The histopathology and ultrastructure of steatitis affecting common dab

**Limanda limanda**

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**ABSTRACT:** This study presents a new description, based on histopathological and ultrastructural studies, of a disease affecting the common dab **Limanda limanda** (L.). The condition can be recognised by the presence of multiple orange or yellow lesions in the pterygophorial region of the fish. The principal histopathological features are necrosis of fat cells, extensive macrophage infiltration leading to the formation of granulomatous structures, and the accumulation of lipopigment by lipid peroxidation. Based on this description, the condition has been diagnosed as steatitis. Although pathology associated with lipid peroxidation is the dominant characteristic of the lesions examined, it is proposed that this process is secondary to necrosis of the adipose tissue. The aetiology is discussed in the light of these observations. In addition, the first record of this condition affecting long rough dab **Hippoglossoides platessoides** (Fabricius) is made.

**KEY WORDS:** Steatitis · Fat cell necrosis · Macrophage infiltration · Lipid peroxidation · Common dab **Limanda limanda**

**INTRODUCTION**

A pathological condition affecting common dab **Limanda limanda** (L.) characterised by the presence of orange or yellow lesions has been recognised for at least 10 yr (A.H.M. unpubl. data) and has been described provisionally as a multiple hypodermal lipoma (Bruno et al. 1991). The condition is known to occur in dab populations from the Firth of Forth, Moray Firth and Orkney areas of the North Sea (Bruno et al. 1991). In a recent study, the overall prevalence on the east coast of Scotland was found to be 6.9% (Begg 1994). During a preliminary histopathological examination of this condition a number of additional observations were made that appeared to extend the description made by Bruno and co-workers. The present study reports the results of a histopathological and ultrastructural investigation that was conducted with the aim of clarifying the nature of this disease. In addition, the first record of this disease affecting long rough dab **Hippoglossoides platessoides** (Fabricius) is made.

**MATERIALS AND METHODS**

**Histology.** Tissue samples for light microscopy were collected from common dab from 3 sites on the east coast of Scotland: the St. Abb's sewage sludge disposal site (56°04.5' N, 02°07.25' W), an adjacent reference site (56°04' N, 02°02' W), and Burghead Bay in the Moray Firth (57°42' N, 03°31' W). Sampling took place between February 1990 and April 1992. In most cases, sampling of the tissue was typically carried out within 3 h after capture of the fish. Areas of affected tissue were removed and fixed in 10% buffered formal saline for at least 24 h. Fixed samples were decalcified in double strength Perenyi's fluid, processed, and paraf-
tin embedded. Sections were cut at 5 μm and stained with Harris's haematoxylin and eosin (H&E). Selected sections were also stained by periodic acid Schiff's (PAS), modified Ziehl-Neelsen, Schmorl's, sudan black B, Gram, and oil red O. The application of the oil red O technique to paraffin-embedded material identifies lipids that, through some modification, are resistant to extraction by ethanol during the processing associated with the preparation of paraffin sections. All staining techniques followed Bancroft & Stevens (1985). A total of 372 samples were examined.

During a survey of the disease conditions of common dab (Begg 1994), lesions similar to the ones of the common dab were incidentally observed in long rough dab. To confirm this observation, samples from 8 long rough dab were processed as above and examined histologically. Sections were stained with H&E only.

**Electron microscopy.** Live common dab were collected from Burghhead Bay, in the Moray Firth, Scotland, and Stonehaven Bay, Aberdeenshire, Scotland, and maintained in aquaria at the Fisheries Research Services Marine Laboratory, Aberdeen. The fish were killed and lesions were teased out from surrounding tissue under a binocular microscope. Small pieces of the lesions (ca 1 mm³) were immediately fixed in 2.5% gluteraldehyde in Millonig's buffer for 1 h at room temperature. Fixed samples were transferred to fresh buffer for 1 h at room temperature, followed by fresh buffer overnight at 4°C. The samples were post-fixed in 1% osmium tetroxide, dehydrated in graded ethanol and propylene oxide, and embedded in Agar 100 resin. Ultrathin sections were cut on a LKB ultramicrotome (LKB 8800 Ultratome III), stained with uranyl acetate and lead citrate, and examined under a Hitachi H-300 electron microscope.

