Vol. 2: 197–204, 1987 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published July 30

Gill x-cell lesions of dab *Limanda limanda* in the North Sea

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ABSTRACT: Up to 5% of dab *Limanda limanda* L. in catches of a research vessel on the Dogger Bank (North Sea) had gills showing the pathological changes of marked swelling and pale colouration. The condition in catches in other parts of the southern North Sea showed a lower prevalence. A light and electron microscope examination showed the lesions to be due to an intra-epidermal accumulation of x-cells. The structure of these cells was atypical of normal dab cells so an infectious aetiology may be indicated. There was evidence of cell motility in peripheral parts of lesions. Variability in structure of x-cells was considered to be mainly associated with degenerative changes, particularly with cells in the centre of lesions. Envelope cells which characteristically encapsulated individual x-cells had features suggesting an epithelial origin. A cellular reaction involving macrophages containing x-cell remnants in phagocytic vacuoles was typical of areas of extensive x-cell degeneration.

INTRODUCTION

Recent investigations into diseases of fish in the North Sea have revealed that dab *Limanda limanda* L. can be affected by a number of external and internal diseases. These include *Lymphocystis,* epidermal hyperplasia or papilloma, skin ulcerations, and hepatosplenic changes (Möller 1979, 1981, Dethlefsen 1980, 1984, Dethlefsen & Watermann 1980, Bucke et al. 1984, Dethlefsen et al. 1984, Wolthaus 1984). During these investigations it had been observed that gills of certain dabs were pale and swollen. The opportunity was taken of collecting material for a study into the pathology of this condition while the authors were participating in an ICES sampling program on board the RV *Anton Dohrn* in January 1984. The study was designed to provide the basis for further investigation into the epidemiology of the condition in the southern North Sea (Knust & Dethlefsen 1986), Scottish waters (Diamant & McVicar in press), and English waters (Bucke unpubl. obs.) and was undertaken because it was possible that the condition might prove useful as an indicator of environmental quality.

MATERIALS AND METHODS

Positions sampled were in the North Sea south of 56 °N and are shown in Fig. 1. A total of 4958 dabs *Limanda limanda* L. were sampled from 21 bottom trawl catches (Table 1). Both opercula from each dab were opened and individual gills examined without magnification for abnormal changes such as oedema and pale or patchy colouration. Where the gills showed an even, bright red colouration they were considered normal.

Samples of both apparently normal and abnormal gills were taken for histological examination by fixing in 8% formol saline. They were processed to paraffin wax sections and stained with haematoxylin and eosin for general morphology, alcian blue/PAS for mucopolysaccharides, or Feulgen's method for nucleic acid. Alternatively, tissues were embedded in LR white acrylic resin (London Resin Co., Basingstoke, U.K.) and 1 µm sections were stained with 1% aqueous toluidine blue in 1% borax. For electron microscopy, samples were fixed in 2.5% glutaraldehyde in Millonig's buffer, post-fixed in 1% osmium tetroxide, and embedded in

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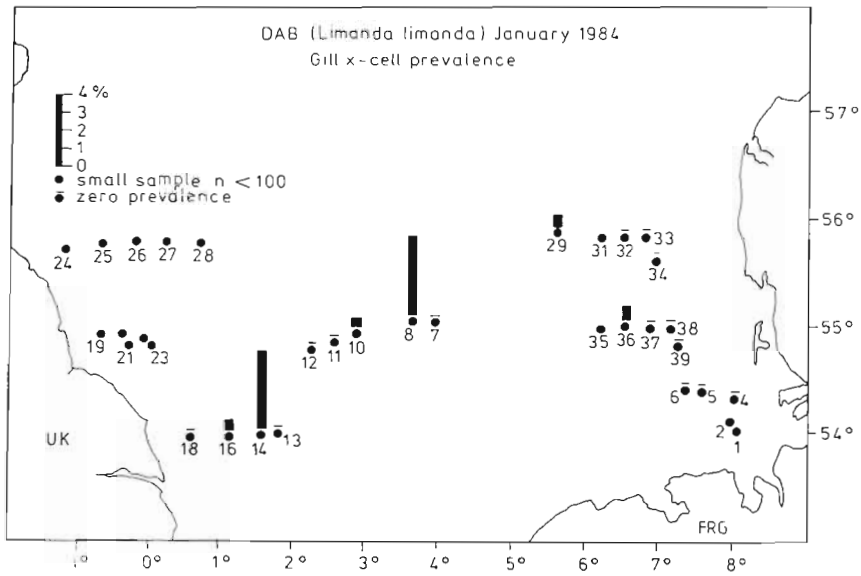


