that following removal of a portion of the lateral line in goldfish, regeneration occurs anteriorly and posteriorly from remnants of canal lateral line epidermis. Work by Merrilees (1975) into the function of the raphe, a structure similar to the epithelial cord, in the walls of the semicircular canals suggested that it was important in maintaining canal morphology. In Merrilees' study sections of semicircular canals were implanted into scale pockets and in the majority of cases a channel or full canal formed on the regenerated scale. The work reported here would suggest that although marked loss of normal lateral line canal morphology can be present in CED cases, enough of the epithelial cord remains for subsequent canal regeneration to occur.

Gross changes noted in the fins of the production fish were not apparent throughout the development trial and although this may have been due to the relative brevity of the experimental period it may also be related to the higher stocking densities used in the normal production tanks. Further studies are needed to establish whether this unusual pattern of fin ulceration is in fact related to the CED condition.

Exposure to putative waterborne contaminants may be episodic rather than continuous, allowing recovery of tissue to occur between episodes. This latter hypothesis is supported by the appearance of normal canal tissue in even severely affected fish, diffuse epidermitis in two later sample groups and the results of several recordings of basic water quality parameters made during the study period (Baily 2003), which revealed moderate fluctuations in electrical conductivity within the groundwater, and reinforce the suggestion that the quality of the groundwater is not consistent.

There is little published work on the response of sensory canal structures to external toxins, although one study in marine teleosts (Gardner & LaRoche 1973) revealed 'swelling and increased cellularity' followed by necrosis in the canal epithelium after exposure for up to 21 days to raised external copper levels. Unfortunately the subsequent progression of these lesions was not described. No hepatic lesions were reported in exposed fish, as might be expected with heavy metal toxicity, although extensive damage of the superficial neuromasts was described. Further studies in marine and estuarine teleosts (Gardner 1975) investigated the responses of sensory and olfactory structures exposed to acute and sub acute levels of pesticides, heavy metals and petroleum products. Responses to heavy metals varied from nothing to severe necrosis of the sensory canal epithelium. The latter was also seen in fish exposed to pesticides. Exposure to petroleum products caused marked hyperplasia of the olfactory epithelium.

The only other report involved apparently identical ulceration of the sensory canals in marine cage-cultured mahi-mahi, *Coryphaena hippurus* L., in Australia (Langdon 1991). A range of staining techniques and bacterial culture of the canal contents yielded no pathogens, nor were viruses recovered from the lesions and internal organs. Repeated water analyses proved unremarkable except for one sample in which high levels of copper were detected. These reports suggest that a variety of compounds are capable of causing lesions in the epithelial lining of the sensory canals but it is also apparent that none of the subsequent changes can be described as pathognomonic.

In the present study, exposure of goldfish to the groundwater confirmed that the development of pathological changes in sensory canal structures is not unique to Murray cod. This has implications for the culture of alternative species on sites affected by CED. Differences in the timing of lesion onset and severity between species may be related to differences in canal structure and in particular to movement of material within the canal.

While the present study suggests that a waterborne factor is the probable cause of CED at this site, several heavy metal and pesticide/insecticide assays conducted on the groundwater since the condition was first observed have failed to identify any significant abnormality (Baily 2003). Small-scale experiments using carbon filters or chelating agents to treat borehole water supplies may help to confirm the presence of a waterborne toxin. Further water testing would still be required, however, to identify the exact causative agent(s) and in this regard, the use of peak fluctuations in electrical conductivity may allow improved timing in sample collection. Alternatively, the monitoring of a biological sentinel species such as daphnia may also allow more meaningful water samples to be taken.

Future difficulties in investigating the cause of this condition lie not only in the timing of sampling but also in the interpretation of results, given that several compounds may be responsible. It is particularly important to consider the potential for interaction between toxins. Pelgrom, Lamers, Haaijman, Balm, Lock & Wendelaar Bonga (1994) demonstrated that a synergistic effect can occur between a variety of heavy metals. This may result in enhanced toxicity from relatively small elevations of several metals making positive identification of the compounds responsible problematic.

Grossly the condition resembles 'head and lateral line erosion' as seen in ornamental species (Wildgoose 2001) and presents as bilaterally symmetrical ulceration and loss of the epidermis and dermis overlying the sensory canals of the head and trunk. As such, therefore, the term *erosive* dermatopathy is a misnomer and the condition should probably more correctly be described as chronic *ulcerative* dermatopathy.

Acknowledgements

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