**RESULTS**

**Gross appearance**

The lesions typically appeared as multiple yellow or orange spots in the pterygophorial region on the ventral surface of the fish. Some small lesions appeared white or cream. The lesions varied in size from minute speckles that were at the limit of resolution by unaided eye to those that involved the entire pterygophorial area. In some instances, the more extensive lesions were accompanied by melanisation of the skin and, in others, by haemorrhagic ulceration (Fig. 1). The lesions of the long rough dab were similar to those of the common dab.

**Light microscopy**

The lesions of the common dab exhibit necrosis of fat cells and foci of macrophages situated principally...
within the sub-dermal and intermuscular adipose tissue. In the smallest lesions the macrophages were situated within the interstitial areas of the adipose tissue. In the more extensive lesions, necrotic fat cells were replaced by large masses of macrophages. The macrophage nuclei were typically small with offset nucleoli. In some cases, the nuclei were pyknotic and there were areas in which the macrophages appeared necrotic (Fig. 2). The macrophage cytoplasm contained numerous granular and refractile inclusions, and occasionally melanin inclusions.

The intracellular granules and refractile droplets of the macrophages were identified as lipopigment, staining positively by sudan black B, modified Ziehl-Neelsen for acid-fast material, and oil red O methods, but negatively by Schmorl’s technique. The intracellular granules also reacted positively to PAS. Both types of inclusion exhibited autofluorescence when tissue smears were viewed under ultraviolet light with exciter filter BG12 and a barrier filter of 490 μm.

The macrophages showed varying degrees of organisation, including the formation of granuloma-type structures. Many of the granulomas consisted of a lacuna surrounded by 1 or several organised layers of cells and collagen (Fig. 3). The cells were predominantly of 1 type; they possessed granular cytoplasmic inclusions similar to those described from the free macrophages. This, in addition to their granuloma-forming behaviour, suggests that these cells were also macrophages. However, they differed from the free macrophages in that they did not possess refractile cytoplasmic inclusions, were not necrotic, and appeared to be active showing signs of pseudopodia. Other granulomas that took the form of enclosed macrophages surrounded by epithelioid type cells and collagen were also frequently observed (Fig. 3). The macrophages associated with these showed degrees of necrosis, which varied within and between granulomas. Necrosis was often greatest in the centres of the granulomas, while active macrophages were evident at the periphery. An intermediate morphology was also evident in some granulomas. These possessed lacunae of varying sizes positioned centrally with macrophages surrounding them. A third type of granuloma was also observed and distinguished from the others by the presence of a clear, colourless, or brown refractile material (Fig. 4). This material was identified as lightly oxidised lipopigment, exhibiting the same staining characteristics as the lightly oxidised lipopigment macrophage inclusions. The walls of these structures were almost exclusively composed of epithelioid-like cells and collagen.

A number of samples demonstrating extensive macrophage infiltration and granuloma formation also
Fig. 3. *Limanda limanda*. Cellular organisation in steatitis lesion showing characteristic granuloma morphology; lacunae surrounded by several layers of active macrophages (I) and encysted macrophages surrounded by epithelioid cells and collagen (II). H&E, scale bar = 100 µm.

Fig. 4. *Limanda limanda*. The third type of granuloma, showing lipopigment (P) encapsulated by epithelial type cells and collagen. Oil red O, scale bar = 100 µm.
exhibited necrosis of the surrounding muscle tissue, including the complete loss of muscle and its replacement by macrophages. In some instances, lesions also possessed areas of collagen deposition, including what appeared to be remnants of granulomas. These areas were devoid of fat cells and macrophages but could be found adjacent to 'active' lesions. Infiltration of lesions by cells other than macrophages, including lymphocytes and eosinophilic granular cells was observed. This was restricted to large lesions with extensive macrophage involvement and was frequently observed in areas of collagen deposition as described above.

Histologically, the lesions of the long rough dab demonstrate all of the features found in the common dab.