Fig. 1 The North Sea showing sampling positions and prevalence of dabs with gill x-cell lesions at each

Agar 100 resin after dehydration in either ethanol or acetone. Survey sections were stained in 1 % methylene blue in 1 % borax and ultrathin sections were examined on a Hitachi H300 electron microscope after staining with uranyl acetate and lead citrate.

RESULTS

Distribution

The prevalence of dab with x-cell gill lesions varied considerably between hauls, even those in close proximity to each other, and reached a maximum of 4 to 5 % in the southwestern part of the Dogger Bank (Fig. 1; Table 1). In areas sampled to the northwest and southeast only occasional dabs with gill lesions were observed. No obvious correlation could be made with other diseases (*Lymphocystis,* skin hyperplasia, skin ulcers) recorded on dab during the cruise, but the sample size was too small to permit extensive analysis.

Clinical signs

Gills of affected dab were thicker (swollen) and appeared white to creamy yellow (Fig. 2). Lesions appeared to be randomly situated on both dorsal and ventral gills. Often the colouration was patchy with a variable proportion (from < 10 % up to 100 %) of the lamellae affected. Occasionally, the swelling led to fusion of the secondary lamellae, but in most cases swelling was only apparent at the base between 2 lamellae extending half-way up the secondary lamellae. The ends of the primary lamellae were sometimes so swollen that they were club-like in appearance. In extreme cases the lesion was sufficiently severe to prevent the operculum from closing; in such cases, the gill filaments were exposed. Gills from apparently normal dab were uniformly bright red in colour.

Light microscopy

In histological sections some of the gill filaments had marked degrees of cellular proliferation, which under low magnification gave the impression of a hyperplastic condition (contrast normal gill in Fig. 3 with that of an affected gill in Fig. 4). Under high magnification, masses of proliferating cells extending from the basal lamina outwards, sometimes extending along the whole length of the lamellae, were observed. These cell masses were enclosed within the gill epithelium. The lesions were composed of 2 cell types:

(1) The predominant cell occurred in different forms ranging from proliferating to highly degenerate. Each cell had a centrally-located nucleus which contained a prominent nucleolus (Fig. 5). The cytoplasm of these cells stained weakly with eosin; there was no reaction to alcian blue but a weak PAS reaction demonstrated the presence of neutral mucopolysaccharides. These cells appeared similar to the x-cells described by a number of workers from various species of marine fish (reviewed by Desser & Khan 1982) and are subsequently referred to as such. There was no evidence of pathological change in areas of gill which did not have the proliferations. Sections of gills from apparently normal dab rarely showed evidence of proliferative cell masses.

(2) Accompanying cells were interspersed between the x-cells and were visible only as a large nucleus with sparse surrounding cytoplasm.

Ultrastructure of gill lesions

The severity of lesions and the frequency of occurrence of abnormal cells varied considerably in different

parts of the gills of fish examined. In severely affected areas, where the secondary filaments were obliterated by the accumulation of lesion cells, the gill tissues were still recognisable and the cells making up the gill tissue showed their normal ultrastructural features (Fig. 6). Although the epithelium in such areas was displaced outwards, there was no evidence of disruption of the respiratory surface layer or of constriction of the blood flow. Two cell types predominated within lesions: large x-cells and interspersed enveloping cells. Because of the similarity of the x-cells to those described by other authors, only those features of particular significance to the condition in dabs are considered in detail here.

A distinction could be made between x-cells located within large accumulations between secondary filaments and those occurring in isolation. When x-cells were located singly in the intercellular space between the respiratory epithelium and the lamellar capillary, or in small groups, they often showed extreme variations in shape with the external membrane being deeply indented or thrown into pseudopodial-like extensions. Such cells were either totally free of enveloping cells or only partially enclosed (Fig. 7). Cytoplasmic inclusions bodies were numerous and variable in structure; they occurred as dense homogeneous vesicles, vesicles with an internal halo and ring configuration, or as complex vesicles containing 2 or more rings. Although some showed superficial resemblance to viral particles, the irregularity in their size and appearance made it unlikely that they were viruses. Bundles of microtubules were occasionally observed within the cytoplasm.

Table 1. *Limanda limanda*. Epidemiological data on numbers of dab investigated. Disease prevalences and sex ratio for Cruise 131 of RV *Anton Dohrn* 3 to 12 Jan 1984

Station No.	No. dab examined	No. dab with gill x-cells	Range in lengths of healthy dab (cm)	Range in lengths of dab with x-cells (cm)	Sex ratio of dab with x-cells		Sex ratio of healthy dab	
					Male	Female	Male	Female
1	•							
2	•							
4	236						127	109
5	269						171	98
6	368						194	174
7	197						97	100
8	816	35	9–32	12–18	21	14	301	480
10	258	1	10–31	17	1		58	199
11	183						44	139
12	293						82	211
13	172						83	89
14	435	19	8–26	9–17	13	6	261	155
16	180	1	9–22	12		1	59	120
18	143						84	59
19	•							
20	•							
21	•							
22	•							
24	•							
25	•							
26	•							
27	•							
28	•							
29	165	1	12–29	18	1		74	90
31	•							
32	215						67	148
33	136						45	91
34	169						65	104
35	52	2	11–25	10+14		2	24	26
36	150	1	11–34	20		1	71	78
37	200						91	109
38	197						93	104
39	124						33	91

• Less than 100 dabs caught and not examined for x-cell lesions

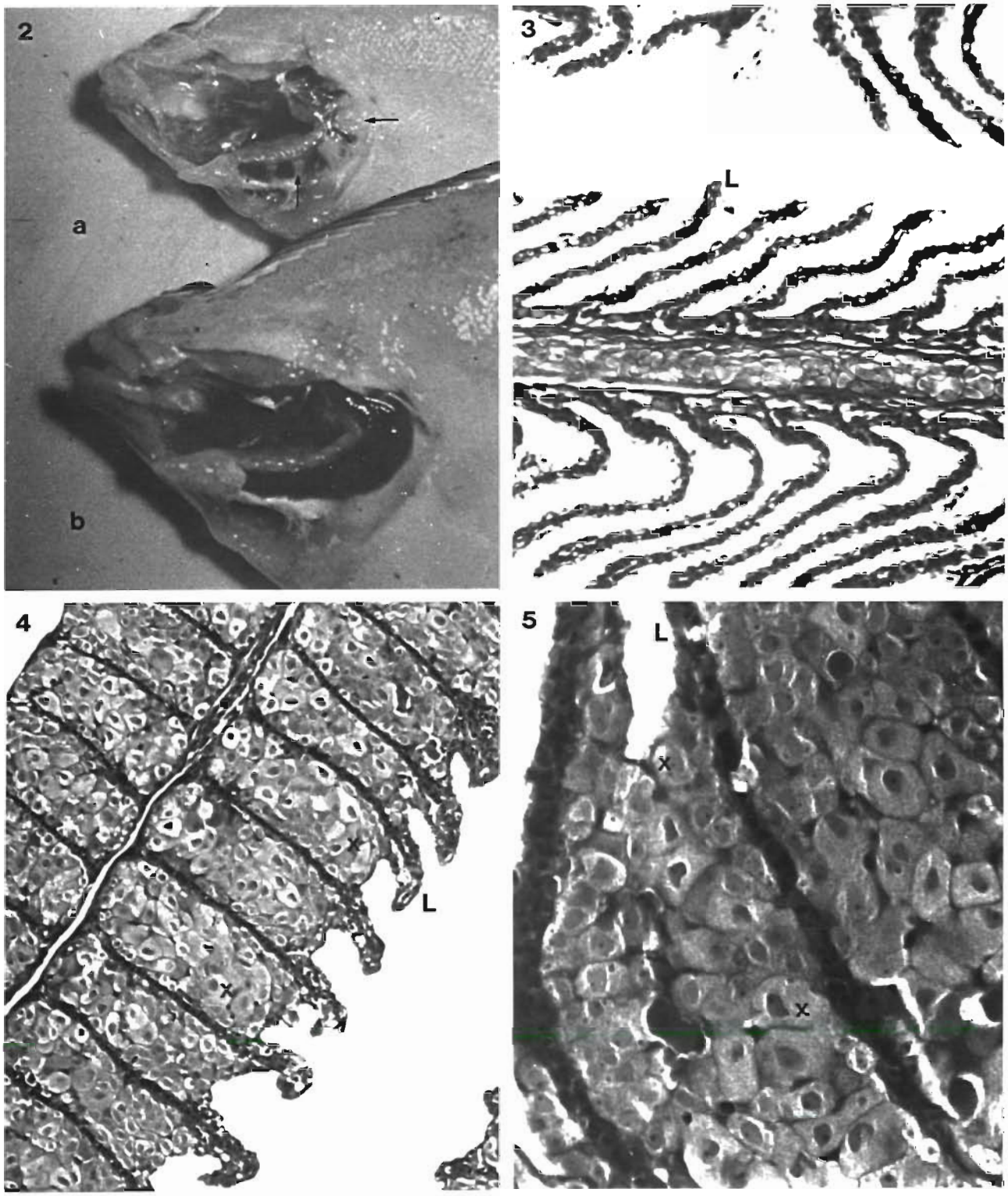


Fig. 2 to 5. *Limanda limanda* gills. Fig. 2. Dabs with the operculum cut away to show the gills. (a) Affected fish with pale swollen areas on the gills (arrowed). (b) Normal fish with dark-coloured gills. Fig. 3 to 5. Light microscopy of gill x-cell lesions. Fig. 3. Low magnification showing lamellar structure (L) of normal dab gill. H & E × 80. Fig. 4. Low magnification of affected dab gill showing proliferation of x-cells (x) between lamellae (L). H & E × 80. Fig. 5. Higher magnification of affected dab gill showing individual x-cells (x) with centrally located nuclei and prominant nucleoli. H & E × 800

Within large lesions each x-cell was more or less spherical and encapsulated by flange-like processes of several enveloping cells (never one) (Fig. 8). Extensive interdigitation or, more frequently, desmosome junctions formed at the meeting points of different enveloping cells, but no such intimate contact was observed between envelope and x-cells. Similarly, in the infrequent areas of direct contact between x-cells, where adjacent areas were not separated by enveloping cells, there were no such junctions.

To a large extent the ultrastructure of the majority of dab x-cells in large lesions showed homogeneity. Lipid droplets and small electron-dense inclusion bodies were randomly scattered throughout the cytoplasm, parallel arrays of endoplasmic reticulum (possibly Golgi) were common and a single spherical granular area, with or without a clear central zone, was frequently present (Fig. 8). Additional features of x-cells clearly distinguishing them from normal dab gill cells were the prominent outer membrane, the spherical mitochondria with sparse tubular cristae, and the nuclei with particularly prominent and numerous nuclear pores and dense nucleoli. Enveloping cells did not show similar structures; mitochondrial cristae were in the form of plates, tonofilaments were often prominent, myelin figures were occasionally present, and, in the nucleus, chromatin was well dispersed marginally and in patches usually without a prominent nucleolus. There was no deposition of collagen in the vicinity of enveloping cells.