**Electron microscopy**

Electron microscopy demonstrated that the lesions were dominated by a cell type with a light and dark form (Fig. 5). The light cells possessed eccentrically positioned nuclei with peripheral and central heterochromatin (Fig. 6). The cytoplasm contained numerous electron-dense granules and membrane-bound droplets of variable electron-density some of which had electron-dense rings at their periphery (Fig. 6). The cytoplasmic granules were variable in size with heterogenous contents consisting of granular material, droplets of varying electron density, electron-dense membranous material, and electron-lucent fibrous areas. Similar material was also evident in membrane-bound structures that had an elongate, convoluted profile in cross section (Fig. 7). Mitochondria were visible in the majority of the light cells; they were swollen and the cristae were often indistinct, and where visible these were tubular (Fig. 7). Other cytoplasmic organelles were evident, including endoplasmic reticulum and golgi apparatus (Fig. 7). The remaining cytoplasmic material was electron-lucent, granular, and in general had a 'frothy' appearance. The cellular membrane was indistinct and cytoplasmic material was extruded from the surface of the cells, suggesting that they were necrotic.

The dark cells were similar to the light cells in size and shape. Their nuclei were more electron-dense within the interchromatin area than those of the light cells, as was the cytoplasmic material which gave rise to the light/dark dichotomy (Fig. 8). In all other respects these cells appeared to be similar to the light cells and again appeared necrotic.

It is clear from the numerical dominance of the light and dark cells that they correspond to cells that were identified by light microscopy as macrophages. The morphological characteristics observed by electron microscopy were consistent with this classification. These included the presence of granules and lipid droplets which were evident as granular lipopigment and refractile inclusions at the light microscope level.

Certain features consistent with the granulomatous structures described by the light microscopy were also identified by electron microscopy. These included the
Fig. 6. *Limanda limanda*. Necrotic light macrophages with nucleus (N), cytoplasmic granules (arrows) and lipid like droplets (L), one of which has an electron-dense perimeter. Scale bar = 2 μm.

DISCUSSION

In their description of a disease condition affecting common dab, Bruno et al. (1991) noted its necrotic and inflammatory nature and described a proliferation of 'encapsulated, greatly distended fat cells'. Based on these findings the condition was described as lipoma (Bruno et al. 1991). In the present study, observations of a larger number of samples of the same condition have shown the principal histopathological characteristics to be: fat cell necrosis, inflammation dominated by macrophages leading to granuloma formation, and the accumulation of lipopigment. These observations are not consistent with a diagnosis of lipoma and it is believed that the 'distended fat cells' described by Bruno et al. (1991) were, in fact, structures similar to the lipopigment-containing granulomas described in the present study. In response to these results we suggest that the term steatitis be adopted to describe the condition. The use of this term, which simply means inflammation of fat tissue, reflects the central role of macrophage infiltration in the pathology, without implying an aetiology.
Fig. 7. *Limanda limanda*. Cytoplasm of light macrophage demonstrating individually membrane-bound elongate-convoluted structures (stars), golgi apparatus (black arrowhead), rough endoplasmic reticulum (white arrowhead), and mitochondria (white arrows). Scale bar = 1 μm

Fig. 8. *Limanda limanda*. Dark macrophage with eccentrically positioned nucleus (arrows), cytoplasmic granules (stars) and electron-dense granular cytoplasm. Scale bar = 2 μm
not observed by light microscopy and consequently could not be identified as lipopigment. However, similar features in atherosclerotic plaques have been identified as lipopigment (Mitchison et al. 1990).

The consensus has developed that lipopigments are the product of lipid peroxidation and the co-polymerisation of proteins (Pearse 1985, Jolly & Dalefield 1990). More recent evidence has shown that, in a group of conditions known as the ceroid-lipofuscinoses, the accumulation of lipopigment may be due to an abnormality in the storage and breakdown of proteins (Jolly & Dalefield 1990). However, as macrophages are known to have the capacity to produce lipopigments by lipid peroxidation (Maeba et al. 1990), their extensive involvement in the pathology of this condition is consistent with lipid peroxidation having taken place. The extensive necrosis of cells and the ready availability of lipids on which the oxidative process may act are further evidence in favour of lipid peroxidation.

Once lipid peroxidation has been initiated, the process is likely to accelerate as a result of the production of organic peroxides, the liberation of lipids, free radicals and enzymes from fat cells, and the release of free radicals from necrotic macrophages. This provides the conditions necessary for the extracellular oxidation of lipids and is responsible for the production of extracellular lipopigments.