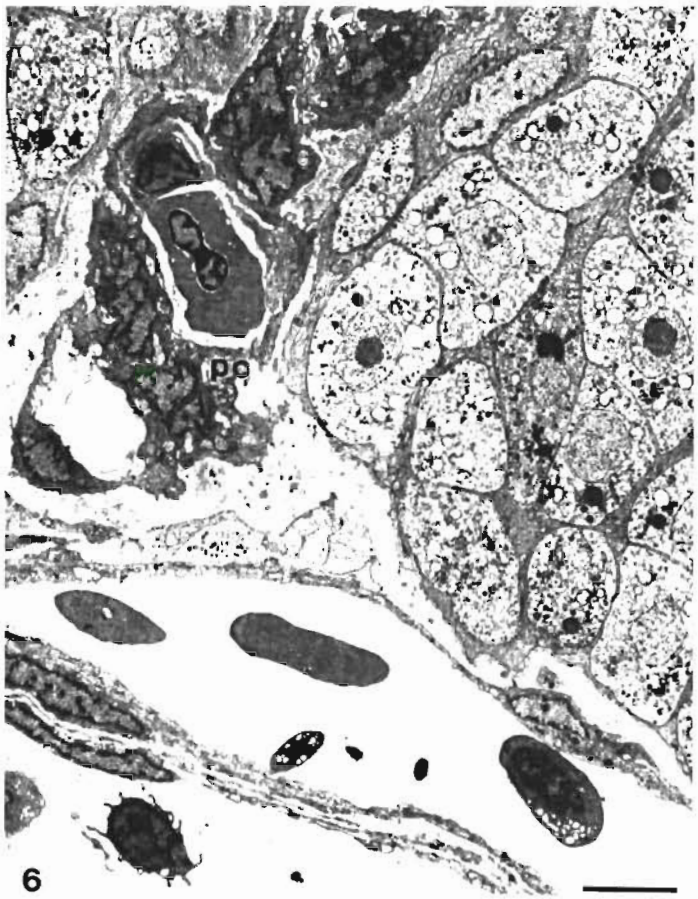
The variability observed in dab x-cells in the present material was not sufficient to classify them into types and was mainly associated with degenerative changes. This was the case particularly in central areas of large lesions where changes included a decrease in cytoplasmic density often accompanied by extensive vacuolation, a reduction in the number of inclusion bodies, irregularity of nuclear shape, and occasionally loss of the outer cell membrane. In these areas only x-cells appeared to be degenerate. Macrophages, with phagocytic vacuoles engorged with degenerative material (occasionally identifiable as remnants of x-cells), frequently occurred within necrotic areas and were sometimes found in direct contact with particularly degenerate x-cells (Fig. 9).

There was no evidence in nuclei of active division of x-cells although the close association of some cells without intrusion of enveloping cells between them (Fig. 9) suggested recent division. Similarly, the common arrangement of x-cells into lines or chains of closely associated cells (Fig. 6) suggested sequential proliferation of cells, although this might have reflected only the linear structural nature of the encompassing secondary gill filaments.

DISCUSSION

The gross morphology of cell structure of the gill lesions in dab was similar to the gill lesions in Chilean hake *Merluccius gayi gayi* (Gorgollon et al. 1982) and eelpout *Lycodes lavalaei* (Desser & Khan 1982). The principal cell types found in these gill conditions closely resembled the x-cells found in epidermal papillomas of pleuronectids (McArn et al. 1968, Brooks et al. 1969, Wellings et al. 1976) and in pseudobranchial tumours in gadoids (McCain et al. 1979, Egidius et al. 1981, Morrison et al. 1982, Watermann & Dethlefsen 1982) from a variety of locations, indicating the possibility of a common cause of the different lesions. It is interesting to note that there may be further links between these conditions, as McCain et al. (1979) described a tumour in a cod gill as having an identical structure to that of pseudobranchial tumours in that species. In addition, as Alpers et al. (1977) point out, the epithelium of the pseudobranch, gill and skin is all developmentally derived from ectoderm. It is possible that dab gill x-cell lesions represent a tissue-specific response to another disease but from the field data available in this study it has not been possible to establish any relationship between the gill lesions and other proliferative cell disease conditions which have been found in dab (Dethlefsen et al. 1986).

The histological features of the dab gill lesions were represented by massive proliferation of 2 cell types (x-cells and enveloping cells). The ultrastructure of dab gill x-cells shows close similarity, whenever detailed descriptions are available, with x-cells from several different fish species (Ito et al. 1976, Wellings et al. 1976, Alpers et al. 1977, Desser & Khan 1982, Watermann 1982). This remarkable structural uniformity of x-cells suggests these cells have a common origin wherever they have been found, but there is considerable controversy in the literature as to what the x-cell represents. Alpers et al. (1977) summarised the most likely possibilities: x-cells are unicellular organisms that induce xenomas; they are neoplastic cells that make up endocrine tumours in the pseudobranch; or they are virally transformed cells. Evidence has been presented by various authors supporting each of these possibilities (reviewed by Desser & Khan 1982, Watermann 1982, Peters et al. 1983) but thus far firm conclusions are not possible. Reasons for this are numerous but include principally (1) the uniformity of structure of x-cells found and the lack of cell forms that lead to or result from x-cells and (2) the degenerating condition of some x-cells as observed in the present study and also noted previously by Peters et al. (1978, 1981) and by Watermann (1982). These observations suggest that in many cases the material examined was taken from well-advanced or even regressing lesions, with any



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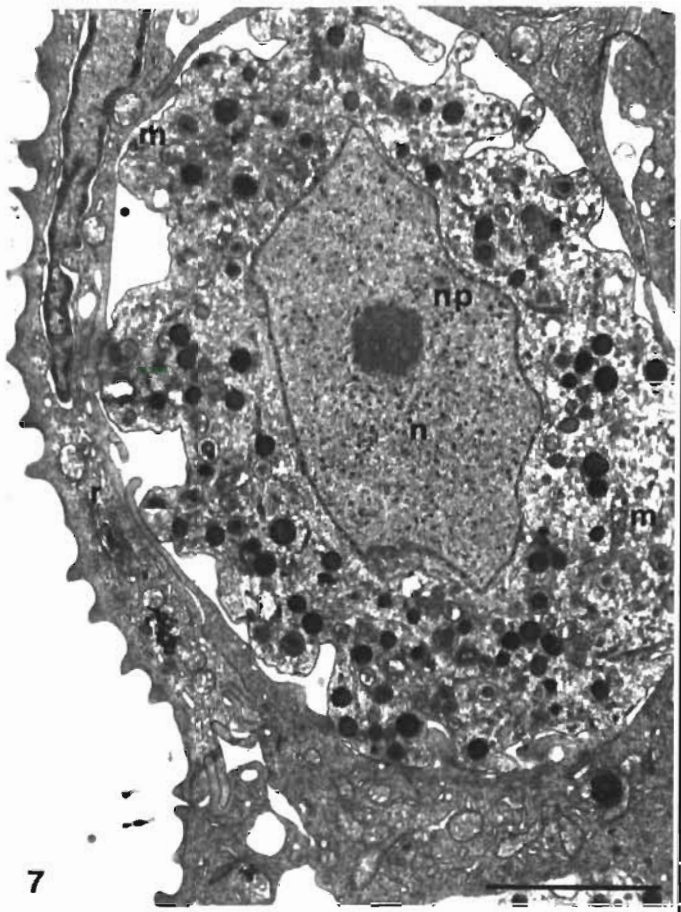

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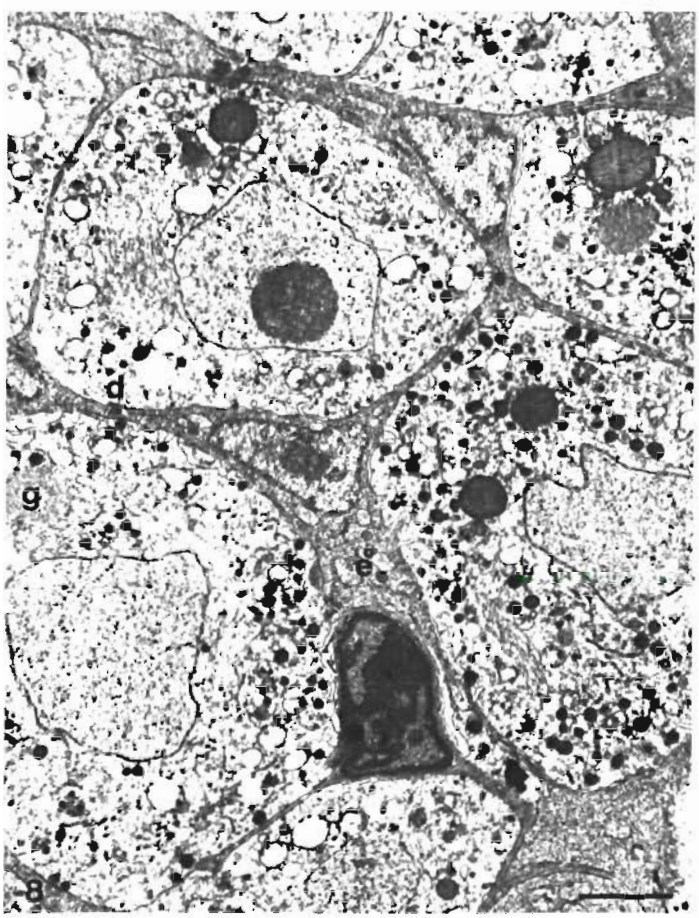