The formation of granulomas in association with the accumulation of lipopigments is known to occur, and ceroid granulomas have been described from a variety of tissues (e.g. uterine ceroid granuloma, Al Nafussi et al. 1992; oral ceroid granuloma, Triantafyllou 1996). In the present study, the examination of a large number of lesions led to the observation of a variety of granulomatous structures. Those exhibiting 'healthy', activated macrophages surrounding a lacuna appear to be a response to extracellular lipid, the lipid having been extracted during the tissue-processing procedure. In other granulomas similarly healthy macrophages were evident at the periphery but the granulomas were filled, entirely in some cases, by macrophages at varying stages of necrosis, which are likely to have been the stimulus for the formation of the granulomas. The necrosis of the encysted macrophages would lead to the release of lipopigment; this is consistent with the
of cell types such as lymphocytes and eosinophilic granular cells was observed. These cell types were also apparent in areas of lesion demonstrating 'scarring', in which the loss of fat cells and macrophages left behind a matrix of collagen. These observations suggest that recovery from the lesions may take place and that lymphocytes and eosinophilic granular cells may be involved in the recovery process. However, the regeneration of adipose tissue was not observed.

The products of the lipid peroxidation process constitute the grossly observable lesion and, consequently, all of the samples examined were of lesions that had entered this stage. As a result it is difficult to establish the sequence of pathological events and in particular the mechanism responsible for initiating the lipid peroxidation process.

An analysis of the livers of common dab from the Firth of Forth sites found the concentration of certain insecticide contaminants (hexachlorocyclohexane, a-chlordane and dieldrin) were high in a UK context (Begg 1994). It is known that exposure to xenobiotics can induce oxidative stress in fish and cause lipid peroxidation (Kelly et al. 1998) and lipid peroxidation can result specifically from exposure to dieldrin (Bachkowski et al. 1998), chlordane (Hasoun et al. 1993), and hexachlorocyclohexane (Barros et al. 1991). However, an extensive literature search revealed no studies to indicate the tissue concentration at which induction of oxidative stress takes place in common dab or other fish species and, consequently, the significance of these exposure levels to the development of this condition is unknown.

Oxidative stress can also be induced by a dietary imbalance, usually excess polyunsaturated fatty acids and insufficient vitamin E (Farwer et al. 1994, De Gritz & Rahko 1995). The resulting pathology, nutritional steatitis, demonstrates histomorphological characteristics identical to those observed in the present study.
and is common in cultured fish (Herman & Kircheis 1985, Wada et al. 1991, Guarda et al. 1997) and other domestic animals (Brooks et al. 1985, Verschuren et al. 1990). However, there is little overlap in the diets of common and long rough dab (Ntiba & Harding 1993), which provides some indication against a dietary role in aetiology of steatitis in these species.

Further evidence against a nutritional aetiology comes from the fact that, in cases of nutritional steatitis, lipopigment may be observed within fat cells prior to inflammation (Jolly & Dalefield 1990). Initiation of lipid peroxidation within the fat cells might also be anticipated as a consequence of xenobiotic-induced oxidative stress, especially if the xenobiotics are lipophilic and assimilated within fat cells. Despite examining a large number of samples, peroxidation in the fat cells was not seen in the present study. This observation suggests that steatitis in common dab is initiated by the necrosis of fat cells with macrophages responding, at least initially, to the constituents of the necrotic fat cells, including lipid. Microbial infection of fat cells has been shown to cause lipid peroxidation and steatitis as a consequence of fat cell necrosis (Hashimoto 1985, Price et al. 1990). Although no infectious agents were detected within the lesions of the common dab, infection of the fat cells prior to the lesion development can not be discounted.

In attempting to establish the aetiology of steatitis in common and long rough dab, efforts should be concentrated on determining the cause of fat cell necrosis, including the possibility of microbial infection and an impact of environmental contaminants.

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LITERATURE CITED

Pearse AEG (1985) Histochemistry theoretical and applied,
Verschuren PM, Houtsmuller UMT, Zevenbergen JL (1990) Evaluation of vitamin E requirement and food palatability in rabbits fed a purified diet with a high fish oil content. Lab Anim 24:164–171

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