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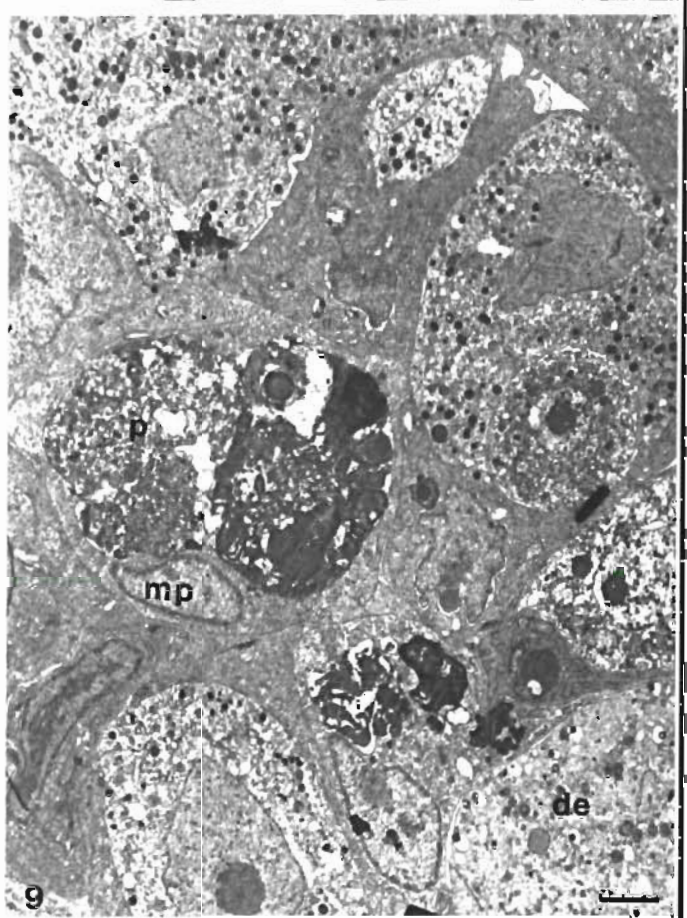

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structural differences found being attributable largely to varying stages of cell necrosis. However, there is now increasing evidence that x-cells are of non-fish origin. Dawe (1981) reported that the DNA content of x-cells in the material he studied was about one third that of fish cells, that lesion material showed an extra isozyme for each of 4 enzyme systems studied, and that such material yielded a protein not present in reference fish tissues. He also reported that many cells were multinucleate, and that frequent mitoses occurred in synchrony. From these data Dawe concluded that x-cells were probably protozoan. Similarly, the mitotic activities shown by Watermann (1982) were also thought to show numerous similarities to special mitotic activities of amoeba.

As the cause of dab gill swelling was unknown at the time of sampling, material suitable for repeating the foregoing studies was not collected. However, several observations in the present study also suggest that x-cells are foreign in nature. The prominence of nuclear pores, appearance of the outer membrane, and the mitochondrial structure differed substantially from normal fish cells. Also, unlike gill cells which form junctions with each other, unencapsulated x-cells did not form intimate connections (desmosomes, tight junctions, interdigitation of extensions) with gill cells. Many were apparently migrating through the sub-epidermal tissues of the gills. The ability of x-cells to form in different tissues, or to migrate from one tissue to another is deduced from the occurrence of the same cells in dermis of the dab (Watermann 1982) and from their presence in the dermis and epidermis of Pacific flatfish (Wellings et al. 1976).

The highly selective degeneration of x-cells within dab gill lesions also strongly suggests differences from adjacent recognizable fish cells. However, fish gill tissue typically responds to the presence of parasitic infection by cell proliferation, focal necrosis, oedema, severe inflammation or more extensive necrosis (Roberts 1978) and the absence of such characteristic pathology associated with dab gill x-cells suggests that caution is warranted in interpreting x-cell as parasites. Morrison et al. (1982) noted that in cod pseudobranchs, microsporidians and other parasites elicited invasion of the affected area by phagocytes and that this was followed by formation of a fibrous capsule. However, they suggested that the envelope cells might be similar to fibroblasts and that they represented a form of host reaction to x-cells. In dab, general ultrastructural characteristics of envelope cells, in particular, the nature of the nuclei, mitochondria, desmosomes, and prominent tonofilaments together with the lack of associated collagen, suggest that they are modified dab cells, possibly epithelial in nature. Consequently, they may be derived from the sub-epithelial proliferative layer of the gills. Alpers et al. (1977) considered the envelope cells of cod pseudobranch tumours to be similar to Schwann cells or myoepithelial cells and it is noteworthy that Peters et al. (1983) considered that the x-cells of the skin papilloma of starry flounder were separated from each other by long, wing-like extensions of Malpighian cells. Such cellular reactions clearly do not fit the normal pattern of fish cellular defence responses to disease agents or tissue damage and warrant further careful and considered investigation. It was noteworthy in the present study that macrophages were present in low numbers and were principally involved with areas of degenerating x-cells. It is possible they were responding to foci of necrosis and tissue leakage, rather than specifically to x-cells. In contrast, Watermann (1982) found x-cells associated with massive inflammatory lesions of subcutaneous connective tissue of dab; Brooks et al. (1969) and Alpers et al. (1977) described macrophage involvement in epidermal tumours of flounders and in pseudobranch tumours of Pacific cod, respectively. These differences have not been resolved but may reflect the age of lesions or the involvement of other agents in the lesions. The studies by Dawe (1981) provided the most convincing evidence that x-cells are protozoan and clearly future research in different species of fish may by fruitfully directed towards investigation of nuclear division in tissue smears. However, it is possible that specific diagnosis of the cell may still be dependent on their successful culture or transmission.

The prevalence of x-cell gill lesions of dab was sufficiently high for the disease to be among those more frequently encountered in the North Sea. No direct reason can be suggested for the areas of higher prevalence but it may be significant that the Dogger Bank

Fig. 6 to 9. *Limanda limanda*. Electron microscopy of gill x-cell lesions. Fig. 6. Low magnification showing relation between x-cell lesion and circulatory components of the primary and secondary lamellae of the gills. pc: pillar cell of secondary lamellae. Bar = 5 µm. Fig. 7. Isolated x-cell beneath the respiratory epithelium (r) partially surrounded by enveloping cells, showing pseudopodia-like extensions and containing a variety of different vesicle types. Mitochondria (m) are spherical with tubular cristae. The nucleus (n) contains a dense nucleolus and prominent nuclear pores (np). Bar = 2 µm. Fig. 8. Typical area of dab gill x-cell lesion showing uniformity of ultrastructure of x-cells and the disposition of enveloping cells (e) closely attached to each other by demosome junctions (d) and interdigitated processes (arrowed). Granular areas (g) were present in the cytoplasm of many x-cells. Bar = 2 µm. Fig. 9. Central region of large x-cell lesion. Macrophages (mp) with large phagocytic vacuoles (p) are present and one is closely applied to a degenerating x-cell (de). Bar = 2 µm

has been shown to be an area of high prevalence of other external lesions, especially *Lymphocystis*, ulcerations, and epidermal hyperplasia/papilloma (Dethlefsen 1984). Further information is being sought on the wider geographical and seasonal distributions of dab gill x-cell lesions (Knust & Dethlefsen 1986). Although gill swelling was occasionally sufficiently advanced in some severely affected fish to prevent the opercula from closing, and to totally obliterate secondary lamella in extensive areas of the gills, clearly to the detriment of respiratory efficiency, no gross changes in the overall condition of affected fish were detected during the present survey. However, additional detailed data on growth, condition factor, and reproductive capacity of more extensive samples of affected dab are being analysed by Knust & Dethlefsen (1986).

Acknowledgements. We thank the captain and crew of the RV *Anton Dohrn* for excellent cooperation.

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Responsible Subject Editor: Dr. T. Evelyn; accepted for printing on May 22, 1987